

# Non-enzymatic lipid peroxidation of microsomes and mitochondria from liver, heart and brain of the bird *Lonchura striata*: Relationship with fatty acid composition

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## Abstract

The aim of this study was to examine the fatty acid composition and non-enzymatic lipid peroxidation (LP) of mitochondria and microsomes obtained from liver, heart and brain of *Lonchura striata*. The percentage of total unsaturated fatty acid was approximately 30–60% in the organelles from all tissues studied. Brain mitochondria and both organelles of liver exhibited the highest percentage of polyunsaturated fatty acid (PUFA) (30 and 18%, respectively). The arachidonic acid (AA) content was 7% in mitochondria of liver and brain and 3% in heart mitochondria. The percentage of docosahexanoic acid (DHA) was 8% in brain mitochondria and approximately 2–3% in heart and liver mitochondria. The peroxidizability index (PI) of brain mitochondria and both organelles from liver was higher than that of organelles from heart and brain microsomes. Liver organelles and brain mitochondria were affected by LP, as indicated by the increase in chemiluminescence and a decrease of AA and DHA. These changes were not observed during LP of brain microsomes and both organelles from heart. These results indicate: 1) PI positively correlates with PUFA percentage and LP; 2) The resistance to LP detected in heart organelles would contribute to the cardiac protection against oxidative damage.

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**Keywords:** Birds; Fatty acids; Lipid peroxidation; Microsomes; Mitochondria

## 1. Introduction

Normal mitochondrial respiration produces small amounts of reactive oxygen species (ROS), which damage important biological molecules such as proteins, nucleic acids and lipids. These adverse effects can be minimized by the antioxidant defense system. The birds have higher metabolic rates, corporal temperature and blood glucose levels than mammals with similar body size (Finch, 1990; Barja et al., 1994; Holmes and Austad, 1995; Holmes and Ottinger, 2003), characteristics associated to premature aging (Holmes et al., 2001). However,

the vast majority of birds are long-lived animals. Experimental data are consistent with the idea that birds have better resistance to oxidative damage, produce fewer reactive oxygen species (ROS) and possess an enhanced antioxidant activity in mitochondrial membranes compared with mammals (Austad, 1997; Barja, 1998; Ogburn et al., 2001). Unsaturated fatty acids are more susceptible to ROS-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).

Previous works of Pamplona et al. (1996, 1999) and recent investigations of our laboratory using 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, as far as we know not much information about the lipid peroxidation of membranes of passerine birds do exist. On

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the other hand, it is also recognized that species with high mass-specific basal metabolic rate (BMR) have highly polyunsaturated membrane and species with low BMR have membranes that are less polyunsaturated (Hulbert, 2005).

The goal of this study was to analyze the fatty acid composition and sensitivity to lipid peroxidation of the “Manon” (*Lonchura striata* var. *domestica*), a small bird with higher BMR than other species studied, belonging to Order Passeriformes.

## 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. Animals

Adult male Manon (*L. striata* var. *domestica*) were obtained from the farm (Agro-80 La Plata). The body mass of the birds was  $13.71 \pm 0.41$  g. The birds were fed on commercial chow (mixed bird seed). Animals were sacrificed by decapitation and the organs were rapidly removed. The tissues of 3 birds per group were bulked. Replicate determinations were made on the fatty acid composition (three in each case; see tables) of each tissue from each bird.

### 2.3. Preparation of microsomes and mitochondria

Liver, heart and brain were cut into small pieces and washed extensively with 0.15 M NaCl. A 30% homogenate (w/v) of each tissue was prepared in a solution containing sucrose 0.25 M, 10 mM Tris–HCl (pH 7.4), PMSF 1 mM, using a Potter–Elvehjem homogenizer (Cole Palmer, Vernon Hills, IL, USA). The homogenate was centrifuged at 10,000 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<sub>3</sub>. 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). Mitochondria were obtained by the method described by Boveris et al. (1999).

### 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 over a 120-min period with 0.05 M phosphate (pH 7.4), 0.4 mM ascorbic acid, final volume 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 lipids from native, control and peroxidized samples 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<sub>3</sub>B 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, Folsom, 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 BSA as a 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 double bond index was calculated as UI = Σ(fatty acid percent) × (number of double bonds). The peroxidizability index was calculated as PI [(% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% hexaenoic × 8)]. Ratio 20:4/18:2 represents the arachidonic acid/linoleic acid ratio and expresses the activity coefficient of enzymes in the biosynthetic pathway of arachidonic acid from linoleic acid. Data were evaluated statistically by one-way analysis of

Table 1  
Fatty acid composition (area %) of dietary lipids

Fatty acid	Diet
16:0	12.84±0.64
16:1n7	0.71±0.43
18:0	18.47±0.27
18:1n9	37.06±0.56
18:2n6	29.92±0.31
18:3n3	0.82±0.06
Saturated	31.31±0.51
Total unsaturated	68.52±0.66
Monounsaturated	37.78±0.99
Polyunsaturated	30.74±0.36
UI	100.09±0.36

Values are means±SD from three analyses.

variance (ANOVA) and Student's *t*-test. Data were expressed as mean±SD. The terms "Native", "control" and "peroxidized" are used to indicate intact organelles (not incubated), incubated with prooxidant and incubated without prooxidant, respectively.

### 3. Results

#### 3.1. Total fatty acid composition of mitochondria and microsomes obtained from liver, heart and brain

Palmitic (C16:0) and stearic (C18:0) were the saturated fatty acids most abundant of total lipids in mitochondria and microsomes from liver, heart and brain (Tables 2–4).

The percentage of total unsaturated fatty acid was approximately 30–60% in liver, heart and brain mitochondria

and microsomes. In both organelles the predominant mono-unsaturated fatty acid was oleic acid. The percentage of polyunsaturated fatty acid was approximately 20% in liver and heart mitochondria and 30% in brain mitochondria. The polyunsaturated fatty content was 18%, 5% and 10% in microsomes of liver, heart and brain, respectively. The percentage of arachidonic acid (C20:4n6) was 7% in mitochondria of liver and brain and 3% in heart mitochondria. The docosahexanoic acid (C22:6n3) percentage was 8% in brain mitochondria and approximately 2–3% in heart and liver mitochondria. Liver and heart mitochondria had a similar UI whereas brain mitochondria had a UI that was 1.5 times higher than in heart (Tables 2–4). The UI of liver microsomes was 2 times higher than that of heart and brain microsomes. The peroxidizability index (PI) of brain mitochondria was 2 times higher than that of liver mitochondria and 3 times higher than that of heart mitochondria. The PI of liver microsomes was 3 and 1.4 times higher than that of heart and brain microsomes, respectively.

#### 3.2. Comparison of fatty acid composition of organelles with the diet

The high percentage of C18:1n9 and C18:2n6 fatty acids and in minor proportion the C18:3n3 fatty acids were responsible of the high UI of diet. Despite of high UI of diet the organelles from liver, heart and brain showed a significantly lower percentage of unsaturated fatty acids.

Since C20:4n6 and C22:6n3 are not present in the diet, it must have been synthesized through a series of elongation and desaturation steps from its dietary precursors, C18:2n6 and C18:3n3, respectively (Fig. 1, Tables 1–4).

Table 2  
Fatty acid composition (area %) of total lipids from liver mitochondria and microsomes of *Lonchura striata*

Fatty acid	Mitochondria			Microsomes		
	Native	Control	Peroxidized	Native	Control	Peroxidized
16:0	25.06±0.07	24.96±0.62	26.07±0.21	36.84±4.56	33.92±3.50	38.25±1.52
16:1n7	1.42±0.32	1.60±0.04	1.49±0.31	2.86±0.29	3.56±0.28	2.78±0.48
18:0	27.57±1.68	29.95±2.18	29.68±0.95	20.78±6.30	27.23±4.02	24.94±4.13
18:1n9	22.98±0.97	22.32±1.49	21.46±1.33	29.14±3.70	23.36±2.83	24.65±1.73
18:2n6	7.28±0.57	7.26±0.26	7.73±1.34	7.74±0.10	5.41±0.31	5.19±0.18
18:3n3	0.32±0.02	0.30±0.03	0.35±0.02	1.13±0.23	1.30±0.10	0.96±0.17
20:4n6	7.78±0.50	7.02±0.63	4.23±0.14**	5.22±0.46	4.48±0.21	1.12±0.45**
22:6n3	3.14±0.24	3.32±0.21	2.27±0.09**	4.13±0.56	4.36±0.28	1.38±0.06***
Saturated	52.63±1.61	54.91±1.57	55.75±1.09	57.63±10.71	61.15±7.37	63.19±3.32
Monounsaturated	24.40±1.08	23.92±1.46	22.95±1.26	31.99±3.61	26.92±2.91	27.44±2.06
Polyunsaturated	18.52±1.13	17.90±0.93	14.59±1.41*	18.22±1.19	15.55±0.55	8.65±0.68**
Total unsaturated	42.92±2.11	41.82±1.81	37.54±0.52*	50.21±3.07	42.47±2.36	36.09±2.73**
Sat/unsaturated	1.23±0.09	1.32±0.07	1.49±0.05*	1.16±0.27	1.45±0.24	1.79±0.22
Polyunsaturated n-3	3.46±0.43	3.61±0.29	2.62±0.12	5.26±0.97	5.66±0.22	2.34±0.24***
Polyunsaturated n-6	15.06±1.08	14.28±1.02	11.96±1.81	12.96±0.50	9.89±0.45	6.31±0.74***
UI	89.87±5.06	87.36±3.72	70.02±1.97**	96.51±4.59	85.73±0.64	53.44±4.21***
PI	64.75±6.79	63.12±5.06	44.30±1.19**	64.79±10.51	61.97±2.77	23.21±1.58***
20:4/18:2	1.07±0.04	0.96±0.09	0.55±0.09**	0.68±0.04	0.83±0.07	0.21±0.11***
22:6/18:3	9.72±0.84	11.29±1.17	6.42±0.19**	3.70±0.40	3.39±0.54	1.42±0.54***

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

Table 3  
Fatty acid composition (area %) of total lipids from heart mitochondria and microsomes of *Lonchura striata*

Fatty acid	Mitochondria			Microsomes		
	Native	Control	Peroxidized	Native	Control	Peroxidized
16:0	30.23±2.16	29.36±1.67	28.76±0.80	39.53±1.70	41.51±1.23	40.86±3.12
16:1n7	2.31±0.07	2.28±0.56	2.46±0.14	6.73±0.45	6.44±0.42	4.84±0.38
18:0	25.57±0.27	25.49±0.13	23.45±1.51	30.39±2.10	29.68±2.27	28.24±3.23
18:1n9	26.54±3.00	28.78±0.72	27.57±1.40	19.92±2.15	22.77±0.70	23.04±3.34
18:2n6	11.52±0.86	11.56±1.23	10.95±0.23	2.49±0.45	2.03±0.08	1.93±0.31
18:3n3	0.45±0.08	0.51±0.08	0.46±0.06	–	–	–
20:4n6	2.83±0.08	2.69±0.07	2.83±0.10	1.20±0.14	1.44±0.16	1.42±0.26
22:6n3	2.05±0.12	2.37±0.13	2.22±0.18	1.41±0.14	1.41±0.15	1.42±0.15
Saturated	55.80±2.04	54.85±1.57	52.21±2.25	69.91±0.98	71.18±3.18	69.10±3.71
Monounsaturated	28.85±2.96	31.06±0.56	30.03±1.49	26.65±2.20	29.21±1.11	27.88±3.19
Polyunsaturated	16.86±1.12	17.12±1.35	16.45±0.32	5.09±0.68	4.88±0.32	4.76±0.72
Total unsaturated	45.71±3.66	48.18±1.58	46.48±1.78	31.74±2.85	34.09±1.42	32.64±2.66
Sat/unsaturated	1.23±0.14	1.14±0.01	1.12±0.01	1.40±0.17	1.40±0.18	1.41±0.17
Polyunsaturated n-3	2.50±0.25	3.12±0.14	2.68±0.25	3.68±0.72	3.47±0.29	3.34±0.70
Polyunsaturated n-6	14.35±1.14	14.25±1.58	13.78±0.21	2.22±0.22	2.09±0.09	2.14±0.30
UI	76.89±5.05	80.65±3.43	77.93±2.94	44.85±4.25	47.47±2.48	45.91±1.97
PI	40.91±3.11	40.02±3.76	41.68±2.83	19.20±3.27	19.89±3.03	19.50±4.37
20:4/18:2	0.25±0.01	0.23±0.03	0.25±0.01	0.48±0.05	0.70±0.06	0.73±0.02
22:6/18:3	4.66±0.79	4.82±1.23	4.92±0.75	–	–	–

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

### 3.3. Total fatty acid profiles and chemiluminescence of mitochondria and microsomes obtained from liver, heart and brain

Light emission: chemiluminescence originated from brain microsomes and organelles from heart was not statistically significant when control and peroxidized samples were compared (Fig. 2). The fatty acid profiles, UI and PI of heart mitochondria and brain and heart microsomes were not modified after lipid peroxidation (Tables 3 and 4).

Light emission was statistically significant in brain mitochondria and in organelles of liver when control and peroxidized samples were compared (Fig. 2). Over the time course studies of brain mitochondria and liver organelles, analysis of chemiluminescence demonstrated that lipid peroxidation is rapid and reaches a maximum at approximately 40–60 min (Fig. 2, inset). The values of light emission of brain and liver mitochondria were approximately 3 times higher and liver microsomes 1.7 times higher when compared with control samples. A significant decrease of C20:4n6, C22:6n3, UI and

Table 4  
Fatty acid composition (area %) of total lipids from brain mitochondria and microsomes of *Lonchura striata*

Fatty acid	Mitochondria			Microsomes		
	Native	Control	Peroxidized	Native	Control	Peroxidized
16:0	20.80±0.06	19.99±0.21	21.65±0.46	31.24±7.32	34.71±2.51	35.21±0.95
16:1n7	2.24±0.17	2.02±0.36	2.83±0.58	1.62±0.27	2.23±0.30	1.99±0.18
18:0	18.68±0.17	19.86±0.39	20.27±0.16	29.45±1.09	38.33±3.58	34.87±4.47
18:1n9	28.66±0.20	29.33±0.38	27.73±0.69	20.90±0.86	18.82±1.46	19.01±1.11
18:2n6	13.60±1.31	12.49±0.69	13.00±1.78	3.12±0.13	2.97±0.25	3.99±0.29
18:3n3	0.77±0.06	0.73±0.04	0.78±0.06	0.71±0.14	0.76±0.16	0.59±0.14
20:4n6	6.68±0.13	6.52±0.23	2.10±0.56***	2.28±0.35	2.40±0.58	1.98±0.22
22:6n3	8.33±0.23	7.84±0.58	2.53±0.33***	3.67±0.18	3.70±0.17	3.83±0.19
Saturated	39.49±0.13	39.85±0.60	41.92±0.38	60.69±6.23	73.03±6.04	70.08±3.68
Monounsaturated	30.90±0.26	31.35±0.39	30.56±0.60	22.52±1.08	21.04±1.20	21.00±1.28
Polyunsaturated	29.38±1.29	27.59±1.07	18.40±1.84**	9.79±0.69	9.84±0.47	10.40±0.51
Total unsaturated	60.29±1.21	58.94±0.72	48.96±2.36**	32.31±1.20	30.88±1.23	31.40±1.51
Sat/unsaturated	0.66±0.01	0.68±0.02	0.86±0.05**	1.89±0.25	2.37±0.24	2.24±0.14
Polyunsaturated n-3	9.10±0.11	8.58±0.70	3.30±0.38***	5.81±3.28	4.47±0.15	4.61±0.36
Polyunsaturated n-6	20.28±1.45	19.01±0.63	15.09±1.93	5.41±0.55	5.37±0.63	5.97±0.36
UI	137.13±2.82	131.67±3.87	82.44±4.95***	62.07±3.02	61.10±2.24	61.69±3.10
PI	114.62±10.84	103.56±5.94	43.92±5.52***	46.61±1.54	44.25±3.15	43.76±2.90
20:4/18:2	0.50±0.08	0.52±0.05	0.17±0.04**	0.72±0.01	0.82±0.38	0.47±0.16
22:6/18:3	10.82±0.87	10.74±1.42	3.27±0.65***	5.37±1.25	5.14±1.73	7.18±1.99

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

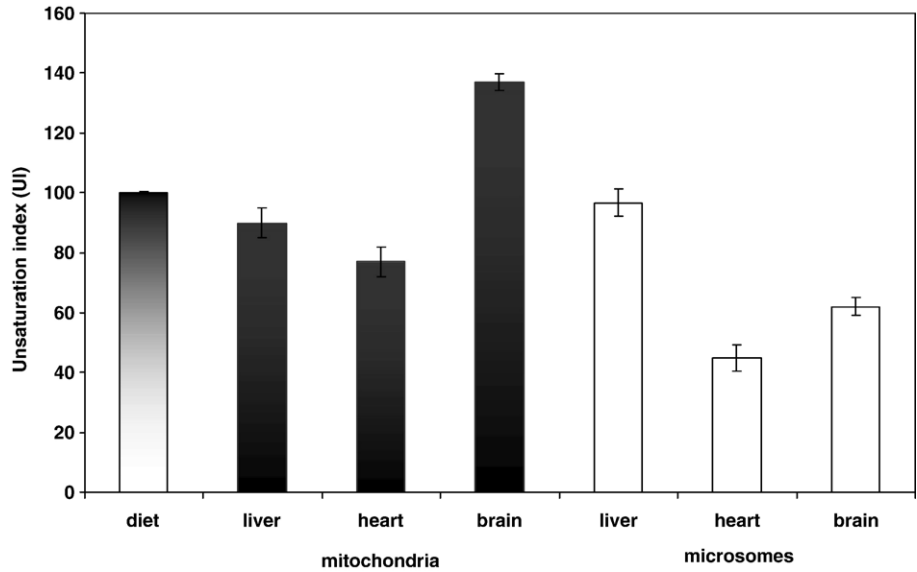


Fig. 1. Unsaturation index (UI) calculated from fatty acids present in the diet, liver, heart and brain mitochondria and microsomes of *Lonchura striata*. The UI was calculated as the sum of the percentage of each unsaturated fatty acid multiplied by its number of double bonds.

PI in brain mitochondria and liver organelles was observed when compared with control (Tables 2 and 4).

**4. Discussion**

In the present study we found that saturated fatty acids of organelles isolated from heart and brain of *L. striata* was slightly higher when compared with the same organelles of other birds studied in our laboratory, such as duck (Gutiérrez et al., 2002) and goose (Gutiérrez et al., 2004). In other words, and

in agreement with recent investigations (Turner et al., 2005), these results are suggesting that the decrease of body size leads to an increase in the total saturated fatty acid content. The percentage of total unsaturated and polyunsaturated fatty acid was different in the organelles of the organs studied. The C18:1n9 was the predominant unsaturated fatty acid in the Manon and high values were also detected in pigeon (Pamplona et al., 1996; Gutiérrez et al., 2000), canary and parakeet (Pamplona et al., 1999), duck, goose and quail (Gutiérrez et al., 2002, 2004, 2006).

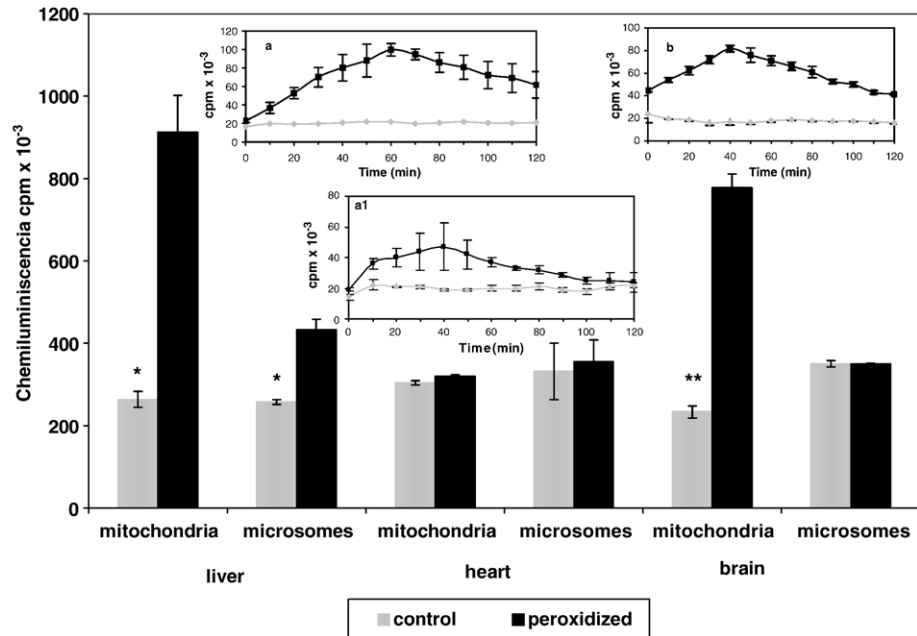


Fig. 2. Total chemiluminescence produced by liver, heart and brain mitochondria and microsomes expressed as cpm/mg protein. Results are expressed as mean±SD of three independent experiments. Statically significant differences between control and peroxidized samples are indicated by \**p*<0.001, \*\**p*<0.0001. Inset: Chemiluminescence as a function of time during lipid peroxidation. a: liver mitochondria; a1: liver microsomes; b: brain mitochondria.

Brain mitochondria from Manon possess a higher content of C20:4n6 and C22:6n3 compared to brain microsomes and liver and heart organelles. Although there are small differences in fatty acid composition between the vertebrates, 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 (Else and Wu, 1999; Farkas et al., 2000; Turner et al., 2005; Gutiérrez et al., 2002, 2004, 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).

The simultaneous study of fatty acid profile in the diet, liver, heart and brain mitochondria and microsomes from Manon demonstrates that the fatty acid composition of the diet is not responsible for the differences observed in the double bond content of the organelles. These differences can be correlated to the homeostatic regulation of each organ principally due to a genetic control of delta-5/-6 desaturases (Pamplona et al., 2002, 1999).

Free radicals are molecules capable of independent existence that contain one or more unpaired electrons (Halliwell and Gutteridge, 1999). An important free radical in biological systems is the superoxide radical,  $O_2^-$ , which is produced as a by-product of normal mitochondrial respiration. Reactive oxygen species (ROS) is the collective term used to describe both radical and non-radical derivatives of oxygen.

Once produced, ROS will oxidatively damage proteins, nucleic acids and lipids. Many of the products of lipid peroxidation are very reactive molecules themselves and are thus very potent damagers of other molecules.

Non-enzymatic lipid peroxidation and formation of lipid peroxides can be initiated by adding ascorbate in the presence of oxygen and either  $Fe^{+3}$  or  $Fe^{+2}$  ions to various tissue preparations such as homogenates, liver mitochondria, hepatic microsomal suspensions and nuclei (Rosa and Catala, 1998; Catalá and Cerruti, 1997).

Lipid peroxidation proceeds by a chain reaction that includes initiation, propagation and termination. Initiation occurs when an oxidant gives rise to an initiating lipid peroxyl radical (LOO.) by reaction with either a lipid (LH) or pre-existing lipid hydroperoxide (LOOH). Propagation is cycled through rounds of LOO. abstraction of the bis-methylene hydrogen atoms of a polyunsaturated fatty acyl chain to generate additional LOO. (after  $O_2$  addition) which results in the net conversion of lipids to LOOHs. Lipid peroxidation termination involves the reaction of LOO. to form non-radical products or the reaction of one LOO. with another terminating radical to generate non-propagating radical species (Dix and Aikens, 1993); the first reaction is particularly interesting as it is accompanied by emission of chemiluminescence (Vladimirov et al., 1980). The requirement of iron in lipid peroxidation has been demonstrated by Minotti and Aust (1992). Thus measurement of lipid peroxidation is one of the most commonly used assays for radical-induced damage (Dmitriev, 2001; Hsieh and Kinsella, 1989).

Individual acyl chains differ greatly in their chemical propensity for oxidative damage (Holman, 1954). The n-3 PUFA are more peroxidation-prone than n-6 PUFA and within each PUFA class there is a 4-fold increase in peroxidizability between the short- and long-chain fats.

C22:6n3 is 320-fold more susceptible to peroxidation than 18:1n9. In the Manon, brain mitochondria and both organelles of liver were susceptible to lipid peroxidation. This high sensitivity can be attributed to the higher 20:4/18:2 and 22:6/18:3 ratios and higher values of PI of those organelles compared to heart organelles. Heart organelles showed a low sensitivity to lipid peroxidation which can be attributed to a low degree of unsaturation and to other factors. This behaviour of heart organelles of Manon was also detected in non-passerine bird species studied in our laboratory (Gutiérrez et al., 2002, 2004, 2006). With respect to microsomes, only the liver microsomes were affected by the process of lipid peroxidation, showing a higher polyunsaturated fatty acid content compared to heart and brain microsomes. Another trait of long-lived mammals and birds is the possession of low degrees of unsaturation in their cellular membranes. This is mainly due to minimizing the presence of highly unsaturated fatty acids such as 22:6n-3 and emphasizing the presence of less unsaturated fatty acids such as 18:2n-6 in long-lived animals, without changing the total amount of polyunsaturated fatty acids. This leads to lower levels of lipid peroxidation and lipoxidation-derived protein modification in long-lived species (Sanz et al., 2006).

The present study describes for the first time the fatty acid composition and sensitivity to lipid peroxidation of mitochondria and microsomes obtained from liver, heart and brain of Manon (*L. striata*). Brain mitochondria and liver organelles were the most susceptible to the process of lipid peroxidation and exhibited the highest values of PI, which showed a positive correlation with the level of long-chain polyunsaturated fatty acid. Another interesting finding in this study is that heart organelles exhibited less sensitivity to in vitro lipid peroxidation and showed lower values of PI than organelles isolated from liver and brain. This fact contributes to the protection of that tissue against oxidative damage and consequently to the preservation of cardiac function. These results together with those previously obtained in other bird species suggest that a low degree of fatty acid unsaturation is a general characteristic of birds with high metabolic rates. This constitutive trait helps to protect their tissues and mitochondria against lipid peroxidation and oxidative protein modification and can be a factor contributing to their slow rate of aging.

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