Original article

Administration of eicosapentaenoic acid may alter high-density lipoprotein heterogeneity in statin-treated patients with stable coronary artery disease: A 6-month randomized trial

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ABSTRACT

Background: Combined statin plus eicosapentaenoic acid (EPA) therapy might be a potentially effective treatment option to prevent coronary artery disease (CAD). The serum EPA/arachidonic acid (AA) ratio has been identified as a potential new risk marker for CAD. Few data exist whether administration of EPA could affect high-density lipoprotein (HDL) particle size. We hypothesized that the addition of EPA to ongoing statin therapy may result in altered HDL heterogeneity.

Methods: We conducted this 6-month, single-center, prospective, randomized open-label clinical trial to investigate the effect of the additional administration of EPA on the HDL heterogeneity (HDL2, HDL3, and HDL2/HDL3 ratio) in stable CAD patients receiving treatment with statins. We assigned stable CAD patients already receiving statin therapy to the EPA group (1800 mg/day: n = 50) or the control group (n = 50).

Results: A significant decrease in the serum HDL3 level (−4.7% vs. −0.5%, p = 0.037), but not of the serum HDL2 level, and a significant increase in the HDL2/HDL3 ratio (5.5% vs. −5.1%, p = 0.032) were observed in the EPA group as compared to the control group. Multiple regression analysis with adjustments for coronary risk factors identified the achieved EPA/AA ratio as an independent and significant predictor of an increase of the HDL2/HDL3 ratio (β = 0.295, p = 0.001). Furthermore, the change in the serum cholesterol ester transfer protein mass was positively correlated with the change in the EPA/AA ratio in the EPA group (r = 0.286, p = 0.044), but not in the control group (r = 0.121, p = 0.401).

Conclusion: Administration of EPA might decrease the serum HDL3 level, resulting in an increase in the HDL2/HDL3 ratio. Furthermore, increased EPA/AA ratio by the addition of EPA to ongoing statin therapy might be an indicator of an increase in the HDL2/HDL3 ratio, thereby regulating HDL particle size.

Clinical Trial Registration: UMIN (http://www.umin.ac.jp/) Study ID: UMIN000010452

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Introduction

Combined statin plus eicosapentaenoic acid (EPA) therapy may be a potentially effective treatment option to prevent atherosclerotic cardiovascular disease (ASCVD) [1,2]. Interventions targeting the serum EPA/arachidonic acid (AA) ratio, particularly those involving an increase in EPA/AA ratio, could be useful for the prevention of ASCVD [3]. Accordingly, it has been suggested in recent years, that the EPA/AA ratio may be useful for ASCVD risk stratification [4,5].

The major antiatherosclerotic effect of high-density lipoprotein cholesterol (HDL-C) is mediated via the reverse cholesterol transport system (RCT). Small particle HDL3, a subclass of HDL, takes up cholesterol from atherosclerotic plaques and converts it to large-particle HDL2, which contains a significant amount of lipid components (cholesterol efflux) [6]. However, there has been no unified view regarding how HDL particle size is related to the suppression of atherosclerosis [7].

A recent study in vitro demonstrated an HDL efflux capacity-activating effect of EPA [8]. Furthermore, it has been reported, primarily from basic research, that EPA, a principal constituent of n-3 polyunsaturated fatty acids (n-3 PUFAs) contained in abundance in edible fishes, has the potential to activate the RCT system [9].
We hypothesized that the regulation of HDL heterogeneity is involved in the suppression of CAD by EPA therapy as follows: the HDL2/HDL3 ratio increases as a result of the absolute reduction of the HDL3 level, resulting in HDL3 conversion to HDL2. Furthermore, there is a possibility that the increase in the HDL2/HDL3 ratio observed after administration of EPA may be correlated with increased EPA/AA ratio.

The aim of this study was to investigate the effect of the addition of EPA to ongoing statin therapy in regulating HDL heterogeneity in CAD patients, in addition to determining whether increased EPA/AA ratio might be a useful indicator of altered HDL heterogeneity.

Methods

Study design and populations

We conducted this 6-month, single-center, prospective, randomised open-label clinical trial to investigate the effect of the additional administration of EPA on the HDL heterogeneity in stable CAD patients receiving treatment with statins. The study was performed at Surugadai Nihon University Hospital between May 1, 2013, and June 30, 2014. The primary efficacy parameter was the changes in the HDL heterogeneity (HDL2, HDL3, and HDL2/HDL3 ratio). The secondary endpoints were to investigate the relationship between HDL2/HDL3 ratio and the EPA/AA ratio in statin-treated patients receiving EPA therapy.

A total of 110 patients participated in the study, all of whom provided consent. The patients were randomized using the simple sealed-envelope method into either an EPA group (n = 55) or a non-EPA control group (n = 55). Subjects in the EPA group received a 900-mg capsule containing EPA ethyl ester of >98% purity twice a day (total daily dose, 1800 mg) (Mochida Pharmaceuticals, Tokyo, Japan). During the 6 months in which the study was conducted, no restrictions were placed on concomitantly used drugs. All patients received dietary counseling according to the American College of Cardiology/American Heart Association guidelines [10].

All subjects were patients undergoing coronary angiography for the evaluation of CAD. The decision to perform coronary angiography was made by the cardiologist in charge, based on the results of non-invasive clinical examinations. Patients who presented acutely with ST-elevation myocardial infarction, non-ST-elevation myocardial infarction, or unstable angina were excluded presented acutely with ST-elevation myocardial infarction, or unstable angina were excluded. All the patients who presented acutely with ST-elevation myocardial infarction, non-ST-elevation myocardial infarction, or unstable angina were excluded.

In addition, we used the following exclusion criteria: a bleeding tendency, hepatic or renal dysfunction (creatinine ≥1.5 mg/dL, alanine aminotransferase and aspartate aminotransferase ≥2 times the upper limit of the normal values), known malignant disease, inflammatory disease, or current treatment with n-3PUFAs, and patients with uncontrolled triglyceride levels (≥400 mg/dL).

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Approval of the study protocol was obtained by the Institutional Review Boards of our institutions (approval number: 20121202), and all participants provided written informed consent. Each subject was verbally confirmed at a clinic interview and/or by checking the number of unused doses of the prescribed drugs at each subject’s bimonthly visit. Furthermore, all of the patients included in this study were followed up at 2-month intervals and exhibited an increase of the serum EPA concentrations following administration of EPA, with no appreciable changes of this parameter thereafter during the study period. Therefore, we believe that there were no

Measurement of laboratory parameters

Fasting blood samples were collected in the early morning after the subjects had fasted for 12 h. The serum PUFAs composition was measured by capillary gas chromatography (SRL Inc., Tokyo, Japan). The HDL2 and HDL3 levels were determined via ultracentrifugation (SRL Inc.) [11]. The serum cholesterol ester transfer protein (CEPT) level was measured by an enzyme-linked immunosorbent assay kit (SEKISUI MEDICAL Co., LTD., Tokyo, Japan) (SRL Inc.). The serum total cholesterol (TC) level was measured by an enzymatic method, the triglyceride (TG) level by an enzyme-free glycerol eliminated method, and the serum HDL-C level by a direct HDL-C method in the central clinical laboratory of our institute. The serum low-density lipoprotein cholesterol (LDL-C) level was estimated using the Friedewald formula [12].

Statistical analysis

We performed all of the statistical analyses using the SPSS Window ver 24.0 software program (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables. Comparison of continuous variables between the groups was conducted by Student’s t-test, and the categoric variables were analyzed by the χ² test. For cases where the data did not show normal distribution, the data were expressed as medians (interquartile: IQR). Mann-Whitney’s U test was used to evaluate the differences in the data between the groups, and Wilcoxon’s signed-rank test was used to analyze the differences in the data within the same group. Regression analysis was performed using linear regression and Pearson’s correlation coefficients. A univariate and multivariate regression analysis were performed to identify the predictors associated with the serum HDL2/HDL3 ratio at 6-month follow-up. All of the variables that were found to be correlated with the serum HDL2/HDL3 ratio at 6-month follow-up at p < 0.05 in the univariate regression analysis were entered into the multivariate model. In this study, we created the following three univariate and multivariate analysis models using the absolute change in EPA/AA ratio (Δ EPA/AA ratio) from the baseline and the EPA/AA ratio achieved following EPA therapy, which was demonstrated as a marker of the coronary artery event-suppressive effect in Japan EPA Lipid Intervention Study (JELIS) [1,3], as independent variables: the 6-month EPA/AA ratio model (model 1), the Δ EPA/AA ratio model (model 2), and a model evaluating both variables in conjunction to ascertain the superior variable (model 3). Values of p less than 0.05 were considered to denote statistical significance.

Results

Patients

In the EPA group, two patients were excluded due to gastrointestinal disorder, and three patients were excluded due to lack of 6-month follow-up data. In the control group, pertinent baseline data were not available for one patient, and four patients lacked 6-month follow-up data. After the exclusion of the above-mentioned patients, data for 100 patients in each of the two groups were subjected to this analysis.

There were no significant differences between the EPA and control groups, and the two groups were well-matched in terms of baseline characteristics (Table 1). There were no changes in diet or other medications used during the study period.

Compliance with the medication used in the study was confirmed at a clinic interview and/or by checking the number of unused doses of the prescribed drugs at each subject’s bimonthly visit. Furthermore, all of the patients included in this study were followed up at 2-month intervals and exhibited an increase of the serum EPA concentrations following administration of EPA, with no appreciable changes of this parameter thereafter during the study period. Therefore, we believe that there were no
Table 1
Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>All Cases (n = 100)</th>
<th>Control group (n = 50)</th>
<th>EPA group (n = 50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n (%)</td>
<td>88 (88)/12 (12)</td>
<td>42 (84)/16 (16)</td>
<td>46 (92)/4 (8)</td>
<td>0.218</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.4 ± 10.6</td>
<td>67.3 ± 10.4</td>
<td>67.5 ± 10.1</td>
<td>0.925</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 3.6</td>
<td>24.8 ± 4.0</td>
<td>24.6 ± 3.2</td>
<td>0.702</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>88 (88)</td>
<td>43 (86)</td>
<td>45 (90)</td>
<td>0.538</td>
</tr>
<tr>
<td>Diabetes Mellitus, n (%)</td>
<td>40 (40)</td>
<td>22 (44)</td>
<td>18 (36)</td>
<td>0.414</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.7 (5.4/6.0)</td>
<td>5.7 (5.4/6.0)</td>
<td>5.7 (5.4/6.1)</td>
<td>0.73</td>
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<tr>
<td>Cigarette smoking, n (%)</td>
<td>8 (8)</td>
<td>5 (10)</td>
<td>3 (6)</td>
<td>0.461</td>
</tr>
<tr>
<td>Hyperuricemia, n (%)</td>
<td>36 (36)</td>
<td>14 (28)</td>
<td>12 (24)</td>
<td>0.692</td>
</tr>
<tr>
<td>CKD, n (%)</td>
<td>36 (36)</td>
<td>19 (38)</td>
<td>17 (34)</td>
<td>0.732</td>
</tr>
</tbody>
</table>

Concomitant drugs n (%)

- Antiplatelets 95 (95) 46 (92) 49 (98) 0.169
- ACEIs/ARBs 55 (55) 26 (52) 29 (58) 0.547
- β blockers 41 (41) 19 (38) 22 (44) 0.542
- Calcium channel blockers 62 (62) 31 (62) >0.999
- Statins 100 (100) 50 (100) 50 (100) >0.999

EPA, eicosapentaenoic acid; BMI, body mass index; Hb, hemoglobin; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2
Laboratory profiles at baseline and 6-month follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6-month follow-up</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n = 50)</td>
<td>EPA group (n = 50)</td>
<td></td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>172 ± 21</td>
<td>167 ± 25</td>
<td>0.262</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>94 ± 18</td>
<td>91 ± 18</td>
<td>0.435</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>114 (90/151)</td>
<td>100 (74/144)</td>
<td>0.100</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>52 ± 15</td>
<td>53 ± 11</td>
<td>0.641</td>
</tr>
<tr>
<td>HDL₂ (mg/dL)</td>
<td>39.0 ± 15.1</td>
<td>41.0 ± 12.3</td>
<td>0.477</td>
</tr>
<tr>
<td>HDL₃ (mg/dL)</td>
<td>20.0 ± 3.5</td>
<td>20.6 ± 3.6</td>
<td>0.343</td>
</tr>
<tr>
<td>HDL₂/HDL₃ ratio</td>
<td>1.966 ± 0.702</td>
<td>2.052 ± 0.776</td>
<td>0.562</td>
</tr>
<tr>
<td>CETP (µg/mL)</td>
<td>1.928 ± 0.383</td>
<td>1.920 ± 0.426</td>
<td>0.922</td>
</tr>
<tr>
<td>EPA (µg/mL)</td>
<td>70 ± 40</td>
<td>71 ± 45</td>
<td>0.909</td>
</tr>
<tr>
<td>AA (µg/mL)</td>
<td>175 ± 47</td>
<td>182 ± 54</td>
<td>0.549</td>
</tr>
<tr>
<td>EPA/AA ratio</td>
<td>0.330 (0.240/0.520)</td>
<td>0.360 (0.200/0.570)</td>
<td>0.914</td>
</tr>
</tbody>
</table>

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; CETP, cholesteryl ester transfer protein; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Fig. 1. Changes in HDL Heterogeneity following EPA Therapy.
HDL, high-density lipoprotein; EPA, eicosapentaenoic acid; SD, standard deviation; IQR, interquartile.
problems in respect of the subjects' adherence to the EPA treatment.

Changes in laboratory profile

No significant differences in the baseline laboratory parameters were observed between the groups. At the 6-month follow-up, the serum levels of TC (p = 0.004), LDL-C (p = 0.028), and TG (p = 0.004) were significantly lower in the EPA group as compared to the control group. There were no significant differences in the serum HDL-C, HDL2, and CETP levels between the groups. Although the HDL2/HDL3 ratio in the EPA group was higher than that in the control group, the difference did not reach statistical significance (p = 0.058). A significantly higher EPA (p < 0.0001) level and EPA/AA ratio (p < 0.0001) were noted in the EPA group as compared to the control group (Table 2). Although there was no significant difference in the degree of change of the serum HDL-C level between the two groups [control group vs. EPA group: −3.4% (−11.8/4.7) vs. −6.0% (−10.4/2.3), p = 0.603], a significant reduction of the serum HDL level (p = 0.037) but not of the serum HDL2 level (p = 0.587), was found in the EPA group as compared to the control group. The percentage change of the HDL2/HDL3 ratio between the groups was found to be statistically significant (p = 0.032) (Fig. 1). No significant difference in the degree of change in the CETP level was observed between the two groups (control group vs. EPA group: 2.2 ± 11.0% vs. 2.4 ± 14.5%, p = 0.923).

Univariate and multivariate regression analyses to identify variables that were independently correlated with the serum HDL2/HDL3 ratio at a 6-month follow-up

At the 6-month follow-up, cigarette smoking was significantly associated with a low serum HDL2/HDL3 ratio. In addition, body mass index was negatively correlated with the serum HDL2/HDL3 ratio. One month EPA/AA ratio was noted in the EPA group, but not in the control group (Fig. 3).

Correlation between change in serum CETP level and change in EPA/AA ratio

No significant difference was seen in the change in serum CETP level between the groups at the 6-month follow-up (Table 2). However, a positive correlation between serum Δ CETP level and Δ EPA/AA ratio was noted in the EPA group, but not in the control group (Fig. 3).

Discussion

The present study yielded the following results: in patients with stable CAD who have already been receiving statins and show a relatively favorable lipid profile, additional EPA therapy brought about a decrease in the serum HDL3 level and increase in HDL2/HDL3 ratio. Furthermore, the results suggest that the achieved EPA/AA ratio, and a better predictor than the absolute change in EPA/AA ratio, observed following administration of EPA may be an independent predictor of elevation of the HDL2/HDL3 ratio.

That is, according to the results of this study, administration of EPA and the concomitant increase in EPA/AA ratio may affect certain processes of the RCT system including changes in HDL particle size, which is the significant anti-atherogenic action of HDL described below; Lecithin cholesterol acyltransferase (LCAT), which catalyzes conversion of free cholesterol to cholesteryl ester,

## Table 3

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>0.338</td>
<td>0.001</td>
</tr>
<tr>
<td>Male gender</td>
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<td>0.200</td>
</tr>
<tr>
<td>Body mass index</td>
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<tr>
<td>Hypertension</td>
<td>0.001</td>
<td>0.990</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>−0.107</td>
<td>0.238</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>−0.210</td>
<td>0.036</td>
</tr>
<tr>
<td>ΔHDL2−C</td>
<td>−0.227</td>
<td>0.023</td>
</tr>
<tr>
<td>ΔHDL3−C</td>
<td>−0.069</td>
<td>0.492</td>
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<tr>
<td>ΔTG</td>
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<tr>
<td>ΔCETP</td>
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<tr>
<td>6 month EPA/AA ratio</td>
<td>0.406</td>
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</tbody>
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## Table 4

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<td>&lt;0.0001</td>
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</table>

The abbreviations are the same as in Table 2; r, correlation coefficient; β, standard partial regression coefficient; Δ, absolute change from baseline; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; CETP, cholesteryl ester transfer protein; EPA, eicosapentaenoic acid; AA, arachidonic acid.
acts intravascularly to induce maturation of HDL particles from pre-βHDL to HDL₃ and then to larger HDL₂. These actions result in irreversible removal of cholesterol from peripheral tissues and the arterial wall. Thereafter, mature HDL₂ particles may transport their cholesterol either directly to the liver or indirectly by transfer of cholesteryl esters via CETP to very-low-density lipoprotein and LDL, which are taken up by hepatic LDL receptors [6]. Although the effect of EPA on HDL metabolism is still unclear, the results of this study suggest that the change in HDL particle heterogeneity is one of the mechanisms underlying the suppression of ASCVD.

The present study results may speculate, at least in part, the dynamic aspects of HDL metabolism. It may be inferred that the decrease in serum HDL₃ level, together with elevation of the serum HDL₂/HDL₃ ratio, occurs as a result of the cholesterol efflux promoting capacity of HDL₃ in response to administration of EPA. This would suggest that small HDL₃ particles that are low in lipid content are converted to large HDL particles that are high in lipid content. The present results may suggest that the dynamic changes of HDL are more relevant to RCT than absolute increase of the serum HDL-C level.

In view of the inverse correlation between serum HDL-C level and CAD morbidity based on a number of epidemiological studies [13], the “HDL-C hypothesis” that an increase in serum HDL-C level results in a decreased risk of CAD has long been supported [14,15]. However, consecutive large-scale clinical studies examining the relation of serum HDL-C level to the reduced occurrence of CAD have offered results disproving this hypothesis [16]. Previously, it has been shown that the more pronounced the HDL cholesterol efflux capacity, the less conspicuous the carotid intima-media thickness [17], and the less frequent the occurrence of CAD events [18]. It is now recognized that for the prevention of atherosclerotic cardiovascular disease, the main concern is not so much to increase...
the serum HDL-C level as it is to improve the HDL cholesterol efflux capacity as a function of HDL. In the EPA group, the change in the EPA/AA ratio was noted to be positively correlated with the change in serum level of CETP (Fig. 2), which serves to transport cholesteryl esters obtained by esterification, via lecithin–cholesterol acyltransferase stored in HDL, of free cholesteryl taken up into HDL via cholesterol efflux to very-low density lipoprotein, intermediate-density lipoprotein, and LDL [6]. This finding may imply possible involvement of smoothening of the RCT consequent upon elevation of the EPA/AA ratio.

Although there is as yet no unified opinion as to the relationship between the serum CETP level and occurrence of CAD, even from the results of large-scale epidemiologic investigations [19–22], this finding may be considered to provide evidence in support of the antiatherosclerotic effect of CETP through increased EPA/AA ratio following administration of EPA. In the EPA group, which showed a positive correlation between the Δ EPA/AA ratio and Δ CETP level, however, no significant increase of the CETP level was noted in about half the patients, and furthermore, increase of the CETP level was observed in nearly half of the patients of the control group. There is no denying that some other factor(s) may be involved in the causal relationship of the EPA/AA ratio with the CETP level.

Furthermore, there is no unified view at present among reports dealing with changes in the CETP following administration of EPA [23–27]. As for the activity of RCT itself, it has been suggested that this parameter is dependent on the study design and also on the lipid profile and degree of progression of atherosclerosis in individual patients [28–30]. The present study population was also not homogeneous in terms of the background characteristics, so that the effect of CETP could vary among individuals. However, the present results demonstrating a positive correlation between the Δ EPA/AA ratio and Δ CETP level would suggest that EPA has a bearing, in some way or the other, upon RCT, which is of profound interest. Further investigation is needed.

It has been reported by Moriyama et al. from their cross-sectional as well as longitudinal studies, that the HDL2/HDL3 ratio shows an inverse correlation with the number of risk factors for metabolic syndrome, as well as with homeostatic model assessment for insulin resistance [31]. This would suggest that elevation of the HDL2/HDL3 ratio has a bearing upon the suppressive effect of EPA on the progression of atherosclerosis, and may also support our contention that increase of the EPA/AA ratio, a target for the treatment of CAD, acts via increasing the HDL2/HDL3 ratio.

Superko et al. indicated in their review that it is important to secure a post-EPA therapy EPA/AA ratio above a certain level, regardless of the baseline EPA/AA ratio, in order for EPA therapy to have an inhibitory effect on cardiac events [32]. The present study results also show that, as a predictor of the increase in the serum HDL2/HDL3 ratio, post-EPA therapy EPA/AA ratio is superior to the amount of change in the serum EPA/AA level. These facts as well as the reported suppression of cardiac events in patients with a post-EPA therapy EPA/AA ratio of >0.75 [2] support the results of the present study.

Although it might appear inappropriate for us to refer unitarily to disparities in respective study results regarding n-3 PUFAs and RCT systems due to the differences in research procedures, it seems reasonable to assume that n-3 PUFAs play some role in improving HDL cholesterol efflux capacity. According to a study by Mori et al., EPA is endowed with an HDL3-lowering effect when administered alone and docosahexaenoic acid has an HDL2-elevating effect when administered alone. It would thus be reasonable to assume that the RCT system-facilitating effect of n-3 PUFAs, as a whole, may become enhanced [33]. We believe that the results of the present study will provide some foundation for the further clarification of the mechanism involved in the cardiovascular event-suppressing effect produced by EPA dosage sufficient to reach an effective EPA/AA ratio [3,32].

### Study limitations and clinical implication

First, this study did not incorporate analysis of changes in the properties of the coronary plaques by means of diagnostic imaging test [34]. Secondly, because this study was not designed to evaluate the drug effect of different class statins, we can make no conclusions about the effects of statin classes on HDL heterogeneity on the basis of the data from this study. Secondly, alteration of the HDL heterogeneity is no more than a single function of HDL that was evaluated. We would also have, in the future, to evaluate the effect of EPA therapy on the HDL cholesterol efflux capacity, which is a central function of HDL. Finally, this study does not demonstrate the relationship between the HDL2/HDL3 ratio, serum EPA/AA ratio, and clinical indices or/and outcomes. Besides HDL2 and HDL3, more detailed classification of HDL by particle size into subclasses may be feasible depending on the method of measurement employed [35,36]. It may be possible to use more refined data for a more in-depth investigation of the changes in HDL heterogeneity and the mechanism of activation of the RCT system in subjects receiving EPA.

### Conclusions

The addition of EPA to ongoing statin therapy significantly increased the HDL2/HDL3 ratio with a corresponding increase in EPA/AA ratio. Furthermore, increased EPA/AA ratio may be possibly associated with facilitating of altering HDL heterogeneity. Combination therapy involving statins and EPA may have the potential to be a viable treatment option in the prevention of CAD, even in stable CAD patients whose serum lipid levels are well controlled.

### Competing interests

The author declares that he/she has no competing interests.

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