Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals

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Abstract The oxidative stress theory of aging predicts a low degree of fatty acid unsaturation in tissues of longevous animals, because membrane lipids increase their sensitivity to oxidative damage as a function of their unsaturation. Accordingly, the fatty acids analyses of liver mitochondria from eight mammals, ranging in maximum life span from 3.5 to 46 years, show that the total number of double bonds and the peroxidizability index are negatively correlated with maximum life span ($r = -0.88$, $P < 0.003$; $r = -0.87$, $P < 0.004$, respectively). This is not due to a low content of unsaturated fatty acids in longevous animals, but mainly to a redistribution between kinds of the polyunsaturated n–3 fatty acids series, shifting from the highly unsaturated docosahexaenoic acid ($r = -0.89$, $P < 0.003$) to the less unsaturated linolenic acid ($r = 0.97$, $P < 0.0001$). This redistribution pattern strongly suggests the presence of a constitutively low Δ^6-desaturase activity in longevous animals ($r = -0.96$, $P < 0.0001$). Thus, it may be proposed that, during evolution, a low degree of fatty acid unsaturation in liver mitochondria may have been selected in longevous mammals in order to protect the tissues against oxidative damage, while maintaining an appropriate environment for membrane function.—Pamplona, R., M. Portero-Otín, D. Riba, C. Ruiz, J. Prat, M. J. Bellmunt, and G. Barja. **Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *J. Lipid Res.* 1998.** 39: **1989–1994.**

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Some evidence indicates that mitochondria and oxidative damage can be implicated both in pathological responses (1, 2) and in the aging process (3–8). The available comparative studies indicate that maximum life span is inversely related to mitochondrial free radical production (3, 9, 10) and DNA oxidative damage (11, 12). While these are very important characteristics consistent with free radical–oxidative stress theories of aging (4, 6), additional factors can also lead to a low level of oxidative damage in long- versus short-lived animal species.

Among cellular macromolecules, polyunsaturated fatty acids (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. As both oxygen consumption and oxygen free radical production occur in mitochondrial membranes, a low degree of fatty acid unsaturation in these membranes would be advantageous, in oxidative stress terms, because it would decrease the sensitivity to lipid peroxidation. This would also protect other molecules against lipid peroxidation-derived damage. In line with this, it has been previously described that liver mitochondria from humans (maximum life span: 122 years) and pigeons (maximum life span: 35 years) have a lower degree of fatty acid unsaturation and a lower sensitivity to lipid peroxidation that rat liver mitochondria (13). This fact also extends to total lipids of long- versus short-lived animal species (R. Pamplona and G. Barja, unpublished results). Finally, a negative correlation between sensitivity to lipid autoxidation and maximum life span in brain and kidney homogenates from different mammalian species has been described by other authors (14).

However, to our knowledge, the possible relationship between the degree of fatty acid unsaturation of mitochondrial membranes and maximum life span among different mammalian species has never been reported. In this work, the fatty acid compositions of liver mitochondrial lipids in eight mammalian species ranging in maximum life span from 3.5 to 46 years have been analyzed. The results obtained show that the degree of fatty acid unsaturation is inversely correlated with maximum life span. Thus, the presence of lower degrees of fatty acid unsatura-

Abbreviations: ACL, average chain lenght; DBI, double bond index; MLSP, maximum life-span; MUFA, monounsaturated fatty acids; PI, peroxidizability index; PUFA, polyunsaturated fatty acids; PUFAn–3, polyunsaturated fatty acids n–3 series; PUFAn–6, polyunsaturated fatty acids n–6 series; SFA, saturated fatty acids; UFA, unsaturated fatty acids; U/S, unsaturated/saturated ratio.

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tion in long- versus short-lived animal species can be involved in the low sensitivity of their tissues to lipid (14) and protein (15) oxidative damage.

MATERIALS AND METHODS

Animals and diets

Animals, namely mouse (*Mus musculus*, n = 8), rat (*Rattus norvergicus*, n = 7), guinea pig (*Cavia porcellus*, n = 5), sheep (*Ovis aries*, n = 7), dog (*Canis familiaris*, n = 4), pig (*Sus scrofa*, n = 7), cow (*Bos taurus*, n = 7), and horse (*Equus caballus*, n = 5), whose maximum life span (MLSP) varies from 3.5 to 46 years (16), were adult specimens selected at an age within 15–30% of their maximum life span. All animals appeared to be healthy, no animal being obese or scraggy. The recorded values of maximum longevity (in years) were: mouse, 3.5; rat, 4; guinea pig, 8; sheep, 20; dog, 21; pig, 27; cow, 30; and horse, 46 (16). The body weight (in kg) of the different animal species ranged between: 0.030–0.040 in mouse; 0.4–0.6 in rat; 0.9–1.1 in guinea pig; 45–55 in sheep; 15–20 in dog; 175–200 in pig; 675–725 in cow; and 625–675 in horse. The animal care protocols were approved by the University of Lleida Animal Experimentation Ethics Committee. Mice, rats, and guinea pigs were killed by decapitation. Dogs were killed in the Lleida city's dog pound by a single succinyl-choline injection. Sheep, pigs, cows, and horses (farm animals) were killed at the abattoir. Diets administered during all the adult life of the animals were obtained when the animals were killed.

Chemicals

Reagents were purchased from Sigma (Sigma, St.Louis, MO) unless otherwise specified. All chemicals were of analytical grade.

Isolation of mitochondrial, lipid extraction, and fatty acid analysis

Tissue samples were taken randomly from the main hepatic lobe and were immediately processed. Mitochondrial fractions were isolated by differential centrifugation as previously described (13). Lipids from mitochondria and diets were extracted into chloroform–methanol 2:1 (v/v) by the method of Folch, Lees, and Sloane Stanley (17), in the presence of 0.01% butylated hydroxytoluene.

Mitochondrial and dietary 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 μl was submitted to gas chromatography/mass spectrometry (GC/MS) analysis.

GC separation was performed in a SP2330 capillary column (30 m × 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 ionization mode. GC/MS conditions were: injector and detector port temperature 220°C and 250°C, respectively; column temperature ranged from 100°C with an increase of 10°C/min, from 200°C to 240°C at 5°C/min, and a final hold of 12 min. Identification of methyl esters was made by comparison with authentic standards and their MS.

Calculations and statistics

The average chain length (ACL) was calculated as ACL = [Σ % $Total_{14}$ × 14) + . . . + (Σ % $Total_n$ × n)] / 100 (n = carbon atom number). The double bond index was calculated as DBI = Σ mol % of unsaturated fatty acids × number of double bonds of each unsaturated fatty acid. The peroxidizability index was calculated as PI = [(%Monoenoic × 0.025) + (%Dienoic × 1) + (%Trienoic × 2) + (%Tetraenoic × 4) + (%Pentaenoic × 6) + (%Hexaenoic × 8)] (18, 19). Saturated fatty acids were calculated as SFA = Σ % (14:0+15:0+16:0+17:0+18:0). Unsaturated fatty acids were calculated as UFA = Σ % (MUFA+PUFA). Monounsaturated fatty acids were calculated as MUFA = Σ % (16:1+17:1+18:1). Polyunsaturated fatty acids were calculated as PUFA = Σ % (PUFAn–3 + PUFAn–6). Polyunsaturated fatty acids n–3 were calculated as PUFAn–3 = Σ % (18:3+20:5+22:5+22:6). Polyunsaturated fatty acids n–6 were calculated as PUFAn–6 = Σ % (18:2+20:3+20:4+22:4). Ratio 20:4/18:2 represents the arachidonic acid/linoleic acid ratio and expresses the activity coefficient of enzymes in the biosynthethic pathway of arachidonic acid from linoleic acid. The ratio 22:6/18:3 represents the docosahexaenoic acid/linolenic acid ratio and expresses the activity coefficient of enzymes in the biosynthethic pathway of docosahexaenoic acid from linolenic acid.

Linear regression equations were obtained, after logarithmic transformation of the variables, with the curve estimation statistic from 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 ± SD.

RESULTS

The fatty acid composition and double bond index of the diets given to the different mammalian species are shown in **Table 1**. The complete fatty acid composition of liver mitochondrial membrane lipids of the mammalian species studied in this work is shown in **Table 2**. Indexes related to the degree of unsaturation, the chain length, or to the main fatty acid types or families appear in **Table 3**. Neither the percentage of total saturated, unsaturated, monounsaturated, and polyunsaturated fatty acids nor the unsaturated/saturated ratio show statistically significant relationships with maximum life-span (**Table 4**). Despite the unchanged content of unsaturated fatty acid, the total number of double bonds and the peroxidizability index show highly significant negative correlations with maximum life span (Table 4 and **Fig. 1a**). This is due to the negative correlation of the highly unsaturated docosahexaenoic acid with maximum life span, and to the positive correlation of the less unsaturated linolenic acid with maximum life span (Table 4 and Figs. 1b and 1c). The 22:6/18:3 ratio that links the fatty acids situated at the beginning and end of the n–3 pathway and the 22:6/22:5 ratio are negatively correlated with maximum life span

TABLE 1. Fatty acid composition (mol% of total lipids) of the dietary fats (n = 3)

	Mouse/Rat	Guinea Pig	Sheep	Dog	Pig	Cow	Horse
16:0	21	19	22	26	31	21	21
16:1n–7	0.5	0.5	2	4	3.5	3	2
18:0	9	3	8	9	9	7	8
18:1n–9	29	24	29	38	35	33	26
18:2n–6	39	49	32	22	25	35	41
18:3n–3	1.5	4.5	7	1	6	1	2
DBI	112	136	116	89	107.5	109	116

TABLE 2. Fatty acid composition (mol%) of liver mitochondrial lipids from mammalian species

	Mouse	Rat	Guinea Pig	Sheep	Dog	Pig	Cow	Horse
14:0	0.21 ± 0.04	0.60 ± 0.18	0.37 ± 0.04	1.25 ± 0.25	1.11 ± 0.34	0.70 ± 0.40	0.57 ± 0.07	—
15:0	0.23 ± 0.08	0.53 ± 0.13	0.82 ± 0.10	0.45 ± 0.08	—	0.41 ± 0.14	0.36 ± 0.10	—
16:0	23.40 ± 0.93	16.02 ± 2.20	14.25 ± 0.95	13.95 ± 0.61	15.67 ± 0.72	12.12 ± 2.05	8.73 ± 0.96	10.31 ± 1.49
16:1n–7	5.27 ± 0.85	3.14 ± 0.88	2.73 ± 0.23	3.20 ± 0.36	2.34 ± 0.21	2.70 ± 0.42	1.47 ± 0.40	3.11 ± 0.21
17:0	0.56 ± 0.06	0.63 ± 0.13	0.75 ± 0.18	1.10 ± 0.22	0.73 ± 0.06	1.71 ± 0.49	1.30 ± 0.41	0.87 ± 0.12
17:1n–7	0.72 ± 0.07	—	0.56 ± 0.12	1.33 ± 0.27	—	1.16 ± 0.33	0.70 ± 0.19	—
18:0	9.53 ± 0.68	14.25 ± 3.80	17.27 ± 1.47	18.83 ± 2.38	21.52 ± 0.87	16.69 ± 3.09	22.99 ± 2.95	17.84 ± 0.82
18:1n–9	18.82 ± 1.40	18.32 ± 1.70	11.35 ± 1.19	18.33 ± 1.84	13.32 ± 1.43	24.70 ± 0.96	19.89 ± 1.86	14.24 ± 0.89
18:2n–6	17.01 ± 2.39	14.75 ± 1.50	36.87 ± 2.39	22.80 ± 1.50	23.09 ± 2.27	18.07 ± 1.94	25.94 ± 2.41	46.74 ± 1.64
18:3n–3	0.24 ± 0.09	0.40 ± 0.04	0.56 ± 0.19	1.09 ± 0.18	1.15 ± 0.17	2.12 ± 0.42	2.34 ± 0.11	2.14 ± 0.05
20:3n–6	1.84 ± 0.23	0.86 ± 0.41	0.57 ± 0.14	0.40 ± 0.19	1.23 ± 0.47	1.30 ± 0.35	0.99 ± 0.88	0.08 ± 0.02
20:4n–6	12.75 ± 0.89	23.46 ± 4.52	10.69 ± 2.29	14.31 ± 1.83	18.55 ± 0.31	13.35 ± 1.40	11.70 ± 3.09	3.14 ± 0.90
20:5n–3	0.49 ± 0.16	0.61 ± 0.25	0.40 ± 0.03	—	—	0.67 ± 1.31	—	0.85± 0.09
22:4n–6	0.13 ± 0.03	0.94 ± 0.44	—	0.51 ± 0.11	—	1.29 ± 0.20	—	—
22:5n–3	0.38 ± 0.09	1.29 ± 0.63	1.23 ± 0.46	1.12 ± 0.16	—	1.14 ± 0.51	1.72 ± 0.85	0.19 ± 0.03
22:6n–3	8.34 ± 0.90	4.20 ± 1.66	1.50 ± 0.62	1.28 ± 0.37	1.25 ± 0.37	1.78 ± 0.59	1.24 ± 0.48	0.42 ± 0.01

Results are expressed as mean ± SD.

(Table 4). The contents of 16:0 and 18:0, decrease or increase, respectively, with maximum life span, but this fact does not influence the total content of saturated fatty acid, the unsaturated/saturated ratio, and the average chain length (Table 4).

DISCUSSION

In this study it is demonstrated that the number of fatty acid double bonds of liver mitochondria is negatively correlated with maximum life span, i.e., the fatty acids of liver mitochondria of longevous mammals have a lower degree of unsaturation than those of short-lived mammals. This is not due to a low content of unsaturated fatty acids in longevous mammals, but mainly to a redistribution between components of the polyunsaturated n–3 fatty acids series, shifting from the highly unsaturated docosahexaenoic acid (22:6n–3) in short-lived animals to the less unsaturated linolenic acid (18:3n–3) in long-lived animals. This leads to a low peroxidizability index in the mitochondrial fatty acids of longevous animals. Further, as average chain length may be seriously altered by the redistribution between 22:6n-3 and 18:3n–3 fatty acids, the decline in 16:0 and rise in 18:0 with maximum life span may be considered as an adaptation to maintain this parameter.

The total content of fatty acid double bonds is strongly regulated in cellular membranes (20, 21). It is well accepted that mammals cannot synthesize the n–3 and n–6 essential fatty acids that are precursors of the long-chain polyunsaturated n–3 and n–6 acids. These precursor acids must be obtained from the diet and then enzymatically elongated and desaturated. This allows the maintenance of an appropriate lipid environment for membrane function, mainly by the modulation of their degree of fatty acid unsaturation and cholesterol content (22). Most of the control of membrane fatty acid unsaturation has been attributed to negative feed-back regulation of transcription of desaturase genes dependent on lipid composition (23, 24) and to the modulation of desaturases by the metabolic–hormonal status (20, 25).

The low double bond index of longevous animals can-

TABLE 3. General indexes related to membrane fatty acid composition

	Mouse	Rat	Guinea Pig	Sheep	Dog	Pig	Cow	Horse
ACL	18.05 ± 0.06	18.32 ± 0.21	17.95 ± 0.08	17.98 ± 0.06	18.00 ± 0.02	18.20 ± 0.13	18.15 ± 0.11	17.82 ± 0.02
SFA	33.95 ± 1.26	32.03 ± 4.82	33.47 ± 1.52	35.59 ± 2.14	39.04 ± 1.49	31.67 ± 4.23	33.97 ± 3.58	29.03 ± 1.25
UFA	66.04 ± 1.26	67.95 ± 4.83	66.52 ± 1.52	64.40 ± 2.14	60.95 ± 1.49	68.32 ± 4.23	66.02 ± 3.58	70.96 ± 1.25
MUFA	24.82 ± 2.12	21.45 ± 2.17	14.65 ± 0.94	22.87 ± 2.05	15.67 ± 1.39	28.57 ± 1.38	22.07 ± 1.97	17.35 ± 0.96
PUFA	41.21 ± 2.40	46.49 ± 5.84	51.86 ± 1.95	41.53 ± 1.53	45.28 ± 2.48	39.75 ± 5.10	43.95 ± 4.09	53.60 ± 0.97
PUFAn–3	9.46 ± 0.97	6.49 ± 1.83	3.71 ± 1.12	3.50 ± 0.40	2.40 ± 0.17	5.72 ± 2.35	5.31 ± 1.22	3.62 ± 0.15
PUFAn–6	31.74 ± 2.28	40.00 ± 4.71	48.15 ± 1.41	38.02 ± 1.28	42.88 ± 2.47	34.02 ± 3.09	38.63 ± 3.60	49.97 ± 0.93
DBI	171.12 ± 6.32	186.96 ± 25.08	151.86 ± 10.97	145.58 ± 6.57	148.23 ± 4.74	153.25 ± 18.17	146.88 ± 15.12	135.94 ± 2.02
PI	145.36 ± 9.42	160.33 ± 14.67	104.20 ± 15.20	102.68 ± 8.43	107.47 ± 3.71	109.28 ± 19.72	100.31 ± 17.04	71.94 ± 2.46
U/S	1.94 ± 0.11	2.17 ± 0.42	1.99 ± 0.13	1.81 ± 0.17	1.56 ± 0.09	2.20 ± 0.41	1.97 ± 0.31	2.44 ± 0.14
20:4/18:2	0.76 ± 0.15	1.60 ± 0.36	0.29 ± 0.08	0.63 ± 0.11	0.80 ± 0.08	0.64 ± 0.09	0.45 ± 0.12	0.06 ± 0.02
22:6/18:3	34.75 ± 0.49	10.48 ± 0.55	2.68 ± 0.40	1.17 ± 0.27	0.53 ± 0.17	0.73 ± 0.13	0.58 ± 0.25	0.20 ± 0.04

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; DBI, double bond index; PI, peroxidizability index; U/S, unsaturated/saturated ratio; 20:4/18:2, arachidonic/linoleic acid ratio; and 22:6/18:3, docosahexaenoic/linolenic acid ratio. For more information, see Materials and Methods.

TABLE 4. Summary of correlations between maximum life span and fatty acid composition (mol%) or fatty acid indexes of mitochondrial membranes in the mammalian species included in this study

	r (d.f.)	*P*		*r* (d.f.)	*P*		*r* (d.f.)	*P*
14:0	0.680 (5)	0.093	20:3n–6	−0.501 (6)	0.205	MUFA	−0.083 (6)	0.849
15:0	0.054 (4)	0.922	20:4n–6	−0.536 (6)	0.171	PUFA	−0.279 (6)	0.502
16:0	−0.820 (6)	0.013	20:5n–3	0.704 (3)	0.184	PUFAn–3	−0.582 (6)	0.130
16:1n–7	−0.590 (6)	0.123	22:4n–6	0.558 (2)	0.442	PUFAn–6	0.339 (6)	0.412
17:0	−0.031 (6)	0.932	22:5n–3	−0.118 (5)	0.804	PI	−0.874 (6)	0.004
17:1n–7	0.504 (3)	0.385	22:6n–3	−0.889 (6)	0.003	U/S	0.104 (6)	0.807
18:0	0.768 (6)	0.026	ACL	−0.401 (6)	0.325	20:4/18:2	−0.574 (6)	0.136
18:1n–9	0.044 (6)	0.918	DBI	−0.886 (6)	0.003	22:5/18:3	−0.779 (5)	0.039
18:2n–6	0.558 (6)	0.150	SFA	−0.126 (6)	0.765	22:6/22:5	−0.670 (5)	0.099
18:3n–3	0.970 (6)	0.000	UFA	0.083 (6)	0.843	22:6/18:3	−0.967 (6)	0.000

Abbreviations: *r*: linear correlation coefficient of Pearson; *P*: statistical significance; d.f.: degree of freedom. For the abbreviations see Table 2.

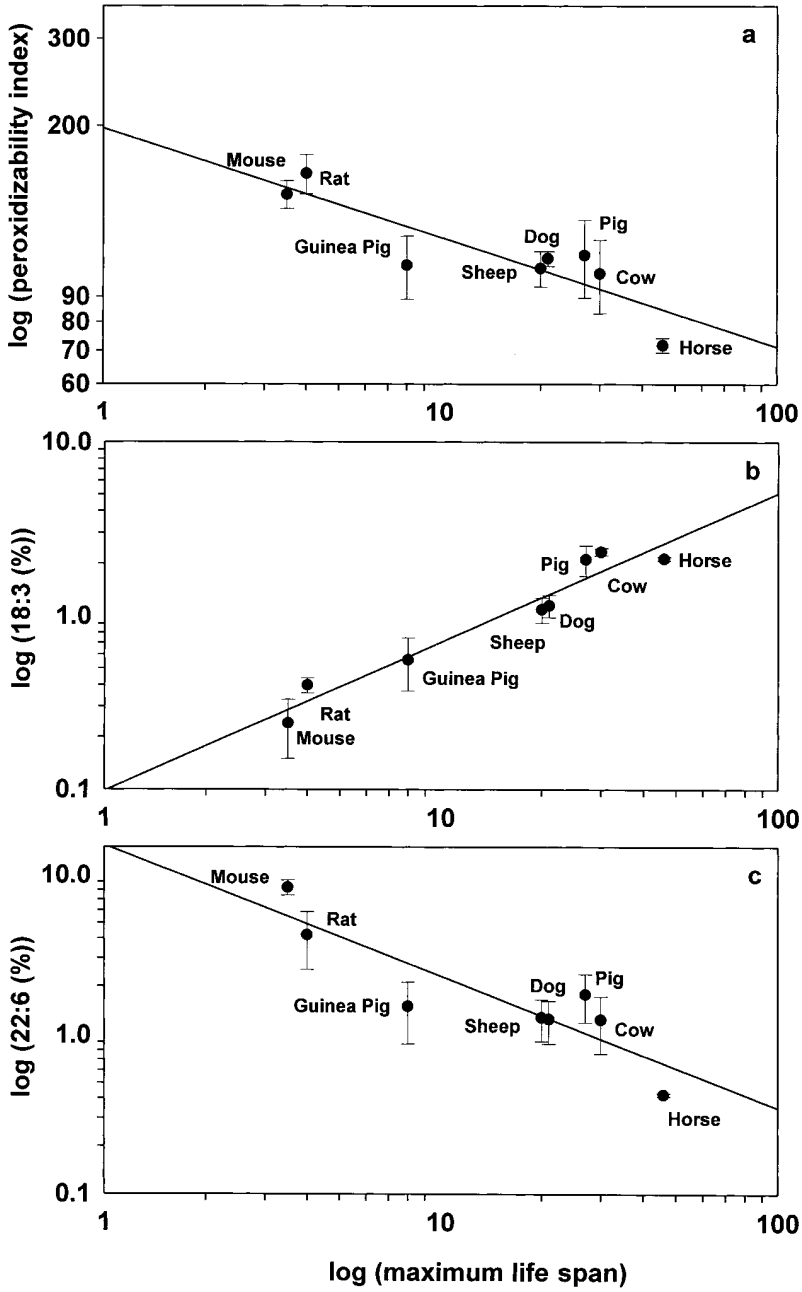

Fig. 1. Relationship between liver mitochondrial fatty acid double bond index (DBI) (a), mol% of linolenic acid (18:3n–3) (b), and mol% of docosahexaenoic acid (22:6n–3) (c) versus maximum life span (MLSP). Regression equations: (1a) $y = 2.29 - 0.09\ x$, $r = -0.88$; $P < 0.003$; (1b) $y' = -1.01 + 0.86\ x$, $r = 0.97$, $P < 0.0001$; (1c) $y'' = 1.18 - 0.82\ x$, $r = -0.89$, $P < 0.003$; where y = log (DBI), y′ = log (mol%18:3n–3), y″ = log (mol%22:6n–3), and x = log (MLSP(yr)). Values are means ± SD.

not be simply attributed to a dietary deficit in essential fatty acids. In such a situation, tissues react to the dietary deficiency either with strong compensatory increases in Mead acid (20:3n–9), a well-known diagnostic marker of essential fatty acid deficiency (26), or with increases in monounsaturated fatty acids (27). Mead acid (n–9 eicosatrienoic acid) was not detected in any animal in our study and the monounsaturated levels were similar in short- and long-lived animals. Moreover, the 22:6n–3 to 18:3n–3 redistribution cannot be obtained by a simple change in the dietary levels of unsaturated fatty acids. Standard diets of laboratory rodents (including those used and analyzed by us in this work) contain 18:3n–3 (1.5% in our case) but do not contain 22:6n–3 (a too easily oxidizable fatty acid to be normally added as a stable food component). Furthermore, normal diets of cows and horses contain 18:3n–3 (28), the precursor in the n–3 series. Nevertheless, the 22:6n–3 levels reached 8.34% in mice and 4.20% in rats but were only 1.24% and 0.42% in cows and horses, respectively. These results might be explained by a decreased conversion of 18:3n–3 to 22:6n–3 by elongation and desaturation in the n–3 pathway in longevous animals. Because the rate-limiting steps in the n–3 biosynthetic pathway are those of desaturation not of elongation (21, 27), it is possible that longevous animals have constitutively low Δ^6 desaturase activities. This may explain the low 22:6/18:3 ratios, as this enzyme controls the n–3 pathway flux both at the beginning of the route (conversion of 18:3n–3 to 18:4n–3 (29) and at the end of the pathway.

A previous comparative study showed that levels of 22:6n–3 in total heart phospholipids strongly decreased in the order: mouse > rat > rabbit > human > whale (30), thus in progressive increase of maximum life span. The only other comparative work performed up to date on mammals (31) found strong progressive significant decreases in the number of double bonds of total heart, skeletal muscle, and kidney phospholipids as body size increased following the order: mouse > rat > rabbit > sheep > cattle. Among the major findings obtained in this work (31) was the negative correlation between body size and the content of 22:6n–3 in heart and skeletal muscle. These authors suggested that small mammals have fatty acids with more double bonds in order to increase the permeability of their membranes. This would increase their membrane ion leaks (32, 33), thus maintaining their high tissue metabolic rates.

The influence of fatty acid unsaturation on the transition temperature, and hence on membrane fluidity is well known (27). 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 (27). This is so because when a double bond is added near the center of the fatty acid chain (first double bond added) the impact on fluidity through the kink (or coiling) of the fatty acyl chain is much larger than when it is added nearer to its extremes (subsequent double bonds added). Thus the change in PUFA composition from the highly unsaturated 22:6n–3 to the less unsaturated 18:3n–3 found in the present work in liver mitochondria from longevous animals may allow a decrease in the double bond content of mitochondrial membranes without greatly changing membrane fluidity, a parameter needed for a proper function of mitochondrial membrane proteins such as enzymes, ion pumps, electron transporters, etc. (21, 34).

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. 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 (35, 36). During aging, a modification of fatty acid unsaturation and oxidative damage in membrane occurs, that can be prevented by food restriction (19, 37–41). Therefore, it is reasonable to assume that the low degree of fatty acid unsaturation in longevous animals could protect their tissues against oxidative damage. In agreement with this, negative correlations between sensitivity to lipid peroxidation of kidney and brain tissues of mammalian species and maximum life span have been described (14). Previous work from our laboratory (13) has also shown that the degree of fatty acid unsaturation and the sensitivity to lipid peroxidation of liver mitochondria are lower in pigeons (maximum life span = 35 years) than in rats (maximum life span = 4 years) in spite of their similar metabolic rates, and that the double bond and 22:6n–3 content is even lower in human (maximum life span = 122 years). Thus, it may be proposed that, during evolution, a low degree of fatty acid unsaturation in liver mitochondria may have been selected in longevous mammals in order to protect the tissues against oxidative damage, while maintaining an appropriate environment for membrane function.

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