An allometric study of fatty acids and sensitivity to lipid peroxidation of brain microsomes and mitochondria isolated from different bird species

A.M. Gutiérrez [a], G.R. Reboredo [a], S.M. Mosca [a,1], A. Catalá [b,*,1]

[a] Cátedra de Fisiología Animal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, CONICET, La Plata, Argentina
[b] Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CONICET, La Plata, Argentina

Keywords:
Birds
Brain
Mitochondria
Microsomes
Fatty acids
Lipid peroxidation
Allometry

A B S T R A C T

The objective of this investigation was to examine the relationship between body size, fatty acid composition and sensitivity to lipid peroxidation of mitochondria and microsomes isolated from the brain of different size bird species: manon, quail, pigeon, duck and goose, representing a 372-fold range of body mass. Fatty acids of total lipids were determined using gas chromatography and lipid peroxidation was evaluated using a chemiluminescence assay. The allometric study of the fatty acids present in brain mitochondria and microsomes of the different bird species showed a small number of significant allometric trends. In mitochondria the percentage of monounsaturated fatty acids, was significantly lower in the larger birds (r=−0.965; P<0.008). The significant allometric increase in 18:2 n-6; linoleic acid (r=0.986; P<0.0143), polyunsaturated (r=0.993; P<0.007) and total unsaturated (r=0.966; P<0.034) in brain microsomes but not in mitochondria may indicate a preferential incorporation of this fatty acid in the brain endoplasmic reticulum of the larger bird species. The brain of all birds studied had a high content of docosahexaenoic acid. However brain mitochondria but not microsomes isolated from all the birds analyzed showed a significant decrease of arachidonic and docosahexaenoic acids during lipid peroxidation. The allometric analyses of chemiluminescence were not statistically significant. In conclusion our results show absence of correlation between the sensitivity to lipid peroxidation of brain mitochondria and microsomes with body size and maximum life span.

1. Introduction

The fatty acid composition of cellular membranes varies thoroughly with body size in mammals (Couture and Hulbert, 1995; Hulbert et al., 2002a). The phospholipids of the tissues of the small mammals possess a major percentage of polyunsaturated fatty acids than the species of the biggest mammals, predominating in the above mentioned over the monounsaturated. This relation is present in various tissues except in brain (Couture and Hulbert, 1995; Hulbert et al., 2002b). Unsaturated fatty acids are more susceptible to reactive oxygen species induced damage and the sensitivity to lipid peroxidation increases as a function of the number of double bonds (North et al., 1994; Pamplona et al., 1998; Catalá, 2006). Although many studies were performed in mammals there are few works in birds (Finch, 1990; Barja et al., 1994; Holmes and Austad 1995; Holmes and Ottinger, 2003; Holmes et al., 2001; Austad, 1997, 2001; Barja, 1998; Ogburn et al., 2001; Hulbert et al., 2007; Pamplona and Barja, 2007; Pamplona et al., 2002). Considering that birds are an exception since they combine high metabolic rates and maximum longevity it is very interesting to study the sensitivity to lipid peroxidation of avian membranes. Previous works of Pamplona et al. (1996, 1999a,b) and recent investigations of our laboratory using passerine non-passerine birds (Gutiérrez et al., 2000, 2002, 2004, 2006) demonstrate that mitochondrial membranes of different tissues of birds compared with mammals of similar body size possess a low degree of fatty acid unsaturation and are more resistant to lipid peroxidation. However, the relationship between sensibility to lipid peroxidation and body size is still unknown and this is the objective of the present investigation. This study was performed using mitochondria and microsomes isolated from the brain of the following birds: manon, quail, pigeon, duck and goose.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT) and phenyl-methyl-sulfonyl fluoride (PMSF) were from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA) (Fraction V) was obtained from Wako Pure Chemical Industries, Japan. L (+) ascorbic acid and boron-trifluoride-methanol complex were from Merck (Darmstadt, Germany).

Standards of fatty acids methyl esters were from Nu Check Prep Inc. (Elysian, MN, USA). All other reagents and chemicals were of analytical grade from Sigma.

2.2. Birds

All birds species examined in the present study were adults of both sexes, were obtained from the farm (Agro-80 La Plata) and were chosen to span as wide a range of body masses as was practical and on the basis of availability (Table 1).

For the manon, quail and pigeon the food was mixed birdseed, and for the ducks and geese it was a commercial mixture of pellets and seeds. Animals were sacrificed by decapitation and the brains were rapidly removed. The recorded values of maximum life span (MLSP) (in years) were as follows: quail, 6; manon, 9; duck, 21; pigeon 35; goose, 35.

Animal treatment protocols were previously approved by the local ethics committee, and are in accordance with the care and treatment of laboratory animals recommended guidelines (U.S. Public Health Service, 1985).

2.3. Preparation of brain mitochondria and microsomes

For manon and Japanese quail the complete brain was used, whereas for pigeons, ducks ad goose, 5 g of sample was used for each mitochondrial preparation. Brain samples were chopped coarsely with scissors, rinsed and suspended in 3 volumes of ice-cold medium, containing sucrose 0.25 M, 10 mM Tris–HCl (pH 7.4), PMFS 0.001 M, then homogenized using six passes of a motorized Teflon/glass homogenizer (Cole Palmer, Vernon Hills, IL, USA). All operations were carried out at 4 °C. Mitochondria were obtained by the method described by Boveris et al. (1999). The homogenate was centrifuged at 1000 *g* for 3 min in a refrigerated centrifuge, and the supernatant was centrifuged at 12,000 *g* for 10 min. The supernatant (3 mL) obtained was applied to a Sepharose 4 B column (1.6×12 cm) equilibrated and eluted with 10 mM Tris–HCl (pH 7.4), 0.01% NaN_3. The microsomal fraction appearing in the void volume (10–16 mL) was brought to 0.25 M sucrose by adding solid sucrose. All operations were carried out at 4 °C. The quality of this microsomal preparation is of similar composition as regards concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation (Tanger et al., 1973).

2.4. Lipid peroxidation of microsomes and mitochondria

Lipid peroxidation of microsomes and mitochondria was measured as described previously (Catalá et al., 1994) with the following modifications: organelles at a concentration of 1 mg protein were incubated at 37 °C for 120 min with 50 mM phosphate (pH 7.4), 0.4 mM ascorbic acid, in a final volume of 1 mL. Phosphate buffer was contaminated with sufficient iron to provide the necessary ferrous or ferric iron for lipid peroxidation (final concentration in the incubation mixture was 2.15 μM) as determined by atomic absorption spectroscopy. Control organelle preparations that lacked ascorbate were carried out simultaneously. Lipid peroxidation was initiated by adding a small amount of stock solution of ascorbate to each vial that was maintained at 37 °C and was measured by monitoring light emission (Wright et al., 1979; Cadenas et al., 1981) with a liquid scintillation analyzer Packard 1900 TR (Meriden, CT, USA). Chemiluminescence was determined over a 120-min period and recorded as cpm every 10 min.

2.5. Fatty acid analyses

Mitochondrial and microsomal brain lipids as well as mixed bird seed were extracted with chloroform/methanol (2:1, v/v containing 0.01% BHT as antioxidant) (Folch et al., 1957). The fatty acids from total lipids were transmethylated with 20% F_3B in methanol at 60 °C for 180 min. Fatty acid methyl esters were analyzed with a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a packed column (1.80 m×4 mm i.d.) (J. and V. Scientific, Folson, CA, USA) GP 10% DEGS-PS on 80/100 Supelcoport. Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 250 °C and the column temperature was held at 200 °C for 60 min. The fatty acid methyl esters were identified by comparison of retention times with standard compounds. All compositions were expressed as % by area of total fatty acid.

2.6. Other methods

Proteins were determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

2.7. Calculations and statistical analyses

Saturated fatty acids were calculated as SFA=Σ% (16:0+18:0). Unsaturated fatty acids were calculated as UFA=Σ%(MUFA+PUFA). The saturated/unsaturated ratio was also calculated. Monounsaturated fatty acids were calculated as MUFA=Σ%(16:1+18:1). Polyunsaturated fatty acids were calculated as PUFA=Σ% (PUFAn3+PUFAn6). The unsaturation index UI is the sum of the percentages of each fatty acid×number of double bonds. Peroxidizability index (PI)=[(0.025×Σmol% monoenoic)+(1×Σmol% dienoic)+(2×Σmol% trienoic)+(4×Σmol% tetraenoic)+(6×Σmol% pentaenoic)+(8×Σmol% hexaenoic)]. Data were expressed as mean±SD and were subjected to the Student's *t*-test.

The relationships between BM (body mass), fatty acids and chemiluminescence were studied by linear regression obtained after the logarithmic transformation of the variables (equation $\log y = \log a + b \log x$). The correlations were analyzed using the Pearson correlation coefficient (r) selecting 0.05 as the point of minimum statistical significance.

3. Results

3.1. Body and brain mass of the birds

The body and brain mass of the birds analyzed in this study are presented in Table 1. The relative percentage brain/body mass shows

Table 1
Body and brain masses of the birds

Common name	Scientific name	Body mass (g)	Brain mass (g)	Relative percentage
Manon (2F/1M)	*Lonchura striata* (3)	13.55±0.50	0.36±0.05	2.65
Quail (4M)	*Coturnix coturnix var japonica* (4)	142.88±15.20	0.78±0.08	0.54
Pigeon (2F/1M)	*Columba livia* (3)	347.50±3.50	1.41±0.26	0.40
Duck (3F)	*Cairina moschata* (3)	2200.0±2.8	5.90±0.36	0.26
Goose (3M)	*Anser anser* (3)	5040.0±1.9	11.16±0.09	0.22

Values are mean±SD. The number of animals used is indicated in parentheses.
The number of specimens for each sex is indicated, where M is male, and F female.

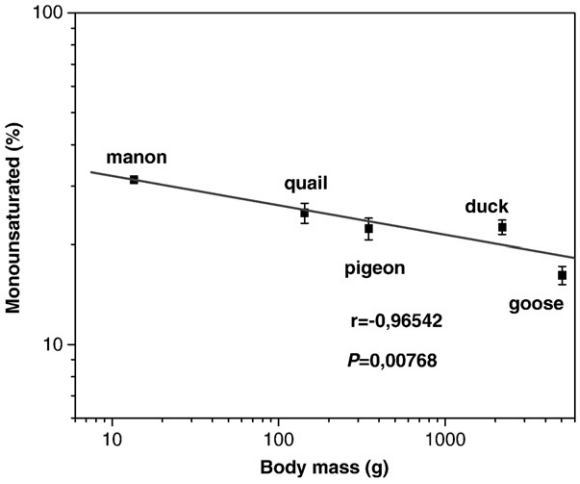

Fig. 1. The allometric relationship between body mass and fatty acids isolated from total lipids of brain mitochondria from avian species The resulting data were fitted to straight line by linear regression $y = 1.60 - 0.09\ x$.

important changes from the smallest (manon) to the largest (goose) bird with values of 2.65 and 0.22 respectively, which represents a 12-fold range of brain with regard to body mass.

3.2. Total fatty acid composition of brain mitochondria and microsomes

The fatty acids present in brain organelles of the different bird species analyzed showed a small number of significant allometric trends. In mitochondria the percentage of monounsaturated fatty acids (MUFAs), was significantly lower in the larger birds ($r = -0.96$) (Fig. 1). Although the content of total saturated fatty acids did not display significant differences in the organelles studied, the content of C18:0 in brain microsomes was significantly smaller ($r = -0.95$) in the birds of greater size (Fig. 2a). A significant allometric increase in C18:2 n6 ($r = 0.98$), polyunsaturated ($r = 0.99$) and total unsaturated ($r = 0.97$) in brain microsomes but not in mitochondria was observed in the larger birds (Tables 2–4; Fig. 2b, c, d). The UI and PI of brain organelles did not show significant differences when it was correlated to body mass of birds (Table 4). The relationship between PI and maximum life span (MLSP) of brain mitochondria (Fig. 4)and microsomes (data not shown) indicated no significant correlations.

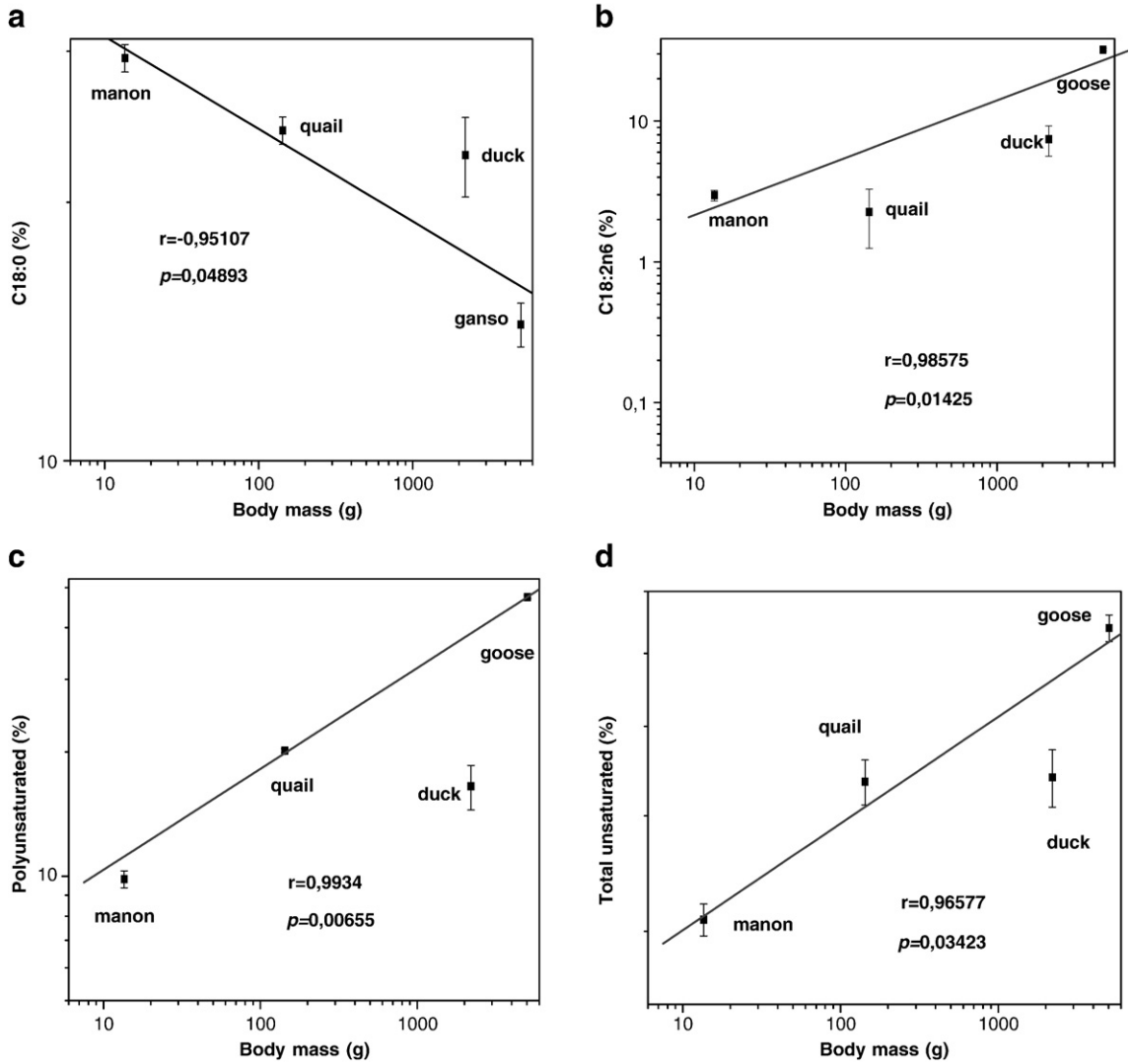

Fig. 2. The allometric relationship between body mass and fatty acids isolated from total lipids of brain microsomes from avian species. a, b, c and d. The resulting data were fitted to straight line by linear regression a- $y = 1.60 - 0.11\ x$; b- $y = -0.001 - +0.41\ x$; c- $y = 0.77 + 0.24\ x$; d- $y = 1.36 + 0.11\ x$.

Table 2
Fatty acid composition (area %) of total lipids from brain mitochondria of the avian species

Fatty acid	Manon		Quail		Pigeon		Duck		Goose	
	Control	Peroxidized	Control	Peroxidized	Control	Peroxidized	Control	Peroxidized	Control	Peroxidized
C16:0	19.99±0.21	21.65±0.46	28.49±0.49	28.75±0.67	24.67±1.14	35.47±0.93	24.8 5±0.32	24.69±3.26	20.61±0.57	26.43±1.01
C16:1 n7	2.02±0.36	2.83±0.58	–	–	1.13±0.07	1.54±0.27	0.78±0.30	8.53±1.07***	2.41±0.22	2.55±0.60
C18:0	19.86±0.39	20.27±0.16	23.36±1.02	23.76±0.81	28.04±2.32	29.17±2.24	20.42±1.73	21.98±0.51	18.19±0.48	19.80±1.04
C18:1 n9	29.33±0.38	27.73±0.69	24.89±1.73	25.33±2.23	21.19±1.75	23.92±2.28	21.78±1.16	22.97±1.80	13.79±1.16	15.52±0.25
C18:2 n6	12.49±0.69	13.00±1.78	2.36±0.07	2.53±0.65	0.60±0.13	0.61±0.15	1.80±0.18	1.74±0.52	31.53±0.89	30.03±0.21
C18:3 n3	0.73±0.04	0.78±0.06	–	–	0.12±0.03	0.13±0.04	–	–	0.30±0.01	0.27±0.01
C20:4 n6	6.52±0.23	2.10±0.56***	7.31±0.40	2.71±0.72**	8.83±1.83	4.62±0.70*	9.12±0.45	5.86±0.68**	6.65±0.20	0.75±0.08***
C22:6 n3	7.84±0.58	2.53±0.33***	8.98±0.68	0.77±0.08**	8.46±0.94	2.78±0.91**	14.99±0.74	7.29±0.47**	8.37±0.08	2.15±0.12***
Saturated	39.85±0.60	41.92±0.38	51.85±1.25	52.51±0.92	52.71±1.18	64.64±2.59**	45.27±1.43	46.66±3.74	38.81±0.26	46.03±1.47**
Monounsaturated	31.35±0.39	30.56±0.60	24.60±1.73	25.33±2.23	22.32±1.68	25.46±2.54	22.56±1.14	31.50±2.86*	16.20±1.01	18.07±0.73
Polyunsaturated	27.59 ±1.07	18.40±1.84**	18.65±0.08	6.06±1.34**	18.02±2.48	8.14±1.73**	25.91±0.69	14.89 ±1.50***	46.85±0.93	33.20± 0.26***
Total unsaturated	58.94±0.72	48.96±2.36**	43.54±2.44	31.39±0.92**	40.33±3.98	36.60±4.14	48.47±1.76	46.39±3.46	63.05±0.21	51.27± 0.89***
UI	131.67±3.87	82.44 ±4.95***	112.72±6.14	45.95±1.84**	109.98±13.09	62.24 ±10.67**	152.58±5.13	102.15±10.32**	157.00±1.21	94.86± 1.38***
PI	114.62±10.84	43.92±5.52***	104.04±6.07	20.23±3.23**	102.98±14.19	42.25±12.37**	158.76±7.19	84.26±16.11**	126.12±2.04	51.25±1.08***

Data are given as the mean±SD of three independent experiments. Statistically significant differences between control and peroxidized groups are indicated by $P<0.05$*, $P<0.01$** y $P<0.001$***. UI=sum of the percentages of each fatty acid×number of double bonds.

3.3. Comparison of lipid peroxidation in brain mitochondria and microsomes isolated from different species of birds

Chemiluminescence was statistically significant in mitochondria, but not in microsomes (Fig. 3). A significant decrease of C20:4 n6 and C22:6 n3 (Table 2) was observed in mitochondria when control and peroxidized samples were compared. However, the fatty acid composition of microsomes did not change (Table 3). The fatty acids present in peroxidized samples of both brain organelles did not show significant allometric trends (Table 4; Fig. 3). The relationship between sensitivity to in vitro lipid peroxidation and maximum life span (MLSP) in brain mitochondria did not show significant differences (Fig. 4).

4. Discussion

Allometric studies of fatty acids and sensitivity to lipid peroxidation of brain microsomes and mitochondria have not previously been measured in vitro in different birds. Our results show that in these five bird species, the sensitivity to lipid peroxidation of brain mitochondria and microsomes did not correlate with body size. The brain organelles of the avian species examined, similarly to other vertebrates have consistently high levels of docosahexaenoic acid, indicating high selective tissue specificity. Fatty acid relative lipid peroxidation rates are largely affected by structural factors such as chain length and the number of double bonds.

In mitochondria, only was detected a significant decrease in the percentage of monounsaturated fatty acids when body size increases. In brain microsomes, a significant allometric decline in C18:0 and significant rises in C18:2 n6, polyunsaturated and total unsaturated with increasing body size were observed. There were not significant allometric relationships in the content of C20:4 n6 and C22:6 n3 in both brain organelles with increasing body mass. Moreover, all species studied maintained substantial amounts of C22:6n3 in both brain organelles which were higher when compared to other tissues (Gutiérrez et al., 2002, 2004, 2006).

Although small differences in fatty acid composition between the vertebrates exist, it is clear that there is a specific and functional requirement for high concentrations of C22:6n3. Indeed, C22:6n3 appears to be prevalent in the brain of most vertebrates (Farkas et al., 2000; Turner et al., 2005a; Gutiérrez et al., 2002, 2004, 2006). This extreme conservation, despite wide genomic changes over 500 million years, testifies to an exclusivity of this molecule in the brain (Crawford, 2006). The emerging consensus is that phospholipids that contain C22:6n3 impart fluidity and permeability to neuronal cell membranes, thereby accommodating the actions of membrane proteins involved in the neuronal transmission mechanism (Salem et al., 2001).

Our findings are in agreement with previous investigations performed in mammals (Couture and Hulbert, 1995) and birds (Turner et al., 2005b; Hulbert et al., 2007) and it have been associated to a specific functional requirement of the brain.

Table 3
Fatty acid composition (area %) of total lipids from brain microsomes of the avian species

Fatty acid	Manon		Quail		Duck		Goose	
	Control	Peroxidized	Control	Peroxidized	Control	Peroxidized	Control	Peroxidized
C16:0	34.71±2.51	35.21±0.95	21.73.± 0.34	24.37±0.78	31.77±3.29	27.08±1.04	20.57±1.60	23.09±1.11
C16:1 n7	2.23±0.30	1.99±0.18	2.19±0.28	2.38±0.31	6.52±0.27	5.28±0.76	3.21±0.62	3.55±1.12
C18:0	38.33±3.58	34.87±4.47	24.25±0.89	22.72±1.61	22.69±2.41	22.48±1.75	14.42±0.85	12.56±0.50
C18:1 n9	18.82±1.46	19.01±1.11	20.16±0.58	20.11±0.33	20.98±1.61	23.60±3.14	13.23±1.49	12.97±2.36
C18:2 n6	2.97±0.25	3.99±0.29	6.66±0.46	6.58±0.29	7.42±1.79	5.36±0.12	32.20±0.65	34.15±0.47
C18:3 n3	0.76±0.16	0.59±0.14	0.91±0.06	0.99±0.23	0.52±017	0.41±0.07	0.28±0.03	0.27±0.03
C20:4 n6	2.40±0.58	1.98±0.22	5.64±0.19	4.97±0.51	3.50±0.60	2.75±0.19	4.24±0.16	4.39±0.35
C22:6 n3	3.70±0.17	3.83±0.19	6.93±0.12	7.04±0.05	5.07±0.10	5.03±0.05	10.73±0.45	10.37±1.06
Saturated	73.03±6.04	70.08±3.68	45.99±0.52	47.09±1.56	54.46±3.55	49.55±1.34	34.99±2.44	33.65±0.79
Monounsaturated	21.04±1.20	21.00±1.28	22.35±0.49	22.49±0.13	27.50±1.83	28.87±2.47	16.44±1.82	16.52±2.40
Polyunsaturated	9.84±0.47	10.40±0.51	20.14±0.24	19.58±0.61	16.51±2.03	13.56±0.39	47.45±0.44	49.19±1.57
Total unsaturated	30.88±1.23	31.40±1.51	42.49±0.61	42.07±0.58	44.02±3.15	42.43±2.67	63.89±2.12	65.71±2.84
UI	61.10±2.24	61.69±3.10	102.54±1.24	100.75±2.08	88.35±5.70	82.02±3.18	163.03±4.21	165.45±7.85
PI	44.25±3.15	43.76±2.90	87.04±1.49	85.32±2.51	63.52±3.31	57.81±1.15	135.96±4.23	135.67±12.24

Data are given as the mean±SD of three independent experiments. UI=sum of the percentages of each fatty acid×number of double bonds.

Table 4
Summary of correlations between body mass and fatty acid composition (area %) or fatty acid indexes of mitochondria and microsomes in the birds included in this study

Fatty acid	Mitochondria				Microsomes			
	Control		Peroxidized		Control		Peroxidized	
	r	*P*	*r*	*P*	*R*	*P*	*r*	*P*
C16:0	0.5787	0.3066	0.6484	0.2365	−0.2987	0.7012	−0.7946	0.2053
C16:1 n9	0.5413	0.4586	0.4884	0.5115	0.8932	0.1067	0.8968	0.1031
C18:0	−0.3363	0.5799	0.5876	0.2974	−0.9510	**0.0490**	−0.8754	0.1245
C18:1 n9	−0.7835	0.1169	−0.7843	0.4260	−0.5300	0.4699	0.1427	0.8572
C18:2 n6	0.6619	0.2236	0.5865	0.2985	0.9857	**0.0143**	0.6643	0.3356
C18:3 n3	−0.9463	0.2094	−0.9636	0.1720	−0.9160	0.0839	−0.8191	0.1809
C20:4 n6	0.2642	0.6674	0.4113	0.4914	−0.7889	0.2110	0.3918	0.6081
C22:6 n3	−0.1248	0.8414	0.3334	0.5834	0.1099	0.8900	−0.7822	0.2177
Saturated	−0.3878	0.5188	0.6678	0.2179	−0.4765	0.5234	−0.7829	0.2170
Monounsaturated	−0.9654	**0.0077**	−0.8756	0.1243	0.3074	0.6925	0.4261	0.5738
Polyunsaturated	0.620	0.2600	0.6894	0.1978	0.9934	**0.0066**	0.7001	0.2999
Total unsaturated	0.5485	0.3384	0.6521	0.2329	0.9657	**0.0343**	0.9171	0.0828
UI	0.8593	0.0619	0.6893	0.1978	0.9194	0.0805	0.5811	0.4188
PI	0.3947	0.5108	0.5190	0.37015	0.7037	0.2962	−0.2257	0.7742

Abbreviations: *r* linear correlation coefficient of Pearson; *P*: statistical significance. Statistically significant numbers are indicated in bold letters.

Lipid peroxidation can alter the cellular structure of membrane-bound enzymes by changing the membrane phospholipids fatty acid composition. Lipid peroxidation has gained renewed attention with increasing evidence showing its biological role in aging animals. The assessment of lipid peroxidation levels in vivo is difficult partly because lipids are oxidized by different oxidants by different mechanisms to give versatile types of products, which may undergo metabolism and secondary reactions (Yoshida et al., 2007).

Chemiluminescence has widely been used as an indicator of reactive oxygen species formation in cells and whole organs, thus allowing the study of a number of pathophysiological conditions related to oxidative stress (Roda et al., 2000).

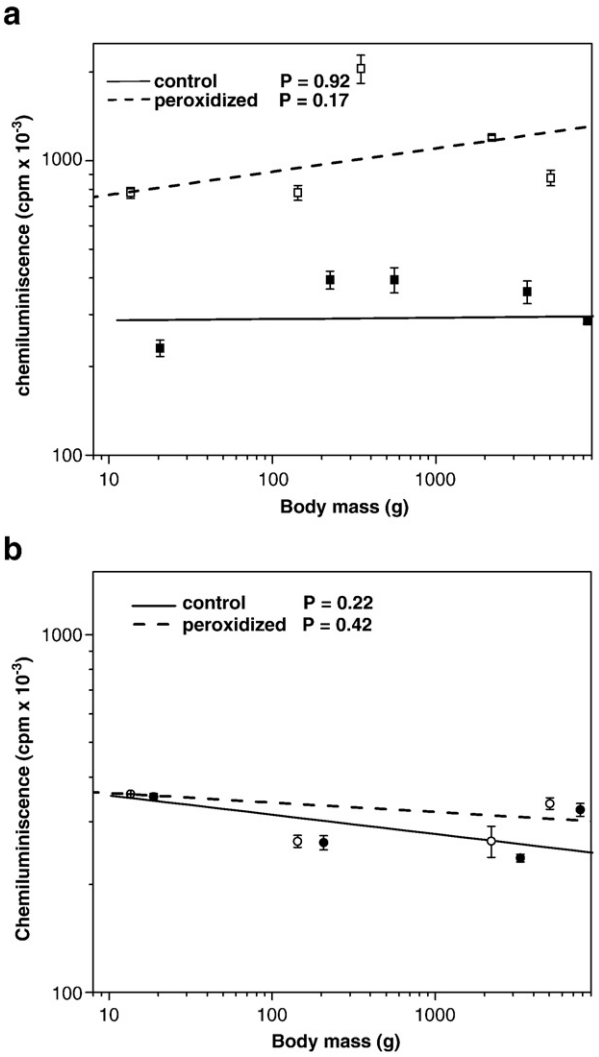

Fig. 3. The allometric relationship between body mass and chemiluminescence rate of mitochondria and microsomes for the five bird species used in the current study. See Table 1 for details of individual species. (a) mitochondria and (b) microsomes.

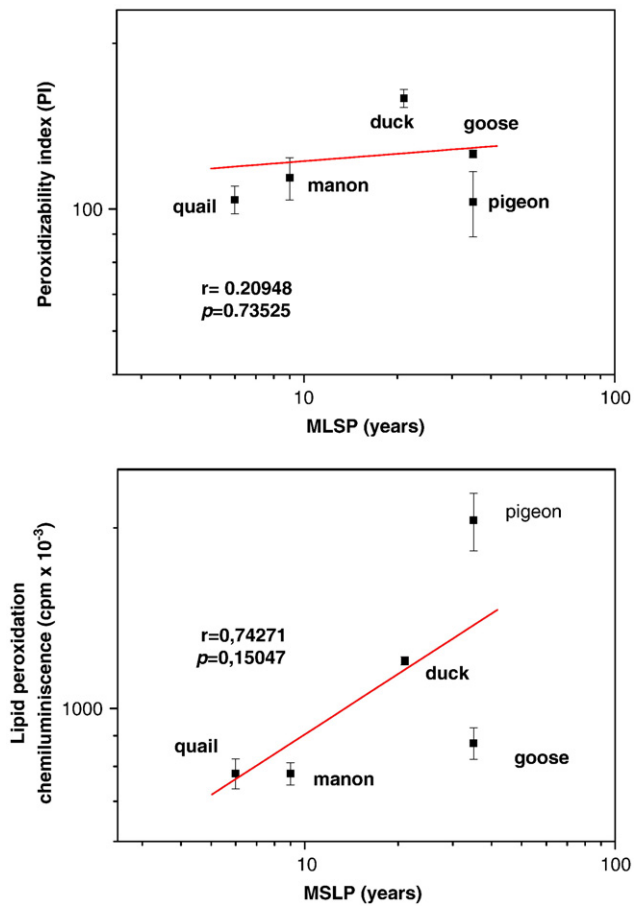

Fig. 4. The allometric relationship between peroxidizability index (PI), sensitivity to in vitro lipid peroxidation and maximum life span (MLSP) in brain mitochondria from bird's species. Values were plotted as a function of MLSP on double logarithmic axes.

Polyunsaturated fatty acids are especially vulnerable to lipid peroxidation and their double bonds are situated in a position susceptible to reactive oxygen species attack, particularly in the mitochondrial and microsomal membrane bilayer (Pamplona et al., 1998). The n-3 PUFA are more peroxidation-prone than n-6 PUFA and within each PUFA class there is 4-fold increase in peroxidisability between the short- and long-chain fats. It has been observed that C22:6n3 is 320-fold more susceptible to peroxidation than 18:1n9. In concordance to bibliographic data (Hulbert et al., 2007) the brain mitochondria of all birds examined in the current study were more susceptible to lipoperoxidation, effect that can be attributed to the high content of C20:4n6 and C22:6n3.

Another interesting finding of this study is that brain mitochondria of all avian species examined into 372-fold range in body mass exhibited a similar sensitivity to lipid peroxidation. Light emission and/or fatty acid composition differences in brain microsomes were not observed when control and peroxidized samples were compared. This lack of lipoperoxidation of microsomes can be due to the lower content of C20:4n6 and C22:6n3, to the higher content of C18:2n6 and the lesser values of unsaturation index compared to mitochondria. These characteristics make to microsomes less vulnerable to oxidative damage. A diminution in C20:4n6/C18:2n6 ratio in microsomes is also indicating a limited activity of Δ5 and Δ6 desaturase enzyme.

The membrane pacemaker theory of aging is an extension of the oxidative stress theory of aging. It emphasizes variation in the fatty acid composition of membranes as an important influence on lipid peroxidation and consequently on the rate of aging and determination of lifespan (Hulbert et al., 2007). However, this asseveration is not valid to all tissues.

In this study we showed that both brain organelles from all birds examined presented similar C22:6n3 content, reinforcing previous evidences about a similar fatty acid composition in brain of homoeothermic vertebrates. We also demonstrated that the sensitivity to lipid peroxidation of brain microsomes and mitochondria was similar and did not correlate with body size of the different avian species studied. Several studies have evaluated the relationship between oxidative stress and MLSP in different vertebrate's species. Available research indicates that there are at least two main characteristics of longevous species: a high rate of DNA repair and a low rate of free radical production. In the present work, we found that the PI in brain lipids from the avian species analyzed did not correlate with body size and MLSP. Thus, our findings of a high degree of unsaturation totally independent of body mass and maximum life span, could explain the maintenance of structural and functional integrity of brain birds.

Acknowledgments

The early phases of this work were done at Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, UNLP. This work was supported by ANPCyT, PICT 13399, granted to AC. We thank Med Vet César Arcemis for the excellent technical assistance and Med Vet. Bernardo Iglesias for performing the statistical analysis.

References

Austad, S.N., 1997. Birds as models of aging in biomedical research. ILAR J. 38, 137–141.
Austad, S.N., 2001. Exceptional cellular resistance to oxidative damage in long-lived birds requires active gene expression. J. Gerontol. A Biol. Sci. Med. Sci. 56, B468–B474.
Barja, G., 1998. Mitochondrial free radical production and aging in mammals and birds. Ann. N. Y. Acad. Sci. 854, 224–238.
Barja, G., Cadenas, S., Rojas, C., Pérez-Campo, R., López-Torres, M., 1994. Low mitochondrial free radical production per unit O2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. Free Radic. Res. 21, 317–328.
Boveris, A., Costa, L.E., Cadenas, E., Poderoso, J.J., 1999. Regulation of mitochondrial respiration by adenosine diphosphate, oxygen, and nitric oxide. Methods Enzymol. 301, 188–198.
Cadenas, E., Varsavsky, A.I., Boveris, A., Chance, B., 1981. Oxygen- or organic hydroperoxide-induced chemiluminescence of brain and liver homogenates. Biochem. J. 198, 645–654.
Catalá, A., 2006. An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. Int. J. Biochem. Cell Biol. 38, 1482–1495.
Catalá, A., Arcemis, C., Cerruti, A., 1994. Interaction of rat liver microsomes containing saturated and unsaturated fatty acids with fatty acid binding protein: peroxidation effect. Mol. Cell. Biochem. 137, 135–139.
Couture, P., Hulbert, A.J., 1995. Membrane fatty acid composition of tissues is related to body mass of mammals. J. Membr. Biol. 148, 27–39.
Crawford, M.A., 2006. Docosahexaenoic acid in neural signaling systems. Nutr. Health 18, 263–276.
Farkas, T., Kitajka, K., Fodor, E., Csengeri, I., Lahdes, E., Yeo, Y.K., Krasznai, Z., Halver, J.E., 2000. Docosahexaenoic acid-containing phospholipid molecular species in brains of vertebrates. Proc. Natl. Acad. Sci. U. S. A. 97, 6362–6366.
Finch, C.A., 1990. Longevity, Senescence and the Genome. University of Chicago Press, Chicago IL.
Folch, J., Lees, N., Sloane Stanley, G.A., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Gutiérrez, A.M., Reboredo, G.R., Arcemis, C.J., Catalá, A., 2000. Non-enzimatic lipid peroxidation of microsomes and mitochondria isolated from liver and heart of pigeon and rat. Int. J. Biochem. Cell Biochem. 32, 73–79.
Gutiérrez, A.M., Reboredo, G.R., Catala, A., 2002. Fatty acid profiles and lipid peroxidation of microsomes and mitochondria from liver, heart and brain of Cairina moschata. Int. J. Biochem. Cell Biol. 34, 605–612.
Gutiérrez, A.M., Reboredo, G.R., Mosca, S.M., Catalá, A., 2004. Fatty acid composition and lipid peroxidation induced by ascorbate-Fe2+ in different organs of goose (*Anser anser*). Comp. Biochem. Physiol. C 137, 123–132.
Gutiérrez, A.M., Reboredo, G.R., Mosca, S.M., Catalá, A., 2006. A low degree of fatty acid unsaturation leads to high resistance to lipid peroxidation in mitochondria and microsomes of different organs of quail (*Coturnix coturnix japonica*). Mol. Cell. Biochem. 282, 109–115.
Holmes, D.J., Austad, S.N., 1995. Birds as animal-models for the comparative biology of aging—a prospectus. J. Gerontol., Ser. A 50, B59–B66.
Holmes, D.J., Fluckiger, R., Austad, S.N., 2001. Comparative biology of aging in birds: an update. Exp. Gerontol. 36, 869–883.
Holmes, D.J., Ottinger, M.A., 2003. Birds as long-lived animal models for the study of aging. Exp. Gerontol. 38, 1365–1375.
Hulbert, A.J., Rana, T., Couture, P., 2002a. The acyl composition of mammalian phospholipids: an allometric analysis. Comp. Biochem. Physiol., B 132, 515–527.
Hulbert, A.J., Faulks, S., Buttemer, W.A., Else, P.L., 2002b. Acyl composition of muscle membranes varies with body size in birds. J. Exp. Biol. 205, 3561–3569.
Hulbert, A.J., Pamplona, R., Buffenstein, R., Buttemer, W.A., 2007. Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87, 1175–1213.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
North, J.A., Spector, A.A., Buettner, G.R., 1994. Cell fatty acid composition affects free radical formation during lipid peroxidation. Am. J. Physiol. 269, R1213–R1224.
Ogburn, C.E., Carlberg, K., Ottinger, M.A., Holmes, D.J., Martín, G.M., Austad, S.N., 2001. Exceptional cellular resistance to oxidative damage in long-lived birds requires active gene expression. J. Gerontol., Ser. A, Biol. Sci. Med. Sci. 56, B468–B474.
Pamplona, R., Barja, G., 2007. Highly resistant macromolecular components and low rate of generation of endogenous damage: two key traits of longevity. Ageing Res. Rev. 6, 189–210.
Pamplona, R., Prat, J., Cadenas, S., Rojas, C., Pérez-Campo, R., López Torres, M., Barja, G., 1996. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and human case. Mech. Ageing Dev. 86, 53–66.
Pamplona, R., Portero-Otin, M., Riba, D., Ruiz, C., Prat, J., Bellmunt, M.J., 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., Requena, J.R., Thorpe, S.R., Herrero, A., 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. Ageing Dev. 106, 283–296.
Pamplona, R., Portero-Otin, M., Ledo, D.F., Gredilla, R., 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., Barja, G., Portero-Otín, M., 2002. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous-longevity adaptation? Ann. N. Y. Acad. Sci. 959, 475–490.
Roda, A., Pasini, P., Guardigli, M., Baraldini, M., Musiani, M., Mirasoli, M., 2000. Bio and chemiluminescence in bioanalysis. J. Anal. Chem. 366, 752–759.
Salem Jr., N., Litman, B., Kim, H.Y., Gawrisch, K., 2001. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 36, 945–959.
Tanger, O., Jonsson, J., Orrenius, S., 1973. Isolation of rat liver microsomes by gel filtration. Anal. Biochem. 54, 597–603.

Turner, N., Haga, K.L., Hulbert, A.J., Else, P.L., 2005a. Relationship between body size, Na+−K+−ATPase+activity, and membrane lipid composition in mammal and bird kidney. Am. J. Physiol., Regul. Integr. Comp. Physiol. 288, R301–R310.

Turner, N., Else, P.L., Hulbert, A.J., 2005b. An allometric comparison of microsomal membrane lipid composition and sodium pump molecular activity in the brain of mammals and birds. J. Exp. Biol. 208, 371–381.

U.S. Public Health Service, 1985. U.S. Public Health Service, Guide to the care and use of laboratory animals. National Institutes of Health, Bethesda, MD, pp. 85–123.

Wright, J.R., Rumbaugh, R.C., Colby, H.D., Miles, P.R., 1979. The relationship between chemiluminescence and lipid peroxidation in rat hepatic microsomes. Arch. Biochem. Biophys. 192, 344–351.

Yoshida, Y., Saito, Y., Hayakawa, M., Imai, Y., Niki, E., 2007. Levels of lipid peroxidation in human plasma and erythrocytes: comparison between fatty acids and cholesterol. Lipids 42, 439–449.