



Basic nutritional investigation

Long-term intake of fish oil increases oxidative stress and decreases lifespan in senescence-accelerated mice

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ABSTRACT

Objective: The effects of fish oil including ω -3 polyunsaturated fatty acids on aging and lifespan are not well understood. In this study, the influence of long-term ingestion of fish oil on lifespan was examined in senescence-accelerated (SAMP8) mice.

Methods: We investigated the effects of dietary fish oil on lifespan and on lipid composition and oxidative stress in plasma and liver in SAMP8 mice. Male mice were fed a fish oil diet (5% fish oil and 5% safflower oil) or a safflower oil diet (10% safflower oil) from 12 wk of age.

Results: The SAMP8 mice fed fish oil did not have a longer maximum lifespan and had a shorter average lifespan than mice fed safflower oil. To examine the mechanism underlying these results, the effects on oxidative stress of long-term ingestion of fish oil were also examined. SAMP8 mice fed fish oil for 28 wk showed strong oxidative stress that caused hyperoxidation of membrane phospholipids and a diminished antioxidant defense system due to a decrease in tocopherol compared with mice fed safflower oil.

Conclusion: These findings suggest that intake of fish oil increases oxidative stress, decreases cellular function, and causes organ dysfunction in SAMP8 mice, thereby promoting aging and shortening the lifespan of the mice.

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Introduction

Fish oil contains high levels of eicosapentaenoic acid (EPA; 20:5 ω -3) and docosahexaenoic acid (DHA; 22:6 ω -3), which are ω -3 polyunsaturated fatty acids. EPA, DHA, and fish oil have been shown to have protective effects against coronary heart disease, thrombosis, inflammatory processes, carcinomatosis, and metabolic syndrome [1–8]. Therefore, fish oils and components of the oils are marketed as health supplements. However, the effects of ω -3 polyunsaturated fatty acids on aging and lifespan are unclear compared with those of ω -6 polyunsaturated fatty acids contained in safflower oil and soybean oil. EPA and DHA are oxidized easily compared with linoleic acid (18:2 ω -6) and oleic acid (18:1 ω -9) in vitro [9]. Therefore, long-term ingestion of EPA and DHA may increase oxidative stress, and this change may be related to disease onset and progression, although it has also been reported that EPA

and DHA are not oxidized as easily in vivo as in vitro [10–12]. Hence, data concerning the safety of long-term ingestion of EPA and DHA are important for proper use of these supplements.

In the present study, the effects on lifespan and oxidative stress of long-term ingestion of fish oil were examined in a mouse model of accelerated senescence. This model was first developed in Japan [13] and has been widely used in the assessment of the aging progress. The model includes senescence-accelerated mouse (SAM) strains (SAMP1, P2, P3, P6, P7, P8, P9, and P10) and control SAMR mouse strains (SAMR1, R4, and R5) with normal aging characteristics. The SAMP8 mouse is often used in aging studies, because it has characteristics of age-associated learning and memory deficits, anxiety, and an impaired immune system; in addition, deposition of amyloid β -protein in the SAMP8 mouse has made it useful as a model of Alzheimer's disease [14]. Because the lifespan of the SAMP8 mouse is about 1 y, it has also been used in studies of the relation between dietary constituents and lifespan [15–17]. Therefore, we used the SAMP8 strain as an experimental animal model. In this study, we investigated the effects of dietary fish oil on lifespan (experiment 1) and on lipid

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composition and oxidative stress in plasma and liver (experiment 2) in SAMP8 mice fed a fish oil diet (5% fish oil and 5% safflower oil) or a safflower oil diet (10% safflower oil).

Materials and methods

Materials

Fish oil and safflower oil were kind gifts from Nippon Oil and Fats, Co. (Tokyo, Japan).

Animals and diets

All procedures were performed in accordance with the animal experiment guidelines of Tohoku University (Sendai, Japan). The animal protocol was approved by the animal use committee at Tohoku University. SAMP8 mice (11 wk of age, male) were obtained from Japan SLC (Hamamatsu, Japan). After acclimatization to a commercial diet for 1 wk (CE-2; Nihon Clear, Tokyo, Japan), the mice were randomly divided into two groups that received different diets containing 10% test oils: a group given safflower oil (77.6% linoleic acid of the total fatty acids in the diet; SO) and a group that received a fish oil/safflower oil mixture (10.4% EPA and 6.8% DHA of the total fatty acids in the diet; FO). The mixture was prepared at a fish oil:safflower oil (v/v) ratio of 50:50 g/kg of diet. The diet composition (grams per kilogram of diet) was casein, 200.0; cornstarch, 100.0; α -cornstarch, 299.5; sucrose, 200.0; cellulose powder, 50.0; AIN-93G mineral mix, 35.0; AIN-93 vitamin mix, 10.0; L-cysteine, 3.0; choline bitartrate, 2.5; and oil (safflower oil or fish oil/safflower oil), 100.0 [18]. Casein, cornstarch, α -cornstarch, cellulose powder, AIN-93G mineral mix, and AIN-93 vitamin mix were obtained from Oriental Yeast Co. (Tokyo, Japan). The diets were stored at -35°C until feeding. Diets with peroxide levels lower than 10 mEq/kg were routinely used. Diets given to mice were exchanged every day.

Two experiments were performed in the study. In each, 10 mice received a 10% safflower oil diet and 10 received a 10% fish oil/safflower oil diet. The mice were housed in individual cages, given free access to food and distilled water, and maintained in a temperature- and humidity-controlled room with light cycles of 12 h on and 12 h off [18,19] throughout their lives in experiment 1 and until they were 40 wk old in experiment 2.

Experiment 1

To examine the average lifespan of mice that received the oil diets, the mice were housed until death. All mice were inspected at least once daily. Mice that died were removed from the cage and the age at death of each mouse was recorded.

Experiment 2

Lipid composition and lipid peroxidation were examined over a period of 28 wk while the mice received the oil diets. During this period, body weight and food intake were measured daily. After this 28-wk period, the mice were sacrificed by decapitation and the liver and plasma were collected and stored at -35°C until performance of the assays [20].

Gas chromatographic analysis

The fatty acid composition of the oil diets was measured using gas chromatography, as described previously [18,21]. The SO diet included 6.6% palmitic acid (16:0), 2.3% stearic acid (18:0), 13.4% oleic acid (18:1 ω -9), 77.6% linoleic acid (18:2 ω -6), and 0.1% α -linolenic acid (18:3 ω -3) (w/w). The FO diet included 4.1% 21myristic acid (14:0), 11.1% palmitic acid (16:0), 5.3% palmitoleic acid (16:1), 2.9% stearic acid (18:0), 14.2% oleic acid (18:1 ω -9), 45.3% linoleic acid (18:2 ω -6), 0.1% α -linolenic acid (18:3 ω -3), 10.4% EPA (20:5 ω -3), and 6.8% DHA (22:6 ω -3).

Determination of vitamin E

To equate the vitamin E (tocopherol) concentration in the two oils, the total tocopherol content was determined using high-performance liquid chromatography with a fluorescent detector [22] and was found to be 20.5 and 2.0 mg/100 g in the safflower oil and fish oil, respectively. Therefore, vitamin E (α -tocopherol) was added to produce a final concentration of 0.0205% in the oils. The total tocopherol levels in liver and plasma were measured using high-performance liquid chromatography with a fluorescent detector, as described previously [23].

Determination of lipid composition

The lipid compositions in the liver and plasma were measured as described previously [24]. Triacylglycerol and total cholesterol levels in plasma were

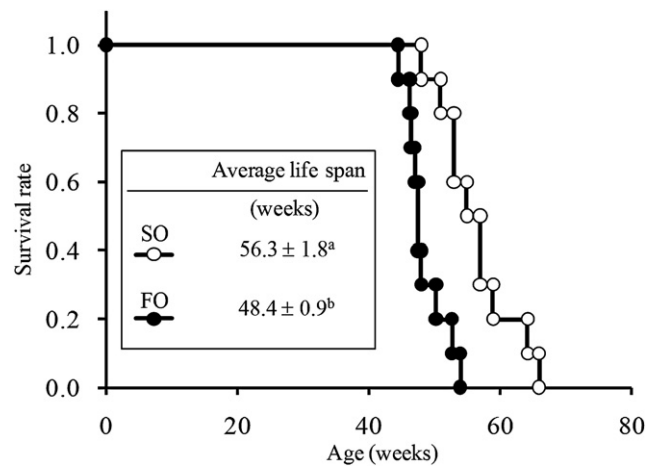


Fig. 1. Effects of safflower oil and fish oil on mean lifespan in SAMP8 mice. Values are means \pm SEs ($n = 10$). Different superscript letters indicate significantly different means at $P < 0.05$. FO, group fed fish oil/safflower oil mixture; SO, group fed safflower oil.

measured using commercially available enzyme kits (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's protocol. The phospholipid levels in the plasma and liver were determined using the method described by Bartlett [25].

Determination of lipid peroxides

To examine oxidative stress caused by fish oil intake, the levels of phospholipid hydroperoxides (PLOOH) and thiobarbituric acid-reactive substances (TBARS) in the liver and plasma were determined as described previously [18,20]. PLOOH was assayed by chemiluminescence detection/high-performance liquid chromatography [18,20].

Statistical analysis

Survival curves were estimated using the Kaplan-Meier test and a comparison of the curves was performed by log-rank test [26]. Results are expressed as means \pm standard error. Statistical analysis was performed using Student's t test for comparisons between two groups after Bartlett's test was used to check that the variances were homogeneous. A difference was considered statistically significant at $P < 0.05$.

Results

Survival curves

The average lifespan was examined for mice in the FO and SO groups. The survival curve for the FO group was shifted to the left (to a younger age) compared with the curve for the SO group (Fig. 1). The average lifespan of the FO group (48.4 ± 0.9 wk) was significantly shorter than that of the SO group (56.3 ± 1.8 wk).

Effects of fish oil on growth parameters

Lipid composition and oxidative stress were compared in SAMP8 mice fed the SO and FO diets for 28 wk (12–40 wk old). Food intake did not differ between the two groups (SO 3.7 ± 0.5 g/d, FO 4.1 ± 0.4 g/d) over this period and there was no significant difference in body weight (SO 38.1 ± 1.0 g, FO 37.1 ± 1.1 g) or liver weight (SO 1.88 ± 0.13 g, FO 1.85 ± 0.06 g) between the groups at the end of the period. These results suggest that fish oil does not influence food intake, body weight, or liver weight of SAMP8 mice.

Table 1

Effects of safflower oil and fish oil on lipid composition, PLOOH, TBARS, and tocopherol levels in plasma and liver of SAMP8 mice*

	SO	FO
Plasma		
Triacylglycerol (mg/mL)	2.66 ± 0.26 ^a	1.73 ± 0.21 ^b
Total cholesterol (mg/mL)	1.06 ± 0.03 ^a	0.89 ± 0.03 ^b
Phospholipid (mg/mL)	2.30 ± 0.09 ^a	2.06 ± 0.08 ^b
PLOOH (mmol/mol PL)	44.9 ± 10.1 ^a	114.9 ± 10.5 ^b
TBARS (nmol/mL)	153 ± 16 ^a	326 ± 49 ^b
Total tocopherol (nmol/mL)	3.62 ± 0.65	2.83 ± 0.74
Liver		
Triacylglycerol (mg/g)	32.8 ± 2.0 ^a	16.6 ± 0.5 ^b
Total cholesterol (mg/g)	3.83 ± 0.08 ^a	2.79 ± 0.07 ^b
Phospholipid (mg/g)	29.5 ± 0.65 ^a	25.7 ± 0.38 ^b
PLOOH (mmol/mol PL)	40.6 ± 6.2 ^a	77.6 ± 8.4 ^b
TBARS (nmol/g liver)	276 ± 45 ^a	399 ± 38 ^b
Total tocopherol (nmol/g)	27.2 ± 5.7 ^a	10.8 ± 1.8 ^b

FO, group fed fish oil/safflower oil mixture; PL, phospholipid; PLOOH, phospholipid hydroperoxide; SO, group fed safflower oil; TBARS, thiobarbituric acid-reactive substances

* Values are means ± SEs (n = 10).

^{a,b} Means in a row with different superscript letters are significantly different at P < 0.05.

Effects of fish oil on lipid composition

Comparison of plasma lipid levels (Table 1) in the FO and SO groups (each expressed as the percentage in the FO group relative to a value of 100% in the SO group) showed that triacylglycerol (65% in the FO group), total cholesterol (84%), and phospholipids (90%) were significantly lower in the FO group. A similar comparison for liver lipid levels also showed that triacylglycerol (51%), total cholesterol (73%), and phospholipids (87%) were significantly lower in the FO group. These results suggest that fish oil suppresses lipid accumulation in SAMP8 mice.

Effects of fish oil on oxidative stress

Comparison of oxidative stress parameters (Table 1) in the FO and SO groups (each expressed as the percentage in the FO group relative to a value of 100% in the SO group) showed that plasma PLOOH (256% in the FO group), plasma TBARS (213%), liver PLOOH (191%), and liver TBARS (145%) were significantly higher in the FO group. The liver total tocopherol level in the FO group was 40% of that in the SO group, with a significant difference between the groups, but the plasma total tocopherol in the FO group (78%) did not differ significantly from that in the SO group. Overall, these results suggest that fish oil strongly promotes oxidative stress in SAMP8 mice.

Discussion

In this study, the effects on lifespan of long-term ingestion of fish oil were examined in senescence-accelerated mice (SAMP8). Mice fed fish oil did not have a longer maximum lifespan and had a shorter average lifespan compared with mice fed safflower oil (Fig. 1). To clarify the cause of these results, the effects on lipid composition and oxidative stress of long-term ingestion of fish oil for 28 wk were also examined in the SAMP8 mice. Fish oil produced very strong oxidative stress that caused hyperoxidation of cell membrane phospholipids and a decrease in the antioxidant defense system due to a decrease in tocopherol compared with mice fed safflower oil (Table 1).

Aging of mice is promoted by an increase in oxidative stress by lipid peroxidation [27] and intake of fish oil has been shown to

increase oxidative stress in vivo [10–12]. Therefore, these findings suggest that fish oil increased oxidative stress in SAMP8 mice, which led to decreased cellular function and increased organ dysfunction. In turn, these changes promoted aging and shortened the lifespan of the mice. The SAMP8 mouse is particularly sensitive to oxidative stress [27], but promotion of lipid peroxidation by fish oil has also been reported in normal animals and in humans [10–12]. Therefore, fish oil intake may also influence the lifespan of humans. For instance, aging is promoted by an increase of oxidative stress in diabetic patients who have ingested a large amount of EPA and DHA. In the present study, half of the fat intake was substituted with fish oil and the EPA and DHA intake levels were relatively low. The intake of EPA and DHA was equivalent to the level in a person who eats a lot of fish and uses EPA and DHA as health food supplements.

In the second part of the study, we showed that SAMP8 mice fed fish oil had decreased lipid accumulation in the plasma and liver compared with mice fed safflower oil (Table 1). Fish oil has been reported to improve lipid metabolism and prevent metabolic syndrome [1–4], and a similar beneficial effect was seen in this study.

Conclusion

Our results suggest that the addition of an antioxidant is required to achieve an overall positive effect of fish oil, because the component oils (EPA and DHA) are oxidized and may shorten lifespan, based on the observation of increased oxidative stress and promotion of aging in mice.

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