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Double bond content of phospholipids and lipid peroxidation negatively correlate with maximum longevity in the heart of mammals

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Abstract

Free radical damage is currently considered a main determinant of the rate of aging. Unsaturated fatty acids are the tissue macromolecules most sensitive to oxidative damage. Therefore, the presence of relatively low degrees of fatty acid unsaturation is expected in the tissues of longevous animals. In agreement with this prediction, fatty acid analyses of heart phospholipids in eight mammals ranging in maximum life span (MLSP) from 3.5 to 46 years showed that their total number of double bonds is negatively correlated with MLSP ($r = -0.78$, $P < 0.02$). The low double content of longevous mammals was not due to a low polyunsaturated fatty acid content. Instead, it was mainly due to a redistribution between types of polyunsaturated fatty acids from the highly unsaturated docosahexaenoic acid (22:6$n-3$) to the less unsaturated linoleic acid (18:2$n-6$) in longevous animals ($r = -0.89$, $P < 0.003$ for 22:6$n-3$ and $r = 0.91$, $P < 0.002$ for 18:2$n-6$ versus MLSP), where n = number of different animals in each species. This redistribution suggests that one of the mechanisms responsible for the low number of fatty acid double bonds is the presence of low desaturase activities in longevous animals, although other causing factors must be involved. In agreement with the low degree of fatty acid unsaturation of longevous mammals, the sensitivity to lipid peroxidation ($r = -0.87$; $P < 0.005$) and the in vivo lipid peroxidation ($r = -0.86$, $P < 0.005$) in the heart were also negatively correlated with MLSP across species. These results, together with previous ones obtained in rodents, birds, and humans, suggest that the low degree of tissue fatty acid unsaturation of longevous homeothermic

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animals could have been selected during evolution to protect the tissues against oxidative damage.

Keywords: Longevity; Aging; Fatty acids; Unsaturation; Lipid peroxidation; Heart

1. Introduction

Available comparative studies indicate that longevous animals have low rates of mitochondrial free radical production (Sohal and Weindruch, 1996; Barja, 1999). While this is a relevant characteristic consistent with the free radical theory of aging (Harman, 1998; Beckman and Ames, 1998), additional factors can also lead to a low oxidative damage in longevous animals. Among tissue macromolecules, polyunsaturated fatty acids (PUFAs) are the ones more sensitive to free radical damage, with their sensitivity increasing as a power function of the number of double bonds per fatty acid. Birds and primates are homeothermic species with extraordinarily high maximum life spans (MLSPs) and slow aging rates in relation to those of the majority of mammals of similar body size and metabolic rate. Previous studies from our laboratories have shown that pigeon (MLSP = 35 years) and human (MLSP = 120 years) heart or liver mitochondria have fatty acids with a lower degree of unsaturation and a lower lipid peroxidation (Pamplona et al., 1996, 1999a) than those of rats (MLSP = 4 years). The degree of fatty acid unsaturation of heart lipids and heart lipid peroxidation was also lower in canaries (MLSP = 24 years) and budgerigars (MLSP = 21 years) than in mice (MLSP = 3.5 years), while these three species share a similar body weight and metabolic rate (Pamplona et al., 1999b). A negative correlation between the degree of fatty acid unsaturation and the MLSP has been also recently described by us in liver mitochondria of different mammalian species (Pamplona et al., 1998).

A low fatty acid unsaturation would be advantageous to longevous animals from the point of view of oxidative stress because it would decrease the sensitivity of their tissues and cellular organelles to lipid peroxidation. In addition, lipid peroxidation products are known to modify and damage other kinds of non-lipidic macromolecules including nearby proteins (Forsmark-Andree et al., 1995; Pamplona et al., 1999a) and DNA (Draper, 1995). Thus, a low fatty acid unsaturation would also protect them against lipid-derived oxidative damage.

To our knowledge, the possible relationship between fatty acid composition/unsaturation and MLSP has never been investigated in total tissues of different mammalian species. In order to clarify if the degree of membrane lipid unsaturation is systematically related to longevity, we studied the fatty acid composition of the heart phospholipids, the sensitivity of the tissue to lipid peroxidation induced in vitro, and heart in vivo lipid peroxidation, in eight species of mammals following the 'rate of living' inverse relationship between the weight-specific basal metabolic rate and MLSP (Pearl, 1928), and progressively differing in their MLSP from 3.5 to 46 years. The heart is an ideal vital organ for aging-related studies due to its essentially post-mitotic character.

2. Materials and methods

2.1. Animals and heart samples

All animals used were young adults with an age within 15–30% of their MLSP. The maximum longevity's of the selected species are well known (Altman and Dittmer, 1972) and progressively vary from 3.5 to 46 years: mouse (*Mus musculus*, $n = 6$, MLSP = 3.5 years), rat (*Rattus norvegicus*, $n = 6$, MLSP = 4 years), guinea pig (*Cavia porcellus*, $n = 7$, MLSP = 8 years), rabbit (*Oryctolagus caniculus*, $n = 5$, MLSP = 13 years), sheep (*Ovis aries*, $n = 3$, MLSP = 20 years), pig (*Sus scrofa*, $n = 7$, MLSP = 27 years), cow (*Bos taurus*, $n = 7$, MLSP = 30 years) and horse (*Equus caballus*, $n = 7$, MLSP = 46 years), where n = number of different animals in each species. All the animals were apparently in good health and no animal was obese or scraggy. Mice, rats, guinea pigs and rabbits were euthanized by decapitation. Sheep, pigs, cows and horses were euthanized at abattoir. Heart samples were taken from ventricles in all animals within the first 10 min after death. They were cut in small pieces, immediately frozen in liquid nitrogen, and transferred before 4 h to a -80°C freezer for storage, to be used later for fatty acid analyses and lipid peroxidation studies.

2.2. Fatty acid analyses

Lipids were extracted from heart samples with chloroform:methanol (2:1 v/v) in the presence of 0.01% butylated hydroxytoluene. Neutral lipids and phospholipids were separated on Supelclean LC-Si SPE silica cartridges (Supelco, Sigma-Aldrich, Madrid, Spain) by successive elusions with chloroform and methanol. Phosphorous analysis confirmed that the phospholipids were exclusively confined to the fraction eluted with methanol, and the absence of neutral lipids in this fraction was confirmed by thin layer chromatography. Phospholipids were transesterified with 5% HCI in methanol at 75°C for 90 min. After esterification the fatty acid methyl esters (FAME) were extracted in n-pentane in the presence of saturated NaCl. After drying under N_2, the FAME were redissolved in 100 μl carbon disulphide (CS_2) and 1 μl was used for gas chromatography/mass spectrometry (GC/MS) analysis. GC separation was performed in a SP2330 capillary column (30m × 0.25 mm × 0.20 μm) in a Hewlett Packard 5890 Series II gas chromatograph. A Hewlett Packard 5989 A mass spectrometer was used as detector in the electron-impact mode. GC/MS conditions were: the injection port was maintained at 220°C, and the detector at 250°C; the temperature program was 2 min at 100°C, then 10°C/min to 200°C, then 5°C/min to 240°C, a finally hold at 240°C for 10 min. Identification of methyl esters was made by comparison with authentic standards (Sigma).

2.3. Lipid peroxidation analyses

In vivo lipid peroxidation was measured in heart samples by a thiobarbituric acid test specially adapted to tissues (Uchiyama and Mihara, 1978) in the presence of

0.07 mM butyl hydroxytoluene which was added as an antioxidant in order to avoid artefactual lipid peroxidation during the assay. In order to estimate the sensitivity of heart lipids to free radical damage, lipid peroxidation was stimulated in vitro by incubating heart homogenates in the presence of 0.4 mM ascorbate and 0.05 mM $FeSO_4$ for 6 h at 37°C. At this incubation time the lipid peroxidation process has reached completion. At the end of the 6 h of incubation the thiobarbituric acid assay (Uchiyama and Mihara, 1978) was performed. The results were expressed as nanomoles of malondialdehyde-MDA/g tissue. Malondialdehydebis (dimethylacetal) (Merck) was used as standard.

2.4. Statistical analyses

Correlations with MLSP of fatty acids, fatty acid indexes, or lipid peroxidation in the different mammalian species were studied by direct linear regression of these values (equation $y = a + bx$) or of their logarithms ($\log y$ against $\log x$; equation $y = a.x^b$), where y is the fatty acid parameter and x is the MLSP. The correlations were analyzed using the Pearson correlation coefficient (r) and the degree of statistical significance of the correlation (P), and the equation (linear or logarithmic) showing better correlation was chosen. The 0.05 level was selected as the point of minimal statistical significance throughout.

3. Results

The results of the analysis of the fatty acid composition of the diets given to the different animal species are shown in Table 1. No significant correlation with MLSP was observed for the total number of double bonds (double bond index, DBI) nor for single fatty acids, except for 16: $1n-7$ (a minor fatty acid) which showed a positive correlation with MLSP ($P < 0.04$; Table 1).

Table 1
Fatty acid composition (mol%) of the dietary fats[a]

	Mouse/rat	Guinea Pig	Rabbit	Sheep	Pig	Cow	Horse	$P<$
16:0	21	19	17	22	31	21	21	0.47 (NS)
16:1$n-7$	0.5	0.5	0.3	2	3.5	3	2	0.04
18:0	9	3	3	8	9	7	8	0.64 (NS)
18:1$n-9$	29	24	24	29	35	33	26	0.57 (NS)
18:2$n-6$	39	49	51	32	25	35	41	0.42 (NS)
18:3$n-3$	1.5	4.5	5	7	6	1	2	0.97 (NS)
DBI	112	136	138	116	107.5	109	116	0.46 (NS)

[a] Double Bond Index (DBI) = Σ% of unsaturated fatty acids x number of double bonds of each unsaturated fatty acid. The values are means from three independent analysis of each diet. No significant correlation (NS) with MLSP was observed for the DBI or the dietary fatty acids except for 16:1$n-7$ ($r = 0.72$, $P < 0.04$).

Table 2
Fatty acid composition (mol%) of total heart phospholipids from eight mammalian species[a]

	Mouse	Rat	Guinea pig	Rabbit	Sheep	Pig	Cow	Horse
n	6	6	7	5	3	7	7	7
14.0	1.53 ± 0.23	1.51 ± 0.15	2.51 ± 0.22	2.57 ± 1.01	1.07 ± 0.33	1.04 ± 0.39	1.21 ± 0.46	1.97 ± 0.8
16	18.58 ± 0.40	20.42 ± 1.34	22.25 ± 2.01	17.83 ± 2.22	18.01 ± 0.74	18.72 ± 1.63	17.33 ± 0.64	17.31 ± 0.79
16:1*n*−7	0.77 ± 0.04	1.47 ± 0.36	2.94 ± 0.41	3.10 ± 0.76	1.6 ± 0.28	0.71 ± 0.57	1.13 ± 0.30	1.9 ± 0.67
18.0	16.01 ± 0.54	23.99 ± 1.73	16.19 ± 2.34	17.37 ± 3.12	24.25 ± 1.40	18.87 ± 0.65	22.34 ± 0.71	19.82 ± 0.76
18:1*n*−9	14.75 ± 0.46	13.81 ± 0.81	21.54 ± 1.14	21.36 ± 0.39	22.3 ± 2.36	19.17 ± 2.96	19.68 ± 0.43	17.43 ± 0.42
18:2*n*−6	14.76 ± 0.8	15.29 ± 1.05	21.81 ± 1.71	24.85 ± 1.34	19.72 ± 0.68	24.6 ± 1.34	28.03 ± 0.31	30.22 ± 3.77
18:3*n*−3	1.95 ± 0.22	0.85 ± 0.06	2.18 ± 0.54	1.63 ± 0.70	2.10 ± 0.57	0.58 ± 0.13	0.56 ± 0.09	2.76 ± 1.79
20:4*n*−6	6.32 ± 0.53	5.69 ± 0.52	6.88 ± 2.62	11.10 ± 2.51	10.05 ± 1.17	15.65 ± 4.28	9.39 ± 0.23	8.32 ± 1.42
22:6*n*−3	25.28 ± 0.96	16.93 ± 1.16	3.65 ± 0.91	0.15 ± 0.12	0.86 ± 0.22	0.6 ± 0.34	0.29 ± 0.03	0.18 ± 0.02

[a] Values are means ± S.D. *n* = No. of different animals in each species.

Table 3
Indices of fatty acid composition in heart phospholipids of eight mammalian species[a]

	Mouse	Rat	Guinea pig	Rabbit	Sheep	Pig	Cow	Horse
ACL	18.68 ± 0.06	18.29 ± 0.06	17.67 ± 0.14	17.70 ± 0.14	17.80 ± 0.03	17.90 ± 0.15	17.78 ± 0.03	17.70 ± 0.04
SFA	36.14 ± 0.54	45.93 ± 1.35	40.96 ± 0.58	37.78 ± 1.02	43.35 ± 1.98	38.64 ± 1.99	40.88 ± 0.46	39.10 ± 0.66
UFA	63.85 ± 0.54	54.06 ± 1.35	59.03 ± 0.58	62.21 ± 1.02	56.64 ± 1.98	61.35 ± 1.99	59.11 ± 0.46	60.84 ± 0.59
MUFA	15.53 ± 0.46	15.29 ± 1.17	24.48 ± 1.52	24.47 ± 0.98	23.90 ± 2.20	19.89 ± 3.52	20.82 ± 0.74	19.34 ± 1.01
PUFA	48.32 ± 0.91	38.77 ± 0.19	34.54 ± 1.71	37.74 ± 1.34	32.74 ± 0.38	41.45 ± 5.38	38.28 ± 0.37	41.50 ± 1.20
PUFA$n-3$	27.24 ± 0.82	17.78 ± 1.10	5.84 ± 0.42	1.78 ± 0.77	2.96 ± 0.43	1.19 ± 0.38	0.86 ± 0.11	2.95 ± 1.77
PUFA$n-6$	21.08 ± 0.50	20.98 ± 1.17	28.70 ± 1.39	35.95 ± 2.10	29.77 ± 0.48	40.26 ± 5.04	37.42 ± 0.48	38.55 ± 2.84
PI	246.65 ± 8.37	175.62 ± 7.72	83.60 ± 14.68	74.36 ± 7.26	71.62 ± 4.75	93.74 ± 10.56	69.60 ± 0.74	70.99 ± 3.96
DBI	227.93 ± 5.47	172.81 ± 3.93	124.16 ± 9.60	124.40 ± 5.40	115.04 ± 1.18	137.12 ± 17.11	117.92 ± 0.69	122.49 ± 2.49

[a] Values are, means ± S.D.; ACL, average chain length = [(Σ% $\text{Total}_{14} \times 14$) + (Σ% $\text{Total}_{16} \times 16$) + (Σ% $\text{Total}_{18} \times 18$) + (Σ% $\text{Total}_{20} \times 20$) + (Σ% $\text{Total}_{22} \times 22$)]/100;SFA, saturated fatty acids = Σ% (14:0 + 16:0 + 18:0); UFA, unsaturated fatty acids = Σ% (16:1 + 18:1 + 18:3 + 20:4 + 22:6); MUFA, monounsaturated fatty acids = Σ% (16:1 + 18:1); PUFA, polyunsaturated fatty acids = Σ% (18:2 + 18:3 + 20:4 + 22:6); PUFA$n-3$ = Σ%(18:3 + 22:6); PUFA$n-6$ = Σ%(18:2 + 20:4);P1,peroxidizabilityindex = [(%Monoenoic × 0.025) + (%Dienoic × 1) + (%Trienoic × 2) + (%Tetraenoic × 4) + (%Penteenoic × 6) + (%Hexaenoic × 8)] (ref. Holman, 1954; Laganiere and Yu, 1993); DBI, double bond index = Σ% of unsaturated fatty acids × number of double bonds of each unsaturated fatty acid.

Table 4
Summary of correlations between maximum life span (MLSP) and fatty acids or indices of fatty acid composition in heart phospholipids of eight mammalian species[a]

Parameter	r	$P<$	Parameter	r	$P<$
14:0	NS	0.585	ACL	−0.74	0.035*
16:0	NS	0.106	SFA	NS	0.840
16: 1$n-7$	NS	0.941	UFA	NS	0.752
18:0	NS	0.510	MUFA	NS	0.224
18: 1$n-9$	NS	0.162	PUFA	NS	0.586
18:2$n-6$	0.91	0.002*	PUFA$n-3$	−0.86	0.006*
18:3$n-3$	NS	0.811	PUFA$n-6$	0.92	0.001*
20:4$n-6$	NS	0.057	PI	−0.84	0.009*
22:6$n-3$	−0.89	0.003*	DBI	−0.78	0.022*

[a] r, linear correlation coefficient of Pearson; P: statistical significance. The r and P values shown in the table correspond to those of log y versus log x (Power function $y = ax^b$) where x is MLSP and y is the fatty acid parameter. For all parameters showing significant correlations (*) r values were higher and P values were lower in the log y versus log x than in the y versus x correlation. NS = non-significant linear correlation of y versus x and of log y versus log x. The abbreviations of fatty acid indices are explained in Table 3.

The fatty acid composition of heart phospholipids in the eight animal species studied in this investigation is shown in Table 2. The average chain length (ACL), the content in saturated (SFA), unsaturated (UFA), monounsaturated fatty acids (MUFA), PUFA, PUFA of the $n-3$ and $n-6$ series, as well as the DBI and peroxidizability index (PI, which takes into account that the sensitivity to peroxidation increases as a function of the number of double bonds per fatty acid) are shown in Table 3.

Table 4 shows the absence or presence of statistically significant correlation with MLSP for each fatty acid or fatty acid-derived parameter, together with the degree of significance (P) and the positive or negative value of the Pearson correlation coefficient (r). Whereas 16:1$n-7$ was the only fatty acid of the diets, which correlated with MLSP, this was not reflected in the tissue since the 16: 1$n-7$ content of heart phospholipids did not show significant correlation with MLSP (Table 4). The DBI (Fig. 1) and PI showed significant negative correlations with MLSP ($P < 0.022$ and 0.009, respectively). This was mainly due to the negative correlation with MLSP of the highly unsaturated docosahexacnoic acid (22:6$n-3$; $P < 0.003$), and to the positive correlation of the less unsaturated linoleic acid (18:2$n-6$; $P < 0.002$) with MLSP (Fig. 2 and Fig. 3 and Table 4), since the rest of the fatty acids were not correlated with MLSP (Table 4). The negative correlation of PUFA$n-3$ with MLSP ($P < 0.006$) was mainly due to the decrease in 22:6$n-3$ as longevity increases, since it was the only fatty acid of the $n-3$ family correlating with longevity. Analogously, the positive correlation of PUFA$n-6$ with MLSP ($P < 0.001$) was mainly due to differences in 18:2$n-6$ content between species, since the other fatty acid of the $n-6$ family (arachidonic acid, 20:4 $n-6$) was not correlated with MLSP. The decrease in PUFA$n-3$ (22:6$n-3$) as a function of

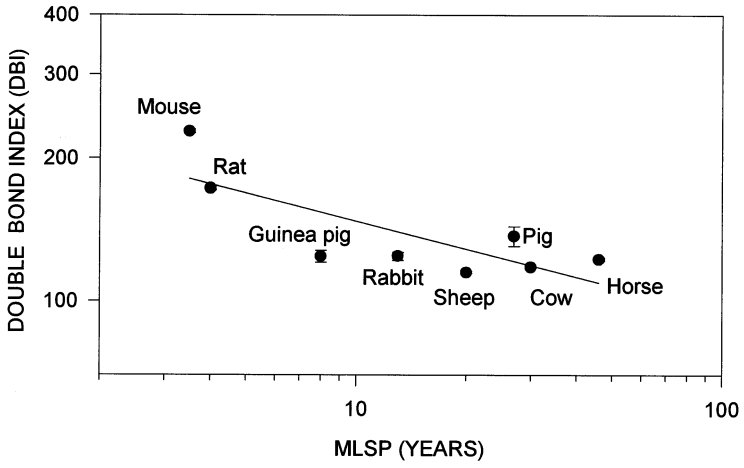

Fig. 1. Relationship between fatty acid double bond index (DBI) and maximum life span(MLSP) in heart phospholipids of eight mammalian species. Values of DBI were plotted as afunction of MLSP, and data were fitted to a straight line by linear regression, $y = 230.7x^{-0.195}$, $r = -0.78$, $P < 0.02$. Values are means $\pm$ SEM. Error bars are not visiblewhen they are smaller than the dot. The number of animals in each species is given in Table 2.

longevity was compensated by the increase in PUFA$n-6$ (18:2$n-6$), since the total PUFA content did not correlate with MLSP (Table 4). The other three indexes of fatty acid unsaturation, MUFA, SFA and UFA, did not show correla-

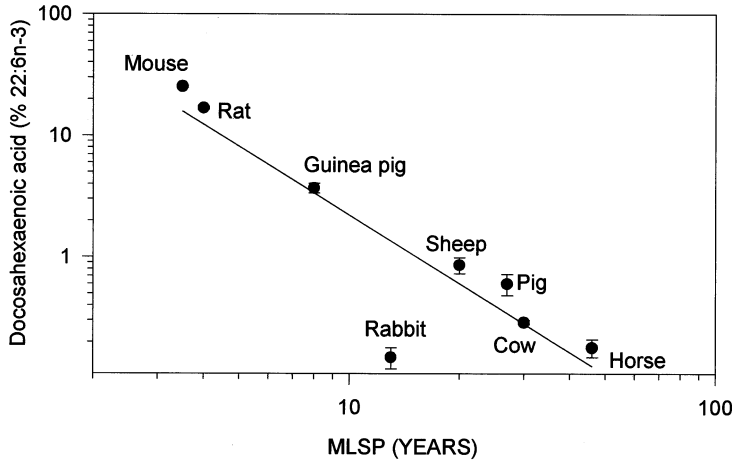

Fig. 2. Relationship between docosahexaenoic acid (22:6$n-3$) content and maximum life span (MLSP) in heart phospholipids of eight mammalian species. Values of docosahexaenoic acid were plotted as a function of MLSP, and data were fitted to a straight line by linear regression, $y = 164.9x^{-0.187}$, $r = -0.89$, $P < 0.003$. Values are means $\pm$ SEM. Error bars are not visible when they are smaller than the dot. The number of animals in each species is given in Table 2.

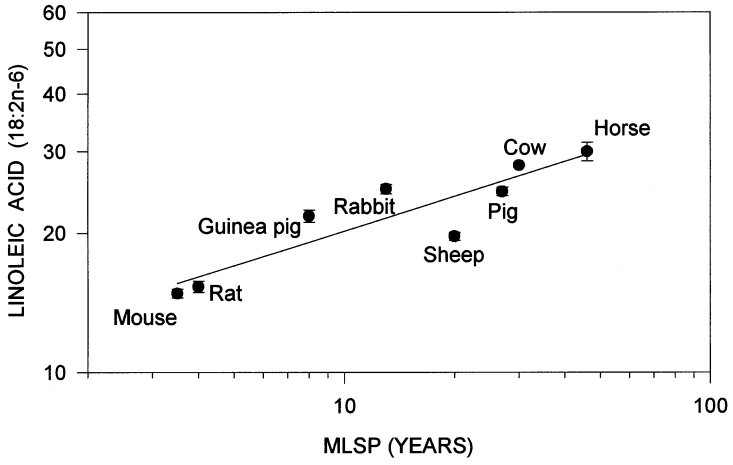

Fig. 3. Relationship between linoleic acid (18:2$n-6$) content and maximum life span (MLSP) in heart phospholipids of eight mammalian species. Values of linoleic acid were plotted as a function of MLSP, and data were fitted to a straight line by linear regression, $y = 11.3x^{0.252}$, $r = 0.91$, $P < 0.002$. Values are means ± SEM. Error bars are not visible when they are smaller than the dot. The number of animals in each species is given in Table 2.

tion with longevity. The average chain length (ACL) was negatively correlated ($P < 0.035$) with MLSP (Table 4).

In agreement with the lower DBI and PI of longevous animals, the sensitivity of the heart lipids to in vitro stimulated lipid peroxidation was negatively correlated

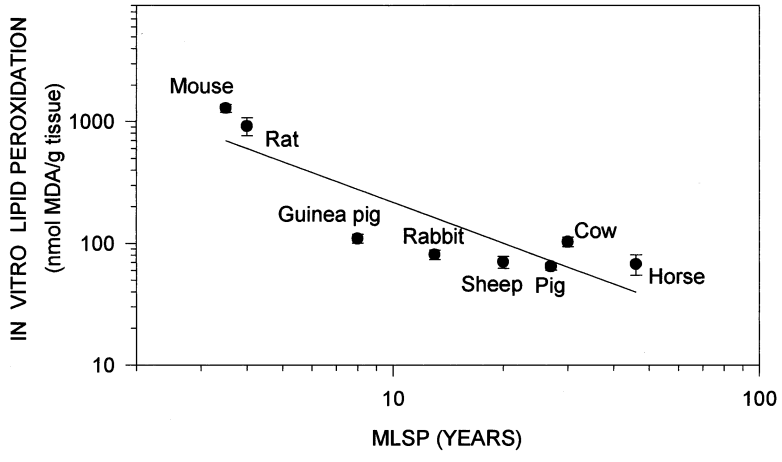

Fig. 4. Relationship between sensitivity to in vitro lipid peroxidation and maximum life span (MLSP) in the heart of eight mammalian species. Values of in vitro lipid peroxidation were plotted as a function of MLSP, and data were fitted to a straight line by linear regression, $y = 2790x^{-0.116}$, $r = -0.87$, $P < 0.005$. Values are means ± SEM. Error bars are not visible when they are smaller than the dot. The number of animals in each species is given in Table 2.

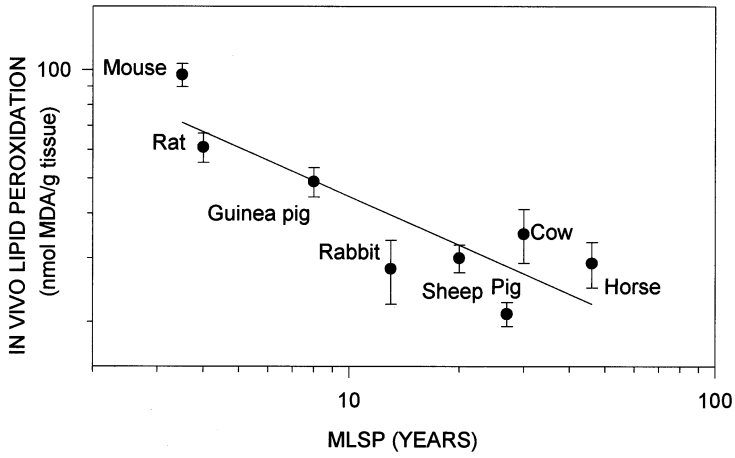

Fig. 5. Relationship between in vivo lipid peroxidation and maximum life span (MLSP) in the heart of eight mammalian species. Values of lipid peroxidation were plotted as a function of MLSP, and data were fitted to a straight line by linear regression, $y = 126.1\ x^{-0.459}$, $r = -0.86$, $P < 0.005$. Values are means $\pm$ SEM. The number of animals in each species is given in Table 2.

with MLSP ($r = -0.87$, $P < 0.005$, Fig. 4). This was also reflected in the endogenous content of lipid peroxidation products, since the in vivo lipid peroxidation of heart samples was also negatively correlated with the MLSP of the animal species ($r = -0.86$, $P < 0.005$, Fig. 5).

4. Discussion

In agreement with previous comparative studies in mitochondria of mammals and birds (Pamplona et al., 1996, 1998, 1999a,b) we found in this investigation that the total number of double bonds (DBI) in mammalian heart phospholipids is negatively correlated with MLSP, i.e., the heart phospholipids of longevous mammals have a lower degree of unsaturation than those of short-lived mammals. This is not due to decreases in unsaturated fatty acids, but to a redistribution between types of unsaturated fatty acids, mainly changing from the highly unsaturated docosahexaenoic (22:6$n-3$) acid in short-lived animals to the less unsaturated linoleic acid (18:2$n-6$) in long-lived ones. This redistribution decreases the DBI of longevous animals without decreasing their UFA and PUFA levels, since the decrease in PUFA$n-3$ (due to the low 22:6$n-3$) in longevous animals is compensated by their higher PUFA$n-6$ (due to high 18:2$n-6$). Similarly, previous studies in different tissues, subcellular fractions, or species usually showed that the low degree of fatty acid unsaturation of longevous animals is obtained by analogous redistributions between types of PUFAS without decreasing the total PUFA or UFA content (Pamplona et al., 1996, 1998, 1999a,b).

The 22:6$n-3$ to 18:2$n-6$ redistribution observed in this study from short- to long-lived animals can be due in part to the presence of low a delta-6 and a delta-5 desaturase activities in the heart of longevous mammals. These enzymes are rate limiting in the multistep pathways of essential fatty acid synthesis from their dietary precursors, 22:6$n-3$ from 18:3$n-3$ and 20:4$n-6$ from 18:2$n-6$. However, remodeling of the acyl substituents of phospholipids via deacylation-reacylation reactions, or catabolic pathways, specific for the different PUFAS, must be also involved, otherwise longevous animals should also show lower 20:4$n-6$ and higher 18:3$n-3$ than short-lived ones.

That the different diets can not be responsible for the different double bond contents of the different mammalian species studied here is shown by the simultaneous study of the fatty acid composition of their diets and their heart tissues. Even though the DBI of the heart phospholipids was negatively correlated with the MLSP of the species studied, this was not true for the DBI of their diets. In fact, any of the main dietary fatty acids showed correlation with MLSP. Only 16:1$n-7$, a minor dietary fatty acid, was positively correlated with MLSP, but this was not reflected in its heart content in the different species, which did not correlate with longevity. The independence of heart DBI on dietary DBI is consistent with the well-known fact that the double bond content of tissue fatty acids is homeostatically regulated (Jeffcoat, 1979; Maresca and Cossins, 1993) by various mechanisms including regulation by PUFA of desaturase enzyme gene expression (Sessier and Ntambi, 1998).

In a first comparative study about fatty acid composition (Gudbjarnason, 1989), levels of 22:6$n-3$ in heart phospholipids strongly decreased in the order: mouse-rat-rabbit-man-whale. This is an order of progressive increase in MLSP. That investigator correlated the decrease in 22:6$n-3$ across species with corresponding progressive decreases in heart rates. He also showed strong increases in 20:4$n-6$ and 22:6$n-3$ and strong decreases in 18:2$n-6$ in the rat heart during aging (Gudbjarnason, 1989). In the only other comparative work performed to date on the subject (Couture and Hulbert, 1995a) it was found, like in our case, that strong progressive significant decreases in the total number of double bonds occurred in the heart, skeletal muscle and kidney phospholipids as body size increased in five mammals following the order: mouse-rat-rabbit-sheep-cattle. Although the degree of statistical correlations of each individual fatty acid against body size was not systematically given. the authors found also as major differences between species that body size negatively correlated with 22:6$n-3$ in heart and skeletal muscle, and positively correlated with 18:2$n-6$ in the heart. These authors suggested that the decrease in fatty acid unsaturation in large mammals is probably a regulated phenomenon, and that it is not the PUFA content what changes between species, but their degree of unsaturation. PUFAs are needed in all animals for various purposes including the proper function of many membrane linked proteins (Lee, 1991).

Concerning the physiological meaning of the decrease in the degree of unsaturation in large mammals, there are various possibilities. First, unsaturation can potentially increase membrane fluidity. Whereas strong increases on lipid fluidity

are observed after introduction of the first double bond to a saturated fatty acid, smaller effects are observed with two double bounds, and negligible effects occur with three or more double bonds (Brenner, 1984). This is so because the kink (or coiling) in the fatty acyl chain due to addition of a double bond has a larger impact on fluidity when the double bond is situated at the center of the fatty acid chain (first double bond) that when it is situated progressively nearer to its extremes (next double bond additions; Brenner, 1984). Thus, a change in heart PUFA composition from the highly unsaturated 22:6$n-3$ to the less unsaturated 18:2$n-6$ would allow a decrease in double bond content of longevous mammals of large body size without a major perturbation of membrane fluidity, a parameter needed for proper function of membrane enzymes, ion pumps, receptors, electron transport, etc. If some moderate decrease in membrane fluidity would still occur in longevous animals due to the 22: 6$n-3$ to 18:2$n-6$ redistribution, it could be compensated with the negative correlation observed here between heart fatty acid chain length and MLSP, since decreases in fatty acid chain length increase the fluidity of membranes. Thus, the global differences in heart fatty acids observed here can represent an 'homeoviscous' evolutionary adaptation between mammals of different body size, metabolic rate and longevity. A different situation concerns lipid permeability, since it increases continuously as a function of the number of double bonds (Stubbs and Smith, 1984; Brand et al., 1994). This led to the proposal that small mammals have more fatty acid double bonds in their tissues in order to increase the permeability of their cellular membranes (Couture and Hulbert, 1995a). This would force them to actively pump more ions per unit time in order to maintain similar transmembrane ion gradients than large mammals, thus increasing their tissue basal metabolic rates through futile cycles leading to higher rates of heat generation. These authors have in fact demonstrated that small animals have liver plasma membranes more leaky to Na^+ and K^+ (Couture and Hulbert, 1995b). Others have found a similar situation concerning H^+ in the liver: proton leak at the inner mitochondrial membrane (the main determinant of respiration in State 4) is negatively correlated with body size in 11 species of mammals from mouse to horse in isolated liver mitochondria and hepatocytes (Porter and Brand, 1993, 1995).

We propose a complementary possible evolutionary reason for the low number of double bonds of mammals with large body size (Pamplona et al., 1998). It is well known that the susceptibility of fatty acids to free radical damage increases exponentially as a function of the number of double bonds per fatty acid molecule, 18:2$n-6$ being relatively stable to oxidative stress in relation to the most highly unsaturated fatty acid found in tissues, 22: 6$n-3$. In fact, many studies have shown that free radical damage and lipid peroxidation increase as a function of the degree of unsaturation of the fatty acid substrates present in the tissues in vivo (Laganiere and Yu, 1990; North et al., 1994; Takeuchi et al., 1991; Bondy and Marwah, 1995; Kobayashi et al., 1996). In addition, lipid peroxidation products are known to damage nearby macromolecules including proteins (Forsmark-Andree et al., 1995; Pamplona et al., 1999a) and DNA (Draper, 1995), with possible long-term consequences for aging. Increases in PI and decreases in 18:2$n-6$ have been described in rodent aging (Gudbjarnason, 1989; Laganiere and Yu, 1993), and the senescent

accelerated prone mouse (SAM-P) has somewhat higher 22:6$n-6$, 20:4/18:2 and PI and lower 18:2$n-6$ in the brain that their SAM-resistant counterparts (Choi et al., 1996). It is reasonable to think that the low degree of fatty acid unsaturation of longevous animals of large body size will protect their tissues against oxidative damage, while at the same time it may also contribute to lowering their basal metabolic rates. The negative correlation observed in this study between heart in vitro lipid peroxidation and MLSP is consistent with such a protective role against oxidative stress. A previous study showed that rates and maximum spontaneous (non-catalyzed) in vitro lipid peroxidation of kidney and brain homogenates are negatively correlated with MLSP in 24 (brain) or nine (kidney) species of mammals from mouse to man (Cutler, 1985). Fatty acid composition was not analyzed in that study, but the author suggested that the major factor determining the lipid peroxidation potential of tissues is the concentration of peroxidizable substrate and that a change in the composition of lipid membranes occurred during the evolution of increased MLSP in mammals, resulting in the tissues being less susceptible to oxygen radical-initiated peroxidation reactions (Cutler, 1985). Our study confirms this last suggestion in the heart of mammals describing the biochemical nature of that change and a previous study is consistent with the same happening in the kidney and skeletal muscle (Couture and Hulbert, 1995a). Our study also shows that the different double bond content of the heart of mammals with different longevities is important not only under conditions o a high level of oxidative stress (in vitro peroxidation), but also under normal basal conditions, since the in vivo levels of lipid peroxidation in the heart were also negatively correlated with MLSP.

Finally, we have previously shown that three different bird species and humans (specially longevous species) also show lower double bond content and sensitivity to lipid peroxidation in heart or liver than rodents (Pamplona et al., 1996, 1999a,b). Thus, while the low DBI of mammals of large body size can be useful to them both to decrease metabolic rate and to protect tissue components against oxidative damage, the low DBI of birds can only be used for the second of these two functions, since the metabolic rate of these birds are high (not low) and similar to those of the rodents used in the comparison. The same kind of concept applies to humans, who are four fold more longevous than expected from its mammalian body size and metabolic rate. A low degree of fatty acid unsaturation seems then to be a characteristic trait of longevous animals, birds, humans, and mammals of large body size, regardless of their possession of large or low metabolic rates.

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References

Altman, P., Dittmer, P., 1972. Life spans: animals. In: Biology Data Book. Fed. Am. Soc. Exp. Biol., Bethesda, pp. 229–235.

Barja, G., 1999. Mitochondrial free radical generation: sites of production in states four and three, organ specificity and relationship with aging rate J. Bioenergetics Biomembr. 31, 347–365.

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Bondy, S.C., Marwah, S., 1995. Stimulation of synaptosomal free radical production by fatty acids: relation to esterification and to degree of unsaturation. FEBS Letters 375, 53–55.

Brand, M.D., Couture, P., Hulbert, A.J., 1994. Liposomes from mammalian liver mitochondria are more polyunsaturated and leakier to protons than those from reptiles. Comp. Biochem. Physiol. 108B, 181–188.

Brenner, R.R., 1984. Effect of unsaturated fatty acids on membrane structure and enzyme kinetics. Progr. Lipid Res. 23, 69–96.

Choi, J.H., Kim, J., Moon, Y.S., Chung, H.Y., Yu, B.P., 1996. Analysis of lipid composition and hydroxyl radicals in brain membranes of senescent-accelerated mice. Age 19, 1–5.

Couture, P., Hulbert, A.J., 1995a. Membrane fatty acid composition of tissues is related to body mass of mammals. J. Membr. Biol. 148, 27–39.

Couture, P., Hulbert, A.J., 1995b. Relationship between body mass, tissue metabolic rate, and sodium pump activity in mammalian liver and kidney. Am. J. Physiol. 268, R645–R650.

Cutler, R.G., 1985. Peroxide-producing potential of tissues: inverse correlation with longevity of mammalian species. Proc. Natl. Acad. Sci. USA 82, 4798–4802.

Draper, H.H., 1995. Effects of peroxidative stress and age on the concentration of a deoxyguanosine-malondialdehyde adduct in rat DNA. Lipids 30, 959–961.

ForsmarkAndree, P., Dallner, G., Ernster, L., 1995. Endogenous ubiquinol prevents protein modification accompanying lipid peroxidation in beef heart submitochondrial particles. Free Rad. Biol. Med. 19, 749–757.

Gudbjarnason, S., 1989. Dynamics of $n-3$ and $n-6$ fatty acids in phospholipids of heart muscle. J. Intern. Med. 225, 117–128.

Harman, D., 1998. Extending functional life span. Exper. Gerontol. 33, 95–112.

Holman, T.R., 1954. Autoxidation of fats and related substances. In: Holman, R.T., Lundberg, W.O., Malkin, T. (Eds.), Proggress in the Chemistry of Fats and other Lipids. Academic Press, New York, pp. 491–514.

Jeffcoat, R., 1979. The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem. 15, 1–36.

Kobayashi, T., Shimizugawa, T., Fukamizu, Y., Huang, M.Z., Watanabe, S., Okuyama, H., 1996. Nutr. Biochem. 7, 542–548.

Laganiere, S., Yu, B.P., 1990. Studies on membrane lipid peroxidation in omega-3 fatty acidified autoimmune mice: effect of vitamin E supplementation. Adv. Exp. Med. Biol. 262, 95–102.

Laganiere, S., Yu, B.P., 1993. Modulation of membrane phospholipid fatty acid composition by age and food restriction. Gerontology 39, 7–18.

Lee, A.G., 1991. Lipids and their effects on membrane proteins: evidence against a role for fluidity. Prog. Lipid Res. 30, 323–348.

Maresca, B., Cossins, A.R., 1993. Fatty acid feedback and fluidity. Nature 365, 606–607.

North, J.A., Spector, A.A., Buettner, G.R., 1994. Cell fatty acid composition affects free radical formation during lipid peroxidation. Am. J. Physiol. 267, C177–C188.

Pamplona, R., Prat, J., Cadenas, S., Rojas, C., Perez-Campo, R., LopezTorres, M., Barja, G., 1996. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from longevous species: the pigeon and human case. Mech. Ageing Dev. 86, 53–66.

Pamplona, R., Portero-Otín, M., Riba, D., Ruiz, C., Prat, J., Bellmunt, M.J., 1998. Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. J. Lipid Res. 39, 1989–1994.

Pamplona, R., PorteroOtín, 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. Agein Dev. 106, 283–296.

Pamplona, R., Portero-Otín, M., Riba, D., Ledo, F., Gredilla, R., Herrero, A., Barja, G, l999b. 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.

Pearl, R., 1928. The Rate of Living. University of London Press, London.

Porter, R.K., Brand, M., 1993. Body mass dependence of H+ leak in mitochondria and its relevance to metabolic rate. Nature 362, 628–629.

Porter, R.K., Brand, M., 1995. Causes of differences in respiration rate of hepatocytes from mammals of different body mass. Am. J. Physiol. 269, R1213–R1224.

Sessier, A., Ntambi, J.M., 1998. Polyunsaturated fatty acid gene expression. J. Nutr. 128, 923–926.

Sohal, R.S., Weindruch, R., 1996. Oxidative stress, caloric restriction and aging. Science 273, 59–63.

Stubbs, C.D., Smith, A.D., 1984. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim. Biophys. Acta 779, 89–137.

Takeuchi, Y., Morii, H., Tamura, M., Hayaishi, O., Watanabe, Y., 1991. A possible mechanism of mitochondrial dysfunction during cerebral ischemia: inhibition of mitochondrial respiratory activity by arachidonic acid. Arch. Biochem. Biophys. 289, 33–38.

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271–278.