Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds

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The proton conductance of isolated liver mitochondria correlates significantly with body mass in mammals, but not in ectotherms. To establish whether the correlation in mammals is general for endotherms or mammal-specific, we measured proton conductance in mitochondria from birds, the other main group of endotherms, using birds varying in mass over a wide range (nearly 3000-fold), from 13 g zebra finches to 35 kg emus. Respiratory control ratios were higher in mitochondria from larger birds. Mitochondrial proton conductance in liver mitochondria from birds correlated strongly with body mass [respiration rate per mg of protein driving proton leak at 170 mV being 44.7 times (body mass in g)$^{-0.19}$], thus suggesting a general relationship between body mass and proton conductance in endotherms. Mitochondria from larger birds had the same or perhaps greater surface area per mg of protein than mitochondria from smaller birds. Hence, the lower proton conductance was caused not by surface area changes but by some change in the properties of the inner membrane. Liver mitochondria from larger birds had phospholipid fatty acyl chains that were less polyunsaturated and more monounsaturated when compared with those from smaller birds. Phospholipid fatty acyl polyunsaturation correlated positively and monounsaturation correlated negatively with proton conductance. These correlations echo those seen in mammalian liver mitochondria, suggesting that they too are general for endotherms.

Key words: allometry, body mass, fatty acid, mitochondria, phospholipid, proton conductance.

INTRODUCTION

Mitochondria isolated from different species are not the same. In particular, liver mitochondria isolated from large mammals are much better coupled than those from smaller mammals [1,2]. The degree of coupling is described by the respiratory control ratio, which expresses how strongly ADP or chemical uncouplers are capable of stimulating oxygen consumption compared with the basal (or state 4) rate. There is an allometric relationship between the respiratory control ratio of mammalian liver mitochondria and body mass: the respiratory control ratio increases by 32 % for every 10-fold increase in body mass. Mitochondria make ATP by pumping protons outwards across the inner membrane and using the energy released by their return to drive ATP synthesis, and hence leakage of protons across the membrane causes inefficiency. The better coupling of liver mitochondria from large mammals is associated with lower proton permeability of the inner membrane; proton leak rate per mg of mitochondrial protein at the same driving force is 4.5-fold lower in the 340 kg horse compared with the 20 g mouse [2].

It is well established that the mass-specific standard metabolic rates of animals decrease with body mass [3–6]. In mammals, this is caused by a lower proportion of metabolically active tissues in large animals, and a lower intensity of metabolism in those organs [7–9]. For example, liver slices and hepatocytes from larger mammals have lower respiration rates than those from smaller mammals [7,9–11]. A result of the lower proton conductance of liver mitochondria from large mammals is that the efficiency of oxidative phosphorylation is maintained despite the strong decrease in ATP demand in larger animals. Hence a constant proportion of metabolic energy is lost through proton cycling across the mitochondrial inner membrane in hepatocytes of all mammals studied, in spite of their quite different metabolic rates [11].

More than 10 years ago, we reported that liver mitochondria from an ectotherm, the bearded dragon lizard, had lower proton conductance than liver mitochondria from the rat, a mammalian endotherm with the same body mass and body temperature [12]. We postulated that lower proton conductance was related to the lower metabolic rates of ectotherms compared with endotherms and was a general property of ectotherm mitochondria. To test this postulate, we recently investigated the proton conductance of liver mitochondria isolated from a variety of ectothermic species [13–15]. Contrary to our postulate, we found that low proton conductance of liver mitochondria was not a general property of ectotherms [15]. However, ectotherm mitochondria generally did have lower rates of proton leak. They achieved this by two different mechanisms: lowered proton conductance (which decreases proton leak rate at any given driving force), or lowered electron-transport-chain activity (which decreases proton leak rate by lowering membrane potential on which it depends). We also investigated the proton conductance of liver mitochondria from large ectotherms (crocodiles), and found that it did not obey the predicted behaviour of lower proton conductance in larger animals that we had found in mammals. Instead, the proton conductance of crocodile liver mitochondria was greater than that of smaller ectotherms, not lower as we had predicted. There was also no allometric relationship between proton conductance and body mass in the disparate ectotherms we studied [15].

In the present study, we examined proton conductance of liver mitochondria from the second major group of endotherms, the birds, to investigate whether the allometric relationships found in mammals are general for all endotherms, or specifically mammalian. We found that, in liver mitochondria from birds,

Abbreviations used: FCCP, carbonylcyanide *p*-(trifluoromethoxy)phenylhydrazone; TPMP, methyltriphenyl phosphonium.

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similar to those from mammals, there is a strong correlation between proton conductance and body mass, suggesting that this correlation is general for endotherms.

There are consistent correlations between mitochondrial proton conductance and phospholipid fatty acyl composition [2,12,13, 15–17]. There are also clear allometric relationships between body mass and the phospholipid fatty acyl composition of isolated mammalian mitochondria [2], of different tissues in mammals [18–20] and of skeletal muscles in birds [21]. In the present study, we also analysed the relationship between phospholipid fatty acyl composition of liver mitochondria, mitochondrial proton conductance and body mass in birds to investigate whether the allometric relationships found in mammals are general for all endotherms, or specifically mammalian. We found that, in mitochondria from birds, similar to those from mammals, there are correlations between fatty acyl composition and body mass or proton conductance, suggesting that these relationships are also general for endotherms.

EXPERIMENTAL

Birds

All experiments were approved by the University of Wollongong Animal Experimentation Ethics Committee. Emus (*Dromaius novaehollandiae* Latham) were purchased from Marayong Park Emu Farm (Falls Creek, NSW, Australia). Zebrafinches (*Taeniopygia guttata* Vieillot), domestic ducks (*Anas platyrhynchos* L.) and domestic geese (*Anser anser* L.) were purchased from local pet shops or the Narellan Aviary Bird Auction (NSW, Australia). Feral pigeons (rock dove, *Columba livia* Gmelin) were from a pigeon breeder (T. Cooper, Corrimal, NSW, Australia). House sparrows (*Passer domesticus* L.), starlings (*Sturnus vulgaris* L.) and pied currawongs (*Strepera graculina* Shaw) were trapped in or near Wollongong (NSW, Australia). All birds were killed by anaesthetic overdose (sodium pentobarbitone, 100 mg/kg body mass; intraperitoneal, except for emus where injection was intrajugular) within a few days of purchase. When birds were kept in captivity, they were provided with water and food *ad libitum* (mixed bird seed for finches and sparrows and a commercial mixture of pellets and seeds for ducks and geese). The diet of birds before their purchase was unknown.

Mitochondria

For finches and sparrows much of the liver from four birds, and for starlings much of the liver from two birds was used for each mitochondrial preparation. For pigeons and currawongs (and rats) most of one liver was used, and for the larger birds a 20 g sample of liver was used for each mitochondrial preparation. Liver samples were chopped coarsely with scissors (or using a household blender for two of the four emus), rinsed and resuspended in 2–10 vol. of ice-cold medium, containing 250 mM sucrose, 5 mM Tris/HCl and 2 mM EGTA (pH 7.4 at 4 °C), then homogenized using six passes of a motorized Teflon/glass homogenizer. The homogenate was centrifuged at 1000 ***g*** for 3 min in a Beckman centrifuge, and the supernatant was centrifuged at 12 000 ***g*** for 10 min. The pellet was then suspended, respun at 12 000 ***g***, resuspended in isolation medium and kept on ice. Mitochondrial protein concentration was determined by Lowry assay with BSA as standard.

Mitochondrial oxygen consumption rates and membrane potentials

Oxygen consumption was measured using a Strathkelvin oxygen electrode connected to a Strathkelvin 781 oxygen meter. Mitochondrial membrane potential was measured using a World Precision Instruments TPP electrode [filled with 10 mM TPMP (methyltriphenyl phosphonium) chloride] and reference inserted into the oxygen electrode chamber through a custom-made Teflon plug, and connected to a Cyberscan 2000 pH meter. The output from the electrodes was fed to an ADI Instruments Powerlab 400 datalogger and Macintosh G3 powerbook. Mitochondria were incubated in a medium containing 120 mM KCl, 5 mM phosphate (potassium), 3 mM Hepes, 1 mM EGTA, 1 mM $MgCl_2$, 5 μM rotenone and 3 mg/ml defatted BSA, in a 1 ml plastic water-jacketted oxygen electrode chamber, magnetically stirred at 500 rev./min and maintained at 37 °C. For measurements of respiration rate, mitochondria were added (approx. 1 mg of mitochondrial protein/ml), followed, at intervals, by 4 mM succinate and 150 nmol of ADP. After state 4 was achieved, 1 μg/ml oligomycin and 2 μM FCCP [carbonylcyanide *p*-(trifluoromethoxy)phenylhydrazone] were added. For measurements of proton-leak kinetics, mitochondria were added (approx. 1 mg of mitochondrial protein/ml), followed by 1 μg/ml oligomycin, 10 nM nigericin and five successive additions of 1 μM TPMP to calibrate the TPMP electrode. Respiration and production of membrane potential were initiated by the addition of 4 mM succinate. After 1–2 min, once steady values were attained, they were titrated through several successive steady states by additions of malonate up to approx. 15 mM. Finally, 2 μM FCCP was added to dissipate fully the membrane potential and allow correction for any small electrode drift. For each species, doubling the nigericin, oligomycin or FCCP concentrations made no difference to the results, to proving that they were in excess. Membrane potentials were calculated assuming a TPMP binding correction of 0.54 mg/μl for all avian species (0.4 for rats) [13]. For details of the determinations of proton-leak kinetics see [2,13–15,22].

Phospholipid fatty acyl composition

Total lipids were extracted from mitochondria by standard methods using ultrapure-grade chloroform and methanol (2:1, v/v), containing butylated hydroxytoluene (0.01 or 0.05 %, w/v) as an antioxidant. Phospholipids were separated from neutral lipids by using silica cartridges. The phospholipid fatty acyl composition was determined as described previously [21]. Total phospholipid content was determined by phosphorus assay [2,23].

RESULTS AND DISCUSSION

Standard metabolic rate

The avian species examined here are listed in Table 1 together with their body masses and standard metabolic rates (see also [21]). Mass-specific standard metabolic rate is known to be lower in birds with greater body mass, as in other animals [5,6,24]. The expected decrease was observed for the species used in the present study, with standard metabolic rate (ml of $O_2 \cdot g^{-1} \cdot h^{-1}$) = $9.3 \times \text{mass (g)}^{-0.37}$, $P < 0.01$. In the present study, avian species were chosen to span as wide a range of body masses as was practical and on the basis of availability. Passerines generally have higher mass-specific standard metabolic rates than non-passerines, and the unusually large negative exponent probably reflects the fact that the four smaller species were passerines and the four larger ones were non-passerines [21]. Nonetheless, this set of species had a wide range of mass-specific standard metabolic rates, more than 20-fold, and was therefore suitable for a further detailed study of the relationships between metabolic rate and mitochondrial properties in birds.

Table 1 Body masses and standard metabolic rates of different avian species

Species	Body mass (g) ± S.E.M. (*n*)*	Mass-specific standard metabolic rate (mol of $O_2 \cdot g^{-1} \cdot h^{-1}$)†
Zebrafinch (*Taeniopygia guttata*)	12.7 ± 0.6 (17)	3.28
House sparrow (*Passer domesticus*)	24.5 ± 0.2 (16)	2.46
Starling (*Sturnus vulgaris*)	74.0 ± 2.4 (8)	2.31
Pied currawong (*Strepera graculina*)	283 ± 19 (4)	1.59
Pigeon (*Columba livia*)	462 ± 35 (4)	0.667
Duck (*Anas platyrhynchos*)	2178 ± 61 (4)	0.626
Goose (*Anser anser*)	4487 ± 341 (4)	0.547
Emu (*Dromaius novaehollandiae*)	34 975 ± 745 (4)	0.152

* Total number of birds used for a total of four mitochondrial preparations per species.
† Data from [21].

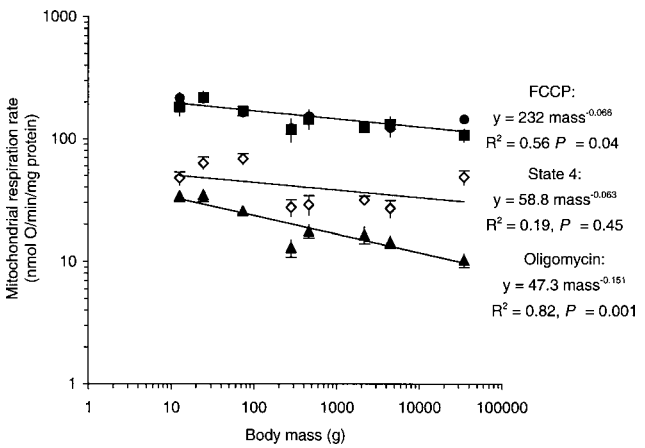

Figure 1 Dependence of the respiration rate of isolated liver mitochondria on body mass in different species of birds

Succinate (4 mM) in the presence of rotenone (5 μM) was used as the substrate; for details see the Experimental section. ■, state 3 respiration after the addition of 150 μM ADP; ◇, state 4 respiration after the consumption of added ADP; ▲, state 4 non-phosphorylating respiration after the addition of 1 μg of oligomycin/mg of protein; ●, uncoupled respiration after the addition of 2 μM FCCP. Experiments were repeated at least twice and averaged for each preparation. Data represent means ± S.E.M. of average results from four independent preparations for each species. Lines are best power fits to the data as described by the inset equations.

Mitochondrial oxygen consumption rates

Liver mitochondria were isolated from each of the avian species and their respiration rates with succinate as substrate were measured using an oxygen electrode (Figure 1). In general, liver mitochondria from the larger species had lower respiration rates (per mg of mitochondrial protein) than those from the smaller species. The rate of oxygen consumption in the presence of the uncoupler FCCP is limited by substrate uptake and the electron-transport chain, and was significantly slower in mitochondria from larger birds. The rate of oxygen consumption at maximum rates of ATP synthesis from added ADP (state 3 respiration) was similar to the uncoupled rates, showing that the phosphorylation of ADP and the exchange of adenine nucleotides across the mitochondrial inner membrane were not strongly rate-controlling in these mitochondria under our experimental conditions. The state 3 rates tended to be lower in the larger species [respiration (nmol of $O \cdot min^{-1} \cdot mg$ of protein^{-1}) $= 233 \times$ mass (g)$^{-0.077}$] but the trend was not quite significant ($P = 0.056$).

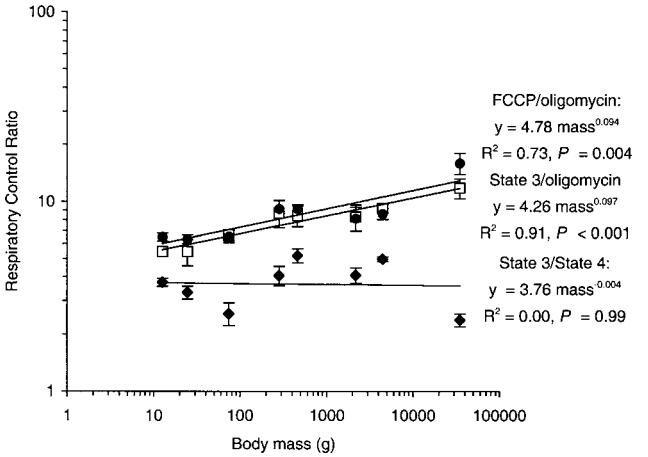

Figure 2 Dependence of the respiratory control ratios of isolated liver mitochondria on body mass in different species of birds

Respiratory control ratios were calculated as follows: ●, uncoupled respiration rate with FCCP divided by non-phosphorylating respiration rate with oligomycin; □, state 3 respiration rate divided by non-phosphorylating respiration rate with oligomycin; ◆, state 3 respiration rate divided by state 4 respiration rate. For details see Figure 1. Experiments were repeated at least twice and averaged for each preparation. Data represent means ± S.E.M. of average results from four independent preparations for each species. Lines are best power fits to the data as described by the inset equations.

The state 4 oxygen consumption rate achieved after all the added ADP had been phosphorylated to ATP was limited mainly by ATP hydrolysis and the leak of protons across the mitochondrial inner membrane; there was no significant relationship with body mass. Oligomycin is an inhibitor of the ATP synthase, and prevents recycling of any ADP formed from ATP by contaminating ATPases in the mitochondrial preparations. Therefore respiration rate in the presence of oligomycin is determined mostly by the proton conductance of the inner membrane. The oxygen consumption rate in the presence of oligomycin was significantly lower in mitochondria from the larger avian species (Figure 1), suggesting that mitochondrial proton conductance was lower in liver mitochondria from the larger avian species (see below). The strong decrease in state 4 respiration rates caused by oligomycin in mitochondrial preparations from the larger species shows that they had considerable ATPase activity. The cause of this ATPase activity was not explored further, but it might reflect more mitochondrial breakage owing to greater mechanical forces generated during the homogenization of the tougher liver tissue from larger birds.

The quality of mitochondrial preparations is often assessed using the respiratory control ratio, i.e. the state 3 rate divided by the state 4 rate. By this criterion, mitochondria from the larger species were no better or worse than those from the smaller species: respiratory control ratios with succinate as substrate ranged from 2.4 to 5.0, with a mean of 3.8, and did not depend on body mass (Figure 2). However, the contaminating ATPase activity of mitochondria from larger birds disguises the fact that these mitochondria are, intrinsically, considerably better coupled. Respiratory control ratios calculated by dividing the uncoupled or state 3 rates by the rates in the presence of oligomycin were much higher than those using the state 4 rates. They ranged from 5.4 to 15.9 and had a strong dependence on body mass (Figure 2). Thus, liver mitochondria from larger bird species are much better coupled than those from smaller bird species, since the non-phosphorylating respiration rates (limited mainly by proton leak rate) decrease more strongly than the phosphorylating

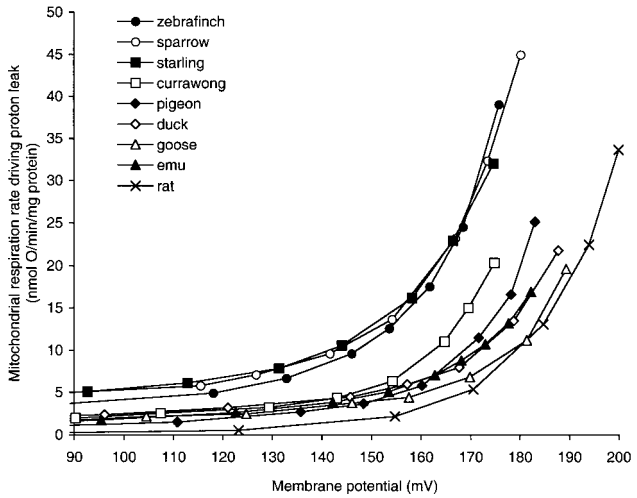

Figure 3 Comparison of the kinetics of proton leak in liver mitochondria isolated from different species of birds

Respiration rate multiplied by the H^+/O ratio of 6.0 for succinate oxidation gives the proton leak rate, which has been plotted as a function of its driving force, namely the membrane potential. For details see the Experimental section. Experiments were repeated at least three times and averaged for each preparation. Average results from four independent preparations for each species (two preparations for rat) have been given. Error bars are omitted for clarity, but S.E.M. values were less than 8 % (mean 4 %) of the values for the membrane potential and less than 19 % (mean 13 %) of the values for the respiration rate in mitochondria from all birds.

and uncoupled rates (limited mainly by substrate and electron transport) as body mass increases. This trend is also apparent in liver mitochondria isolated from mammalian species of different body mass [1,2], suggesting that it is a general feature for endotherms.

Proton conductance of liver mitochondria from different avian species

To test directly whether proton conductance does indeed decrease in liver mitochondria isolated from larger birds, we measured the kinetics of the proton leak. Figure 3 shows the rate of proton leak (measured as the rate of oxygen consumption used to drive the leak) plotted as a function of its driving force, namely the mitochondrial membrane potential. A clear trend can be observed: mitochondria from smaller species had the highest proton leak rates, and those from the larger species tended to have progressively lower rates of proton leak at any particular driving force. This trend is the same as that seen for mammalian liver mitochondria [1,2]. This observation is not influenced by any size-related differences in ATPase activity in the mitochondrial preparations, since it was measured in the presence of oligomycin.

To allow comparison between kinetic curves, Figure 4 analyses the proton leak rate at a single value of the membrane potential, 170 mV (the highest membrane potential common to both the published mammalian data and the avian data presented here). Figure 4(a) shows that there was a strong and significant negative dependence of proton leak rate at 170 mV on body mass in birds, with an exponent of -0.19, similar to the exponent of -0.13 seen in mammalian mitochondria [1,2]. There was also a strong positive correlation between proton leak rate and mass-specific standard metabolic rate, with an exponent of 0.47 (Figure 4b), showing that the relationship did not depend on the size distribution of the passerines and non-passerines.

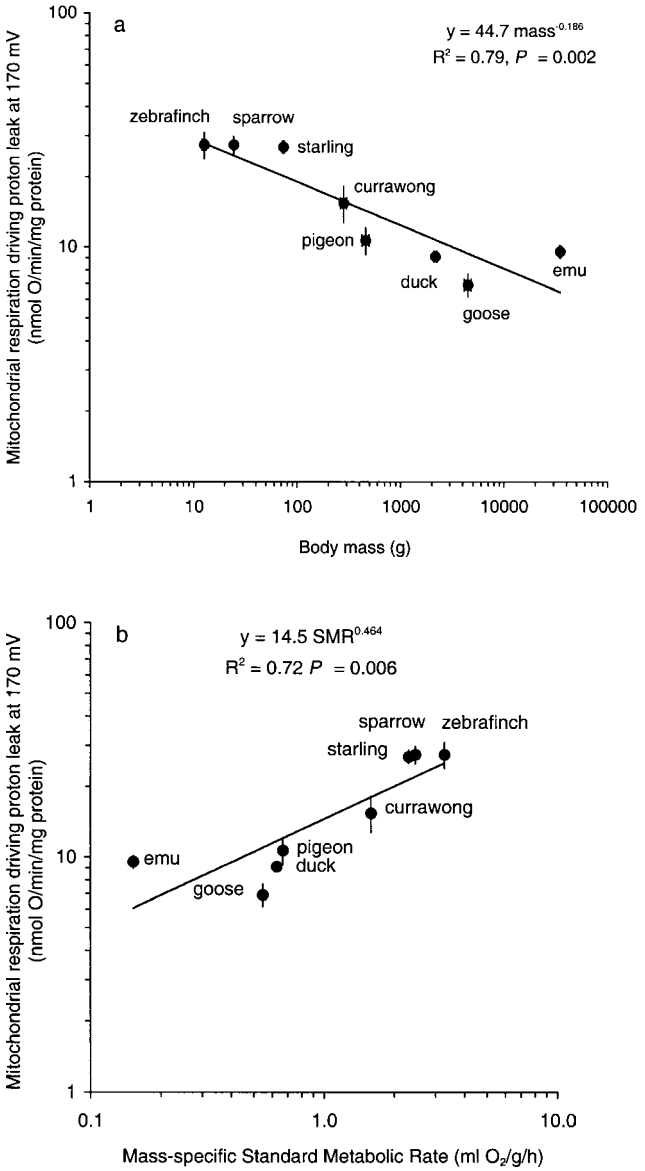

Figure 4 Allometric relationship between mitochondrial proton leak rate at 170 mV and either body mass or mass-specific standard metabolic rate for different bird species

Relationship between proton leak rate and (**a**) body mass and (**b**) mass-specific standard metabolic rate for different bird species. Proton leak rate was measured as respiration rate at 170 mV in the presence of oligomycin. Data from Table 1 and Figure 3. The lines are best power fits to the data as described by the inset equations.

The elevation constant describing the relationship between proton leak rate per mg of protein at 170 mV and body mass was 44.7 in avian mitochondria (Figure 4a) and 61.3 in mammalian mitochondria (after adjusting the value of 150.7 given in [2] to the units used here), suggesting that liver mitochondria from a 100 g bird would have only approx. 56 % of the proton conductance of liver mitochondria from a 100 g mammal. However, the leak measurements in mammals were performed in the absence of bovine serum albumin, whereas the avian measurements were performed in the presence of albumin. Albumin binds fatty acids that increase the proton leak rate [25], and hence the calculated proton conductances from the two studies cannot

be compared. Figure 3 includes the proton-leak kinetics for rat liver mitochondria measured at the same time as the kinetics of avian liver mitochondria, showing that the rat liver mitochondria were not leakier than the comparable bird liver mitochondria but were, in fact, less leaky. Similarly, Brookes et al. [13] found that, in the presence of albumin, the proton-leak kinetic curves of rat and pigeon liver mitochondria overlapped. Thus the proton conductance of avian liver mitochondria depends strongly on body mass, but does not differ much from the proton conductance of mitochondria from mammals of comparable body mass.

Overall, liver mitochondria from larger birds had lower oxygen consumption rates in the absence of ATP synthesis compared with mitochondria from smaller birds (Figure 1, oligomycin present); that is, under resting conditions, they had to pump less protons to counter the proton leak across the inner membrane. Similar to the mitochondria from ectotherms when compared with endotherms [15], they achieved this by two separate mechanisms: first (Figure 4a), by a decrease in proton conductance (which lowers the proton leak rate at any given membrane potential) and secondly (Figure 1, FCCP present), by a decrease in the capacity for substrate entry and electron transport, measured as the uncoupled respiration rate (which tends to lower the membrane potential, to which the proton leak rate is very sensitive). Together, these two mechanisms cause the resting respiration rate of liver mitochondria from larger birds to be less than that of smaller birds, reflecting the lower mass-specific standard metabolic rate of larger birds. The decrease in proton conductance tends to increase the membrane potential and the decrease in respiratory chain activity tends to decrease it. The trend for higher resting potentials in liver mitochondria from larger birds (Figure 3: $P = 0.080$ for regression of maximum potential on log body mass) suggests that the dominant effect is the decreased proton conductance of mitochondria from larger birds.

The proton leak rates discussed so far were calculated per mg of mitochondrial protein. As a result, changes in mitochondrial inner-membrane surface area per mg of protein could contribute to changes in calculated proton conductance. Indeed, in mammalian liver mitochondria, most of the mass-dependent differences in proton conductance per mg of protein could be explained by mass-dependent differences in inner-membrane surface area [2]. The amount of phospholipid that can be extracted from isolated mitochondria is one measure of the total phospholipid surface area of the inner and outer mitochondrial membranes, and Porter et al. [2] found that the yield of mitochondrial phospholipid decreased significantly with body mass in mammals. Table 3 lists the amount of phospholipid extracted from avian mitochondria from different species in the present study. Unlike mammals, there was no decrease in liver mitochondrial phospholipid content with body mass in birds. Instead, there was a trend for higher phospholipid yields from mitochondria from larger birds [μg of phospholipid/mg of mitochondrial protein $= 52.2 \times$ body mass $(\mathrm{g})^{0.077}$], although this failed to reach statistical significance ($P = 0.15$).

In mammals, the correlation between body mass and mitochondrial proton leak at 170 mV did not reach statistical significance when leak was expressed per mg of mitochondrial phospholipid [2]. In birds, however, due to the trend for higher phospholipid yields in mitochondria from larger birds, this correlation was very significant [mitochondrial proton leak at 170 mV expressed as nmol of $\mathrm{O} \cdot \mathrm{min}^{-1} \cdot \mu\mathrm{g}^{-1}$ mitochondrial phospholipid $= 0.85 \times$ mass $(\mathrm{g})^{-0.262}$, $P = 0.017$]. Nevertheless, both mammals and birds show a significant allometric relationship between body mass and proton conductance per mg of protein. In mitochondria from mammals, the changes in proton conductance per mg of protein can be mostly explained by changes in the inner-membrane surface area without having to invoke large changes in the conductance properties of that membrane [2]. However, in avian mitochondria there is a constant or increased surface area in larger birds, and we need to invoke changes in some property of the inner membrane to explain the changes in proton conductance per mg of protein.

Phospholipid fatty acyl composition of liver mitochondria from different avian species

Previous studies have demonstrated consistent correlations between mitochondrial proton conductance and phospholipid fatty acyl composition [2,12,13,15–17]. There are some differences between studies, but over a wide range of different vertebrate species and experimental treatments we recently calculated that there is a positive correlation with $n-3$ polyunsaturate content, particularly 22:6n3, and a negative correlation with monounsaturate content, particularly 18:1n9 [15]. Since the proton conductance of liposomes prepared from mitochondrial phospholipids is only approx. 5 % of the proton conductance of rat liver mitochondria [26], and the proton conductance of these liposomes is independent of phospholipid fatty acyl composition [27], it is unlikely that these correlations reflect an enhanced proton conductance through the mitochondrial membrane lipid bilayer itself. It remains possible, however, that they reflect an influence of the mitochondrial bilayer on mitochondrial membrane proteins.

To examine this question further, we measured the phospholipid fatty acyl composition of liver mitochondria isolated from different bird species. Table 2 reports the values for individual fatty acyl species, and in Table 3 values for the derived global indices are presented. Our values for pigeon are very similar to previously reported values from our own [27] and other [28] studies.

There were several significant correlations between mitochondrial fatty acyl composition and log body mass in birds (Tables 2 and 3). There was a positive correlation between the content of 18:1n9 and log body mass, leading to positive correlations for monounsaturates and n9 fatty acyl groups. There was a negative correlation between the content of polyunsaturates (mainly 20:4n6, 18:2n6 and 22:6n3) and log body mass, leading to a significant correlation for n6 fatty acyl groups. The unsaturation index was also negatively correlated with log body mass. These correlations, particularly the negative correlation between unsaturation index and log body mass and the positive correlation between monounsaturates and log body mass, echo those seen in whole muscle from these avian species [21] and in mammalian mitochondria [2], suggesting that, irrespective of their basis (see e.g. [18,28–34]), there are strong evolutionary pressures to maintain them in mitochondria from these two distinct endotherm lineages. The relationships between mitochondrial phospholipid fatty acyl unsaturation and body mass in avian liver mitochondria are highlighted using log–log plots in Figure 5. The mole fraction of total unsaturates in mitochondrial membranes remained constant with changes in body mass. However, the mitochondrial membranes of small bird species were highly polyunsaturated, with relatively few monounsaturated acyl chains. As body mass increased, the fraction of polyunsaturates decreased, whereas the fraction of monounsaturates increased, and in the largest bird studied (emu), they were approximately equal.

The correlations between phospholipid fatty acyl composition and proton conductance were generally not as strong as those involving body mass. However, there was a significant positive correlation between proton conductance and polyunsaturates (Table 3). There were weaker (not quite significant) correlations

Table 2 Phospholipid fatty acyl group composition of liver mitochondria from different bird species: individual fatty acyl chains

Values are means ± S.E.M. of measurements of fatty acyl mole fraction from four independent preparations for each avian species. Fatty acyl groups contributing less mole fraction than 0.015 in any avian species are not shown. All fatty acyl parameters were plotted as a function of log body mass (g) (data from Table 1) or of mitochondrial respiration rate driving proton leak at 170 mV (nmol of $O \cdot min^{-1} \cdot mg\ of\ protein^{-1}$) (data from Figure 4). *P* values for the significance of correlation (linear regression: slopes are indicated) were determined using critical values of the correlation coefficient *r*. $^{*}P < 0.1$; $^{**}P < 0.05$; $^{***}P < 0.01$. NS: not significant ($P > 0.1$).

Species	16:0	16:1*n*7	18:0	18:1*n*9	18:1*n*7	18:2*n*6	20:3*n*6	20:4*n*6	20:5*n*3	22:3*n*3	22:4*n*6	22:6*n*3
Zebrafinch	0.125 ± 0.006	0.010 ± 0.001	0.218 ± 0.007	0.102 ± 0.007	0.009 ± 0.000	0.228 ± 0.000	0.018 ± 0.003	0.237 ± 0.008	0.003 ± 0.001	0.011 ± 0.002	0.003 ± 0.000	0.021 ± 0.003
House sparrow	0.097 ± 0.033	0.011 ± 0.004	0.206 ± 0.020	0.151 ± 0.017	0.017 ± 0.001	0.176 ± 0.003	0.014 ± 0.001	0.222 ± 0.016	0.000 ± 0.000	0.028 ± 0.004	0.002 ± 0.001	0.059 ± 0.002
Starling	0.140 ± 0.006	0.005 ± 0.002	0.242 ± 0.010	0.114 ± 0.010	0.012 ± 0.004	0.129 ± 0.003	0.008 ± 0.001	0.207 ± 0.010	0.002 ± 0.001	0.026 ± 0.001	0.006 ± 0.000	0.093 ± 0.007
Currawong	0.110 ± 0.008	0.008 ± 0.004	0.209 ± 0.015	0.143 ± 0.020	0.018 ± 0.001	0.134 ± 0.007	0.010 ± 0.001	0.261 ± 0.007	0.000 ± 0.000	0.010 ± 0.004	0.003 ± 0.002	0.066 ± 0.005
Pigeon	0.101 ± 0.005	0.023 ± 0.004	0.243 ± 0.011	0.147 ± 0.017	0.030 ± 0.003	0.255 ± 0.011	0.008 ± 0.001	0.132 ± 0.015	0.006 ± 0.002	0.003 ± 0.001	0.005 ± 0.002	0.013 ± 0.001
Duck	0.187 ± 0.010	0.006 ± 0.001	0.193 ± 0.007	0.143 ± 0.007	0.017 ± 0.006	0.097 ± 0.005	0.011 ± 0.003	0.232 ± 0.006	0.001 ± 0.000	0.027 ± 0.003	0.016 ± 0.003	0.042 ± 0.006
Goose	0.155 ± 0.005	0.014 ± 0.005	0.184 ± 0.034	0.242 ± 0.036	0.008 ± 0.003	0.155 ± 0.004	0.009 ± 0.001	0.148 ± 0.010	0.024 ± 0.013	0.005 ± 0.003	0.004 ± 0.001	0.019 ± 0.002
Emu	0.138 ± 0.002	0.017 ± 0.005	0.168 ± 0.015	0.329 ± 0.031	0.016 ± 0.005	0.134 ± 0.007	0.008 ± 0.003	0.137 ± 0.009	0.002 ± 0.001	0.002 ± 0.000	0.004 ± 0.000	0.013 ± 0.001
Relationship to log body mass	NS	NS	$^{*}P = 0.059$	$^{***}P = 0.005$	NS	NS	$^{**}P = 0.049$	NS	NS	NS	NS	NS
Slope			−0.016	0.055			−0.002					
Relationship to proton leak at 170 mV	NS	NS	NS	NS	NS	NS	$^{*}P = 0.093$	NS	NS	NS	NS	NS
Slope							0.0003					

Table 3 Phospholipid fatty acyl group composition of liver mitochondria from different bird species: parameter values

Values are means ± S.E.M. of measurements from four independent preparations for each avian species. Unsaturation index: number of double bonds per 100 acyl chains. All fatty acyl parameters were plotted as a function of log body mass (g) (data from Table 1) or of mitochondrial respiration rate driving proton leak at 170 mV (nmol of $O \cdot min^{-1} \cdot mg\ of\ protein^{-1}$) (data from Figure 4). *P* values for the significance of correlation (linear regression: slopes are indicated) were determined using critical values of the correlation coefficient *r*. $^{*}P < 0.1$; $^{**}P < 0.05$; $^{***}P < 0.01$. NS: not significant ($P > 0.1$). Values in columns 3–11 are expressed in mole fractions.

Species	Total phospholipid (μg/mg of protein)	Saturates	Monounsaturates	Polyunsaturates	Unsaturates	Unsaturation index	*n*−9	*n*−7	*n*−6	*n*−3	Average chain length (carbon atoms)
Zebrafinch	62.5 ± 5.4	0.34 ± 0.01	0.13 ± 0.01	0.53 ± 0.01	0.66 ± 0.01	178.8 ± 2.5	0.10 ± 0.01	0.02 ± 0.00	0.50 ± 0.01	0.04 ± 0.00	18.40 ± 0.02
House sparrow	56.4 ± 2.8	0.31 ± 0.02	0.18 ± 0.02	0.51 ± 0.02	0.69 ± 0.02	193.4 ± 6.2	0.15 ± 0.02	0.03 ± 0.00	0.42 ± 0.02	0.09 ± 0.00	18.62 ± 0.10
Starling	64.3 ± 4.9	0.38 ± 0.01	0.14 ± 0.01	0.48 ± 0.01	0.62 ± 0.01	193.9 ± 4.7	0.12 ± 0.01	0.02 ± 0.00	0.36 ± 0.01	0.12 ± 0.01	18.66 ± 0.05
Currawong	76.0 ± 8.6	0.32 ± 0.02	0.18 ± 0.03	0.50 ± 0.01	0.68 ± 0.02	200.4 ± 5.1	0.14 ± 0.02	0.03 ± 0.01	0.42 ± 0.01	0.08 ± 0.01	18.64 ± 0.05
Pigeon	91.9 ± 6.3	0.35 ± 0.02	0.21 ± 0.02	0.44 ± 0.02	0.65 ± 0.02	146.6 ± 5.8	0.15 ± 0.02	0.06 ± 0.00	0.41 ± 0.02	0.03 ± 0.01	18.13 ± 0.05
Duck	172.0 ± 13.8	0.38 ± 0.02	0.17 ± 0.01	0.44 ± 0.01	0.62 ± 0.02	178.1 ± 2.3	0.15 ± 0.01	0.03 ± 0.01	0.36 ± 0.01	0.08 ± 0.01	18.48 ± 0.03
Goose	115.6 ± 5.7	0.35 ± 0.03	0.27 ± 0.04	0.38 ± 0.00	0.65 ± 0.03	151.9 ± 0.6	0.25 ± 0.04	0.02 ± 0.01	0.32 ± 0.01	0.06 ± 0.01	18.16 ± 0.04
Emu	72.2 ± 5.8	0.32 ± 0.01	0.37 ± 0.03	0.31 ± 0.02	0.68 ± 0.01	135.2 ± 3.0	0.33 ± 0.03	0.04 ± 0.01	0.29 ± 0.01	0.02 ± 0.00	18.06 ± 0.03
Relationship to log body mass	NS	NS	$^{***}P = 0.004$	$^{***}P = 0.000$	NS	$^{**}P = 0.041$	$^{***}P = 0.004$	NS	$^{***}P = 0.004$	NS	$^{*}P = 0.070$
Slope			0.058	−0.059		−15.10	0.055		−0.047		−0.139
Relationship to proton leak at 170 mV	NS	NS	$^{*}P = 0.071$	$^{**}P = 0.027$	NS	$^{*}P = 0.067$	NS	NS	NS	NS	$^{*}P = 0.071$
Slope			−0.006	0.006		1.834					0.018

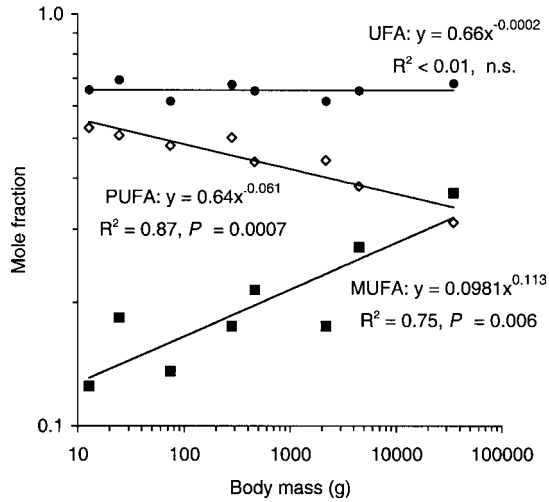

Figure 5 Allometric relationship between log body mass and log mole fraction of total unsaturated, monounsaturated and polyunsaturated phospholipid acyl chains in liver mitochondria from different bird species

Values are means ± S.E.M. of measurements of fatty acyl mole fraction from four independent preparations for each species. Data from Tables 1 and 3. Note that fatty acyl correlations with body mass are linear–log in Table 3, whereas in this Figure they are log–log. The lines are best power fits to the data as described by the inset equations. ●, UFA (total unsaturated fatty acyl chains); ■, MUFA (total monounsaturated fatty acyl chains); ◇, PUFA (total polyunsaturated fatty acyl chains).

with 20:3*n*6, unsaturation index, average chain length and monounsaturates. These correlations are very similar to those seen in mammalian mitochondria [2] and in mitochondria from a wide range of organisms [15]. Whether such correlations reflect a causal relationship is, however, not currently known.

We conclude that liver mitochondria from birds show an allometric relationship between proton conductance and body mass. In this respect, they are similar to those from the other main endothermic group, the mammals [1], and unlike those from ectotherms in general [15]. Unlike mammals, where lower proton conductance per mg of mitochondrial protein can be mostly explained by decreases in inner-membrane surface area and less by alterations in the properties of the mitochondrial inner membrane [2], birds would appear to achieve lower proton conductance by altering the basal proton conductance property of the inner membrane itself rather than by altering the amount of membrane. Exactly how they do this remains to be precisely determined. The changes in conductance may be related to properties associated with their body mass-related changes in mitochondrial phospholipid acyl composition or to other potential mechanisms (see [25,35–37]).

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