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Fowl play and the price of petrel: long-living Procellariiformes have peroxidation-resistant membrane composition compared with short-living Galliformes

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The membrane pacemaker hypothesis predicts that long-living species will have more peroxidation-resistant membrane lipids than shorter living species. We tested this hypothesis by comparing the fatty acid composition of heart phospholipids from long-living Procellariiformes (petrels and albatrosses) to those of shorter living Galliformes (fowl). The seabirds were obtained from by-catch of commercial fishing operations and the fowl values from published data. The 3.8-fold greater predicted longevity of the seabirds was associated with elevated content of peroxidation-resistant monounsaturates and reduced content of peroxidation-prone polyunsaturates and, consequently, a significantly reduced peroxidation index in heart membrane lipids, compared with fowl. Peroxidation-resistant membrane composition may be an important physiological trait for longevous species.

Keywords: longevity; lipid peroxidation; polyunsaturates; petrels; fowl

1. INTRODUCTION

Sexually reproducing animals must balance the competing nutritional needs of growth and self-maintenance with those of reproduction. This creates a fecundity–longevity trade-off (Kirkwood & Holliday 1979). Extrinsic mortality rates will shape the type of life history that best manages these conflicts and promotes the highest reproductive potential. Species having low rates of adult survivorship will benefit from high rates of fecundity at an early age, which competes with self-maintenance. Longevity traits are not likely to evolve in such species, because individuals with genes promoting longer lifespans will not realize higher inclusive fitness due to the high levels of extrinsic mortality. By contrast, species with low rates of extrinsic mortality will benefit from traits promoting long-term survivorship, if lifetime reproductive success is improved by living longer. This, in turn, will select for a complementary suite of physiological, morphological and behavioural traits that foster an increased maximum lifespan potential (MLSP). Among birds, the fowl (Galliformes) are good examples of the former, while petrels and albatrosses (Procellariiformes) exemplify the latter case.

Although there are many physiological processes that influence rates of ageing, those associated with oxidative stress, particularly from mitochondrial free-radical production, are proposed to be important (Harman 1956; Beckman & Ames 1998). A way to reduce free-radical production would be to reduce mitochondrial oxygen consumption. The first identified physiological correlate of lifespan was metabolic intensity (Rubner 1908), whereby the lifespans and mass-specific metabolic rate had similar inverse relations with body mass in mammals ranging widely in size and lifespan. This 'live fast, die young' interpretation of ageing, however, does not accord with birds living about twice as long as comparably sized mammals, yet having higher metabolic rates (Lindstedt & Calder 1976).

Comparison of long-living birds and shorter living mammals have identified traits that may contribute to their vastly different MLSP: birds have lower rates of mitochondrial reactive oxygen species (ROS) formation per unit of oxygen consumed (Barja *et al.* 1994; Herrero & Barja 1998) and have mitochondrial and cell membranes that are much less susceptible to damage through lipoxidation (Pamplona *et al.* 1999*a*,*b*). Owing to ROS attack mainly involving carbon atoms situated between two double-bonded carbons along an acyl chain, the susceptibility of membranes to oxidative damage is determined by their fatty acid composition (Halliwell & Gutteridge 1999). Saturated (SFA) and monounsaturated fatty acyl chains (MUFA) lack such carbon configurations and are largely unaffected by ROS, whereas polyunsaturated acyl chains (PUFA) are highly vulnerable. Importantly, because the products of membrane lipid peroxidation are themselves ROS (Halliwell & Gutteridge 1999), the process is autocatalytic and can propagate irreversible damage to the membrane and nearby cellular structures. Thus, species having a greater proportion of SFA and MUFA in their membranes will be more resistant to oxidative damage than species having proportionately more PUFA and will, therefore, be less prone to ageing effects associated with oxidative damage (Hulbert *et al.* 2007). This is the basis of the membrane pacemaker hypothesis of ageing, which predicts that MLSP will correlate with membrane lipid composition (Hulbert 2005).

If the membrane pacemaker hypothesis were robust, one would expect species from taxonomic groups with high MLSP to have membrane lipids with lower peroxidation susceptibility than those of shorter lived groups. We recently acquired a range of petrel and albatross specimens that were collected by the New Zealand Department of Conservation (NZDOC) as by-catch of commercial fisheries. The coincidence of receiving these birds with high MLSP (Holmes & Austad 1995) and a recent publication characterizing cardiac muscle lipids of birds emphasizing much shorter living gallinaceous species (Szabo *et al.* 2006) provided the opportunity to test the membrane pacemaker hypothesis of ageing.

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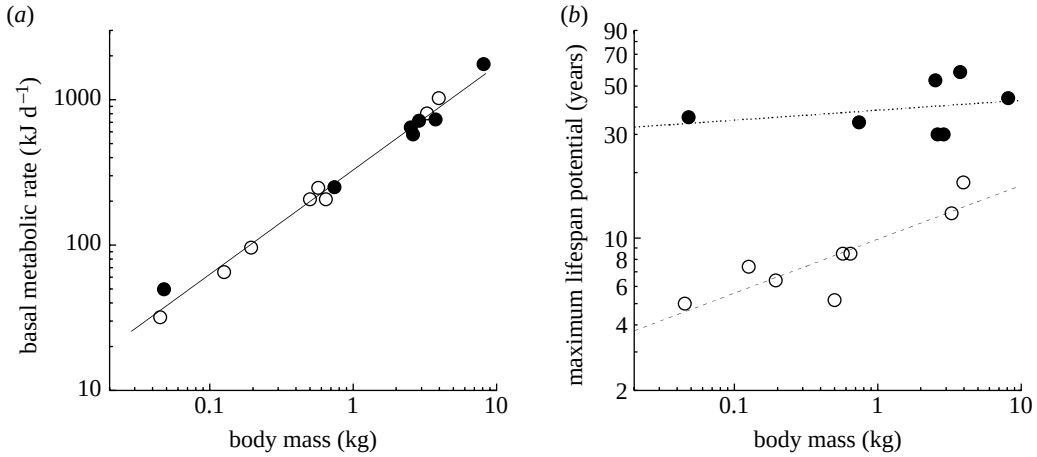

Figure 1. (*a*) BMR and (*b*) MLSP of petrels ($n=7$ species, closed circles) and fowl ($n=8$ species, open circles) in relation to body mass. The lines in both plots represent best-fit least-squares regression through all data points (see tables for data sources in the electronic supplementary material).

2. MATERIAL AND METHODS

(a) *Experimental animals*

All albatrosses and petrels used in our study were frozen upon collection and returned to shore for validation of identification and weighing. Birds taken in this manner are in excellent condition and were made available to us by C. J. R. Robertson for our research purposes. Upon thawing, we extracted the heart from the following species: fairy prion (*Pachyptila turtur*, 122 g); diving petrel (*Pelecanoides urinatrix*, 125 g); broad-billed prion (*Pachyptila vittata*, 180 g); two grey-faced petrels (*Pterodroma macroptera gouldi*, 480 and 531 g); two flesh-footed shearwaters (*Puffinus carneipes*, 586 and 605 g); white-chinned petrel (*Procellaria aequinoctialis*, 1.13 kg); two Buller's albatrosses (*Thalassarche bulleri*, 2.99 and 3.15 kg); Campbell albatross (*Thalassarche impavida*, 3.21 kg); and royal albatross (*Diomedea epomophora*, 8.70 kg).

(b) *Heart phospholipid analysis*

Total lipids were isolated, phospholipids separated and their fatty acid composition determined using techniques described previously (Hulbert *et al.* 2006; see appendix in the electronic supplementary material). Fatty acids were grouped according to the number of double bonds (noted by the suffix -enoics) and peroxidation index (PI) calculated: PI = (0.025 × % monoenoics) + (1 × % dienoics) + (2 × % trienoics) + (4 × % tetraenoics) + (6 × % pentaenoics) + (8 × % hexenoics), based on empirical measurements of Holman (1954). We similarly determined the PI for four gallinaceous species from published fatty acid composition of myocardial phospholipids (Szabo *et al.* 2006).

(c) *Metabolic rate and MLSP*

Because many of the Procellariiformes and Galliformes used in our evaluations of cardiac muscle PI lacked data for both MLSP and basal metabolic rate (BMR), we surveyed the literature to find suitable measures of both MLSP and BMR for species comprising a wide mass distribution for both groups. The species selected along with associated MLSP and BMR data and their sources are listed in the tables of the electronic supplementary material.

(d) *Statistical analyses*

Statistical procedures were performed using SPSS v. 13 (SPSS Inc., Chicago, IL, USA; see appendix for details in the electronic supplementary material). All data are presented as means ±1 s.e. unless specified otherwise.

3. RESULTS

The relationship between body mass and BMR was very similar in the selected Procellariiformes and Galliformes in having statistically indistinguishable intercept values ($t=0.24$; $p>0.7$), but slightly different slopes ($t=2.44$; $p<0.05$). As a consequence, metabolic rates differed by 10% or less over the size range of 500–3000 g (figure 1*a*). By contrast, the average MLSP of Procellariiformes (41.6 ± 3.0 years) was 4.6-fold greater than the average MLSP of the Galliformes (9.0 ± 1.6 years; figure 1*b*). There was a significant effect of body mass on MLSP in fowl ($F_{1,6}=17.29$; $p=0.006$) but not in petrels ($F_{1,5}=0.454$; $p=0.53$). Because the groups have unequal size distributions, the average MLSP is biased towards small individuals in the fowl. This bias can be avoided by comparing MLSP of similar-sized birds. The MLSP predicted for a 2.9 kg fowl (i.e. mean mass of petrels) is 12.8 years for such a fowl, which is approximately 30% of average petrel longevity.

The fatty acid composition of cardiac phospholipids also differed conspicuously between these groups. The petrels had significantly more MUFA in their membranes ($F_{1,11}=100.6$; $p<0.001$), whereas fowl had significantly more PUFA ($F_{1,11}=115.7$; $p<0.001$; figure 2*a*). Among the PUFA, the ratio of *n*-6 : *n*-3 PUFA was 9 : 1 in the fowl and 3 : 1 in the petrels (figure 2*a*). Because MUFAs are resistant to oxidative damage compared with PUFAs and similarly *n*-6 PUFA are generally less peroxidizable than *n*-3 PUFA (figure 2*b*), the heart membrane phospholipids of petrels had a significantly lower PI than those of the fowl ($F_{1,11}=9.48$; $p=0.01$). PI values ranged from 71 to 113 in the petrels and from 116 to 223 in the fowl and, after log transformation, showed a significant inverse relation with body mass in fowl ($r=-0.89$; $p<0.05$), but not in petrels. Overall, the PI of petrels was 36% lower than that of fowl (figure 2*c*).

4. DISCUSSION

The metabolic rates of these two groups are remarkably similar, yet their MLSPs are vastly different. Although total lifetime energy expenditure is more relevant to lifetime cumulative oxidative damage than is BMR, most birds of a given size have a similar ratio of BMR to daily energy expenditure (Daan *et al.* 1990). This makes it very unlikely that the variation in MLSP between these groups is related to differences in energy expenditure. Apart from MLSP, the most striking difference between these groups was the peroxidation susceptibility of their cardiac membranes. The significantly lower PI values in the Procellariiformes are consistent with predictions of

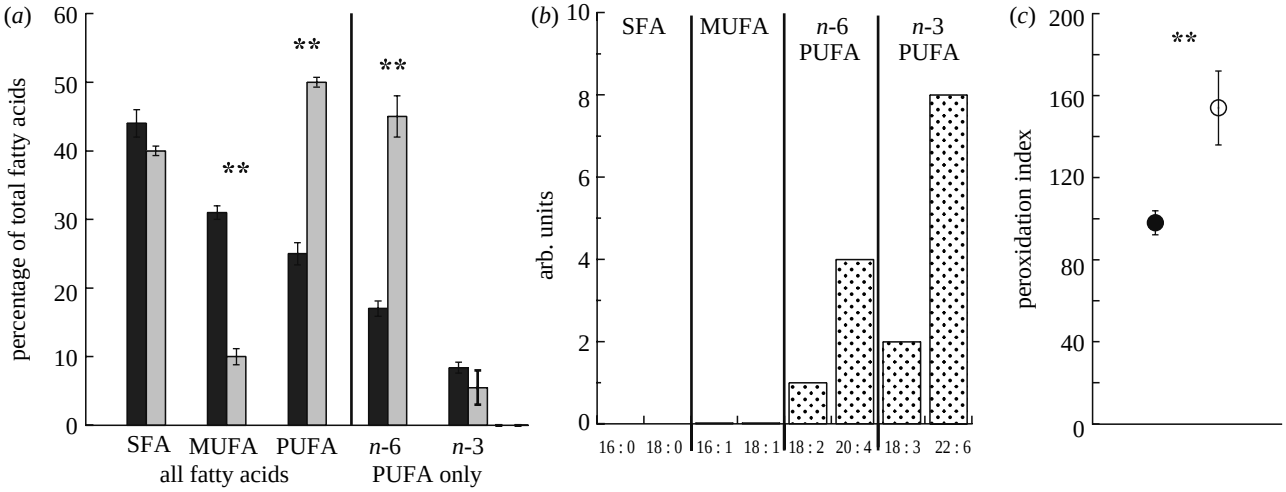

Figure 2. (*a*) Fatty acid composition of cardiac phospholipids of (black bars) petrels ($n=9$ species) and (grey bars) fowl ($n=4$ species; from Szabo *et al.* 2006). (*b*) The relative rate of peroxidation of select fatty acids. Individual fatty acids identified as 'acyl chain carbons: double bonds'. The values empirically determined by Holman (1954). (*c*) The average PI of cardiac phospholipids of (closed circle) petrels ($n=9$ species) and (open circle) fowl ($n=4$ species; after Szabo *et al.* 2006). All error bars are ± 1 s.e.m. **Significant difference ($p \leq 0.01$) between petrels and fowl.

the membrane pacemaker hypothesis of ageing. Even more striking, however, is that the magnitude of the differences in PI accords well with the differences in MLSP between these groups. Based on the evaluations of PI of skeletal muscle phospholipids in relation to MLSP for birds and mammals, a decrease of only 19% in PI is associated with every doubling of MLSP (Hulbert *et al.* 2007). Applying this relation to the current comparison, the 36% lower PI in petrels predicts a 4.5-fold greater MLSP for petrels compared with fowl. This value is the same order of magnitude as the 4.6-fold average difference and similar to the 3.2-fold size-adjusted difference in MLSP that we determined for these groups.

It is quite obvious that the lower PI found among petrels is mainly the result of selective incorporation of MUFA into their membranes at the expense of PUFA. This is because MUFA are 40-fold more resistant to peroxidation than a PUFA containing two double bonds and 320 times more peroxidation-resistant than a PUFA containing six double bonds (figure 2*b*). Although we do not know the fatty acid composition of petrel diets, we did characterize the fatty acids of several birds' storage fats. These were much higher in MUFA than SFA, and also had a fivefold higher content of *n*-3 versus *n*-6 PUFA, which is expected from their fish and cephalopod diet. The lower proportion of *n*-3 to *n*-6 PUFA in petrel cardiac membranes compared with their diet demonstrates the highly selective nature of fatty acid incorporation into membrane lipids. Such discrimination of dietary fats is especially important with diets rich in *n*-3 PUFA, as, for a given chain length, these PUFA have a higher PI than their *n*-6 counterparts (figure 2*b*).

We stated earlier that species benefiting from longevity should be less prone to oxidative stress than short-lived species. We also proposed that membrane fatty acid composition would be more peroxidation resistant in longer lived than in shorter lived species. Our characterization of membrane fatty acids in long-living Procellariiformes compared with Galliformes supports these predictions. These preliminary observations are intriguing and a more extensive comparison of avian taxonomic groups that differ in lifespan is currently underway.

This study was done in accordance with the NHMRC Australian code of practice for the care and use of animals for scientific purposes.

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