ISSN : 0974 - 7427 Volume 6 Issue 5

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 6(5), 2012 [162-168]

A low degree of fatty acid unsaturation protects against lipid peroxidation in mitochondria of different organs of gentoo penguin *Pygoscelis papua*

Mónica Marmunti[1], Ana María Gutiérrez[1,2], Diego Montalti[3]*, Mariana Gavazza[1], Alejandro Palacios[1]

[1]Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 60 y 118, 1900-La Plata, (ARGENTINA)

[2]Cátedra Fisiología Animal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque, FWA1900-La Plata, (ARGENTINA)

[3]Instituto Antártico Argentino-CONICET, Cerrito 1248, C1010AAZ-Buenos Aires, (ARGENTINA)

E-mail : dmontalti@fcnym.unlp.edu.ar

Received: 19th July, 2012 ; Accepted: 22nd September, 2012

ABSTRACT

Lipid peroxidation is one of the main events induced by oxidative stress and is particularly active in those tissues whose membranes are rich in polyunsaturated fatty acids. The aim of the present study was examine fatty acid profiles and the susceptibility to lipid peroxidation in mitochondria obtained from liver and heart of Gentoo penguin *Pygoscelis papua*. When we analysed the fatty acid composition of Gentoo penguin we observed that the amount of saturated and unsaturated fatty acids obtained from liver mitochondria was 53% and 45%, whereas in heart mitochondria was 40% and 60% respectively. In liver as in heart the predominant unsaturated fatty acid was C18:1n9. The polyunsaturated fatty acid percentages of mitochondria obtained from liver was 16% whereas in heart was 18%. The rate between C20:4n6/C18:2n6 in liver and heart mitochondria was 2.17 and 0.34 respectively. The mitochondria obtained from liver and heart is not susceptible to lipid peroxidation. The most interesting finding of our study is the low sensitivity to lipid peroxidation observed in both organs; this fact may be involved in the protection to oxidative stress observed in these organelles.

KEYWORDS

Mitochondria;
Oxidative stress;
Liver;
Heart;
Penguin;
Antarctica.

INTRODUCTION

Birds are characterized by low free radical production than mammals of similar body size and metabolic rates[1]. Previous studies have shown that the degree of unsaturation of fatty acids and the sensitivity to lipid peroxidation of liver and heart mitochondria from birds are lower when compared with mammals[2-5]. Therefore it has been shown that the heart lipids of canaries (*Serinus canarius* Linnaeus, 1758) and parakeets (*Melopsittacus undulatus* Shaw, 1805) have a lower fatty acid double bond content than those of mice

(*Mus musculus* Linnaeus, 1758)[4]. Recently, we reported a relationship between tissue sensitivity to lipid peroxidation *in vitro* and long chain polyunsaturated fatty acid concentration in liver, heart and brain mitochondria from Goose (*Anser anser* Linnaeus, 1758)[6].

All cellular membranes are especially vulnerable to oxidation due to their high concentration of polyunsaturated fatty acids (PUFAs) which are abundant in nature and are generated by action of desaturases on saturated fatty acids[7].

Long chain polyunsaturated fatty acids such as arachidonic acid (C20:4 n6) and docosahexaenoic acid (C22:6 n3) play important roles in a variety of biological functions[8]. PUFAs are essential components in higher eukaryotes that confer fluidity, flexibility and selective permeability to cellular membranes. PUFAs affect many cellular and physiological processes in both plants and animals. Animal biosynthesis of high polyunsatutated fatty acids from linoleic, a-linolenic and oleic acids is mainly modulated by the delta-6 and delta-5 desaturases through dietary and hormonal stimulated mechanisms[9].

Lipid peroxidation is generally thought to be a major mechanism of cell injury in aerobic organism subjected to oxidative stress. Non enzymatic lipid peroxidation and formation of lipid-peroxides can be initiated by adding ascorbate in the presence of oxygen and Fe^{2+} or Fe^{3+} ions to various tissues preparations such as homogenates, mitochondria, microsomes and nuclei obtained from various tissues and species[10,11,12]. Among cellular macromolecules, PUFAs exhibit the highest sensitivity to oxidative damage. It is accepted that their sensitivity increases as a power function of the number of double bonds per fatty acid molecule[13]. Membranes phospholipids are particularly susceptible to oxidation not only because of their highly polyunsaturated fatty acid content but also because of their association in the cell membrane with non-enzymatic and enzymatic systems capable of generating pro-oxidative-free radical species. The measurement of lipid peroxidation is one of the most commonly used assays for radical induced damage[1,14].

A combination of ascorbate plus iron can trigger a Fenton-reaction with formation of highly reactive hydroxyl radicals, which can cause chain-initiation reaction of lipid peroxidation and secondary protein oxidation[15]. Ascorbate may enhance the process by keeping iron in the reduced state. In crude tissue fractions iron in reduced form can degrade preformed lipid hydroperoxides forming radicals that can catalyze the chain propagation phase of lipid peroxidation, without involving directly the hydroxyl radicals or other active oxygen species[16]. Lipid peroxidation termination involves the reaction of $LOO^{\cdot}$ to form non-radical products or the reaction of one $LOO^{\cdot}$ with another terminating radical to generate non-propagating radical species[17]. Some lipid peroxidation products are light-emitting species and their spontaneous chemiluminiscence can be used as an internal marker of oxidative stress[18].

Penguins are capable of storing great reserves of energy in their layer of subcutaneous fat, which means that they can survive long periods without feeding at all. The length of time a bird can go without food depends basically on its weight at the beginning of the fasting period; the fatter the bird, the longer it can survive[19].

Gentoo penguins *Pygoscelis papua* Forster, 1781 have a wide latitudinal distribution (46-65°S) compared to other species of Antarctic and Subantarctic penguins. It feeds mainly on euphausiid crustaceans and fish, with large foraging trips[20].

In the present work we have studied the fatty acid profiles and non enzymatic lipid peroxidation of mitochondria obtained from liver and heart of Gentoo penguin. Therefore, the potential relationships between the fatty acid composition of the food and any changes in those of the tissues were examined.

Liver was selected because of its ability of high production of free radicals and heart due to its high oxygen partial pressure.

MATERIALS AND METHODS

Animals

The samples for this study were obtained from three adults Gentoo penguin (*Pygoscelis papua*) from Hope Bay (63°23´S 54°00´W), Antarctic Peninsula, Antarctica. The organs were removed and the stomach contents were collected and transferred to our laboratory maintained at -20°C. Liver and heart were analyzed, and the rest of the organs were distributed in different laboratories for other scientific studies. The

Regular Paper

mean body weight of the birds was 5,287 ± 728.5 g.

Preparation liver and heart mitochondria

Mitochondria were prepared as described by Schneider and Hogeboom[21]. The organs were cut into small pieces and washed extensively with 0.15 M NaCl. An homogenate of the tissue was prepared in solution containing 0.25 M sucrose, 10 mM Tris-HCl pH 7.4, PMSF 0.1 mM, 3 ml of solution per gram of tissue, using the Potter-Elvejhem homogeneizer. The homogenate was spun at 1,000 x g, pellet was discarded, and the supernatant was spun at 20,000 x g for 10 min to obtain mitochondria. All operations were performed at 4 °C. Mitochondria was stored at –83 °C and used within a week of its preparation, after one cycle of freezing and thawing.

Non-enzymatic lipid peroxidation of mitochondria

Chemiluminescence and lipid peroxidation were initiated by adding ascorbate-Fe^{2+} to mitochondrial preparations[22]. Mitochondria (1 mg of protein) were incubated at 37°C with 0,01 M phosphate buffer (pH 7.4), 0.4 mM ascorbate, final volume 1 ml. Phosphate buffer is contaminated with sufficient iron to provide the necessary ferrous or ferric iron for lipid peroxidation, (final concentration in the incubation mixture was 2.15 mM)[23]. Mitochondria preparations which lacked ascorbate-Fe^{2+} (control) were carried out simultaneously. Chemiluminescence was measured as counts per min in a liquid scintillation analyzer Packard 1900 TR. Membrane light emission was determined over 120 min period, and recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein.

Measurement of fatty acid composition

Mitochondrial lipids were extracted with chloroform/methanol (2:1 v/v containing 0.01 % BHT as antioxidant) from peroxidized membranes[24]. Fatty acids were transmethylated with 10% F_3B in methanol at 60 °C for 3 h. Fatty acid methyl esters were analyzed with a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a packed column (1.80 m x 4 mm i.d.) 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, the column temperature was held at 200 °C. Fatty acid methyl ester peaks were identified by comparison of the retention times with those of standards. All compositions were expressed as % by area of total fatty acids.

Unsaturation index (UI)

UI was calculated according to the formula, UI = sum (fatty acid percent) x (number of double bonds)[25].

Protein determination

Proteins were determined by the method of Lowry et al.[26] using BSA as standard.

Chemicals

Butylated hydroxytoluene (BHT) and phenyl-methyl-susfonyl fluoride (PMSF) were from Sigma (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. 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.

Statistical analysis

Data were expressed as means ± S.D. Statistical analysis utilized was Student's t-test. Statistical criterion for significance was selected at different p values and indicated in each case.

RESULTS

Fatty acid composition and chemiluminescence of liver and heart mitochondria submitted to lipid peroxidation

The saturated long chain fatty acids present in liver and heart mitochondria obtained from Gentoo penguin were mainly C16:0 and C18:0 in a percentage of approximately 50% and 40 % respectively. The concentration of unsaturated fatty acids of liver and heart mitochondria was approximately 50 % and 60 %, respectively, with a prevalence of oleic acid C18:1 n9.

In liver mitochondria the content of polyunsaturated long chain fatty acid decrease in the order C22:6 n3 > C20:4 n6 > C18:2 n6. Whereas in heart mitochondria were in the order C18:2 n6 > C20:4 n6 > C22:6 n3.

Regular Paper

TABLE 1 : Fatty acid composition of total lipids from liver mitochondria of Gentoo penguin *Pygoscelis papua*.

Fatty acid	Control	Ascorbate-Fe^{++}
C16:0	24.40 ± 4.64	30.32 ± 0.25
C18:0	28.72 ± 0.66	29.56 ± 1.56
C18:1 n9	29.28 ± 2.36	27.25 ± 0.51
C18:2 n6	2.84 ± 0.22	2.56 ± 0.77
C20:4 n6	6.04 ± 1.70	3.69 ± 1.01
C22:6 n3	7.26 ± 0.82	6.51 ± 0.94
Saturated	53.12 ± 5.02	59.88 ± 1.32
Monounsaturated	29.28 ± 2.36	27.25 ± 0.51
Polyunsaturated	16.14 ± 2.25	12.75 ± 1.19
Total unsaturated	45.42 ± 4.59	40.00 ± 1.37
Saturated/unsaturated	1.19 ± 0.23	1.50 ± 0.09
UI	102.68 ± 13.34	86.15 ± 8.28
PUFA n3	7.26 ± 0.82	6.51 ± 0.94
PUFA n6	8.87 ± 1.50	6.24 ± 0.31
C20:4 n6/C18:2 n6	2.17 ± 0.76	1.62 ± 0.91

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

UI= sum of the percentages of each fatty acid x number of double bonds.

TABLE 2 : Fatty acid composition of total lipids from heart mitochondria of Gentoo penguin *Pygoscelis papua*.

Fatty acid	Control	Ascorbate-Fe^{++}
C16:0	19.35 ± 0.52	18.47 ± 0.76
C18:0	22.83 ± 1.35	25.92 ± 1.52
C18:1 n9	39.12 ± 1.09	36.16 ± 0.50
C18:2 n6	12.33 ± 0.32	13.26 ± 1.20
C20:4 n6	4.46 ± 0.44	5.05 ± 0.54
C22:6 n3	1.44 ± 0.18	1.30 ± 0.29
Saturated	42.17 ± 1.85	43.69 ± 1.82
Monounsaturated	39.12 ± 1.09	36.16 ± 0.50
Polyunsaturated	18.23 ± 0.71	19.61 ± 1.71
Total unsaturated	57.36 ± 1.79	55.77 ± 2.21
Saturated/unsaturated	0.74 ± 0.05	0.79 ± 0.06
UI	90.26 ± 3.94	90.69 ± 5.81
PUFA n3	1.44 ± 0.18	1.30 ± 0.29
PUFA n6	17.46 ± 0.92	20.68 ± 0.55
C20:4 n6/C18:2 n6	0.34 ± 0.02	0.57 ± 0.18

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

UI= sum of the percentages of each fatty acid x number of double bonds.

The main polyunsaturated fatty acid profiles (C20:4 n6 and C22:6 n3) of heart and liver mitochondria were not modified after lipid peroxidation process (TABLES 1 and 2).

The rate C20:4 n6 / C18:2 n6 in liver and heart mitochondria was 2.17 and 0.34 respectively. Therefore the unsaturation index was not similar.

Light emission equal to chemiluminescence originating from liver and heart was not statistically significant when control and peroxidized samples were compared (Figure 1).

Comparison of fatty acid composition of organelles with the diet

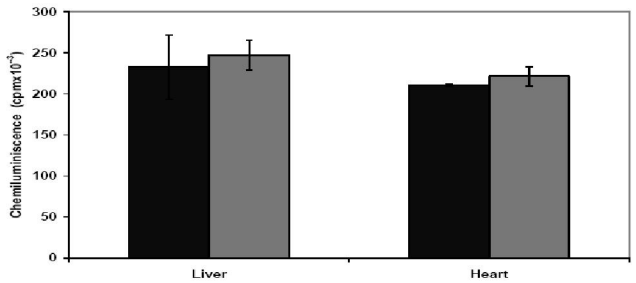

Figure 1 : Total chemiluminescence produced by liver and heart mitochondria obtained from Gentoo penguin (*Pygoscelis papua*) ■ control and □ peroxidized (in the presence of ascorbate-Fe^{++}). Results are expressed as mean ± S.D.

TABLE 3 : Fatty acid composition of total lipids from stomach content of Gentoo penguin *Pygoscelis papua*

Fatty acid	Area %
C16:0	23.83 ± 1.14
C 16:1 n7	7.93 ± 0.12
C18:0	0.90 ± 0.10
C18:1 n9	20.53 ± 1.37
C18:2 n6	1.93 ± 0.05
C18:3 n3	0.63 ± 0.06
C20:4 n6	30.37 ± 0.47
C22:6 n3	14.58 ± 1.49
Saturated	24.73 ± 1.21
Monounsaturated	28.47 ± 1.42
Polyunsaturated	47.50 ± 1.55
Total unsaturated	75.96 ± 0.21
Saturated/unsaturated	0.33 ± 0.02
UI	243.13 ± 7.62
PUFA n3	15.00 ± 1.85
PUFA n6	32.29 ± 0.52
C20:4/ C18:2	15.76 ± 0.15
C22:6/ C18:3	23.31 ± 1.85

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

UI= sum of the percentages of each fatty acid x number of double bonds.

Regular Paper

When we analyzed the penguin stomach content we observed that this diet is very rich source of long chain n3 and n6 polyunsaturated fatty acids such as C22:6 n3 and C20:4 n6, and is also characterized by high proportions of long chain monounsaturated like was C18:1 n9 (TABLE 3).

Consequently the fatty acid profile is characterized by a high percentage of polyunsaturated fatty acids. The unsaturation index: UI, which is an indication of the number of double bonds present in lipids was higher in liver than heart mitochondria, 102.68 ± 13.34 and 90.26 ± 3.94 respectively. Turn in stomach content the UI value was 243.13 ± 7.62, a value that is 2.4 and 2.8 times higher than that obtained in liver and heart, respectively. The main reason for this difference was the higher C20:4 n6 and C22:6 n3 percentages found in the stomach content compared to liver and heart mitochondria (TABLES 1, 2 and 3).

DISCUSSION

Previous studies have shown that the degree of unsaturation of fatty acids of mitochondria from birds is lower when compared to mammals. Barja et al.[27] and Ku and Sohal[28] have observed that free radical production in Rock pigeon (*Columba livia* Gmelin, 1789) mitochondria was lower than in rat (*Rattus* sp) mitochondria.

In this study we described for the first time the fatty acids profiles and non enzymatic lipid peroxidation of mitochondria obtained from liver and heart of Gentoo penguin.

The Gentoo penguin inhabits the southern Antarctic region and breeds during the summer in large colonies on remotes islands. The adult feeds exclusively at sea and their diet during the breeding season consist almost enterily of Krill (*Euphausia superba* Dana, 1852 and E. *crystalorophias*)[29].

The simultaneous study of fatty acid profile in diet and in liver and heart mitochondria obtained from grain-eating birds like goose *Anser anser* Linnaeus, 1758 and quails *Coturnix coturnix japonica* Linnaeus, 1758 demonstrate that the fatty acid composition of the diet is not responsible for the differences observed in the double bond content of the organelles[6,30]. This analysis is in concordance with the observations of Pamplona et al.[3]. We also show that although the polyunsaturated fatty acids were similar in all the tissues examined the contribution of each fatty acid was different.

Liver mitochondria from Gentoo penguin possess a higher content of polyunsaturated fatty acids, mainly C20:4 n6 and C22:6 n3 and a lower content of C18:1 n9 and C18:2 n6, compared with heart mitochondria. This distribution in the tissues analyzed was similar to that observed in previous studies[2,5,11,6,30]. These authors have also demonstrated that different bird species show a low degree of unsaturation despite differences in their diet composition. Taking this observation into account we considered that the low content of double bonds observed in penguin organelles would be independent of diet.

Docosahexaenoic C22:6 n3 and arachidonic C20:4 n6 acids are synthesized from its dietary precursor 18:3 n3 and 18:2 n6, respectively[31] and its content was different in the tissues studied. Liver mitochondria exhibited a high C20:4/C18:2 ratios and a higher content of PUFA n3 compared with heart mitochondria. These results indicate that the level of unsaturated fatty acids is homeostatically regulated in tissues[32].

The control of membrane fatty acid unsaturation has been attributed to negative feed-back regulation of transcription of desaturase genes dependent on lipid composition[32,33] and to the modulation of desaturases by the metabolic-hormonal status[34].

Many studies have shown that free radical damage and lipid peroxidation increase as a function of the degree of unsaturation of 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[13,35,36].

However, these observations cannot be taken as a general rule. Previous results have demonstrated that fatty acid profiles of mitochondria obtained from several bovine tissues are not responsible for their different susceptibility to free radical degradation[37].

Studies conducted over the last decade have shown that birds – particularly long-lived species – have better defenses against reactive oxygen species (ROS) damage, and that they probably utilise a com-

bination of cellular protective mechanisms[38,39]. These defenses include lower mitochondrial ROS production, inducible defenses, like antioxidant enzymes and constitutive or structural defenses[40,41]. In the mitochondria of heart and liver of penguins dominate monounsaturated fatty acids, mainly C18:1n9. High values of those fatty acids were also detected in pigeon[2,5], canary and parakeet[4], duck, goose and quail[11,6,30,42], all of them essentially unable of peroxidase. C22:6n3 is 320-fold more susceptible to peroxidation than18:1n9[43]. The low degree of nonsaturation that these cellular membranes present, is principally due to their lower content of highly unsaturated fatty acids, such as C22:6n3, and higher presence of less unsaturated fatty acids, as the C18:1n9 and C18:2n6. This redistribution of fatty acids without changes in the total amount of polyunsaturated fatty acids, leads to lower levels of lipid peroxidation.

The most interesting finding of our study is the low sensitivity to lipid peroxidation detected in heart and liver penguin organelles, especially liver that is an organ that in mammals produces great quantity of radicals free. The results obtained in heart and liver mitochondria contribute to the protection of that tissues against oxidative damage and consequently to the preservation of its 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.

ACKNOWLEDGEMENTS

The authors wish to thank César Arcemis for excellent technical assistance. Instituto Antártico Argentino gave logistic and financial support of the work and its Program of Environmental Management and Tourism gave D. Montalti the approval and the protocol of ethical conditions under which this work was carried out.

REFERENCES

[1] R.Pamplona, M.Portero-Otin, D.Riba, C.Ruiz, J.Prat, M.J.Bellmunt, G.Barja; Journal of Lipid Research, **39**, 1989-1994 **(1998)**.

[2] R.Pamplona, J.Prat, S.Cadenas, C.Rojas, R.Pérez-Campo, M.López Torres, G.Barja; Mechanisms and Ageing Development, **86**, 53-66 **(1996)**.

[3] R.Pamplona, M.Portero-Otin, J.R.Requena, S.R.Torpe, A.Herrero, G.Barja; Mechanisms of Ageing and Development, **106**, 283-296 **(1999a)**.

[4] R.Pamplona, M.Portero-Otin, D.F.Ledo, R.Gredilla, G.Barja; Aging Clinical and Experimental Research, **11**, 44-49 **(1999b)**.

[5] A.M.Gutiérrez, G.R.Reboredo, C.J.Arcemis, A.Catalá; International Journal of Biochemical and Cell Biology, **32**, 73-79 **(2000)**.

[6] A.M.Gutiérrez, G.R.Reboredo, S.M.Mosca, A.Catalá; Comparative Biochemistry and Physiology C, **137**, 123-132 **(2004)**.

[7] H.G.Enoch, A.Catalá, P.Strittmatter; Journal of Biological Chemistry, **251**, 5095-5103 **(1976)**.

[8] A.A.Spector; Lipids, **34**, 51-53 **(1999)**.

[9] R.R.Brenner; Prostaglandins Leukotrienes and Essential Fatty Acids, **68**, 151-162 **(2003)**.

[10] M.Marmunti, A.Catalá; International Journal of Biochemistry and Cell Biology, **30**, 967-672 **(1998)**.

[11] A.M.Gutiérrez, G.R.Reboredo, A.Catalá; International Journal of Biochemical and Cell Biology, **34**, 605-612 **(2002)**.

[12] M.Gavazza, A.Catalá; International Journal of Biochemical and Cell Biology, **35**, 359-366 **(2003)**.

[13] B.A.Wagner, G.R.Buettner, C.P.Burns; Biochemistry, **33**, 4449-4453, **(1994)**.

[14] S.Ozgova, J.Hermanek, I.Gut; Biochemical Pharmacology, **66**, 1127-1137 **(2003)**.

[15] G.Spiteller; Medical Hypotheses, **60**, 69-83 **(2003)**.

[16] H.Chakraborty, S.N.Ray, S.Chakrabarti; Neurochemistry International, **39**, 311-317 **(2001)**.

[17] T.A.Dix, J.Aikens; Chemical Research on Toxicology, **6**, 2-18 **(1993)**.

[18] M.Havaux; Trends in Plant Science, **8**, 409-413 **(2003)**.

[19] I.Martínez; Family Spheniscidae (penguins), In: Handbook of the birds of the world, J.del Hoyo, A.Elliot, J.Sargatal, (Eds); Ostrich to Ducks, (Barcelona, Lynx Edicions), **1**, 140-160 **(1992)**.

[20] T.D.Williams; The Penguins, New york: Oxford University press, 328 **(1995)**.

[21] W.C.Schneider, H.G.Hogeboom; Journal of Biological Chemistry, **183**, 123-128 **(1950)**.

[22] J.R.Wright, R.C.Rumbaugh, H.D.Colby, P.R.Miles; Archives of Biochemistry and Biophysics, **192**, 344-351 **(1979)**.

[23] A.Terrasa, M.Guajardo, A.Catalá; Molecular and Cellular Biochemistry, **211**, 39-45, **(2000)**.

[24] J.Folch, N.Lees, G.A.Sloane, G.A.Stanley; Journal of Biological Chemistry, **226**, 497-509 **(1957)**.

Regular Paper

[25] M.Llanillo, J.Sánchez-Yagüe, A.Checa, E.M.Martín-Valmaseda, A.Felipe; Experimental Hematology, **23**, 258-264 **(1995)**.
[26] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; Journal of Biological Chemistry, **193**, 265-275 **(1951)**.
[27] G.Barja, S.Cadenas, C.Rojas, R.Perez–Campo, M.Lopez Torres; Free Radical Research, **21**, 317-328 **(1994)**.
[28] H.H.Ku, R.S.Sohal; Mechanisms of Ageing and Development, **72**, 67-76 **(1993)**.
[29] M.M.Libertelli, G.A.Daneri, U.Piatowski, N.R.Coria, A.R.Carlini; Polish Polar Research, **25**, 267-274 **(2004)**.
[30] A.M.Gutiérrez, G.R.Reboredo, S.M.Mosca, A.Catalá; Molecular Cell Diochemistry, **282**, 109-115 **(2006)**.
[31] M.T.Nakamura, T.Y.Nara; Prostaglandins Leukotrienes and Essential Fatty Acids, **68**, 145-150 **(2003)**.
[32] B.Maresca, A.R.Cossins; Nature, **365**, 606-607 **(1993)**.
[33] L.Vigh, D.A.Los, I.Horvath, N.Muirata; Proceedings of the National Academy of Science, **90**, 9090-9094 **(1993)**.
[34] F.Guéraud, A.Paris; Biochimica et Biophysica Acta, **1329**, 97-110 **(1997)**.
[35] A.Catalá; International Journal of Biochemistry & Cell Biology, **38**, 1482-1495 **(2006)**.
[36] A.Catalá; Chemistry and Physics Lipids, **157**, 1-11 **(2009)**.
[37] D.Rosa, A.Catalá; Archives of Physiology and Biochemistry, **106**, 33-37 **(1998)**.
[38] A.Herrero, G.Barja; Mechanisms of Ageing and Development, **103**, 133-146 **(1998)**.
[39] C.E.Ogburn, K.Carlberg, M.A.Ottinger, D.J.Holmes, G.M.Martín, S.N.Austad; Journal of Gerontology, Series A: Biological Science and Medical Science, **56**, 468-474 **(2001)**.
[40] G.Barja; Annals of the New York Academy of Science, **854**, 224-238 **(1998)**.
[41] D.J.Holmes, R.Fluckiger, S.N.Austad; Experimental Gerontology, **36**, 869-883 **(2001)**.
[42] A.M.Gutiérrez, G.R.Reboredo, S.M.Mosca, A.Catalá; Comparative Biochemistry and Physiology A, **146**, 415–421 **(2007)**.
[43] A.J.Hulbert; Journal of Theoretical Biology, **234**, 277-288 **(2005)**.