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Fatty acid profiles and lipid peroxidation of microsomes and mitochondria from liver, heart and brain of *Cairina moschata*

Ana M. Gutiérrez^b, Guillermo R. Reboredo^b, Angel Catalá^{a,*},¹

^a Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CC 296, B1900 AVW, La Plata, Argentina

^b Cátedra de Fisiología Animal, Facultad de Cs. Naturales y Museo, La Plata, Argentina

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Abstract

Studies were done to analyze the fatty acid composition and sensitivity to lipid peroxidation (LP) of mitochondria and microsomes from duck liver, heart and brain. The fatty acid composition of mitochondria and microsomes was tissue-dependent. In particular, arachidonic acid comprised 17.39 ± 2.32 , 11.75 ± 3.25 and $9.70 \pm 0.40\%$ of the total fatty acids in heart, liver and brain mitochondria respectively but only 13.39 ± 1.31 , 8.22 ± 2.43 and $6.44 \pm 0.22\%$ of the total fatty acids in heart, liver and brain microsomes, respectively. Docosahexaenoic acid comprised 17.02 ± 0.78 , 4.47 ± 1.02 and $0.89 \pm 0.07\%$ of the total fatty acids in brain, liver and heart mitochondria respectively but only 7.76 ± 0.53 , 3.27 ± 0.73 and $1.97 \pm 0.38\%$ of the total fatty acids in brain, liver and heart microsomes. Incubation of organelles with ascorbate- Fe^{2+} at 37°C caused a stimulation of LP as indicated by the increase in light emission: chemiluminescence (CL) and the decrease of arachidonic acid to: 5.17 ± 1.34 , 8.86 ± 0.71 and $5.86 \pm 0.68\%$ of the total fatty acids in heart, liver and brain mitochondria, respectively, and to 4.10 ± 0.61 in liver microsomes. After LP docosahexaenoic acid decrease to 7.29 ± 1.47 , 1.36 ± 0.18 and $0.30 \pm 0.11\%$ of the total fatty acids in brain, liver and heart mitochondria. Statistically significant differences in the percent of both peroxidable fatty acids (arachidonic and docosahexaenoic acid) were not observed in heart and brain microsomes and this was coincident with absence of stimulation of LP. The results indicate a close relationship between tissue sensitivity to LP in vitro and long chain polyunsaturated fatty acid concentration. Nevertheless, any oxidative stress in vitro caused by ascorbate- Fe^{2+} at 37°C seems to avoid degradation of arachidonic and docosahexaenoic acids in duck liver and brain microsomes. It is possible that because of the important physiological functions of arachidonic and docosahexaenoic acids in these tissues, they are protected to maintain membrane content during oxidative stress. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Lipid peroxidation; Microsomes; Mitochondria; Fatty acids; Ducks

1. Introduction

The amount of fatty acids and the level of antioxidants found in biological membranes are different

between species and tissues of the same species. This variation of peroxidable long chain fatty acids and antioxidants found in membranes made them more or less vulnerable to lipid peroxidation (LP). The polyunsaturated fatty acids located in mitochondrial membranes are excellent targets for peroxidation. Conversely, it is known that the lipid environment can affect membrane function [1], including mitochondrial electron transport, which could influence reactive oxygen species production. Microsomes as

* Corresponding author. Fax: +54-221-5257980.

E-mail address: acatala@fcv.medvet.unlp.edu.ar (A. Catalá).

¹ Angel Catalá is Member of Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

well as mitochondria obtained from several mammals [2–5] and different bird species [6–8] are susceptible to lipid-peroxidation. The measurement of LP is one of the most commonly used assays for radical induced damage [9,10]. Non enzymatic LP and formation of lipid peroxides can be initiated by adding ascorbate in the presence of oxygen and Fe^{3+} or Fe^{2+} ions to various tissue preparations such as homogenates, mitochondria, microsomes and nuclei obtained from various tissues and species [5,11]. Recent data showed that pigeon mitochondria produce oxygen radicals at a rate much slower than rat mitochondria, in spite of showing similar levels of oxygen consumption [1,6]. The production of reactive oxygen species by mitochondria was lower in pigeon than in rat mitochondria in the three tissues (two times lower in liver an lung and four times lower in the brain), the difference was maximal in the brain, an organ of special importance in the aging process [8]. In a previous study, it has been shown that the heart lipids of canaries and parakeets have a lower fatty acid double bond content than those of mice [12] and the same occurs in liver mitochondria of pigeons in relation to those of rats [1]. The aim of the present study is to examine the fatty acid profiles and non-enzymatic LP of mitochondria and microsomes obtained from liver, heart and brain of duck (*Cairina moschata*).

2. Material and methods

2.1. Animals

Young adult (1-year-old) female ducks (*C. moschata*) were obtained from farm. The birds were acclimatized at the laboratory for 1 week at 20 °C. The ducks were fed on commercial chow. The diet contained 3.97% of total lipid with a fatty acid composition of 14.79% palmitic acid C16:0, 2.01% stearic acid C18:0, 26.59% oleic acid C18:1 n9, 53.59% linoleic acid C18:2 n6 and 1.12% linolenic acid C18:3 n3. Animals were sacrificed by decapitation and the organs were rapidly removed.

2.2. 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) was prepared in a solution containing sucrose 0.25 M, 10 mM Tris-HCl (pH 7.4) PMFS 0.001 M, using a potter-Elvehjem homogenizer. The homogenate was centrifuged at $10,000 \times g$ for 10 min. The supernatant (3 ml) obtained was applied to a Sepharose 4 B column (1.6 cm \times 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 [13]. Mitochondria were obtained by the method described by [14].

2.3. Chemiluminescence and lipid peroxidation of microsomes and mitochondria

LP of microsomes and mitochondria, were measured as described previously [4] with the following modifications; organelles at a concentration 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 2 ml. Phosphate buffer is contaminated with sufficient iron to provide the necessary ferrous or ferric iron for LP (final concentration in the incubation mixture was 2.15 μM). Control organelle preparations that lacked ascorbate were carried out simultaneously. Chemiluminescence (CL) and LP were initiated by adding a small amount of stock solution of ascorbate to each vial that was maintained at 37 °C in a water bath during 5–10 min prior to starting the experiment. CL was measured as counts per minute (cpm) in Packard 1900 TR equipment with a program for chemiluminescence. Measurements of lipid-peroxidation induced by ascorbate were made on the same samples from which CL was determined by measuring the fatty acid composition of organelles peroxidized with or without ascorbic acid at the indicated times.

2.4. Fatty acid analysis

Lipids from samples peroxidized in the presence or in the absence of ascorbic acid were extracted with chloroform/methanol (2:1, v/v) [15]. The fatty acids from total lipids were transmethylated with 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 DB-225 capillary column (30 m × 0.32 mm i.d., J. and V. Scientific, Folsom, CA, USA.). Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 250 °C, the column temperature were held at 90 °C for 1 min 90–180 °C at 15 °C/min, 180–200 °C at 3 °C/min, 200–220 °C at 3 min, 220 °C for 7 min. Fatty acid methyl esters peaks were identified by comparing retention times to those of standards. Standards of fatty acids methyl esters were from Nu Chek Prep Inc., Elysian, MN, USA.

2.5. Unsaturation index

Was calculated according to the formula, UI = sum (fatty acid percent) × (number of double bonds) [16].

2.6. Other methods

Proteins were determined by the method of [17].

2.7. Statistical analysis

The data were subjected to the Student's *t*-test. Data were expressed as mean ± S.D. Statistical criterion

for significance was selected at different *P*-values and indicated in each case.

3. Results

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

Mitochondria and microsomes from duck liver, heart and brain contain long chain fatty acids, approximately 50% are unsaturated with a prevalence of oleic acid C18:1 n9. In liver and heart the content of the polyunsaturated long chain fatty acids decrease in the order C20:4 n6 > C18:2 n6 > C 22:6 n3. The saturated long chain fatty acids were mainly C16:0 and C18:0. (Tables 1–6).

3.2. Fatty acid profiles and chemiluminescence of mitochondria and microsomes obtained from duck liver

The fatty acid composition of the total native lipids isolated from liver mitochondria and microsomes was different. The rate 18:2/20:4 was 1.3 times higher in microsomes than in mitochondria and the rate 20:4/22:6 was 1.0 times higher in

Table 1
Fatty acid composition of total lipids from liver mitochondria of duck, *C. moschata*

Fatty acid	Native	Control	Peroxidized
C16:0	24.22 ± 2.94	26.25 ± 4.44	32.32 ± 1.31
C16:1 n7	1.30 ± 0.49	1.43 ± 0.95	1.41 ± 0.44
C18:0	12.72 ± 1.10	17.36 ± 2.79	18.94 ± 2.39
C18:1 n9	22.48 ± 2.86	21.45 ± 2.91	26.00 ± 3.32
C18:2 n6	6.91 ± 1.15	6.72 ± 1.28	6.36 ± 1.48
C18:3 n3	–	–	–
C20:4 n6	11.75 ± 3.25	13.46 ± 1.40	8.86 ± 0.71*
C22:6 n3	4.47 ± 1.02	3.92 ± 1.41	1.36 ± 0.18*
Saturated	36.94 ± 2.60	43.61 ± 1.75	51.26 ± 1.14
Monounsaturated	23.78 ± 3.06	22.55 ± 3.17	27.41 ± 3.37
Polyunsaturated	23.12 ± 4.12	24.09 ± 2.78	16.58 ± 1.65*
Total unsaturated	46.91 ± 2.59	46.64 ± 2.05	43.98 ± 1.72
Saturated/unsaturated	0.70 ± 0.13	0.94 ± 0.04	1.17 ± 0.06**
UI ^a	111.43 ± 14.04	113.36 ± 11.29	83.71 ± 0.70*

Data are given as the mean ± S.D. of three independent experiments. Statistically significant differences between control and peroxidized groups are indicated by **P* < 0.05, ***P* < 0.001.

^a UI = sum of the percentages of each fatty acid × number of double bonds.

Table 2

Fatty acid composition of total lipids from liver microsomes of duck, *C. moschata*

Fatty acid	Native	Control	Peroxidized
C16:0	29.39 ± 0.93	29.17 ± 0.99	39.86 ± 0.95
C16:1 n7	3.90 ± 0.65	1.63 ± 0.28	–
C18:0	13.82 ± 2.39	14.59 ± 2.16	14.37 ± 3.50
C18:1 n9	31.27 ± 3.04	29.31 ± 4.14	32.01 ± 4.00
C18:2 n6	6.45 ± 0.40	5.42 ± 0.91	3.98 ± 0.87
C18:3 n3	–	0.39 ± 0.35	0.20 ± 0.06
C20:4 n6	8.22 ± 2.43	9.76 ± 2.58	4.10 ± 0.61*
C22:6 n3	3.27 ± 0.73	3.49 ± 1.03	0.89 ± 0.48*
Saturated	43.22 ± 1.95	43.74 ± 1.16	54.23 ± 4.45
Monounsaturated	35.18 ± 3.48	30.94 ± 4.33	32.66 ± 4.32
Polyunsaturated	17.28 ± 2.79	19.09 ± 3.18	9.41 ± 1.11*
Total unsaturated	53.13 ± 1.10	50.04 ± 1.20	42.10 ± 3.18*
Saturated/unsaturated	0.81 ± 0.05	0.86 ± 0.03	1.28 ± 0.18*
UI ^a	101.00 ± 7.80	102.86 ± 10.14	62.57 ± 2.44**

Data are given as the mean ± S.D. of three independent experiments. Statistically significant differences between control and peroxidized groups are indicated by * $P < 0.05$, ** $P < 0.001$.

^a UI = sum of the percentages of each fatty acid × number of double bonds.

mitochondria than in microsomes (Tables 1 and 2) when both types of organelles were compared. Light emission (CL) was 4.8 and 2.3 times higher in microsomes than in mitochondria when subjected to LP, Fig. 1. (Tables 1 and 2) shows the fatty acid composition of total lipids of control and peroxi-

dized mitochondria and microsomes. A significant decrease of C20:4 n6 and C22:6 n3 in liver mitochondria and microsomes was observed when compared with control organelles. The unsaturation index of peroxidized liver microsomes was lower than in mitochondria.

Table 3

Fatty acid composition of total lipids from heart mitochondria of duck, *C. moschata*

Fatty acid	Native	Control	Peroxidized
C16:0	20.58 ± 0.66	22.14 ± 1.18	28.06 ± 0.71
C16:1 n7	3.05 ± 0.77	1.52 ± 0.47	2.41 ± 1.06
C18:0	11.15 ± 0.43	11.96 ± 0.58	8.15 ± 0.99
C18:1 n9	28.20 ± 4.06	31.23 ± 3.00	37.45 ± 2.36
C18:2 n6	15.01 ± 0.83	13.51 ± 3.06	9.71 ± 1.83
C18:3 n3	0.51 ± 0.24	0.11 ± 0.03	0.12 ± 0.03
C20:4 n6	17.39 ± 2.32	18.10 ± 0.94	5.17 ± 1.34***
C22:6 n3	0.89 ± 0.07	0.83 ± 0.07	0.30 ± 0.11**
Saturated	31.75 ± 0.81	34.10 ± 1.47	36.22 ± 1.70
Monounsaturated	31.25 ± 4.49	32.76 ± 3.61	39.87 ± 2.85
Polyunsaturated	33.82 ± 2.61	32.56 ± 4.05	15.32 ± 2.47**
Total unsaturated	65.07 ± 2.13	65.32 ± 5.69	55.18 ± 3.96
Saturated/unsaturated	0.48 ± 0.02	0.52 ± 0.06	0.68 ± 0.06
UI ^a	137.72 ± 5.69	137.50 ± 11.21	82.21 ± 7.30**

Data are given as the mean ± S.D. of three independent experiments. Statistically significant differences between control and peroxidized groups are indicated by ** $P < 0.01$, *** $P < 0.001$.

^a UI = sum of the percentages of each fatty acid × number of double bonds.

Table 4
Fatty acid composition of total lipids from heart microsomes of duck, *C. moschata*

Fatty acid	Native	Control	Peroxidized
C16:0	29.84 ± 2.26	30.65 ± 1.75	30.21 ± 2.46
C16:1 n7	4.66 ± 0.47	3.46 ± 0.23	3.41 ± 0.37
C18:0	13.52 ± 0.79	12.88 ± 0.84	8.41 ± 1.11
C18:1 n9	26.71 ± 2.18	24.95 ± 0.70	24.65 ± 0.61
C18:2 n6	7.76 ± 1.40	8.11 ± 1.40	8.35 ± 1.04
C18:3 n3	0.95 ± 0.10	–	–
C20:4 n6	13.39 ± 1.31	12.97 ± 1.59	13.89 ± 0.67
C22:6 n3	1.97 ± 0.38	1.68 ± 0.40	2.37 ± 0.20
Saturated	43.36 ± 1.60	43.53 ± 1.48	39.29 ± 2.52
Monounsaturated	30.71 ± 2.05	28.42 ± 0.47	28.07 ± 0.26
Polyunsaturated	24.08 ± 2.27	22.79 ± 2.49	24.58 ± 1.52
Total unsaturated	54.79 ± 0.48	51.21 ± 2.52	52.65 ± 1.46
Saturated/unsaturated	0.79 ± 0.03	0.84 ± 0.07	0.74 ± 0.05
UI ^a	114.93 ± 3.92	106.65 ± 6.36	114.39 ± 3.69

Data are given as the mean ± S.D. of three independent experiments.

^a UI = sum of the percentages of each fatty acid × number of double bonds.

3.3. Fatty acid profiles and chemiluminescence of mitochondria and microsomes obtained from duck heart

The fatty acid composition of total native lipids of heart mitochondria and microsomes was different. The rate 18:2/20:4 was 2.0 times higher in mitochondria than in microsomes and the rate 20:4/22:6 was

2.9 times higher in mitochondria than in microsomes (Tables 3 and 4) when both types of organelles were compared.

The unsaturation index was 1.2 times higher in mitochondria than in microsomes (Tables 3 and 4). Light emission was statistically significantly in mitochondria but not in microsomes when control and peroxidized samples were compared Fig. 1.

Table 5
Fatty acid composition of total lipids from brain mitochondria of duck, *C. moschata*

Fatty acid	Native	Control	Peroxidized
C16:0	21.97 ± 0.63	24.85 ± 0.32	24.69 ± 3.26
C16:1 n7	1.30 ± 0.45	0.78 ± 0.30	8.53 ± 1.07***
C18:0	19.47 ± 0.31	20.42 ± 1.73	21.98 ± 0.51
C18:1 n9	20.45 ± 0.50	21.78 ± 1.16	22.97 ± 1.80
C18:2 n6	0.70 ± 0.36	1.80 ± 0.18	1.74 ± 0.52
C18:3 n3	–	–	–
C20:4 n6	9.79 ± 0.40	9.12 ± 0.45	5.86 ± 0.68**
C22:6 n3	17.02 ± 0.78	14.99 ± 0.74	7.29 ± 1.47**
Saturated	41.44 ± 0.92	45.27 ± 1.43	46.66 ± 3.74
Monounsaturated	21.75 ± 0.26	22.56 ± 1.14	31.50 ± 2.86*
Polyunsaturated	27.51 ± 0.71	25.91 ± 0.69	14.89 ± 1.50***
Total unsaturated	49.25 ± 0.51	48.47 ± 1.76	46.39 ± 3.46
Saturated/unsaturated	0.84 ± 0.01	0.93 ± 0.03	1.01 ± 0.01*
UI ^a	164.41 ± 4.82	152.58 ± 5.13	102.15 ± 10.32**

Data are given as the mean ± S.D. of three independent experiments. Statistically significant differences between control and peroxidized groups are indicated by * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

^a UI = sum of the percentages of each fatty acid × number of double bonds.

Table 6

Fatty acid composition of total lipids from brain microsomes of duck, *C. moschata*

Fatty acid	Native	Control	Peroxidized
C16:0	34.21 ± 0.33	31.77 ± 3.29	27.08 ± 1.04
C16:1 n7	4.24 ± 0.24	6.52 ± 0.27	5.28 ± 0.76
C18:0	18.33 ± 1.06	22.69 ± 2.41	22.48 ± 1.75
C18:1 n9	21.30 ± 1.29	20.98 ± 1.61	23.60 ± 3.14
C18:2 n6	7.93 ± 0.14	7.42 ± 1.79	5.36 ± 0.12
C18:3 n3	0.46 ± 0.07	0.52 ± 0.17	0.41 ± 0.07
C20:4 n6	6.44 ± 0.22	3.50 ± 0.60	2.75 ± 0.19
C22:6 n3	7.76 ± 0.53	5.07 ± 0.10	5.03 ± 0.05
Saturated	52.54 ± 0.85	54.46 ± 3.55	49.55 ± 1.34
Monounsaturated	25.54 ± 1.36	27.50 ± 1.83	28.87 ± 2.47
Polyunsaturated	22.58 ± 0.45	16.51 ± 2.03	13.56 ± 0.39
Total unsaturated	48.12 ± 0.98	44.02 ± 3.15	42.43 ± 2.67
Saturated/unsaturated	1.09 ± 0.04	1.24 ± 0.04	1.17 ± 0.10
UI ^a	115.06 ± 1.49	88.35 ± 5.70	82.02 ± 3.18

Data are given as the mean ± S.D. of three independent experiments.

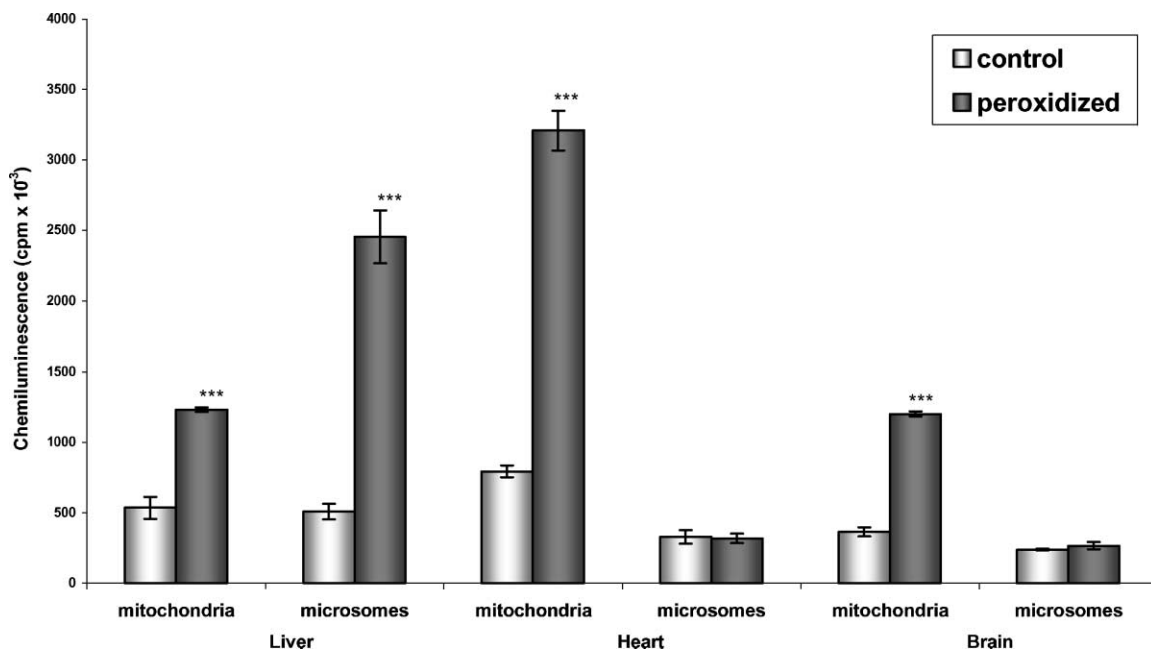
^a UI = sum of the percentages of each fatty acid × number of double bonds.

Fig. 1. Lipid peroxidation ascorbate-Fe²⁺ of microsomes and mitochondria from duck liver, heart and brain. Chemiluminescence was determined over a 120 min period and recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein. Results are expressed as mean ± S.D. of three independent experiments. Statistically significant differences between control and peroxidized are indicated by *** $P < 0.0001$.

Tables 3 and 4 shows the fatty acid composition of total lipids of control and peroxidized mitochondria and microsomes. A significant decrease of C20:4 n6 and C22:6 n3 in mitochondria was observed when compared with control. No statistically significant differences due to peroxidation were observed in microsomes when fatty acid profiles and light emission were compared. The unsaturation index of peroxidized heart mitochondria was lower than in microsomes.

3.4. Fatty acid profiles and chemiluminescence of mitochondria and microsomes obtained from duck brain

The fatty acid composition of total native lipids of brain mitochondria and microsomes was different. The major difference, from the point of view of unsaturation, was the content of the highly unsaturated C22:6 n3. The rate 18:2/20:4 was 18 times higher in microsomes than in mitochondria and the rate 20:4/22:6 was 1.5 times higher in microsomes than in mitochondria (Tables 5 and 6) when both types of organelles were compared. The unsaturation index was 1.4 times higher in mitochondria than in microsomes (Tables 5 and 6). Light emission was statistically significantly in mitochondria but not in microsomes when control and peroxidized samples were compared Fig. 1. Tables 5 and 6 shows the fatty acid composition of total lipids of control and peroxidized mitochondria and microsomes. A significant decrease of C20:4 n6 and C22:6 n3 in mitochondria was observed when compared with control. No significant differences due to peroxidation were observed in microsomes when fatty acid profiles and light emission were compared.

4. Discussion

Previous investigations have shown that the degree of unsaturation of fatty acids and the sensitivity to LP of liver mitochondria is lower in pigeons than in rats [1,6]. The fatty acid composition of total lipids isolated from heart of pigeon, canary and parakeet showed lower degree of unsaturated and LP level when compared with mouse heart [6,7,12]. Barja et al. [8] and Ku and Sohal [18] have observed that free radical production by pigeon mitochondria was lower

than in rat brain mitochondria. In addition it has been demonstrated that the pigeon showed the lowest level of many tissue antioxidants, because their rates of free radical production are low, whereas the rat showed very high levels of antioxidants that compensated the high rates of free radical production [1]. Many studies have shown that free radical damage and LP increase as a function of the degree of unsaturation of the fatty acids present in the phospholipids of biological membranes. In this regard it has been demonstrated that the number of bis-allylic positions contained in the cellular lipids of intact cells determines their susceptibility, i.e. oxidizability, to free radical mediated peroxidative events [19]. However, these observations can not be taken as a general rule. Previous results from our laboratory have demonstrated that fatty acids profiles of microsomes and mitochondria obtained from several bovine tissues are not responsible for their different susceptibility to free radical degradation [5,6]. Mitochondria and microsomes from heart, liver and brain of *C. moschata* show a high content of 18:1 n9. This fatty acid is also found in high percent in pigeon [1,6], canary and parakeet [12]. Mitochondria and microsomas from duck liver possess a high content of polyunsaturated fatty acids, mainly 20:4 n6 and 22:6 n3 that are vulnerable to the LP process. Heart duck mitochondria and microsomes has approximately the same unsaturated fatty acid content than heart pigeon mitochondria and microsomes [6], with a high percent of 20:4 n6 which is vulnerable to peroxidation. However, there was not correlation between the polyunsaturated fatty acid content and light emission when heart microsomes from both species were compared. The lack of relationship between fatty acid unsaturation and sensitivity to peroxidation observed suggest that other factor/s may be involved in the protection to LP observed in heart microsomes from both species. Duck brain mitochondria possess a high content of 22:6 n3, which correlates, with a high production of free radicals. Duck brain microsomes possess a high content of polyunsaturated fatty acids mainly C20:4 n6 and C22:6 n3, however, there is not correlation between the polyunsaturated fatty acid content and light emission. It is possible that because of the important physiological functions of arachidonic and docosahexaenoic acids in these tissues, they are protected to maintain membrane content of fatty acids during oxidative stress.

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