The Change in Low-Density Lipoprotein Cholesterol Concentration is Positively Related to Plasma Docosahexaenoic Acid but not Eicosapentaenoic Acid

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**Aim:** The Japan EPA Lipid Intervention Study (JELIS) reported a 19% reduction of the risk for coronary artery disease after long-term use of pure eicosapentaenoic acid (EPA) in Japanese patients with hypercholesterolemia. The variation in plasma fatty acid composition influenced the risk of coronary events. The aim of this study was to examine in JELIS participants the possible correlation of changes in plasma fatty acids with those of serum lipids.

**Methods:** The coefficient for the correlation between the absolute change in plasma fatty acid concentrations and the changes in serum lipids was calculated in 13,901 JELIS participants.

**Results:** Low-density lipoprotein (LDL) cholesterol exhibited a positive correlation with docosahexaenoic acid \( (r=0.117 \text{ in control group, } r=0.155 \text{ in EPA group}) \) and linoleic acid \( (r=0.139 \text{ in control group, } r=0.177 \text{ in EPA group}) \), but the correlation coefficients with EPA \( (r=0.097 \text{ in control group, } r=-0.032 \text{ in EPA group}) \) were less than 0.1. We distributed the patients into 9 groups according to tertiles of the change in EPA and DHA. The average absolute decrease of LDL cholesterol and L/H ratio in each group was significantly smaller \( (p<0.001) \) in the DHA-high tertile, but not in any EPA tertile.

**Conclusion:** The changes in DHA, but not in EPA, showed a positive correlation with the changes in LDL-cholesterol.

**Key words:** Eicosapentaenoic acid, Docosahexaenoic acid, Low-density lipoprotein cholesterol, Triglycerides

**Introduction**

Previous nutritional surveys reported that lower serum triglycerides, higher high-density lipoprotein (HDL) cholesterol, and a lower atherosclerotic mortality rate were observed in Greenland Inuits than in
Danish subjects, although the fat proportion in their total energy intake was nearly the same. It was suggested that a high blood eicosapentaenoic acid (EPA) concentration brought such benefits to the Inuit, who have different eating habits from the European population\(^1\)\(^-\)\(^3\). Furthermore, serum EPA and HDL cholesterol concentrations correlated positively in a nutrition survey of Kohama-Island residents in Okinawa, Japan\(^4\). Some clinical trials intervening in food intake have demonstrated that consuming polyunsaturated fatty acid (PUFA)-rich oil or fish-oil supplements decreased the levels of serum very low-density lipoprotein (VLDL) and triglycerides\(^5\)\(^-\)\(^7\). Moreover, some clinical trials demonstrated that diet control affected changes in serum lipids, and that consuming n-3 PUFA was known to bring health benefits. Docosahexaenoic acid (DHA) and EPA are two major n-3 PUFAs, together with alpha-linoleic acid and docosapentaenoic acid (DPA), but it is unclear which n-3 PUFA is responsible for the benefits. Pure EPA was confirmed to have a cholesterol-lowering effect\(^8\)\(^,\)\(^9\) and it was approved by the Japanese Ministry of Health, Labour, and Welfare for the treatment of hyperlipidemia and peripheral artery diseases. The Japan EPA Lipid Intervention Study (JELIS) reported a 19% reduction of the risk for CAD after long-term use of pure EPA in Japanese patients with hypercholesterolemia under treatment with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (pravastatin or simvastatin). However, although JELIS did not confirm a low-density lipoprotein (LDL) cholesterol-lowering effect beyond statin therapy, a tri-glyceride-lowering effect was observed with EPA at a daily dose of 1800 mg\(^9\)\(^8\%). Local physicians monitored compliance with dietary instructions and the use of medications at each clinic visit. The design and inclusion and exclusion criteria were described in detail elsewhere\(^1\)\(^1\). At registration, 16,397 patients (control group, \(n=8,076\); EPA group, \(n=8,321\)) gave their informed consent to annual blood sampling to test plasma fatty acids\(^1\)\(^2\). We obtained 13,901 samples (control group, \(n=6,844\); EPA group, \(n=7,057\)) at baseline and final follow-up paired data.

**Lipid Determinations**

Serum lipids (total cholesterol, HDL cholesterol and triglycerides) were measured at 6 and 12 months and every year thereafter. LDL cholesterol was calculated using Friedewald’s equation. Plasma total fatty acid concentrations were measured annually at the central laboratory of BML Inc. (Saitama, Japan). Plasma fatty acid composition was determined by capillary gas chromatography. Briefly, plasma lipids were extracted by Folch’s procedure. Then, using tricosanoic acid (C23:0) as the internal standard, fatty acids were methylated with boron trifluoride and methanol, and methylated fatty acids were analyzed using a SHIMAZU GC-17A gas chromatograph (Shimazu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.25 mm ID \(\times\) 30 m; SGE International Ltd., Melbourne, Australia). The following major fatty acids data were used for subanalysis: saturated fatty acids (C16:0 palmitic acid, C18:0 stearic acid), a monounsaturated fatty acid (C18:1 oleic acid), n-6 PUFAs (C18:2 linoleic acid, C20:4 arachidonic acid), n-3 PUFAs (C20:5 EPA, C22:6 DHA).

**Statistical Analysis**

Absolute changes in serum lipids and plasma fatty acids were the difference between the value at baseline and that at the final follow-up visit. The LDL

**Methods**

**Patients**

This investigation was a subanalysis of JELIS data. Eligibility criteria were total cholesterol level of 250 mg/dL or greater, which corresponds to a low-density lipoprotein cholesterol level of \(\geq 170 \) mg/dL at baseline. The minimum age was 40 years for men; women were required to be postmenopausal. Maximum patient age was 75 years. The review board of each institute approved the study protocol, and all patients provided written informed consent. The enrollment period in JELIS was from November 1996 to November 1999. The planned duration of follow-up was 5 years, with actual monitoring for a mean of 4.6 (SD1.1) years. All patients received 10mg pravastatin or 5mg simvastatin once daily as first-line treatment and were counseled to follow the National Cholesterol Education Program step I diet. In total, 18,645 patients complied with the inclusion and exclusion criteria for JELIS.

**Study Design**

The study population was randomly assigned to receive EPA (EPA group) or not (control group) after a 4- to 8-week washout of antihyperlipidemic drugs. In the EPA group, we prescribed a daily dose of 1800 mg EPA, that is, 6 capsules containing 300 mg each of pure (\(>98\%)\) EPA ethyl ester. Local physicians monitored compliance with dietary instructions and the use of medications at each clinic visit. The design and inclusion and exclusion criteria were described in detail elsewhere\(^1\)\(^1\). At registration, 16,397 patients (control group, \(n=8,076\); EPA group, \(n=8,321\)) gave their informed consent to annual blood sampling to test plasma fatty acids\(^1\)\(^2\). We obtained 13,901 samples (control group, \(n=6,844\); EPA group, \(n=7,057\)) at baseline and final follow-up paired data.

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Fatty Acids and LDL Cholesterol

cholesterol/HDL cholesterol (L/H) ratio and EPA/ara-chidonic acid (AA) ratio were calculated from serum lipid and plasma fatty acid data. Spearman’s correlation coefficients were used for the analyses. In particular, partial correlation coefficients were used to analyze the correlation between the change in serum LDL cholesterol or L/H ratio and the change in plasma fatty acids. The Kruskal-Wallis test was used to compare the absolute change in LDL-cholesterol and L/H ratio with the change in plasma fatty acids. Probability values of 5% or less (two-sided) were considered significant. Analyses were performed using SAS statistical software version 9.1 (SAS Institute, Inc, Cary, NC).

Results

Plasma Fatty Acid Profiles by Age and Sex

The average concentrations at registration of serum lipoproteins and plasma fatty acids distributed by age and sex are shown in Fig. 1. LDL cholesterol and HDL cholesterol levels were similar in all age brackets regardless of sex. Triglyceride levels were high in the <50 y.o. group, especially in men. The same trend was observed for palmitic acid, stearic acid, and oleic acid levels, especially in men in the <50 y.o. group. N-6 PUFA (linoleic acid and arachidonic acid) levels were also high in the <50 y.o. group, both in men and women. In contrast, n-3 PUFA (EPA and DHA) levels and the EPA/AA ratio were lower in the <50 y.o. group than in the <60-69 and ≥70 y.o. groups, and slightly higher in men of the same generation. However, no marked differences in the relative amount (mol%) of plasma fatty acids were found among age groups, regardless of sex.

Correlations between the Absolute Change in the Concentration of Serum Lipids and Plasma Fatty Acids

Table 1 shows the Spearman’s correlation coefficients for the absolute change in serum lipids and change in the absolute amount of plasma fatty acids. There were positive correlations between triglycerides...
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HDL-cholesterol

LDL-cholesterol

Table 1. The correlation coefficients between the change in serum lipid and the change in absolute amount of plasma fatty acid (μg/mL)

<table>
<thead>
<tr>
<th></th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1n-9</th>
<th>C18:2n-6</th>
<th>C20:4n-6</th>
<th>C20:5n-3</th>
<th>C22:6n-3</th>
<th>EPA/AA</th>
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<tr>
<td>LDL-cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.055***</td>
<td>0.039**</td>
<td>0.005</td>
<td>0.139***</td>
<td>0.086***</td>
<td>0.097***</td>
<td>0.117***</td>
<td>0.058***</td>
</tr>
<tr>
<td>EPA group</td>
<td>0.080***</td>
<td>0.048***</td>
<td>0.036**</td>
<td>0.177***</td>
<td>0.143***</td>
<td>-0.032*</td>
<td>0.155***</td>
<td>-0.101***</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.099***</td>
<td>-0.064***</td>
<td>-0.125***</td>
<td>-0.030*</td>
<td>0.029*</td>
<td>0.029*</td>
<td>-0.020</td>
<td>0.018</td>
</tr>
<tr>
<td>Control group</td>
<td>-0.086***</td>
<td>-0.041***</td>
<td>-0.111***</td>
<td>-0.022</td>
<td>0.060***</td>
<td>-0.002</td>
<td>-0.018</td>
<td>-0.029*</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.408***</td>
<td>0.378***</td>
<td>0.418***</td>
<td>0.258***</td>
<td>0.173***</td>
<td>0.039*</td>
<td>0.195***</td>
<td>-0.031**</td>
</tr>
<tr>
<td>Control group</td>
<td>0.429***</td>
<td>0.389***</td>
<td>0.434***</td>
<td>0.295***</td>
<td>0.205***</td>
<td>-0.030*</td>
<td>0.215***</td>
<td>-0.090***</td>
</tr>
</tbody>
</table>

***p<0.001, **p<0.01, *p<0.05

Table 2. The correlation coefficients between the change in serum lipid and the change in relative amount of plasma fatty acid (mol%)

<table>
<thead>
<tr>
<th></th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1n-9</th>
<th>C18:2n-6</th>
<th>C20:4n-6</th>
<th>C20:5n-3</th>
<th>C22:6n-3</th>
<th>EPA/AA</th>
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<tr>
<td>LDL-cholesterol</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>-0.037**</td>
<td>-0.099***</td>
<td>-0.120***</td>
<td>0.096***</td>
<td>-0.007</td>
<td>0.054***</td>
<td>0.057***</td>
<td>0.058***</td>
</tr>
<tr>
<td>EPA group</td>
<td>-0.031*</td>
<td>-0.147***</td>
<td>-0.094***</td>
<td>0.136***</td>
<td>0.038**</td>
<td>-0.094***</td>
<td>0.087***</td>
<td>-0.101***</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.074***</td>
<td>0.059***</td>
<td>-0.148***</td>
<td>0.086***</td>
<td>0.130***</td>
<td>0.070***</td>
<td>0.058***</td>
<td>0.018</td>
</tr>
<tr>
<td>Control group</td>
<td>-0.069***</td>
<td>0.084***</td>
<td>-0.145***</td>
<td>0.086***</td>
<td>0.150***</td>
<td>0.014</td>
<td>0.046***</td>
<td>-0.029*</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.213***</td>
<td>-0.023</td>
<td>0.285***</td>
<td>-0.212***</td>
<td>-0.300***</td>
<td>-0.153***</td>
<td>-0.160***</td>
<td>-0.032**</td>
</tr>
<tr>
<td>Control group</td>
<td>0.225***</td>
<td>-0.049***</td>
<td>0.294***</td>
<td>-0.163***</td>
<td>-0.268***</td>
<td>-0.170***</td>
<td>-0.148***</td>
<td>-0.090***</td>
</tr>
</tbody>
</table>

***p<0.001, **p<0.01, *p<0.05

and each fatty acid except EPA. Correlation coefficients were larger for saturated fatty acids and oleic acid than for PUFA. There were positive but weak correlations between LDL cholesterol and saturated fatty acids and oleic acid. In contrast, PUFA, except EPA, were more positively correlated with LDL cholesterol than with saturated fatty acids and oleic acid. Oleic acid was negatively correlated with HDL cholesterol. There were weak correlations between HDL cholesterol and other fatty acids. There were weak correlations between HDL cholesterol and other fatty acids.

Table 2 shows the Spearman’s correlation coefficients for the absolute change in serum lipids and change in the relative amount of plasma fatty acids. There were negative correlations between triglycerides and PUFA, and positive correlations with palmitic acid and oleic acid. The trends for correlations were different between the absolute change and relative change in saturated fatty acids and oleic acid. Oleic acid was negatively correlated while arachidonic acid was positively correlated with HDL cholesterol. However, there were weak correlations between HDL cholesterol and other fatty acids. There were poor correlations between the change in EPA/AA ratio and serum lipids. The correlation coefficients were similar in both the absolute change and relative change.

Interrelationship between EPA and DHA with the LDL Cholesterol Change

We distributed the patients into 9 groups according to tertiles of the absolute change in EPA and those in DHA (Fig. 2). We then calculated the average change in LDL cholesterol concentrations and L/H ratios in each group. As a result, the decrease in LDL cholesterol and L/H ratio was smaller (p<0.001) in the DHA-high tertile than in the DHA-low tertile in any EPA tertile. Additionally, in all patients, the partial correlation coefficient between the changes in
molecular structures, recent evidence suggests that the effects of EPA on the concentration of plasma and membrane lipids differ from those of DHA. Davidson and co-workers reported that 0.0–2.5 g DHA/day increased LDL-cholesterol concentration in a dose-related manner\(^{13}\). Moreover, Leigh-Firbank and colleagues, who performed a multiple regression analysis of changes in platelet lipids as dependent variables with changes in plasma phospholipid EPA and DHA as independent variables, found that changes in DHA but not in EPA emerged as an independent dominant factor of the rise in LDL-cholesterol\(^{14}\). According to a recent report, the addition of an n-3 PUFA formula-tion (a mixture of EPA and DHA) to statin therapy was associated with an increase in LDL cholesterol in patients with dyslipidemia\(^{15-18}\). This subanalysis also indicated that the change in DHA but not in EPA emergence showed a positive correlation with the change in LDL cholesterol. As a result of EPA intervention, the simple correlation between the change in LDL cholesterol and EPA became slightly negative but the correlation between the change in LDL cholesterol and DHA showed the same trend despite EPA intervention. We speculated that the change in DHA concentration strongly influenced the relationship between the change in LDL cholesterol and EPA in the control group, because both EPA and DHA are n-3 PUFA present in food. A recent interventional trial suggested the retroconversion from DHA to EPA in LDL particles\(^{19}\), but we speculated that DHA intake will increase serum LDL-cholesterol because fish oil (EPA + DHA) intervention increased LDL-cholesterol significantly\(^{20}\) and pure EPA intervention

**Discussion**

In the JELIS population, serum triglycerides and absolute amount of plasma fatty acid concentrations decreased with aging, and these trends were marked in men. This might be a consequence of differences in eating habits occurring with age between men and women. It is important to consider age and sex as confounding factors to discuss the relationship between fatty acids in blood and clinical events, such as coronary artery disease. In addition, we previously investigated the quantities of change in serum lipids and plasma fatty acids, and the average age and sex distribution were also the same in any allocation group\(^{12}\).

We determined the relative amount (mol%) and the absolute amount (μg/mL) of plasma fatty acids. Since the absolute amount of plasma fatty acids was influenced by the total amount of fat, we used the absolute amount of fatty acids to discuss the correlations between the change in LDL-cholesterol and that in EPA or DHA after adjustment (Fig. 2). The coefficients for the correlations of change in the EPA/AA ratio with those of serum lipids were quite identical in both absolute change and relative change, thus, these discrepancies can be disregarded.

Although both EPA and DHA have similar LDL cholesterol and the change in EPA (adjusted for DHA) was \(-0.007 (p=0.416)\), and in DHA (adjusted for EPA) was \(0.131 (p<0.001)\). Similarly, the partial correlation coefficient between the changes in L/H ratio and the change in EPA was \(0.0004 (p=0.965)\), and in DHA was \(0.099 (p<0.001)\).

**Fig. 2.** The change in LDL-cholesterol and in L/H ratio by the tertiles of the change in EPA and DHA.

EPA T1, \(<-1.20\); EPA T2, \(-1.20\) to \(<59.60\); EPA T3, \(\geq59.60\); DHA T1, \(<-25.30\); DHA T2, \(-25.30\) to \(<11.00\); DHA T3, \(\geq11.00\)
did not influence the change in serum LDL-cholesterol\textsuperscript{10}.

This subanalysis indicated that the absolute change in palmitic acid and oleic acid correlated with triglycerides more significantly than the absolute change in PUFAs. Therefore, the relative change in PUFAs showed a negative correlation with triglycerides. In a similar way, the relative change in stearic acid showed a slightly negative correlation. Unlike DHA, the correlation coefficient of EPA was very small. The triglyceride-lowering effect of EPA has already been reported\textsuperscript{10}, but we could not figure out why among all PUFAs only EPA showed a very weak correlation with triglycerides. However, we did not consider the difference in food intake of the participants. The plasma free fatty acid level is influenced by food intake and other confounding factors such as insulin signaling. From the Omacor Carotid Endarterectomy Intervention (OCEAN) trial\textsuperscript{21}, advanced atherosclerotic plaques appear to readily incorporate EPA from an n-3 PUFA formulation and a higher content of EPA in carotid plaques is associated with a reduced number of foam cells and T cells, less inflammation and increased stability. Nevertheless, the concentration of DHA in the phospholipid fraction of carotid plaques did not differ between the group prescribed an n-3 PUFA formulation and the control group. These results suggest that EPA and DHA play different roles in atherosclerosis-related tissues. DHA is well known as an important structural fatty acid in the brain and eyes, among other tissues. We consider that EPA is a functional fatty acid, and that a small increase in concentration brings a significant benefit such as reduction of the risk for coronary artery disease.

A science advisory nutrition subcommittee from the American Heart Association (AHA) supports an n-6 PUFA, particularly linoleic acid, intake of at least 5% to 10% of energy in the context of other AHA lifestyle and dietary recommendations, because there is clinical evidence that n-6 PUFA has an LDL cholesterol-lowering effect\textsuperscript{22}. But changes in the absolute amount of linoleic acid concentration showed a positive correlation with the change in LDL cholesterol in this subanalysis. Saturated fatty acids and oleic acid are major fatty acids that constitute triglycerides, and their role is energy accumulation. Changes in SFAs and oleic acid show positive correlations with changes in triglycerides, but the coefficient for the correlation with changes in LDL cholesterol was relatively small. The results of this analysis also indicated that changes in SFAs were almost unrelated to changes in cholesterol.

As a study limitation, we used low doses of statins for all participants that are recommended by Japan’s Ministry of Health, Labour, and Welfare. Therefore, our results are only applicable for hypercholesterolemia with statin treatment. In conclusion, the relationship between the changes in serum lipoprotein and plasma fatty acid was variable. Among n-3 polyunsaturated fatty acids, changes in the absolute amount of DHA, but not in that of EPA, exhibited a positive correlation with the changes in LDL cholesterol.

Acknowledgements

We thank all trial participants and the large numbers of physicians, nurses, and hospital staff members who made long-term commitments to the study, as well as the patients who participated in the trial.

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Mochida Pharmaceutical Co. Ltd. (Tokyo, Japan) bore the expenses for the determination of plasma fatty acid concentration. The marketed capsules containing 300 mg EPA ethyl ester were supplied by Mochida Pharmaceutical Co. Ltd. None of the authors have a conflict of interest regarding this study.

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