The Journal of Experimental Biology 205, 3561–3569 (2002)
Printed in Great Britain © The Company of Biologists Limited
JEB4204

Acyl composition of muscle membranes varies with body size in birds

A. J. Hulbert[1,2,*], S. Faulks[2], W. A. Buttemer[2] and P. L. Else[1,3]

[1]*Metabolic Research Centre,* [2]*Department of Biological Science* and [3]*Department of Biomedical Science, University of Wollongong, Wollongong, NSW 2522, Australia*

*Author for correspondence (e-mail: hulbert@uow.edu.au)

Accepted 20 August 2002

Summary

The acyl composition of phospholipids from pectoral muscle of eight species of birds, ranging in size from the 13 g zebra finch to the 34 kg emu, were measured and combined with recent published results for a 3 g hummingbird. This represents an approximately 11 000-fold range in body mass. Muscle phospholipids, and thus muscle membrane bilayers, from birds had a relatively constant unsaturated acyl chain content of 62% but exhibited a significant allometric decline in unsaturation index (number of double bonds per 100 acyl chains) with increasing body mass. There was a significant allometric increase in the percentage of mono-unsaturates and a significant allometric decline in the percentage of *n*-3 polyunsaturates with increasing body mass, whilst there were no significant allometric trends in either percentage of *n*-6 or percentage of total polyunsaturates in bird muscle. The relative content of the highly polyunsaturated docosahexaenoic acid (22:6 *n*-3) showed the greatest scaling with body mass, having an allometric exponent of –0.28. The contribution of this *n*-3 polyunsaturate to the unsaturation index varied with body size, ranging from less than a 6% contribution in the emu to approximately 70% in the hummingbird. Such allometric variation in the acyl composition of bird muscle phospholipids is similar to that observed in mammals, although birds have fewer *n*-3 polyunsaturates and more *n*-6 polyunsaturates than do mammalian phospholipids. This allometric variation in phospholipid acyl composition is discussed with respect to both the metabolic intensity and lifespan of different sized bird species.

Key words: muscle phospholipid, polyunsaturate, docosahexaenoic acid, allometry, basal metabolism, maximum lifespan, bird.

Introduction

Gudbjarnason et al. (1978) published a fascinating correlation between the heart rate of mammals, ranging in size from mice to whales, and the docosahexaenoic acid content of their heart phospholipids, but the functional significance of this strong positive correlation between heart rate and the content of the *n*-3 polyunsaturate, docosahexaenoic acid (22:6) in heart membranes was not known at the time. Resting heart rate is an indicator of the mass-specific basal metabolic rate (BMR) of the different mammalian species (Brody, 1945), and BMR has been known for 70 years to be allometrically related to the body mass of mammals (Kleiber, 1932; Brody and Procter, 1932).

Intrigued by Gudbjarnason's observation, coupled with the knowledge that the high metabolic rate of endothermic rats, compared to the low metabolic rate of ectothermic lizards, was also associated with a high 22:6 content of both liver and kidney phospholipids (Hulbert and Else, 1989) as well as liver mitochondrial phospholipids (Brand et al., 1991), Couture and Hulbert (1995a) investigated whether Gudbjarnason's observation might be part of a more general body-size-related phenomenon. They measured the acyl composition of tissue phospholipids from five species of mammal of different sizes, ranging from mice to cattle. They confirmed Gudbjarnason's original observation and found it was indeed part of a more general relationship and not restricted to the heart. Tissue phospholipids from the small mammals were more polyunsaturated than those from the large mammal species whilst the phospholipids from large mammals were more mono-unsaturated than those from small mammals. This relationship was present in all tissues examined (heart, liver, kidney and skeletal muscle) except the brain. Brain phospholipids were highly polyunsaturated in all the mammal species examined, irrespective of the species body size.

More recently a collation and review of published data for mammals (Hulbert et al., 2002) has shown that, excluding the brain, the tissue phospholipids of different sized mammals show no allometric trend in their total percentage of unsaturated acyl chains, but statistically significant allometric declines in their degree of unsaturation (i.e. in their unsaturation index; the total number of double bonds per 100 acyl chains) with increasing body size. This is predominantly due to very significant and substantial allometric decreases in the content of the highly polyunsaturated *n*-3 acyl chain, docosahexaenoic acid (22:6 *n*-3). The allometric exponents for

the 22:6 relationships varied from –0.19 for liver phospholipids to –0.40 for skeletal muscle phospholipids. These probably represent the largest body-size-related variation of body composition recorded for mammals and the allometric exponents obtained are similar to those for mass-specific metabolic rate of mammals (Kleiber, 1961).

Over the last decade it has become obvious that much of the energetic cost of life is associated with the maintenance, by various membrane-associated transport systems, of thermodynamically unfavourable transmembrane ion gradients. Two important examples are the plasmalemmal Na^+ gradient and the mitochondrial H^+ gradient (e.g. Clausen et al., 1991; Brand et al., 1994; Rolfe and Brown, 1997). Recently, it has been suggested that the acyl composition of membranes has an important influence on the molecular activity of many membrane proteins, including membrane transport systems, and that in this way membrane lipids may act as pacemakers for metabolism of different species (Hulbert and Else, 1999). The functional significance of the finding of more polyunsaturated membranes in small mammals and its potential role in the allometric variation in metabolic rate of mammals have recently been discussed (Hulbert and Else, 2000), and the effects of membrane lipids on the molecular activity of membrane proteins, such as the Na^+,K^+-ATPase, may be related to their effects on the physical aspects of molecular packing in membranes (Wu et al., 2001).

If the degree of membrane polyunsaturation is indeed a pacemaker for metabolic rate, then similar allometric variation in membrane acyl composition should exist in other groups of animals that also show body-size-related variation in their metabolism. One such group are the birds. Relatively little is known of the acyl composition of tissue phospholipids from birds. Here we report the findings of a study of the acyl composition of phospholipids from the skeletal muscle of eight species of bird ranging in mass from the 13 g zebra finch to the 34 kg emu. The results for these eight species have been combined with those recently published for a 3 g hummingbird (Infante et al., 2001) and allometric relationships determined. These species represent an approximately 11 000-fold difference in body mass between the smallest and largest birds.

Materials and methods

Pectoral muscle samples were obtained from four individuals of each of the following species: zebra finch (*Taeniopygia guttata* Vieillot; mean mass approx. 13 g), sparrow (*Passer domesticus* L.; mean mass approx. 25 g), starling (*Sturnus vulgaris* L.; mean mass approx. 73 g), pied currawong (*Strepera graculina* Shaw; mean mass approx. 283 g), rock dove (feral pigeon) (*Columba livia* Gmelin; mean mass approx. 462 g), domestic duck (*Anas platyrhynchos* L.; mean mass approx. 2.18 kg), domestic goose (*Anser anser* L.; mean mass approx. 4.44 kg) and emu (*Dromaius novaehollandiae* Latham; mean mass approx. 35 kg). The emus were purchased from Marayong Park Emu Farm (Falls Creek, NSW, Australia), whilst the zebra finches, ducks and geese were purchased from local pet shops or the Narellan Aviary Bird Auction (NSW, Australia). The pigeons were obtained from a local pigeon breeder (T. Cooper, Corrimal, NSW, Australia) and the sparrows, starlings and currawongs were trapped locally in the Wollongong environs. All birds were killed by anaesthetic overdose (sodium pentobarbitone, 100 mg kg^{-1} body mass; intraperitoneal, except in the case of the emus where injection was intrajugular) either on the day of purchase or within a few days of purchase. For the few days that some birds were kept in captivity, they were provided with access to water and food *ad libitum*. For the finches and sparrows the food was mixed bird seed, and for the ducks and geese it was a commercial mixture of pellets and seed. The diet of birds before their purchase was generally unknown. Pectoral muscle samples were immediately excised after death and placed in liquid nitrogen storage until lipid extraction and acyl analysis approximately 4 weeks later.

Total lipids were extracted from the muscle samples by standard methods (Folch et al., 1957) using ultrapure-grade chloroform and methanol (2:1, v/v) containing butylated hydroxytoluene (0.01% w/v) as an antioxidant. For each preparation phospholipids were separated from neutral lipids using Sep-pak silica cartridges (Waters, Milford, MA, USA). The acyl composition of each phospholipid fraction was determined by methods described in detail elsewhere (Pan and Storlien, 1993).

The results obtained were combined with those for breast muscle phospholipids from the ruby-throated hummingbird *Archilochus colubris* taken from Table 2 of Infante et al. (2001). In this table there are some misprints; the correct values for 20:2 *n*-6, 20:3 *n*-6 and 20:4 *n*-6 are, respectively, 0%, 0.11% and 5.87% (J. T. Brenna, personal communication). Body mass and BMR values for this hummingbird species were taken from Lasiewski (1963). The mass-specific BMR values for the other species were either calculated from published allometric equations (Lasiewski and Dawson, 1967), obtained from the literature or from as-yet-unpublished studies. All figures and allometric equations were produced using KaleidaGraph (version 3.0.5) software (Abelbeck Software). The statistical significance of each relationship was determined from the correlation coefficient given with each allometric equation. All experiments were approved by the University of Wollongong Animal Experimentation Ethics Committee.

Results

The species examined in this study comprised both passerine and non-passerine birds. Apart from the hummingbird, the next four smallest species are passerines whilst the largest four species are all non-passerines. Passerine birds are reported to generally possess higher rates of basal metabolism than non-passerines (Lasiewski and Dawson, 1967). The body mass and basal metabolic rates are presented in Table 1 and plotted allometrically in Fig. 1. For all species the original empirical BMR values are used. For the pied currawong we were unable to obtain the original BMR values from the literature and for

Table 1. *Body mass, docosahexaenoate content and unsaturation index of skeletal muscle phospholipids and basal metabolic rates of the nine species of birds used in the present study*

Species	Body mass (g)	22:6 content (%)	Unsaturation index	BMR (ml O_2 g^{-1} h^{-1})	Source of BMR value
Ruby-throated hummingbird *Archilochus colubris*	3.2	28.0±4.3*	245*	4.3	Table 1 of Lasiewski (1963)
Zebra finch *Taeniopygia guttata*	12.7	7.6±2.0	177±7	3.28	Unpublished observations of W. Buttemer, C. Bech, M. Chappell and L. Astheimer
House sparrow *Passer domesticus*	24.5	11.8±2.5	190±6	2.46	Chappell et al. (1999)
Starling *Sturnus vulgaris*	74.0	13.6±1.6	180±13	2.31	Unpublished observations of W. Buttemer
Pied currawong *Strepera graculina*	283	6.1±0.6	163±5	1.59	Calculated from passerine equation of Lasiewski and Dawson (1967)
Pigeon *Columba livia*	462	2.4±0.4	160±4	0.667	Lasiewski and Dawson (1967)
Duck *Anas platyrhynchos*	2 178	7.0±2.7	180±7	0.626	Lasiewski and Dawson (1967)
Goose *Anser anser*	4 487	2.5±0.3	163±1	0.547	Lasiewski and Dawson (1967)
Emu *Dromaius novaehollandiae*	34 975	1.4±0.2	144±10	0.152**	Maloney and Dawson (1993)

Unsaturation index, number of double bonds per 100 acyl chains.
BMR, basal metabolic rate.
*Values for 22:6 content and unsaturation index of hummingbird muscle phospholipids taken or calculated from Infante et al. (2001).
**Values for emus are for male emus during winter.

this species a BMR value was calculated from the passerine equation of Lasiewski and Dawson (1967) (see Table 1). As can be seen from Fig. 1, the allometric equation describing the power relationship between body mass and BMR, for the species used in this study, has an exponent of –0.35; this is a much steeper relationship than the allometric relationships normally ascribed to birds, which have exponents of approximately –0.25.

When the acyl composition of skeletal muscle phospholipids from these nine species were compared the phospholipids from the smaller species were significantly more unsaturated (as represented by the unsaturation index, Fig. 2A); however, there was no significant body-size-related variation in the total percentage of unsaturated acyl chains (Fig. 2B). On average, the skeletal muscle phospholipids of birds possess 62% unsaturated acyl chains. There was a significant allometric increase in the mono-unsaturate content in the larger species, whilst the total polyunsaturate content was relatively constant irrespective of species body mass (Fig. 2C). Whilst there was a non-significant trend for *n*-6 polyunsaturated fatty acid (PUFA) content to increase, *n*-3 PUFA content of skeletal muscle phospholipids showed a significant decrease with increasing body mass (Fig. 2D). From the allometric slopes of the statistically significant relationships in Fig. 2 we can calculate that for every doubling of bird body mass, there is on average a 2.7% decrease in the number of double bonds per 100 acyl chains, a 5.0% increase in the mono-unsaturate content and a 8.0% decrease in *n*-3 PUFA content of skeletal phospholipids.

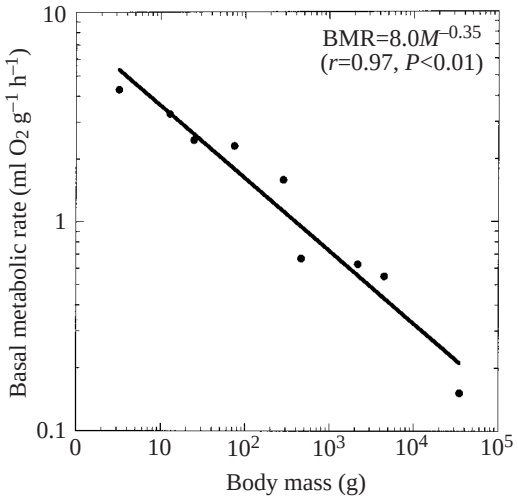

Fig. 1. The allometric relationship between body mass and basal metabolic rate for the nine bird species used in the current study. See Table 1 for details of individual species.

In Fig. 3 are plotted the allometric relationships for some of the major individual acyl chains. For the saturated acyl chains (Fig. 3A) there was no significant trend in stearic acid (18:0) content but there was a significant allometric decline in

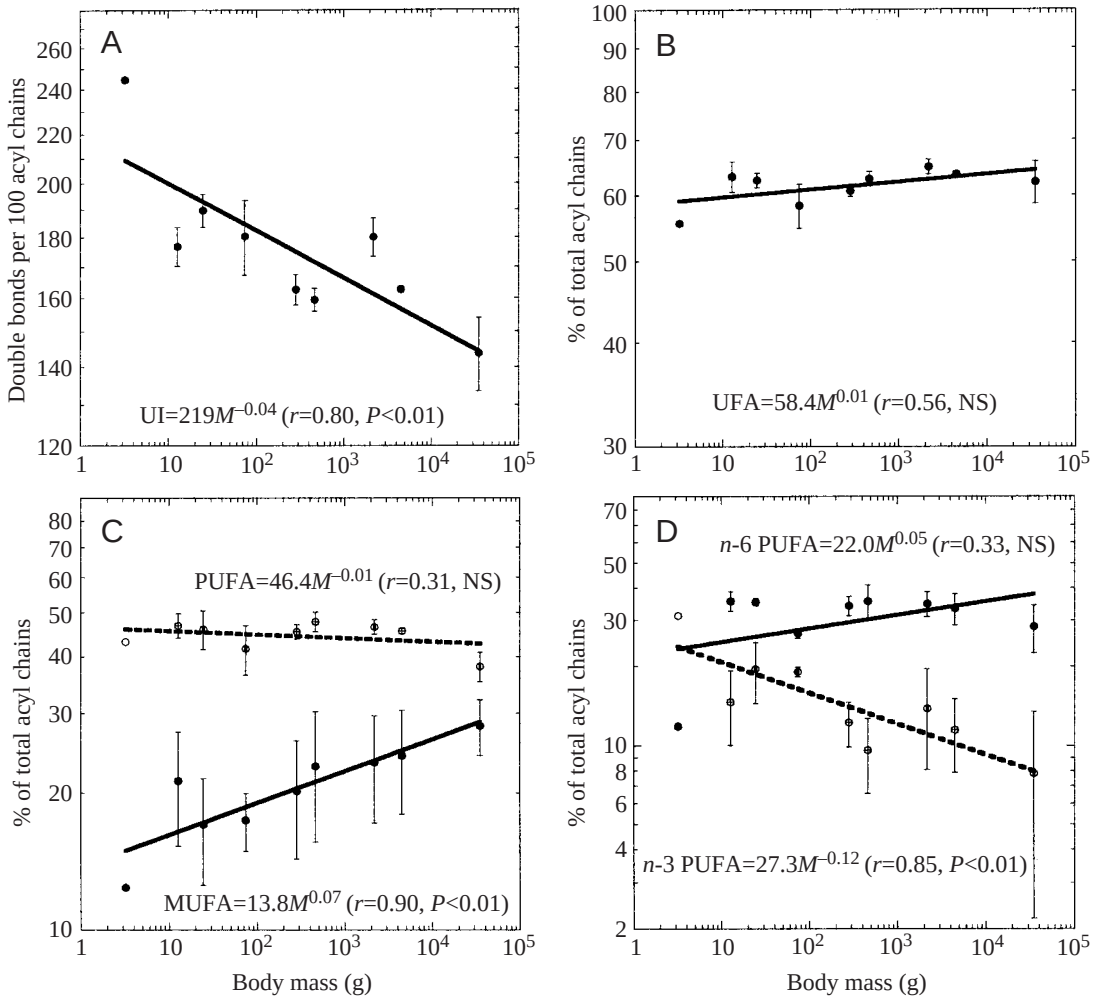

Fig. 2. Allometric plots of the acyl composition of skeletal muscle phospholipids of birds. (A) Unsaturation index, UI (total number of double bonds per 100 acyl chains), (B) unsaturated acyl chains, UFA (C) polyunsaturated (PUFA; open circles) and mono-unsaturated (MUFA; closed circles) acyl chains, and (D) *n*-6 (closed circles) and *n*-3 (open circles) polyunsaturated acyl chains. Values are means ± S.E.M. N=4 for all values with error bars; NS, not significant. M, body mass.

palmitic acid (16:0). For the two 18C mono-unsaturates (Fig. 3B) there were opposing trends but only the relationship for the more common *n*-9 oleic acid was statistically significant. Both of these mono-unsaturates can be synthesised from the saturated palmitic acid (16:0) by the sequential action of the elongase and the Δ9-desaturase enzyme systems. Whether vaccenic acid (18:1 *n*-7) or oleic acid (18:1 *n*-9) is formed will depend on the sequence that these two enzyme systems act on 16:0. When 16:0 is elongated before being Δ9-desaturated, 18:1 *n*-9 is the product, whereas when Δ9-desaturation occurs before elongation then 18:1 *n*-7 is the resultant product. The significant allometric increase in 18:1 *n*-9 may indicate a greater elongase activity in the larger bird species. Although it was not statistically significant there was a tendency for the ratio of 18:0/16:0 (which is sometimes used as an indicator of elongase activity) to be greater with increasing body mass (results not shown).

The allometric relationships describing the relative content of the two main *n*-6 PUFAs are shown in Fig. 3C. The relationship for linoleic acid (18:2 *n*-6) was not statistically significant and the apparent slope of this relationship is solely due to the very low 18:2 *n*-6 content reported for the hummingbird by Infante et al. (2001). This value seems anomalous compared to those for the other species. There is a significant positive allometric relationship for arachidonic (20:4 *n*-6) content of skeletal muscle in birds. In most species the 20:4 *n*-6 content was substantially less than the content of its precursor 18:2 *n*-6. This was not the case for the *n*-3 PUFAs, where in all species the content of the highly polyunsaturated docosahexaenoic acid (22:6 *n*-3) was greater than the content of its precursor *n*-3 PUFAs. In Fig. 3D are plotted the allometric relationships for two *n*-3 polyunsaturates. Eicosapentanoic acid (20:5 *n*-3) content did not vary with body mass but docosahexaenoic acid (22:6 *n*-3) showed a very significant negative allometric relationship with an exponent of –0.28. Such an exponent means that for every doubling of body mass in birds there is a 17.6% decrease in the content of 22:6 *n*-3 in skeletal muscle phospholipids. This relationship

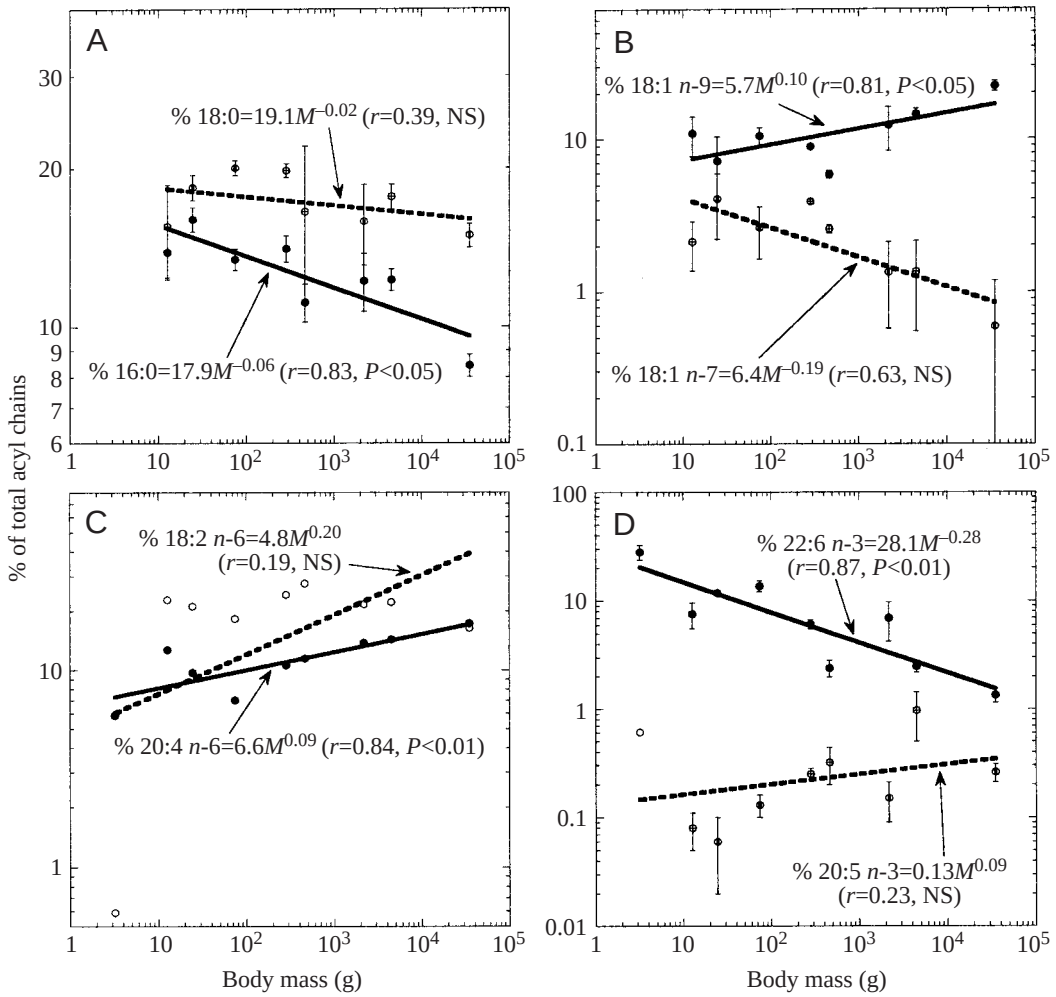

Fig. 3. Allometric plots of acyl composition of skeletal muscle phospholipids of birds. (A) Palmitic acid (closed circles) and stearic acid (open circles), (B) oleic acid (closed circles) and vaccenic acid (open circles), (C) linoleic acid (open circles) and arachidonic acid (closed circles), (D) eicosapentanoic acid (open circles) and docosahexaenoic acid (closed circles). Values are means ± S.E.M. N=4 for all values with error bars; NS, not significant. M, body mass. For further details, see text.

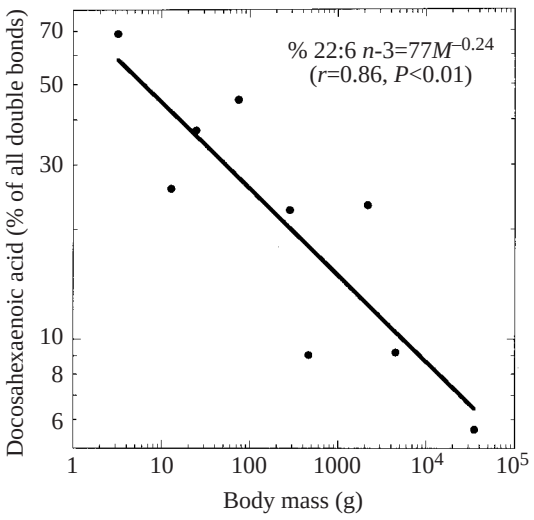

Fig. 4. Contribution of docosahexaenoic acid (22:6 n-3) to the total number of double bonds in the skeletal muscle phospholipids of birds. N=4 for all values.

represented the steepest allometric relationship of any acyl chain examined. Because of its highly desaturated nature, when present in substantial amounts this acyl chain is a major contributor to the overall unsaturation index of skeletal muscle phospholipids. In Fig. 4 is plotted the calculated percentage contribution of 22:6 n-3 to the unsaturation index for each species and this is inversely related to body mass, varying from approximately 70% in the hummingbird to less than 6% in the emu.

Discussion

The body-size-related variation in the composition of membrane bilayers of skeletal muscle previously described for mammals (Couture and Hulbert, 1995; Hulbert et al., 2002) also exists in birds. The acyl composition of skeletal muscle phosholipids in these two vertebrate groups that have independently evolved endothermy are very similar. In both groups, although there is no significant allometric variation in the percentage of total unsaturates, total polyunsaturates and n-6 polyunsaturates, there is a significant allometric increase in the percentage of mono-unsaturates and significant allometric decreases in the percentage of n-3 polyunsaturates

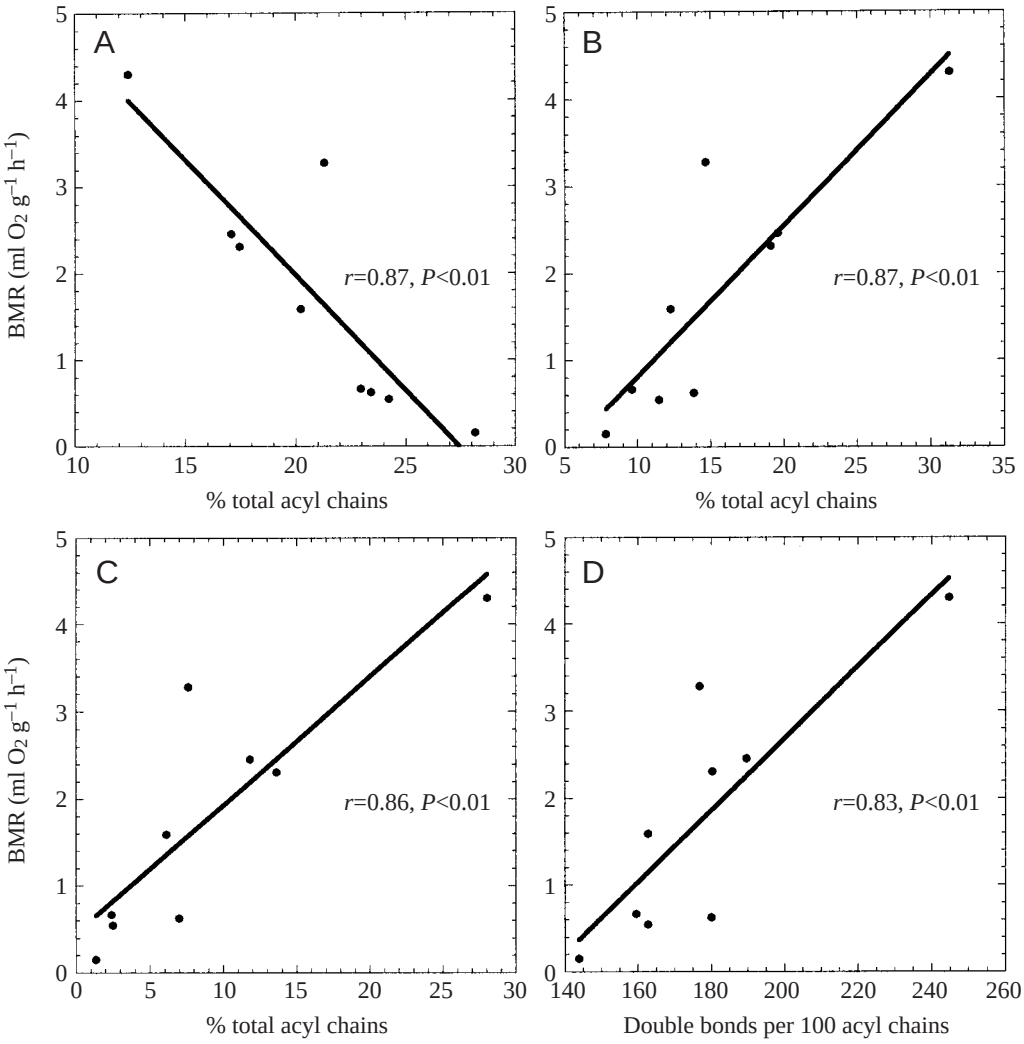

Fig. 5. Linear correlations between basal metabolic rate BMR of birds and (A) mono-unsaturate content, (B) *n*-3 polyunsaturate content, (C) docosahexaenoic acid, 22:6 *n*-3 content, and (D) unsaturation index of skeletal muscle phospholipids.

and docosahexaenoic acid with increasing body size. In both mammals and birds the allometric slope describing the 22:6 *n*-3 content of skeletal muscle phospholipids is the steepest of all components describing acyl composition of the phospholipids and is not too dissimilar to the allometric slope for mass-specific BMR. These relationships for docosahexaenoic acid probably describe the greatest body-size-related variation in chemical composition yet described for birds and mammals.

The results of this study further collaborate the observation that, in vertebrates, metabolically active systems have polyunsaturated membranes, whilst in metabolically inactive systems, membranes are less polyunsaturated but more mono-unsaturated (Hulbert and Else, 1999). This can be observed in Fig. 5, where there is a significant negative correlation between the mono-unsaturate content of skeletal muscle phospholipids of the bird species and their mass-specific BMR and significant positive correlations of the unsaturation index, *n*-3 polyunsaturate content and 22:6 content of skeletal muscle phospholipids and BMR. In view of the strong input from 22:6 into unsaturation index, the three positive correlations are all describing the same phenomenon.

In the present study we have extrapolated our finding in pectoral muscle to skeletal muscle in general. Nothing is known about the phospholipid acyl composition of different muscles in birds. In the rat there are only small differences in the acyl composition and no significant difference in the unsaturation index and docosahexaenoic acid content of phospholipids from soleus or EDL muscle, although these muscles differ substantially in their fibre type composition (Ayre and Hulbert, 1996; Blackard et al., 1997).

Whether other tissues of birds show the same body-size-related variation in phospholipid acyl composition that is observed in skeletal muscle, as in mammals, is not yet known. Similarly, the degree of allometric variation in the acyl composition of subcellular membranes of tissues from birds has not yet been studied systematically and is thus not known, although liver mitochondrial membranes from birds show an allometric decline in their degree of unsaturation with body size (M. D. Brand, P. L. Else and A. J. Hulbert, unpublished observations), similar to our observations for total phospholipids from skeletal muscle.

Higher animals lack the Δ12- and Δ15-desaturases found in plants and thus both the *n*-6 and *n*-3 polyunsaturated acyl chains must either be obtained from their diet or from their gut

microbes (which presumably are capable of *de novo* PUFA synthesis). Most knowledge in this area comes from research on mammals, and whilst very little is known for birds it is assumed that the same processes occur. Once in the body the 18C chain versions of both of these types of polyunsaturates can generally be both elongated and further desaturated by appropriate enzyme systems, although this is not universally true for all animal species. The relative occurrence of different acyl chains in membranes is a regulated phenomenon, and although dietary deficiency of particular types of fatty acids will have an influence, it is generally difficult to substantially change membrane acyl composition by dietary manipulation. In some situations, there seems to be simple competition between *n*-6 and *n*-3 polyunsaturates, and their relative abundance in muscle membranes is strongly influenced by their relative presence in the diet (e.g. Pan and Storlien, 1993). Some of the variation observed in the present study is probably related to the generally small influence of diet on membrane acyl composition. None of the birds would appear to have had a diet deficient in polyunsaturates, in that mead acid (20:3 *n*-9) was absent in most samples and present in negligible amounts (<0.1%) in the currawong and the goose. This unusual acyl chain is only synthesised in significant amounts when normal *n*-6 and *n*-3 polyunsaturates are absent and its appearance is used as an indicator of such dietary essential fatty acid (PUFA) deficiency. The fatty acyl composition of the diet can influence the acyl composition of phospholipids, but not to the same degree that it influences the composition of triglycerides. However, differences in diet are unlikely to explain the allometric variation in docosahexaenoic content of phospholipids observed in the current study, as this long-chain *n*-3 polyunsaturate would not be expected to be a significant component of the diet of any of the bird species examined.

Whilst the synthesis and modification to many acyl chains appears to occur in the endoplasmic reticulum, the synthesis of docosahexaenoic acid appears to be more complex and is not currently agreed. It has been suggested that, whilst Δ5 and Δ6-desaturases definitely exist, the Δ4-desaturase (which in some proposed schemes is necessary for synthesis of 22:6 *n*-3) does not in fact exist, and that the synthesis of 22:6 *n*-3 involves a single cycle of β-oxidation of 24:6 *n*-3 in peroxisomes (for a review, see Sprecher, 2000). The synthesis of this important highly unsaturated acyl chain appears to be different and more complex than that of most other acyl chains, and regulated differently.

Membrane acyl composition is also regulated by constant membrane remodelling. In rat liver cells only four molecular species of phosphatidylcholine and phosphatidylethanolamine are synthesised *de novo*; all other molecular species are produced by deacylation–reacylation processes (Schmid et al., 1995). These processes are very rapid, in that within minutes of being added to the culture medium, labelled acyl chains appear in the plasma membrane phospholipids of cultured cells (Chakravarthy et al., 1986). The roles of any of these enzyme systems in determining the allometric variation in membrane acyl composition is currently unknown for both mammals and birds.

The functional significance of this body-size-related variation in membrane acyl composition is probably related to the long-known allometric variation in metabolism. For example, the activity of the sodium pump varies allometrically with body size in mammalian liver and kidney slices (Couture and Hulbert, 1995b), the molecular activity of the Na^+,K^+-ATPase from endothermic vertebrates is several times that from ectothermic vertebrates when measured at the same temperature (Else et al., 1996) and in 'species-crossover' experiments between rats and toads it has been demonstrated that the membrane lipids are major determinants of this difference in molecular activity (Else and Wu, 1999). Using preparations from two tissues that have a high tissue density of sodium pumps, namely kidney and brain, it has recently been shown that the higher polyunsaturate content of rat compared to toad membranes may be influencing the high molecular activity in mammalian tissues *via* effects on the molecular packing of membrane lipids (Wu et al., 2001). Similarly, a number of studies have now suggested a connection between liver mitochondrial membrane polyunsaturation and liver mitochondrial proton leak between both endothermic and ectothermic vertebrates (Brand et al., 1991; Brookes et al., 1998) as well as between mammals of different body size (Porter et al., 1996). Even the difference in mitochondrial proton leak between the different tissues of the rat appears to be related to differences in the degree of polyunsaturation of mitochondrial membranes, with skeletal muscle having the greatest unsaturation index, the highest docosahexaenoic acid content and the greatest proton leak of all tissues examined (Rolfe et al., 1994). Another significant component of metabolism, especially in muscle, is the cost of maintaining trans-membrane Ca^{2+} gradients, and docosahexanoate-containing phospholipids have been suggested to be important for very active Ca^{2+}-ATPases (Infante, 1987; Infante et al., 2001).

Polyunsaturated acyl chains in membranes may be important determinants not only of the pace of life but also for the length of life. Not only do they probably result in a greater consumption of oxygen but they are also important substrates for damage by the free radicals produced by this enhanced oxygen consumption, resulting in lipid peroxides. Polyunsaturates are especially susceptible to lipid peroxidation and their double bonds are located in the very place that most reactive oxygen species are produced, namely deep in the mitochondrial membrane bilayer. In mammals, the body-size-related variation in acyl composition of heart phospholipids (Pamplona et al., 1999c) and liver mitochondrial phospholipids (Pamplona et al., 1998) have been shown to be strongly correlated with maximum lifespan. The low level of phospholipid unsaturation in larger mammalian species has been related to a lower level of lipid peroxidation and lipoperoxidative damage to tissue proteins in these larger mammals, and has been suggested as a mechanism involved in their longer maximum lifespans (Pamplona et al., 2000).

Although birds and mammals show similar body-size-related relationships in the acyl composition of skeletal muscle phospholipids, there are some differences in composition between these two endothermic groups of vertebrates. If we compare a medium-sized species of bird and mammal (say 250 g body mass), the skeletal muscle phospholipids of the bird will possess 95% of the unsaturated acyl content of the mammal but have an unsaturation index that is 82% of the value for the mammal. The bird skeletal muscle phospholipids will have 50% more mono-unsaturated and 15% less polyunsaturated acyl chains than the mammal. The bird will have 12% more *n*-6 PUFA and 26% less *n*-3 PUFA than the mammal, with the consequence that the ratio of *n*-3/*n*-6 in the bird will be about half the value calculated for the mammalian muscle phospholipids. The docosahexaenoic acid content of the bird muscle membranes will, on average, be approximately two-thirds of that in the mammal. Whether these differences have functional consequences is not known, but it is tempting to speculate that the *n*-3 and *n*-6 differences may be related to the longer lifespan of birds compared to mammals (Holmes and Austad, 1995). It has been proposed that the low unsaturation of pigeon mitochondria compared to rat mitochondria, for both liver and heart, protects against lipid peroxidation in the pigeon and is related to the much longer lifespan in the pigeon than the rat (Pamplona et al., 1996, 1999a). A comparison of heart phospholipid acyl composition of the canary and the parakeet with the mouse yielded similar results (Pamplona et al., 1999b).

Whether the allometric relationships found for the phospholipid acyl composition of skeletal muscle of birds (this study) and mammals (Hulbert et al., 2002) also exist in skeletal muscle of ectothermic vertebrates is unknown. The bird and mammal studies both involved vertebrates that have approximately the same body temperature within each group. Any comparison of ectotherms of differing body size would need to take the body temperature of the species into account, as modification of membrane acyl composition is known to be one of the main mechanisms of temperature acclimation in ectotherms (e.g. Hazel and Williams, 1990; Hazel, 1995).

We would like to dedicate this paper to Dr Juan Infante for his long interest in docosahexaenoic acid. He unfortunately passed away on 26th December, 2001. We would also like to thank Nigel Turner and Parisa Abolhasan for technical assistance. This work was supported by a grant from the Australian Research Council (to A.J.H. and P.L.E.).

References

Ayre, K. J. and Hulbert, A. J. (1996). Dietary fatty acid profile influences composition of skeletal muscle phospholipids in rats. *J. Nutr.* **126**, 653-662

Blackard, W. G., Li, J., Clore, J. N. and Rizzo, W. B. (1997). Phospholipid fatty acid composition in type I and type II rat muscle. *Lipids* **32**, 193-198.

Brand, M. D., Chien, L.-F., Ainscow, E. K., Rolfe, D. F. S. and Porter, R. K. (1994). The causes and function of mitochondrial proton leak. *Biochim. Biophys. Acta* **1187**, 132-139.

Brand, M. D., Couture, P., Else, P. L., Withers, K. W. and Hulbert, A. J. et al. (1991). Evolution of energy metabolism: proton permeability of the inner membrane of liver mitochondria is greater in a mammal than in a reptile. *Biochem. J.* **275**, 81-86.

Brody, S. (1945). *Bioenergetics and Growth*. Canada: Hafner Press. 1023 pp.

Brody, S. and Procter, R. C. (1932). Relation between basal metabolism and mature body weight in different species of mammals and birds. *University Missouri Agriculture Experimental Station Res. Bull.* **no. 166**.

Brookes, P. S., Buckingham, J. A., Tenreiro, A. M., Hulbert, A. J. and Brand, M. D. (1998). The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty acid composition. *Comp. Biochem. Physiol.* **119B**, 325-334.

Chakravarty, B. R., Spence, M. W. and Cook, H. W. (1986). Turnover of phospholipid fatty acid chains in cultured neuroblastoma cells: involvement of deacylation–reacylation and de novo synthesis in plasma membranes. *Biochim. Biophys. Acta* **879**, 264-277.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Clausen, T. C., van Hardeveld, C. and Everts, M. E. (1991). Significance of cation transport in control of energy metabolism and thermogenesis. *Physiol. Rev.* **71**, 733-775.

Couture, P. and Hulbert, A. J. (1995a). Membrane fatty acid composition is related to body mass in mammals. *J. Membr. Biol.* **148**, 27-39.

Couture, P. and Hulbert, A. J. (1995b). Relationship between body mass, tissue metabolic rate and sodium pump activity in mammalian liver and kidney. *Am. J. Physiol.* **268**, R641-R650.

Else, P. L., Windmill, D. J. and Markus, V. (1996). Molecular activity of sodium pumps in endotherms and ectotherms. *Am. J. Physiol.* **271**, R1287-R1294.

Else, P. L. and Wu, B. J. (1999). Do membranes contribute to the high sodium pump molecular activity of mammals. *J. Comp. Physiol. B* **169**, 296-302.

Folch, J., Lees, M. and Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Gudbjarnason, S., Doell, B., Oskardottir, G. and Hallgrimsson, J. (1978). Modification of cardiac phospholipids and catecholamine stress tolerance. In *Tocopherol, Oxygen and Biomembranes* (ed. C. deDuve and O. Hayaishi), pp. 297-310. Amsterdam: Elsevier.

Hazel, J. R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **57**, 19-42.

Hazel, J. R. and Williams, E. E. (1990). The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* **29**, 167-177.

Holmes, D. J. and Austad, S. N. (1995). Birds as animal-models for the comparative biology of aging – a prospectus. *J. Gerontol. Series A* **50**, B59-B66.

Hulbert, A. J. and Else, P. L. (1989). The evolution of endothermic metabolism: mitochondrial activity and changes in cellular composition. *Am. J. Physiol.* **256**, R1200-R1208.

Hulbert, A. J. and Else, P. L. (1999). Membranes as possible pacemakers of metabolism. *J. Theor. Biol.* **199**, 257-274.

Hulbert, A. J. and Else, P. L. (2000). Mechanisms underlying the cost of living in animals *Ann. Rev. Physiol.* **62**, 207-235.

Hulbert, A. J., Rana, T. and Couture, P. (2002). The acyl composition of mammalian phospholipids: an allometric analysis. *Comp. Biochem. Physiol. B* **132**, 515-527.

Infante, J. P. (1987). Docosahexanoate-containing phospholipids in sarcoplasmic reticulum and retinal photoreceptors. A proposal for a role in Ca^{2+}-ATPase calcium transport. *Mol. Cell. Biochem.* **74**, 111-116.

Infante, J. P., Kirwan, R. C. and Brenna, J. T. (2001). High levels of docosahexaenoic acid (22:6 *n*-3)-containing phospholipids in high-frequency contraction muscles of hummingbirds and rattlesnakes. *Comp. Biochem. Physiol. B* **130**, 291-298.

Kleiber, M. (1932). Body size and metabolism. *Hilgardia* **6**, 315-353.

Kleiber, M. (1932). *The Fire of Life*. 454pp. New York: Academic Press.

Lasiewski, R. C. (1963). Oxygen consumption of torpid, resting, active, and flying hummingbirds. *Physiol. Zool.* **36**, 122-140.

Lasiewski, R. C. and Dawson, W. R. (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. *Condor* **69**, 13-23.

Maloney, S. and Dawson, T. J. (1993). Sexual dimorphism in BMR and T_b of a large bird, the Emu. *Condor* **95**, 1034-1037.

Pamplona, R., Portero-Otin, M., Requena, J. R., Thorpe, S. R., Herrero, A. and Barja, G. (1999a). A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in

heart mitochondria of the longevous pigeon than in the short-lived rat. *Mech. Age. Dev.* **106**, 283-296.

Pamplona, R., Portero-Otin, M., Riba, D., Ledo, F., Gredilla, R., Herrero, A. and Barja, G. (1999b). Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the canary and the parakeet than in the mouse. *Aging Clin. Exp. Res.* **11**, 44-49.

Pamplona, R., Portero-Otin, M., Riba, D., Requena, J. R., Thorpe, S. R., Lopez-Torres, M. and Barja, G. (2000). Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in mammalian species with long life spans. *J. Gerontol.* **55A**, B286-B291.

Pamplona, R., Portero-Otin, M., Riba, D., Ruiz, C., Prat, J. and Barja, G. (1998). Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *J. Lipid Res.* **39**, 1989-1994.

Pamplona, R., Portero-Otin, M., Ruiz, C., Gredilla, R., Herrero, A. and Barja, G. (1999c). Double bond content and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals. *Mech. Age. Dev.* **112**, 169-183.

Pamplona, R., Prat, J., Cadenas, S., Rojas, C., Perez-Campo, R., Lopez-Torres, M. and Barja, G. (1996). Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and the human case. *Mech. Age. Dev.* **86**, 53-66.

Pan, D. A. and Storlien, L. H. (1993). Dietary lipid profile is a determinant of tissue phospholipid fatty acid composition and rate of weight gain in rats. *J. Nutr.* **123**, 512-519.

Porter, R. K., Hulbert, A. J. and Brand, M. D. (1996). Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition. *Am. J. Physiol.* **271**, R1550-R1560.

Rolfe, D. F. S. and Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77**, 731-758.

Rolfe, D. F. S., Hulbert, A. J. and Brand, M. D. (1994). Characteristics of mitochondrial proton leak and control of oxidative phosphorylation in the major oxygen-consuming tissues of the rat. *Biochim. Biophys. Acta* **1188**, 405-416.

Schmid, P. C., Deli, E. and Schmid, H. H. O. (1995). Generation and remodelling of phopholipid molecular species in rat hepatocytes. *Arch. Biochem. Biophys.* **319**, 168-176.

Sprecher, H. (2000). Metabolism of highly unsaturated *n*-3 and *n*-6 fatty acids. *Biochim. Biophys. Acta* **1486**, 219-231.

Wu, B. J., Else, P. L., Storlien, L. H. and Hulbert, A. J. (2001). Molecular activity of Na^+,K^+-ATPase from different sources is related to the packing of membrane lipids. *J. Exp. Biol.* **204**, 4271-4280.