Integrative and Comparative Biology

Integrative and Comparative Biology, pp. 1–17
doi:10.1093/icb/icz017

Society for Integrative and Comparative Biology

SYMPOSIUM

Does Cellular Metabolism from Primary Fibroblasts and Oxidative Stress in Blood Differ between Mammals and Birds? The (Lack-thereof) Scaling of Oxidative Stress

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From the symposium "Beyond the powerhouse: integrating mitonuclear evolution, physiology, and theory in comparative biology" presented at the annual meeting of the Society of Integrative and Comparative Biology, January 3–7, 2019 at Tampa, Florida.

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Synopsis As part of mitonuclear communication, retrograde and anterograde signaling helps maintain homeostasis under basal conditions. Basal conditions, however, vary across phylogeny. At the cell-level, some mitonuclear retrograde responses can be quantified by measuring the constitutive components of oxidative stress, the balance between reactive oxygen species (ROS) and antioxidants. ROS are metabolic by-products produced by the mitochondria that can damage macromolecules by structurally altering proteins and inducing mutations in DNA, among other processes. To combat accumulating damage, organisms have evolved endogenous antioxidants and can consume exogenous antioxidants to sequester ROS before they cause cellular damage. ROS are also considered to be regulated through a retrograde signaling cascade from the mitochondria to the nucleus. These cellular pathways may have implications at the whole-animal level as well. For example, birds have higher basal metabolic rates, higher blood glucose concentration, and longer lifespans than similar sized mammals, however, the literature is divergent on whether oxidative stress is higher in birds compared with mammals. Herein, we collected literature values for whole-animal metabolism of birds and mammals. Then, we collected cellular metabolic rate data from primary fibroblast cells isolated from birds and mammals and we collected blood from a phylogenetically diverse group of birds and mammals housed at zoos and measured several parameters of oxidative stress. Additionally, we reviewed the literature on basal-level oxidative stress parameters between mammals and birds. We found that mass-specific metabolic rates were higher in birds compared with mammals. Our laboratory results suggest that cellular basal metabolism, total antioxidant capacity, circulating lipid damage, and catalase activity were significantly lower in birds compared with mammals. We found no body-size correlation on cellular metabolism or oxidative stress. We also found that most oxidative stress parameters significantly correlate with increasing age in mammals, but not in birds; and that correlations with reported maximum lifespans show different results compared with correlations with known aged birds. Our literature review revealed that basal levels of oxidative stress measurements for birds were rare, which made it difficult to draw conclusions.

Introduction

Mitonuclear communication is at the heart of metabolic regulation, especially in fundamental processes such as cellular respiration (Sunnucks et al. 2017). All endothermic organisms have evolved high metabolic rates for increased heat production (Hulbert et al. 2007). However, birds and mammals evolved endothermy independently of each other, and demonstrate some stark differences (Hulbert et al. 2007). Birds live significantly longer lives compared with mammals of similar body size (see Jimenez [2018] and references therein), despite having higher metabolic rates (Fig. 1A), body temperatures, and blood glucose concentration (Ricklefs 2010). The

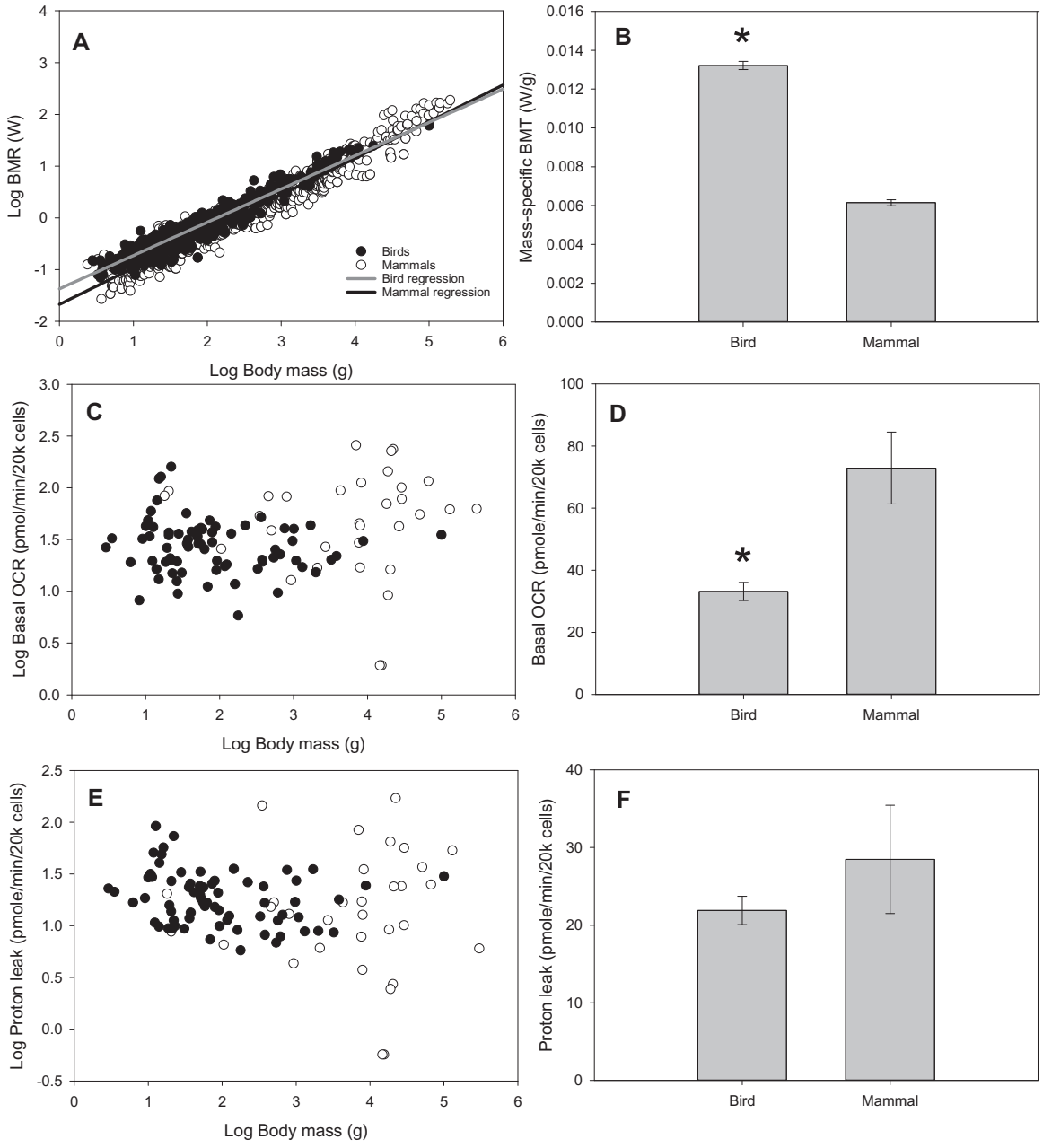

Fig. 1 (**A**) Mammalian and bird whole-animal BMR showed a significant positive correlation with body size (mammals: $y = 0.70665x - 1.6718$, $P < 0.0001$, $r^2 = 0.945$; birds: $y = 0.6430x - 1.3702$, $P < 0.0001$, $r^2 = 0.934$). (**B**) Mass-specific metabolism was significantly higher in birds compared with mammals ($F = 753.4$, $P < 0.0001$). (**C**) Basal OCR showed no correlation with body size in either mammals ($y = -0.0029x + 1.6673$, $P = 0.9751$, $r^2 = 0.0000329$) nor birds ($y = -0.0405x + 1.501$, $P = 0.2589$, $r^2 = 0.0177$). (**D**) Basal OCR was significantly higher in mammals compared with birds ($F = 18.05$, $P < 0.0001$). (**E**) Proton leak did not correlate with body size in either group (mammals: $y = 0.0095x + 1.0910$, $P = 0.9293$, $r^2 = 0.0003$; birds: $y = -0.0654x + 1.3835$, $P = 0.0709$, $r^2 = 0.0479$). but (**F**) proton leak was not different between mammals and birds ($F = 1.46$, $P = 0.228$).

underlying physiological mechanisms that explain differences between mammals and birds are varied, and include differences at tissue- and cell-levels. For both of these groups, mass-specific basal metabolic rate (BMR) decreases with body size and body size accounts for much of the variation in BMR, however, much variation among species still remains to be explained (Raichlen et al. 2010). Because BMR is defined fundamentally as the sum of tissue metabolic rates, it follows that variation in BMR may relate to the relative size of central organs (Daan et al. 1990; Konarzewski and Diamond 1995; Piersma et al. 1996; Hammond et al. 2000; Nespolo et al. 2002; Brzek et al. 2007; Wiersma et al. 2012; but see Tieleman et al. 2006). Krebs (1950) demonstrated that O_2 consumption of liver slices from mice was

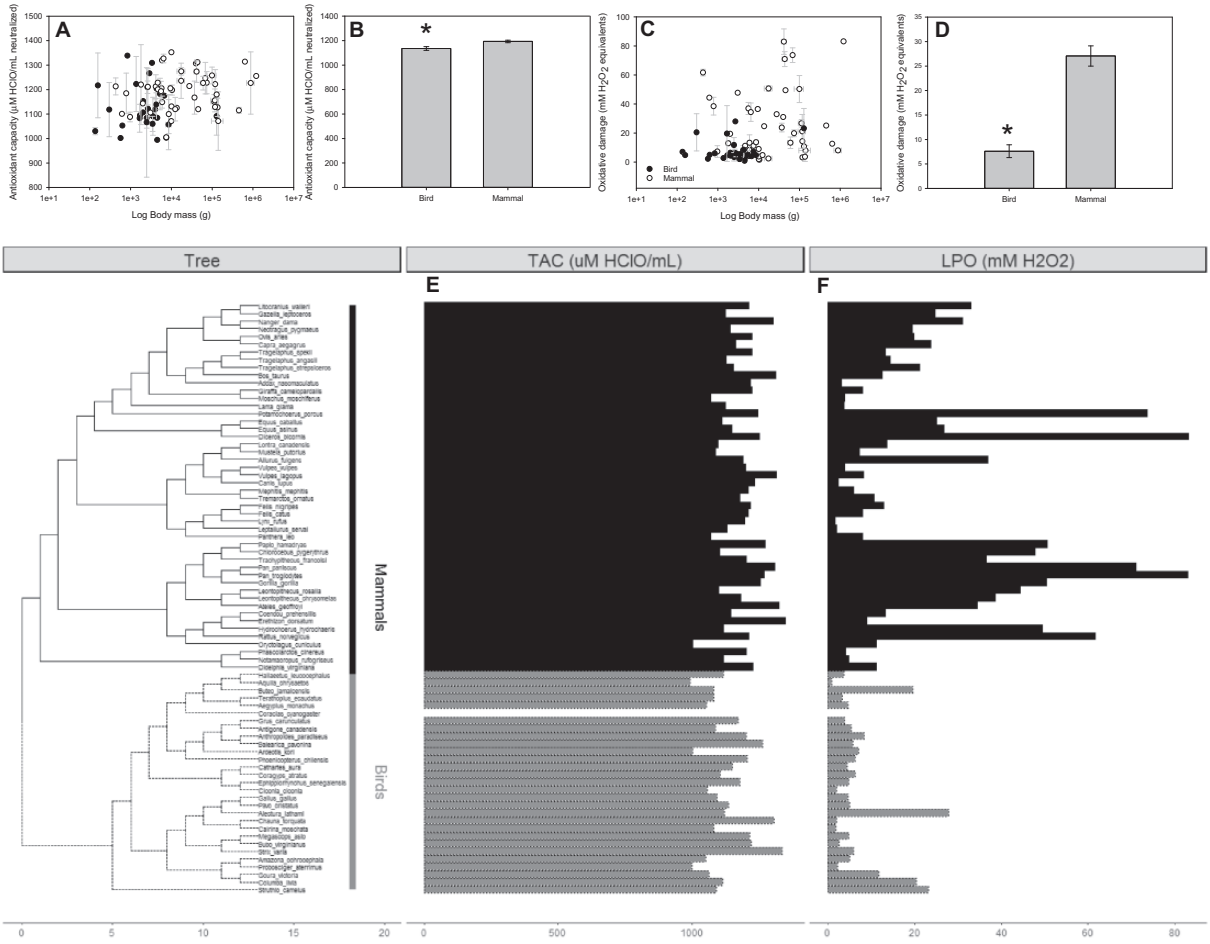

Fig. 2 (**A**) Linear models showed that TAC does not change with body mass ($F = 1.45$, $P = 0.230$), but (**B**) mammals have a 4.8% higher TAC than birds ($F = 7.13$, $P = 0.009$). (**C**) LPO damage increases with body mass when both groups are considered together ($\beta = 0.02 \pm 0.01$; $F = 8.12$, $P = 0.005$) and (**D**) mammals have 194% more LPO damage than birds ($F = 13.3$, $P < 0.001$). Phylogenetically-corrected analyses support these results and showed that phylogeny explains much of the variation in LPO damage (**F**), but not TAC (**E**), among species.

7.4 times higher than that of liver slices from horses, a result supported by work of Porter and Brand (1995) on hepatocytes. Additionally, different tissues scale differently in animals, so that mouse brain and kidney oxygen consumption are twice that of horse brain and kidney, but differences in liver and spleen mass are four-fold in these two species (Krebs 1950), implying that the size of the tissue may make up for tissue-wide differences within whole-animal BMR. Thus, it may follow that the remaining variation in BMR between mammals and similar-sized birds is explained by relative organ sizes.

Alternatively, cellular machinery of the tissues of birds and mammals may differ. Metabolic intensity of tissues is thought to vary because of differences in numbers of mitochondria within cells (Else and Hulbert 1985; Suarez 1996; Moyes 2003), concentrations of metabolic enzymes (March and Dawson 1982; Garrido et al. 1996; Vezina and Williams 2005), activity or quantity of the membrane sodium–potassium ATPase pump (Wu et al. 2004; Jimenez et al. 2013), and the number of double bonds in fatty acids of cell membranes (Hulbert and Else 2005; Brzek et al. 2007). At the cell-level, West et al. (1997, 2003) argued that metabolic scaling followed a fractal-like design, and predicted that the metabolic rate of cells isolated from an animal would be uniform and independent of animal body mass. Others have similarly argued that cells in culture would converge to similar rates of energy assimilation due to standardized media (Wheatley 2007). To test this hypothesis, Brown et al. (2007) measured oxygen consumption in primary dermal fibroblasts of 10 species of different-sized mammals and found no correlation between cellular metabolism and body size. On the other hand, the quantum metabolism theory, which attempts to infer whole organism metabolic rates from the metabolic activities of

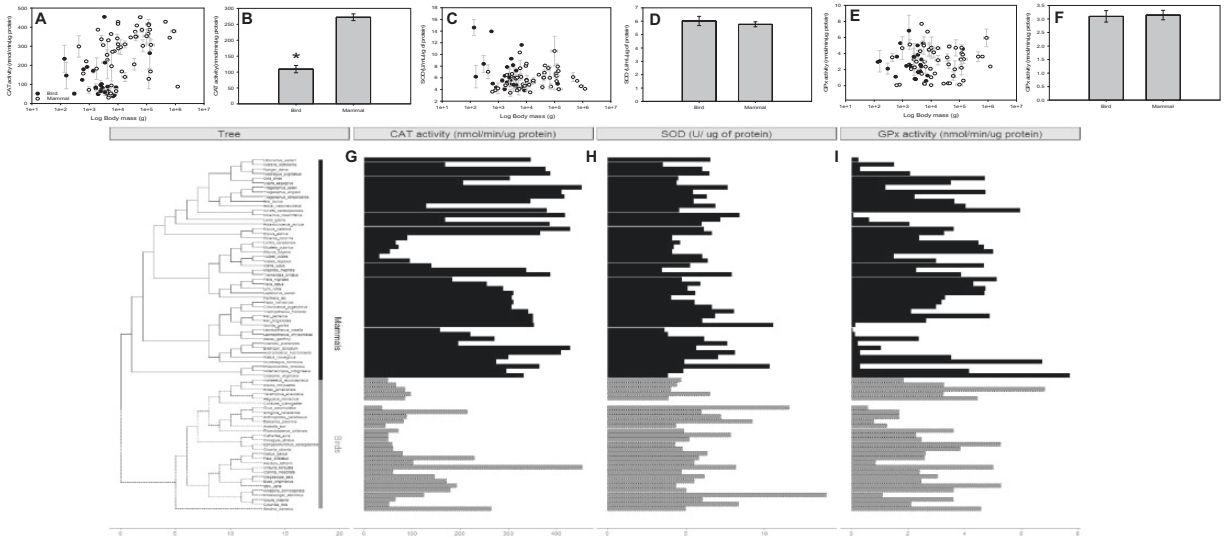

Fig. 3 (**A**) Traditional linear mixed effects models showed that CAT activity increases with body mass when both groups are considered together ($\beta = 0.01 \pm 0.06$; $F = 24.7$, $P < 0.001$) and (**B**) mammals have a 139% higher CAT activity than birds ($F = 24.3$, $P < 0.001$); that GPx activity did not change with body mass ($F = 0.32$, $P = 0.001$, **C**) or class ($F = 0.13$, $P = 0.73$, **D**); and that SOD activity did change with body mass ($F = 0.01$, $P = 0.92$, **E**) or with class ($F = 0.24$, $P = 0.63$, **F**). Phylogenetically-informed analyses supported these results and showed that phylogeny accounts for 0.48–0.77% of variation in CAT activity (**G**), 8–16% of variation in SOD activity (**H**), and 29–46% of variation of GPx activity (**I**).

component cells, posits that cells isolated from larger animals with low rates of whole-organism metabolism will maintain a low rate of cellular metabolism (Demetrius 2006). However, direct comparisons of cellular metabolism between birds and mammals are largely unexplored.

Because of differences in whole-organism metabolic rate, we may also expect differences within the rates of cellular processes, including oxidative stress, that contribute to differences in the metabolism of birds and mammals is oxidative stress. Broadly defined, oxidative stress is the balance between pro-oxidants produced during aerobic metabolism mainly by mitochondria, and antioxidants, enzymatic and non-enzymatic molecules capable of thwarting pro-oxidants before cellular damage occurs (Skrip and McWilliams 2016). Pro-oxidants in the form of reactive oxygen species (ROS) cause damage to cells. If ROS production becomes unabated, they will cause oxidative damage to proteins, DNA, and lipids. Lipids are among the molecules most affected, and two of the most prevalent ROS that can initiate damage to lipid membranes are hydroxyl radicals (OH•) and hydroperoxyl radicals (OOH•) (Ayala et al. 2014). The process of lipid peroxidation (LPO) continues unabated until the propagation of damage is halted by an antioxidant molecule (Halliwell and Chirico 1993; Ayala et al. 2014). Enzymatic antioxidants, such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), function by catalyzing the oxidation of less biologically insulting molecules. Other antioxidant molecules, such as Vitamins E and C, act as chain-breaking antioxidants; they scavenge for ROS, remove them once they are formed, and further halt propagation of peroxidation (Halliwell and Chirico 1993). The concept of oxidative stress has been implicated as the underlying cellular determinant for life-history trade-offs (Dowling and Simmons 2009) and thus it can potentially have cellular effects on aging and growing rates, which may be linked to aerobic function (Jimenez 2018).

When comparing birds and mammals in the context of oxidative stress, much of the previous work has focused on determining physiological mechanisms that may allow for longer lifespans in birds of a given mass despite their higher metabolic rate. The infamous "rat–pigeon" comparison has led the way in determining potential cellular mechanism that may allow for the longer lives of birds, a seemingly concomitant feature of lower BMR (Ku and Sohal 1993; Barja et al. 1994; Montgomery et al. 2011). Several studies highlight that mitochondria isolated from brain, heart, and kidney of the pigeon had significantly lower production of O_2^- and H_2O_2 compared with the rat, whereas only SOD activity increased in the pigeon compared with the rat (Ku and Sohal 1993). Surprisingly, CAT activity was significantly higher in the rat compared with the pigeon (Ku and Sohal 1993), potentially providing a

mechanism for oxidative stress differences across mammals and birds. A re-visit of this comparison demonstrated that most oxidative stress parameters between the rat and the pigeon may not be different at all, but that cellular membrane composition could lead to differences in rates of oxidative damage accumulation (Montgomery et al. 2011). Although this long-standing comparison has been useful, it lacks a broad phylogenetic perspective into the matter of oxidative stress differences between birds and mammals. To bridge the gap from a two-species comparison, here, we utilize the concept of scaling across three levels of organization: whole-animal, cellular, and enzymatic, to determine body size relationships in components of oxidative stress and to compare how these relationships differ between birds and mammals. We collected whole-animal BMR data from the literature, collected published and new cellular metabolic rate data on primary fibroblasts isolated from birds and mammals, and collected new and published oxidative stress data from blood products on a phylogenetically diverse group of birds and mammals, under "basal" conditions to shed light on whether higher metabolic rates of birds come at an increased cost due to oxidative stress.

Materials and methods

Whole-animal BMRs

We compiled body masses (g) and BMR (W) from birds and mammals from the literature following Johnson et al. (2018). Briefly, we compiled data on whole-animal BMRs and body masses from the primary literature and several existing reviews (White et al. 2006; Makarieva et al. 2008; Sieg et al. 2009; Londono et al. 2015; Uyeda et al. 2017). New data (i.e., those not previously included in a review) were only included if they were collected from individuals that thermoneutral, resting, and post-absorptive and sample sizes $\geq$3 (McKechnie and Wolf 2004). We converted VO_2 consumed to heat production using a conversion factor of 20.1 J/mL O_2 and a respiratory quotient (RQ) of 0.72. In their study, Gessaman and Nagy (1988) examine the best conversion factor for converting respiration data to energetic equivalents. The RQ of 0.72 we used empirically has the smallest error with "actual" nutrient catabolism across diet types and nitrogenous waste end product; we converted VCO_2 produced using a factor of 27.3 J/mL CO_2 and an RQ of 0.72 (Gessaman and Nagy 1988). Measures of heat conversion were then converted into watts. Mass-specific metabolic rates were multiplied by body mass to obtain whole-animal metabolic rates. When possible we used the body mass from the same study from which we obtained BMR, otherwise we used a published species mean.

Cellular metabolic rates on fibroblasts

We compared whole-organism metabolic rate data with cellular-level data. To do so, we compiled measured cellular metabolic rate data, specifically basal oxygen consumption rate (OCR) and proton leak, from primary fibroblast cells isolated from wild birds (Jimenez et al. 2014; Jimenez and Williams 2014), dogs, and rats (Jimenez et al. 2018a, 2018b; Winward et al. 2018). We further measured cellular metabolic rates from several mammalian cell lines purchased from the Aging Cell Depository of the National Institute on Aging maintained at the Coriell Institute for Medical research in Camden, New Jersey (Supplementary Table S1). We used a Seahorse XF96e oxygen flux analyzer at Colgate University (Agilent Scientific Instruments) as in Jimenez et al. (2014). In brief, we grew primary fibroblast obtained from Coriell. We then plated these primary fibroblasts on a 96-well plate from Agilent technologies and allowed them to attach overnight in an atmosphere of 37°C, 5% O_2/5% CO_2. Each cell line was plated in duplicate. OCRs were determined using XF-96 FluxPaks from Agilent Technologies. We measured OCRs after cells were equilibrated to running media for 1 h, which contained 10 mM glucose, 1 mM sodium pyruvate, and 2 mM glutamine, pH =7.4. Baseline measurements of OCRs were made three times prior to injecting a final well concentration of 2 μM oligomycin, to obtain a proxy for proton leak. After measurements were completed, we trypsinized cells from each well and used a Countess II FL cell counter to count the actual final concentration of cells in each well and normalized all rates to 20,000 cells (Jimenez et al. 2018a, 2018b; Winward et al. 2018).

It should be noted that all cellular measurements for birds, dogs, and rats were done at Passage 2 (P2), and that these cells were all grown in our laboratory (Jimenez and Williams 2014; Jimenez et al. 2014, 2018a, 2018b; Winward et al. 2018). However, cellular measurements from mammalian cells purchased from the Coriell Institute were measured at various passages based on availability of the cell line (Supplementary Table S1). Morphology of primary fibroblast changes with passage number (Calhoon et al. 2014), which may have implications for cellular metabolic rates. Although other have testes the effect of passage number on cellular metabolic rates and found no significant changes across passage numbers (Brown et al. 2007). There is no accepted way to

correct for differences in passage numbers, thus, we did not correct for passage number.

Oxidative stress measurements in blood from captive birds and mammals

We collected blood samples from zoos and veterinarians while animals were under "basal" conditions. We defined basal as "adult, nonbreeding, nonmanipulated (e.g., no LPS, no diet change, no exercise, etc.), chronically non-stressed, resting animals." Blood samples were collected, spun immediately to separate plasma from red blood cells (RBCs), and frozen immediately. Samples were transported to our laboratory at Colgate University on dry ice and stored at −80°C until used. For each individual, we collected information regarding body mass, sex, and age. We collected blood samples from 28 bird species, and 48 mammal species (Supplementary Table S2). We recognize that zoo animals may not face the same physiological requirements as their wild counterparts, however, the general oxidative stress physiology of each species should still be similar between wild and captive populations.

We determined circulating antioxidant capacity using the OXY-adsorbent test as the ability of plasma to neutralize hypochlorous acid (Diacron International, Grosseto, Italy; Costantini 2011); and measured oxidative damage as the presence of circulating hydroperoxides, including products of lipid oxidation, using the d-ROMs test in plasma (Diacron International; Costantini 2011). Using these methods, we aimed to measure circulating whole-organism markers of oxidative stress.

To estimate CAT (Cat. No. 707002), SOD (Cat. No. 706002), and GPx (Cat. No. 703102) activities in RBCs, we used commercially available kits (Cayman Chemical Company, Ann Harbor, MI, USA). We used 2 µL of RBC into 198 µL of 20 mM HEPES, 1 mM EGTA, and 90 mM mannitol buffer solution. After dilution, samples were vortexed prior to each assay. We then followed the manufacturer's protocol to determine each of these enzyme activities. All enzyme assays were run on the same day as sample dilution. Furthermore, we quantified total protein in each diluted RBC sample using a protein determination kit (Cayman Chemicals Cat. No. 704002) to standardize from sample to sample.

Literature review of oxidative stress in birds and mammals

We, first, used Web of Science for papers to compile a list of primary literature sources published prior to June 1, 2018. Searches included papers of total antioxidant capacity (TAC); ROS production; SOD, CAT, and GPx; LPO damage; DNA oxidative damage; and protein carbonyl damage in birds and mammals. We excluded the terms "chicken, turkey, rats, and mice" from all of our searches due to the high volume of literature in this topic including these usually laboratory-reared species. We also used the "cited by" function for googlescholar.com to find any additional papers, not included in our original search. After each search, we inspected all resulting abstracts and retained papers that included animals at basal conditions, as defined above. We compiled data for each parameter and classified it by body mass (g), age of individuals (if given), and captive/non-captive. We standardized to common units for each parameter. The most commonly measured tissue in the literature was blood, thus we report our findings on this tissue and its component parts. We excluded any data points from species with vastly varying body masses within the species (such as dogs), when the original weight of the animals used was not reported. For all other species, we obtained an average body mass of the species from AnAge when body masses were not reported. We obtained averages of each parameter per species prior to statistical analysis.

Statistics

Whole-animal metabolism and cellular metabolic rates on fibroblast

Whole-animal metabolic rates gathered from the literature and cellular metabolic rates were analyzed using a linear regression as a function of body size, first. Then, we used an ANOVA to test differences between mass-specific metabolic rates of mammals and birds, and an ANCOVA to test differences between mammals and bird for basal cellular metabolism and proton leak.

Oxidative stress—zoo samples

We performed phylogenetically-informed general linear mixed models that accounted for within species variation to determine whether taxonomic class (bird vs. mammal), species mean body mass (species mass), within species deviation in body mass from the mean (individual mass), and the interaction between mean mass and taxonomic class predicted TAC, GPx, CAT, SOD, and LPO. Body mass was $\log_{10}$-transformed to improve the normality of its distribution. Models were fit using the MCMCglmm package in Program R v.3.5.1 (Hadfield and Nakagawa 2010; Hadfield 2010; R Development Core Team 2018). The phylogenetic

covariance matrix for this analysis was estimated based on a phylogenetic tree constructed using NCBI molecular data and phyloT (Letunic 2015). Polytomies were excluded using the randomization process in phyloT. Using this tree to model phylogenetic dependence, all mixed models were fit using a weak inverse-Gamma prior with shape and scale parameters set to 0.01 for the random effect of phylogenetic variance. Default priors for all other fixed effects were used. Model chains were run for 7.8×10^5 iterations, an 180,000 iteration burn-in, and a 600 iteration thinning interval. Chain length was sufficient to yield negligible autocorrelation. We took an information theoretic framework to determine which parameters were important predictors for each response variable. Relative support for each model was determined based on deviance information criterion (DIC) values and differences among models (ΔDIC). Models within five ΔDIC of the top model were considered indistinguishable and informative, and those greater than 10 ΔDIC of the top model were not informative.

We estimated the importance of phylogenetic signal as lambda (de Villemereuil and Nakagawa 2014). We then calculated marginal R^2 and conditional R^2 following Nakagawa and Schielzeth (2013). The marginal R^2 describes how much of the total variation was explained by the fixed effects included in a particular model, whereas the conditional R^2 describes how much variation was explained by the complete models (i.e., both fixed and random effects).

For comparative purposes, we also performed linear mixed effects models without phylogenetic corrections for TAC, GPx, CAT, SOD, and LPO. Log_{10}-transformed body mass and taxonomic class were included as fixed effects and species was included as a random effect to account for multiple samples from the same species. In preliminary analyses, we tested for an interaction between taxonomic class and body mass, but did not include it in final analyses because it was not significant in any model and did not improve fit of any model. These models were fit using package nlme in Program R (Pinheiro et al. 2011).

Oxidative stress—literature data

Data from every assay were first tested for normality using a Kolmogorov–Smirnov test. If not normal, the data were log-transformed and re-tested using a Kolmogorov–Smirnov test prior to other statistical analyses to meet assumptions of an ANCOVA. Because of the small number of published studies on basal oxidative stress, we were only able to compare bird and mammal data for TAC, GPx, CAT, SOD, and LPO damage from the literature. Log transforming did not normalize GPx activity data, thus, we ran an independent samples Mann–Whitney *U*-test.

Age and maximum lifespan

When accurate ages were provided for samples from zoos, we used linear regressions between age and each of the parameters we measured above ($N = 28$ species of birds and $N = 43$ species of mammals). Additionally, we used linear regressions to correlate maximum lifespan (MLSP) collected from AnAge (Tacutu et al. 2012, 2018) and each of the parameters measured above ($N = 21$ species of birds, and $N = 45$ species of mammals; Supplementary Table S2).

Results

Whole-animal metabolic rates

Log-transformed mammalian and bird whole-animal BMR showed a significant positive correlation with body size (mammals: $y = 0.706\times -1.671$, $P < 0.0001$, $r^2 = 0.945$; birds: $y = 0.643\times -1.370$, $P < 0.0001$, $r^2 = 0.934$). Mass-specific metabolism was significantly higher in birds compared with mammals ($F = 753.4$, $P < 0.0001$; Fig. 1B).

Cellular metabolic rates on fibroblasts

Log-transformed basal OCR showed no correlation with body size in either mammals ($y = -0.0029x + 1.6673$, $P = 0.9751$, $r^2 = 0.0000329$) nor birds ($y = -0.0405x + 1.501$, $P = 0.2589$, $r^2 = 0.0177$). Similarly, log-transformed proton leak did not correlate with body size in either group (mammals: $y = 0.0095x + 1.0910$, $P = 0.9293$, $r^2 = 0.0003$; birds: $y = -0.0654x + 1.3835$, $P = 0.0709$, $r^2 = 0.0479$). Basal OCR was significantly higher in mammals compared with birds ($F = 18.05$, $P < 0.0001$; Fig. 1D), but proton leak was not different between mammals and birds ($F = 1.46$, $P = 0.228$; Fig. 1F).

Oxidative stress

Samples collected from zoos

Phylogenetically-informed general linear mixed effects models. The list of informative models for LPO damage, TAC, CAT activity, GPx activity, and SOD activity included all of the potential models, including the null model (e.g., intercept only) (Table 1). For all of these response variables, all of the fixed effects in all of the models had 95% CIs overlapped zero and the marginal R^2 was low indicating that none of the fixed effects predicted the responses well

Table 1 DIC, ΔDIC, marginal and conditional R^2, and lambda values for models of lipid peroxidase damage (LPO, mM H_2O_2), TAC (μM HClO/mL), CAT activity (nmol/min/μg protein), SOD activity (nmol/min/μg protein), and GPx (nmol/min/μg protein) activity

Response variable	Model No.	Fixed effects	DIC	Delta DIC	Conditional R^2	Lambda	Marginal R^2
LPO	1	Null	1419.5	0.4	0.963	0.951	0
					(0.905–0.982)	(0.899–0.983)	(0, 0)
	2	Species mass + individual mass + class	1422.2	3.1	0.976	0.953	0.005
					(0.925–0.990)	(0.905–0.981)	(0–0.528)
	3	Class	1419.1	0.0	0.972	0.953	0.003
					(0.927–0.991)	(0.910–0.983)	(0–0.508)
	4	Species mass + individual mass	1422.9	3.8	0.968	0.947	0.000
					(0.890–0.980)	(0.879–0.982)	(0–0.013)
	5	Species mass + individual mass + class + species mass × class	1422.3	3.2	0.971	0.954	0.011
					(0.923–0.990)	(0.912–0.985)	(0.001–0.515)
TAC	1	Null	2317.67	0	0.001	0.104	0
					(0–0.300)	(0–0.300)	(0, 0)
	2	Species mass + individual mass + class	2320.779	3.3	0.059	0.059	0.997
					(0.003–0.380)	(0–0.290)	(0.088–0.999)
	3	Class	2317.511	0.0	0.049	0.046	0.995
					(0–0.341)	(0–0.230)	(0.021–1)
	4	Species mass + individual mass	2320.467	3.0	0.067	0.115	0.009
					(0–0.345)	(0–0.333)	(0–0.935)
	5	Species mass + individual mass + class + species mass × class	2321.1	3.6	0.084	0.068	0.996
					(0.013–0.397)	(0–0.293)	(0.127–1)
CAT	1	Null	2114.36	0	0.919	0.861	0
					(0.762–0.960)	(0.692–0.963	(0, 0)
	2	Species mass + individual mass + class	2116.284	1.9	0.916	0.765	0.010
					(0.752–0.977)	(0.400–0.958)	(0–0.648)
	3	Class	2114.716	0.4	0.934	0.859	0.002
					(0.802–0.984)	(0.629–0.972)	(0–0.603)
	4	Species mass + individual mass	2116.180	1.8	0.868	0.794	0.001
					(0.719–0.961)	(0.538–0.966)	(0–0.154)
	5	Species mass + individual mass + class + species mass × class	2116.9	2.5	0.934	0.768	0.018
					(0.747–0.979)	(0.377–0.971)	(0.002–0.634)
GPx	1	Null	699.107	0	0.481	0.206	0
					(0.284–0.688)	(0.001–0.575)	(0, 0)
	2	Species mass + individual mass + class	701.272	2.2	0.546	0.310	0.022
					(0.378–0.820)	(0.002–0.693)	(0–0.342)
	3	Class	699.587	0.5	0.531	0.291	0.002
					(0.302–0.787)	(0.001–0.655)	(0–0.316)
	4	Species mass + individual mass	701.043	1.9	0.507	0.234	0.001
					(0.319–0.721)	(0.001–0.599)	(0–0.111)
	5	Species mass + individual mass + class + species mass × class	701.9	2.8	0.549	0.320	0.044
					(0.343–0.814)	(0.001–0.680)	(0.006–0.387)

(continued)

Table 1 Continued

Response variable	Model No.	Fixed effects	DIC	Delta DIC	Conditional R^2	Lambda	Marginal R^2
SOD	1	Null	773.099	0	0.344	0.144	0
					(0.118–0.573)	(0.001–0.468)	(0, 0)
	2	Species mass + individual mass + class	776.782	3.7	0.428	0.238	0.021
					(0.177–0.719)	(0.001–0.621)	(0–0.426)
	3	Class	774.306	1.2	0.357	0.228	0.002
					(0.153–0.689)	(0.001–0.562)	(0–0.428)
	4	Species mass + individual mass	776.17	3.1	0.294	0.161	0.002
					(0.146–0.618)	(0.001–0.498)	(0–0.155)
	5	Species mass + individual mass + class + species mass × class	775.7	2.6	0.343	0.197	0.103
					(0.177–0.653)	(0.001–0.499)	(0.015–0.483)

Species mass and individual mass were $\log_{10}$ transformed.

(Table 1, Supplementary Table S3). Models for TAC had lambdas and conditional R^2 that were low or had large 95% CIs for all models indicating that the models had low explanatory power for TAC (Table 1). All of the models for LPO damage each explained >95% of the variation and had lambdas >0.95 damage models indicating that most of the variation explained by the models were explained by the phylogeny. Models for CAT activity explained >86% of the overall variation and phylogeny explained between was 0.76 and 0.87 that variation (Table 1). Models for GPx activity explained 48–55% of the overall variation and lambdas indicate that most of the variation explained by the model was explained by the phylogeny (Table 1). The models for SOD activity explained 34–43% of the overall variation, most of which was explained by phylogeny (Table 1).

Linear mixed effects models without phylogenetic corrections. LPO damage increases with body mass when both groups are considered together ($\beta = 1.67 \pm 2.69$; $F = 5.29$, $P = 0.023$) and mammals have 192% more LPO damage than birds ($F = 10.1$, $P = 0.002$). TAC does not change with body mass ($F = 1.4$, $P = 0.233$), but mammals have a 5.2% higher TAC than birds ($F = 7.16$, $P = 0.009$). CAT activity increases with body mass ($\beta = 33.7 \pm 15.2$; $F = 25.9$, $P < 0.001$) and mammals have a 141% higher CAT activity than birds ($F = 24.3$, $P < 0.001$). GPx activity did not change with body mass ($F = 0.35$, $P = 0.55$) or class ($F = 0.17$, $P = 0.68$). SOD activity did change with body mass ($F = 0.02$, $P = 0.90$) or with class ($F = 0.23$, $P = 0.63$).

Literature data

TAC capacity was not significantly different between a single bird species and mammals ($F = 2.774$, $P = 0.134$; Fig. 4A). LPO damage was also not significantly different between birds and mammals ($F = 0.191$, $P = 0.669$; Fig. 4B). CAT and SOD activities were not significantly different between birds and mammals ($F = 0.077$, $P = 0.783$; $F = 0.006$, $P = 0.941$, respectively; Fig. 4C, D), however, GPX activity is marginally higher in mammals compared with birds (Mann–U; $P = 0.059$; Fig. 4E).

Age

When TAC is correlated with age, there is a significant positive correlation in mammals (R^2 =0.042, $P = 0.020$, $r = 3.0831$; Fig. 4A), but not in birds ($R^2 = 0.039$, $P = 0.145$; Fig. 4A). LPO damage was not significantly correlated with age in birds (R^2 = 0.005, $P = 0.592$; Fig. 4B), but there was a positive significant correlation in mammals (R^2 =0.081, $P = 0.001$, $r = 0.8482$; Fig. 4B). We found a significantly negative correlation between age and CAT activity in birds (R^2 =0.081, $P = 0.035$, $r = -2.4431$; Fig. 4C), but no relationship between age and CAT activity in mammals (R^2 =0.016, $P = 0.157$; Fig. 4C), though there seems to be a positive trend with age. We see a marginally significant negative correlation between age and SOD activity in birds (R^2 =0.065, $P = 0.061$; Fig. 4D), but we see a significantly positive correlation between mammalian age and SOD activity (R^2 =0.065, $P = 0.002$, $r = 0.0703$; Fig. 4D). We found no correlation between bird and mammalian age and GPx

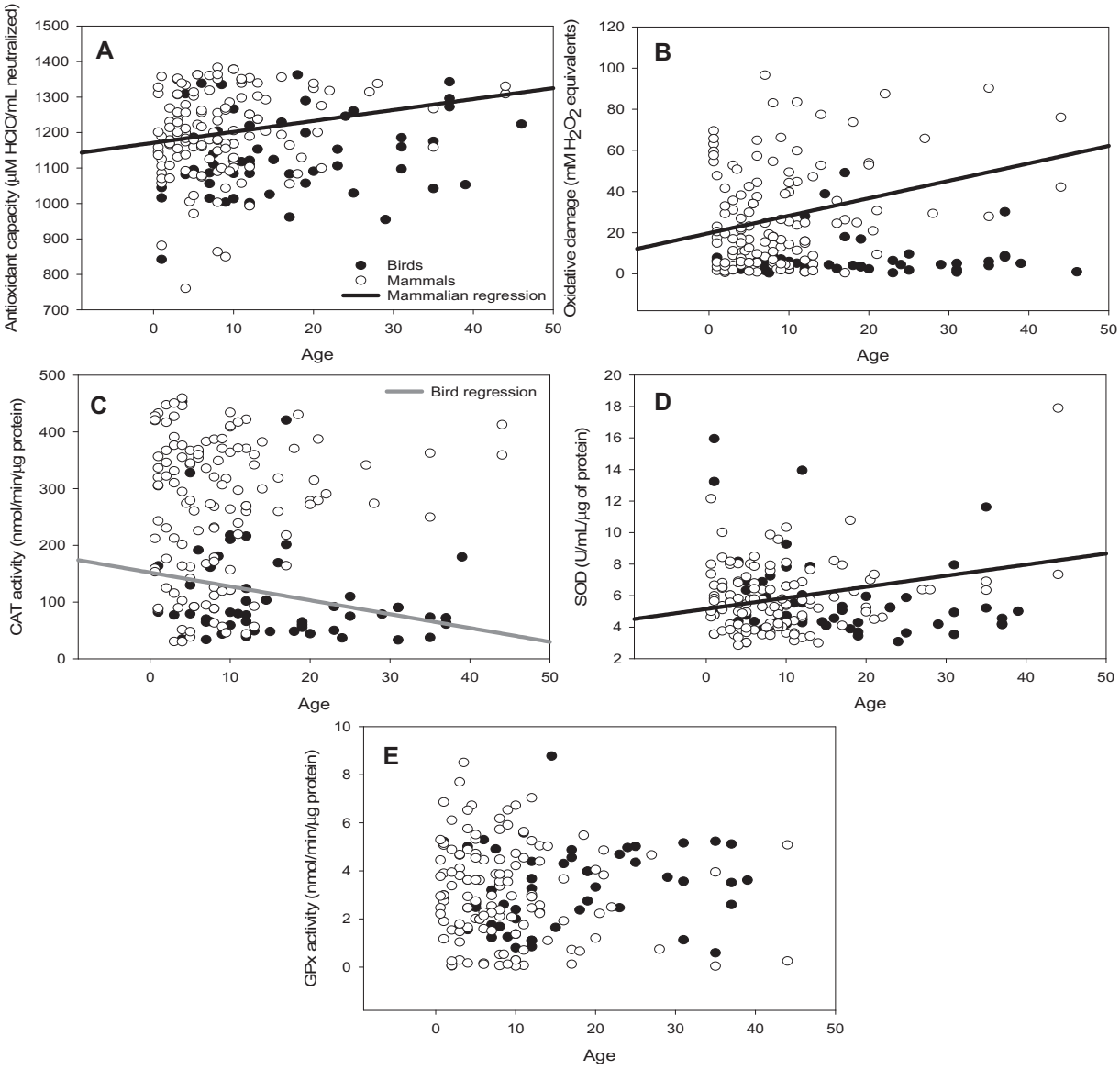

Fig. 4 Regression lines are included only when the relationship is significant. Black lines represent significant relationships in mammals, while gray lines depict significant relationships in birds. (**A**) When TAC is correlated with age, there is a significant positive correlation in mammals ($R^2 = 0.0424$, $P = 0.020$), but not in birds (r^2=0.039, $P = 0.145$). (**B**) LPO damage was not significantly correlated with in birds (r^2=0.0053, $P = 0.5923$), but there was a positive significantly correlation in mammals (r^2=0.0809, $P = 0.0010$). (**C**) There was a significant negative correlation between age and CAT activity in birds ($r^2 = 0.0811$, $P = 0.0351$), but no relationship between age and CAT activity in mammals ($r^2 = 0.0159$, $P = 0.1572$). (**D**) We see a marginally significant negative correlation between age and SOD activity in birds ($r^2 = 0.0646$, $P = 0.0612$), but we see a significant positive correlation between mammalian age and SOD activity ($r^2 = 0.0645$, $P = 0.0023$). (**E**) We found no correlation between bird and mammalian age and GPx activity ($r^2 = 0.0376$, $P = 0.1563$; $r^2 = 0.0141$, $P = 0.1842$, respectively).

activity (R^2 =0.038, $P = 0.156$; R^2 = 0.014, $P = 0.184$, respectively; Fig. 4E).

Maximum lifespan

There was a significantly negative correlation between bird TAC and MLSP (R^2 =0.353, $P = 0.005$, r =−3.7280; Fig. 5A), but no significant correlation between mammalian TAC and MLSP (R^2 =0.062, $P = 0.098$; Fig. 5A). Bird LPO damage and MSLP did not show a significant correlation (R^2 =0.065, $P = 0.264$; Fig. 5B). Mammal LPO was significantly positively correlated with MLSP (R^2 =0.1727, $P = 0.005$, r = 0.6778; Fig. 5B). There was no correlation between bird or mammal CAT activity and MLSP (R^2 =0.004, $P = 0.785$; R^2 =0.030, $P = 0.254$, respectively; data not shown). Bird and SOD activity were also not significantly correlated with MLSP (R^2 =0.046, $P = 0.352$; R^2 =0.079, $P = 0.0619$,

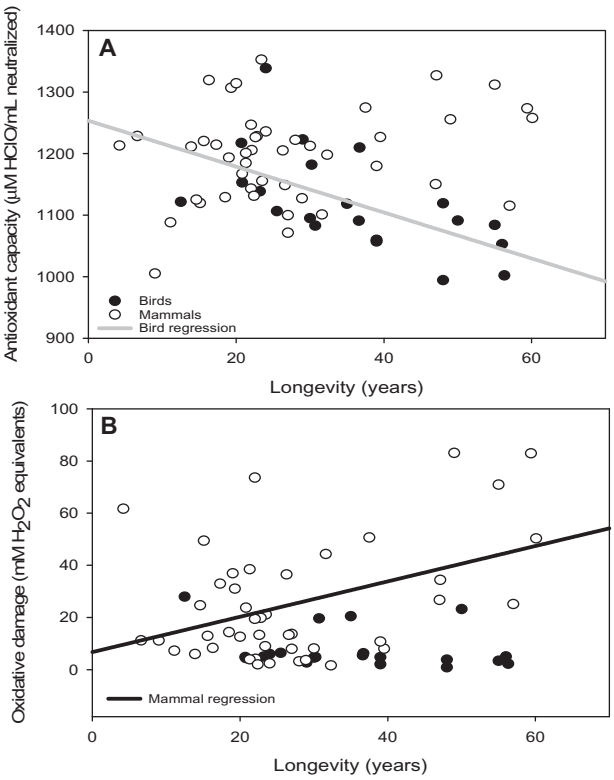

Fig. 5 Regression lines are included only when the relationship is significant. Black lines represent significant relationships in mammals, while gray lines depict significant relationships in birds. (**A**) There was a significant negative correlation between bird TAC and MLSP ($r^2 = 0.3531$, $P = 0.0045$), but no significant correlation between mammalian TAC and MLSP ($r^2 = 0.0622$, $P = 0.0984$). (**B**) Bird LPO damage and MSLP did not show a significant correlation ($r^2 = 0.0652$, $P = 0.2638$). Mammal LPO was significantly positively correlated with MLSP ($r^2 = 0.1727$, $P = 0.0045$).

respectively; data not shown). Bird and mammal GPx activity were not significantly correlated with MLSP (R^2 =0.002, $P = 0.857$; R^2 =0.010, $P = 0.506$; data not shown).

Discussion

Oxidative stress is a balance, inherent to all aerobic organisms, between the potential damage that could be accrued by ROS and the resources cells have to thwart that damage through the antioxidant system (Skrip and McWilliams 2016). This process has gained momentum in the ecological physiology literature because it has been implicated in determining rates of aging and life-histories trade-offs (Dowling and Simmons 2009). Here, we sought to quantify parts of the oxidative stress system in a diverse group of birds and mammals. Our question was two-fold: does oxidative stress (a product of aerobic respiration and thus BMR) scale with body mass in these two groups? And are there differences in oxidative stress between birds and mammals? Our study is novel in that it spans from whole-animal organization to a cellular process and incorporates phylogenetically-informed analyses of data from several different species of birds and mammals and because we focus on basal oxidative stress. Additionally, because our blood samples were collected from zoos, we were able to look at implications between the animal's age and its oxidative status, and match those predictions to each species' reported longevity.

Our first finding is that cellular metabolism and every parameter that we measured to quantify oxidative stress in birds and mammals does not scale with body mass. This implies that differences at the cellular level might make small contributions to scaling at the organ level, pointing to the fact that scaling of metabolism may reside in higher levels of organization (Agutter and Wheatley 2004). An obvious explanation may be that organ sizes between similarly-sized birds and mammals may be disproportionally larger in birds compared with mammals, leading to higher BMR. In birds, heart and kidney mass showed a positive correlation with BMR (Daan et al. 1991), and in even within a single species (mice), individuals selected for higher BMRs showed a positive correlation with the size of metabolically active organs including heart, kidney, liver, and small intestine (Konarzewski and Diamond 1995). Furthermore, tropical birds that demonstrate lower BMR have also been found to have significantly smaller metabolically active organs compared with temperate birds that have higher BMR (Wiersma et al. 2012). Interestingly, the total mass represented by metabolically active organs often seems negligible (17% according to Konarzewski and Diamond 1995). It follows that even small changes in the mass of metabolically active organs may have a tremendous impact on BMR costs. Heart size was two-fold greater in parakeets compared with similar-sized mice (Herrero and Barja 1998), and others have generalized this trend across multiple species (Schmidt-Nielsen 1984). Though it would be of interest to compare organ size data and BMR data for both mammals and birds.

Birds showed significantly lower basal cellular oxygen consumption, lipid oxidative damage, and lower activities of CAT. These results together imply several possible physiological mechanisms, none of which are mutually exclusive: (i) birds may have cells with significantly fewer mitochondria or with mitochondria that are more uncoupled; (ii) birds may be less burdened by ROS production compared with mammals; or (iii) birds may have membranes with

lower membrane polyunsaturation compared with mammals. To address the first point, pigeon heart and liver seem to have less mitochondrial protein per gram of tissue compared with rat tissue, but more mitochondrial protein in skeletal muscle compared with rats (Montgomery et al. 2011). Thus, mitochondrial concentration may be tissue dependent, and hard to generally quantify between birds and mammals. The efficiency of mitochondria between these two groups can be addressed. Comparisons between phylogenetically-paired species of tropical and temperate birds showed that tropical birds have significantly less mitochondrial lipid per cell, suggesting that tropical birds have fewer mitochondria per cell or less inner mitochondrial membrane (Calhoon et al. 2014). Cellular oxygen consumption of tropical birds is also significantly lower compared with temperate birds (Jimenez et al. 2014). Thus, these findings strongly imply that cellular metabolism is linked to mitochondrial content. To some, it may be surprising that birds demonstrate lower cellular oxygen consumption, especially considering that birds have higher whole-animal BMRs. Seventy percent of the cellular oxygen consumed in all cells in the presence of oligomycin is controlled by proton leak activity, however, the remaining 30% is controlled by the activity of the electron transport chain. A change in the activity or concentration of protein complexes in the ETC could also affect differences in basal OCR and proton leak (Kikusato et al. 2010). We calculated an index of mitochondrial efficiency by subtracting non-mitochondrial respiration from basal OCR and proton leak values, and then we calculated a ratio between basal OCR/proton leak. We found that birds had a ratio of 7.2 ± 1.6 and mammals had a ratio of 8.24 ± 3.27, though these ratios are similar, we may assume that the lower ratio belonging to birds could be due to a lower activity of the ETC compared with mammals, thus suggesting another explanation for birds' lower basal OCR compared with mammals.

Following the predictions of symmorphosis (Weibel et al. 1991) that birds demonstrate lower levels of TAC, low lipid oxidative damage, and low CAT activity indicates a potential decrease in ROS in birds compared with mammals. Differences in ROS production across species are still not well understood, but may include mechanisms such as efficiency of the ETC (as mentioned above), mitochondrial membrane composition, coenzyme Q amounts, and regulation of electrons at key entry points within the ETC (Csiszar et al. 2012). The long-studied rat–pigeon comparison demonstrates support for our general findings across different bird and mammal species. Mitochondria isolated from brain, heart, and kidney of the pigeon had significantly lower production of O_2 and H_2O_2 compared with the rat (Ku and Sohal 1993). In another study, mitochondria from liver, whole brain, and lungs were isolated from pigeons and rats and mitochondrial ROS were found to be significantly higher in the rat tissues compared with the pigeon (Barja et al. 1994). Resting State 4 respiration (ADP absent respiration) in mitochondria isolated from pigeon heart and nonsynaptic brain showed a significant decrease in free radical production compared with mitochondria isolated from rat (Herrero and Barja 1997). Similarly, in heart mitochondrial of two bird species, the parakeet and canary, there were lower rates of state 3 (ADP stimulated respiration) and 4 H_2O_2 production compared with isolated heart mitochondria from mice (Herrero and Barja 1998). Thus, some evidence suggests that low rate of mitochondrial free radical production is a general property of birds, likely due to a decrease in free radical leak, which is independently regulated from VO_2 and BMR (Herrero and Barja 1998; Barja 2007) potentially due to mild uncoupling in birds (Skulachev 2004). However, others caution that inter-specific comparisons of mitochondrial rates standardized to per gram of protein are physiologically inappropriate due to the variation in ETC morphology (Montgomery et al. 2011).

Antioxidant capacity should closely match the pro-oxidant state of the animal. Previous work shows that only SOD activity increased in the pigeon compared with the rat (Ku and Sohal 1993). CAT activity was significantly higher in the rat compared with the pigeon (Ku and Sohal 1993; Montgomery et al. 2011), similar to our interspecies findings. Plasma TAC did not differ between rats and pigeons (Montgomery et al. 2011), unlike our findings; and, there were significant differences in plasma GPx activities between the rat and the pigeon, unlike our findings (though we measured all enzymatic activities in RBC, not plasma) (Montgomery et al. 2011). That SOD and GPx activities do not differ between birds and mammals may not be surprising considering that the antioxidant system is costly to maintain and enzymatic antioxidants are only upregulated when needed (Vágási et al. 2019). GPx and CAT have similar functions within the cells (Skrip and McWilliams 2016), thus, similar levels of GPx in birds and mammals may indicate that this enzyme is dealing with the ROS load, rather than CAT.

Polyunsaturated membrane lipids can be the source of "secondary ROS" molecules; these free radicals are generated when an unpaired electron from superoxide or hydrogen peroxide reacts with another

within membrane polyunsaturated fatty acids (Montgomery et al. 2011). These secondary ROS molecules carry across membranes during LPO and can often be responsible for damaging not just lipids, but also proteins; thus, more susceptible membranes (unsaturated) are said to create increases in oxidative damage due to the increased number of unpaired electrons. It may be that low levels of ROS do not translate to lower oxidative damage if an animal's membranes have more unsaturated lipids (Montgomery et al. 2011). Low ROS production is said to be coupled with cellular membranes that are more resistant to LPO in birds compared with mammalian membranes, in a variety of different tissues including heart, liver, and skeletal muscle (Pamplona et al. 1999; Hulbert et al. 2007; Montgomery et al. 2011). Indirect evidence of this fact includes the reduced capacity of MDA production in mitochondria isolated from birds compared with mammals (Hulbert et al. 2007). Membrane unsaturation is largely regulated and often linked with longevity, so that mice fed similar diets but selected for differing longevities have differing membrane unsaturation levels (Hulbert et al. 2006), pointing to the fact that this is an internally regulated mechanism that does not deviate with diet. Additionally, in rat and pigeon liver mitochondria, there seems to be a decrease in highly unsaturated fatty acids, and an increase in less unsaturated lipids, lending support to the idea that unsaturation level in membranes may play a role in longevity (Pamplona et al. 1996).

Our literature search demonstrated no significant differences between birds and mammals in most oxidative stress parameters of interest. This may be a function of small sample sizes from the literature that could be interconverted to similar units, or because many species are over-represented (cattle, camels, goats, sheep, pigs, dogs), while birds of any kind are under-represented. Additionally, we were hard-pressed to find standardized methods, thus, we report values that included different methods for sampling one parameter, which adds to variation. Furthermore, some papers did not specify whether any of these parameters were measured in whole blood, serum/plasma, and/or RBC, thus, so we could not control for the component of blood tissue used. Despite these complications, we found significantly lower activity of GPx in birds than mammals, similar to Montgomery et al. (2011). It is worrisome that basal-level oxidative stress measurements, especially for birds, are so scarce in the literature. We would suggest that, although basal measurements are not as exciting as potentially measuring great feats in athleticism (migration), peak metabolism or breeding, they are still important to report because they may be related to important life history characteristics (Dowling and Simmons 2009).

The bird–mammal aging paradox is striking, as it provides evidence that prolonged lifespans are not a function for slower whole-animal metabolic rates. It was originally thought that longevous species would have an increased antioxidant capacity compared with short-lived species. Multiple tissues from longevous species, however, demonstrated either a negative correlation with MLSP or no correlation at all (Perez-Campo et al. 1998; Barja 2004), suggesting that antioxidant levels do not explain differences in MLSP (Costantini 2008; Jimenez 2018), but low free radical production may (Perez-Campo et al. 1998). Indeed, low free radical production in mitochondria as a potential mechanism for longevous species to maintain their higher MLSP has been demonstrated widely (Lambert et al. 2007; Csiszar et al. 2012). For example, skin fibroblast from primates showed an inverse correlation with longevity, however, when these data are corrected for body mass and phylogeny, this correlation disappears suggesting that both of these factors have a significant impact on mitochondrial biology (Csiszar et al. 2012). Although others have found that under physiologically challenging conditions, long-lived species have higher non-enzymatic antioxidant levels compared with shorter-lived species of birds (Vágási et al. 2019). In plasma from known-aged birds and mammals, we found a significant positive correlation between age of the animal and TAC and oxidative damage in mammals, but not in birds. Although age explained a small amount of the variation in the data, these results indicate that although older mammals show higher lipid damage, increases in TAC reduce the associated costs. As birds aged, there was a significant decrease in CAT activity in RBCs, but a significant increase in SOD activity in mammals. When we correlated our data with MLSP, we found a negative correlation between TAC and longevity in birds that explained ~35% of the variation in the data, and no correlation with oxidative damage (Sanz et al. 2006; Barja 2013). Our findings imply that longer lifespan in birds is associated with decreases in TAC, but not concomitant increases in oxidative damage. This may be due to the fact that enzymatic antioxidants do not change with MLSP, and oxidative damage can still be abated. Previously, we have found that in primary fibroblasts isolated from birds, there was no correlation between cellular metabolism and oxidative stress with MLSP (Jimenez 2018). Our current findings suggest that variations with MLSP may be tissue dependent, and generalizations

between and across species should not be done lightly. We also found a positive correlation between oxidative damage and MLSP in mammals that explained ~17% of the variation in the data, but no correlation between mammalian MLSP and TAC or enzymatic antioxidants. These results imply that mitochondrial efficiency may decrease with lifespan in mammals and more ROS production could overwhelm the antioxidant system and cause more accumulated damage in longer-lived mammals or that the antioxidant system is not sufficient to keep up with existing ROS production. The correlation between antioxidants and MLSP across the literature shows positive, negative, and no correlation at all depending on tissue type and measurement (reviewed in Barja 2004). The fact that known age and MSLP patterns differed may be a cautionary sign for correlating unknown aged animals to MSLP, especially when it comes to birds. However, we note that more data should be collected suing a comparative framework to fully test this hypothesis. Perhaps the most accurate depiction of these data is to obtain a fraction of the current age of each animal to its species' MLSP. When we do this, we find no correlations in any of our oxidative stress parameters in birds or mammals (data not shown), possibly demonstrating that oxidative stress is not the main determinant for aging or longevity. However, from our data, it is generalizable that birds demonstrate overall lower levels of oxidative stress, which may or may not be linked to their longer lifespans.

An important caveat when interpreting our results is the bias in our sampling of species. We obtained samples from zoo opportunistically, and because of this, we have samples from 20 more mammal species than bird species. In addition, we have under sampled small animals including passerines and our mammal samples are biased toward Primates, Artiodactylas (Cetartiofactyla and Perissofactyla), and Feliformia. As a result of these biases, our conclusions may not be applicable to endotherms of all body sizes and they may be driven, in part, by the less accurate estimates of birds than mammals. The slope of the relationship between body mass and BMR is shallower in small endotherms (Dodds et al. 2001) and passerines have a higher BMR than non-passerines (McKechnie et al. 2006), suggesting that there might be a different relationship with underlying cellular mechanisms of oxidative stress than the species we studied. Further investigation is needed. Despite the error that will arise in the incomplete sampling of birds and mammals, our analyses still provide some intriguing results and also highlight gaps in the literature. Additionally, it is our understanding, at least in the bird literature, that blood oxidative stress values are widely accepted as a "global" representation of the oxidative stress status of birds (Costantini 2008, 2011, 2016); though there may be some oxidative stress differences associated with mitotic and post-mitotic tissues (Selman et al. 2000), blood tissue serves as a reservoir for oxidative products to gather the overall oxidative status of the animal.

Acknowledgments

We are grateful to Richard Sim at the Birmingham Zoo; Michelle Goodman at the Elmwood Park Zoo; June Olds, Kerry Doyen, and Megan Wright from Blankpark Zoo; James Gilchrist and Morgan Peppenelli from Waterville Veterinary Clinic; Janey Kramlik and Lynn Klein from the Brandywine Zoo; Heather Scott Shaub from the North Carolina Zoo; Alexander Ernst from the Cape May County Park Zoo; Chelsea Bennett, Chris Smith, and Laura Keener from the San Diego Zoo. Dr Jennie Hayes for collecting blood from horses and donkeys for us. Additionally, we would like to thank Morgan Wynkoop, Aidan Harrington, and Sarah Aldrich for their help entering data and Isabelle DiBiase, Sadie Ray, and Sydney Ziatek for their help running assays. We are also grateful to the symposium organizers, Drs. Geoff Hill and Justin Havird, for providing funding to A.G.J. to present this work.

Funding

This research was partially funded by the National Science Foundation [grant NSF-IOS 1656551 to C.J.D.] and Hamilton College's Dean of Faculty [to C.J.D.]. Additionally, Colgate University also funded part of this research [to A.G.J.].

Supplementary data

Supplementary data are available at *ICB* online.

References

Agutter PS, Wheatley DN. 2004. Metabolic scaling: consensus or controversy? Theor Biol Med Model 1:13.

Ayala A, Muñoz MF, Argüelles S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014:360438.

Barja G. 2004. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism? Biol Rev 79:235–51.

Barja G. 2007. Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuvenation Res 10:215–23.

Barja G. 2013. Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. Antioxid Redox Signal 19:1420–45.

Barja G, Cadenas S, Rojas C, Pérez-Campo R, López-Torres M. 1994. Low mitochondrial free-radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic-rate in birds. Free Radic Res 21:317–27.

Brown MF, Gratton TP, Stuart JA. 2007. Metabolic rate does not scale with body mass in cultured mammalian cells. Am J Physiol Regul Integr Comp Physiol 292:R2115–21.

Brzek P, Bielawska K, Ksiazek A, Konarzewski M. 2007. Anatomic and molecular correlates of divergent selection for basal metabolic rate in laboratory mice. Physiol Biochem Zool 80:491–9.

Calhoon EA, Jimenez AG, Harper JM, Jurkowitz MS, Williams JB. 2014. Linkages between mitochondrial lipids and life history in temperate and tropical birds. Physiol Biochem Zool 87:265–75.

Costantini D. 2008. Oxidative stress in ecology and evolution: lessons from avian studies. Ecol Lett 11:1238–51.

Costantini D. 2011. On the measurement of circulating antioxidant capacity and the nightmare of uric acid. Methods Ecol Evol 2:321–5.

Costantini D. 2016. Oxidative stress ecology and the d-ROMs test: facts, misfacts and an appraisal of a decade's work. Behav Ecol Sociobiol 70:809–20.

Csiszar A, Podlutsky A, Podlutskaya N, Sonntag WE, Merlin SZ, Philipp EER, Doyle K, Davila A, Recchia FA, Ballabh P, et al. 2012. Testing the oxidative stress hypothesis of aging in primate fibroblasts: is there a correlation between species longevity and cellular ROS production? J Gerontol A Biomed Sci Med Sci 67:841–52.

Daan S, Masman D, Strijkstra A, Kenagy G. 1991. Daily energy turnover during reproduction in birds and mammals: its relationship to basal metabolic rate. Int Ornithol Congr 20:1976–87.

Daan S, Masman D, Strijkstra AM, Kenagy GJ. 1990. Daily energy turnover during reproduction in birds and mammals: its relationship to basal metabolic rate. Acta XX Congr Int Ornithol 4:1976–87.

de Villemereuil P, Nakagawa S. 2014. General quantitative genetic methods for comparative biology. In: Modern phylogenetic comparative methods and their application in evolutionary biology. Berlin, Heidelberg: Springer. p. 287–303.

Demetrius L. 2006. The origin of allometric scaling laws in biology. J Theor Biol 243:455–67.

Dodds PS, Rothman DH, Weitz JS. 2001. Re-examination of the "3/4-law" of metabolism. J Theor Biol 209:9–27.

Dowling DK, Simmons LW. 2009. Reactive oxygen species as universal constraints in life-history evolution. Proc R Soc B Biol Sci 276:1737–45.

Else P, Hulbert A. 1985. An allometric comparison of the mitochondria of mammalian and reptilian tissues—the implications for the evolution of endothermy. J Comp Physiol B 156:3–11.

Garrido G, Guzman M, Odriozola J. 1996. Effects of physical training on fatty acid metabolism in liver and skeletal muscle of rats fed four different high-carbohydrate diets. J Nutr Biochem 7:348–55.

Gessaman JA, Nagy KA. 1988. Energy metabolism: errors in gas-exchange conversion factors. Physiol Zool 61:507–13.

Hadfield JD. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. J Stat Softw 33:1–22.

Hadfield JD, Nakagawa S. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. J Exp Biol 23:494–508.

Halliwell B, Chirico S. 1993. Lipid-peroxidation—its mechanism, measurement, and significance. Am J Clin Nutr 57:715–25.

Hammond KA, Chappell MA, Cardullo RA, Lin R-S, Johnsen TS. 2000. The mechanistic basis of aerobic performance variation in red junglefowl. J Exp Biol 203:2053–64.

Herrero A, Barja G. 1997. ADP-regulation of mitochondrial free radical production is different with complex I- or complex II-linked substrates: implications for the exercise paradox and brain hypermetabolism. J Bioenerg Biomembr 29:241–9.

Herrero A, Barja G. 1998. H_2O_2 production of heart mitochondria and aging rate are slower in canaries and parakeets than in mice: sites of free radical generation and mechanisms involved. Mech Ageing Dev 103:133–46.

Hulbert AJ, Else PL. 2005. Membranes and the setting of energy demand. J Exp Biol 208:1593–9.

Hulbert AJ, Faulks SC, Harper JM, Miller RA, Buffenstein R. 2006. Extended longevity of wild-derived mice is associated with peroxidation-resistant membranes. Mech Ageing Dev 127:653–7.

Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. 2007. Life and death: metabolic rate, membrane composition, and life span of animals. Physiol Rev 87:1175–213.

Jimenez AG. 2018. "The same thing that makes you live can kill you in the end": exploring the effects of growth rates and longevity on cellular metabolic rates and oxidative stress in mammals and birds. Integr Comp Biol 58:544–58.

Jimenez AG, Winward JD, Smith DM, Ragan CM. 2018. Effects of short-term clomipramine on anxiety-like behavior, cellular metabolism, and oxidative stress in primary fibroblast cells of male and female rats. Physiol Rep 6:e13615.

Jimenez AG, Dillaman RM, Kinsey ST. 2013. Large fibre size in skeletal muscle is metabolically advantageous. Nat Commun 4:2150.

Jimenez AG, Van Brocklyn J, Wortman M, Williams JB. 2014. Cellular metabolic rate is influenced by life-history traits in tropical and temperate birds. PLoS ONE 9:e87349.

Jimenez AG, Williams JB. 2014. Cellular metabolic rates from primary dermal fibroblast cells isolated from birds of different body masses. Comp Biochem Physiol A Mol Integr Physiol 176:41–8.

Jimenez AG, Winward J, Beattie U, Cipolli W. 2018. Cellular metabolism and oxidative stress as a possible determinant for longevity in small breed and large breed dogs. PLoS ONE 13:e0195832.

Johnson MA, Francis CD, Miller ET, Downs CJ, Vitousek MN. 2018. Detecting Bias in Large-Scale Comparative Analyses: Methods for Expanding the Scope of Hypothesis-Testing with HormoneBase. Integr Comp Biol 58:720–8.

Kikusato M, Ramsey AJJ, Amo T, Toyomizu M. 2010. Application of modular kinetic analysis to mitochondrial oxidative phosphorylation in skeletal muscle of birds exposed to acute heat stress. FEBS Lett 584:3143–8.

Konarzewski M, Diamond J. 1995. Evolution of basal metabolic rate and organ masses in laboratory mice. Evolution 49:1239–48.

Krebs H. 1950. Body size and tissue respiration. Biochim Biophys Acta 4:249–69.

Ku H, Sohal R. 1993. Comparison of mitochondrial prooxidant generation and antioxidant defenses between rat and pigeon—possible basis of variation in longevity and metabolic potential. Mech Ageing Dev 72:67–76.

Lambert AJ, Boysen HM, Buckingham JA, Yang T, Podlutsky A, Austad SN, Kunz TH, Buffenstein R, Brand MD. 2007. Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. Aging Cell 6:607–18.

Letunic I. 2015. phyloT: phylogenetic tree generator [online].

Londono GA, Chappell MA, Castaneda MR, Jankowski JE, Robinson SK. 2015. Basal metabolism in tropical birds: latitude, altitude, and the 'pace of life'. Funct Ecol 29:338–46.

Makarieva AM, Gorshkov VG, Li BL, Chown SL, Reich PB, Gavrilov VM. 2008. Mean mass-specific metabolic rates are strikingly similar across life's major domains: evidence for life's metabolic optimum. Proc Natl Acad Sci U S A 105:16994–9.

March R, Dawson W. 1982. Substrate metabolism in seasonally acclimatized American goldfinches. Am J Physiol 242:R563–9.

McKechnie A, Freckleton RP, Jetz W. 2006. Phenotypic plasticity in the scaling of avian basal metabolic rate. Proc R Soc B Biol Sci 273:931–7.

McKechnie A, Wolf B. 2004. The allometry of avian basal metabolic rate: good predictions need good data. Physiol Biochem Zool 77:502–21.

Montgomery MK, Hulbert AJ, Buttemer WA. 2011. The long life of birds: the rat–pigeon comparison revisited. PLoS ONE 6:e24138.

Moyes C. 2003. Controlling muscle mitochondrial content. J Exp Biol 206:4385–91.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–42.

Nespolo RF, Bacigalupe LD, Sabat P, Bozinovic F. 2002. Interplay among energy metabolism, organ mass and digestive enzyme activity in the mouse-opossum Thylamys elegans: the role of thermal acclimation. J Exp Biol 205:2697–703.

Pamplona R, Prat J, Cadenas S, Rojas C, Perez CR, Torres ML, Barja G. 1996. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and human case. Mech Ageing Dev 86:53–66.

Pamplona R, Portero-Otin M, Requena J, Thorpe S, Herrero A, Barja G. 1999. A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat. Mech Ageing Dev 106:283–96.

Perez-Campo R, Lopez-Torres M, Cadenas S, Rojas C, Barja G. 1998. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. J Comp Physiol B Biochem Syst Environ Physiol 168:149–58.

Piersma T, Bruinzeel L, Drent R, Kersten M, van der Meer J, Wiersma P. 1996. Variability in basal metabolic rate of a long-distance migrant shorebird (red knot, Calidris canutus) reflects shifts in organ sizes. Physiol Zool 69:191–217.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Team. 2011. nlme: linear and nonlinear mixed effects models. R package version 3.1-101.

Porter R, Brand M. 1995. Causes of differences in respiration rate of hepatocytes from mammals of different body-mass. Am J Physiol Regul Integr Comp Physiol 269:R1213–24.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Raichlen DA, Gordon AD, Muchlinski MN, Snodgrass JJ. 2010. Causes and significance of variation in mammalian basal metabolism. J Comp Physiol B Biochem Syst Environ Physiol 180:301–11.

Ricklefs RE. 2010. Insights from comparative analyses of aging in birds and mammals. Aging Cell 9:273–84.

Sanz A, Pamplona R, Barja G. 2006. Is the mitochondrial free radical theory of aging intact? Antioxid Redox Signal 8:582–99.

Schmidt-Nielsen K. 1984. Scaling: why is animal size so important? Cambridge: Cambridge University PressID: 56.

Selman C, McLaren JS, Himanka MJ, Speakman JR. 2000. Effect of long-term cold exposure on antioxidant enzyme activities in a small mammal. Free Radic Biol Med 28:1279–85.

Sieg AE, O'Connor MP, McNair JN, Grant BW, Agosta SJ, Dunham AE. 2009. Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models matter? Am Nat 174:720–33.

Skrip MM, McWilliams SR. 2016. Oxidative balance in birds: an atoms-to-organisms-to-ecology primer for ornithologists. J Field Ornithol 87:1–20.

Skulachev V. 2004. Mitochondria, reactive oxygen species and longevity: some lessons from the barja group. Aging Cell 3:17–9.

Suarez R. 1996. Upper limits to mass-specific metabolic rates. Annu Rev Physiol 58:583–605.

Sunnucks P, Morales HE, Lamb AM, Pavlova A, Greening C. 2017. Integrative approaches for studying mitochondrial and nuclear genome co-evolution in oxidative phosphorylation. Front Genet 8:25.

Tacutu R, Craig T, Budovsky A, Wuttke D, Lehmann G, Taranukha D, Costa J, Fraifeld VE, de Magalhães JP. 2012. Human ageing genomic resources: integrated databases and tools for the biology and genetics of ageing. Nucleic Acids Res 41:D1027–33.

Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, Craig T, Diana E, Lehmann G, Toren D, Wang J, et al. 2018. Human ageing genomic resources: new and updated databases. Nucleic Acids Res 46:D1083–90.

Uyeda JC, Pennell MW, Miller ET, Maia R, McClain CR. 2017. The evolution of energetic scaling across the vertebrate tree of life. Am Nat 190:185–99.

Tieleman BI, Dijkstra TH, Lasky JR, Mauck RA, Visser GH, Williams JB. 2006. Physiological and behavioural correlates of life-history variation: a comparison between tropical and temperate zone House Wrens. Funct Ecol 20:491–9.

Vágási CI, Vincze O, Pătraș L, Osváth G, Pénzes J, Haussmann MF, Barta Z, Pap PL. 2019. Longevity and life history coevolve with oxidative stress in birds. Funct Ecol 33:152–61.

Vezina F, Williams T. 2005. Interaction between organ mass and citrate synthase activity as an indicator of tissue maximal oxidative capacity in breeding European starlings: implications for metabolic rate and organ mass relationships. Funct Ecol 19:119–28.

Weibel E, Taylor C, Hoppeler H. 1991. The concept of symmorphosis—a testable hypothesis of structure–function relationship. Proc Natl Acad Sci U S A 88:10357–61.

West GB, Brown JH, Enquist BJ. 1997. A general model for the origin of allometric scaling laws in biology. Science 276:122–6.

West GB, Savage VM, Gillooly J, Enquist BJ, Woodruff WH, Brown JH. 2003. Why does metabolic rate scale with body size? Nature 421:713.

Wheatley DN. 2007. Convergence of metabolic rate of cultured cells from animals of different sizes. Am J Physiol Regul Integr Comp Physiol 292:R2113–4.

White CR, Phillips NF, Seymour RS. 2006. The scaling and temperature dependence of vertebrate metabolism. Biol Lett 2:125–7.

Wiersma P, Nowak B, Williams JB. 2012. Small organ size contributes to the slow pace of life in tropical birds. J Exp Biol 215:1662–9.

Winward JD, Ragan CM, Jimenez AG. 2018. Cellular metabolic rates and oxidative stress profiles in primary fibroblast cells isolated from virgin females, reproductively experienced females, and male Sprague–Dawley rats. Physiol Rep 6:e13909.

Wu B, Hulbert A, Storlien L, Else P. 2004. Membrane lipids and sodium pumps of cattle and crocodiles: an experimental test of the membrane pacemaker theory of metabolism. Am J Physiol Regul Integr Comp Physiol 287:R633–41.