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Abstract

We have examined Na^+,K^+-ATPase molecular activity and membrane fatty acid composition in the heart of six mammalian and eight avian species ranging in size from 30 g in mice to 280 kg in cattle and 13 g in zebra finches to 35 kg in emus, respectively. Na^+,K^+-ATPase activity scaled negatively with body mass in both mammals and birds. In small mammals, the elevated enzyme activity was related to allometric changes in both the concentration and molecular activity (turnover rate) of Na^+,K^+-ATPase enzymes, while in small birds, higher Na^+,K^+-ATPase activity appeared to result primarily from an increased molecular activity of individual enzymes. The unsaturation index of cardiac phospholipids scaled negatively with body mass in both groups, while a significant allometric increase in monounsaturate content was observed in the larger mammals and birds. In particular, the relative content of the highly polyunsaturated docosahexaenoic acid (22:6n-3) displayed the greatest variation, scaling negatively with body mass and varying greater than 40-fold in both mammals and birds. Membrane fatty acid profile was correlated with Na^+,K^+-ATPase molecular activity in both mammals and birds, suggesting a potential association between membrane lipid composition and the activity of membrane-bound enzymes in the hearts of endotherms.

Keywords

Scaling, ATPase, molecular, activity, membrane, fatty, acid, composition, mammalian, avian, hearts

Disciplines

Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

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Scaling of Na^+,K^+-ATPase Molecular Activity and Membrane Fatty Acid Composition in Mammalian and Avian Hearts

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ABSTRACT

We have examined Na^+,K^+-ATPase molecular activity and membrane fatty acid composition in the heart of six mammalian and eight avian species ranging in size from 30 g in mice to 280 kg in cattle and 13 g in zebra finches to 35 kg in emus, respectively. Na^+,K^+-ATPase activity scaled negatively with body mass in both mammals and birds. In small mammals, the elevated enzyme activity was related to allometric changes in both the concentration and molecular activity (turnover rate) of Na^+,K^+-ATPase enzymes, while in small birds, higher Na^+,K^+-ATPase activity appeared to result primarily from an increased molecular activity of individual enzymes. The unsaturation index of cardiac phospholipids scaled negatively with body mass in both groups, while a significant allometric increase in monounsaturate content was observed in the larger mammals and birds. In particular, the relative content of the highly polyunsaturated docosahexaenoic acid (22:6n-3) displayed the greatest variation, scaling negatively with body mass and varying greater than 40-fold in both mammals and birds. Membrane fatty acid profile was correlated with Na^+,K^+-ATPase molecular activity in both mammals and birds, suggesting a potential association between membrane lipid composition and the activity of membrane-bound enzymes in the hearts of endotherms.

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Introduction

In a fascinating report more than 20 years ago, Gudbjarnarson et al. (1978) showed that the heart rate of mammals, ranging from mice to whales, was strongly correlated with the docosahexaenoic acid (22:6n-3) content of the cardiac phospholipids. Since resting heart rate is correlated with basal metabolic rate (BMR) in mammals (even after the removal of body mass effects; Brody 1945; White and Seymour 2004), this intriguing relationship suggested that variations in membrane fatty acid composition may be associated with the well-documented allometric variation of BMR in mammals (Kleiber 1961).

Recently, it has been shown that Gudbjarnarson's observation of allometric variation in cardiac fatty acid composition is part of a more general body size–related phenomenon. With the exception of the brain, tissue phospholipids of different-sized mammals show a significant allometric decline in their degree of unsaturation (i.e., unsaturation index: the number of double bonds per 100 fatty acid chains) despite there being no allometric trend in their total percentage of unsaturated fatty acids (Couture and Hulbert 1995*a*; Hulbert et al. 2002*b*). This variation is predominantly due to the significant and substantial allometric decline in the content of the highly polyunsaturated n-3 fatty acid (22:6n-3), which displays allometric exponents ranging from −0.19 in liver phospholipids to −0.40 in skeletal muscle phospholipids (Hulbert et al. 2002*b*). Interestingly, while these allometric trends exist in most mammalian tissues (heart, kidney, liver, and skeletal muscle), brain phospholipids are highly polyunsaturated, with very high levels of 22:6n-3, regardless of species body size.

Birds represent the other major class of endotherms, and similar to mammals, BMR in birds is allometrically related to body mass (Lasiewski and Dawson 1967). Hulbert et al. (2002*a*) have also recently shown that the fatty acid composition of skeletal muscle membranes varies with body size in birds. Their results are very similar to those observed in mammalian muscle and show that in birds ranging from hummingbirds to emus, there is no allometric trend in the total percentage of unsaturated fatty acids; however, there is a significant allometric decline in the unsaturation index of muscle phospholipids (Hulbert et al. 2002*a*). Again, these trends are primarily the result of a significant and substantial allometric decline in the content of 22:6n-3, which scaled with an allometric exponent of −0.28 (Hulbert et al. 2002*a*). Collectively, these data suggest that body size–related variation in fatty acid composition, especially 22:6n-3, is an allometric trend that is common to en-

Table 1: Body mass, heart mass, heart protein concentration, and basal metabolic rate (BMR) of the mammals and birds

Common Name	Scientific Name	Body Mass (g)	Heart Mass (g)	Heart Protein Concentration (mg g wet wt^{-1})	BMR (kcal g^{-1} d^{-1})
Mammals:					
Mouse	*Mus musculus*	37.9 ± 1.1 (16)	.19 ± .01 (16)	215 ± 5 (12)	.171
Rat	*Rattus norvegicus*	281 ± 6 (12)	.90 ± .02 (12)	201 ± 12 (12)	.100
Rabbit	*Oryctolagus cuniculus*	2,450 ± 117 (4)	5.7 ± .27 (4)	212 ± 8 (4)	.048
Sheep	*Ovis aries*	38,500 ± 1,380 (8)	209 ± 12 (8)	198 ± 7 (8)	.028
Pig	*Sus scrofa*	88,300 ± 6,990 (8)	344 ± 9 (8)	228 ± 7 (7)	.019
Cow	*Bos taurus*	277,000 ± 20,800 (8)	994 ± 71 (8)	208 ± 8 (7)	.015
Birds:					
Zebra finch	*Taeniopygia guttata*	12.6 ± .9 (4)	.17 ± .01 (4)	172 ± 12 (4)	.370
Sparrow	*Passer domesticus*	25.9 ± .9 (4)	.32 ± .01 (4)	172 ± 13 (4)	.278
Starling	*Sturnus vulgaris*	75 ± 3 (4)	.92 ± .02 (4)	175 ± 7 (4)	.260
Currawong	*Strepera graculina*	283 ± 19 (4)	2.06 ± .06 (4)	175 ± 12 (4)	.179
Pigeon	*Columba livia*	462 ± 35 (4)	5.03 ± .17 (4)	180 ± 12 (4)	.075
Duck	*Anas platyrhynchos*	2,178 ± 61 (4)	15.7 ± .60 (4)	174 ± 8 (4)	.071
Goose	*Anser anser*	4,444 ± 360 (4)	32 ± 6 (4)	191 ± 5 (4)	.062
Emu	*Dromaius novaehollandiae*	34,975 ± 745 (4)	308 ± 8 (4)	152 ± 3 (4)	.017

Note. Values show means ± SEM, with the number of animals measured in parentheses. BMR values for mammals are from Kleiber (1961); BMR values for birds are from Hulbert et al. (2002*a*) and were converted from mL O_2 g^{-1} h^{-1} to kcal g^{-1} h^{-1} using a conversion factor of 4.7, which assumes a respiratory quotient of 0.7.

dotherms despite mammals and birds evolving endothermy independently.

The functional significance of the elevated 22:6n-3 levels in the membranes of small mammals and birds is potentially related to their high mass-specific metabolic rate compared with larger endotherms. In a similar correlation, the high metabolic rate of an endothermic rat compared with an ectothermic reptile (of the same size and body temperature) has been shown to be associated with a high 22:6n-3 content in liver and kidney phospholipids of the rat (Hulbert and Else 1989). It has been recently proposed that, because a substantial proportion of basal metabolism is associated with membrane-linked processes (Rolfe and Brown 1997), membrane lipids, and particularly 22:6n-3, may play a role in determining the metabolic rate of different species via an influence on the molecular activity (turnover rate of substrate) of membrane-bound enzymes (Hulbert and Else 1999, 2000).

One such enzyme is the Na^+,K^+-ATPase (sodium pump), which is a ubiquitous enzyme that is found in all animal cells and is an important contributor to BMR (Rolfe and Brown 1997). In humans and rats, the in vivo activity of the Na^+,K^+-ATPase is estimated to account for approximately 20% of basal metabolism (Rolfe and Brown 1997); however, its energy use varies among different tissues, accounting for up to 50%–70% of metabolic activity in brain and kidney (Clausen et al. 1991). Previous comparisons of metabolically diverse species have shown that the higher metabolic rate of endotherms compared with ectotherms is associated with an increased molecular activity of the Na^+,K^+-ATPase in endothermic tissues (Else et al. 1996). Furthermore, these differences in molecular activity have been causally linked to the fatty acid composition of the surrounding membrane (Else and Wu 1999; Wu et al. 2004), with high levels of membrane polyunsaturated fatty acids (PUFAs) associated with high enzyme molecular activity. We recently showed a similar relationship exists in the kidney of mammals of different body size (Turner et al. 2005); however, it is unknown whether the same trends are present in other tissues of endotherms of different body size.

In this article, we have explored this relationship and examined Na^+,K^+-ATPase molecular activity in the heart of mammals and birds of different body size in order to investigate whether the high mass-specific metabolic rate of small endotherms is associated with an increased molecular activity of the Na^+,K^+-ATPase. Furthermore, we have also measured membrane acyl composition to examine whether variations in fatty acid profile may be underpinning differences in Na^+,K^+-ATPase molecular activity in the different species.

Material and Methods

Animals

The mammals and birds examined in our study were adults of either sex and are listed in Table 1. Conditions relating to the

procurement, care, and death of the animals have been described in detail elsewhere (Turner et al. 2005). All procedures were performed in accordance with the National Health and Medical Research Council Guidelines for Animal Research in Australia and were approved by the Animal Experimentation Ethics committee of the University of Wollongong.

Na^+,K^+-ATPase Molecular Activity

Following the death of the animals, hearts were immediately removed and sections of the ventricle were used to determine Na^+,K^+-ATPase molecular activity. All measurements were conducted at 37°C in mammals and 40°C in birds to approximate in vivo conditions. Details of the experimental techniques were previously reported (Turner et al. 2005). Briefly, Na^+,K^+-ATPase concentration was determined in myocardial samples by [^{3}H]ouabain binding, while Na^+,K^+-ATPase activity was measured as the ouabain-inhibitable ATPase activity in detergent-treated (sodium deoxycholate, 1 mg mL^{-1}) homogenates (10% w/v). Molecular activity, which is defined as the maximal rate of substrate turnover by a protein, was derived by dividing maximal Na^+,K^+-ATPase activity (expressed as pmol Pi mg wet $weight^{-1}$ min^{-1}) by the Na^+,K^+-ATPase concentration (in pmol mg wet $weight^{-1}$) for the same preparation. The net result was expressed as the number of ATP molecules hydrolyzed by each Na^+,K^+-ATPase molecule per minute.

Preparation of Microsomal Membranes

All lipid measurements were conducted using microsomal membranes, prepared from heart homogenates (10% in 250 mM sucrose, 20 mM imidazole, 1 mM EDTA; pH 7.4) that were centrifuged at 3,000 *g* for 3 min and a further 10 min at 10,000 *g* to remove nuclei and mitochondria, respectively. The supernatant was then centrifuged at 98,000 *g* for 35 min and the resultant pellet, designated "microsomal membranes," was resuspended in 25 mM imidazole, 2 mM EDTA (pH 7.5). In this investigation, we examined microsomal membranes in preference to whole tissue because although microsomes represent a mixed membrane preparation, the phospholipids isolated from microsomes are more representative of the lipids that are associated with the Na^+,K^+-ATPase rather than whole-tissue phospholipids, which would also contain nuclear and mitochondrial membrane fractions. One microsomal fraction was prepared per animal except for the mouse heart, where one pooled sample from nine animals was prepared, and the zebra finch and sparrow hearts, where two pooled microsomal fractions were prepared, each containing tissue from two birds. Thus, while there was only $n = 1$–2 for the lipid measurements in these species, the sample was considered to be representative for the species. No lipid measurements were conducted on the rabbits. The protein contents of microsomal preparations (and tissue homogenates) were determined by the Lowry method with bovine serum albumin as the standard.

Analysis of Phospholipid Fatty Acid Composition

All solvents used in the lipid extractions were of ultrapure grade. Total lipids were extracted from the microsomal preparation by standard methods (Folch et al. 1957) using chloroform : methanol (2 : 1 v/v) containing butylated hydroxytoluene (0.01% w/v) as an antioxidant. Phospholipids were separated from neutral lipids by solid phase extraction on Strata SPE SI-2 silica columns (Phenomenex, New South Wales, Australia). Fatty acid composition of the phospholipid fraction was determined as described in detail elsewhere (Pan and Storlien 1993). Briefly, phospholipid fractions were transmethylated with 14% w/v boron trifluoride in methanol and fatty acid methyl esters were separated by gas-liquid chromatography on a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) with a fused silica capillary column. Individual fatty acids were identified by comparing each peak's retention time to those of external standards.

Data Analyses

Data were analyzed using both conventional methods and phylogenetically independent contrasts (Felsenstein 1985; Garland et al. 1992). All statistical comparisons were determined and tested for significance using the mean value for each species (i.e., $n = 5$–6 for mammals; $n = 8$ for birds). Conventional allometric equations were determined by linear regression (least-squares method) of log-transformed values using JMP 4.0.1 software (SAS Institute, Cary, NC), which was also used to determine linear correlation coefficients comparing lipid pa-

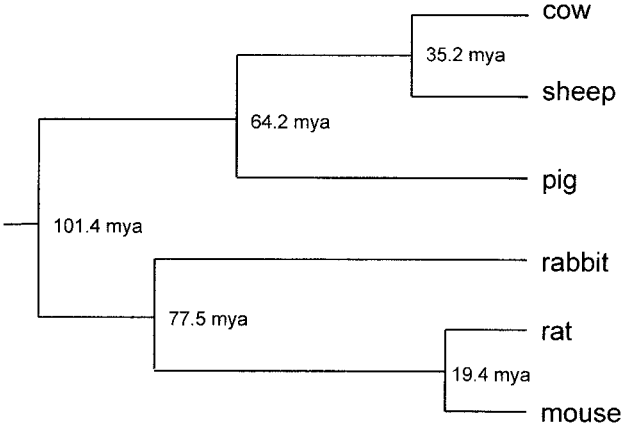

Figure 1. Phylogeny for the mammalian species used in this study, constructed using phylogenetic data from sources listed in the text. Divergence dates, in millions of years (mya), are listed at the internal nodes.

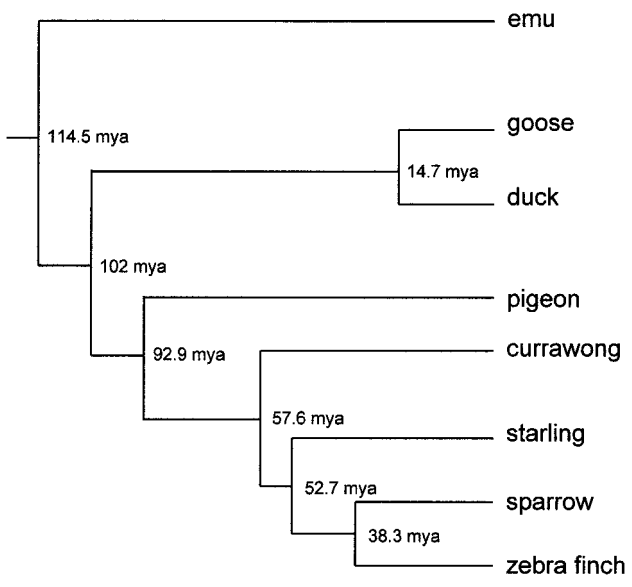

Figure 2. Phylogeny for the avian species used in this study, constructed using phylogenetic data from sources listed in the text. Divergence dates, in millions of years (mya), are listed at the internal nodes.

rameters and molecular activity. For phylogenetic analyses, we constructed phylogenetic trees for the mammals (Fig. 1) and birds (Fig. 2) based on the divergence times and phylogenies of Sibley and Ahlquist (1990), Waddel et al. (1999), Cao et al. (2000), Murphy et al. (2001), Van Tuinen and Hedges (2001), Smith and Peterson (2002), Hasegawa et al. (2003), and Springer et al. (2003). Independent contrasts were computed with the PDTREE module of the PDAP suite of programs (Garland et al. 1993). Because regressions and correlations using independent contrasts are computed through the origin, the degrees of freedom are the same as for conventional statistics (i.e., $n-2$). Significance for all relationships was accepted at the level of $P<0.05$, and results are reported as means ± SEM. All figures were produced using KaleidaGraph software (ver. 3.51; Synergy Software, Reading, PA).

Results

Table 1 presents the body mass and mass-specific BMR of the mammals and birds examined in this study. The experimental species were chosen to span as wide a range of body mass as was practical and on the basis of availability. In both mammals and birds, conventional analysis showed that mass-specific BMR (kcal g^{-1} d^{-1}) was significantly lower in the larger species, with mammalian BMR = 0.45 × mass $(g)^{-0.27}$ ($P<0.01$) and avian BMR = 1.06 × mass $(g)^{-0.37}$ ($P<0.01$). Independent contrasts analysis also revealed significant relationships for both groups, with an almost identical equation seen in the mammals (BMR = 0.46 × mass $[g]^{-0.28}$, $P<0.01$), while for birds, a slightly different equation was seen with independent contrasts (BMR = 0.86 × mass $[g]^{-0.33}$, $P<0.01$). The allometric slope describing mammalian mass-specific BMR in this study is close to the value of −0.25 generally found to describe BMR in mammals (Kleiber 1961; Peters 1983); however, the allometric slope observed in the birds is steep and reflects the fact that the four largest birds were nonpasserines, which generally possess lower rates of basal metabolism than passerines, which comprised the four smaller bird species (Lasiewski and Dawson 1967).

Birds had larger hearts than those of similar-sized mammals (Table 1). The mass of the heart in mammals represented a fairly constant proportion of body mass (~0.5%), while the smaller birds had relatively bigger hearts than the larger birds (1.3% vs 0.7%). Protein concentration showed no relationship with body size in either the mammals or birds. The mean protein concentration in mammals was 210 ± 4 mg g wet wt^{-1}, which was significantly ($P<0.001$) higher than the mean protein concentration (174 ± 4 mg g wet wt^{-1}) found in birds (Table 1).

Na^+,K^+-ATPase activity values determined in the mammals and birds were examined relative to body mass (Fig. 3), and there was a significant allometric decline in Na^+,K^+-ATPase activity in the larger mammals with both conventional (exponent −0.04, $P<0.05$) and independent contrasts analysis

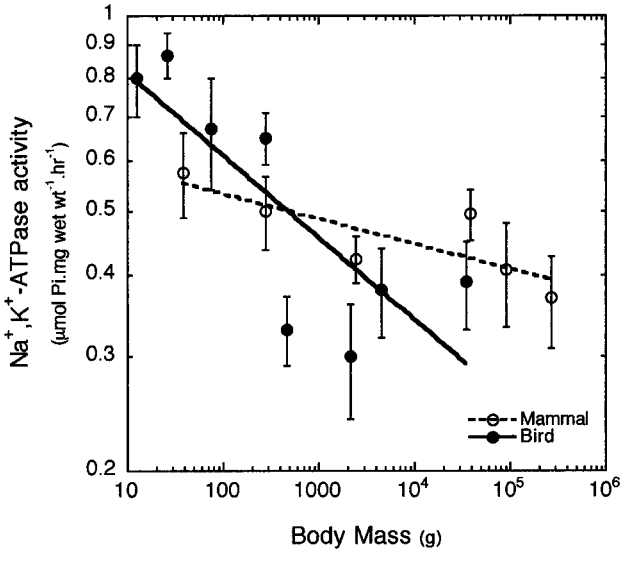

Figure 3. Relationship between body mass and Na^+,K^+-ATPase activity in mammalian and avian hearts. Values are means ± SEM of Na^+,K^+-ATPase measurements at 37°C in mammals and 40°C in birds. n = 4–8 in mammals and $n=4$ in birds. The lines show the best power fits to the data, using conventional statistics. The conventional allometric equations are, for mammals, $y = 0.64 \times \text{mass}^{-0.04}$ ($r=0.82$, $P<0.05$) and, for birds, $y = 1.09 \times \text{mass}^{-0.13}$ ($r=0.80$, $P<0.05$), while the corresponding independent contrasts equations are, for mammals, $y = 0.74 \times \text{mass}^{-0.06}$ ($r=0.82$, $P<0.05$) and, for birds, $y = 0.66 \times \text{mass}^{-0.08}$ ($r=0.50$, $P=0.21$).

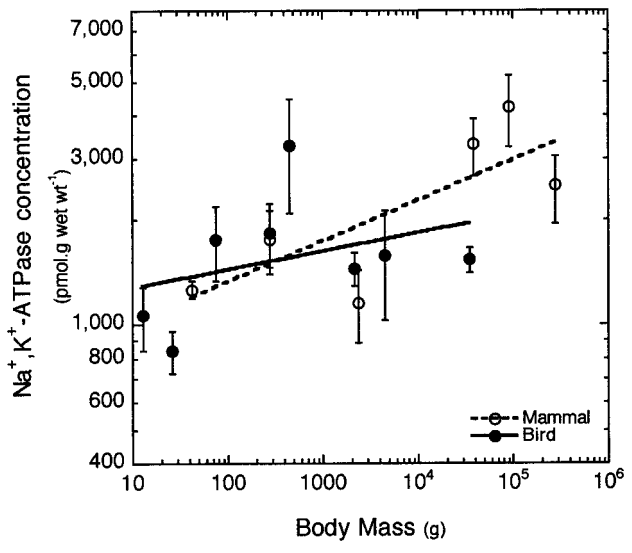

Figure 4. Relationship between body mass and Na^+,K^+-ATPase concentration in mammalian and avian hearts. Values are means ± SEM; n = 4–8 in mammals and n = 3–4 in birds. The lines show the best power fits to the data, using conventional statistics. The conventional allometric equations are, for mammals, $y = 776 \times \text{mass}^{0.12}$ ($r = 0.77$, $P = 0.07$) and, for birds, $y = 1{,}128 \times \text{mass}^{0.05}$ ($r = 0.36$, $P = 0.36$), while the corresponding independent contrasts equations are, for mammals, $y = 889 \times \text{mass}^{0.10}$ ($r = 0.58$, $P = 0.30$) and, for birds, $y = 1{,}019 \times \text{mass}^{0.07}$ ($r = 0.36$, $P = 0.36$).

(exponent −0.06, $P < 0.05$). For birds, a significant allometric relationship for Na^+,K^+-ATPase activity was observed using conventional analysis (exponent −0.13, $P < 0.05$) but not independent contrasts (exponent −0.08, $P = 0.21$). Na^+,K^+-ATPase concentration was higher in the hearts of larger mammals (Fig. 4), and although the conventional analysis revealed a close to significant allometric increase in this parameter (exponent 0.12, $P = 0.07$), the allometric exponent determined in the independent contrasts analysis was not significantly different from 0 (exponent 0.10, $P = 0.30$). There was large variation in Na^+,K^+-ATPase concentration in bird hearts, with both conventional and independent contrasts analysis revealing that body mass explained only approximately 13% of the variability in this parameter (Fig. 4). Molecular activity values were quite similar in the two groups (Fig. 5) and varied approximately fivefold in mammalian heart (1,700–7,900 ATP min^{-1}), while in bird hearts there was substantially greater variation between species (2,000–18,850 ATP min^{-1}). It should be noted that the molecular activity values for birds were calculated using Na^+,K^+-ATPase activities measured at 40°C and are thus approximately 25% higher than if measured at the same temperature (37°C) as the values for the mammals (assuming a thermal quotient [Q_{10}] of 2.0). When molecular activity values were considered with respect to body mass (Fig. 5), conventional analysis showed a significant allometric decline in molecular activity in the larger mammals (exponent −0.14, $P < 0.05$), with a similar, nearly significant trend observed in the birds (exponent −0.17, $P = 0.08$). With independent contrasts analysis, molecular activity values were not significantly related to body size in either the mammals (exponent −0.11, $P = 0.18$) or the birds (exponent −0.13, $P = 0.33$).

The fatty acid profiles of heart microsomal phospholipids from mammals and birds are presented in Tables 2 and 3, respectively. In both mammals and birds, the most abundant fatty acids were palmitate (16:0), stearate (18:0), oleate (18:1n-9), linoleate (18:2n-6), arachidonate (20:4n-6), and docosahexaenoate (22:6n-3). When fatty acid parameters were considered with respect to body mass in mammals, the unsaturation index (Fig. 6*A*) showed a significant allometric decline with body mass (conventional $y = 391 \times \text{mass}^{-0.05}$ [$r = 0.96$, $P < 0.01$]; independent contrasts $y = 369 \times \text{mass}^{-0.04}$ [$r = 0.89$, $P < 0.05$]) despite there being a slightly lower percentage of total unsaturated fatty acid chains in the smaller mammals (Table 2). Monounsaturated fatty acid (MUFA) content (Fig. 6*B*) showed a significant allometric increase in the larger mammals with conventional analysis ($y = 5.3 \times \text{mass}^{0.07}$ [$r = 0.95$, $P = 0.01$]) but was not significantly related to body size with independent contrasts analysis ($y = 6.7 \times \text{mass}^{0.05}$ [$r = 0.63$,

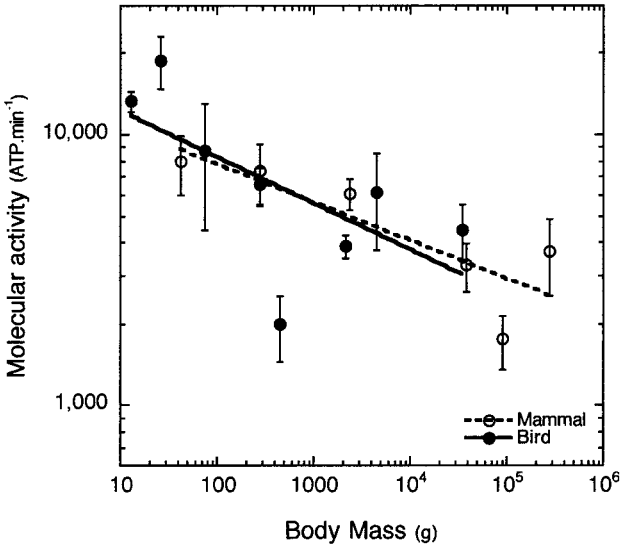

Figure 5. Relationship between body mass and Na^+,K^+-ATPase molecular activity in mammalian and avian hearts. Values are means ± SEM; n = 4–8 in mammals and n = 3–4 in birds. Molecular activity values were calculated by dividing maximal Na^+,K^+-ATPase activity by the Na^+,K^+-ATPase concentration for the same animal. The lines show the best power fits to the data, using conventional statistics. The conventional allometric equations are, for mammals, $y = 14{,}920 \times \text{mass}^{-0.14}$ ($r = 0.85$, $P < 0.05$) and, for birds, $y = 18{,}185 \times \text{mass}^{-0.17}$ ($r = 0.65$, $P = 0.08$), while the corresponding independent contrasts equations are, for mammals, $y = 10{,}765 \times \text{mass}^{-0.11}$ ($r = 0.63$, $P = 0.18$) and, for birds, $y = 10{,}593 \times \text{mass}^{-0.13}$ ($r = 0.40$, $P = 0.33$).

Table 2: Phospholipid fatty acid profile of mammalian heart microsomes

	Mouse	Rat	Sheep	Pig	Cow
Fatty acid:					
16:0	11.8	7.5 ± .1	6.8 ± .3	11.0 ± .7	7.6 ± .9
18:0	13.4	14.7 ± .8	10.7 ± .6	9.5 ± .4	11.0 ± 1.1
18:1n-9	7.5	3.0 ± .2	8.9 ± .3	9.0 ± .3	9.8 ± .6
18:1n-7	.0	3.8 ± .3	2.2 ± .1	3.4 ± .3	2.4 ± .3
18:2n-6	10.7	13.8 ± .6	32.6 ± 1.8	30.8 ± 1.5	26.1 ± 2.2
18:3n-3	.1	.2 ± .0	6.3 ± .5	.6 ± .0	5.0 ± .8
20:2n-6	2.3	.6 ± .3	1.2 ± .2	2.0 ± .3	.9 ± .1
20:3n-6	.4	.4 ± .0	1.1 ± .1	1.0 ± .1	2.4 ± .3
20:4n-6	8.4	27.9 ± .6	14.8 ± 1.4	25.2 ± 1.3	18.1 ± 2.8
20:5n-3	.0	.1 ± .0	6.0 ± .4	.6 ± .1	8.1 ± 1.3
22:4n-6	1.4	1.5 ± .3	.3 ± .1	1.8 ± .4	.3 ± .1
22:5n-6	1.8	.9 ± .1	.1 ± .0	.3 ± .0	.0 ± .0
22:5n-3	1.9	3.1 ± .1	4.0 ± .7	2.4 ± .0	4.4 ± .8
22:6n-3	39.2	21.8 ± .7	3.0 ± .4	.9 ± .1	1.0 ± .2
% saturates	25.5	22.5 ± .7	18.0 ± .9	20.7 ± 1.1	19.0 ± 2.1
% MUFAs	7.8	6.9 ± .5	11.8 ± .2	12.6 ± .6	13.6 ± 1.0
% PUFAs	66.7	70.6 ± .5	70.2 ± 1.1	66.7 ± 1.6	67.4 ± 3.1
% n-6	25.3	45.2 ± .3	50.2 ± 1.0	61.1 ± 1.6	48.0 ± .9
% n-3	41.2	25.2 ± .7	19.7 ± .5	4.6 ± .1	19.1 ± 2.4
% unsaturates	74.5	77.5 ± .7	82.0 ± .9	79.3 ± 1.1	81.0 ± 2.1
Unsaturation index	330	307 ± 4	233 ± 7	216 ± 4	235 ± 18
Chain length	19.8	19.5 ± .0	18.6 ± .1	18.6 ± .0	18.7 ± .1
C20+22 PUFAs	55.6	56.5 ± .8	31.2 ± 2.3	35.3 ± 1.0	36.1 ± 5.2
n-6/n-3	.6	1.8 ± .1	2.5 ± .1	13.4 ± .4	2.6 ± .3

Note. Microsomal phospholipid fatty acid profile, expressed as mole percentage of total fatty acid chains. Unsaturation index is the average number of double bonds per 100 fatty acid chains. Chain length is the average chain length of each fatty acid. Values are means ± SEM ($n = 4$) for all preparations except for mouse, where a pooled preparation from nine animals was used ($n = 1$). Fatty acids contributing less than 1.5% in all mammalian species are not shown. MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

$P = 0.30$]). Total PUFA content showed no relationship with body mass using either conventional or independent contrasts analysis (Fig. 6*C*). The content of 22:6n-3 showed the greatest variation of any individual fatty acid (Fig. 6*D*), displaying a highly significant allometric decline with body mass in conventional ($y = 219 \times \text{mass}^{-0.44}$ [$r = 0.98$, $P < 0.01$]) and independent contrasts analyses ($y = 186 \times \text{mass}^{-0.42}$ [$r = 0.96$, $P < 0.01$]). With both methods, body mass explained >90% of the variability in 22:6n-3 levels, and it can be calculated that for every doubling in body mass, there would be an approximate 25% decrease in 22:6n-3 content in heart microsomal phospholipids in mammals.

In bird microsomal phospholipids, there were trends for higher unsaturation index (Fig. 6*A*) and a greater proportion of total polyunsaturates (Fig. 6*C*) in smaller species; however, neither relationship reached statistical significance with either conventional or independent contrasts analysis ($P > 0.05$). There was a significant allometric increase in MUFA content in the larger birds (Fig. 6*B*) with conventional analysis ($y = 3.1 \times \text{mass}^{0.16}$ [$r = 0.91$, $P < 0.01$]); however, this relationship was not significant when using independent contrasts ($y = 4.4 \times \text{mass}^{0.10}$ [$r = 0.57$, $P = 0.14$]). Similar to the mammals, the greatest variation in a bird individual fatty acid was seen in the content of 22:6n-3 (Fig. 6*D*), which showed a significant and substantial decline with body size using both conventional ($y = 74 \times \text{mass}^{-0.45}$ [$r = 0.92$, $P < 0.01$]) and independent contrasts analysis ($y = 48 \times \text{mass}^{-0.38}$ [$r = 0.74$, $P < 0.05$]).

To assess whether membrane lipid composition may have been associated with the molecular activity of the Na^+,K^+-ATPase in the mammals and birds, linear correlation coefficients were determined between the mean values for all individual lipid parameters and Na^+,K^+-ATPase molecular activities. In mammalian hearts, there were positive correlations between Na^+,K^+-ATPase molecular activity and unsaturation index (conventional $r = 0.99$, $P < 0.01$; independent contrasts $r = 0.95$, $P < 0.02$; Fig. 7*A*), the percentage of 22:6n-3 (conventional $r = 0.93$, $P < 0.03$; independent contrasts $r = 0.73$, $P = 0.15$; Fig. 7*B*), the average fatty acid chain length (con-

Table 3: Phospholipid fatty acid profile of avian heart microsomes

	Zebra Finch	Sparrow	Starling	Currawong	Pigeon	Duck	Goose	Emu
Fatty acid:								
16:0	8.1 ± .6	8.4 ± .4	7.2 ± 1.0	6.1 ± .3	8.8 ± 1.0	8.0 ± .4	7.9 ± .5	8.0 ± .8
18:0	10.5 ± .7	8.8 ± .6	8.2 ± 1.2	7.8 ± .3	9.1 ± 1.2	6.4 ± .3	7.9 ± .8	5.9 ± .5
18:1n-9	3.6 ± .5	3.5 ± .1	5.5 ± .6	5.7 ± .1	5.1 ± .3	12.6 ± .7	10.4 ± 1.0	11.5 ± .7
18:2n-6	27.6 ± 1.2	21.2 ± 1.2	18.2 ± .8	18.5 ± .4	30.6 ± .8	17.8 ± .1	22.3 ± .9	24.2 ± .6
18:3n-3	.7 ± .0	.7 ± .0	1.1 ± .0	1.3 ± .1	.7 ± .2	1.2 ± .1	5.8 ± 2.9	.4 ± .0
20:4n-6	30.4 ± 1.7	20.1 ± 1.7	29.3 ± 1.0	42.5 ± .7	28.6 ± 1.1	42.9 ± .7	32.9 ± 3.5	44.8 ± 2.3
20:5n-3	.1 ± .1	.0 ± .0	.9 ± .1	1.1 ± .1	1.6 ± .4	.3 ± .0	4.4 ± 2.2	.4 ± .0
22:4n-6	1.8 ± .1	1.4 ± .1	1.1 ± .1	1.6 ± .1	1.5 ± .1	1.9 ± .1	1.0 ± .3	.9 ± .0
22:5n-6	3.2 ± .7	7.9 ± 1.5	2.1 ± .1	1.4 ± .1	.7 ± .1	1.5 ± .1	.4 ± .1	.3 ± .1
22:5n-3	.7 ± .1	1.0 ± .2	2.7 ± .3	2.7 ± .1	6.5 ± .8	.9 ± .1	2.4 ± .5	.3 ± .1
22:6n-3	8.5 ± 2.4	24.1 ± 1.0	21.6 ± 1.7	8.3 ± .5	3.6 ± .4	2.7 ± .8	1.3 ± .1	.5 ± .1
% saturates	20.9 ± 2.0	17.2 ± 1.0	15.6 ± 2.2	14.0 ± .5	17.9 ± 2.1	14.4 ± .8	16.1 ± .9	14.3 ± 1.3
% MUFAs	4.7 ± .6	4.7 ± .3	6.6 ± .7	6.9 ± .1	6.9 ± .4	15.9 ± .7	11.9 ± 1.2	13.5 ± 1.0
% PUFAs	74.4 ± 1.4	78.2 ± 1.3	77.8 ± 2.8	79.2 ± .6	75.2 ± 2.2	69.6 ± 1.1	72.0 ± 1.5	72.2 ± 2.3
% n-6	64.1 ± 1.2	52.3 ± 2.5	51.5 ± .9	65.5 ± .9	62.7 ± 1.9	64.6 ± 1.0	57.1 ± 4.3	70.6 ± 2.2
% n-3	10.3 ± 2.6	25.8 ± 1.2	26.3 ± 2.1	13.7 ± .7	12.4 ± 1.7	5.1 ± 1.0	14.9 ± 5.4	1.6 ± .1
% unsaturates	79.1 ± 2.0	82.8 ± 1.0	84.4 ± 2.2	86.0 ± .5	82.1 ± 2.1	85.6 ± .8	83.9 ± .9	85.7 ± 1.3
Unsaturation index	266 ± 7	328 ± 6	328 ± 15	304 ± 2	260 ± 9	265 ± 7	258 ± 6	255 ± 9
Chain length	19.0 ± .1	19.6 ± .1	19.5 ± .1	19.3 ± .0	18.9 ± .1	19.0 ± .1	18.8 ± .0	18.8 ± .1
C20+22 PUFAs	46.0 ± .2	56.2 ± 2.5	58.4 ± 3.4	59.3 ± .6	43.8 ± 2.1	50.7 ± 1.5	43.9 ± 1.6	47.6 ± 2.5
n-6/n-3	6.7 ± 1.8	2.0 ± .2	2.0 ± .1	4.8 ± .3	5.4 ± .9	14.1 ± 2.4	6.7 ± 2.7	45.5 ± 2.6

Note. Microsomal phospholipid fatty acid profile, expressed as mole percentage of total fatty acid chains. Unsaturation index is the average number of double bonds per 100 fatty acid chains. Chain length is the average chain length of each fatty acid. Values are means ± SEM ($n = 4$) for all preparations except the zebra finch and sparrow, where pooled samples were used ($n = 2$). Fatty acids contributing less than 1.5% in all avian species are not shown. MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

ventional $r = 0.97$, $P < 0.01$; independent contrasts $r = 0.90$, $P < 0.05$), and the percentage of C20-22 PUFAs (conventional $r = 0.94$, $P < 0.02$; independent contrasts $r = 0.94$, $P < 0.02$). Considering the dramatic variation in membrane 22:6n-3 in the mammals, many of the significant correlations observed in the composite parameters are related to the changes in 22:6n-3 content. Additionally, in the mammalian hearts, negative correlations were seen between Na^+,K^+-ATPase molecular activity and the percentage of 18:2n-6 (conventional $r = 0.99$, $P < 0.01$; independent contrasts $r = 0.96$, $P < 0.01$) and total MUFAs (conventional $r = 0.90$, $P < 0.05$; independent contrasts $r = 0.60$, $P = 0.28$). In avian hearts, the percentage of n-6 PUFAs was significantly negatively correlated with Na^+,K^+-ATPase molecular activity with conventional analysis ($r = -0.71$, $P < 0.05$) but not with independent contrasts ($r = -0.54$, $P = 0.17$). Similarly, there were discrepancies between conventional and independent contrasts analysis for correlations observed between molecular activity and total n-3 PUFAs (conventional $r = 0.72$, $P < 0.05$; independent contrasts $r = 0.53$, $P = 0.18$) and the percentage of 22:6n-3 (conventional $r = 0.77$, $P < 0.05$; independent contrasts $r = 0.61$, $P = 0.10$; Fig. 8*B*), while the positive correlation observed between molecular activity and the percentage of 22:5n-6 was significant with both methods (conventional $r = 0.92$, $P < 0.01$; independent contrasts $r = 0.80$, $P < 0.02$; Fig. 8*A*).

Discussion

In this study, we have investigated the relationship between body size, Na^+,K^+-ATPase molecular activity, and membrane lipid composition in the hearts of mammals and birds. We analyzed data using both conventional methods and phylogenetically independent contrasts. In the mammals, the conventional and independent contrasts statistics were generally fairly similar, with just a few exceptions. In birds, there were a number of discrepancies between the conventional and independent contrasts analysis, with a reduced number of significant relationships observed with independent contrasts analysis. The results of both methods have been presented to demonstrate the differences between conventional and independent contrasts analyses and highlight the importance of considering phylogeny when conducting allometric analyses.

In hearts from both mammals and birds, the activity of the Na^+,K^+-ATPase enzyme was higher in the smaller species and

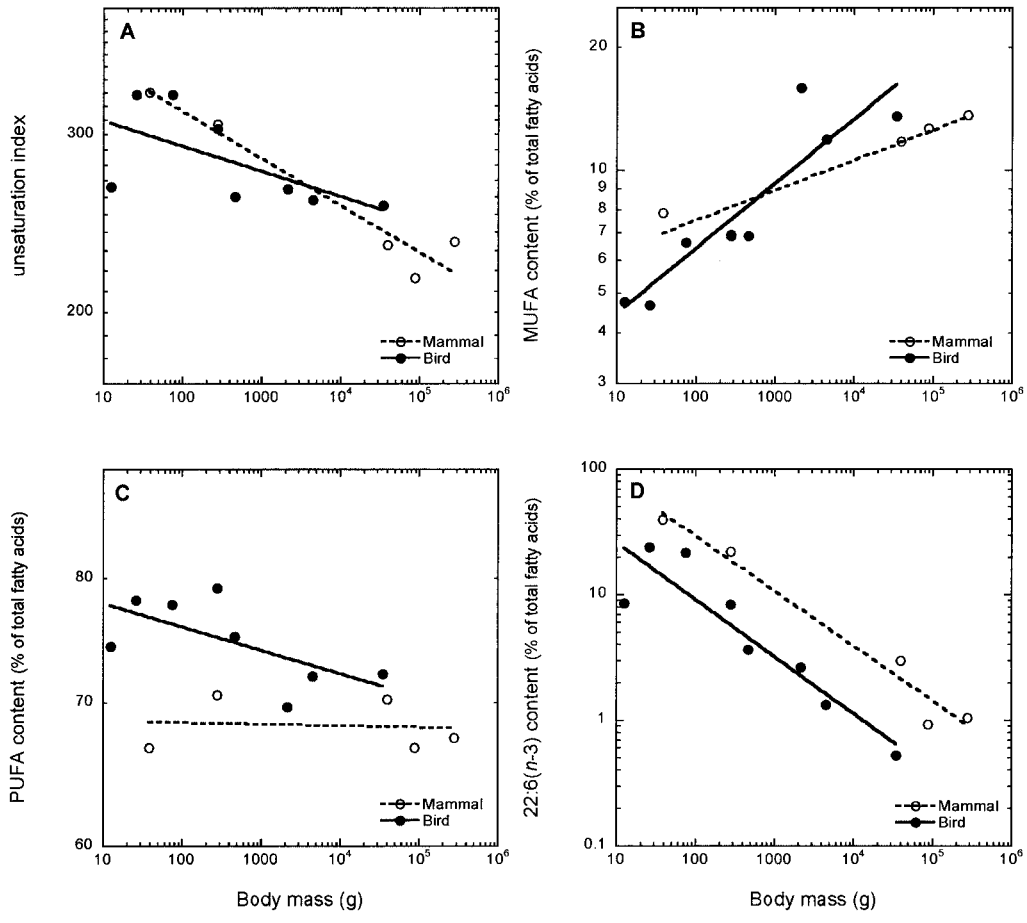

Figure 6. Relationship between body mass and the fatty acid composition of heart microsomal phospholipids in mammals and birds. *A*, Unsaturation index (number of double bonds per 100 fatty acid chains); *B*, monounsaturated fatty acids content; *C*, polyunsaturated fatty acids content; *D*, 22:6n-3 content. Fatty acids are expressed as the mole percentage of total fatty acids. Data are from Tables 1–3. The lines show the best power fits to the data, using conventional statistics. Conventional and independent contrasts allometric equations are presented in the text (see "Results").

displayed a significant allometric decline with body size (Fig. 3). These findings are similar to those reported in the kidney of these animals (Turner et al. 2005) and in liver and kidney slices in mammals (Couture and Hulbert 1995*b*), suggesting that the activity of the Na^+,K^+-ATPase enzyme is elevated in species with a high mass-specific metabolism.

In the heart, the Na^+,K^+-ATPase enzyme functions to maintain the electrochemical gradient for sodium and also plays an important role in Ca^{2+} homeostasis (Wang et al. 1996). Following the contraction cycle, the removal of intracellular Ca^{2+} is accomplished by a number of mechanisms including active transport by the Ca^{2+} ATPase of the sarcoplasmic reticulum and Na^+/Ca^{2+} exchange at the sarcolemma, which is in turn serviced by the Na^+,K^+-ATPase (Lingrel et al. 2003). Considering that heart rate is elevated in smaller mammals and birds (Grubb 1983; Peters 1983), the higher Na^+,K^+-ATPase activity observed in these species (Fig. 3) seems to reflect an increased capacity for the rapid reestablishment of appropriate ionic concentrations following each heartbeat.

In addition to measuring Na^+,K^+-ATPase activity, we also determined Na^+,K^+-ATPase concentration to see whether the greater Na^+,K^+-ATPase activity observed in small endotherms resulted from a higher Na^+,K^+-ATPase concentration, an increased molecular activity per enzyme, or a combination of both. Compared with large mammals, small mammals had both a lower Na^+,K^+-ATPase concentration (Fig. 4) and an increased molecular activity in individual Na^+,K^+-ATPase enzymes (Fig. 5), indicating that a combination of allometric changes in both of these variables contributed to the body size–related variation observed in Na^+,K^+-ATPase activity (Fig. 3). In birds, the allometric exponents determined for Na^+,K^+-ATPase activity and molecular activity were quite similar with both conventional (-0.13 and -0.17) and independent contrasts analyses (-0.08 and -0.13), suggesting that variation in Na^+,K^+-ATPase activ-

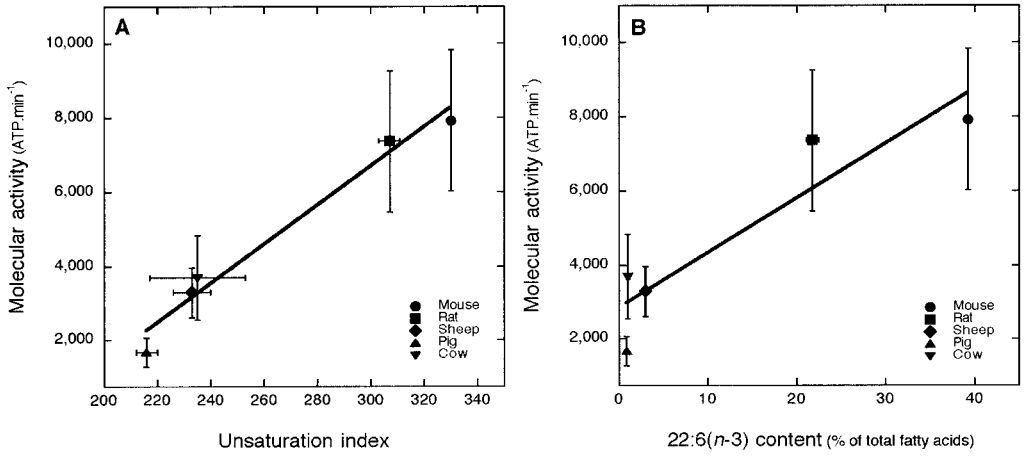

Figure 7. Linear correlations between Na^+,K^+-ATPase molecular activity and (*A*) unsaturation index (number of double bonds per 100 fatty acid chains) and (*B*) 22:6n-3 content in mammalian hearts. Values are means ± SEM. Fatty acids are expressed as the mole percentage of total fatty acids. Data are from Table 2 and Figure 5. The lines show best linear fit to the data, using conventional statistics. Correlation coefficients are as follows: for conventional statistics, unsaturation index $r = 0.99$, $P < 0.01$, and 22:6n-3 $r = 0.93$, $P < 0.03$; for independent contrasts, unsaturation index $r = 0.95$, $P < 0.02$, and 22:6n-3 $r = 0.73$, $P = 0.15$.

ity in avian myocardium is largely associated with changes in the molecular activity of individual enzyme molecules rather than enzyme concentration.

The molecular activity values determined for mammalian myocardium in this study ranged between 1,700 and 7,900 ATP min^{-1}, while in birds, values were between 2,000 and 18,850 ATP min^{-1} (Fig. 5). The Na^+,K^+-ATPase enzyme has not been extensively studied in avian hearts, but the molecular activity of the Na^+,K^+-ATPase in mammalian hearts from a variety of sources (rat, rabbit, dog, cow) is reported to be between 4,000 and 10,000 ATP min^{-1} (Pitts and Schwartz 1975; Choi and Akera 1978; Akera 1984; Else et al. 1996). These values have been determined for a variety of preparations (homogenates to purified enzymes), using a number of different experimental techniques. The molecular activity values determined for mammalian myocardium in this study are relatively similar to the literature values, and it is worth noting that a major strength of this investigation is that all the mammals and birds were examined using the same assay techniques, thus allowing direct comparison between all species.

In both endothermic groups, the smaller species tended to have an increased molecular activity (Fig. 5), and a question that arises is whether this variation observed in molecular activity in the different species may have resulted from the specific expression of various isoforms. Cardiac tissue expresses up to three isoforms of the Na^+,K^+-ATPase ($\alpha1$, $\alpha2$, and $\alpha3$), with the proportion of these isoforms varying with both age (Lingrel 1992) and species (Sweadner 1989). In canine heart, different molecular activity values have been observed in the high-affinity ($\alpha3$) and low-affinity ($\alpha1$) isoforms of the Na^+,K^+-ATPase (Maixent and Berribi-Bertrand 1993). These molecular activity values, however, vary only between 8,800 ATP min^{-1} in $\alpha3$ and 5,300 ATP min^{-1} in $\alpha1$ (Maixent and Berribi-Bertrand 1993) and thus cannot completely explain the 4.5-fold and 9.5-fold ranges observed in the mammals and birds, respectively, in this study (Fig. 5).

In the second part of the investigation, we investigated whether microsomal membrane acyl composition varied with body size in mammalian and avian hearts to determine whether differences in membrane fatty acid profile may be associated with differences in Na^+,K^+-ATPase molecular activity. Previous studies have shown that phospholipid fatty acid composition varies with body size in mammalian tissues (heart, skeletal muscle, liver, kidney; Couture and Hulbert 1995*a*; Hulbert et al. 2002*b*), in bird skeletal muscle (Hulbert et al. 2002*a*), and in liver mitochondria from mammals (Porter et al. 1996) and birds (Brand et al. 2003). Collectively, these studies show that phospholipids of smaller species are generally less monounsaturated and have a higher unsaturation index and much higher concentrations of 22:6n-3 than their larger counterparts.

In this study, phospholipids from cardiac microsomes in the mammals and birds showed the same allometric trends (Fig. 6), indicating that body size–related variation in membrane fatty acid composition is manifest in all subcellular membranes. In particular, the content of 22:6n-3 displayed dramatic variation in both groups, with allometric exponents of −0.44 and −0.45 (conventional analysis) for the mammals and birds, respectively (Fig. 6*D*). These relationships for 22:6n-3 probably describe the greatest body size–related variation in chemical composition yet measured for mammals and birds. It should be noted, however, that the limited number of species examined in this study may have resulted in a potential overestimation

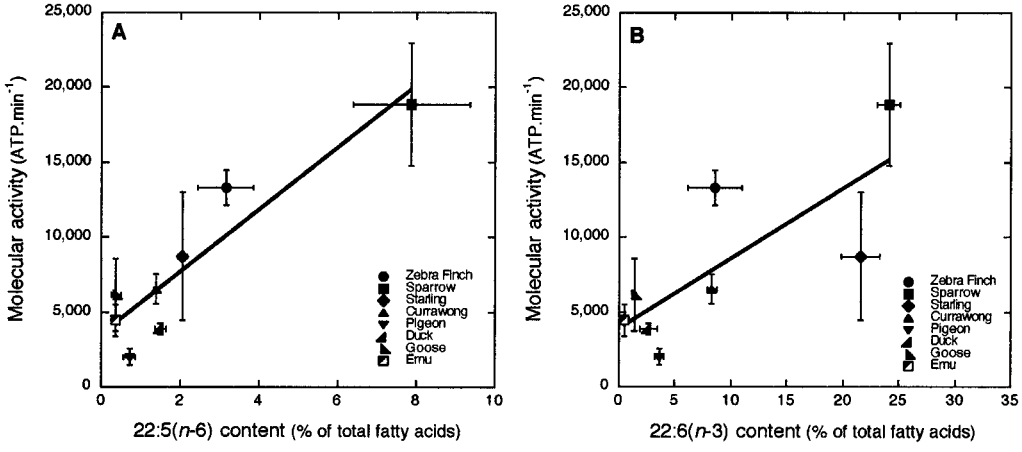

Figure 8. Linear correlations between Na^+,K^+-ATPase molecular activity and (*A*) 22:5n-6 content and (*B*) 22:6n-3 content in avian hearts. Values are means ± SEM. Fatty acids are expressed as the mole percentage of total fatty acids. Data are from Table 3 and Figure 5. The lines show best linear fit to the data, using conventional statistics. Correlation coefficients are as follows: for conventional statistics, 22:5n-6 $r = 0.92$, $P < 0.01$, and 22:6n-3 $r = 0.77$, $P < 0.05$; for independent contrasts, 22:5n-6 $r = 0.80$, $P < 0.02$, and 22:6n-3 $r = 0.61$, $P = 0.10$.

of some of the allometric relationships. For example, heart phospholipids from the very metabolically active shrew (*Sorex araneus*) have been shown to contain ~28% 22:6n-3 (Käkelä and Hyvärinen 1995). Using the current allometric equation for mammals and the body mass of the shrew from the above study (7.7 g), the predicted content of 22:6n-3 is 91%, which is obviously an overestimation.

The exact mechanisms that determine the allometric variation of fatty acid composition with body size are currently unknown. Membrane composition is highly regulated, and it is difficult to substantially alter membrane composition through dietary manipulation (Hulbert et al. 2005). The main membrane parameter affected by the diet is the relative percentage of n-6 and n-3 PUFAs (Hulbert et al. 2005), and although the dietary intake of many animals in our study was not known, the content of n-6 and n-3 PUFAs in the cardiac phospholipids may provide insight into the food habits of some species. For example, the high proportion of n-3 PUFAs in the heart of the sheep and cattle (Table 2) suggests a predominantly pasture-based diet because forage crops tend to be high in 18:3(n-3) (Cordain et al. 2002). A similar finding was also observed in the geese hearts, where n-3 PUFA levels were 24.1% in two geese that were "free-living" animals trapped in the local area, compared with 5.7% in geese that were purchased from a local aviary auction. Furthermore, the zebra finches consumed mainly seeds, which are rich in n-6 PUFAs (Harwood 1998), and their membranes contained substantially lower levels of n-3 PUFAs compared with the other small birds, particularly 22:6n-3 (Table 3).

Despite these findings, it is unlikely that variation in dietary fatty acid profile was a major determinant of the body size–related variation in fatty acid composition because the rats and mice in this study consumed exactly the same diet; however, the rat hearts contained high proportions of n-6 PUFAs, while the mice phospholipids were dominated by n-3 PUFAs, particularly 22:6n-3. Thus, it appears that other regulatory mechanisms such as elongase and desaturase enzyme systems and membrane deacylation-reacylation cycles are probably important in determining the specific membrane composition of each animal. It is also possible that the significant and substantial variation observed in 22:6n-3 in both groups (Fig. 6*D*) may be related to its unusual synthetic pathway, which involves a single β oxidation of 24:6n-3 in peroxisomes (Sprecher 2000); however, this requires further investigation.

Comparisons of endotherms and ectotherms have recently shown that the membrane lipid environment is a major determinant of the higher Na^+,K^+-ATPase molecular activity observed in endotherms (Else and Wu 1999; Wu et al. 2004). In this investigation, we found in both mammalian and avian hearts that Na^+,K^+-ATPase molecular activity was positively correlated with a number of fatty acid parameters that are all indicators of membrane PUFA levels (Figs. 7, 8). Although these results are only correlative, they suggest that Na^+,K^+-ATPase molecular activity is higher in membranes containing higher levels of PUFAs, and they support the findings of several other studies (Else and Wu 1999; Turner et al. 2003, 2005). It is also interesting to note that similar relationships have been reported between liver mitochondrial membrane PUFAs and mitochondrial proton leak (Porter et al. 1996; Brookes et al. 1998), which represents another significant contributor to BMR (Rolfe and Brown 1997).

Overall, considering the dramatic and highly significant variation of 22:6n-3 with body size in both mammals and birds and its positive correlation with Na^+,K^+-ATPase molecular activity in both groups (with conventional statistics), it would appear that of all of the fatty acids, 22:6n-3 is potentially the

most functionally important. Muscles with high respiration rates have elevated membrane levels of 22:6n-3 (Infante et al. 2001), and there is a strong positive correlation between the content of 22:6n-3 in cardiac phospholipids and heart rate in mammals (Gudbjarnarson et al. 1978). Resting heart rate is correlated with BMR (Brody 1945; White and Seymour 2004), and such evidence coupled with the current results supports the suggested role for 22:6n-3 in metabolic variation between species (Hulbert and Else 1999, 2000).

In conclusion, our results show that higher mass-specific metabolism in both small mammals and birds is associated with increased molecular activity in the Na^+,K^+-ATPase enzyme. Furthermore, both endothermic groups display similar body size–related variations in heart microsomal fatty acid composition, with the substantial variation observed in the content of 22:6n-3 appearing to be particularly important. The current finding that heart Na^+,K^+-ATPase molecular activity correlates with membrane fatty acid composition and reports that the activity of the Ca^{2+}-ATPase, a particularly important component of cardiac metabolism (Clausen et al. 1991), is higher in membranes containing 22:6n-3 (Infante 1987; Infante et al. 2001) suggest that membrane lipid composition may be an important factor influencing the activity of membrane bound enzymes in the heart of endotherms.

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