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ORIGINAL ARTICLES

Effects of dietary restriction on age-related changes in the phospholipid fatty acid composition of various rat tissues

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ABSTRACT. ***Background and aims:*** *Polyunsaturated fatty acids (PUFAs) are essential components of the cell lipid bilayer and are involved in membrane fluidity and normal functioning, but they are vulnerable to free radical attack. Given the role of oxidative stress in the aging process, age-related changes in phospholipid fatty acid (PLFA) composition in rat liver, kidney and heart were assessed in 3-, 12- and 24-month-old rats fed either ad libitum but only every other day, or daily but only 60% of the quantity normally consumed by age-matched controls.* ***Methods:*** *Lipids were extracted and phospholipids (PLs) were separated using the solid phase extraction technique, then transesterified and assayed by gas-liquid chromatography.* ***Results:*** *Saturated fatty acids (FAs) did not change significantly with age; mono- and bi-unsaturated FAs decreased in the liver and heart, and the ratio of the former to the latter increased in the liver, kidney and heart. PUFAs increased in the liver and heart. As regards individual FAs, 20:1(n-9) decreased in all organs, 14:1 and 18:1(n-7) increased in the kidney and heart, 18:1(n-9) increased in the kidney, 20:2(n-6), 18:2(n-6) and 22:5(n-3) decreased in the liver and heart, 20:3(n-6) decreased in the kidney and increased in the heart. The most abundant PUFAs, 20:4(n-6) and 22:6(n-3), either remained the same or increased with age. The N-9 family increased in the kidney, the N-7 family increased in the kidney and heart, the N-6 family decreased in all three organs, and the N-3 family increased in the liver and kidney. Dietary restriction (DR) significantly counteracted most of these changes, but changes in some FAs [20:2(n-6) in the heart] were magnified by DR and may not be age-related.* ***Conclusions:*** *Most age-related changes (that occurred in the rat liver, kidney and heart and were counteracted by the two different types of DR) may be involved in the mechanism of aging.*
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INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are essential components of the cell lipid bilayer and may have a pivotal role in free radical metabolism (1) and in maintaining the membrane structure and fluidity required for normal functioning. A number of reports have shown that fluidity affects signal transduction (2), transmembrane transport (3), membrane enzyme activities (4, 5), eicosanoid metabolism and physiological activities (6, 7). PUFAs are highly vulnerable to free radical attack due to their susceptibility to hydrogen removal, and they may be converted into lipid hydroperoxides. Most cells contain antioxidant enzymes (such as superoxide dismutase and catalase) and non-enzymatic defenses (such as vitamin E, vitamin C and glutathione), but reports have shown that the concentrations of several of these antioxidant defense molecules may only suffice to cope with normal free radical production, and an imbalance between free radical production and their removal may develop with age, enabling progressive damage to occur (7-9).

Significant changes have been reported in the 20:4(n-6) and 22:6(n-3) fatty acids (FAs) in phospholipids (PLs) from the liver, kidney and heart of aged rats, and dietary supplementation with thyme oil provided significant protection (7). The present study investigated the effects of aging and anti-aging dietary restriction (DR) on changes in the phospholipid fatty acid (PLFA) composition of various tissues during the rat's life span. Two different, equally-ef-

Key words: Aging, dietary restriction, heart, kidney, liver, membrane function, polyunsaturated fatty acid, rat.

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fective regimens of DR were used, i.e. 40% calorie restriction (40% CR) and *ad libitum* feeding every other day (EOD). DR can delay and/or blunt most age-related changes in physiological systems (10, 11), possibly by enhancing the rates of autophagy and membrane turnover and maintenance (12).

METHODS

Animals

Male Sprague-Dawley rats were housed five in a cage. Ambient temperature was kept at 21-22°C with 12-hour cycles of dark and light (the lights were turned on from 6:00 a.m. to 6:00 p.m.). Two-month-old rats were randomly divided into three dietary groups: 20 control rats were fed AL on a standard pellet diet (Teklad, Harlan Italy: Table 1), six 40% CR rats received 60% of the amount of food consumed by the age-matched controls (AL group), and six EOD rats received food ad libitum every other day. Fresh water was freely available. Body weights are shown in Table 2. The AL-fed control rats had median life-span of approximately 24 months.

Tissue samples were taken in the morning under anesthesia with nembutal (50 mg in 1 ml saline solution per kg body weight) from 3-, 12- and 24-month-old AL rats and from 24-month-old 40% CR and EOD rats (on the day of feeding). A 16 h (overnight) fasting period always preceded tissue sampling.

Parts of liver, kidney and left ventricle of heart were excised, placed immediately in liquid nitrogen and stored at -80°C until analysis.

Table 1 - *FA % composition of dietary lipids.*

FATTY ACIDS	DIETARY CONTENT
14:0	0.78
16:0	13.9
18:0	3.3
20:0	0.32
22:0	0.39
24:0	0.22
Saturated	19.0
14:1	0.11
18:1(n-9)	18.5
18:1(n-7)	1.2
20:1(n-9)	0.00
18:2(n-6)	49.2
20:2(n-6)	0.07
Mono- and bi-unsaturated	69.1
Mono-/bi-unsaturated	0.40
18:3(n-3)	0.02
18:3(n-6)	0.01
20:3(n-6)	0.04
20:4(n-6)	0.02
20:5(n-3) / 22:4(n-6)	0.84
22:5(n-3)	0.13
22:6(n-3)	1.2
Polyunsaturated	2.3
Not identified peaks	9.6
Family abundance	
N-9	18.5
N-7	1.2
N-6	49.3
N-3	1.4

Lipid extraction and fatty acid analysis

Lipids were extracted with a chloroform/methanol (2:1, v/v) mixture containing 0.05 g/l butylated hydroxytoluene as an antioxidant according to Folch et al. (13).

PLs were separated from neutral lipids and glycolipids using Sep-Pak silica gel cartridges (Supelco, Sigma-Aldrich) by elution with chloroform/acetic acid (100:1, v/v), acetone/acetic acid (100:1, v/v) and methanol/chloroform/water (100:50:40, v/v/v) for neutral lipids, glycolipids and PLs, respectively (14).

PLs were transesterified with 5% H_2SO_4 in methanol at 80°C for 180 min. After esterification the fatty acid methyl esters (FAMEs) were extracted in hexane, dried under N_2 and redissolved in 50 µl hexane. Two µl were used for gas-liquid chromatography (GC) analysis (7, 15). GC separation was performed with a SP2340 capillary column (Supelco 60 m×0.25 mm I.D., film thickness 0.20 µm) using a gas-liquid chromatograph (Shimadzu GC-17A) equipped with a flame ionization detector. Helium was used as the carrier gas; the flow rate was 0.9 mL/min and the split ratio was 1:100. The injector and detector temperatures were 250°C and 260°C, respectively. Initial column temperature (185°C) was maintained for 25 min; temperature was increased at a rate of 10°C/min to 205°C, maintained at 205°C for 1 min and increased at a rate of 5°C/min to 215°C. The final temperature was maintained for 12 min. The peaks were checked against the retention times of standard FAMEs (Sigma-Aldrich). FA composition is expressed as a percentage of the total FAs.

Table 2 - *Body weights (g) of AL, 40% CR and EOD rats.*

	AL 3 months	AL 12 months	AL 24 months	40% CR 24 months	EOD 24 months
Body weights	390±3.4	557±13.5	718±36.3	456±4.7	444±11.1

Results are expressed as means ±SEM. The number of animals considered in each case was 6 (except for the 24-month-old AL-fed control rats, of which there were 5).

Table 3 - *Effect of age and anti-aging dietary restriction (40% CR or EOD) on PLFA composition in the liver of young, adult and old rats.*

FATTY ACIDS	AL 3 months	AL 12 months	AL 24 months	40% CR 24 months	EOD 24 months
14:0	0.12±0.007	0.12±0.004	0.14±0.006	0.14±0.003	0.16±0.012^{A,C}
16:0	17.2±0.22	18.7±0.16^{A}	17.0±0.75^{C}	18.6±0.27^{E}	20.1±0.15^{A,E,X}
18:0	21.5±0.40	20.3±0.27	23.2±0.95^{C}	20.0±0.26^{E}	19.3±0.42^{A,E}
20:0	0.11±0.005	0.10±0.004	0.04±0.018^{A,C}	0.08±0.002	0.06±0.012^{A,C}
22:0	0.27±0.010	0.30±0.010	0.15±0.038^{A,C}	0.27±0.010^{E}	0.24±0.012^{E}
24:0	0.84±0.027	0.89±0.017	0.68±0.023^{A,C}	0.80±0.018^{C,E}	0.78±0.019^{C,E}
Saturated	40.1	40.4	41.3	39.9	40.7
14:1	0.22±0.020	0.30±0.024	0.28±0.043	0.31±0.015	0.35±0.007^{A}
18:1(n-9)	2.1±0.07	2.5±0.06	2.1±0.15	3.2±0.13^{A,C,E}	3.6±0.12^{A,C,E}
18:1(n-7)	2.3±0.08	2.5±0.12	2.6±0.30	2.2±0.12	2.0±0.12
20:1(n-9)	0.21±0.025	0.10±0.009^{A}	0.01±0.015^{A,C}	0.14±0.008^{C,E}	0.12±0.011^{C,E}
18:2(n-6)	15.8±0.21	12.3±0.51^{A}	12.0±0.72^{A}	18.4±0.33^{A,C,E}	17.3±0.48^{C,E}
20:2(n-6)	0.44±0.023	0.27±0.013^{A}	0.20±0.069^{A}	0.22±0.003^{A}	0.20±0.013^{A}
Mono- and bi-unsaturated	21.1	18.0^{A}	17.2^{A}	24.5^{A,C,E}	23.6^{A,C,E}
Mono-/bi-unsaturated	0.30±0.006	0.43±0.025^{A}	0.41±0.008^{A}	0.32±0.011^{C,E}	0.35±0.005^{C,E}
18:3(n-3)	0.04±0.020	0.06±0.004	0.01±0.012^{D}	0.06±0.002	0.03±0.012
18:3(n-6)	0.07±0.003	0.08±0.006	0.13±0.038	0.16±0.009^{A,C}	0.21±0.016^{A,C,E}
20:3(n-6)	0.77±0.059	0.64±0.034	0.88±0.083^{C}	1.0±0.02^{A,C}	1.3±0.05^{A,C,E,X}
20:4(n-6)	25.3±0.35	22.4±0.40^{A}	24.1±0.50	19.2±0.43^{A,C,E}	17.3±0.62^{A,C,E}
20:5(n-3)/22:4(n-6)	0.45±0.033	0.28±0.016	0.29±0.038	0.85±0.040^{A,C,E}	1.0±0.08^{A,C,E,X}
22:5(n-3)	1.2±0.06	1.2±0.06	0.87±0.074^{A,C}	1.6±0.09^{A,C,E}	1.9±0.07^{A,C,E,X}
22:6(n-3)	7.8±0.25	13.4±0.50^{A}	13.0±1.01^{A}	10.0±0.61^{C,E}	11.0±0.71^{A}
Polyunsaturated	35.6	38.0	39.2A	32.8^{C,E}	32.7^{C,E}
Not identified peaks	3.2±0.12	3.4±0.08	2.3±0.20^{D}	2.8±0.10	3.0±0.45
Family abundance					
N-9	2.4	2.6	2.1^{C}	3.3^{A,C,E}	3.7^{A,C,E}
N-7	2.3	2.5	2.6	2.2	2.0
N-6	42.3	35.8^{A}	37.2^{A}	38.9^{A,C}	36.3^{A,X}
N-3	9.1	14.6^{A}	13.9^{A}	11.7^{A,C}	12.9^{A}

Results are expressed as means ±SEM of the % composition of PLFAs. The number of animals considered in each case was 6 (except for the 24-month-old AL-fed control rats, of which there were 5).
The statistical analysis is given as follows: difference between aged or treated rats and young (3-month-old) control rats $^{A}p<0.01$, $^{B}p<0.05$; adult (12-month-old) and older or treated rats $^{C}p<0.01$, $^{D}p<0.05$; old (24-month-old) control rats and DR rats $^{E}p<0.01$, $^{F}p<0.05$; difference between EOD and CR rats $^{X}p<0.01$, $^{Y}p<0.05$.

Statistical analysis

Data are given as means ±SEM. The statistical significance of the effects of age and diet was tested by analysis of variance (ANOVA), followed by Tukey's test for pairwise comparisons taking $p<0.05$ as the limit of significance. p-values <0.01 were considered highly significant.

RESULTS

Changes in the composition of FAs in liver PLs with age and DR are shown in Table 3. Older AL-fed rats were lower in 20:0, 22:0, 24:0, 20:1(n-9), 18:2(n-6), 20:2(n-6) and 22:5(n-3) than younger rats. DR significantly counteracted these age-related changes, except in the case of 20:0 and 20:2(n-6). In contrast, 22:6(n-3) was higher in the older than in the younger AL-fed rats; this increase was contained to some degree by 40% CR. The proportions of 16:0 and 18:0 were affected significantly by DR in opposite directions. The percentage of 20:4(n-6) tended to decline with age and was significantly reduced by DR. The tendency to higher 18:3(n-6) and 20:3(n-6) levels with aging was not significant, but it was magnified by EOD DR.

Table 4 shows changes in the FA levels in kidney PLs. The percentages of 14:0, 18:0, 20:1(n-9) and 20:3(n-6) were lower in older than in younger AL-fed rats. DR significantly counteracted these age-related variations with the sole exception of 20:1(n-9). In contrast, the proportions of 22:0, 14:1, 18:1(n-9), 18:1(n-7), 18:3(n-3) and 22:6(n-3) were higher in older than in younger AL-fed rats and such an increase was prevented by DR in all cases, except for 22:0, 14:1 and 18:3(n-3). The percentages of 18:2(n-6) and 20:5(n-3)/22:4(n-6) tended to decline with age and were increased by DR. The proportion of 20:4(n-6) tended to decrease with age and the decrease was magnified by EOD DR. The tendency towards higher 18:3(n-6) levels with aging was not significant, but it was magnified by DR.

Comparisons between FA compositions in the PLs from the left ventricle of the hearts of young and aged rats

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Table 4 - *Effect of age and anti-aging dietary restrictions (40% CR or EOD) on PLFA composition in the kidney of young, adult and old rats.*

FATTY ACIDS	AL 3 months	AL 12 months	AL 24 months	40% CR 24 months	EOD 24 months
14:0	0.16±0.004	0.15±0.007	0.12±0.005^{A,C}	0.15±0.007^{E}	0.16±0.005^{E}
16:0	17.3±0.18	18.3±0.32	17.5±0.25	17.2±0.26^{D}	18.0±0.24
18:0	17.7±0.11	17.4±0.14	16.1±0.15^{A,C}	16.8±0.07^{A,C,E}	16.2±0.073^{A,C,X}
20:0	0.24±0.005	0.34±0.028^{B}	0.30±0.011	0.29±0.010	0.31±0.029
22:0	0.54±0.011	0.68±0.025^{A}	0.67±0.014^{A}	0.67±0.007^{A}	0.72±0.023^{A}
24:0	4.3±0.03	4.1±0.25	4.1±0.15	4.4±0.12	4.2±0.26
Saturated	40.2	40.9	38.9^{C}	39.5^{C}	39.6^{C}
14:1	1.3±0.16	1.6±0.14	1.9±0.08^{A}	1.8±0.14	2.1±0.09^{A}
18:1(n-9)	5.1±0.05	5.5±0.14	6.4±0.21^{A,C}	5.6±0.06^{E}	5.7±0.13^{A,E}
18:1(n-7)	1.8±0.01	2.2±0.04^{A}	2.6±0.10^{A,C}	2.1±0.05^{A,E}	2.1±0.04^{A,E}
20:1(n-9)	0.24±0.017	0.11±0.006^{A}	0.10±0.002^{A}	0.09±0.003^{A}	0.08±0.004^{A}
18:2(n-6)	14.2±0.12	12.1±0.50^{A}	13.5±0.34	15.5±0.13^{C,E}	15.1±0.47^{C,E}
20:2(n-6)	0.35±0.010	0.39±0.042	0.28±0.015	0.28±0.006	0.30±0.045
Mono- and bi-unsaturated	23.0	21.9	24.8^{A,C}	25.3^{A,C}	25.3^{A,C}
Mono-/bi-unsaturated	0.58±0.011	0.76±0.048^{A}	0.80±0.032^{A}	0.61±0.014^{C,E}	0.65±0.033^{E}
18:3(n-3)	0.03±0.014	0.11±0.012^{A}	0.10±0.005^{A}	0.10±0.002^{A}	0.11±0.011^{A}
18:3(n-6)	0.03±0.001	0.03±0.001	0.04±0.003	0.05±0.002^{A,C,E}	0.05±0.002^{A,C,E}
20:3(n-6)	0.80±0.025	0.65±0.021^{A}	0.68±0.018^{A}	0.81±0.018^{C,E}	0.93±0.036^{A,C,E,X}
20:4(n-6)	26.7±0.17	25.8±0.48	25.5±0.24	24.5±0.10^{A,C}	23.9±0.22^{A,C,E}
20:5(n-3) / 22:4(n-6)	0.59±0.024	0.39±0.030^{A}	0.45±0.044	0.65±0.019^{C,E}	0.73±0.041^{A,C,E}
22:5(n-3)	0.56±0.011	0.60±0.027	0.50±0.023	0.53±0.022	0.55±0.056
22:6(n-3)	2.1±0.05	2.5±0.07	2.5±0.16^{A}	2.1±0.08^{E}	2.3±0.08
Polyunsaturated	30.8	30.0	29.8	28.8^{A,C}	28.6^{A,C,E}
Not identified peaks	6.1±0.05	7.1±0.36	6.4±0.16	6.4±0.34	6.5±0.30
Family abundance					
N-9	5.4	5.6	6.5^{A,C}	5.7^{E}	5.8^{E}
N-7	1.8	2.2^{A}	2.6^{A,C}	2.1^{A,E}	2.1^{A,E}
N-6	42.0	38.9^{A}	40.1^{A}	41.1^{C}	40.3
N-3	2.7	3.2^{A}	3.1^{A}	2.8^{C}	3.0

Results are expressed as the mean ±SEM of the % composition of PLFAs. The number of animals considered in each case was 6 (except for the 24-month-old AL-fed control rats, of which there were 5).
The statistical analysis is given as follows: difference between aged or treated rats and young (3-month-old) control rats $^{A}p<0.01$, $^{B}p<0.05$; adult (12-month-old) and older or treated rats $^{C}p<0.01$, $^{D}p<0.05$; old (24-month-old) control rats and DR rats $^{E}p<0.01$, $^{F}p<0.05$; difference between EOD and CR rats $^{X}p<0.01$, $^{Y}p<0.05$.

are shown in Table 5. The percentage of 20:0, 22:0, 20:1(n-9) and 18:2(n-6) decreased in old AL rats and the decrease was prevented by DR except in the case of 20:1(n-9). The levels of 24:0, 14:1, 18:1(n-7), 20:3(n-6) and 20:4(n-6) were significantly higher in older than in younger control rats, and the increase was prevented by DR with the exception of 24:0 and 18:1(n-7). The proportion of 20:5(n-3)/22:4(n-6) tended to decrease with age and was increased by DR. The level of 22:6(n-3) tended to increase with age and was reduced by EOD DR. The percentage of 20:2(n-6) declined with age and was significantly reduced by DR. The levels of 16:0 tended to increase with age and DR magnified this effect.

DISCUSSION

FAs, and C20 and C22 PUFAs in particular, are important components of membrane PLs. PUFAs confer fluidity, flexibility and selective permeability to cell membranes and affect many cellular and physiological processes including adaptation to cold and survival (16, 17), ion channel modulation (18), endocytosis/exocytosis (19), and the activities of membrane-bound enzymes that are sensitive to the biophysical properties of membrane lipids (7). Most of these processes are known to undergo dramatic changes with increasing age. For example, 20:3(n-6), 20:4(n-6) and 20:5(n-3) FAs are important precursors of eicosanoids, which exhibit a wide variety of highly-desirable (e.g. anti-inflammatory, anti-thrombotic and vasodilatory) actions.

The levels of 19 FAs (accounting for 95% of the total PLFAs) in the liver, kidney and heart of young, mature and old male Sprague Dawley rats are reported here. Significant age-related changes were observed in 8 FAs in the liver and 10 in the kidney and heart. The abundance of 11 FAs in the same organs of male Wistar rats has been reported elsewhere, with similar results (7) - the minor dif-

ferences with respect to our findings are probably attributable mainly to strain and diet. Different results were obtained with muscle PLs of female Wistar rats (20). Gender-related differences in longevity are well recognized but, to our knowledge, very little is known about gender-related differences in FA metabolism.

The composition of PLFAs differs remarkably in organs from the same rats at different ages. PLFA composition showed different age-related changes in organs, which were sometimes, but not always, sensitive to anti-aging DR. Two saturated and four unsaturated FAs were significantly affected by the aging process in the liver, as opposed to two and three in the kidney and heart. PUFAs, dolichol and vitamin E have antioxidant properties and may interact with each other to form a highly-matched free-radical transfer chain (1). The molar ratios of these molecules are known to change with age (21), and the observed age-related changes in the abundance of unsaturated FAs may influence free-radical metabolism in older membranes. An involvement of oxidative stress as a causal factor in the aging process is quite likely (22).

The saturated FA contents of the liver and kidney were similar and higher than those of the heart (mainly because of the lower proportion of 16:0 in this latter organ). In all three organs, saturated FAs did not change with age as a total because changes in individual FAs may have compensated each other. Age-related changes were found consistently in the mono- and bi-unsaturated FA levels (which decreased in the liver and heart), in their ratio (which increased in all three older organs), and in PUFA levels (which increased in the liver and heart), with a consequent increase in the peroxidability index. These changes were counteracted by DR, and are therefore likely to be involved in the basic mechanisms of the aging process.

DR is known to extend median and maximum lifespan, delaying the onset of age-associated diseases, counteracting many effects of age on metabolism and regulation (23), and changing the PUFA levels in mitochondrial and

Table 5 - *Effect of age and anti-aging dietary restrictions (40% CR or EOD) on PLFA composition in the left ventricle of the heart of young, adult and old rats.*

FATTY ACIDS	AL 3 months	AL 12 months	AL 24 months	40% CR 24 months	EOD 24 months
14:0	0.07±0.004	0.08±0.003	0.08±0.003	0.08±0.002	0.08±0.003^{B}
16:0	9.8±0.05	11.1±0.19^{A}	10.3±0.09^{C}	12.0±0.20^{A,C,E}	12.7±0.12^{A,C,E,X}
18:0	21.1±0.08	20.2±0.06^{A}	21.1±0.24^{C}	19.6±0.08^{A,C,E}	18.6±0.10^{A,C,E,X}
20:0	0.30±0.006	0.27±0.006	0.21±0.013^{A,C}	0.25±0.007^{A,E}	0.25±0.009^{A,E}
22:0	0.36±0.005	0.36±0.009	0.29±0.012^{A,C}	0.34±0.005^{E}	0.32±0.008^{A,C}
24:0	0.28±0.012	0.30±0.009	0.40±0.026^{A,C}	0.36±0.005^{A}	0.39±0.014^{A,C}
Saturated	31.9	32.3	32.5	32.7^{B}	32.4
14:1	1.5±0.11	1.3±0.26	2.4±0.06^{A,C}	1.6±0.17^{E}	2.1±0.07^{C}
18:1(n-9)	2.3±0.02	2.4±0.04	2.3±0.08	2.3±0.06	2.5±0.04^{B}
18:1(n-7)	2.9±0.03	3.1±0.03^{A}	3.3±0.11^{A}	3.2±0.07^{A}	3.3±0.08^{A}
20:1(n-9)	0.21±0.008	0.14±0.007^{A}	0.11±0.003^{A,C}	0.11±0.004^{A,C}	0.11±0.004^{A,C}
18:2(n-6)	22.1±0.30	19.4±0.35^{A}	17.1±0.65^{A,C}	21.0±0.29^{E}	22.3±0.32^{C,E}
20:2(n-6)	0.23±0.007	0.19±0.006^{A}	0.19±0.007^{A}	0.13±0.006^{A,C,E}	0.13±0.008^{A,C,E}
Mono- and bi-unsaturated	29.2	26.5^{A}	25.3^{A}	28.4^{C,E}	30.5^{C,E,X}
Mono-/bi-unsaturated	0.31±0.008	0.35±0.017	0.47±0.025^{A,C}	0.34±0.007^{E}	0.36±0.007^{E}
18:3(n-3)	0.01±0.009	0.02±0.009	0.03±0.001	0.03±0.001^{A}	0.04±0.001^{A}
18:3(n-6)	0.01±0.000	0.01±0.001	0.02±0.002	0.02±0.000^{B}	0.01±0.003
20:3(n-6)	0.35±0.009	0.33±0.014	0.49±0.026^{A,C}	0.36±0.005^{E}	0.41±0.012^{A,C,E}
20:4(n-6)	17.1±0.17	17.6±0.54	19.9±0.29^{A,C}	16.1±0.11^{C,E}	16.0±0.21^{C,E}
20:5(n-3) / 22:4(n-6)	0.20±0.008	0.15±0.011^{A}	0.17±0.015	0.28±0.009^{A,C,E}	0.29±0.011^{A,C,E}
22:5(n-3)	2.4±0.12	2.2±0.10	2.1±0.07^{B}	2.3±0.04	2.1±0.05
22:6(n-3)	14.1±0.14	15.5±0.37^{A}	14.5±0.39	14.9±0.31	13.3±0.21^{C,E,X}
Polyunsaturated	34.2	35.8	37.2^{A}	34.0^{C,E}	32.1^{A,C,E,X}
Not identified peaks	4.7±0.05	5.3±0.13^{A}	5.0±0.11	5.0±0.12	5.0±0.03
Family abundance					
N-9	2.5	2.5	2.4	2.4	2.6^{X}
N-7	2.9	3.1^{A}	3.3^{A}	3.2^{A}	3.3^{A}
N-6	39.8	37.5^{A}	37.7^{A}	37.6^{A}	38.8
N-3	16.6	17.8^{A}	16.6	17.2	15.4^{C,X}

Results are expressed as the mean ±SEM of the % composition of PLFAs. The number of animals considered in each case was 6 (except for the 24-month-old AL-fed control rats, of which there were 5).
The statistical analysis is given as follows: difference between aged or treated rats and young (3-month-old) control rats $^{A}p<0.01$, $^{B}p<0.05$; adult (12-month-old) and older or treated rats $^{C}p<0.01$, $^{D}p<0.05$; old (24-month-old) control rats and DR rats $^{E}p<0.01$, $^{F}p<0.05$; difference between EOD and CR rats $^{X}p<0.01$, $^{Y}p<0.05$.

microsomal membranes (24). The anti-aging mechanism of DR may involve the stimulation of liver autophagy and membrane degradation (25, 26). Two DR regimens were used in our experiments, producing different effects on metabolism and similar effects on longevity (a similarity of their effects could substantiate an involvement in the basic aging mechanism). DR consistently influenced the abundance of saturated FAs, mono- and bi-unsaturated FAs and PUFAs in all three organs, and some of their effects counteracted the age-related changes, while others appeared to be unrelated with the aging process. DR always reduced the abundance of 20:4 (n-6). Surprisingly, this effect of both types of DR was also observed in the liver and kidney, and magnified the age-related declining trend of this FA. Another finding that may deserve further investigation is that both types of DR overcompensated for the age-related decrease or declining trend of 18:2(n-6) and of 20:5 (n-3)/22:4 (n-6), as well as for the age-related increase or rising trend of 22:6(n-3), in all three organs. Most of the observed anti-aging effects of the two types of DR on PLFAs were similar (including those on the mono-/bi-unsaturated ratio and on 18:2(n-6), 20:4(n-6), 20:5(n-3)/22:4(n-6) and 22:6(n-3) levels) and might be a suitable target for further studies. The effects of EOD were sometimes stronger than those of 40% CR.

The exact reason(s) for changes in saturated FAs, in mono- and bi-unsaturated FAs and PUFAs with aging is not clear. Desaturase activity, and delta-9 (28) and delta-6 (27) desaturase activities in particular, may decline with age (27, 28), but other studies have indicated that desaturase changes do not necessarily decline constantly with age (29). Mammals obtain 18:2(n-6) and 18:3(n-3) FAs from their diets from which they produce a range of FAs including arachidonic acid [20:4(n-6)] and docosahexaenoic acid [22:6(n-3)] (DHA). Our data suggest that the synthesis of longer-chained PUFAs from 18:2(n-6) might be more influenced by age than in the case of 18:3(n-3). An age-related increase in the proportion of DHA was observed in the liver by the ages of 12 and 24 months, and was accompanied by a significant decrease in the proportion of 22:5(n-3). The synthesis of DHA by 18:3(n-3) requires alternate desaturation (by delta-6 and delta-5 desaturases) and elongation steps to synthesize 24:6(n-3) in the endoplasmic reticulum, followed by chain shortening brought about by one cycle of beta-oxidation in the peroxisome. Liver peroxisomes may be significantly altered in older animals (30, 31); indeed, autophagic function [the cell repair mechanism that can selectively break down liver peroxisomes (32)] declines in old tissue cells (12).

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