Original Article

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 (DHA; $r=0.117$ in control group, $r=0.155$ in EPA group) and linoleic acid ($r=0.139$ in control group, $r=0.177$ in EPA group), but the correlation coefficients with EPA ($r=0.097$ in control group, $r=-0.032$ 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.**

J Atheroscler Thromb, 2012; 19:673-679.

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

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Received: August 31, 2011
Accepted for publication: February 9, 2012

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 triglyceride-lowering effect was observed with EPA at a daily dose of 1800 mg[10]. The aim of this study was to examine in JELIS participants the possible correlation between changes in plasma fatty acids with those of serum lipids, with special focus on the differences between EPA and DHA, because the effects of which are controversial.

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 ≥ 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[11]. 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[12]. 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 × 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

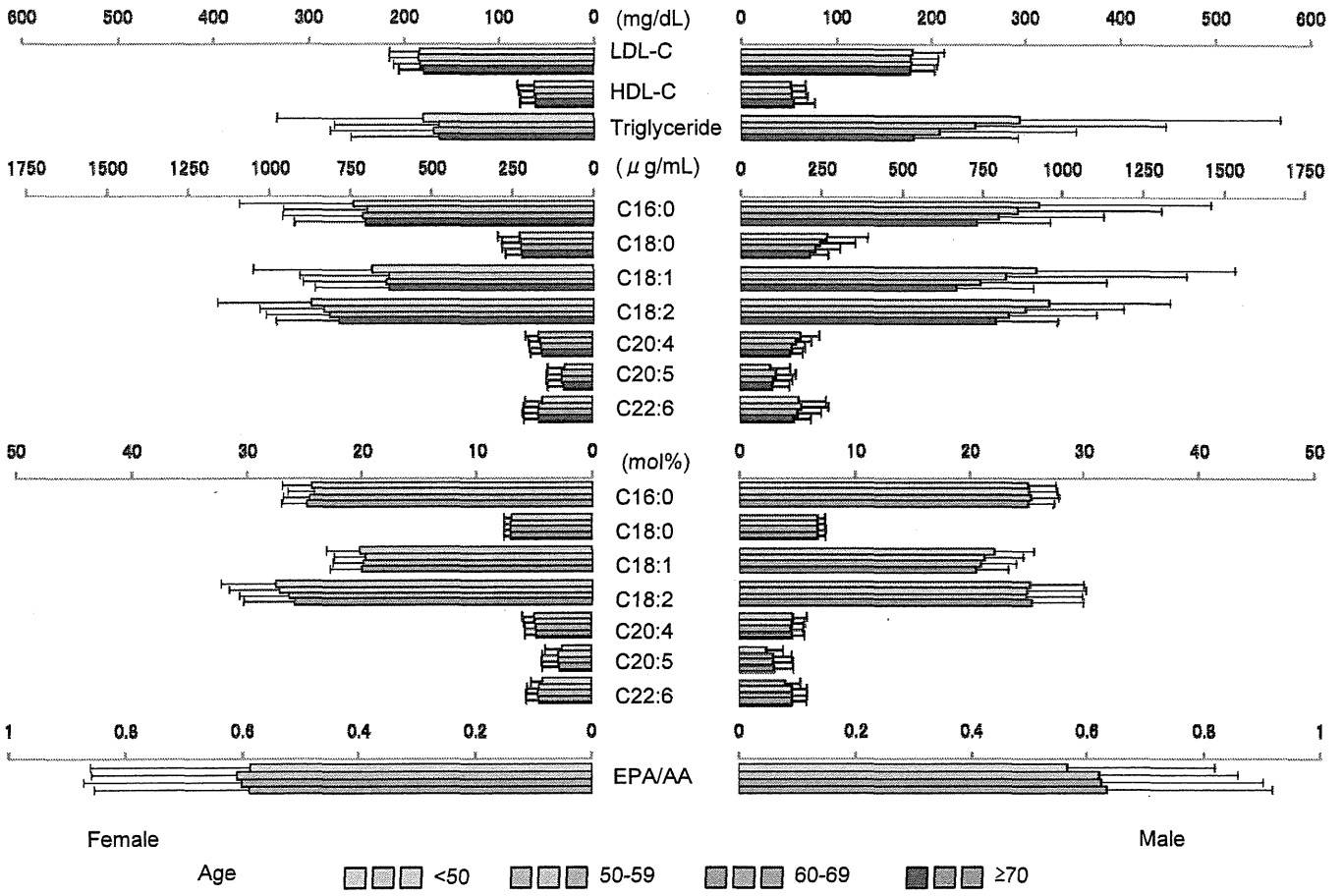

Fig. 1. Baseline serum lipid and plasma fatty acids according to sex and age.

cholesterol/HDL cholesterol (L/H) ratio and EPA/arachidonic 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

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

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

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

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

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

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

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, PUFAs, 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.

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 PUFAs, and positive correlations with palmitic acid and oleic acid. The trends for correlations were different between the absolute change and relative change in stearic acid and PUFAs. There were negative but weak correlations between LDL cholesterol and saturated fatty acids as well as monounsaturated 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

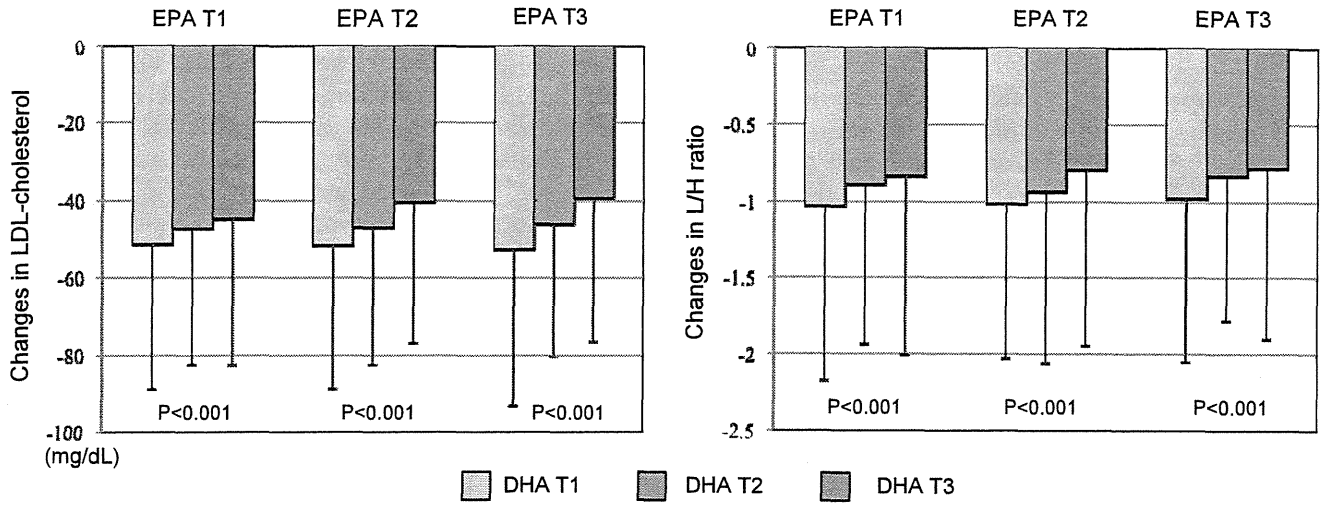

Fig. 2. The change in LDL-cholesterol and in L/H ratio by the tertiles of the change in EPA and DHA.
EPA T1, <−1.20; EPA T2, −1.20 to <59.60; EPA T3, ≥59.60; DHA T1, <−25.30; DHA T2, −25.30 to <11.00; DHA T3, ≥11.00

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).

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 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 formulation (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 concentration 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

did not influence the change in serum LDL-cholesterol[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[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[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[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.

Sources of Funding

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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Original Article

Relationship between Coronary Artery Disease and Non-HDL-C, and Effect of Highly Purified EPA on the Risk of Coronary Artery Disease in Hypercholesterolemic Patients Treated with Statins: Sub-Analysis of the Japan EPA Lipid Intervention Study (JELIS)

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Aim: The present study examined the importance of reducing non-high-density lipoprotein cholesterol (non-HDL-C) for the primary prevention of the occurrence of coronary artery disease (CAD) in the JELIS, and the effects of EPA.

Methods: The patients were distributed into 4 subgroups using the lipid management goal for LDL-C recommended by the Japan Atherosclerosis Society guideline (2007) and the goal for non-HDL-C defined as 30 mg/dL higher than LDL-C: A) achieved both goals; B) achieved the LDL-C but not non-HDL-C goal; C) achieved the non-HDL-C but not LDL-C goal; and D) did not attain either goal. The incidences of CAD in the 4 subgroups were compared, and the effects of eicosapentaenoic acid (EPA) on the risk of CAD in these subgroups were examined.

Results: In the non-EPA group, the incidence of CAD in patients who did not achieve the goals for LDL-C or non-HDL-C was higher than in patients who achieved those goals. Patients in subgroups B, C, and D were at higher risk for CAD than those in subgroup A (B, HR 2.31; C, HR 1.90; D, HR 2.47). EPA reduced the risk of CAD by 38% in subgroups B, C, and D ($p=0.007$).

Conclusion: We reconfirmed non-HDL-C as a predictor of the risk for CAD and a residual risk marker of CAD after LDL-C-lowering therapy. EPA was useful to reduce the occurrence of CAD in patients who did not achieve the goals for LDL-C and/or non-HDL-C.

J Atheroscler Thromb, 2012; 19:194-204.

Key words; Eicosapentaenoic acid, Non HDL-C, Coronary artery disease, Residual risk

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Received: January 2, 2011
Accepted for publication: September 20, 2011

Introduction

Coronary artery disease (CAD) is one of the major causes of death in developed countries. This is a disease based on atherosclerosis whose onset and progression are closely related to serum lipids. Low density lipoprotein cholesterol (LDL-C) is considered a

very important risk factor for CAD, and lowering of LDL-C has been adopted as a treatment goal[1, 2]. The LDL-C goals for the primary prevention group in categories I (Low risk), II (Intermediate risk), and III (High risk), and for the secondary prevention group are less than 160, 140, 120 and 100 mg/dL, respectively, by the Japan Atherosclerosis Society (JAS) established in 2007[3].

Although the results of many randomized controlled trials (RCTs) using statins have shown the usefulness of LDL-C-lowering therapy, the extent of CAD suppression did not exceed 30%[4-6]; therefore, the residual risk of CAD has become a problem. Recently, non-high density lipoprotein cholesterol (non-HDL-C) has begun to attract attention as a new predictor of CAD risk[2, 7-10]. Tanabe *et al.* reported the results of the Japan Arteriosclerosis Longitudinal Study (JALS), which stated that the risk of acute myocardial infarction is more reliably predicted by serum non-HDL-C than by serum total cholesterol (TC)[9]. Robinson *et al.* found that the percent of non-HDL-C lowering correlates with coronary heart disease reduction[10]. Furthermore, Kastelein at el. reported that on-treatment levels of non-HDL-C and apolipoprotein B are more closely associated with cardiovascular outcome than with LDL-C levels in patients receiving statin therapy[11]. Non-HDL-C levels reflect the amount of remnant lipoproteins and small-dense LDL, which also are atherogenic. Since these atherogenic lipoproteins are known to increase in patients with hypertriglyceridemia or low levels of HDL-C, the levels of non-HDL-C may reflect an abnormal lipid metabolism associated with metabolic syndrome, obesity, and insulin resistance.

The Adult Treatment Panel III (ATP III) recommends that the goals for non-HDL-C in the high, intermediate, and low risk groups should be less than 130, 160, and 190 mg/dL, respectively; these values are 30 mg/dL higher than the recommended level of LDL-C[12]. Based on clinical data, Shimano *et al.* confirmed the same management goals for non-HDL-C[13]; however, there is no evidence that the goals for non-HDL-C (LDL-C plus 30 mg/dL) are useful for reducing CAD in Japanese patients with dyslipidemia.

On the other hand, an epidemiological study of Innuits in Greenland[14] and analyses of the fatty acid composition in their diet[15] and blood[16] showed a long time ago that n-3 polyunsaturated fatty acids (PUFAs) contained in fish oil suppressed the development of CAD. Many subsequent studies including epidemiological studies, clinical studies, and RCTs have provided evidence of the suppression of CAD by n-3 PUFAs[17-22].

In the Japan EPA Lipid Intervention Study (JELIS), a large-scale RCT of highly purified eicosapentaenoic acid (EPA), we demonstrated that EPA reduced the occurrence of major coronary events (MCE) independent of LDL-C reduction[23]. We also reported that patients with abnormal serum triglyceride (TG) and HDL-C levels (TG ≥150 mg/dL; HDL-C <40 mg/dL) had a significantly higher CAD risk than those with normal serum TG and HDL-C levels, and intervention with EPA markedly reduced the risk of CAD in this high-risk population in subanalysis of primary prevention cases from the JELIS[24]. Sugimoto *et al.* reported that non-HDL-C had a positive correlation with TG concentration[25]. The present study examined the importance of non-HDL-C for prevention of the occurrence of CAD and the effects of EPA.

Methods

Study Design and Patients

Details of the design of JELIS have been reported in a previous paper[26]. Briefly, patients with a serum TC level ≥250 mg/dL (men: 40-75 years; women: postmenopausal-75 years) were followed for up to 5 years (mean: 4.6 years) using a prospective, randomized, open-label, blinded-endpoint evaluation (PROBE) method. A total of 18,645 patients,, including 3,664 with a history of CAD were registered and randomly assigned to either an EPA with statin (EPA group; $n=9,326$) or a statin-alone (non-EPA group; $n=9,319$) group using a central registration system.

The study population was randomly assigned to receive EPA or not after a 4- to 8-week washout period of antihyperlipidemic drugs. In the EPA group, we administered a daily dose of 1800 mg EPA as 6 capsules, each containing 300 mg of highly purified (>98%) EPA ethylester. The primary endpoint of JELIS was the cumulative incidence of MCE, including sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina pectoris with documented myocardial ischemia, and angioplasty/stenting or coronary artery bypass grafting. Clinical endpoints were reviewed by expert cardiologists belonging to the Event Evaluation Committee and without knowledge of treatment allocation. Local physicians monitored compliance with dietary instructions and the use of medications at each hospital visit. Patients (non-EPA group: $n=5,806$, EPA group: $n=5,863$) with the fasting serum lipid determined at one year and without a history of CAD were the subjects of this report (**Fig. 1**).

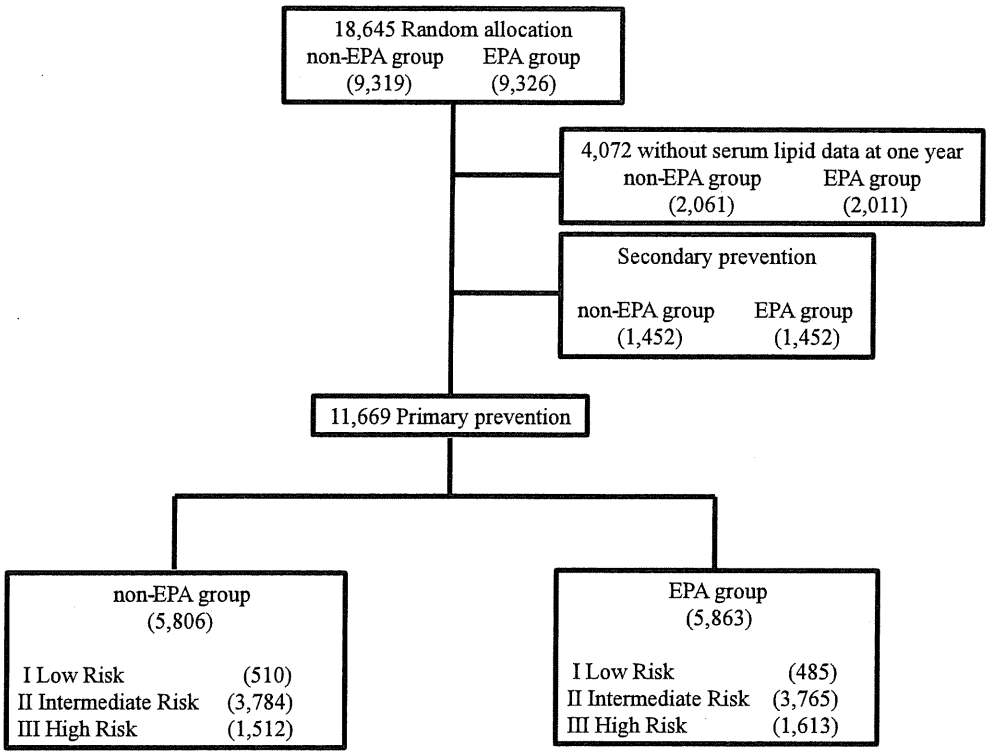

Fig. 1. Randomization and analysis set.

Categorization to Prevent CAD in Reference to the JAS Guideline (2007)[3]

Patients in the primary prevention group were categorized as low risk, intermediate risk or high risk depending on the number of CAD risk factors. Gender, aging, hypertension, impaired glucose metabolism (IGM) [including diabetes mellitus (DM)], smoking, and low HDL-C (<40 mg/dL) were defined as risk factors.

Patients with DM, a history of stroke, or with arteriosclerosis obliterans (ASO) were classified as category Ⅲ. The patients were divided into Categories I, Ⅱ, and Ⅲ according to the JAS Guideline (2007)[3] and randomization of the study population is shown in **Fig. 1**.

Lipid Management Goal Levels for LDL-C According to the JAS Guideline (2007)

The serum LDL-C concentration was calculated using the Friedewald formula. The goal levels for LDL-C in categories I, Ⅱ, and Ⅲ in the primary prevention group were <160 mg/dL, <140 mg/dL, and 120 mg/dL, respectively[3].

Lipid Management Goal levels for Non-HDL-C

The goal level for non-HDL-C was 30 mg/dL higher than that of LDL-C according to the ATP Ⅲ recommendation[12].

All subjects were distributed into the four subgroups below, based on their serum LDL-C and non HDL-C levels. We examined the incidence of CAD in these four subgroups and the effects of EPA on CAD during the follow-up period.

Subgroup A: The patients achieved the goals for both LDL-C and non-HDL-C

Subgroup B: The patients achieved the goals for LDL-C but not for non-HDL-C

Subgroup C: The patients did not achieve the goals for LDL-C but achieved those for non-HDL-C

Subgroup D: The patients did not achieve the goals for LDL-C and non-HDL-C

We investigated the incidence of CAD during the follow-up period in patients who achieved and those who did not achieve the goals for LDL-C and/or non-HDL-C after treatment for one year.

Statistical Analysis

All analyses were for intention-to-treat with the level of significance set at $p<0.05$ (2-sided). The Wilcoxon two-sample test was used to compare continuous variables. The chi-square test was used to compare class variables. The Kaplan-Meier method, log-rank

Table 1. Proportion of patients with risk factors for coronary artery disease between those who did and did not achieve the goals for LDL-C or non-HDL-C

(A) LDL-C

Goals for LDL-C	non-EPA					EPA				
	Achieved % total n=2,853		Did not achieve % total n=2,953		p value	Achieved % total n=2,975		Did not achieve % total n=2,888		p value
Age (male ≥ 45 years, female ≥ 55 years)	2,273	79.7%	2,522	85.4%	<0.001	2,431	81.7%	2,450	84.8%	0.001
Hypertension	939	32.9%	1,013	34.3%	0.26	1,039	34.9%	981	34.0%	0.44
IGM (including DM)	442	15.5%	847	28.7%	<0.001	477	16.0%	868	30.1%	<0.001
DM	251	8.8%	597	20.2%	<0.001	279	9.4%	591	20.5%	<0.001
Smoking	372	13.0%	531	18.0%	<0.001	405	13.6%	538	18.6%	<0.001
Low HDL-C (<40 mg/dL)	155	5.4%	224	7.6%	<0.001	156	5.2%	264	9.1%	<0.001
Stroke	93	3.3%	167	5.7%	<0.001	122	4.1%	191	6.6%	<0.001
ASO	11	0.4%	15	0.5%	0.48	9	0.3%	23	0.8%	0.009

(B) non-HDL-C

Goals for non-HDL-C	non-EPA					EPA				
	Achieved % total n=2,841		Did not achieve % total n=2,965		p value	Achieved % total n=3,067		Did not achieve % total n=2,796		p value
Age (male ≥ 45 years, female ≥ 55 years)	2,285	80.4%	2,510	84.7%	<0.001	2,516	82.0%	2,365	84.6%	0.009
Hypertension	934	32.9%	1,018	34.3%	0.24	1,048	34.2%	972	34.8%	0.63
IGM (including DM)	436	15.3%	853	28.8%	<0.001	489	15.9%	856	30.6%	<0.001
DM	260	9.2%	588	19.8%	<0.001	292	9.5%	578	20.7%	<0.001
Smoking	315	11.1%	588	19.8%	<0.001	380	12.4%	563	20.1%	<0.001
Low HDL-C (<40 mg/dL)	92	3.2%	287	9.7%	<0.001	103	3.4%	317	11.3%	<0.001
Stroke	94	3.3%	166	5.6%	<0.001	136	4.4%	177	6.3%	0.001
ASO	13	0.5%	13	0.4%	0.91	10	0.3%	22	0.8%	0.02

IGM, impaired glucose metabolism; DM, diabetes mellitus; HDL-C, high density lipoprotein cholesterol; ASO, arteriosclerosis obliterans.

test, and Cox proportional hazard model were used for the analysis of survival. All analyses were conducted using version 5.0.1a of the JMP statistical software program (SAS Institute, Inc., Cary, NC).

Results

At baseline, the numbers of patients with aging, hypertension, IGM (including DM), DM, smoking, low HDL-C, stroke, and ASO were 4,795 (82.6%), 1,952 (33.6%), 1,289 (22.2%), 848 (14.6%), 903 (15.6%), 379 (6.5%), 260 (4.5%), and 26 (0.4%) in the non-EPA group (n=5,806), respectively, and 4,881 (83.3%), 2,020 (34.5%), 1,345 (22.9%), 870 (14.8%), 943 (16.1%), 420 (7.2%), 313 (5.3%), and 32 (0.5%) in the EPA group (n=5,863), respectively. Only the proportion of stroke was significantly different between the two groups (p=0.03).

Table 1 shows the proportion of patients with risk factors for CAD with reference to the JAS Guideline (2007)[3] between achieved and not achieved goals for LDL-C or non-HDL-C. The proportion of patients who achieved the goals for both LDL-C and non-HDL-C in patients with aging, IGM, DM, smoking, low HDL-C, or stroke was significantly lower than those who did not achieve the goals in both groups, and in patients with ASO, the proportion was significantly lower only in the EPA group.

The distribution of patients in the non-EPA group by risk category was 8.8% (n=510) in category I, 65.2% (n=3,784) in category II, and 26.0% (n=1,512) in category III. In the EPA group, 8.3% (n=485) of patients were in category I, 64.2% (n=3,765) were in category II, and 27.5% (n=1,613) were in category III (**Fig. 1**). The proportion of patients who achieved the goals for LDL-C in the non-EPA and EPA groups was 49.1% (2,853/5,806) and 50.7% (2,975/5,863), respectively (p=0.08). The proportion of patients who achieved the goals for non-HDL-C in the non-EPA and EPA groups was 48.9%

Table 2. Incidence of CAD compared between patients who did and did not achieve goals for LDL-C

(A) non-EPA

Goals for LDL-C	Incidence of CAD (Events/n, %)				HR	(95%CI)	*p* value
	Achieved		Did not achieve				
Total	36/2,853	1.3%	74/2,953	2.5%	2.02	(1.36-3.03)	<0.001
I Low Risk	3/356	0.8%	1/154	0.6%			
II Intermediate Risk	15/2,017	0.7%	32/1,767	1.8%			
III High Risk	18/480	3.8%	41/1,032	4.0%			

(B) EPA

Goals for LDL-C	Incidence of CAD (Events/n, %)				HR	(95%CI)	*p* value
	Achieved		Did not achieve				
Total	43/2,975	1.4%	44/2,888	1.5%	1.06	(0.70-1.62)	0.78
I Low Risk	3/339	0.9%	0/146	0.0%			
II Intermediate Risk	23/2,093	1.1%	22/1,672	1.3%			
III High Risk	17/543	3.1%	22/1,070	2.1%			

CAD, coronary artery disease; HR, hazard ratio; 95%CI, 95% confidence interval.

Table 3. Incidence of CAD compared between patients who achieved and did not achieve the goals for non-HDL-C

(A) non-EPA

Goals for non HDL-C	Incidence of CAD (Events/n, %)				HR	(95%CI)	*p* value
	Achieved		Did not achieve				
Total	34/2,841	1.2%	76/2,965	2.6%	2.18	(1.46-3.30)	<0.001
I Low Risk	2/344	0.6%	2/166	1.2%			
II Intermediate Risk	17/2,036	0.8%	30/1,748	1.7%			
III High Risk	15/461	3.3%	44/1,051	4.2%			

(B) EPA

Goals for non HDL-C	Incidence of CAD (Events/n, %)				HR	(95%CI)	*p* value
	Achieved		Did not achieve				
Total	41/3,067	1.3%	46/2,796	1.6%	1.24	(0.81-1.89)	0.32
I Low Risk	3/351	0.9%	0/134	0.0%			
II Intermediate Risk	22/2,169	1.0%	23/1,596	1.4%			
III High Risk	16/547	2.9%	23/1,066	2.2%			

CAD, coronary artery disease; HR, hazard ratio; 95%CI, 95% confidence interval

(2,841/5,806) and 52.3% (3,067/5,863), respectively ($p<0.001$).

In the non-EPA group, the incidence of CAD in patients who did not achieve the goals for LDL-C was significantly higher than in patients who achieved the goals [hazard ratio (HR), 2.02; 95% confidence interval (CI), 1.36-3.03; $p<0.001$] (**Table 2A**). On the other hand, it was not higher in the EPA group (HR, 1.06; 95%CI, 0.70-1.62; $p=0.78$) (**Table 2B**). In the non-EPA group, the incidence of CAD in patients who did not achieve the goals for non-HDL-C was significantly higher than in patients who achieved those goals (HR, 2.18; 95%CI, 1.46-3.30; $p<0.001$) (**Table 3A**). On the other hand, it was not higher in the EPA group (HR, 1.24; 95%CI, 0.81-1.89; $p=0.32$) (**Table 3B**).

Table 4 shows the relationships between serum lipid levels and the incidence of CAD in the non-EPA

Table 4. Hazard ratio of the risk of CAD for 1SD increased serum lipids in the first year in the non-EPA group

	1SD change	HR	95% confidence interval	*p* value
TC	35 mg/dL	1.01	(0.82-1.24)	0.91
LDL-C	35 mg/dL	1.19	(1.00-1.42)	0.05
ln TG	0.5 ln(mg/dL)	1.08	(0.88-1.32)	0.46
HDL-C	17 mg/dL	0.60	(0.47-0.76)	<0.001
non-HDL-C	37 mg/dL	1.35	(1.11-1.66)	0.003

CAD, coronary artery disease; HR, hazard ratio; SD, standard deviation; ln TG, logarithm of TG
The given hazard ratio was for a 1SD change adjusted for gender, age, hypertension, diabetes mellitus, and smoking.

Table 5. Patients' background

	A subgroup		B subgroup			C subgroup			D subgroup		
	LDL-C Achieved	non-HDL-C Achieved	LDL-C Achieved	non-HDL-C Did not achieve	*p* value	LDL-C Did not achieve	non-HDL-C Achieved	*p* value	LDL-C Did not achieve	non-HDL-C Did not achieve	*p* value
	(*n*=5,077)		(*n*=751)			(*n*=831)			(*n*=5,010)		
Male	1,104	21.7%	294	39.1%	<0.001	163	19.6%	0.16	1,516	30.3%	<0.001
Age	60.9±8.4		59.2±8.8		<0.001	61.5±7.6		0.14	60.2±8.3		<0.001
BMI (kg/m²)	23.6±3.2		24.9±3.2		<0.001	23.5±3.1		0.47	24.4±3.2		<0.001
Smoking	595	11.7%	182	24.2%	<0.001	100	12.0%	0.80	969	19.3%	<0.001
Drinker	986	19.4%	243	32.4%	<0.001	155	18.7%	0.60	1,332	26.6%	<0.001
Clinical history											
Diabetes	422	8.3%	108	14.4%	<0.001	130	15.6%	<0.001	1,058	21.1%	<0.001
Hypertension	1,722	33.9%	256	34.1%	0.93	260	31.3%	0.13	1,734	34.6%	0.46
IGM	734	14.5%	185	24.6%	<0.001	191	23.0%	<0.001	1,524	30.4%	<0.001
Blood pressure											
Systolic (mmHg)	134.8±18.0		136.5±17.8		0.02	134.5±18.4		0.97	136.3±18.4		<0.001
Diastolic (mmHg)	79.3±10.8		80.8±10.8		0.003	78.7±11.2		0.12	80.0±10.9		0.02
Lipid profile											
Total cholesterol (mg/dL)	270.0±19.5		273.4±23.5		0.002	273.2±20.8		<0.001	281.9±28.9		<0.001
LDL cholesterol (mg/dL)	174.6±24.9		171.4±28.0		<0.001	183.9±23.4		<0.001	191.4±31.7		<0.001
HDL cholesterol (mg/dL)	64.4±18.9		51.5±13.2		<0.001	64.2±15.8		0.34	55.2±14.8		<0.001
non HDL cholesterol (mg/dL)	205.7±26.2		222.3±25.7		<0.001	208.8±23.6		0.007	226.7±31.9		<0.001
Triglycerides (mg/dL)	164.2±113.9		289.7±178.4		<0.001	124.4±56.6		<0.001	182.8±107.7		<0.001
Fatty acid profile											
C20:5 (mol%)	2.90±1.58		2.41±1.30		<0.001	3.02±1.62		0.04	2.85±1.55		0.11
C18:1/C18:0 ratio	2.83±0.56		3.28±0.61		<0.001	2.72±0.45		<0.001	3.01±0.58		<0.001
C16:1/C16:0 ratio	0.118±0.035		0.128±0.038		<0.001	0.114±0.033		0.002	0.122±0.036		<0.001

Data are reported as a percentage or the mean ± standard deviation. BMI, body mass index; IGM, impaired glucose metabolism; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; C20:5, eicosapentaenoic acid; C18:1, oleic acid; C18:0, stearic acid; C16:1, palmitoleic acid; C16:0, palmitic acid.
p value vs. A subgroup.

group. Non-HDL-C was more strongly positively associated with the incidence of CAD than LDL-C (HR, 1.35; 95%CI, 1.11-1.66; $p=0.003$ and HR, 1.19; 95%CI, 1.00-1.42; $p=0.05$, respectively). HDL-C level showed a negative correlation with CAD, but serum TC and the logarithm of the TG level did not.

Table 5 shows the baseline characteristics of

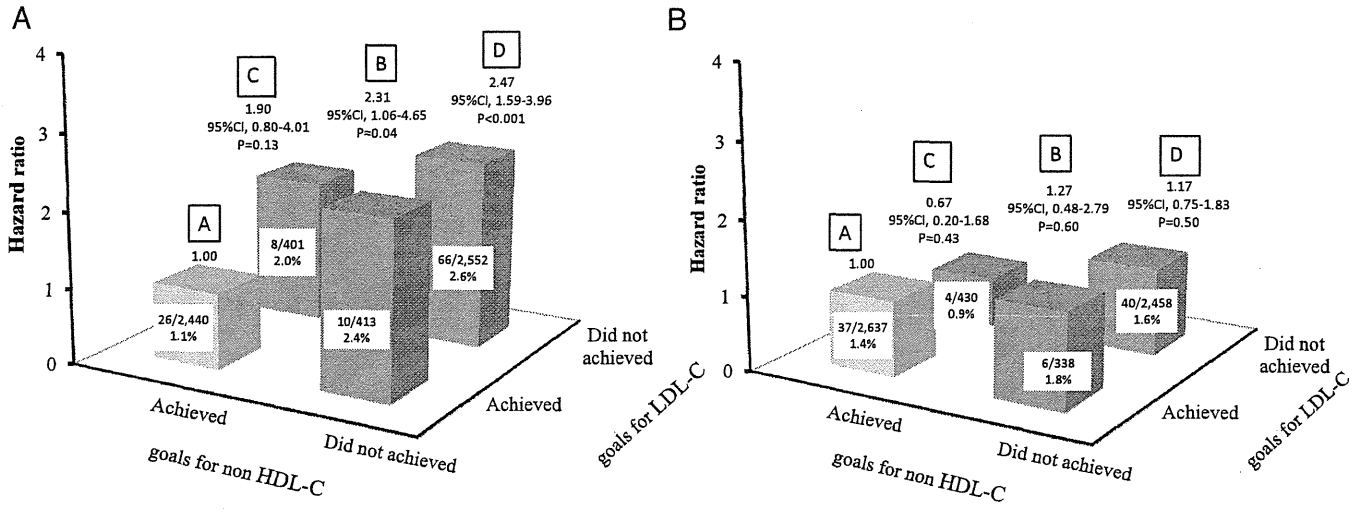

Fig. 2. Relationships between the risk of CAD and the goals for LDL-C and non-HDL-C.

(A) Non-EPA group; (B) EPA group

CAD, coronary artery disease; LDL-C, low density lipoprotein cholesterol; non-HDL-C, non-high density lipoprotein cholesterol; 95%CI, 95% confidence interval; A, achievement of goals in LDL-C and non-HDL-C groups; B, achievement of goals in LDL-C group and failure to achieve goals in the non-HDL-C group; C, failure to achieve goals in the LDL-C group and achievement of goals in the non-HDL-C group; D, failure to achieve goals in the LDL-C and non-HDL-C groups. Incidences of CAD in each selfgroups were shown in each box (events, %).

patients in the 4 subgroups. In subgroups B, C, and D, the prevalence of DM, IGM, TC, and non-HDL-C at baseline was significantly higher than in subgroup A. On the other hand, in subgroups B and D who did not achieve the goals for non-HDL-C, the proportion of men, smoking and drinking, the mean body mass index (BMI), systolic and diastolic blood pressure, TG, oleic acid (C18:1)/stearic acid (C18:0) ratio and palmitoleic acid (C16:1)/palmitic acid (C16:0) ratio at baseline were significantly higher and HDL-C at baseline was significantly lower than in subgroup A (**Table 8**).

In the non-EPA group, HRs for CAD in subgroups B and D (B subgroup, HR, 2.31; 95%CI, 1.06-4.65; $p=0.04$; D subgroup, HR, 2.47; 95%CI, 1.59-3.96; $p<0.001$) were significantly higher than in subgroup A. The HR in subgroup C was not higher than in subgroup A (C subgroup, HR, 1.90; 95%CI, 0.80-4.01; $p=0.13$) (**Fig. 2A**). In the EPA group, the HRs for CAD in patients with B, C and D subgroups were not higher than in subgroup A (B subgroup, HR, 1.27; 95%CI, 0.48-2.79; $p=0.60$; C subgroup, HR, 0.67; 95%CI, 0.20-1.68; $p=0.43$; D subgroup, HR, 1.17; 95%CI, 0.75-1.83; $p=0.50$) (**Fig. 2B**).

In patients who did not achieve the goals for LDL-C and/or non-HDL-C (subgroups B, C, and D), EPA treatment significantly suppressed the risk of CAD by 38% (HR, 0.62; 95%CI, 0.43-0.88; $p=0.007$) (**Fig. 3**).

Other than MCE, the incidence of stroke was 1.8% (61/3,366) in the non-EPA group and 1.5% (50/3,226) in the EPA group, and the all-cause mortality was 1.9% (63/3,366) in the non-EPA group and 2.0% (66/3,226) in the EPA group in patients who did not achieve the goals for LDL-C and/or non-HDL-C. There were no differences between the two treatment groups. The occurrence rate of gastrointestinal disturbance and skin abnormality in the EPA group was significantly higher than in the non-EPA group.

Discussion

In the present sub-analysis, we found that patients in the non-EPA group who did not achieve the goals for LDL-C recommended by the JAS Guideline (2007)[3] were at a significantly higher risk of developing CAD than those who achieve them. These results suggested that the goals for LDL-C were useful to reduce the risk of CAD in Japanese patients with dyslipidemia.

It is well known that hypertriglyceridemia is strongly correlated with high levels of non-HDL-C[25] and that non-HDL-C levels reflect remnant lipoprotein and small-dense LDL, which are also atherogenic. The present analysis demonstrated that non-HDL-C levels were positively associated with the risk of CAD, the same as LDL-C levels, and that patients in the

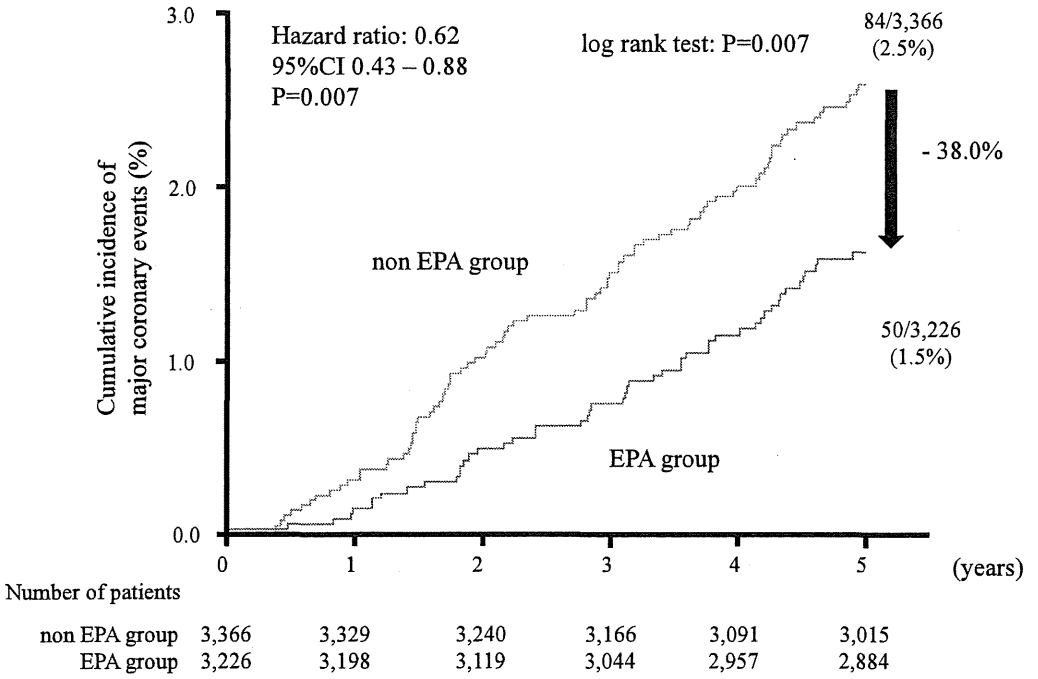

Fig. 3. Effects of EPA on CAD in patients who did not achieve the goals for LDL-C and/or non-HDL-C (B, C, and D subgroups).

CAD: coronary artery disease, LDL-C: low density lipoprotein cholesterol, non-HDL-C: non-high density lipoprotein cholesterol, 95%CI: 95% confidence interval, B: achievement of goals in the LDL-C group and failure to achieve goals in the non-HDL-C group, C: failure to achieve goals in the LDL-C group and achievement of goals in the non-HDL-C group, D: failure to achieve goals in the LDL-C group and non-HDL-C group.

non-EPA group who could not achieve the goals for non-HDL-C had a significantly higher risk of developing CAD than those who could achieve them. It might be that patients who did not achieve the lipid management goals comprised a higher proportion of the risk of CAD than those who did achieve the goals (**Table 1**). These findings suggest that non-HDL-C is one of the residual risk factors for CAD after LDL-C-lowering therapy and that the goals for non-HDL-C are useful to reduce the risk of CAD in Japanese patients with dyslipidemia. EPA treatment may be a useful strategy to reduce the risk of CAD in patients undergoing lipid-lowering therapy who do not achieve the goals for LDL-C or non-HDL-C.

The present analysis shows that patients in subgroups B, C and D of the non-EPA group were at a higher risk of developing CAD than the patients in subgroup A; however, this was not the case in the EPA group.

In subgroups B and D, the number of patients who did not achieve the goals for non-HDL-C, the proportion of patients with IGM, DM, high BMI, hypertension, and high levels of non-HDL-C and TG at baseline were significantly higher than in subgroup A, and the number of patients with a high level of HDL-C was significantly lower. Thus, these subgroups seemed to include patients with metabolic syndrome. It seems that the goals for LDL-C and non-HDL-C can serve to reduce the risk for CAD associated with metabolic syndrome. We have already reported that EPA treatment markedly reduced the risk for CAD by 53% in patients with high TG and low HDL-C, who had many features of metabolic syndrome[24], similarly to patients in subgroups B and D.

Although EPA mildly reduced the level of non-HDL-C in this study, the proportion of patients who achieve the goal levels for non-HDL-C in the EPA group was significantly higher than in the non-EPA group ($p < 0.001$). Furthermore, even in patients who did not achieve the goals for LDL-C and/or non-HDL-C, EPA treatment significantly reduced the risk of CAD. These results suggested that EPA may be a useful basic drug to prevent the risk of CAD in patients with dyslipidemia.

In patients who did not achieve the goals for non-HDL-C (subgroups B and D), the plasma EPA

levels at baseline were significantly lower and C18:1/C18:0 and C16:1/C16:0 ratios were significantly higher than in patients who achieved both goals (subgroup A). These results may reflect the decreased activity of liver stearoyl-CoA desaturase 1 (SCD-1). Suppression of SCD-1 is considered useful therapy against metabolic syndrome[27, 28] and insulin resistance[29, 30]. It is possible that EPA suppressed liver lipogenesis associated with metabolic syndrome. Recently, Sato *et al.* reported that in mice given a high-fat/high-sucrose diet, EPA suppressed sterol regulatory element binding protein-1, fatty acid synthase, and SCD-1 in the liver[31], indicating that EPA was appropriate for the treatment of metabolic syndrome as it suppressed hepatic lipogenesis and steatosis. Further clinical trials are needed to investigate the relationship between EPA treatment and the development of metabolic syndrome.

In addition, the results may reflect the anti-arteriosclerosis effects of EPA, such as anti- platelet aggregation[32, 33], plaque stabilization[34, 35], anti-inflammation[31, 36-39] nitric oxide production[40, 41], small-dense LDL[38] and remnant-like particle cholesterol[42, 43] -lowering effects.

Conclusion

This analysis indicates that non-HDL-C is a predictor of the risk of CAD and that a high non-HDL-C level is one of the residual risk factors for CAD after LDL-C-lowering therapy. EPA significantly reduced the risk of CAD in patients who did not achieve the goals for LDL-C and/or non-HDL-C. Consequently, EPA may be a useful basic drug to reduce the risk of CAD in patients who resist LDL-C or non-HDL-C lowering.

Limitations

We planned to emulate an evaluation in the real world of medical care, so we did not use a placebo in the non-EPA group, and for ethical reasons we adopted the additional design parameter of treating hypercholesterolemia in all patients with statin administration.

Acknowledgements

This study was supported by grants from Mochida Pharmaceutical Co. Ltd, Tokyo, Japan. We thank all trial participants and the large numbers of physicians, nurses, and hospital staff who made long-term commitments to the study.

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Hindawi Publishing Corporation
International Journal of Vascular Medicine
Volume 2012, Article ID 657025, 6 pages
doi:10.1155/2012/657025

Review Article

Pentraxin 3: A Novel Biomarker for Inflammatory Cardiovascular Disease

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Received 1 July 2011; Revised 1 October 2011; Accepted 4 October 2011

Academic Editor: Rajeev Gupta

Numerous studies have recently examined the role of pentraxin 3 (PTX3) in clinical situations. The pentraxin family includes C-reactive protein (CRP); however, unlike CRP, PTX3 is expressed predominantly in atherosclerotic lesions that involve macrophages, neutrophils, dendritic cells, or smooth muscle cells. Interestingly, PTX3 gene expression in human endothelial cells is suppressed to a greater extent by pitavastatin than the expression of 6,000 other human genes that have been examined, suggesting that PTX3 may be a novel biomarker for inflammatory cardiovascular disease. The expression and involvement of PTX3 in cardiovascular diseases are discussed in this paper, along with the characteristics of PTX3 that make it a suitable biomarker; namely, that the physiological concentration is known and it is independent of other risk factors. The results discussed in this paper suggest that further investigations into the potential novel use of PTX3 as a biomarker for inflammatory cardiovascular disease should be undertaken.

1. Introduction

Biomarkers are measurable and quantifiable biological parameters that can have an important impact on clinical situations. Ideal biomarkers are those that are associated with disease clinical endpoints in observational studies and clinical trials, and in some cases, they may even be used as surrogate endpoints. Biomarkers must also be both independent of established risk factors and recognized to be a factor in the disease for which they are a marker. The normal physiological expression of a potential biomarker must also be known in order to interpret results, as well as to generalize results to various population groups. Finally, potential biomarkers must also have the ability to improve overall prediction beyond that of traditional risk factors, while assays to detect them must have an acceptable cost and be subject to standardization in order to control for the variability of measurements [1].

Basic research over the past decades has identified numerous candidate genes and proteins as biomarkers for cardiovascular disease. In the cardiovascular field, such biomarkers are useful not only for diagnosis but also as indicators of disease trait (risk factor or risk marker), disease state (preclinical or clinical), or disease rate (progression or prognosis) [2]. One protein that has the potential to be a viable biomarker for inflammatory vascular disease is pentraxin 3 (PTX3).

2. Pentraxin 3

PTX3 is an evolutionarily conserved, multimeric acute phase inflammatory glycoprotein in the same family as the well-established cardiovascular biomarker C-reactive protein (CRP) [3, 4]. PTX3 also shares 98% identity with tumor necrosis factor- (TNF-) stimulated gene 14 (TSG14) [5, 6]. PTX3 has been successfully identified by Breviario et al. using differential screening of a cDNA library from human umbilical vein endothelial cells (HUVECs) stimulated by interleukin-1 beta [5], as well as by Gustin et al. using the 2D-DIGE approach to detect PTX3 in HUVECs stimulated by lysophospholipids [7]. Our group also identified PTX3 when we were investigating statin as a target gene in HUVECs

incubated with pitavastatin for 24 hours prior to RNA extraction [8]. Interestingly, chip analysis has demonstrated that, of the 6,000 human genes that have been investigated for response to pitavastatin treatment, PTX3 gene expression is suppressed in human endothelial cells to the greatest extent.

PTX3 synthesis is stimulated in endothelial cells, macrophages, myeloid cells, and dendritic cells by cytokines and endotoxins such as bacterial products, interleukin-1, and TNF [9–11]. The role of PTX3 in neutrophils has also been gradually elucidated by a number of studies. Once synthesized, PTX3 is predominantly organized into covalent octamers through disulfide bonds [12]. Although PTX3 is mainly localized in lactoferrin positive-specific granules [13, 14], it is translocated to the surface of late apoptotic neutrophils upon stimulation, where it accumulates in blebs and is rapidly released. PTX3 then binds with the high-affinity complement component C1q to initiate the classical pathway of complement activation and facilitate pathogen recognition by macrophages.

3. Suitability of PTX3 as a Biomarker

3.1. PTX3 Expression in Cardiovascular Diseases

3.1.1. Acute Coronary Syndrome (ACS). The expression of PTX3 has been found to be increased in patients with acute myocardial infarction (AMI). For instance, Peri et al. observed that patients (n = 37) with AMI who were admitted to the coronary care unit within 3.2 ± 3.2 hours of the onset of symptoms had increased plasma PTX3 over time [15]. In this study, plasma PTX3 levels were found to peak at a median of 7.5 hours after AMI, and to return to normal levels after 3 days. Similarly, in murine models of AMI, PTX3 mRNA is expressed within 4 hours of the ligation of the coronary artery, reaches peak levels after 24 hours, and returns to normal levels 3 days later [16]. We have also found that plasma PTX3 levels are increased in patients (n = 16) with unstable angina pectoris (UAP; 6.20 ng/mL) [17]. Such findings have led to the investigation of PTX3 expression levels as a potential prognostic indicator of disease. Matsui et al. found that the expression of more than 3.1 ng/mL of PTX3 in patients with UAP/non-ST-elevation MI (n = 204) was predictive of the occurrence of a 6-month cardiac event, including cardiac death, rehospitalization for ACS, and rehospitalization for worsening heart failure [18], while Latini et al. have shown that the expression of more than 10.73 ng/mL of PTX3 predicted 3-month mortality in patients with AMI (n = 724) [19].

3.1.2. Congestive Heart Failure. PTX3 has also been implicated as a predictor of adverse clinical outcomes in patients with heart failure (n = 196) in a study with a median follow-up period of 655 days and an ejection fraction of less than 50% [20]. In a further study by Matsubara et al. that focused on patients with heart failure with normal ejection fraction (HFNEF), plasma PTX3 levels were also found to be increased (3.26 (2.36–4.35) ng/mL). This was observed even in patients with HFNEF, although B-type natriuretic peptide (BNP) was within normal limits [21].

3.1.3. Sleep Apnea Syndrome. Plasma PTX3 levels have also been suggested to be a good marker for the response to treatment of patients with obstructive sloop apnea (OSA). Kasai et al. demonstrated that not only did patients with OSA (n = 50) express higher levels of plasma PTX3 than individuals in an age- and body mass index-matched control group, but also that continuous positive airway pressure (CPAP) therapy led to a significant reduction in plasma PTX3 levels. While high sensitive CRP has previously been suggested to be a highly sensitive candidate biomarker that can reflect the status of patients with OSA, the findings of this study led the authors to conclude that plasma PTX3 levels seem to be a more suitable biomarker to monitor treatment effects in patients with OSA [22].

3.1.4. Heart Valvular Disease. In a study by Naito et al. that investigated PTX3 expression patterns in patients with aortic valve stenosis (AS) or regurgitation (AR), it was found that the expression of plasma PTX3 was significantly increased in patients with AS. Furthermore, PTX3 was found to be expressed predominantly in macrophage cells in the aortic valves of these patients [23].

3.2. PTX3 Involvement in Cardiovascular Diseases. Several studies have examined why plasma PTX3 levels are increased in patients with cardiovascular disease, and those that have targeted the PTX3 gene in mice suggest that plasma PTX3 levels may increase in order to confer protection against cardiac tissue damage [16, 24]. For instance, in a model of AMI caused by coronary artery ligation, PTX3-knockout mice showed exacerbated heart damage with a greater no-reflow area and increased inflammatory response, including increased neutrophil infiltration, a decreased number of capillaries, and an increased number of apoptotic cardiomyocytes [16]. This phenotype was reversed by the expression of exogenous PTX3.

PTX3 expression has also been examined using double knockout mice in which PTX3 and apolipoprotein E have been targeted. When gene expression in the aortic arches of these mice was analyzed using gene chip, it was found that several transcription factors involved in intracellular proinflammatory signaling, such as nuclear factor-kappa B and the related proteins Irak1, Fos, Jun, GATA3, GATA4, Egr2, and Egr3, were upregulated after the mice had been fed an atherogenic diet for 16 weeks. The mRNA expression levels of intracellular adhesion molecule, vascular cell adhesion molecule-1, endothelial leukocyte adhesion molecule-1, and platelet/endothelial cell adhesion molecule were also found to be increased in the vascular wall of double knockout mice when compared to those of wild-type mice [24]. Furthermore, the lack of PTX3 in a proatherogenic background may be associated with an increased inflammatory status in the vascular wall, which in turn contributes to the atherogenic process. In contrast, the transgenic overexpression of PTX3 has been found to result in greater resistance to lipopolysaccharide toxicity and cecal ligation and puncture [26]. There is also evidence that PTX3 may modulate inflammation-associated tissue damage.