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Modulation of Membrane Phospholipid Fatty Acid Composition by Age and Food Restriction

Abstract

Phospholipids from liver mitochondrial and microsomal membrane preparations were analyzed to further assess the effects of age and lifelong calorie restriction on membrane lipid composition. Results showed that the major phospholipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol and cardiolipin did not vary significantly with age or diet. The fatty acid composition of the phospholipids was determined in PC and PE and ages of 6, 12 and 24 months. The data revealed characteristic patterns of age-related changes in ad libitum (AL) fed rats: membrane levels of long-chain polyunsaturated fatty acids, 22:4 and 22:5, increased progressively, while membrane linoleic acid (18:2) decreased steadily with age. Levels of 18:2 fell by approximately 40%, and 22:5 content almost doubled making the peroxidizability index increase with age. In addition, levels of 16:1 and 18:1 decreased significantly with age, indicating a possible change in Δ^9-desaturase activity coefficient. Food restriction resulted in a significant increase in levels of essential fatty acids while attenuating levels of 22:4, 22:5, 22:6 and peroxidizability. We concluded that the membrane-stabilizing action of long-term calorie restriction relates to the selective modification of membrane long-chain polyunsaturated fatty acids during aging.

Key Words
Fatty acids
Aging
Food restriction
Δ^9-Desaturase
Fischer 344 rats
Peroxidizability
Mitochondria
Microsomes
Phospholipids

Introduction

In recent years, many investigators have successfully demonstrated the modulation of the aging processes by restricting the food intake of experimental animals [1–3]. The influence of food restriction on the propitious modulation of aging has been shown to alter most physiological functions that have been studied and to reduce the incidence of many

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age-related diseases. Although several hypotheses have been proposed to account for life span extension and the antiaging action of food restriction [reviewed in ref. 4], the biochemical mechanisms underlying such diverse benefits are not well understood.

In an attempt to define possible cellular and molecular mechanisms underlying the antilipoperoxidative action of dietary restriction, we have explored free radical-induced lipid peroxidation of liver subcellular membranes [5]. The concentration of membrane lipid hydroperoxides increased with age, however, the membranes of rats maintained on the restricted regimen contained less lipid hydroperoxides, and in vitro lipid peroxidation was largely suppressed. In a subsequent study, we were able to relate the increased membrane lipid peroxidation during age with the increased highly oxidizable long-chain polyunsaturated fatty acids in mitochondrial and microsomal membranes of ad libitum (AL)-fed rats [6]. More recently, the modulation of age-related changes in the fluidity of these membranes was reported by our laboratory [7].

There are few studies reported in the gerontological literature on membrane phospholipid changes with age, and almost no information can be found regarding the effect of long-term calorie restriction on membrane phospholipids. In the present study, individual phospholipids from liver mitochondrial and microsomal membrane fractions were analyzed to further characterize the pattern of changes in membrane lipid composition with age and food restriction. Our results indicate clearly that chronic restriction of calorie intake can radically modify the fatty acid composition of phospholipids during the course of aging and attenuate the peroxidizability of the membrane lipid.

Materials and Methods

Animals and Diet

Male specific pathogen-free Fischer 344 rats (Charles River Laboratories, Portage, Mich., USA) of 28 ± 2 days were maintained in a barrier facility. They were housed singly in plastic cages suspended on the Hazelton-Enviro Rack System (Hazelton Systems, Aberdeen, Md., USA). The procedures for the monitoring and the operating of the barrier facility, and the composition of the diets were the same as reported in detail previously [8]. Briefly, rats were fed a semisynthetic diet (Ralston Purina, Richmond, Ind., USA) ad libitum (AL) until 6 weeks of age. Rats belonging to the AL-fed group continued the same regimen until they were sacrificed. Food-restricted (FR) rats were fed 60% of the mean caloric intake of the AL-fed group. FR rats were provided their daily food allotment about 1 h before the start of the dark phase of a 12-hour-light/12-hour-dark cycle.

Membrane Preparations

Rats were sacrificed by exsanguination while under anesthesia with ether. The liver was quickly removed and stored in homogenizing buffer 10 m*M* Hepes (pH 7.4) containing 220 m*M* mannitol, 60 m*M* sucrose and 10 m*M* KCl at –70 °C. Mitochondrial and microsomal membranes were prepared and purified by differential centrifugation as described previously [6]. Purity of the membrane preparations was assessed using marker enzyme activities as described previously [5]. Protein was determined using the Folin reagent [9].

Lipid Analysis

Membrane lipids were extracted with a mixture of chloroform/methanol (2:1; v/v) by the method of Folch et al. [10]. Lipids were dried and resuspended in a 2:1 (v/v) chloroform/methanol solution. Membrane phospholipid classes were separated by two-dimensional thin-layer chromatography on precoated preparative silica gel G plastes (Analtech) using the solvent systems described by others [11]. Individual components on plates were detected under a UV lamp following exposure to 6-*p*-toluidino-2-naphthalene sulfonic acid [12] and identified by comparison with standards (Avanti, Birmingham, Ala., USA). Phosphatidylserine was reliably separated using a chromatographic system already described [11]. However, because phosphatidylserine was detected as only a mior component of our membrane preparations, it is therefore not reported further. Spots were scraped, eluted with a mixture of chloroform/methanol/H_2O (50:30:5; v/v/v) and transferred into acid-washed tubes. Aliquots of the

eluted lipids were analyzed for phospholipid phosphorus [13]. Fatty acid methyl esters were derived in sealed ampoules at 90 °C for 2 h using methanolic HCl (Alltech-Applied Science, Deerfield, Ill., USA). The prepared esters were extracted with heptane and analyzed on a Perkin-Elmer Model 8420 gas-liquid chromatograph equipped with fused silica capillary column (DB-225, J&W Scientific, Folsom, Calif., USA). Chromatographic conditions were set as follows: Injection port temperature, 250 °C; oven and detector temperatures, 210 and 250 °C, respectively, and helium carrier gas at 0.9 ml/min. Peak identification was obtained by comparison of retention times with mixtures of fatty acid standards (Nu-Check-Prep, Elysian, Minn., USA). Peroxidizability index was calculated as described [14]. The ratio of monounsaturates/saturates was calculated as 16:1 + 18:1 divided by 16:0 + 18:0 [15].

Data Analysis

Differences in fatty acid levels were compared by multivariate analysis of variance. Posthoc analysis of individual group means was performed with a matrix using the Systat Statistical System (Evanston, Ill., USA).

Results

The data pertaining to the quantitative analysis of the mitochondrial and microsomal phospholipid composition are presented in figure 1. The major phospholipid classes, phospatidylcholine (PC) and phosphatidylethanolamine (PE), accounted for almost 80% of the membrane total phospholipid content. Results indicated that the concentrations of PC, PE, phosphatidylinositol and cardiolipin did not vary significantly with age or diet.

The fatty acid composition of the subcellular phospholipids was determined in PC and PE and ages of 6, 12 and 24 months, and the data revealed striking age- and diet-related changes. As shown in tables 1, and 2, linoleic acid (18:2), docosatetraenoic acid (22:4) and docosapentaenoic acid (22:5) from mitochondrial (table 1) and microsomal (table 2, fig. 2, 3) membrane fractions showed an interesting pattern of changes with age in the AL-fed rats. While levels of 18:2 decreased steadily with age (PC, $p < 0.02$; PE, $p < 0.001$), levels of 22:4 and of 22:5 increased concomitantly (PC, $p < 0.05$; PE, $p < 0.001$, and PC, $p < 0.002$; PE, $p < 0.05$, respectively). The levels of 18:2 fell by approximately 40%, whereas 22:5 levels most doubled during the age interval from 6 to 24 months. It

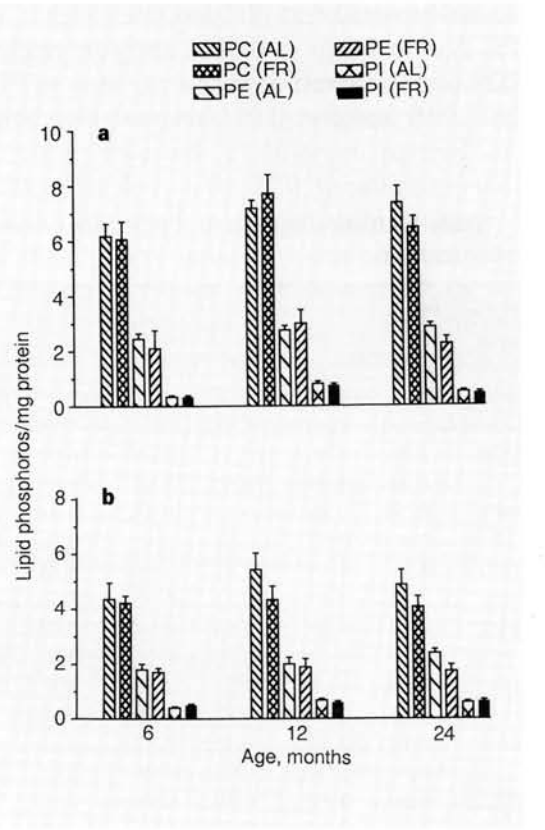


Fig. 1. Effects of age and food restriction on the distribution of PC, PE, phosphatidylinositol (PI) and cardiolipin (CL) in microsomal (**a**) and mitochondrial (**b**) membrane fractions. Values represent means ± SEM of 5 rats per group. No age- or diet-related differences were significant.

should be noted that 18:2 was much higher in PC than in PE ($p < 0.001$), and conversely, 22:4 and 22:5 were lower in PC than in PE ($p < 0.01$ and $p < 0.001$, respectively). Several other fatty acids were also significantly modulated by age in the AL-fed rats. For instance, arachidonic acid (20:4), which is the most abundant polyunsaturated long-chain

Table 1. Effects of age and food restriction on fatty acid composition of phospholipids: mitochondrial membrane fraction

Fatty acid	PC						PE					
	6 months		12 months		24 months		6 months		12 months		24 months	
	AL	FR	AL	FR	AL	FR	AL	FR	AL	FR	AL	FR
16:0	18.03 ±0.84	18.06 ±0.29	17.23 ±0.63	20.07 ±0.66	16.79 ±1.09	18.48 ±0.64	16.48 ±0.51	16.28 ±0.57	16.36 ±0.68	17.10 ±0.37	16.68 ±1.12	16.09 ±0.73
16:1	0.79 ±0.12	0.86 ±0.07	0.77 ±0.05	0.95 ±0.12	0.35 ±0.06	0.68 ±0.08	0.59 ±0.12	0.74 ±0.11	0.51 ±0.08	0.74 ±0.06	0.33 ±0.06	0.52 ±0.07
18:0	25.71 ±1.14	24.33 ±0.67	27.41 ±0.61	22.20 ±0.55	29.63 ±1.44	25.72 ±0.98	26.74 ±1.69	25.48 ±1.08	26.74 ±0.84	23.05 ±0.82	26.19 ±1.91	24.67 ±0.76
18:1	4.64 ±0.39	5.37 ±0.23	4.64 ±0.22	6.04 ±0.27	3.93 ±0.01	5.41 ±0.38	4.13 ±0.36	5.39 ±0.39	3.98 ±0.21	5.37 ±0.23	3.43 ±0.40	5.11 ±0.33
18:2	8.04 ±0.73	10.08 ±0.58	7.2 ±0.37	12.48 ±0.36	6.38 ±0.66	11.51 ±1.00	3.74 ±0.34	6.02 ±0.43	3.52 ±0.24	6.99 ±0.43	2.68 ±0.28	6.69 ±0.50
18:3	0.10 ±0.03	0.24 ±0.07	0.33 ±0.03	0.52 ±0.06	0.13 ±0.04	0.29 ±0.05	0.08 ±0.02	0.12 ±0.05	0.31 ±0.08	0.25 ±0.03	0.05 ±0.02	0.23 ±0.03
20:2	0.30 ±0.02	0.37 ±0.02	0.30 ±0.02	0.41 ±0.05	0.20 ±0.03	0.47 ±0.06	0.30 ±0.05	0.34 ±0.04	0.24 ±0.02	0.30 ±0.05	0.22 ±0.03	0.39 ±0.04
20:4	31.04 ±0.75	29.87 ±0.71	31.75 ±0.48	27.16 ±0.88	30.43 ±1.38	27.58 ±0.28	27.03 ±0.97	25.51 ±2.04	25.93 ±0.64	25.14 ±0.58	22.79 ±0.94	25.12 ±1.06
22:4	0.57 ±0.03	0.59 ±0.03	0.94 ±0.08	0.72 ±0.09	0.86 ±0.07	0.71 ±0.08	1.33 ±0.09	1.24 ±0.12	1.96 ±0.27	1.98 ±0.16	2.27 ±0.13	2.69 ±0.06
22:5	2.93 ±0.31	2.43 ±0.28	4.49 ±0.63	2.34 ±0.29	5.39 ±0.07	2.05 ±0.03	6.15 ±0.05	4.97 ±0.63	8.00 ±0.52	5.25 ±0.73	12.10 ±1.31	4.35 ±0.64
22:6	3.10 ±0.27	2.46 ±0.12	2.34 ±0.17	2.53 ±0.25	3.61 ±0.36	2.04 ±0.05	6.34 ±0.37	5.22 ±0.17	5.93 ±0.37	5.49 ±0.65	6.90 ±0.34	4.84 ±0.12

Results are expressed as percent composition. Values represent means ± SEM of 5 rats/group.
Age effect in AL-fed rats (2-way multivariate analysis of variance, MANOVA): 16:1, PC ($p < 0.001$), PE ($p < 0.01$); 18:0, PC ($p < 0.05$), PE (NS); 18:1, PC ($p < 0.05$), PE (NS); 18:2, PC ($p < 0.02$), PE ($p < 0.001$); 18:3 (NS); PC (NS); 20:4, PC (NS), PE ($p < 0.002$); 22:4, PC, ($p < 0.002$), PE ($p < 0.05$); 22:5, PC ($p < 0.001$), PE ($p < 0.001$), 22:6 PC ($p < 0.05$), PE (NS).
Age effect in FR rats (2-way MANOVA): 16:1, PC ($p < 0.02$), PE ($p < 0.01$); 18:2, PC ($p < 0.01$), PE ($p < 0.001$); 18:3, PC ($p < 0.005$), PE ($p < 0.05$); 20:4, PC ($p < 0.002$), PE (NS); 22:4, PC ($p < 0.05$), PE ($p < 0.001$); 22:6, PC ($p < 0.02$), PE ($p < 0.05$).
Diet effect (3-way MANOVA): 16:0, PC ($p < 0.01$), PE (NS); 16:1, PC ($p < 0.01$), PE ($p < 0.06$); 18:0, PC ($p < 0.001$), PE ($p < 0.001$); 18:1, PC ($p < 0.001$), PE ($p < 0.001$); 18:2, PC ($p < 0.001$), PE ($p < 0.001$); 18:3, (motochondria, PC ($p < 0.01$), PE ($p < 0.025$); microsomes, NS); 20:4, PC ($p < 0.01$), PE (NS); 22:4, PC (NS), PE ($p < 0.01$); 22:5, PC ($p < 0.001$), PE ($p < 0.001$); 22:6, PC ($p < 0.001$), PE ($p < 0.001$).

fatty acid in subcellular membrane lipids, decreased in PC ($p < 0.001$) and in PE ($p < 0.01$). There was no significant age-related change in linolenic acid (18:3), the precursor of docosahexaenoic aicd (22:6), however, levels of 22:6 increased steadily in membrane PC ($p < 0.05$). The main saturated fatty acids found in PC and PE were palmitic (16:0) and stearic (18:0) acids. Stearic acid was slightly increased in membrane PC ($p < 0.05$), but not in PE, whereas 16:0 remained constant with age. On the other hand, 16:1 decreased steadily with age in PC ($p < 0.001$) and in PE ($p < 0.01$), whereas 18:1 content decreased only in PC ($p < 0.05$).

The data pertaining to the modulation of fatty acid composition by chronic food restriction reflected a different pattern of changes (table 1, 2, fig. 2, 3). In contrast to the pattern observed in AL-fed rats, mitochondrial and microsomal 18:2 was progressively increased in PC ($p < 0.01$) and PE ($p < 0.001$) with age, and the diet-related differences in 18:2 content were highly significant between 6 and 24 months of age in PC and PE. For instance, 18:2 content was approxi-

Table 2. Effects of age and food restriction on fatty acid compositon of phospholipids: microsomal membrane fracton

Fatty acid	PC						PE					
	6 months		12 months		24 months		6 months		12 months		24 months	
	AL	FR	AL	FR	Al	Fr	AL	FR	AL	FR	AL	FR
16:0	18.55 ±0.54	19.36 ±0.33	20.18 ±1.57	21.44 ±0.72	16.79 ±1.09	18.48 ±0.64	16.74 ±0.61	17.56 ±0.24	20.10 ±1.52	19.03 ±0.69	17.16 ±0.72	17.31 ±0.92
16:1	0.80 ±0.09	0.78 ±0.07	0.66 ±0.07	0.74 ±0.03	0.35 ±0.06	0.68 ±0.12	0.58 ±0.07	0.74 ±0.03	0.64 ±0.12	0.54 ±0.03	0.37 ±0.09	0.55 ±0.03
18:0	24.73 ±1.83	24.74 ±0.25	27.50 ±1.31	23.37 ±0.90	27.08 ±2.15	23.75 ±0.41	24.94 ±1.15	23.37 ±0.89	27.57 ±2.10	24.12 ±0.74	25.84 ±1.19	22.98 ±0.91
18:1	4.46 ±0.21	5.48 ±0.13	4.59 ±0.08	5.74 ±0.27	4.04 ±0.31	6.04 ±0.17	4.23 ±0.24	5.74 ±0.27	3.52 ±0.41	5.69 ±0.23	3.60 ±0.41	5.88 ±0.13
18:2	9.07 ±0.76	10.46 ±0.81	8.23 ±0.48	12.30 ±0.51	6.71 ±0.78	13.34 ±0.74	5.09 ±0.25	7.10 ±0.44	4.66 ±0.24	7.63 ±0.31	3.06 ±0.37	9.09 ±0.14
18:3	0.20 ±0.05	0.22 ±0.04	0.31 ±0.04	1.24 ±0.08	0.24 ±0.06	0.52 ±0.07	0.18 ±0.02	0.24 ±0.05	0.18 ±0.04	0.33 ±0.03	0.21 ±0.07	0.26 ±0.02
20:2	0.07 ±0.07	0.29 ±0.04	0.28 ±0.04	0.34 ±0.11	0.29 ±0.04	0.18 ±0.12	0.15 ±0.11	0.27 ±0.30	0.11 ±0.05	0.30 ±0.05	0.25 ±0.25	0.16 ±0.18
20:4	30.80 ±1.15	29.43 ±0.63	29.05 ±0.77	25.94 ±0.46	32.95 ±2.41	27.45 ±0.37	27.44 ±0.55	25.94 ±0.46	21.83 ±1.77	21.92 ±0.98	21.81 ±0.82	24.97 ±0.77
22:4	0.56 ±0.04	0.57 ±0.03	0.68 ±0.05	0.57 ±0.11	0.80 ±0.05	0.70 ±0.03	1.88 ±0.12	1.69 ±0.11	2.54 ±0.32	2.06 ±0.20	2.54 ±0.18	2.18 ±0.05
22:5	2.68 ±0.32	2.32 ±0.28	2.81 ±0.09	1.91 ±0.19	5.52 ±0.53	1.88 ±0.21	5.94 ±0.61	4.38 ±0.64	5.56 ±0.66	4.51 ±0.67	12.43 ±1.15	4.96 ±0.42
22:6	3.07 ±0.23	2.27 ±0.16	3.24 ±0.20	2.21 ±0.08	3.51 ±0.62	1.99 ±0.05	6.34 ±0.43	4.51 ±0.15	6.37 ±0.51	4.80 ±0.39	6.04 ±0.42	4.43 ±0.29

Results are expressed as percent composition. Values represent means ± SEM of 5 rats/group. For significances, see table 1.

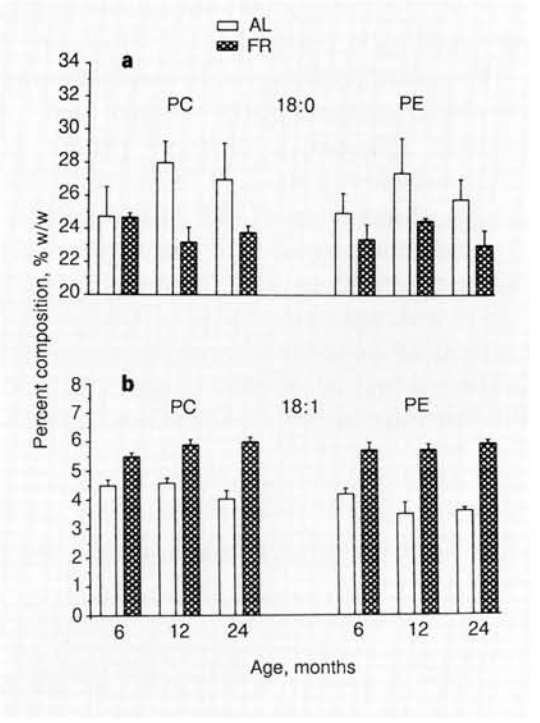


Fig. 2. Effect of age and food restriction on levels of stearic (18:0) and oleic acid (18:1) in PC and PE of liver microsomal membrane fraction of 6, 12 and 24-month-old Fischer 344 rats. Results are expressed as percent composition. Refer to table 2 for details.

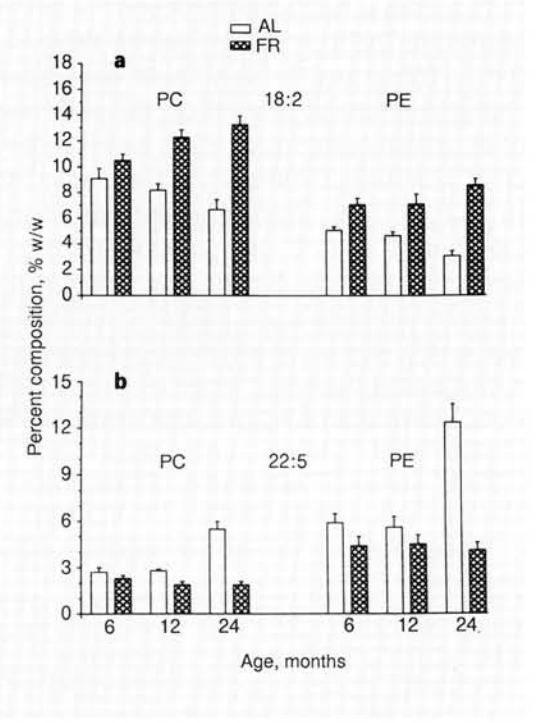


Fig. 3. Effect of age and food restriction on levels of linoleic (18:2) and docosapentaenoic acid (22:5) in PC and PE of liver microsomal membrane fraction of 6, 12 and 24-month-old Fischer 344 rats. Results are expressed as percent composition. Refer to table 2 for details.

mately twice as high in FR rats than in AL-fed rats at 24 months of age. Data showed that the content of 22:5, which is derived from 18:2, exhibited a strong tendency to decrease with age in PC and PE of both membranes, although the trend was not statistically significant. Diet-related differences in 22:5 content were also highly significant in PC and PE, in that 22:5 was approximately 3 times lower in PE of 24-month-old FR rats than in AL-fed rats of the same age.

The suppression of high levels of membrane polyunsaturates by food restriction in aging rats was also observed in the content of 22:4 in membrane PE ($p < 0.01$) but not in PC. As presented in table 2, arachidonic content of FR rats remained constant with age in PE, but fell in PC ($p < 0.002$). Therefore, 20:4 content was markedly lower in FR rat membrane PC ($p < 0.01$), whereas no significant difference was observed in membrane PE. Regardless of the dietary regimen, 20:4 content was higher in membrane PC than PE ($p < 0.001$). Linolenic acid (18:3) content was modulated by food restriction, as shown in table 2. As observed with 18:2, linolenic content increased with age in microsomal PC ($p < 0.005$) and in mitochondrial PE ($p < 0.05$). Linolenic content was higher in the microsomal membranes of FR rats (PC, $p <$

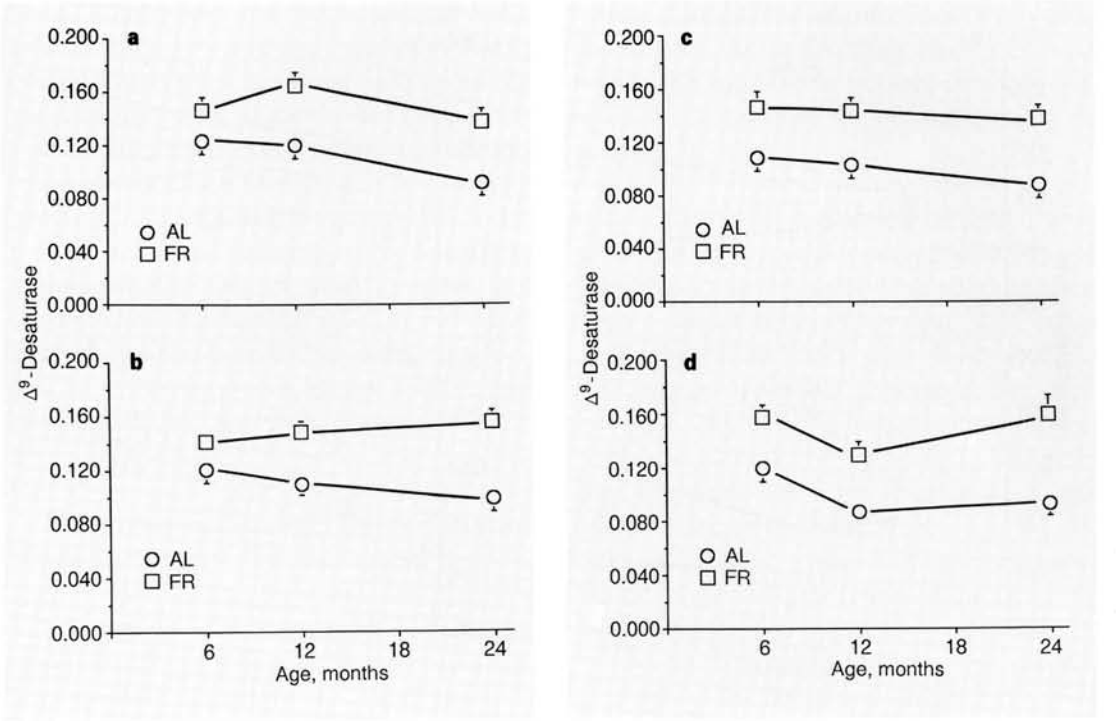
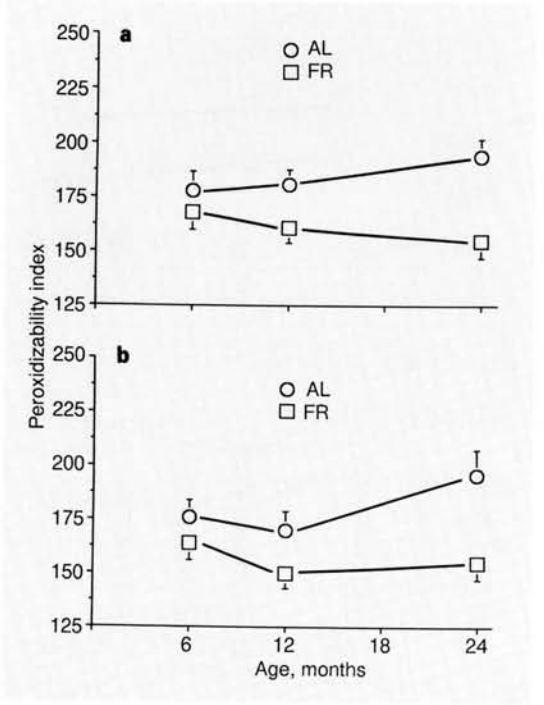


Fig. 4. Effects of age and food restriction on Δ^9 fatty acid desaturase coefficient of activity in PC (**a**) and PE (**b**) from liver mitochondrial membrane fraction, and in PC (**c**) and PE (**d**) from liver microsomal membrane fraction. Coefficient of activity was calculated as following: sum of 16:0 and 18:0 divided by sum of 16:1 and 18:1 (table 1). Values represent means ± SEM of 5 rats per group. Age effect was tested by 2-way Manova (PC, PE): AL-fed rats, mitochondria ($p < 0.001$); microsomes ($p < 0.003$). FR rats: no significant age effect. Diet effect (3-way Manova): mitochondria ($p < 0.001$); microsomes ($p < 0.001$).

0.01; PE, $p < 0.025$), but not in mitochondrial membranes. The levels of 22:6 were actually decreased with age in membrane PC of FR rats ($p < 0.05$). Thus, levels of 22:6 were consistently lower in FR rat membrane (PC, $p < 0.001$; PE, $p < 0.001$), between 6 and 24 months of age.

Tables 1 and 2 also show that the saturates 16:0 and 18:0 remained constant with age in FR rats. Accordingly, levels of 16:0 and 18:0 were lower than in AL-fed rat membranes (PC, $p < 0.01$; and PE, $p < 0.001$; PC, $p < 0.001$, respectively). On the other hand, although levels of 16:1 decreased with age in PC ($p < 0.02$) and PE ($p < 0.02$), 16:1 content remained higher in PC ($p < 0.01$) and PE ($p < 0.06$) of FR rat membranes. Food restriction suppressed the age-related increase in 18:0 and maintained lower levels of this fatty acid in PC ($p < 0.001$) and PE ($p < 0.001$). Levels of 18:1 were much higher in FR rat membrane PC and PE ($p < 0.001$). The conversion of saturated fatty acids into monounsaturates is catalyzed by a Δ^9-desaturase. The changes in Δ^9-desaturase were examined by calculating the ratio of monounsaturates/saturates. Figure 4 indicates a steady decrease with age in mitochondrial ($p < 0.001$) and microsomal ($p < 0.003$) desaturase coefficient of activity of AL-fed rats. Interestingly



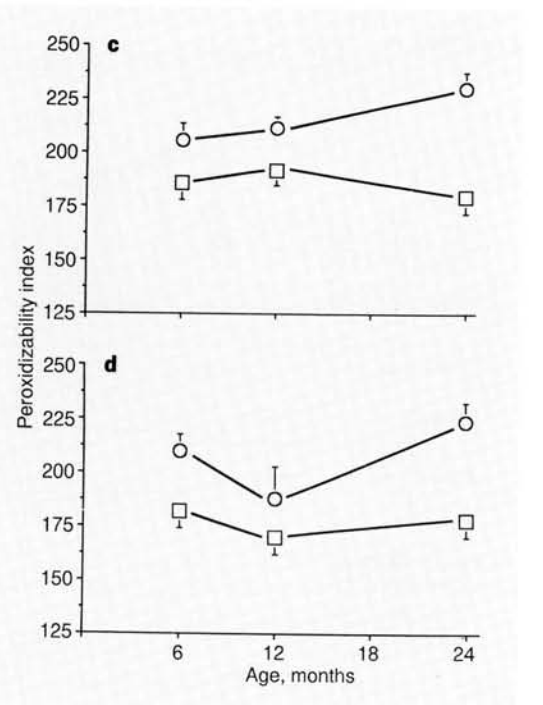


Fig. 5. Effects of age and food restriction on the peroxidizability index of the fatty acyls chains in PC (**a**) and PE (**b**) from liver mitochondrial membrane fraction, and in PC (**c**) and PE (**d**) from liver microsomal membrane fraction. Coefficient was calculated as following: (% monounsaturate × 0.025) + (% diunsaturate × 1) + (% triunsaturate × 2) + (% tetraunsaturate × 4) + (% pentaunsaturate × 6) + (% hexaunsaturate × 8). Values represent means ± SEM of 5 rats per group. Age effect was tested by 2-way Manova (PC, PE): AL-fed rats, mitochondria ($p < 0.004$); microsomes ($p < 0.015$). FR rats: no significant age effect. Diet effect (3-way Manova): mitochondria ($p < 0.001$); microsomes ($p < 0.001$).

the coefficient of Δ^9-desaturase activity displayed no age-related decrease in FR rats, but was consistently higher than in AL-fed rats ($p < 0.001$).

The analysis of the data presented in tables 1 and 2 suggests strongly the modulation of membrane composition in aging AL-fed rats with a characteristic accretion in levels of oxidizable fatty acids. To quantitate this membrane change, we utilized the peroxidizability index to canvass the overall changes in membrane characteristics which make membranes more susceptible to peroxidation. Figure 5 illustrates that peroxidizability was greatly increased in both mitochondrial and microsomal membranes of aging AL-fed rats ($p < 0.004$, $p < 0.015$, respectively). In contrast, the restricted regimen prevented this age-related phenomenon in membrane PC and PE, and peroxidizability was consistently lower in FR rats ($p < 0.001$).

Discussion

The dynamic adaptation of membrane lipids to dietary manipulation has been demonstrated experimentally under many nutri-

tional conditions, such as acute starvation and refeeding [16, 17], and changes in dietary composition [18]. The salient findings emerging from our study indicate that (1) the substantial modification of the fatty acid composition of subcellular membrane phospholipids observed in aging animals supports the notion that the enhanced vulnerability of membrane peroxidizability occurs during aging and (2) that these age-related phenomena are effectively counteracted by long-term calorie restriction. This unique action of food restriction, which has not been previously recognized, has become more evident recently [6, 19].

When Fischer rats are rigorously maintained on the same diet throughout their life span in a well-controlled environment, the modulation of membrane fatty acids during aging appears to delineate the following pattern: membrane levels of precursor fatty acids (18:2 and 18:3) are steadily decreased, whereas the content in long-chain polyunsaturated derivatives is progressively increased with age [6, 19, 20]. Our results have clearly demonstrated that food restriction does not only prevent this phenomenon, but triggers a significant reversal by increasing the content of precursor fatty acids and decreasing polyunsaturated derivatives with age in liver subcellular membranes. This pattern of modulation is expected to enhance membrane stability in general, as reported in recent studies [21, 22]. It should also be pointed out that this pattern is not limited to the liver. A similar action of food restriction has been reported in tissue as varied as plasma [23], kidney [19], splenocytes and bone marrow cells [20]. Additionally, serum lipoprotein from FR rats contained higher levels of 18:2 and lower levels of 20:4 at 6, 12 and 18 months [S. Laganiere, B.P. Yu, unpubl. data]. Altogether, the patterns of age- and diet-related changes in lipids lead to the conclusion that the antioxidative action of long-term calorie restriction is associated with the preferential modification of membrane long-chain polyunsaturated fatty acids as indicated by the peroxidizability index (fig. 5).

In earlier studies, we have shown an age-related rise in concentration of lipid hydroperoxides in subcellular membranes [5, 24]. The immediate targets of these alterations are likely to be long-chain fatty acids with multiple double bonds which were shown to make membranes vulnerable to peroxidative damage [25, 26]. The age-related increase in polyunsaturates during aging [6, 27, 28] probably underlies the high incidence of age-related diseases that was observed in AL-fed rats [29, 30]. This notion is in agreement with the rationale and prediction of the free-radical theory [31, 32].

The mechanisms underlying the progressive change in membrane fatty acids during aging are poorly undertood. Although fatty acid composition is markedly different in PC and PE, our results indicate that the choline and ethanolamine phosphoglycerides were by and large equally affected by age. It has been known from studies on the metabolism of essential fatty acids that long-chain fatty acids are biosynthesized by the alternate reactions of desaturation-elongation processes [18, 33, 34]. Our results indicate that desaturation of saturates (e.g. 16:0, 18:0) into monosaturates (e.g. 16:1, 18:1, respectively) by the action of Δ^9-desaturase may be altered with age. As illustrated in figure 4, the Δ^9-desaturase activity coefficient decreased steadily throughout the life span of AL-fed rats. This finding may reveal that reduced amounts of 16:1 and 18:1 are synthesized during aging. By using individual phospholipids in the present study, we have provided a clue regarding the status of Δ^9-desaturase, which, to the best of our knowledge, has not been previously investigated in aging animals. Our data show clearly

that liver subcellular membranes of FR rats contain higher proportions of monosaturates than those of the controls, especially in the aged animals. This result may produce a significant advantage in membrane signal transduction, in the light of recent studies indicating that oleic acid acts as a potent regulator of protein kinase C [35]. Higher levels of membrane oleic acid coupled to lower saturated fatty acids are expected to increase membrane fluidity [18]. However, the extent to which the physicochemical properties of the lipid bilayer are influenced by aging and food restriction are still unknown.

Pertinent information accounting for the action of food restriction upon the regulation of membrane fatty acid biosynthesis, particularly during aging, is completely absent from the availble literature at present. Recent reviews [34, 36] have emphasized that membrane fatty acid desaturase rates can be markedly modulated by experimental conditions involving dietary manipulation, such as food deprivation and starvation. Although one is tempted to speculate that a similar mechanism could be operative in lifelong calorie-restricted rats, however, such an outcome is unlikely because FR rats are at steady state in term of energy consumption and expenditure. Their daily caloric intake (kcal/lean body mass) is the same as that of AL-fed rats [8, 37]. One key enzyme that could contribute to further understanding membrane fatty acid synthesis under food restriction is the Δ^6 fatty acid desaturase activity, which is the rate-limiting enzyme responsible for conversion of 18:2 and 18:3 [33]. Previous studies have shown that Δ^6-desaturase activity is lowered in aged animals [38–40]. Preliminary data from our laboratory have confirmed this finding in the liver of AL-fed Fischer rats, however, the age-related decline was significantly suppressed by long-term dietary restriction [41]. Although Δ^6-desaturase activity was found to decrease with age, levels of n–6 and n–3 polyunsaturated fatty acids increased instead of decreased in the AL-fed rats. This means that the Δ^5 and Δ^4-desaturases, or factors other than desaturation activity, such as de novo membrane lipid synthesis and degradation, fatty acid turnover and oxidation, must also be involved in the regulation of the fatty acid composition of liver membranes during aging. Full exploration of mechanisms underlying the regulation of desaturation of membrane lipids is beyond the scope of this present study. However, it can be speculated, based on the analysis of fatty acid profile and the preliminary data on Δ^6- and Δ^6-desaturates, that the regulation of desaturases is directed toward maintaining the most optimal membrane physical status, such as fluidity in which cellular homeostasis is properly maintained. Irrespective of the exact mechanism, food restriction blunted the age-related changes.

In conclusion, our study substantiated clearly that membrane phospholipid peroxidizability is progressively increased during the course of aging. The mitigation of such a phenomenon by chronic food restriction provides further evidence that the antiaging action of dietary restriction may primarily be promoted by stabilizing membrane integrity throughout the organism. Further investigation is needed to define the important factor(s) involved in the regulation of membrane biosynthetic activity during aging and long-term calorie restriction.

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