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Effect of dietary oils enriched with n-3 fatty acids on survival of mice

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Abstract

Female mice were fed a conventional diet, shifted at 119 days of age to a diet supplemented with 10 wt % lard (Lar), high-linoleic (n-6) safflower oil (Saf), rapeseed oil (low-erucic, Rap), high-α-linolenic (n-3) perilla oil (Per) or a mixture (1:9) of ethyl docosahexaenoate (n-3) and soybean oil (DHA/Soy). Weight gain was less in the Per group than in the other groups at 497 days of age. In the Rap group, proteinuria was more severe than in the Saf, Per and DHA/Soy group, and hepatic triacylglycerol accumulation was greater than in the other groups. The mean survival time of the DHA/Soy group (753 days) was significantly longer than in the Lar group (672 days) and Saf group (689 days); the differences among other groups (e.g., 701 days in the Per group and 712 days in the Rap group) were not statistically significant. Although DHA is more susceptible to auto-oxidation than other major fatty acids in the air, an oil containing DHA was found to increase the survival of mice. Rapeseed oil that decreases the survival time of SHRSP rats was found to be safe in the mouse strain used in this study when survival was an end point.

Keywords: Mouse; Longevity; Docosahexaenoic acid (DHA); Rapeseed oil; Renal function

1. Introduction

Classical recommendations for the prevention of elderly-onset diseases are based on the apparent hypocholesterolemic activity of vegetable oils enriched with linoleic acid (LA, 18:2n-6) compared with animal fats enriched with saturated and monounsaturated fatty acids. However, the short-term effects of animal fats and high-LA-vegetable oils were not observed after long-term dietary manipulations [1,2]. Responses of animals to short-term and long-term dietary changes are often different, and lipid nutrition for the prevention of elderly-onset diseases and aging should be based on the consequences of long-term dietary manipulation [3,4].

The free radical theory of aging originally proposed by Harman and coworkers [5,6] was based mainly on in vivo observations in rodents that learning ability in a maze test was lower when the degree of unsaturation and the amounts of unsaturated fatty acids in diets were higher; the fish oil group was the lowest in learning ability when compared with groups fed lard, olive oil, corn oil or safflower oil. The survival rate was not affected significantly by these fats and oils but some inhibitors of free radical reactions extended the survival rate [7]. In support of this theory, membranes from animals fed fish oils exhibit enhanced autoxidizability in vitro [8,9]. However, the observed extension of life span by some free radical reaction inhibitors could well be due to caloric restriction, a condition known to prolong the life span of rodents [10], because weight losses were noted in such cases [7]. Furthermore, phospholipid hydroperoxide levels in freshly prepared erythrocytes tend to be lower in fish oil- and perilla oil-fed rats than in safflower oil-fed rats when measured using a chemiluminescence method [11,12]. Thus, the autoxidizability of major fatty acids in diets does not appear to be reflected directly in lipid peroxide levels of tissues.

Previously, we demonstrated that brightness-discrimination learning ability is significantly higher in rats fed perilla oil enriched with α-linolenic acid (ALA, 18:3n-3) compared with those fed safflower oil enriched with LA through two generations. DHA-rich fish oil also maintained the learning ability at a high level [13,14], indicating that it is not the autoxidizability of dietary fatty acids but the type of unsaturated fatty acids (n-6 vs n-3) that is critical for the maintenance of brain functions [4,15]. Thus, more in vivo data are necessary to understand the relationship between dietary polyunsaturated fatty acids and aging [4]. The present experiments were designed to determine if, (a) a long-term

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Table 1
Fatty acid composition of experimental diet[a]

Fatty acid	Lar	Saf	Rap	Per	DHA/Soy
		Weight % of total fatty acids			
14:0	1.2	0.3	0.3	0.2	0.2
16:0	23.2	9.4	7.5	8.4	10.9
18:0	12.1	2.6	2.3	1.8	3.4
20:0	0.2	0.3	0.5	0.2	0.3
22:0	0.1	0.4	0.3	0.1	0.3
24:0	0.1	0.1	0.2	0.1	0.1
Total SFA[c]	36.9	13.2	11.1	10.9	15.3
16:1	1.9	0.4	0.6	0.4	0.3
18:1n-9[b]	37.1	17.7	51.7	17.9	20.7
20:1	1.2	0.7	1.5	0.8	0.6
22:1	0.4	0.4	0.3	0.3	0.2
Total MUFA[c]	40.5	19.3	54.2	19.3	21.8
18:2n-6	19.4	64.5	26.2	23.5	48.6
20:4n-6	0.2	0.1	0.1	0.1	0.1
Total n-6[c]	19.6	64.6	26.2	23.6	48.7
18:3n-3	1.5	1.4	7.2	44.6	6.4
20:5n-3	0.8	0.9	0.7	0.8	1.1
22:6n-3	0.8	0.7	0.6	0.8	6.7
Total n-3[c]	3.0	3.0	8.6	46.2	14.2
n-6/n-3 Ratio	6.5	21.6	3.1	0.5	3.4
PI[d]	34.7	78.9	51.4	125.0	122.0

[a] The fatty acid composition of the diet (% of total fatty acids) was analyzed by gas-chromatography.
[b] The position of the double bond numbered from the methyl terminus is designated as n-9, n-6 or n-3.
[c] SFA = saturated fatty acids. MUFA = monounsaturated fatty acids. n-6 = n-6 polyunsaturated fatty acids. n-3 = n-3 polyunsaturated fatty acid.
[d] PI, peroxidizability index calculated as described in the text [19].

feeding of oils enriched with n-3 fatty acids shortens survival rate and (b) rapeseed oil (low-erucic type) with a relatively low n-6/n-3 ratio but with an unusual survival time-shortening activity in stroke-prone spontaneously hypertensive (SHRSP) rats [16–18] also affects the survival rate of mice.

2. Materials and methods

2.1. Animals and diets

Female C57BL/6 mice (a conventional strain) at 35 days of age were purchased from SLC Japan, Inc., Tokyo. This strain is known to develop hair-loss along with aging. The mice were initially fed for up to 119 days of age a conventional diet (CE2; Central Laboratory for Experimental Animal (Clea) Japan, Inc., Tokyo) containing 4.4% (w/w) lipids (lipids contained in the materials and supplemented soybean oil) and defined amounts of nutrients. Then, mice were divided randomly into five groups of sixty animals each. Average body weight of the 5 dietary groups was 24.8 ± 0.6 g and the maximum deviation from the mean was 3.7%. Six mice were put into each cage. In each dietary group, 48 mice were assigned for survival time measurement, and the rest were sacrificed for biochemical analysis at 497 days of age. The mice were housed in a room specified for Special Pathogen-Free animals with a room temperature (24 ± 2°C), humidity (55 ± 5%) and lighting (from 06:00 to 18:00), and given free access to water and an experimental diet. Lard (commercially available for human use, Lar), safflower oil (high-LA type, commercially available for human use, Saf), rapeseed oil (low-erucic Canola type, Rap), perilla oil (from seeds of beefsteak plant, prepared for human use, Per) and a 1:9 mixture of DHA ethylester (>95% pure, kindly supplied from Harima Chemicals Inc., Tokyo and Shiseido Co., Tokyo) and soybean oil (DHA/Soy) were used. The amount of DHA in the DHA/Soy diet (2 energy %) was set between the intakes of DHA and EPA (eicosapentaenoic acid) by average Japanese (0.7 energy %) and Greenland natives (5.1 energy %).

The basal diet (CE2) and experimental fat or oil was mixed at a weight ratio of 9 to 1. The final lipid content was calculated to be 14.0 wt % (31.4 energy %). The fatty acid composition of the experimental diet is shown in Table 1. Peroxidizability index (PI) [19] in Table 1 was calculated as, PI = (% monoenoate × 0.025) + (% dienoate × 1) + (% trienoate × 2) + (% tetraenoate × 4) + (% pentaenoate × 6) + (% hexaenoate × 8). Experimental diets were made into pellets by Clea Japan Co., Ltd., and kept at 4°C for less than 1 month, except for the DHA diet which was prepared in our laboratory, quickly sealed under nitrogen gas, kept frozen at –20°C and served within 10 days. The diets were replaced every day in the case of the DHA/Soy

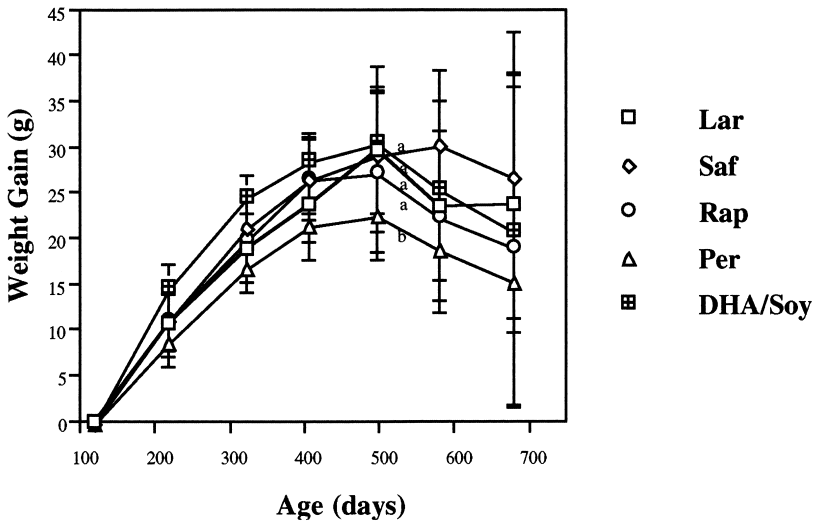

Fig. 1. Weight gain of mice fed Lar, Saf, Rap, Per or DHA/Soy diet was calculated from the body weight at 119 days from which test diets were given. The numbers of animals were 42 ~ 47 at 497 days of age, but at 679 days of age, they were 27 for the Lar group, 23 for the Saf group, 29 for the Rap group, 30 for the Per group and 36 for the DHA/Soy group. Values with different letters indicate significant difference according to ANOVA followed by Bonferroni/Dunn test at $p < 0.05$.

diet, and every two days for other diets to keep the peroxide values of the diets below 10 meq/kg.

2.2. Analysis of urinary protein

Urinary protein in mice from 2 p.m. to 3 p.m. was determined directly using a test paper (Pretest, Wako Pure Chemicals Co., Osaka). The developed color was determined semi-quantitatively using standard tetrabromophenol blue solutions as described by Free et al. [20]

2.3. Determination of fatty acid composition

Mice originally assigned for biochemical analysis were sacrificed at 497 days of age, and tissue samples were stored at −80°C until analysis. Lipids were extracted from diets or tissues with chloroform/methanol according to Bligh and Dyer's method [21]. Fatty acids were converted to methyl-esters, which were analyzed by gas-liquid chromatography with a capillary column (DB-225; J&W Scientific, Folsom, CA) using heptadecanoic acid as an internal standard.

2.4. Statistical analysis

Data are presented as means ± SD. Statistical analysis of the survival rates was performed by Log-rank and Wilcoxon Signed Rank method (a non-parametric method) using a computer program JMP 3.0, Statistic Made Visual (SAS Institute, Cary, NC). Other data were analyzed using Bonferroni's multiple comparison (Stat View J-4.11; Abacus Concepts, Inc., Berkeley, CA).

3. Results

3.1. Weight gain and food intake

The experimental diet had a significant effect on weight gain. Weight gains in the DHA/Soy group (when 46/48 survived), Lar group (when 42/48 survived), Saf group (when 44/48 survived) and Rap group (when 47/48 survived) at 497 days of age were significantly higher than that in the Per group (when 42/48 survived) (Figure 1). The average intakes of experimental diets were not significantly different among the dietary groups. They were 5.2, 5.1, 5.1, 5.0, and 4.9 (g/per day) in the DHA/Soy group, Saf group, Per group, Rap group, and Lar group, respectively at 497 days of age. The body weight of the DHA, Lar, Saf, Rap and Per group increased by 54.6%, 54.3%, 54.1%, 52.4% and 48.5%, respectively.

3.2. Loss of hair and skin inflammation

When the conventional diet containing 4.4% total lipids (CE2) was shifted to the experimental diets containing 14% lipids at 119 days of age, mice in all the dietary groups developed hair loss on the dorsal and abdominal skin, and inflammatory erythema appeared on the naked skin. Young test mice kept in the same room on the control diet (CE2) were confirmed to be free of specific pathogens indicating that the loss of hair and inflammation on the skin are not due to specific pathogens. The number of mice with apparently good, healthy hair was relatively less in the Per group compared with the DHA/Soy and Rap group.

Table 2
Fatty acid composition of hepatic triacylglycerol[1]

Fatty acid	Lar	Saf	Rap	Per	DHA/Soy
14:0	0.4 ± 0.1[a 2]	0.2 ± 0.0[b]	0.4 ± 0.1[a]	0.2 ± 0.1[b]	0.3 ± 0.0[b]
16:0	22.8 ± 0.7[a]	17.8 ± 1.2[b]	18.6 ± 2.3[b]	13.8 ± 0.4[c]	19.8 ± 0.6[b]
18:0	1.8 ± 0.2[ab]	1.9 ± 0.5[a]	1.3 ± 0.2[b]	1.9 ± 0.3[a]	1.4 ± 0.1[ab]
20:0	0.4 ± 0.5	0.0 ± 0.1	0.1 ± 0.1	0.3 ± 0.5	0.0 ± 0.0
24:0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Total SFA	25.5 ± 1.2[a]	20.0 ± 1.2[b]	20.4 ± 2.2[b]	16.3 ± 0.7[c]	21.5 ± 0.6[b]
16:1	1.8 ± 0.5[b]	1.1 ± 0.1[c]	2.5 ± 0.5[a]	1.0 ± 0.1[c]	1.3 ± 0.2[bc]
18:1	47.7 ± 4.3[a]	18.3 ± 1.8[c]	51.7 ± 3.1[a]	23.9 ± 1.7[b]	23.5 ± 3.2[bc]
20:1	0.5 ± 0.1[a]	0.2 ± 0.1[b]	0.6 ± 0.1[a]	0.2 ± 0.1[b]	0.2 ± 0.1[b]
Total MUFA	50.0 ± 4.1[a]	19.6 ± 1.9[c]	54.8 ± 3.5[a]	25.1 ± 1.8[b]	24.9 ± 3.4[bc]
18:2n-6	18.4 ± 2.3[d]	52.6 ± 1.6[a]	19.7 ± 3.9[d]	27.6 ± 2.4[c]	39.8 ± 3.1[b]
18:3n-6	0.4 ± 0.1[b]	1.2 ± 0.2[a]	0.4 ± 0.1[b]	0.4 ± 0.2[b]	0.4 ± 0.1[b]
20:3n-6	0.3 ± 0.1[b]	1.0 ± 0.2[a]	0.3 ± 0.1[b]	0.3 ± 0.1[b]	0.5 ± 0.1[b]
20:4n-6	0.8 ± 0.2[b]	1.7 ± 0.3[a]	0.5 ± 0.1[c]	0.5 ± 0.1[c]	0.6 ± 0.1[bc]
22:4n-6	0.1 ± 0.1[b]	0.2 ± 0.1[a]	0.0 ± 0.0[bc]	0.0 ± 0.0[c]	0.0 ± 0.0[c]
22:5n-6	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Total n-6	20.1 ± 2.6[d]	56.7 ± 2.0[a]	20.9 ± 4.3[d]	28.8 ± 2.3[c]	41.3 ± 3.0[b]
18:3n-3	0.8 ± 0.2[b]	0.7 ± 0.1[b]	1.8 ± 1.0[b]	23.8 ± 4.1[a]	3.1 ± 0.4[b]
20.5n-3	0.7 ± 0.1[c]	0.5 ± 0.2[c]	0.6 ± 0.3[c]	2.4 ± 0.2[a]	1.6 ± 0.2[b]
22:5n-3	0.5 ± 0.1[c]	0.4 ± 0.1[cd]	0.4 ± 0.1[d]	1.3 ± 0.1[a]	0.9 ± 0.1[b]
22:6n-3	2.4 ± 0.4[b]	2.2 ± 0.3[b]	1.1 ± 0.3[c]	2.2 ± 0.2[b]	6.6 ± 0.4[a]
Total n-3	4.4 ± 0.8[c]	3.7 ± 0.3[c]	3.9 ± 0.8[c]	29.7 ± 4.1[a]	12.2 ± 0.7[b]
n-6/n-3 Ratio	4.6 ± 0.4[bc]	15.3 ± 1.3[a]	5.6 ± 1.6[b]	1.0 ± 0.2[d]	3.4 ± 0.4[c]
PI	53 ± 8[c]	89 ± 4[b]	43 ± 9[c]	119 ± 5[a]	119 ± 3[a]
TG[3]	89 ± 19[b]	72 ± 17[b]	140 ± 24[a]	71 ± 15[b]	94 ± 23[b]

[1] Fatty acid composition of hepatic triacylglycerol (% of total fatty acids) is shown as mean ± SD (n = 6).
[2] Values with different superscripts are significantly different from each other at $p < 0.05$.
[3] The unit of TG is μmol/g wet hepatic weight.

3.3. The amount and fatty acid composition of hepatic triacylglycerol

For the assessment of dietary manipulation, fatty acid composition of hepatic triacylglycerol was determined. Fatty acid composition of hepatic triacylglycerol roughly reflected that of the diet (Table 2). The hepatic triacylglycerol level was significantly higher in the Rap group than in the other groups; almost 2-fold higher than in the Per group.

3.4. Total urinary protein

As rapeseed oil accelerated renal injury in SHRSP rats compared with DHA-rich fish oil, perilla oil and soybean oil [22], total urinary protein was measured at 647 days of age (Figure 2). The level of total urinary protein in the Rap group was significantly higher than in the DHA/Soy, Per and Saf groups, but the differences among the DHA/Soy, Per, Saf and Lar groups were not statistically significant.

3.5. Survival time

The mean survival time decreased in the order of the DHA/Soy (753 days), Rap (712 days), Per (701 days), Saf (689 days) and Lar group (672 days) as shown in Table 3. Statistically, the survival time of the DHA/Soy group was longer by 12% (vs Lar group, $p < 0.006$ in Log-rank) and 9.3% (vs Saf group, $p < 0.009$ in Log-rank) but no significant differences were observed among the other dietary groups.

Comparing the survival rates (Figure 3), the DHA/Soy group exhibited a greater survival rate than in other dietary groups particularly at the early period after the onset of

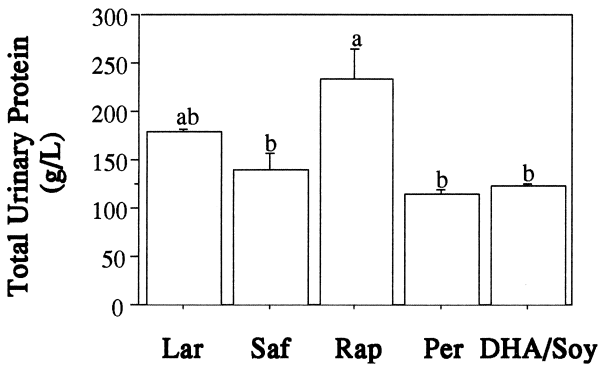

Fig. 2. Effect of dietary fats and oils on total urinary protein of mice. Values are means ± SD for the mice at 627 days of age (experimental diets were fed from 119 days of age). The numbers of mice were 27 for the Lar group, 35 ~ 37 for the Saf group, 29 ~ 30 for the Rap group, 34 for the Per group, and 38 for the DHA/Soy group. The bars with different superscripts are significantly different from each other by ANOVA followed by Bonferroni/Dunn test at $p < 0.05$.

Table 3
Survival time of female C57BL/6 mice fed different fats and oils

Dietary groups	Lar	Saf	Per	Rap	DHA/Soy
Survival day (±SD)	672 ± 142	689 ± 131	701 ± 149	712 ± 125	753 ± 128
P value by					
Log-rank		0.597 vs. Lar	0.196 vs. Lar	0.159 vs. Lar	0.006 vs. Lar
Wilcoxon		0.824 vs. Lar	0.370 vs. Lar	0.193 vs. Lar	0.007 vs. Lar
Log-rank			0.268 vs. Saf	0.297 vs. Saf	0.009 vs. Saf
Wilcoxon			0.867 vs. Saf	0.503 vs. Saf	0.022 vs. Saf
Log-rank				0.865 vs. Per	0.202 vs. Per
Wilcoxon				0.884 vs. Per	0.185 vs. Per
Log-rank					0.083 vs. Rap
Wilcoxon					0.105 vs. Rap

48 mice were assigned for survival time measurement in each dietary group.

deaths. The Per group, another n-3 enriched diet group, tended to exhibit relatively lower survival time at the beginning but in the later period the survival rate was improved compared with Lar and Saf group. The Rap diet showed no survival time-shortening activity in this strain of mouse compared with Per, Saf and Lar diets. The maximum survival times were relatively similar; 963 days in the DHA/Soy group, 913 days in the Rap group, 974 days in the Per group, 912 days in the Saf group and 923 days in the Lar group.

4. Discussion

Caloric restriction has been established to be effective for the extension of life span of rodent [10]. Although the weight gains were different among the dietary groups, no significant difference in food intakes has been observed among the 5 dietary groups. n-3 Fatty acids such as DHA and ALA are known to be β-oxidized by peroxisomal enzymes in preference to LA, oleic, palmitic and stearic acids. Mitochondrial β-oxidation system utilizes ALA effectively, and these n-3 fatty acids accumulate as depot fat in relatively less amounts than saturated (palmitic, stearic), oleic acid and LA [23,24]. This β-oxidation system mechanism of ALA may explain the lower weight gain of the Per group containing 14 energy % ALA (Figure 1) compared with other dietary groups. In fact, perilla oil and fish oil tended to lower body weights of mice compared with lard and safflower oil in C57BL/6J mice [27]. In the DHA/Soy diet, the DHA content may have been too small to exert the effect observed with the Per diet; DHA content was 1 wt % while LA was 7 wt % in the DHA/Soy diet.

These diets affected the skin lesion differently but no specific fatty acid or fatty acid groups (e.g., saturated, monounsaturated, n-6 and n-3) accounted for the differential effects. If the amount of ALA in the Per diet was above the capacity of β-oxidation system in this strain, the observed most severe skin lesion of the Per group might be due to ALA, although other unidentified factors may be involved. The skin lesion in this strain of mice, e.g., hair loss and inflammatory erythema, is known to be age-related, and anti-inflammatory activities of the perilla oil observed in other animal models [25] were not observed in the skin of this mouse.

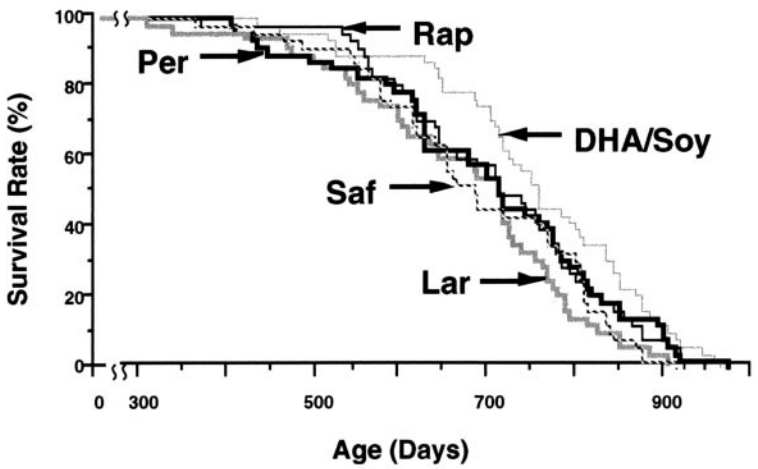

Fig. 3. Effect of dietary fats and oils on survival rate of mice. Mice were fed experimental diets from 119 days of age. The numbers of mice were 48 in each group.

Supplementation with n-3 fatty acids (fish oil) has been reported to protect against renal lesions, although Per with very low n-6/n-3 ratio was detrimental for a crescent-type nephritis model of mice [26]. However, the renal lesion caused by rapeseed oil in SHRSP rats was not related to its relatively low n-6/n-3 ratio but was ascribed to a factor other than fatty acids [18,22]. In this mouse strain, rapeseed oil caused accelerated proteinuria, which was not related to the n-6/n-3 ratio of dietary lipids [18]. Unusually high amounts of hepatic triacylglycerol accumulation in this group (Table 2) might also be related to the presumed survival time-shortening factor in rapeseed oil and some other vegetable oils [16–18]. Unlike the case of SHRSP rats, the rapeseed oil used did not shorten the survival time of this mouse strain despite these unusual properties observed.

Survival of mice in the DHA/Soy group was longer by ca 10% than the Lar and Saf group but the maximum survival times were relatively similar. Although soybean oil group was not included in the present experiment, none of major fatty acids of soybean oil (palmitic, oleic, LA and ALA) appeared to be beneficial for this strain of mice because diets enriched with these fatty acids were not beneficial. DHA-rich fish oil and perilla oil prolonged the survival time of SHRSP rats by >10% compared with safflower oil, but perilla oil was not effective in this strain of mice. Other strains of rodents or other species should be examined under appropriate nutritional conditions to assess if the dietary n-6/n-3 ratio affects survival rates in general. However, the prolongation of life span of on the order of 10% should not be overlooked because only a 4% extension of the human life span would be expected even if all types of cancer were conquered.

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References

[1] Ishihara, A., Ito, A., Sakai, K., Watanabe, S., Kobayashi, T., and Okuyama, H, Dietary high-linoleate safflower oil is not hypocholesterolemic in aged mice after a long-term feeding-comparison with lard, perilla oil and fish oil, Biol Pharm Bull 18, 485–490 (1995).

[2] Strandberg, T.E., Salomaa, V.V., Naukkarinen, V.A., Vanhanen, H.T., Sarna, S.J., and Miettinen, T.A, Long-term mortality after 5-year multifactorial primary prevention of cardiovascular diseases in middle-aged men, JAMA 266, 1225–1229 (1991).

[3] de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J. L., Monjaud, I., Guidollet, J., Touboul, P., and Delaye, J, Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease, Lancet 343, 1454–1459 (1994).

[4] Okuyama, H., Kobayashi, T., and Watanabe, S, Dietary fatty acids—the n-6/n-3 balance and chronic elderly diseases. Excess linolenic acid and relative n-3 deficiency syndrome seen in Japan, Prog Lipid Res 35, 409–457 (1997).

[5] Harman, D, A theory based on free radical and radiation chemistry, J Gerontol 11, 298–300 (1956).

[6] Harman, D., Hendricks, S., Eddy, D.E., and Seibold, J, Free radical theory of aging: effect of dietary fat on central nervous system functoin, J Am Geriatr Soc 24, 301–307 (1976).

[7] Harman, D, Free radical theory of aging: effect of free radical reaction inhibitors on the mortality rate of male LAF mice, J Gerontol 23, 476–482 (1968).

[8] Chautan, M., Calaf, R., Leonardi, J., Charbonnier, M., Andre, M., Portugal, H., Pauli, A.M., Lafont, H., and Nalbone, G, Inverse modifications of heart and liverα-tocopherol status by various dietary n-6/n-3 polyunsaturated fatty acid ratios, J Lipid Res 31, 2201–2208 (1990).

[9] Gonzalez, M.J., Gray, J.I, Schemmel, R.A., Dugan, L.Jr., and Welsch, C.W, Lipid peroxidation products are elevated in fish oil diets even in the presence of added antioxidants, J Nutr 122, 2190–2195 (1992).

[10] Yu, B.P., Masoro, E.J., Murata, I., Bertrand, H.A., and Lynd, F.T, Life span study of SPF Fisher 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease, J Gerontol 37, 130–141 (1982).

[11] Ando, K., Nagata, K., Beppu, M., Kikugawa, K., Kawabata, T., Hasegawa, K., and Suzuki, M, Effect of n-3 fatty acid supplementation on lipid peroxidation and protein aggregation in rat erythrocyte membranes, Lipids 33, 505–512 (1998).

[12] Miyazawa, T, Polyunsaturated oil ingestion and lipid hydroperoxides in biomembranes, J Jpn Oil Chem Soc 40, 876–884 (1991).

[13] Okuyama, H., Nishizawa, Y., Isoda, Y., Kageyama, H., and Sakai, K, Japan Patent Publication, 1990–1, 53629 (1990).

[14] Fujimoto, K., Kanno, T., Koga, H., Onodera, K., Hirono, H., Nishikawa, M., and Maruyama, K. (1993), Effects of n-3 fatty acid deficiency during pregnancy and lactation on learning ability of rats. In Advances in Polyunsaturated Fatty Acid Research (T. Yasugi, H. Nakamura, M. Soma , ed.), pp, 257–260, Amsterdam: Elsevier Science Publishers B.V.

[15] Yoshita, S., Sato, A., and Okuyama, H, Pathophysiological effects of dietary essential fatty acid balance on neural systems, Jpn J Pharmacol 77, 11–22 (1998).

[16] Huang, M.Z., Naito, Y., Watanabe, S., Kobayashi, T., Kanai, H., Nagai, H., and Okuyama, H, Effect of rapeseed and dietary oils on the mean survival time of stroke-prone spontaneously hypertensive rats, Biol Pharm Bull 19, 554–557 (1996).

[17] Huang, M.Z, Watanabe, S., Kobayashi, T., Nagatsu, A., Sakakibara, J., and Okuyama, H, Unusual effects of some vegetable oils on the survival time of stroke-prone spontaneously hypertensive rats, Lipids 32, 745–751 (1997).

[18] Miyazaki, M., Huang, M.Z., Takemura, N., Watanabe, S., and Okuyama, H, Free fatty acid fractions from some vegetable oils exhibit reduced survival time-shortening activity in stroke-prone spontaneously hypertensive rats, Lipids 33, 655–661 (1998).

[19] Witting, L.A. and Horwitt, M.K, Effect of degree of fatty acid unsaturation in tocopherol deficiency-induced creatinuria, J Nutr 82, 19–33 (1964).

[20] Free, A.H., Rupe, C.O., and Metzler, I, Studies with a new colorimetric test for proteinuria, Clin Chem 3, 716–727 (1957).

[21] Bligh, E.G. and Dyer, W.J, A rapid method of total lipid extraction and purification, Can J Biochem Physiol 37, 911–917 (1959).

[22] Miyazaki, M., Takemura, N., Watanabe, S., Hata, N., Misawa, Y., and Okuyama, H, Dietary docosahexaenoic acid ameliorates, but

rapeseed oil and safflower oil accelerate renal injury in stroke-prone spontaneously hypertensive rats as compared with soybean oil, which is associated with expression for renal transforming growth factor-β, fibronectin and renin, Biochim Biophys Acta 1483, 101–110 (2000).

[23] Leyton, J., Drury, P.J., and Crawford, M.A, Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat, Br J Nutr 57, 383–393 (1987).

[24] Clouet, P., Niot, I., and Bezard, J, Pathway of alpha-linolenic acid through the mitochondrial outer membrane in the rat liver and influence on the rate of oxidation. Comparison with linoleic and oleic acids, Biochem J 263, 867–873 (1989).

[25] Oh-hashi, K., Takahashi, T., Tanabe, A., Watanabe, S., and Okuyama, H, Dietaryα-linolenate suppresses endotoxin-induced platelet-activating factor production in rat kidney, Lipids 34, 31–37 (1999).

[26] Watanabe, S., Suzuki, E., Kojima, R., Suzuki, Y., and Okuyama, H, Effect of dietary alpha-linolenate/linoleate balance on crescent type-anti-glomerular basement membrane nephritis in rats, Lipids 25, 267–272 (1990).

[27] Ikemoto, S., Takahashi, M., Tsunoda, N., Maruyama, K., Itakura, H., and Ezaki, O, High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils, Metabolism 45, 1539–1546 (1996).