Comparison of the Increment in Plasma Eicosapentaenoate Concentrations by Fish Oil Intake between Young and Middle-Aged Volunteers

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Summary The effect of age on eicosapentaenoic acid (20:5 n-3; EPA) incorporation into plasma lipids was investigated in young volunteers (8 males, 19±1 yr) and middle-aged volunteers (6 males, 53±7 yr). They were asked to take 5.4 g fish oil per day for one week. The increment in EPA in the cholesteryl ester fraction after the supplementation was significantly greater in the middle-aged group (Δ=1.69%) than in the young group (Δ=0.44%) (p<0.05). The food intake analyzed for 3 consecutive days just before the supplementation revealed that the young group took more linoleate (17 vs. 10 g) than the middle-aged group. There was a significant inverse correlation between the increment in EPA in the cholesteryl ester fraction after the supplementation and daily linoleate intake among all the volunteers combined (r = −0.63, p<0.02). The higher increment in EPA in cholesteryl ester in the middle-aged group might be due to less intake of linoleate and not due to the difference in age itself.

Key Words fish oil, eicosapentaenoic acid, fatty acid composition of plasma lipids, age, food analysis, n-3 fatty acids, linoleic acid

Intake of marine oils has been shown to be beneficial to prevent thrombotic disorders both epidemiologically (1–3) and experimentally (see reviews 4, 5, and 6). According to Kromhout et al. (2), consumption of at least 30 g of fish per day may be enough to reduce mortality from myocardial infarction by half compared to people who did not eat fish at all. Dehmer et al. (7) showed that the incidence of

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early vessel restenosis after coronary angioplasty was 36% in the control patients, who were administered conventional antiplatelet drugs, and only 16% in the patients treated with a similar regimen supplemented with fish oil. In our previous paper (3) we showed that arteriosclerosis estimated by a pulse wave velocity method in fishers in a coastal area was significantly less severe than in farmers in a mountainous area, where mortality from ischemic heart disease was significantly higher than in the coastal area.

However, it is very difficult to determine how much and what kind of fats and oils, including n-3 fatty acids in particular, we should ingest for a high quality of life and longevity. Indeed, there are very few recommendations with regard to daily intake of these fatty acids (8). Consequently, it is important to begin to study how much and what kind of lipids to ingest, with special emphasis on n-3 fatty acids. For this kind of study, we thought that it was necessary to investigate whether or not n-3 fatty acid composition in blood behaved differently between young and old people after fish oil supplementation and if so, why. Insofar as we perform a marine oil experiment with volunteers, we have to take into account ages of subjects to be studied if there is a significant difference in n-3 fatty acid behavior between young and old people.

It was previously reported that the increment in eicosapentaenoic acid (20:5 n-3; EPA) in total plasma lipids in old people was twice as much as in young people after ingestion of a can of mackerel (2.3 g of EPA) per day for one week (9). The reason why there was a difference in EPA increment between young and old people was not investigated (9).

This report discusses a possibility that age itself does not affect an increment in EPA content after fish oil supplementation but that the difference in the intake of fats, linoleate in particular, between a young and a middle-aged group affects the increment in EPA.

METHODS

Study design. Eight young male volunteers [19 ± 1 yr (mean ± SD), 169 ± 3 cm, 61 ± 5 kg] and 6 middle-aged volunteers (53 ± 7 yr, 167 ± 7 cm, 69 ± 8 kg) participated in the present study. The young volunteers were all healthy. The middle-aged volunteers were all slightly hyperlipidemic (2 of type IIa, 2 of type IIb, and 2 of type IV), but otherwise normal. None of these 14 volunteers were heavier than 120% of his standard body weight [(length − 100) × 0.9]. All the volunteers did not take any drugs for at least 2 weeks before and during the study. They were asked to take 18 capsules of fish oil concentrate (5.4 g of fish oil containing 1.5 g of EPA and 1.0 g of docosahexaenoic acid) in three divided doses each with a meal for one week. The fatty acid composition of the fish oil is shown in Table 1. Blood samples were taken just before and at the end of fish oil supplementation in the early morning while the subjects were fasting. Food intake was monitored for 3 consecutive days just before fish oil supplementation. They were asked to continue their usual life-style,
Table 1. Fatty acid composition of fish oil concentrate used in the study.

| Fatty acids | mol% |
|-------------|------|
| 14:0        | 5.5  |
| 16:0        | 7.6  |
| 16:1        | 9.3  |
| 18:0        | 0.9  |
| 18:1 n-9    | 9.7  |
| 18:2 n-6    | 6.1  |
| 18:3 n-3    | 0.2  |
| 18:4 n-3    | 4.7  |
| 20:4 n-6    | 1.3  |
| 20:5 n-3    | 28.0 |
| 22:5 n-3    | 3.1  |
| 22:6 n-3    | 13.2 |

including diets during the food-monitoring period and the fish oil supplementation period. They were asked to record what and how much they ate at each meal during the food-monitoring period. Their records were checked by expert dietitians. The amount of ingested food was calculated by a computer program (10). The computer program had a list of fatty acid composition of over 600 food materials including about 60 fishes and shellfishes with seasonal fluctuations of their fatty acid content which could cover almost all food material data collected during the food study. When we could not find the food material which had been reported by a subject in the food material list of the computer program, we substituted the most similar material in the food material list for the unlisted material. The present study was performed according to the Helsinki declaration.

Measurement. Total cholesterol, cholesteryl esters (measured as total cholesterol minus free cholesterol), triglycerides, phospholipids and free fatty acids were measured enzymatically with serum. High-density lipoprotein (HDL)-cholesterol in serum was measured after precipitation of apo B-containing lipoproteins with heparin, calcium, and nickel. Plasma was separated from EDTA-anticoagulated (1.5 mg/ml) blood and frozen at −20°C until fatty acid analysis of plasma lipids, which was done within two months after blood sampling. Total lipids were extracted from plasma according to Folch et al. (11), and applied to a thin-layer plate (0.25 mm silica gel 60, Merck, Darmstadt, F.R.G.). The plate was developed with petroleum ether: diethyl ether: acetic acid (80:20:1) as a solvent system. After development, lipid fractions were visualized with iodine vapor, and the origin (taken as phospholipid fraction) and areas on the plate corresponding to externally applied standards of cholesteryl esters, triglycerides, and free fatty acids were scraped off the plate. Silica gel was incubated with 6% sulfuric acid in methanol at 70°C for 45 min without prior extraction of lipids. Methylated fatty acids were separated by a capillary column (Supelcowax-10, 30 m × 0.32 mm, Supelco, Bellefonte, PA) at-
tached to a Shimadzu GC 14A gas chromatograph (Shimadzu, Kyoto) with a C-
R6A Chromatopac recorder (Shimadzu). The injection port was at 250°C, column
temperature was set at 180°C for the first 5 min then increased to 234°C at 6°C/min
and hold for 16 min. Detection was by flame ionization at 250°C. Helium was used
as a carrier gas with an inlet pressure at 0.56 kg/cm². Results are reported as percent
of total fatty acids (mol%) because many of the previous results have been reported
as percent (4). Data were expressed as means ± SD and treated by a Student’s t-test
(pairied or unpaired) except where otherwise described.

RESULTS

Compliance with the schedule of fish oil administration was checked with
leftover capsules. It was perfect in the young group and more than 90% in the old
group.

As shown in Table 2 there were significant differences in the serum levels of
total cholesterol, cholesterol esters, and triglycerides between the two groups before
the supplementation of fish oil. No significant changes were observed in any serum
lipid levels due to the fish oil supplementation in either group except for HDL-
cholesterol levels, which increased significantly in the middle-aged group after the
fish oil supplementation. However, the changes in HDL-cholesterol levels occurring
in the two groups were not significantly different.

Changes in the fatty acid composition in plasma phospholipids, choleseryl
esters, triglycerides, and free fatty acids are shown in Tables 3–6, respectively. The
base-line EPA level in each lipid fraction was significantly higher in the middle-aged
group than in the young group, except for the triglyceride fraction. The base-line
linoleate (18:2 n-6) level in each lipid fraction was significantly lower in the middle-
aged group than in the young group. The base-line palmitate level in each lipid

| Lipids                | Young Before | Young After | Middle-aged Before | Middle-aged After |
|-----------------------|--------------|-------------|---------------------|-------------------|
| Total cholesterol     | 150 ± 13     | 153 ± 12    | 226 ± 20³          | 218 ± 18          |
| Cholesteryl esters    | 105 ± 9      | 107 ± 11    | 138 ± 32*²         | 129 ± 15          |
| HDL-cholesterol       | 51 ± 6       | 51 ± 7      | 40 ± 10            | 45 ± 10*          |
| Triglycerides         | 93 ± 33      | 94 ± 26     | 195 ± 92*¹         | 191 ± 78          |
| Phospholipids         | 203 ± 20     | 206 ± 22    | 201 ± 42           | 195 ± 18          |
| Free fatty acids (mEq/liter) | 0.46 ± 0.17 | 0.39 ± 0.14 | 0.44 ± 0.14       | 0.42 ± 0.20       |

Significant differences in base-line values between the two groups are shown by
* p < 0.05; * * p < 0.02; and * * * p < 0.001. Significant change due to fish oil supplementation
within the same group is shown by * p < 0.05.

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Table 3. Fatty acid composition in plasma total phospholipids in the two groups before and after one-week supplementation with fish oil concentrate (mol%).

| Fatty acids | Young Before | Young After | Middle-aged Before | Middle-aged After |
|-------------|--------------|-------------|---------------------|-------------------|
| 16:0        | 36.3 ± 2.3   | 30.4 ± 1.8B | 39.3 ± 1.4a         | 34.0 ± 1.7B       |
| 18:0        | 15.2 ± 1.1   | 13.1 ± 0.8B | 15.2 ± 0.8          | 14.4 ± 1.1        |
| 18:1 n-9    | 8.7 ± 0.9    | 7.2 ± 0.9c  | 7.4 ± 0.6b          | 7.0 ± 1.1         |
| 18:2 n-6    | 20.3 ± 2.7   | 17.8 ± 2.3A | 15.9 ± 2.4b         | 15.9 ± 2.9        |
| 20:4 n-6    | 4.7 ± 1.2    | 5.3 ± 0.8   | 4.8 ± 0.9           | 5.9 ± 0.9B        |
| 20:5 n-3    | 0.4 ± 0.2    | 2.7 ± 0.7c  | 1.5 ± 0.8c          | 3.9 ± 1.4c        |
| 22:6 n-3    | 3.0 ± 0.5    | 4.2 ± 0.7c  | 5.1 ± 0.7d          | 7.0 ± 1.2B        |

Significant differences in base-line values between the two groups are shown by a p<0.05; b p<0.02; c p<0.01; and d p<0.001. Significant changes due to fish oil supplementation within the same group are shown by A p<0.05; B p<0.005; and C p<0.001. See Table 7 for the comparison of changes in fatty acid composition between the two groups.

Table 4. Fatty acid composition in plasma cholesteryl esters in the two groups before and after one-week supplementation with fish oil concentrate (mol%).

| Fatty acids | Young Before | Young After | Middle-aged Before | Middle-aged After |
|-------------|--------------|-------------|---------------------|-------------------|
| 16:0        | 11.3 ± 1.3   | 15.0 ± 3.9A | 18.1 ± 0.8c         | 17.2 ± 1.2        |
| 16:1        | 2.6 ± 1.0    | 3.3 ± 1.2A  | 4.8 ± 1.0b          | 4.2 ± 1.0         |
| 18:1 n-9    | 19.3 ± 2.5   | 23.4 ± 3.2B | 19.8 ± 0.7          | 17.9 ± 1.5A       |
| 18:2 n-6    | 52.5 ± 4.9   | 42.6 ± 7.0A | 41.7 ± 4.2c         | 44.0 ± 4.4        |
| 20:4 n-6    | 2.9 ± 0.6    | 1.7 ± 0.7c  | 2.8 ± 1.0           | 3.5 ± 0.9         |
| 20:5 n-3    | 0.4 ± 0.1    | 0.9 ± 0.5A  | 1.1 ± 0.6a          | 2.8 ± 1.9A        |

Significant differences in base-line values between the two groups are shown by a p<0.01; b p<0.005; and c p<0.001. Significant changes due to fish oil supplementation within the same group are shown by A p<0.05; B p<0.02; and C p<0.005. See Table 7 for the comparison of changes in fatty acid contents between the two groups.

Fraction was significantly higher in the middle-aged group than in the young group.

Changes in polyunsaturated fatty acid composition in 4 lipid fractions are summarized and compared between the two groups in Table 7 and Fig. 1 (with regard to EPA alone). In each lipid fraction the decrease in linoleate level due to the fish oil supplementation in the young group was significantly different from the change in the middle-aged group, which was actually an increment in any fraction (Table 7). There was a significant difference in EPA increment between the two
Table 5. Fatty acid composition in plasma total triglycerides in the two groups before and after one-week supplementation with fish oil concentrate (mol%).

| Fatty acids | Young Before | Young After | Middle-aged Before | Middle-aged After |
|-------------|--------------|-------------|---------------------|-------------------|
| 16:0        | 28.7 ± 3.3   | 28.5 ± 7.7  | 34.1 ± 3.2A         | 30.3 ± 3.0A       |
| 16:1        | 4.0 ± 1.0    | 3.8 ± 1.0   | 5.1 ± 1.1           | 4.6 ± 1.0         |
| 18:0        | 3.7 ± 0.8    | 4.2 ± 1.2   | 4.0 ± 0.7           | 3.9 ± 0.9         |
| 18:1 n-9    | 33.0 ± 4.4   | 36.3 ± 5.8B | 32.3 ± 4.2          | 30.2 ± 2.6        |
| 18:2 n-6    | 14.0 ± 2.2   | 13.2 ± 4.8  | 10.0 ± 1.5B         | 14.3 ± 2.0C       |
| 20:5 n-3    | 0.4 ± 0.2    | 0.5 ± 0.4   | 0.2 ± 0.1           | 0.8 ± 0.4B        |

Significant differences in base-line values between the two groups are shown by *p < 0.02 and #p < 0.005. Significant changes due to fish oil supplementation within the same group are shown by ^p < 0.05; p < 0.01; and c p < 0.005. See Table 7 for the comparison of changes in fatty acid contents between the two groups.

Table 6. Fatty acid composition in plasma free fatty acids in the two groups before and after one-week supplementation with fish oil concentrate (mol%).

| Fatty acids | Young Before | Young After | Middle-aged Before | Middle-aged After |
|-------------|--------------|-------------|---------------------|-------------------|
| 16:0        | 24.9 ± 1.2   | 23.8 ± 2.6  | 27.5 ± 1.6b         | 24.4 ± 2.2        |
| 18:0        | 11.7 ± 1.8   | 10.9 ± 2.3  | 11.2 ± 1.8          | 10.5 ± 2.5        |
| 18:1 n-9    | 34.1 ± 5.2   | 24.5 ± 7.7B | 31.2 ± 2.1          | 29.0 ± 5.5        |
| 18:2 n-6    | 14.1 ± 1.4   | 8.7 ± 2.6C  | 11.0 ± 2.2A         | 12.9 ± 1.2        |
| 20:4 n-6    | 0.6 ± 0.4    | 0.5 ± 0.4   | 0.4 ± 0.1           | 0.5 ± 0.1         |
| 20:5 n-3    | 0.1 ± 0.1    | 0.3 ± 0.2A  | 0.3 ± 0.02c         | 0.7 ± 0.3A        |

Significant differences in base-line values between the two groups are shown by *p < 0.02; #p < 0.01; and c p < 0.001. Significant changes due to fish oil supplementation within the same group are shown by ^p < 0.05; p < 0.02; and c p < 0.001. See Table 7 for the comparison of changes in fatty acid contents between the two groups.

groups in the cholesteryl ester fraction.

Daily intake of total fats, linoleate, and EPA is shown in Table 8. Intake of total fats and linoleate was significantly higher in the young group than in the middle-aged group. On the contrary, EPA intake was significantly higher in the middle-aged group than in the young group.

There was a significant inverse correlation between the increment in EPA content in cholesteryl ester fraction after the fish oil supplementation and daily linoleate intake among all the participants combined (Fig. 2).
Table 7. Comparison of changes (%) in plasma n-6 and n-3 fatty acid levels due to fish oil supplementation between the two groups.

| Lipids fractions | Young       | Middle-aged |
|------------------|-------------|-------------|
| Total phospholipids |             |             |
| 18:2 n-6        | -2.53 ± 2.47 | 0.01 ± 1.42a* |
| 20:4 n-6        | 0.56 ± 0.84  | 1.17 ± 0.39  |
| 20:5 n-3        | 2.22 ± 0.69  | 2.39 ± 0.66  |
| 22:6 n-3        | 1.19 ± 0.53  | 1.88 ± 0.86  |
| Cholesteryl esters |             |             |
| 18:2 n-6        | -9.98 ± 9.10 | 2.26 ± 3.64b |
| 20:4 n-6        | -1.24 ± 0.78 | 0.67 ± 0.52c |
| 20:5 n-3        | 0.44 ± 0.47  | 1.69 ± 1.31a |
| Total triglycerides |             |             |
| 18:2 n-6        | -0.86 ± 3.96 | 4.32 ± 1.48b |
| 20:5 n-3        | 0.06 ± 0.44  | 0.52 ± 0.31  |
| Free fatty acids |             |             |
| 18:2 n-6        | -5.46 ± 2.59 | 1.88 ± 1.98c |
| 20:4 n-6        | -0.08 ± 0.46 | 0.13 ± 0.12  |
| 20:5 n-3        | 0.17 ± 0.16  | 0.34 ± 0.30  |

Values indicate differences in fatty acid levels in a given lipid fraction before and after one-week supplementation of fish oil concentrate. Significant differences in changes between the two groups are shown by a p < 0.05; b p < 0.02; and c p < 0.001. * Data were treated by Wilcoxon rank sum test.

Fig. 1. Comparison of changes (Δ%) in plasma EPA content due to fish oil supplementation between the two groups. Open columns, the young group; hatched columns, the middle-aged group. * p < 0.05.
Table 8. Comparison of averaged daily intake of fatty acids (g/day).

| Fatty acids | Young          | Middle-aged   |
|-------------|----------------|---------------|
| Total fat   | 56.2 ± 15.0    | 38.9 ± 8.8**  |
| 18:2 n-6    | 17.1 ± 5.6     | 9.8 ± 2.6b    |
| 20:5 n-3    | 0.06 ± 0.08    | 0.32 ± 0.29   |
|             | 0 (0–0.2)      | 0.3 (0–0.8)*  |

Significant differences between the two groups are shown by *p < 0.05 and **p < 0.02. With regards to EPA (20:5 n-3), medians and ranges in parentheses are also shown under means ± SD, and Wilcoxon rank sum test (*) was performed because data were not normally distributed.

DISCUSSION

The most important findings in the present study were that there was a significant difference in the increase in EPA level in the cholesteryl ester fraction between the two groups (Table 7 and Fig. 1), and that the increase in EPA content in the cholesteryl ester fraction was inversely correlated to daily intake of linoleate (Fig. 2).

It has already been reported that following fish or fish oil diets, the plasma cholesteryl ester fraction is markedly increased in EPA level in humans (12, 13).
Because EPA in the 2-position of plasma phosphatidylcholine (PC) seems to be a precursor of cholesteryl EPA (13), occupation of the 2-position of PC by linoleate may competitively inhibit EPA incorporation into the cholesteryl ester fraction. Although we did not analyze fatty acid composition of plasma PC, it seems likely that, judging from higher linoleate level in plasma total phospholipids in the young group, linoleate level in plasma PC in the young group was higher than that in the middle-aged group. This might cause a smaller increase in EPA content in cholesteryl esters in the young group after fish oil supplementation.

Cholesteryl EPA might also be synthesized through the action of acyl-CoA cholesterol acyltransferase in the intestine. In the young group, whose intake of linoleate was larger than in the middle-aged group (Table 8), the synthesis of cholesteryl EPA through this pathway was probably more inhibited by the competition with linoleate-CoA than in the middle-aged group.

The correlation shown in Fig. 2 is similar to the finding reported by Moilanen et al. (14) that EPA in the cholesteryl ester fraction in Finnish youngsters had a strong negative correlation with the dietary P/S ratio, which reflected mostly linoleate intake because the amount of n-3-containing marine food was very low in the Finnish diet (14). It is also reported that 8-year-old Finnish boys using butter on their bread had higher proportions of serum EPA in cholesteryl ester and phospholipid fractions than boys using vegetable margarine, which contained more linoleate than butter (15). In these two Finnish studies, differences in fatty acid compositions in blood lipids were studied as a function of daily intake of fats and not that of age, the studied subjects all being youngsters. Consequently, the difference in EPA increase in the cholesteryl ester fraction between the two groups in the present study might be due to the difference in daily linoleate intake rather than to the difference in ages.

As shown in Table 8, the young subjects ingested more fats including linoleate and less EPA than did the middle-aged subjects. This is comparable with a general trend in Japan that people eat more fish and less fats with age (16). The fatty acid composition in plasma lipid fractions before fish oil supplementation (Tables 3 to 6) is consistent with the data of daily fatty acid intake.

Hirai et al. (9) reported that the increase in EPA contents in total plasma lipids in old subjects (mean age = 65) was twice as much as that in young subjects (ages: 20 to 30) after the supplementation of a can of mackerel (water 146 g, crude fats 38 g including 2.3 g EPA, crude protein 39 g) per day for one week. In their study the daily total intake of fats by volunteers was neither controlled nor calculated. The difference in daily linoleate intake between young and old subjects might have been even exaggerated during their study, because old Japanese people, who usually do not like oily meals, might not have eaten any more oily stuff in addition to the can of oily mackerel per day. Consequently, we believe that there was also less competition between linoleate and EPA in lipid metabolism in the old subjects than in the young subjects in their study.

Suzuki et al. (17) investigated the effect of age on the modification of plasma
fatty acid composition by feeding rats of 3 and 14 months of age on a fish oil-supplemented diet. Both young and old rats ate comparable amounts of the fish oil-supplemented diet for 30 days. They found a greater EPA increase in total plasma lipids in the aged rats than in the young rats. However, it is very difficult to extrapolate their data to a human situation. There is not only the problem of species difference but also that of a gigantic difference in body weights between young and old rats. The body weights of the aged rats were probably double those of the young rats, which would cause a difference in the ratios of surface area to body weight of rats. Energy saving probably took place in the aged rats by a smaller heat radiation from the body surface in terms of kilograms of body weight due to a smaller ratio of surface area to body weight and thicker subcutaneous adipose tissue layers. This energy-saving effect might cause accumulation of fatty acids in adipose tissues, which preferred saturated fatty acids to polyunsaturated fatty acids (18, 19), resulting in concentration of polyunsaturated fatty acids in plasma.

There were significant differences in changes in linoleate levels in all lipid fractions between the two groups (Table 7). It is not clear whether these differences were due to difference in age, daily linoleate intake, or some other factor.

In conclusion, our data suggest that the difference in changes in EPA levels in plasma lipids after fish oil supplementation between young and aged people might be due not to the difference in age itself but to that in daily linoleate intake. Control of daily intake of linoleate and possibly other fatty acids appears to be very important in fish oil supplementation studies.

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