RESEARCH ARTICLE | *Respiration*

Untangling life span and body mass discrepancies in canids: phylogenetic comparison of oxidative stress in blood from domestic dogs and wild canids

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Jimenez AG, Downs CJ. Untangling life span and body mass discrepancies in canids: phylogenetic comparison of oxidative stress in blood from domestic dogs and wild canids. *Am J Physiol Regul Integr Comp Physiol* 319: R203–R210, 2020. First published July 1, 2020; doi:10.1152/ajpregu.00067.2020.—Canids are a morphological and physiological diverse group of animals, with the most diversity found within one species, the domestic dog. Underlying observed morphological differences, there must also be differences at other levels of organization that could lead to elucidating aging rates and life span disparities between wild and domestic canids. Furthermore, small-breed dogs live significantly longer lives than large-breed dogs, while having higher mass-specific metabolic rates and faster growth rates. At the cellular level, a clear mechanism underlying whole animal traits has not been fully elucidated, although oxidative stress has been implicated as a potential culprit of the disparate life spans of domestic dogs. We used plasma and red blood cells from known aged domestic dogs and wild canids, and measured several oxidative stress variables: total antioxidant capacity (TAC), lipid damage, and enzymatic activities of catalase, superoxide dismutase, and glutathione peroxidase (GPx). We used phylogenetically informed general linear mixed models and nonphylogenetically corrected linear regression analysis. We found that lipid damage increases with age in domestic dogs, whereas TAC increases with age and TAC and GPx activity increases as a function of age/maximum life span in wild canids, which may partly explain longer potential life spans in wolves. As body mass increases, TAC and GPx activity increase in wild canids, but not domestic dogs, highlighting that artificial selection may have decreased antioxidant capacity in domestic dogs. We found that small-breed dogs have significantly higher circulating lipid damage compared with large-breed dogs, concomitant to their high mass-specific metabolism and higher growth rates, but in opposition to their long life spans.

body mass; domestic dog; life span; oxidative stress; wild canids

INTRODUCTION

Canidae is one of the most diverse groups of mammals, with a single species, the domestic dog, demonstrating the most phenotypic plasticity (i.e., body size, coat lengths, color, and limb structure). In this group, body sizes range from the 2-kg Chihuahua to the 90-kg Great Dane (23). Smaller dogs tend to live significantly longer than larger dogs across all breeds (9, 23, 30, 35), and they have also been positively correlated to lower cancer risks (30), with demonstrated lower prevalence of age-related diseases such as cataracts (47). A single insulin growth factor-1 (*IGF1*) haplotype seems to substantially con-

tribute to size variation in dogs (42), and serum IGF-1 is reduced in small dogs relative to concentrations in large breeds, providing a potential link to their longer lives (12). The small dog phenotype is, however, also associated with significantly higher mass-specific metabolic rates and significantly faster relative growth rates compared with large dog breeds (20, 21, 23, 36). It is perhaps surprising that small-breed dogs have longer life spans than large-breed dogs. However, large-breed dogs tend to have longer developmental trajectories compared with small-breed dogs (23). On the other hand, wild canids do not seem to demonstrate the same whole animal traits as their domesticated counterparts. For example, gray wolves, the domestic dog's closest ancestor, at ~40 kg can live up to 20.6 yr of age (43, 44), whereas a similarly sized domestic dog (i.e., Cane corso), lives on average 10–12 yr of age (American Kennel Club). Thus, from whole animal differences, we may assume that domestic dogs and their wild canid counterparts may differ in key physiological and cellular processes that may yield differences in life span.

The process of oxidative stress could contribute to differing life spans in the domestic dog, and differences in aging rates across wild canids and domestic dogs. Broadly defined, oxidative stress is the balance between prooxidants produced during aerobic metabolism mainly by mitochondria, and antioxidants, enzymatic and nonenzymatic molecules capable of thwarting prooxidants before cellular damage occurs (4, 19). Lipids are among the molecules most affected, and two of the most prevalent prooxidants that can initiate damage to lipid membranes are hydroxyl radicals ($\cdot$OH) and hydroperoxyl radicals ($\cdot$OOH) (4). The process of lipid peroxidation continues unabated until the propagation of damage is halted by an antioxidant molecule (4, 19). 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 vitamin E and C, act as chain-breaking antioxidants; they scavenge for reactive oxygen species (ROS), remove them once they are formed, and further halt propagation of peroxidation (19). At low levels, ROS are essential in gene regulation, cell signaling, and apoptosis. At high levels, ROS can overwhelm the antioxidant system, which can lead to damage (11). The concept of oxidative stress has been implicated as the underlying cellular determinant for life-history trade-offs (11) and, thus, this process may potentially have cellular effects on aging and growth rates, which may be linked to aerobic function in wild and domestic dogs (23).

Previous work on primary fibroblast cells of large- and small-breed domestic dogs, as they aged, found that dogs with shorter mean life spans have significantly higher DNA oxidative damage compared with dogs with longer mean life spans. And, that large-breed dogs have higher rates of glycolysis across their life span, a potential precancerous phenotype, which may shorten their life spans (25). Many cells that are or become cancerous are associated with higher rates of glycolysis due to the Warburg effect (25). Because of the nature of collecting skin tissue to grow primary cell lines, this work only encompassed two disparate ends of the physiological spectrum: very young and very old dogs. Arguably, a criticism of this approach is that both of those phenotypes are not the predominant phenotype of the animal during the majority of its life span. Here, we address two questions *1*) how does blood oxidative stress associate with body mass and age in "middle"-aged domestic dogs? and *2*) are oxidative stress patterns in blood from wild canids similar to those found in domestic dogs? This approach provides evolutionary data on aging pathways present in this group of mammals. Our current study includes data on the oxidative stress system in blood of domestic dogs of different sizes from 2 to 8 yr of age, and wild canids of different sizes and ages. This comparison allows us to start elucidating whether domestication and artificial selection has changed oxidative stress patterns in domestic dogs.

MATERIALS AND METHODS

Oxidative stress measurements in blood from domesticated and wild canids. We collected blood samples of domestic and wild canids from zoos and veterinarians, as blood serves as a reservoir of metabolic products, including oxidative stress. Blood was collected from two veterinarian offices and nine zoos as part of routine veterinary care for all animals included. Owners of domestic dogs gave informed consent for the blood collection from their animal and participation in this study. Sample collection was done under the guidelines of the Colgate University Institutional Animal Care and Use Committee and the Animal Welfare Act. Only healthy, not actively reproducing individuals of known age, were included. Blood samples were collected, spun 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 further use. For each individual, we collected information regarding body mass, sex, and age at blood draw. Domestic dog samples included individuals that were out of the "growing" phase (~2 yr old) and not yet in the "aging" phase (up to ~8 yr old). Domestic dogs were categorized into three size classes based on their body weight: small (up to ~10 kg), medium (~10 to ~20 kg), and large (~20 kg and up) (23). Veterinary staff at nine zoos collected samples from wild canid species and shipped these samples to Colgate University on dry ice. We used species from zoos because these zoo animals receive veterinary care, extra care when they display signs of sickness, and access to a consistent and healthy diet. Thus, by using samples from zoo animals, we are minimizing confounding variables that would be associated with free-ranging individual animals. We did not control for diet of each animal in this study.

We determined circulating antioxidant capacity using the OXY-Adsorbent (TAC) test as the ability of plasma to neutralize hypochlorous acid (Diacron International, Grosseto, Italy), and we measured oxidative damage as the presence of circulating hydroperoxides, including products of lipid oxidation, using the d-ROMs test in plasma (Diacron International). 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 Chemicals, Ann Harbor, MI). We added 4 μL of RBC into 396 μL of 20 mM HEPES, 1 mM EGTA, and 90 mM mannitol buffer solution. After dilution, samples were vortexed before 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 across samples (24). Sample sizes for each species are listed in Table 1.

Statistics. Each variable was analyzed using linear regressions as a function of body size and age, first. Additionally, we used linear regressions to correlate the ratio between age and maximum life span (MLSP). We obtained life spans of each American Kennel Club (AKC)-recognized breed from the AKC website, and maximum life span of wild canids from Anage. Using these numbers, we estimated a ratio of age/MLSP per species and AKC recognized breed. Mixed domestic dog breeds were not included in age/MLSP correlations. This ratio provides a more accurate estimation of where each individual is relative to the potential life span of each species and, thus, provides a measure of age scaled by species longevity. Additionally, we used an ANOVA to test differences in each variable across different domestic dog sizes.

Species and breeds are not evolutionarily independent, so we performed phylogenetically informed analyses to account for the evolutionary relatedness of our samples (13, 16). We also wanted to account for the variation within each species because of the wide range of morphological variation demonstrated within domestic dogs, so we used all of our data in our analyses rather than species means (10). Specifically, we performed phylogenetically informed general linear mixed models that accounted for within-species/breed variation to determine whether history (wild vs. domestic), 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 lipid damage. 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 (17, 18, 36a). The phylogenetic covariance matrix for this analysis was estimated on the basis of a cladogram that included wild Canidae and domestic dog breeds. Briefly, we started with the cladogram published for domestic dogs (34), and we constructed a phylogenetic tree for wild Canidae species included in our data set using NCBI molecular data and phyloT (27); polytomies were excluded using the randomization process in phyloT. To create a consensus tree, we merged the two trees at the closest living ancestor for domestic dogs, the gray wolf, a merging point supported by previous work (34) (Supplemental Fig. S1, https://doi.org/10.6084/m9.figshare.12573914.v1). Using this tree to model phylogenetic dependence, all mixed models were fit using a weak inverse-Gamma

Table 1. *Samples sizes of each wild canid specie and domestic dog sizes*

Species	Plasma N	RBC N
African wild dog	3	0
Arctic fox	5	4
Bush dog	1	1
Coyote	27	23
Dhole	1	1
Domestic dog, large breeds	66	66
Domestic dog, medium breeds	16	16
Domestic dog, small breeds	139	139
Fennec fox		3
Gray wolf	5	5
Maned wolf	6	3
New Guinea singing dog	1	0
Red Fox	1	1

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 approach to determine which parameters were important predictors for each response variable. Relative support for each model was determined on the basis of Deviance Information Criterion (DIC) values and differences among models (ΔDIC). Models within five ΔDIC values of the top model were considered indistinguishable and informative, and values greater than 10 ΔDIC of the top model were not informative.

We estimated the importance of phylogenetic signal as Pagel's lambda (8). Larger numbers indicate that more variation is explained by the phylogeny. We then calculated marginal R^2 and conditional R^2 following (32). 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).

RESULTS

Linear regressions for domestic dogs. We aimed to address whether middle-aged domestic dogs demonstrated changes in blood oxidative stress due to body mass and age. Considering all size classes together, older domestic dogs had higher lipid damage, but age was not correlated with any of the other measures of oxidative stress ($y = 0.32x + 2.87$; $P = 0.008$; Fig. 1 and Table 2). We found no correlation between the age/MLSP ratio and lipid damage, TAC, CAT, GPx, and SOD (Table 2). We found that smaller dog breeds had higher lipid damage compared with larger breeds, but body mass was not correlated with any other measure of oxidative stress ($y = -0.037x + 5.13$; $P = 0.04$; Fig. 2 and Table 2).

Linear regressions for wild canids. We also wanted to address whether, across different species of canids, oxidative stress changed due to age and body mass, and how these patterns may compare between wild and domestic canids. We found that older wild canids showed significant increases in TAC ($y = 19.6x + 971$; $P = 0.03$; Fig. 1 and Table 2), but no other oxidative stress measure correlated with age (Table 2). We also found that wild canids with higher Age/MLSP ratios had significantly higher TAC, and GPx activity (TAC: $y = 366.7x + 974.2$; $P = 0.02$; GPx: $y = 8.07x + 5.04$; $P = 0.04$; Fig. 1 and Table 2), but no other measure correlated with Age/MLSP (Table 2). Larger wild canids had significantly higher TAC and GPx (TAC: $y = 6.5x + 969.6$; $P = 0.03$; GPx: $y = 0.15x + 4.68$; $P = 0.02$; Fig. 2 and Table 2), but no other
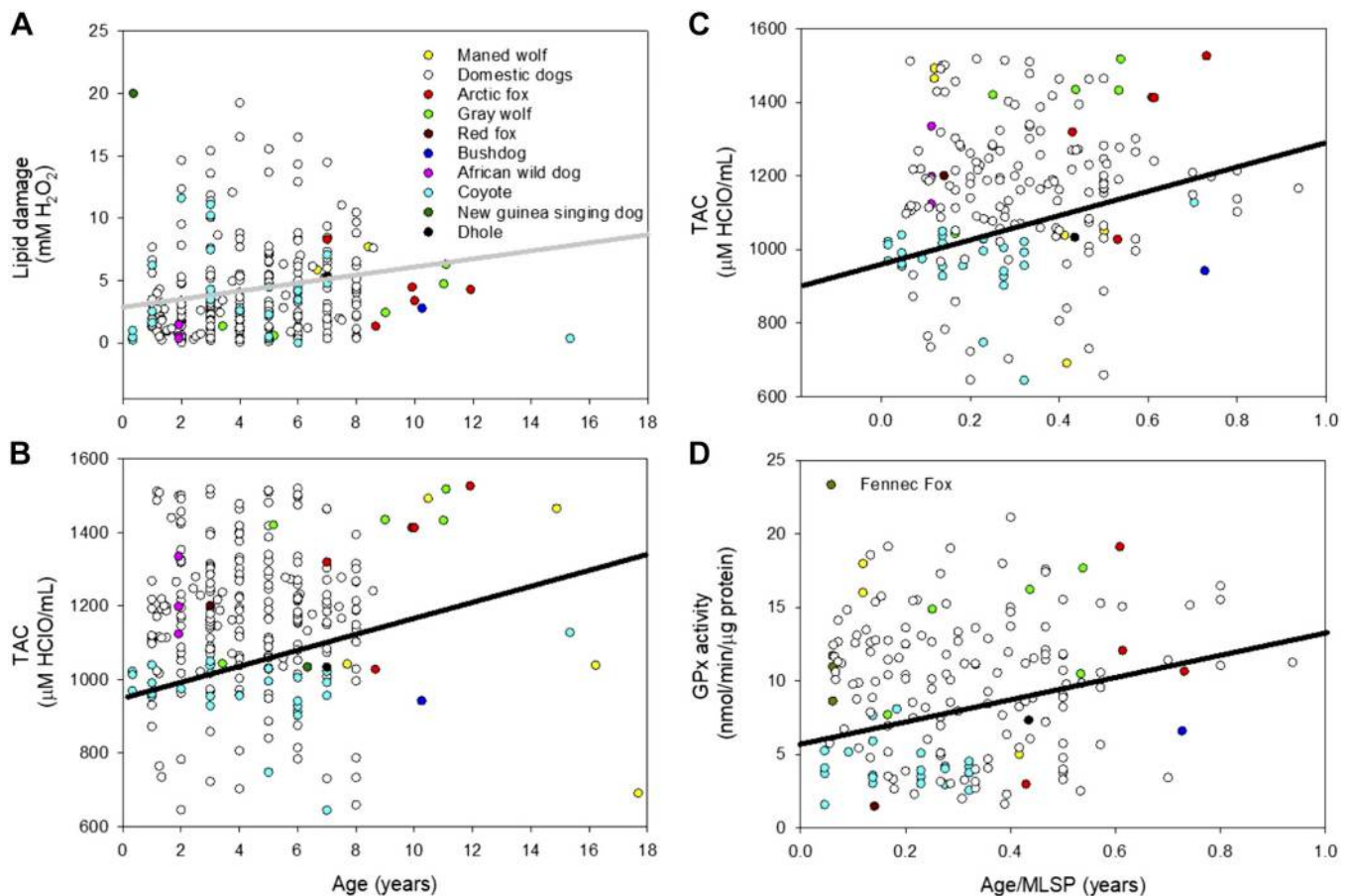

Fig. 1. *A*: there was a significantly positive correlation between age and lipid damage in domestic dogs, but not in wild canids ($r^2 = 0.18$, $y = 0.32x + 2.87$; $P = 0.0078$). *B*: there was a significantly positive relationship between age and total antioxidant capacity (TAC) in wild canids ($r^2 = 0.10$, $y = 19.57x + 971$; $P = 0.029$). *C*: there was a significantly positive correlation between the age/MLSP ratio and TAC in wild canids ($r^2 = 0.12$, $y = 366.67x + 974.22$; $P = 0.0197$), and glutathione peroxidase (GPx; *D*) in wild canids ($r^2 = 0.096$, $y = 8.07x + 5.04$; $P = 0.048$). Gray line represents domestic dog regression, black line represents wild canid regression. Samples sizes are listed in Table 1. Nonsignificant linear regressions were not plotted.

Table 2. *Linear regression results*

	TAC, μm HClO/mL			Lipid Damage, mM H_2O_2			CAT Activity, nmol·min^{-1}·μg protein^{-1}			SOD, U·mL^{-1}·μg protein^{-1}			GPx Activity, nmol·min^{-1}·μg protein^{-1}		
	r^2	Equation	P	r^2	Equation	P	r^2	Equation	P	r^2	Equation	P	r^2	Equation	P
Domestic dog regressions															
Age	0.055	$y = -4.8x + 1199.6$	0.4	0.18	$y = 0.32x + 2.87$	**0.008**	0.057	$y = -1.44x + 128.12$	0.4	0.09	$y = 0.055x + 4.19$	0.17	0.07	$y = 0.15x + 10.71$	0.3
Age/MLSP	0.0009	$y = 0.1x + 1166.9$	0.9	0.06	$y = 1.30x + 3.97$	0.5	0.025	$y = 7.72x + 117.86$	0.8	0.16	$y = 1.15x + 4.08$	**0.007**	0.014	$y = 0.333x + 9.80$	0.9
Body mass	0.015	$y = 0.19x + 1174.4$	0.8	0.14	$y = 0.037x + -5.13$	**0.04**	0.087	$y = 0.032x + 114.07$	0.2	0.06	$y = -0.005x + 4.55$	0.4	0.0007	$y = 0.0002x + 10.09$	0.9
Wild canid regressions															
Age	0.1	$y = 19.6x + 971$	**0.03**	0.002	$y = 0.05x + 4.04$	0.8	0.024	$y = -4.74x + 109.77$	0.3	0.02	$y = -0.033x + 3.91$	0.4	0.07	$y = 0.41x + 5.06$	0.09
Age/MLSP	0.12	$y = 366.7x + 974.2$	**0.02**	0.002	$y = 0.57x + 3.8$	0.8	0.012	$y = -56.37x + 100.51$	0.5	0.02	$y = -0.58x + 3.89$	0.4	0.09	$y = 8.07x + 5.04$	**0.04**
Body mass	0.1	$y = 6.5x + 969.6$	**0.03**	0.0002	$y = 0.0053x + 4.40$	0.9	0.012	$y = -0.95x + 100.88$	0.5	0.000.4	$y = -0.0015x + 3.76$	0.4	0.13	$y = 0.15x + 4.68$	**0.02**

Significant results are bolded, $P < 0.05$. CAT, catalase; GPx, glutathione peroxidase; MLSP, maximum life span; SOD, superoxide dismutase; TAC, total antioxidant capacity.

oxidative stress measure correlated with body mass in wild canids (Table 2).

Comparisons between domestic dog size classes. Log-Lipid damage was significantly different across sizes where small-breed dogs had higher damage compared with large-breed dogs ($F_{2,218} = 6.998$; $P = 0.001$; Fig. 3); however, TAC, CAT, GPx, and SOD were not significantly different across sizes ($F_{2,218} = 0.141$; $P = 0.868$; $F_{2,218} = 1.864$; $P = 0.160$; $F_{2,218} = 0.795$; $P = 0.453$; $F_{2,218} = 0.933$; $P = 0.395$, respectively).

Phylogenetically informed general linear mixed models. We performed phylogenetically informed analyses to account for the fact that species are not independent because of their shared evolutionary history (13). Three models for lipid damage had a ΔDIC within five points of the top model and were considered to be informative (Supplemental Table S2, https://doi.org/10.6084/m9.figshare.12573908.v1). Species mass (log$_{10}$-transformed) was the only parameter with a credible interval (CI) that did not overlap 0, indicating strong support for this factor as a predictor of lipid damage. Lipid damage decreased with mean species mass (mean β = −37.8 to −27.9), (Supplemental Table S1). The 95% CI for all other fixed effects in the contender models overlapped 0, indicating that these parameters are important for describing the results but were not supported as factors driving the results. The fixed effects, the phylogeny, and the overall models explained little of the variation in the lipid damage data (Table 1).

The top model set for CAT activity included the full model (*model 5* on Supplemental Table S1), the null model, and the model with history as the only fixed effect. The inclusion of the null model in the list of top models indicates weak support that species mass is an important predictor of CAT activity. In the top model, which included species mass, individual mass, history, and the interaction species mass by individual mass, the fixed effects explained 27% (95% CI: 10–49%) of the variation in the data, and phylogeny explained almost none of the variation in the data. In contrast, the null model, which does not include any fixed effects, explains ~50% of the variation. Thus, a comparison of all of the top models indicates that mass and history are conflated with phylogeny.

In contrast, the list of informative models TAC, GPx activity, and SOD activity included all of the potential models, including the null model (e.g., intercept-only) (Supplemental Table S2). Pagel's lambda values indicate that models for TAC, GPx activity, and SOD activity explained less than 0.01% of variation in TAC and GPx activity and <15% of the variation in SOD activity (Supplemental Table S2). Thus, fixed effects in these models had low explanatory power. In addition, Pagel's lambda values indicate that models for TAC, GPx activity, and SOD activity explained less than 0.01% of variation in TAC and GPx activity and <15% of the variation in SOD activity, indicating that phylogeny also had relatively low explanatory power for (Supplemental Table S2).

DISCUSSION

Here, using plasma and RBCs from domestic dogs and wild canids of different ages and body sizes, we found that wild and domestic dogs exhibit different patterns in components of oxidative stress, which may contribute to their different patterns in aging with respect to body size. We
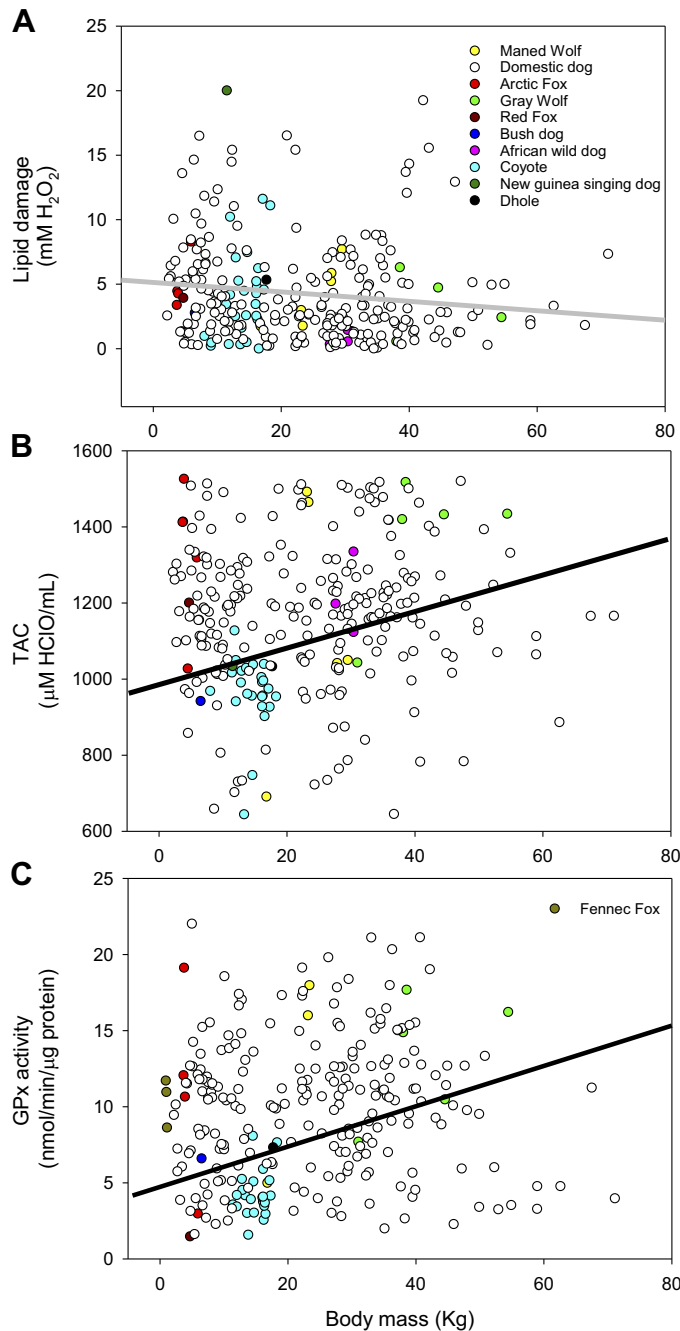
Fig. 2. *A*: there was a significantly negative correlation between body mass and lipid damage in domestic dogs ($r^2 = 0.14$, $y = -0.037x + 5.13$; $P = 0.039$). However, there was a significantly positive correlation between body mass and total antioxidant capacity (TAC) in wild canids (*B*; $r^2 = 0.10$, $y = 6.47x + 969.62$; $P = 0.027$) and a positive correlation with body mass and glutathione peroxidase (GPx) in wild canids (*C*; $r^2 = 0.13$, $y = 0.16x + 4.68$; $P = 0.022$). Gray line represents domestic dog regression, whereas black line represents wild canid regression. Samples sizes are listed in Supplemental Table S1. Nonsignificant linear regressions were not plotted.

we found that small-breed dogs have significantly higher circulating lipid damage on average compared with large-breed dogs. These data suggest that artificial selection in domestic dogs may have selected for a decrease in antioxidant capacity and that small-breed dogs may be "surviving" with increased oxidative damage.

To address our first question, whether oxidative stress changes in blood from "middle"-aged domestic dogs due to body mass and age, we show here that the domestic dogs have an increase in lipid damage with age. Previous work on the oxidative status of the dog showed that lipid peroxidation increased and reduced glutathione decreased with age, suggesting an imbalance of prooxidants, leading to more damage in some studies (45, 48). Whereas others have found no change in TAC in blood plasma with increasing age (5), although most of this work has been done on a single breed and not taking body mass into account. Others have found an age-related increase in SOD activity in domestic dog blood (46, 48). In primary fibroblasts from small and large dog breeds as they age, there was a significant increase in DNA oxidative damage in shorter-lived breeds, but no differences in lipid peroxidation damage in this cell type (25), pointing to the variability in oxidative stress across tissues. For example, previous work on Wistar rats has shown that lipid damage occurs with age in liver and brain, but not in the heart or lungs in male rats (37).

Small-breed dogs have higher mass-specific metabolic rates and higher growth rates (23, 38), a phenotype that some may say would be prone to higher rates of ROS production. Although the relationship between whole animal metabolism and ROS production is not linear (41), it is often thought that increasing metabolic rates during thermal challenges would also increase ROS production. Small-breed dogs show no increases in any circulating antioxidant we measured; however, they live longer lives and are less cancer-prone than large-breed dogs (23, 30, 31). This phenotype seems to be a physiological discrepancy, however, not a particularly unique one. Long-lived vampire bats, and long-lived birds have higher oxidative DNA damage than their mammalian counterparts (28). Similarly, the naked mole rat (NMR) is a mouse-sized subterranean hystricognath that can live in captivity for ~28 yr,
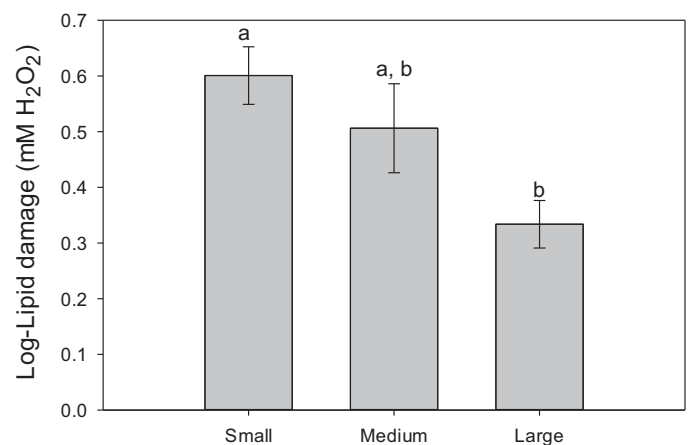

Fig. 3. Comparison of lipid damage across domestic dogs of different sizes. Log-lipid damage was significantly different across sizes where small breed dogs had higher damage compared with large breed dogs ($F = 6.998$; $P = 0.001$). Letters indicate subset differences. Values are expressed as means $\pm$ SE. Samples sizes are listed in Supplemental Table S1.

found that lipid damage increases with age in domestic dogs. In contrast, TAC increases with age in wild canids, and TAC and GPx activity increase as a function of age/MLSP in wild canids. As body mass increases, TAC and GPx activity increase in wild canids, but not domestic dogs in models without phylogenetic information. Surprisingly,

and in the wild for ~17 yr, greatly exceeding its body mass-predicted life span (2, 6). Antioxidant enzyme activities in NMRs do not change with age, as we have shown in dogs; however, those of similarly sized mice showed a decline with age in CAT and cellular glutathione peroxidase (cGPx) (2). NMRs also exhibit significantly higher oxidative damage to every biologically relevant molecule, including lipids, proteins, and DNA (1, 3). Higher levels of lipid peroxidation in NMRs seem to not be associated with their membrane peroxidation index (28). Similarly, cell membranes of primary fibroblast cells from small- and large-breed dogs did not show any differences in peroxidation index or saturation levels (26). It is suspected that the higher lipid peroxidation level in NMR comes from an abundance of ROS production during the growing process that is not properly thwarted, and, thus, builds up with age, over time (28). This may be a similar case in small-breed dogs, considering that they have faster growth rates in comparison to larger breeds (23). These cellular-level changes in NMR are accompanied by whole animal resistance to change with age in body composition and basal metabolism (33). These whole animal patterns are unlike those of dogs, which demonstrate differing body composition changes with age depending on breed size. For example, Great Danes increase body fat with age, and the smallest breeds (Papillons) increase their lean mass as they age (40). Additionally, dogs show a decrease in resting metabolism and mass-specific metabolism with age (23, 39).

To address our second question, whether oxidative stress patterns in blood from wild canids are similar to those found in domestic dogs, we found that TAC increases with age, and TAC and GPx increase as a function of age/MLSP in wild canids. A study looking at the scaling of oxidative stress in blood of 48 different mammal species also found a positive correlation between TAC and age, although older mammals also showed increases in lipid damage (24), which we did not see in wild canids. A correction of age/MLSP across these mammals resulted in no correlations in any of the oxidative stress measurements included (24), unlike the wild canids included in the current study. Furthermore, as body mass increases, TAC and GPx activity increase in wild canids, but not in domestic dogs. The scaling of the oxidative stress system with body mass is underrepresented in the literature (24). However, when data from Jimenez et al. (24) are limited to only mammals that are similar sizes as wild canids included in the present study (TAC: $r^2 = 0.0069$; $P = 0.45$; GPx: $r^2 = 0.013$; $P = 0.30$; Fig. 4), only the wild canids show a positive trend with body mass. This may imply that, as a whole, this group of animals has evolutionary traits that have yielded increases in antioxidant protection. However, artificial selection of the domestic dog seems to have selected against retaining this trait. To this point, others have found total antioxidant status, bilirubin, and glutathione metabolites to be significantly lower in domestic dog small breeds compared with large breeds (30). That large wild canids, such as the gray wolf, increase antioxidant capacity with age may, in part, explain their long potential life span and elucidate a unique physiological trait within this group that differs from other mammalian species. Additionally, this may also elucidate one potential mechanism via which the gray wolf has a longer life span compared with a similarly sized domestic dog. However, it also should be noted that some argue the diversity of extant wolves does not represent the diversity of wolves at the time domestic dogs split from them (14).

Aging remains one of the most poorly understood biological phenomena, and how metabolic processes are linked to aging still remain unclear (6). The concept of oxidative stress as it relates to aging has yielded variable results with seemingly every variable changing in contradiction across species and tissues (7). Oxidative status in domestic dogs can vary with diet and exercise, as well as across organs (28, 38); thus, some of the variability within our data set could stem from these factors. However, others have demonstrated that even strenuous exercise did not change the plasma TAC and antioxidant enzymes in blood of dogs (22). Other model organisms of aging generally show that increases in life span are linked to an increased concentration of antioxidants and a decrease in oxidative damage to most molecules, as it is the case of the dwarf Ames mice (23).
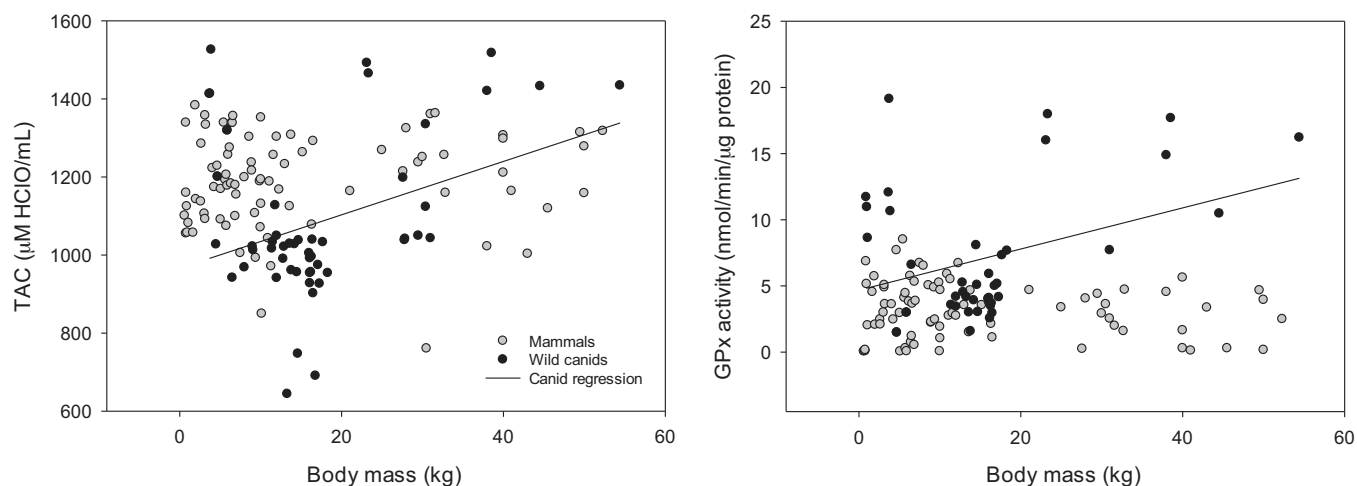

Fig. 4. Comparison of total antioxidant capacity (TAC; *left*) and glutathione peroxidase (GPx; *right*) activity between wild canids (this study) and similar-sized mammals found by Jimenez et al. (24). When mammals of similar size to wild canids are compared, only the wild canids show a positive trend with body mass in TAC ($r^2 = 0.0069$; $P = 0.45$) and GPx ($r^2 = 0.013$; $P = 0.30$). Black line indicates canid linear regression.

Perspectives and Significance

Here, we found that long-lived small-breed dogs seem to have more circulating lipid damage compared with short-lived larger breeds, in opposition of other model systems. Additionally, we found that wild canids, as a group, seem to have increases in antioxidant defenses with increased body mass and increased age, unlike domestic dogs, which demonstrate an overall increase in lipid damage as they age, unlike other mammals. These patterns could be due to antagonistic pleiotropy, as dogs were selected for earlier and more rapid reproduction (15). Broadly speaking, our study points to evolutionary physiological implications that may have taken place during rigorous artificial selection to make the domestic dog have fewer antioxidant defenses than their wild counterparts. Mechanistically speaking, it would be intriguing for future work to hone down how mitochondria in these animals are working differently to produce these results.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.G.J. and C.J.D. conceived and designed research; A.G.J. performed experiments; A.G.J. and C.J.D. analyzed data; A.G.J. and C.J.D. interpreted results of experiments; A.G.J. and C.J.D. prepared figures; A.G.J. drafted manuscript; A.G.J. and C.J.D. edited and revised manuscript; A.G.J. and C.J.D. approved final version of manuscript.

REFERENCES

1. **Andziak B, Buffenstein R.** Disparate patterns of age-related changes in lipid peroxidation in long-lived naked mole-rats and shorter-lived mice. *Aging Cell* 5: 525–532, 2006. doi:10.1111/j.1474-9726.2006.00246.x.

2. **Andziak B, O'Connor TP, Buffenstein R.** Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. *Mech Ageing Dev* 126: 1206–1212, 2005. doi:10.1016/j.mad.2005.06.009.

3. **Andziak B, O'Connor TP, Qi W, DeWaal EM, Pierce A, Chaudhuri AR, Van Remmen H, Buffenstein R.** High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5: 463–471, 2006. doi:10.1111/j.1474-9726.2006.00237.x.

4. **Ayala A, Muñoz MF, Argüelles S.** Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014: 360438, 2014. doi:10.1155/2014/360438.

5. **Blount DG, Heaton PR, Pritchard DI.** Changes to levels of DNA damage and apoptotic resistance in peripheral blood mononuclear cells and plasma antioxidant potential with age in labrador retriever dogs. *J Nutr* 134, *Suppl*: 2120S–2123S, 2004. doi:10.1093/jn/134.8.2120S.

6. **Buffenstein R.** Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. *J Comp Physiol B* 178: 439–445, 2008. doi:10.1007/s00360-007-0237-5.

7. **De Quiroga GB, Lopez-Torres M, Perez-Campo R.** Relationship between antioxidants, lipid peroxidation and aging. In: *Free Radicals and Aging*. Basel, Switzerland: Birkhäuser, 1992, p. 109–123.

8. **de Villemereuil P, Nakagawa S.** General quantitative genetic methods for comparative biology. In: *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*, edited by Garamszegi LZ. Heidelberg, Germany: Springer, p. 287–303.

9. **Deeb BJ, Wolf NS.** Studying longevity and morbidity in giant and small breeds of dogs. *Vet Med* 89: 702–713, 1994.

10. **Dingemanse NJ, Dochtermann NA.** Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J Anim Ecol* 82: 39–54, 2013. doi:10.1111/1365-2656.12013.

11. **Dowling DK, Simmons LW.** Reactive oxygen species as universal constraints in life-history evolution. *Proc Biol Sci* 276: 1737–1745, 2009. doi:10.1098/rspb.2008.1791.

12. **Eigenmann JE, Amador A, Patterson DF.** Insulin-like growth factor I levels in proportionate dogs, chondrodystrophic dogs and in giant dogs. *Acta Endocrinol (Copenh)* 118: 105–108, 1988. doi:10.1530/acta.0.1180105.

13. **Felsenstein J.** Phylogenies and the comparative method. *Am Nat* 125: 1–15, 1985. doi:10.1086/284325.

14. **Freedman AH, Gronau I, Schweizer RM, Ortega-Del Vecchyo D, Han E, Silva PM, Galaverni M, Fan Z, Marx P, Lorente-Galdos B, Beale H, Ramirez O, Hormozdiari F, Alkan C, Vilà C, Squire K, Geffen E, Kusak J, Boyko AR, Parker HG, Lee C, Tadigotla V, Wilton A, Siepel A, Bustamante CD, Harkins TT, Nelson SF, Ostrander EA, Marques-Bonet T, Wayne RK, Novembre J.** Genome sequencing highlights the dynamic early history of dogs. *PLoS Genet* 10: e1004016, 2014. doi:10.1371/journal.pgen.1004016.

15. **Galis F, Van der Sluijs I, Van Dooren TJM, Metz JAJ, Nussbaumer M.** Do large dogs die young? *J Exp Zoolog B Mol Dev Evol* 308: 119–126, 2007. doi:10.1002/jez.b.21116.

16. **Garamszegi LZ.** Uncertainties due to within-species variation in comparative studies: measurement errors and statistical weights. In: *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*, edited by Garamszegi LZ. Heidelberg, Germany: Springer, 2014, p. 157–199.

17. **Hadfield JD.** MCMC methods for multi-response generalized linear mixed models: the MCMCglmm *R* package. *J Stat Softw* 33: 2010. doi:10.18637/jss.v033.i02.

18. **Hadfield JD, Nakagawa S.** General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. *J Evol Biol* 23: 494–508, 2010. doi:10.1111/j.1420-9101.2009.01915.x.

19. **Halliwell B, Chirico S.** Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr* 57, *Suppl*: 715S–724S, 1993. doi:10.1093/ajcn/57.5.715S.

20. **Hawthorne AJ, Booles D, Nugent PA, Gettinby G, Wilkinson J.** Body-weight changes during growth in puppies of different breeds. *J Nutr* 134, *Suppl*: 2027S–2030S, 2004. doi:10.1093/jn/134.8.2027S.

21. **Helmsmüller D, Wefstaedt P, Nolte I, Schilling N.** Ontogenetic allometry of the Beagle. *BMC Vet Res* 9: 203, 2013. doi:10.1186/1746-6148-9-203.

22. **Hinchcliff KW, Reinhart GA, DiSilvestro R, Reynolds A, Blostein-Fujii A, Swenson RA.** Oxidant stress in sled dogs subjected to repetitive endurance exercise. *Am J Vet Res* 61: 512–517, 2000. doi:10.2460/ajvr.2000.61.512.

23. **Jimenez AG.** Physiological underpinnings in life-history trade-offs in man's most popular selection experiment: the dog. *J Comp Physiol B* 186: 813–827, 2016. doi:10.1007/s00360-016-1002-4.

24. **Jimenez AG, O'Connor ES, Tobin KJ, Anderson KN, Winward JD, Fleming A, Winner C, Chinchilli E, Maya A, Carlson K, Downs CJ.** Does cellular metabolism from primary fibroblasts and oxidative stress in blood differ between mammals and birds? The (lack-thereof) scaling of oxidative stress. *Integr Comp Biol* 59: 953–969, 2019. doi:10.1093/icb/icz017.

25. **Jimenez AG, Winward J, Beattie U, Cipolli W.** Cellular metabolism and oxidative stress as a possible determinant for longevity in small breed and large breed dogs. *PLoS One* 13: e0195832, 2018. doi:10.1371/journal.pone.0195832.

26. **Jimenez AG, Winward JD, Walsh KE, Champagne AM.** Effects of membrane fatty acid composition on cellular metabolism and oxidative stress in dermal fibroblasts from small and large breed dogs. *J Exp Biol* 223: jeb221804, 2020. doi:10.1242/jeb.221804.

27. **Letunic I.** phyloT: phylogenetic tree generator, https://phylot.biobyte.de/.

28. **Lewis KN, Andziak B, Yang T, Buffenstein R.** The naked mole-rat response to oxidative stress: just deal with it. *Antioxid Redox Signal* 19: 1388–1399, 2013. doi:10.1089/ars.2012.4911.

30. **Michell AR.** Longevity of British breeds of dog and its relationships with sex, size, cardiovascular variables and disease. *Vet Rec* 145: 625–629, 1999. doi:10.1136/vr.145.22.625.

31. **Middleton RP, Lacroix S, Scott-Boyer M-P, Dordevic N, Kennedy AD, Slusky AR, Carayol J, Petzinger-Germain C, Beloshapka A, Kaput J.** Metabolic differences between dogs of different body sizes. *J Nutr Metab* 2017: 4535710, 2017. doi:10.1155/2017/4535710.

32. **Nakagawa S, Schielzeth H.** A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol Evol* 4: 133–142, 2013. doi:10.1111/j.2041-210x.2012.00261.x.

33. **O'Connor TP, Lee A, Jarvis JUM, Buffenstein R.** Prolonged longevity in naked mole-rats: age-related changes in metabolism, body composition and gastrointestinal function. *Comp Biochem Physiol A Mol Integr Physiol* 133: 835–842, 2002. doi:10.1016/S1095-6433(02)00198-8.

34. **Parker HG, Dreger DL, Rimbault M, Davis BW, Mullen AB, Carpintero-Ramirez G, Ostrander EA.** Genomic analyses reveal the influence of geographic origin, migration, and hybridization on modern dog breed development. *Cell Reports* 19: 697–708, 2017. doi:10.1016/j.celrep.2017.03.079.

35. **Patronek GJ, Waters DJ, Glickman LT.** Comparative longevity of pet dogs and humans: implications for gerontology research. *J Gerontol A Biol Sci Med Sci* 52: B171–B178, 1997. doi:10.1093/gerona/52A.3.B171.

36. **Posada OS, Gomez OL, Rosero NR.** Application of the logistic model to describe the growth curve in dogs of different breeds. *Rev Mvz Cordoba* 19: 4015–4022, 2014. doi:10.21897/rmvz.121.

36a.**R Core Team.** *The R Project for Statistical Computing.* https://www.R-project.org/.

37. **Rikans LE, Hornbrook KR.** Lipid peroxidation, antioxidant protection and aging. *Biochim Biophys Acta* 1362: 116–127, 1997. doi:10.1016/S0925-4439(97)00067-7.

38. **Rubner M.** Ueber den einfluss der Korpergrosse auf Stoffund Kaftwechsel. *Z Biol* 19: 535–562, 1883.

39. **Speakman JR, Blount JD, Bronikowski AM, Buffenstein R, Isaksson C, Kirkwood TBL, Monaghan P, Ozanne SE, Beaulieu M, Briga M, Carr SK, Christensen LL, Cochemé HM, Cram DL, Dantzer B, Harper JM, Jurk D, King A, Noguera JC, Salin K, Sild E, Simons MJP, Smith S, Stier A, Tobler M, Vitikainen E, Peaker M, Selman C.** Oxidative stress and life histories: unresolved issues and current needs. *Ecol Evol* 5: 5745–5757, 2015. doi:10.1002/ece3.1790.

40. **Speakman JR, van Acker A, Harper EJ.** Age-related changes in the metabolism and body composition of three dog breeds and their relationship to life expectancy. *Aging Cell* 2: 265–275, 2003. doi:10.1046/j.1474-9728.2003.00061.x.

41. **Stier A, Massemin S, Criscuolo F.** Chronic mitochondrial uncoupling treatment prevents acute cold-induced oxidative stress in birds. *J Comp Physiol B* 184: 1021–1029, 2014. doi:10.1007/s00360-014-0856-6.

42. **Sutter NB, Bustamante CD, Chase K, Gray MM, Zhao K, Zhu L, Padhukasahasram B, Karlins E, Davis S, Jones PG, Quignon P, Johnson GS, Parker HG, Fretwell N, Mosher DS, Lawler DF, Satyaraj E, Nordborg M, Lark KG, Wayne RK, Ostrander EA.** A single IGF1 allele is a major determinant of small size in dogs. *Science* 316: 112–115, 2007. doi:10.1126/science.1137045.

43. **Tacutu R, Craig T, Budovsky A, Wuttke D, Lehmann G, Taranukha D, Costa J, Fraifeld VE, de Magalhães JP.** Human Ageing Genomic Resources: integrated databases and tools for the biology and genetics of ageing. *Nucleic Acids Res* 41: D1027–D1033, 2013. doi:10.1093/nar/gks1155.

44. **Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, Craig T, Diana E, Lehmann G, Toren D, Wang J, Fraifeld VE, de Magalhães JP.** Human Ageing Genomic Resources: new and updated databases. *Nucleic Acids Res* 46: D1083–D1090, 2018. doi:10.1093/nar/gkx1042.

45. **Todorova I, Simeonova G, Kyuchukova D, Dinev D, Gadjeva V.** Reference values of oxidative stress parameters (MDA, SOD, CAT) in dogs and cats. *Comp Clin Pathol* 13: 190–194, 2005. doi:10.1007/s00580-005-0547-5.

46. **Tomsič K, Seliškar A, Lukanc B, Nemec Svete A.** Plasma total antioxidant capacity and activities of blood glutathione peroxidase and superoxide dismutase determined in healthy dogs by using commercially available kits. *Acta Vet (Beogr)* 66: 534–548, 2016. doi:10.1515/acve-2016-0046.

47. **Urfer SR, Greer K, Wolf NS.** Age-related cataract in dogs: a biomarker for life span and its relation to body size. *Age (Dordr)* 33: 451–460, 2011. doi:10.1007/s11357-010-9158-4.

48. **Vajdovich P, Gaál T, Szilágyi A, Harnos A.** Changes in some red blood cell and clinical laboratory parameters in young and old Beagle dogs. *Vet Res Commun* 21: 463–470, 1997. doi:10.1023/A:1005929801735.