Correlation of Fatty Acid Unsaturation of the Major Liver Mitochondrial Phospholipid Classes in Mammals to Their Maximum Life Span Potential

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ABSTRACT: Free radical damage is considered a determinant factor in the rate of aging. Unsaturated fatty acids are the tissue macromolecules that are most sensitive to oxidative damage. Therefore, the presence of low proportions of fatty acid unsaturation is expected in the tissues of long-lived animals. Accordingly, the fatty acid compositions of the major liver mitochondrial phospholipid classes from eight mammals, ranging in maximum life span potential (MLSP) from 3.5 to 46 yr, show that the total number of double bonds is inversely correlated with MLSP in both phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) ($r = 0.757$, $P < 0.03$, and $r = 0.862$, $P < 0.006$, respectively), but not in cardiolipin ($P = 0.323$). This is due not to a low content of unsaturated fatty acids in long-lived animals, but mainly to a redistribution between kinds of fatty acids on PtdCho and PtdEtn, shifting from arachidonic ($r = 0.911$, $P < 0.002$, and $r = 0.681$, $P = 0.05$, respectively), docosahexaenoic ($r = 0.931$ and $r = 0.965$, $P < 0.0001$, respectively) and palmitic ($r = 0.944$ and $r = 0.974$, $P < 0.0001$, respectively) acids to linoleic acid ($r = 0.942$, $P < 0.0001$, for PtdCho; and $r = 0.957$, $P < 0.0001$, for PtdEtn). For cardiolipin, only arachidonic acid showed a significantly inverse correlation with MLSP ($r = 0.904$, $P < 0.002$). This pattern strongly suggests the presence of a species-specific desaturation pathway and deacylation-reacylation cycle in determining the mitochondrial membrane composition, maintaining a low degree of fatty acid unsaturation in long-lived animals.

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Several lines of evidence indicate that mitochondria and oxidative damage can be implicated both in pathological responses and in the aging process (1). The available comparative studies indicate that maximum life span potential (MLSP) is inversely related to mitochondrial free radical production (2,3) and mitochondrial DNA oxidative damage (4,5). Although these very important characteristics are consistent with free radical–oxidative stress theories of aging (6,7), additional factors related to other macromolecules also can lead to a low level of oxidative damage in long- *vs.* short-lived animal species.

Among cellular macromolecules, polyunsaturated fatty acids (PUFA) 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. Since both oxygen consumption and reactive oxygen species formation occur predominantly in mitochondrial membranes, a low degree of fatty acid unsaturation in these membranes may be advantageous, in oxidative stress terms, by decreasing their sensitivity to lipid peroxidation. This would also protect other molecules against lipoxidation-derived damage. In line with this, it has been suggested that in long-lived species a low degree of total tissue and mitochondrial fatty acid unsaturation (low double-bond content) is accompanied by a low sensitivity to lipid peroxidation and a low concentration of the lipoxidation-derived adducts malondialdehyde-lysine and N^{ε}-(carboxymethyl)lysine in several tissues and mitochondrial proteins (8–12). Independent experiments have also demonstrated a negative correlation between sensitivity to lipid autoxidation and MLSP in brain and kidney homogenates from different mammalian species (13).

Mitochondria from different mammalian tissues are similar with respect to their phospholipid distribution (14). The major phospholipids, phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn), together account for about 70–80% of total phospholipids; cardiolipin constitutes 10–20% of total mitochondrial phospholipids.

Phospholipids play multiple roles in mitochondria including establishing a permeability barrier, providing the matrix for the assembly and function of a wide variety of catalytic processes, acting as donors in the synthesis of macromolecules, and actively influencing the functional properties of membrane-associated processes. The wide range of processes in which specific involvement of phospholipids has been documented explains the need for diversity in phospholipid structure and fatty acid composition (15).

In this work, the fatty acid compositions of the phospho-

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Abbreviations: AA, arachidonic acid; ACL, average chain length; DBI, double bond index; DHA, docosahexaenoic acid; GC–MS, gas chromatography–mass spectrometry; LA, linoleic acid; MLSP, maximum life span potential; MSE buffer, mannitol, sucrose, and EGTA at pH 7.4; MUFA, monounsaturated fatty acids; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids; TLC, thin-layer chromatography; UFA, unsaturated fatty acids.

lipids PtdCho, PtdEtn, and cardiolipin from liver mitochondria of eight mammalian species ranging in MLSP from 3.5 to 46 yr were analyzed. The results obtained show that the degree of fatty acid unsaturation is inversely correlated with MLSP in PtdCho and PtdEtn, while there is no relationship between cardiolipin composition and MLSP.

MATERIALS AND METHODS

Chemicals. Phospholipid standards were obtained from Avanti Polar Lipids (Alabaster, AL); fatty acid methyl ester standards, from Sigma (St. Louis, MO); gas chromatography columns, from Teknokroma (Teknokroma SCCL, Barcelona, Spain); silica gel thin-layer chromatography (TLC) plates, from Whatman (Fisher, Cincinnati, OH); other reagents were purchased from Sigma unless otherwise specified. All chemicals were of analytical grade.

Animals and diets. All animals, namely, mouse (*Mus musculus, n* = 6), hamster (*Critecus critecus, n* = 4), guinea pig (*Cavia porcellus, n* = 4), rabbit (*Oryctolagus cuniculus*, *n* = 6), sheep (*Ovis aries, n* = 6), pig (*Sus scrofa, n* = 5), cow (*Bos taurus, n* = 4), and horse (*Equus caballus, n* = 5), whose MLSP vary from 3.5 to 46 yr, were adult specimens with an age at 15–30% of their MLSP (equivalent to an age ranging from 0.5 to 6 yr). The recorded values of MLSP (in yr) were: mouse, 3.5; hamster, 4; guinea pig, 8; rabbit, 13; sheep, 20; pig, 27; cow, 30; and horse, 46 (16). The animal care protocols were approved by the University of Lleida Animal Experimentation Ethics Committee. Mice, hamsters, guinea pigs, and rabbits were killed by decapitation. Sheep, pigs, cows, and horses (farm animals) were sacrificed at the abattoir. Samples of diet administered during the adult life of the animals were obtained at the sacrifice.

Mitochondrial isolation. Tissue samples were taken from the main hepatic lobe and were immediately processed. Mitochondrial fractions were isolated by standard methods of homogenization and differential centrifugation as previously described (8). Liver samples (2–3 g) were briefly and gently homogenized with a loose fitting pestle hand-operated glass-glass homogenizer in 10 mL of MSE buffer (225 mM mannitol, 75 mM sucrose and 1 mM EGTA, pH 7.4) containing 5 mg of nagarse, a bacterial proteinase (EC 3.4.21.14) from Fluka (product no. 82518, Sigma Co.), and 25 mg of albumin. After standing for 1 min, 25 mL of additional MSE buffer containing 25 mg of albumin was added, and homogenization was gently performed again with a tighter-fitting pestle. The homogenates were centrifuged for 3 min at 1,500 × *g* (5°C) in a RC5C Sorvall centrifuge. The supernatants were centrifuged 10 min at 9,800 × *g,* the pellets were resuspended, and the procedure was repeated two more times. All procedures were performed at 0–4°C.

The purity of the mitochondrial fractions was tested by determining 5′-nucleotidase (as marker for plasma membrane), peroxidase (for peroxisomes), glucose-6-phosphatase (for endoplasmic reticulum), acid phosphatase (for lysosomes), and cytochrome c oxidase (for mitochondria) activities according to published methods (17). Based on the specific activities of the different markers, our mitochondrial preparations contains approximately 1% of nonmitochondrial subcellular membranes.

Lipid extraction and phospholipid classes separation. Lipids from mitochondria were extracted into chloroform/methanol (2:1 vol/vol) by the method of Folch *et al.* (18) in the presence of 0.01% butylated hydroxytoluene. The phospholipid classes were separated by TLC on silica gel plates. Development with *n*-hexane/1,2-dichloroethane/methanol/formic acid (16:14:4:1 by vol) was performed, followed by 1,2-dichloroethane solvent system (19). Fractions were made visible by spraying with 0.02% 8-anilino-1-naphthalenesulfonic acid in ethanol. The bands on the plates corresponding to major mitochondrial phospholipids (PtdCho, PtdEtn, and cardiolipin), identified by comparison with authentic standards, were scraped, transferred to screw-capped tubes, and transesterified.

To quantify the percentages of major phospholipid classes, lipid samples were separated on Silica gel 60A LK6D TLC plates (Whatman, Clifton, NJ), by using the solvent system described above. Separated lipid fractions were detected using a 10% cupric sulfate in 8% phosphoric acid solution, followed by charring at 160°C for 20 min, and were quantified by scanning densitometry using a Shimadzu CS-9001PC dual-wavelength flying spot scanner (Shimadzu Europe GmbH, Duisburg, Germany).

Fatty acid analysis. Mitochondrial phospholipid fatty acids were transesterified in 2.5 mL of 5% methanolic HCl at 75°C for 90 min. The resulting methyl esters were extracted by adding 2.5 mL *n*-pentane and 1 mL saturated NaCl. The *n*-pentane phase was separated and evaporated under N_2, and the fatty acid methyl esters were redissolved in 100 μL of carbon disulfide. One microliter was submitted to gas chromatography–mass spectrometry (GC–MS) analysis. GC separation was performed on an SP2330 capillary column (30 m × 0.25 mm × 0.20 μm) in a Hewlett-Packard 6890 Series II gas chromatograph (Hewlett-Packard, S.A., Barcelona, Spain). A Hewlett-Packard 5973 mass spectrometer was used as detector in the electron-impact mode. 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, and finally hold at 240°C for 10 min. Identification of methyl esters was made by comparison with authentic standards and on the basis of mass spectra. Results are expressed as mol%.

Calculations and statistics. The average chain length is calculated as ACL= $[(\Sigma\ \%\text{Total}_{16} \times 16) + \cdots + (\Sigma\ \%\text{Total}_{n} \times n)]/100$ (*n* = carbon atom number); the double bond index as DBI = (Σ %Monoenoic × 1) + (Σ %Dienoic × 2) + (Σ %Trienoic × 3) + (Σ %Tetraenoic × 4) + (Σ %Pentaenoic × 5) + (Σ %Hexaenoic × 6); saturated fatty acids as SFA = Σ % (16:0 + 18:0); unsaturated fatty acids as UFA = Σ % (16:1 + 18:1 + 18:2 + 18:3 + 20:3 + 20:4 + 22:6); monounsaturated fatty acids as MUFA = Σ % (16:1 + 18:1); polyunsaturated fatty acids as PUFA = Σ % (18:2 + 18:3 + 20:3 + 20:4 + 22:6); n-3 polyunsaturated fatty acids as PUFAn-3 = Σ % (18:3 + 22:6); and finally, n-6 polyunsaturated fatty acids as PUFAn-6 = Σ% (18:2 + 20:3 + 20:4).

Regression equations were obtained by nonlinear regression analyses with the curve estimation statistic by SPSS/PC software for Windows (SPSS, Chicago, IL). These regressions were determined and tested for significance using the mean values for each species. The 0.05 level was selected as the point of minimal statistical significance. Values in tables and figures are expressed as mean ± SEM.

RESULTS

The fatty acid composition of the diets of the different animal species showed that the DBI of the dietary fats was not correlated with MLSP (Table 1). From analytical TLC, mitochondrial phospholipid distribution was calculated. In all the mammalian species studied in this work, the major phospholipid was PtdCho, in the range of 40–50%, followed by the PtdEtn fraction in the range of 23–35%. Cardiolipin was present in the range of 14–21%. Phospholipid distribution among species was not correlated with MLSP (data not shown). The complete fatty acid composition and indexes related to the degree of unsaturation, the chain length, or to the main fatty acid types or series of the major liver mitochondrial phospholipids, namely, PtdCho, PtdEtn, and cardiolipin, are shown in Tables 2, 3, and 4. Summaries of correlations between MLSP and fatty acid composition or fatty acid indexes of different phospholipid classes from liver mitochondria in the mammalian species are included in Tables 2, 3 and 4.

Neither the ACL nor the percentage of total MUFA shows statistically significant correlation with MLSP in any of the phospholipid classes analyzed. In contrast, total SFA and UFA contents show highly significant correlations with MLSP, in a negative and positive way, respectively, for PtdCho and PtdEtn, whereas no relationship was observed for the cardiolipin fraction. The decrease in the percentage of SFA with increases in MLSP is due to the negative correlation of palmitic acid (16:0) with MLSP in both PtdCho and PtdEtn (Figs. 1A, 1B); in contrast, no significant correlations were observed with cardiolipin. In the PtdCho and PtdEtn fractions docosahexaenoic acid (22:6n-3, DHA) was negatively correlated with maximum longevity, whereas the contrary was true

TABLE 1
Fatty Acid Composition (mol%) and Double Bond Index (DBI) of the Dietary Fats (*n* = 3)

	Mouse	Hamster	Guinea pig	Rabbit	Sheep	Pig	Cow	Horse
16:0	21	26	19	18	22	31	21	21
16:1n-7	0.5	4	0.5	0.5	2	3.5	3	2
18:0	9	9	3	3	8	9	7	8
18:1n-9	29	22	24	23.5	29	35	33	26
18:2n-6	39	36	49	50	32	25	35	41
18:3n-3	1.5	3	4.5	5	7	6	1	2
DBI[a]	112	107	136	139	116	107.5	109	116

[a]DBI = (Σ % Monoenoic × 1) + (Σ % Dienoic × 2) + (Σ % Trienoic × 3).

TABLE 2
Fatty Acid Composition (mol%) and General Indexes Related to Membrane Fatty Acid Composition of Liver Mitochondrial Phosphatidylcholine from Mammalian Species[a]

	Mouse	Hamster	Guinea pig	Rabbit	Sheep	Pig	Cow	Horse	
	3.5	4	8	13	20	27	30	46	r^b
16:0	33.97 ± 1.03	33.11 ± 0.62	26.90 ± 1.00	24.59 ± 0.81	18.49 ± 0.47	20.10 ± 1.13	19.52 ± 0.85	13.39 ± 0.50	0.944[d,g]
16:1n-7	0.21 ± 0.01	0.21 ± 0.001	0.59 ± 0.06	0.75 ± 0.12	1.84 ± 0.54	1.40 ± 0.27	0.45 ± 0.007	0.47 ± 0.12	0.225
18:0	7.92 ± 0.53	7.81 ± 0.42	18.22 ± 1.03	11.25 ± 0.67	18.04 ± 0.25	19.22 ± 0.46	17.30 ± 0.41	17.62 ± 1.08	–0.850[d,f]
18:1n-9	15.46 ± 0.71	19.07 ± 1.31	16.57 ± 0.97	15.27 ± 0.81	18.69 ± 0.38	14.96 ± 0.62	15.72 ± 0.26	14.31 ± 0.67	–0.518
18:2n-6	17.93 ± 0.45	21.72 ± 2.15	27.67 ± 0.59	36.18 ± 2.28	32.90 ± 0.48	32.77 ± 0.48	41.18 ± 1.20	52.58 ± 1.24	0.942[e,g]
18:3n-3	0.17 ± 0.01	0.14 ± 0.006	0.47 ± 0.46	0.58 ± 0.10	0.56 ± 0.01	0.48 ± 0.01	0.08 ± 0.001	0.14 ± 0.03	–0.187
20:3n-6	2.12 ± 0.03	2.24 ± 0.11	0.25 ± 0.02	0.46 ± 0.11	0.31 ± 0.006	1.22 ± 0.30	0.07 ± 0.001	0.12 ± 0.02	0.852[d,f]
20:4n-6	14.38 ± 0.81	11.13 ± 1.43	8.71 ± 0.89	10.58 ± 0.61	8.62 ± 0.28	8.93 ± 0.74	5.45 ± 0.09	1.20 ± 0.04	–0.911[c,f]
22:6n-3	7.79 ± 0.81	4.54 ± 0.86	0.57 ± 0.11	0.30 ± 0.02	0.50 ± 0.01	0.85 ± 0.03	0.19 ± 0.003	0.12 ± 0.02	0.931[d,g]
ACL	17.95 ± 0.06	17.78 ± 0.05	17.65 ± 0.003	17.72 ± 0.01	17.79 ± 0.007	17.80 ± 0.03	17.71 ± 0.01	17.75 ± 0.01	–0.246
SFA	41.89 ± 0.50	40.93 ± 1.05	45.13 ± 0.33	35.85 ± 1.39	36.54 ± 0.40	39.33 ± 1.58	36.82 ± 1.25	31.02 ± 0.60	–0.804
UFA	58.10 ± 0.50	59.06 ± 1.05	54.86 ± 0.33	64.14 ± 1.39	63.45 ± 0.40	60.66 ± 1.58	63.17 ± 1.25	68.97 ± 0.60	0.804
MUFA	15.68 ± 0.69	19.28 ± 1.31	17.17 ± 0.92	16.02 ± 0.79	20.53 ± 0.54	16.37 ± 0.64	16.17 ± 0.27	14.79 ± 0.60	–0.397
PUFA	42.41 ± 1.19	39.78 ± 0.26	37.69 ± 1.05	48.12 ± 2.02	42.92 ± 0.29	44.28 ± 1.40	46.99 ± 1.21	54.18 ± 1.18	0.820
PUFAn-3	7.97 ± 0.82	4.68 ± 0.87	1.05 ± 0.32	0.88 ± 0.11	1.07 ± 0.02	1.34 ± 0.05	0.28 ± 0.004	0.27 ± 0.05	0.933[d,g]
PUFAn-6	34.44 ± 0.43	35.09 ± 0.61	36.64 ± 0.78	47.23 ± 1.93	41.85 ± 0.28	42.93 ± 1.44	46.71 ± 1.21	53.91 ± 1.20	0.900[e,f]

[a]The maximum life span potential (MLSP, in years) of each mammal is indicated under the species name in the column headings.

[b]For all variables, statistical significance intergroups was $P < 0.0001$, r, nonlinear regression coefficient vs. MLSP (for each regression equation the degrees of freedom were 6), (c) $y = a + bx$; (d) $y = a + b/x$; (e) $y = a \cdot x^b$; (f) $P < 0.01$; (g) $P < 0.001$. Abbreviations: ACL, average chain length; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; PUFAn-3, polyunsaturated fatty acids n-3 series; PUFAn-6, polyunsaturated fatty acids n-6 series. For more information, see the Materials and Methods section.

TABLE 3
Fatty Acid Composition (mol%) and General Indexes Related to Membrane Fatty Acid Composition of Liver Mitochondrial Phosphatidylethanolamine from Mammalian Species[a]

	Mouse 3.5	Hamster 4	Guinea pig 8	Rabbit 13	Sheep 20	Pig 27	Cow 30	Horse 46	*r*[b]
16:0	22.80 ± 0.51	18.09 ± 1.09	11.08 ± 0.96	10.88 ± 0.13	8.11 ± 0.20	7.05 ± 0.10	8.08 ± 0.55	3.89 ± 0.45	0.974[d,f]
16:1n-7	0.27 ± 0.01	2.35 ± 0.49	0.37 ± 0.05	0.17 ± 0.01	1.03 ± 0.03	0.84 ± 0.01	0.33 ± 0.01	0.30 ± 0.02	–0.327
18:0	14.81 ± 0.43	17.29 ± 1.28	26.51 ± 0.44	23.99 ± 0.75	20.23 ± 0.53	24.21 ± 0.64	24.26 ± 0.13	18.90 ± 0.49	0.181
18:1n-9	12.47 ± 0.75	14.93 ± 1.09	23.62 ± 0.47	17.11 ± 0.17	18.20 ± 0.35	20.64 ± 0.78	17.85 ± 0.65	22.02 ± 0.35	0.533
18:2n-6	8.52 ± 0.17	11.45 ± 0.63	21.25 ± 0.51	30.52 ± 0.15	24.96 ± 0.77	32.01 ± 0.63	39.54 ± 0.65	49.57 ± 1.32	0.957[e,f]
18:3n-3	0.24 ± 0.12	0.49 ± 0.22	0.33 ± 0.03	1.05 ± 0.01	0.22 ± 0.03	0.69 ± 0.03	0.14 ± 0.004	0.02 ± 0.005	–0.382
20:3n-6	0.95 ± 0.01	1.22 ± 0.07	0.86 ± 0.25	0.17 ± 0.01	0.26 ± 0.04	0.91 ± 0.02	0.16 ± 0.02	0.02 ± 0.005	0.747
20:4n-6	20.60 ± 0.50	20.11 ± 0.55	14.02 ± 0.99	15.02 ± 0.80	26.06 ± 0.89	12.73 ± 0.71	9.42 ± 0.63	5.18 ± 0.10	–0.681
22:6n-3	19.30 ± 0.74	14.01 ± 0.64	1.93 ± 0.31	1.04 ± 0.10	0.88 ± 0.04	0.88 ± 0.01	0.18 ± 0.01	0.07 ± 0.01	0.965[d,f]
ACL	18.74 ± 0.04	18.57 ± 0.005	18.14 ± 0.01	18.12 ± 0.02	18.37 ± 0.02	18.15 ± 0.01	18.03 ± 0.02	18.02 ± 0.007	–0.714
SFA	37.61 ± 0.53	35.39 ± 0.19	37.59 ± 0.54	34.88 ± 0.88	28.35 ± 0.62	31.26 ± 0.59	32.35 ± 0.55	22.80 ± 0.90	–0.897
UFA	62.38 ± 0.53	64.60 ± 0.19	62.40 ± 0.54	65.11 ± 0.88	71.64 ± 0.62	68.73 ± 0.59	67.64 ± 0.55	77.19 ± 0.90	0.897
MUFA	12.75 ± 0.74	17.29 ± 0.60	24.00 ± 0.52	17.29 ± 0.15	19.24 ± 0.38	21.48 ± 0.78	18.18 ± 0.67	22.32 ± 0.37	0.485
PUFA	49.63 ± 1.15	47.31 ± 0.79	38.40 ± 0.26	47.85 ± 0.72	52.40 ± 0.89	47.24 ± 1.09	49.45 ± 0.89	54.86 ± 1.26	0.582
PUFAn-3	19.54 ± 0.61	14.51 ± 0.86	2.27 ± 0.32	2.10 ± 0.08	1.11 ± 0.06	1.57 ± 0.03	0.32 ± 0.02	0.09 ± 0.01	0.967[d,f]
PUFAn-6	30.08 ± 0.66	32.80 ± 0.06	36.13 ± 0.26	45.72 ± 0.64	51.29 ± 0.90	45.66 ± 1.11	49.13 ± 0.90	54.77 ± 1.25	0.956[e,f]

[a]For footnotes and abbreviations see Table 2.

TABLE 4
Fatty Acid Composition (mol%) and General Indexes Related to Membrane Fatty Acid Composition of Liver Mitochondrial Cardiolipin from Mammalian Species[a]

	Mouse 3.5	Hamster 4	Guinea pig 8	Rabbit 13	Sheep 20	Pig 27	Cow 30	Horse 46	*r*[b]
16:0	9.68 ± 0.63	10.90 ± 0.29	11.39 ± 0.63	6.59 ± 0.50	15.53 ± 1.35	6.04 ± 0.27	14.32 ± 1.76	18.22 ± 0.32	0.544
16:1n-7	5.12 ± 0.64	3.36 ± 0.04	1.89 ± 0.05	1.16 ± 0.22	2.87 ± 0.25	1.18 ± 0.05	0.48 ± 0.08	0.61 ± 0.11	–0.876[d,f]
18:0	5.32 ± 0.38	4.68 ± 0.49	8.06 ± 0.22	4.21 ± 0.43	8.68 ± 0.94	4.31 ± 0.40	7.13 ± 0.96	2.08 ± 0.13	–0.372
18:1n-9	19.66 ± 0.37	17.58 ± 0.51	14.67 ± 0.41	8.84 ± 0.39	11.92 ± 0.89	16.86 ± 0.75	11.96 ± 1.38	22.97 ± 0.56	0.256
18:2n-6	52.60 ± 2.17	57.25 ± 0.45	59.27 ± 0.45	68.45 ± 1.91	52.94 ± 2.54	62.16 ± 0.75	59.50 ± 2.90	55.84 ± 0.49	–0.000
18:3n-3	3.04 ± 0.53	3.61 ± 0.13	2.29 ± 1.11	9.01 ± 0.39	6.26 ± 1.08	9.16 ± 1.35	5.73 ± 0.88	0.15 ± 0.004	–0.000
20:3n-6	0.95 ± 0.01	1.22 ± 0.07	0.86 ± 0.25	0.17 ± 0.01	0.26 ± 0.04	0.91 ± 0.02	0.16 ± 0.02	0.02 ± 0.005	0.747
20:4n-6	4.54 ± 0.46	2.59 ± 0.16	1.68 ± 0.04	1.71 ± 0.05	1.77 ± 0.21	0.26 ± 0.02	0.85 ± 0.1	0.10 ± 0.008	0.904[d,f]
ACL	17.79 ± 0.07	17.76 ± 0.01	17.76 ± 0.01	17.87 ± 0.01	17.66 ± 0.03	17.86 ± 0.01	17.72 ± 0.03	17.62 ± 0.01	–0.509
SFA	15.01 ± 0.94	15.58 ± 0.78	19.45 ± 0.71	10.80 ± 0.94	24.21 ± 2.27	10.36 ± 0.36	21.45 ± 1.39	20.30 ± 0.18	0.296
UFA	84.98 ± 0.94	84.41 ± 0.78	80.54 ± 0.71	89.19 ± 0.94	75.78 ± 2.27	89.63 ± 0.36	78.54 ± 1.39	79.69 ± 0.18	–0.296
MUFA	24.78 ± 0.90	20.94 ± 0.49	16.57 ± 0.46	10.01 ± 0.60	14.79 ± 0.67	18.04 ± 0.80	12.45 ± 1.43	23.58 ± 0.66	0.000
PUFA	60.20 ± 1.77	63.46 ± 0.60	63.96 ± 1.02	79.18 ± 1.54	60.98 ± 2.34	71.59 ± 0.80	66.09 ± 2.44	56.10 ± 0.49	–0.202
PUFAn-3	3.04 ± 0.53	3.61 ± 0.13	2.29 ± 0.06	9.01 ± 0.39	6.26 ± 1.08	9.16 ± 1.35	5.73 ± 0.88	0.15 ± 0.01	–0.094
PUFAn-6	57.15 ± 2.15	59.85 ± 0.52	61.66 ± 1.07	70.16 ± 1.92	54.71 ± 2.49	62.43 ± 0.73	60.35 ± 3.00	55.94 ± 0.49	–0.246

[a]For footnotes and abbreviations see Table 2.

for linoleic acid (18:2n-6, LA) (Figs. 1A, 1B). Cardiolipin also showed a significant negative correlation with MLSP in the arachidonic acid (20:4n-6, AA) content, while LA was not affected. As result of this fatty acid redistribution, the total content of double bonds showed significant negative correlations with MLSP in PtdCho and PtdEtn, and was not affected in cardiolipin (Fig. 2).

DISCUSSION

In agreement with previous comparative studies of mitochondria in mammals and birds (8–12) we found in this investigation that the number of fatty acid double bonds of the major phospholipid classes from liver mitochondria is negatively correlated with MLSP, i.e., the fatty acids of mitochondrial PtdCho and PtdEtn of long-lived mammals have a lower degree of unsaturation than those of short-lived ones. This is due to the redistribution between components of the polyunsaturated n-3 and n-6 fatty acid series, shifting from the highly unsaturated DHA and AA in short-lived animals to the less unsaturated LA in long-lived ones. This leads to a low DBI in the mitochondrial PtdCho and PtdEtn of long-lived animals. Further, since ACL may be seriously altered by the redistribution between DHA/AA and LA fatty acids, the decline in 16:0 and rise in 18:0 with increases in MLSP may be considered as an adaptation to maintain this parameter. In contrast, the cardiolipin fatty acid composition and fatty acid indexes show that only AA and palmitoleic acid (16:1) shows a signifi-

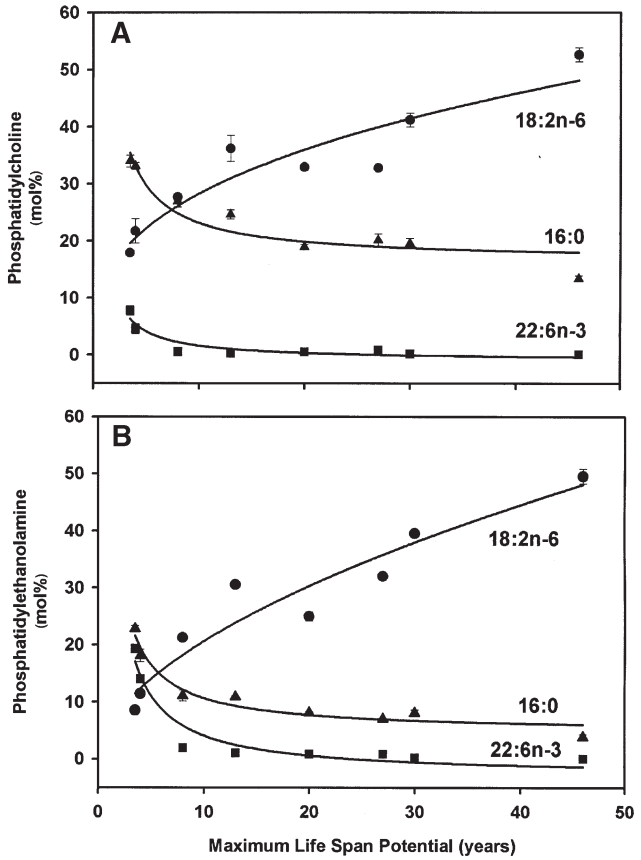

FIG. 1. Relationship between maximum life span (MLSP) and mol% of linoleic acid (18:2n-6), mol% of docosahexaenoic acid (22:6n-3), and mol% of palmitic acid (16:0), in liver mitochondrial phosphatidylcholine (A) and phosphatidylethanolamine (B). Regression equations: In A, for phosphatidylcholine, (i) 18:2n-6 (mol%) = 12.65 · MLSP $^{0.348}$, $r = 0.93$; $P < 0.0008$; (ii) 22:6n-3 (mol%) = –0.931 + (25.35/MLSP), $r = 0.93$, $P < 0.0008$; (iii) 16:0 (mol%) = 16.53 + (66.17/MLSP), $r = 0.94$, $P < 0.0004$. In B, for phosphatidylethanolamine, (i) 18:2n-6 (mol%) = 5.72 · MLSP$^{0.555}$, $r = 0.96$; $P < 0.0002$; (ii) 22:6n-3 (mol%) = –2.91 + (70.05/MLSP), $r = 0.96$, $P < 0.0001$; (iii) 16:0 (mol%) = 4.76 + (58.93/MLSP), $r = 0.97$, $P < 0.0001$. Values are means ± SEM.

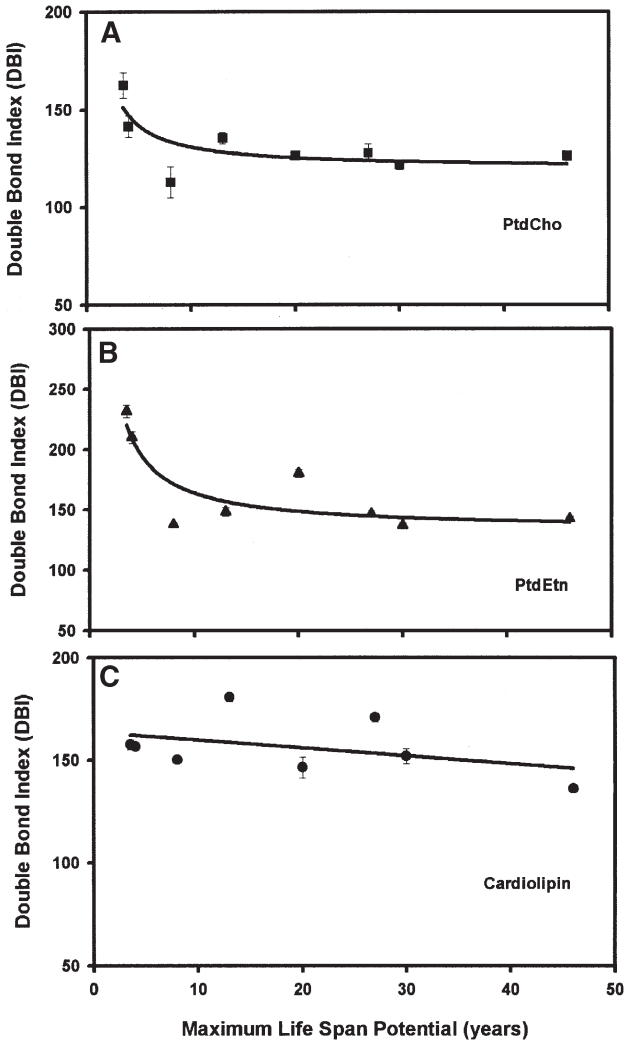

FIG. 2. Relationship between maximum life span potential (MLSP) and double bond index (DBI) of liver mitochondrial phosphatidylcholine (PtdCho, A), phosphatidylethanolamine (PtdEtn, B), and cardiolipin (C) in mammalian species. Regression equations: (A) $DBI_{PtdCho} = 119.81 + (110.81/MLSP)$, $r = 0.75$, $P < 0.03$; (B) $DBI_{PtdEtn} = 133.25 + (304.95/MLSP)$, $r = 0.86$, $P < 0.006$; (C) $DBI_{Cardiolipin} = 163.53 - 0.38 \cdot MLSP$, $r = 0.40$, $P = 0.323$. Values are means ± SEM.

cant negative correlation with MLSP. Similarly, previous studies in different tissues, subcellular fractions, or species usually showed that the low degree of fatty acid unsaturation of long-lived animals is obtained by analogous redistributions between types of PUFA without decreasing the total PUFA or UFA content (8–12). These results were later confirmed by another independent laboratory (20). While this had never been described as a function of MLSP, two previous comparative reports exist in mammals in relation to body size (21,22). In the first one (21), DHA decreased sharply as body size increased in the order mouse–rat–rabbit–man–whale, which is also an order of increasing MLSP although the authors did not comment on this. In the second report (22), DBI was found to correlate negatively with body size in the heart, skeletal muscle, and kidney cortex of five species, mouse–rat–rabbit–sheep–cattle, whereas in the liver the negative trend did not reach statistical significance and in the brain a low DBI was observed only in cattle. The fatty acids mainly responsible for these differences were again DHA and AA, which decreased as body size increased, and LA, which showed progressively larger levels in animals of larger size.

These results suggest that cellular and/or subcellular mechanisms exist to bring about the observed distinctive distribution of acyl groups in the mitochondrial membrane phospholipids among different mammals. Two mechanisms may be implied in determining the fatty acid profile observed in the major liver mitochondrial phospholipid classes: (i) the fatty acid desaturation pathway and (ii) the deacylation–reacylation cycle. With respect to (i), these results might be explained by the metabolic characteristics of the recently postulated mitochondrial desaturation pathways (23), which would make the n-6 and n-3 fatty acids available *in situ* to phospholipid acyltransferases in order to remodel the phospholipid

acyl groups and lead to the postulation of a constitutively decreased species-specific desaturase activity in the long-lived animals. Furthermore, since fatty acid composition differs between the zwitterionic (PtdCho and PtdEtn) and anionic (cardiolipin) phospholipids, perhaps there is differential remodeling activity among mammals. With respect to (ii), in order to maintain the appropriate molecular species composition of the different phospholipids, the deacylation–reacylation of mitochondrial phospholipids based on the presence of both phospholipase A_2 and acyl-CoA:lysophospholipid acyltransferase activities may be species-specific and rate-limiting for the molecular remodeling of PtdCho, PtdEtn, and cardiolipin in liver mitochondria. The conservation among mammals of the cardiolipin DBI probably reflects its importance as modulator of the activity of a number of mitochondrial membrane enzymes involved in the oxidative generation of ATP (24). Furthermore, special attention must be addressed to the decreased AA content in cardiolipin with MLSP. A recent report demonstrates that AA interaction with the mitochondrial electron transport chain promotes generation of reactive oxygen species (25). The lower AA content in long-lived species is consistent with the lower rate of free radical production in these animal species (26).

The presence of constitutively whole membrane remodeling activities in long-lived animals can explain why feeding corn oil (rich in LA) to primates (marmoset monkeys, *Callithrix jacchus*) increases mainly LA (to 30% of total fatty acids) instead of AA (to only 10% of total) in their tissues (27), whereas in short-lived rodents dietary LA leads to strong increases in AA. Similarly (28), members of human monastic communities that chronically consume only corn oil as the main dietary fat source (67% rich in LA) have lipid profiles with around 30% LA but only 9% AA in their high density lipoproteins (29). Moreover, the diets of all the animals studied contain LA and linolenic acid (18:3n-3), the precursors in the n-3 and n-6 series, but do not contain AA and/or DHA. Nevertheless, the DHA levels reached 8 and 20% in mice, but were only 0.12 and 0.07% in horses, for PtdCho and PtdEtn, respectively. In any case, the double-bond content of the diets was not correlated to MLSP.

Concerning the physiological meaning of the decrease in the degree of unsaturation in long-lived animals, there are various possibilities. Other authors have proposed (22) that mammals of large body size have a low DBI to decrease their metabolic rates, because the lower the DBI of a membrane, the lower is its permeability to ions (ion pumping is one of the main determinants of metabolic rate). The permeability to Na^+ and K^+ in liver hepatocytes (30) and to H^+ in inner mitochondrial membranes (31) also correlates negatively with body size. Although this possibility may be true for mammals of different sizes, it cannot explain the low DBI from birds because they have a metabolic rate similar to or higher than that of mammals of similar size. But the studied birds and the mammals of large body size share a common trait; they have a long life span. We thus hypothesized that the low phospholipid DBI of long-lived homeotherms (mammals or birds) could have been selected during evolution to decrease membrane lipid peroxidation and its peroxidative consequences to other cellular macromolecules including proteins (32–34) and DNA (35). In agreement with this, a low degree of total tissue and mitochondrial fatty acid unsaturation, accompanied by a low sensitivity to lipid peroxidation and a low concentration of the lipoxidation-derived adducts malondialdehydelysine and N^{ε}-carboxymethyllysine in tissue and mitochondrial proteins have been described (8–12). A negative correlation between sensitivity to lipid autoxidation and MLSP in brain and kidney homogenates from different mammalian species has also been described (13). Furthermore, during aging, a modification of fatty acid unsaturation and oxidative damage in membranes occurs, which is prevented by food restriction (36–41). Thus, the low fatty acid unsaturation of long-lived mammals of large body size would protect their tissues against oxidative damage while at the same time it could contribute to lower their metabolic rates. But the more general relationship in all homeotherms is that between DBI and MLSP, not between DBI and metabolic rate, because the low DBI of birds does not fit with their very high metabolic rates. Undoubtedly, other factors must be responsible for the high metabolic activity of these last animals.

The influence of fatty acid unsaturation on the transition temperature, and hence in the membrane fluidity, is well known (42). Whereas strong increases in lipid fluidity are observed after introduction of the first double bonds to a saturated fatty acid, progressively smaller effects are observed after the introduction of additional double bounds. Thus, the change in PUFA composition from the highly unsaturated AA and DHA to the less unsaturated LA found in the present work in PtdCho and PtdEtn from long-lived animals may allow them to decrease their double bond content without greatly changing their membrane fluidity. The membrane fluidity is a parameter needed for a proper function of mitochondrial membrane proteins such as enzymes, ion pumps, or electron carriers (43,44).

Thus, it may be proposed that, during evolution, a low degree of fatty acid unsaturation in liver mitochondria may have been selected for in long-lived mammals in order to protect their tissues against oxidative damage while maintaining an appropriate environment for membrane function.

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