Effects of Eicosapentaenoic Acid on the Levels of Inflammatory Markers, Cardiac Function and Long-Term Prognosis in Chronic Heart Failure Patients with Dyslipidemia

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Aims: The effects of eicosapentaenoic acid (EPA) on the levels of inflammatory markers, cardiac function and long-term prognosis in chronic heart failure (CHF) patients with dyslipidemia remain unclear.

Methods: A total of 139 CHF patients with a mean left ventricular ejection fraction (LVEF) of 37.6±8.0% were divided into two groups based on whether EPA was included in their treatment regimen: the EPA group (n=71) and the no EPA group (n=68). Only patients with dyslipidemia at baseline (entry) were treated with EPA. The monocyte chemoattractant protein (MCP)-1 and asymmetric dimethylarginine (ADMA) levels were measured at baseline and after 12 months of treatment.

Results: At 12 months, in the EPA group, the LVEF had improved and the MCP-1 and ADMA levels had decreased (respectively, p<0.001); however, in the no EPA group, the LVEF had worsened, while the MCP-1 and ADMA levels had increased (respectively, p<0.001). Fifty-five patients experienced cardiac events, including 15 cardiac deaths and 40 readmissions for worsening of CHF during a median follow-up period of 28.0 months. The percent change in LVEF from baseline was found to be significantly associated with the percent change in ADMA (r=−0.462, p<0.001). A multivariate Cox hazard analysis showed EPA treatment (hazard ratio: 0.21, 95% confidence interval: 0.05-0.93, p=0.031) to be an independent predictor of cardiac events.

Conclusions: These data indicate that EPA treatment may improve the cardiac function and long-term prognosis of CHF patients with dyslipidemia, at least in part, due to reductions in inflammation and improvements in the endothelial function.


Key words: Eicosapentaenoic acid, Cytokine, Heart failure, Dyslipidemia
Eicosapentaenoic acid (EPA) is a long-chain omega-3 polyunsaturated fatty acid (PUFA) contained in fish oil. A meta-analysis showed that EPA treatment decreases the triglyceride level by approximately 25% and slightly increases the high-density lipoprotein (HDL)-cholesterol level; however, it has no significant effect on the total cholesterol level. Prostaglandins and thromboxanes, are produced following the release of arachidonic acid (AA) from the plasma membrane by phospholipases and subsequently metabolized by cyclooxygenases and specific isomerases. In general, AA-derived prostanoids act in a proinflammatory manner.

Eicosapentaenoic acid (EPA) is a long-chain omega-3 polyunsaturated fatty acid (PUFA) contained in fish oil. A meta-analysis showed that EPA treatment decreases the triglyceride level by approximately 25% and slightly increases the high-density lipoprotein (HDL)-cholesterol level; however, it has no significant effect on the total cholesterol level. A recent clinical study showed that EPA therapy reduces the incidence of major coronary events in Japanese hypercholesterolemic patients when given in addition to a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin). Furthermore, treatment with PUFA significantly lowers the incidence of death from any cause and hospitalization due to cardiovascular factors in comparison to a placebo in patients with CHF.

Recent studies suggest that EPA treatment may have independent lipid-lowering effects (pleiotropic effects), including improving endothelial dysfunction, inhibiting inflammation and reducing oxidative stress. Such pleiotropic effects may be beneficial for patients with CHF.

However, there have thus far been few studies regarding the effects of EPA treatment on the inflammatory marker expression, cardiac function and/or long-term prognosis in CHF patients with dyslipidemia.

**Methods**

**Patient Population**

The present study was a prospective study that included 139 selected patients with CHF (119 men and 20 women; mean age: 70.2 ± 9.0 years), with a mean left ventricular ejection fraction (LVEF) of 37.6 ± 8.0%. All patients were recruited from an outpatient clinic at Tama-Nagayama Hospital, Nippon Medical School between January 2007 and December 2010. After discharge, follow-up data (every four to eight weeks) were obtained through direct contact with the patients at an outpatient clinic of our hospital until September 2012.

The etiology of CHF was dilated cardiomyopathy (DCM) in 25 patients and ischemic cardiomyopathy (ICM) in 114 patients. DCM was defined as the presence of a normal coronary arteriogram together with severe hypokinesis of the left ventricular wall motion as determined on left ventriculography and typical pathological findings on an endomyocardial biopsy of the left ventricle. ICM was defined as a history of myocardial infarction with significant coronary artery disease (>70% luminal stenosis in at least two major coronary arteries). No patients had a history of hypertensive heart disease, valvular heart disease or ischemic heart disease with multivessel coronary disease without myocardial infarction. The LVEF was measured using echocardiography with a LOGIQ 7 GE (Milwaukee, WI) within one week of measuring the levels of biochemical markers. The patient was placed in the left lateral recumbent position, and the LVEF was calculated according to the Simpson method using apical four- and two-chamber views. All echocardiograms of the eligible patients were analyzed by two blinded trained cardiologists. We enrolled CHF patients who had been stabilized using standard medical treatment for CHF, as follows: all eligible patients had received standard medical therapies, including angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor β-blockers (ARBs), β-blockers, furosemide, spironolactone, aspirin, clopidogrel or any combination of these medications for at least three years prior to enrollment in the present study. In addition, 23 patients had received statins (18 patients: 10 mg/day pravastatin, five patients: 5 mg/day simvastatin) for at least five years before enrollment into the present study.

At baseline (entry), the patients were considered to have dyslipidemia if they had an overnight fasting serum total cholesterol (TC) level of ≥ 220 mg/dL, a triglyceride (TG) level of ≥ 150 mg/dL, a low-density lipoprotein (LDL)-cholesterol level of ≥ 140 mg/dL or a high-density lipoprotein (HDL) cholesterol level of ≤ 40 mg/dL. The low-density lipoprotein (LDL)-cholesterol level was calculated using the Friedewald formula. The patients were divided into two groups based on whether EPA was included in their treatment regimen: the EPA group (n = 71) and the no EPA group (n = 68). Only patients with dyslipidemia at
entry, a primary indication for EPA use, were treated with EPA. A total of fourteen patients in the EPA group and nine patients in the no EPA group had received statin therapy before enrollment in the present study. None of the patients had received EPA before enrollment. In addition, none of the patients in the no EPA group received EPA during the follow-up period. All 71 patients in the EPA group were treated with 1,800 mg/day of EPA after enrollment, all of whom tolerated the treatment well, without any serious side effects during the follow-up period. None of the patients received any additional treatment for CHF during the follow-up period.

Exclusion Criteria

All patients with clinical signs of acute infection, autoimmune disorders, severe renal (serum creatinine >2.0 mg/dL) or hepatic disease or suspected malignancy were excluded from the present series. In addition, patients in the acute decompensated stage of CHF during the follow-up period. The patients received any additional treatment for CHF. None of the patients had received EPA before enrollment in the present study. None of the patients had received EPA treatment at the time of enrollment. In addition, none of the patients in the no EPA group received EPA during the follow-up period. The no EPA group received EPA during the follow-up period. None of the patients had received EPA. The high-sensitivity C-reactive protein (hsCRP) levels were measured using an immunoturbidimetry assay. The plasma levels of asymmetric dimethylarginine (ADMA) were measured using high-performance liquid chromatography (HPLC) with ortho-phthalaldehyde for fluorescence determination (SRL, Tokyo, Japan). Briefly, HPLC was performed on a Hitachi L-7100 system equipped with a Jasco FP-2025 fluorescence detector for excitation at 348 nm and emission at 450 nm with a PEGASIL ODS (4.6 mm i.d. × 250 mm; Chemical Inspection and Testing Institute). The samples were eluted with 75 mmol/L of aqueous acetate buffer.

The serum levels of EPA and AA in fasting blood samples were measured at SRL Co., Ltd. (Tokyo, Japan). In brief, total lipids were extracted according to the Folch method, and the fatty acids were directly transmethylated with a 14% boron trifluoride methanol solution (Sigma Aldrich Japan, Tokyo, Japan) at 90°C for 90 minutes. The fatty acid levels were measured using a GC-FID system (6890N; Agilent Technologies, Tokyo, Japan) equipped with a fused silica capillary column (Omegamax 250, 30 m × 0.25 mm i.d.; 0.25 μm film thickness; Supelco, USA), with tri-cosenoic acid (C23:0) methyl ester as an internal standard. The injector and detector temperatures were both set at 270°C, and the column temperature was held at 205°C. Helium was used as the carrier gas at a flow rate of 2.0 mL/min. The split ratio was 50:1.

Follow-Up and Determination of Outcomes

The outcome data were collected via serial direct contact with each patient (every four to eight weeks) at an outpatient clinic of Tama-Nagayama Hospital, Nippon Medical School until September 2012. We were able to provide follow-up for all patients, for a median period of 28.0 months (range: 12 to 60 months) in order to determine the incidence of cardiac events, including cardiac death and readmission due to worsening of CHF. None of the patients dropped out from the present study.

Statistical Analysis

The results are presented as the mean ± SD for continuous variables and the percentage of the total number of patients for categorical variables. Student’s t-test for independent samples and the chi-square test were used for comparisons of continuous and categorical variables, respectively. The TNF-α, MCP-1, TG, hsCRP, AA and EPA levels and the EPA/AA ratio had skewed distributions. Therefore, the Mann-Whitney U test was used for unpaired comparisons between groups and Wilcoxon’s signed-rank test was used for paired comparisons within groups. Bivariate correlations between parameters were assessed using Spearman correlation (r) coefficients. In order to determine the optimal cut-off values for the body mass index (BMI) and the serum levels of MCP-1 and LVEF as
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Patients Treated with or without EPA, the patients with dilated cardiomyopathy treated with or without EPA, and the patients with ischemic cardiomyopathy treated with or without EPA and the groups with high (≥37.4 μg/mL) or low (<37.4 μg/mL) EPA levels estimated according to the median EPA level at one year, and the log-rank test was used to compare these groups. Univariate and multivariate Cox regression analyses were employed to calculate the estimated hazard ratio (HR) and 95% confidence interval (CI), where appropriate. The variables were entered into a multivariate model for factors with a p-value of ≤0.05 in the univariate analysis. The examined variables included the BMI, LVEF, eGFR and the hsCRP, BNP and hemoglobin (Hb) levels, as well as the serum TNF-α and MCP-1 levels at baseline, the plasma ADMA levels at baseline and the use of EPA, ACEIs, aspirin, clopidogrel, furosemide or spironolactone. The Statistical Package for Social Sciences (SPSS) software program for Windows, version 22.0 (IBM, Japan), was used for all statistical analyses. A p-value of <0.05 was considered to be statistically significant.

Results

Study Population

The baseline clinical characteristics of the patients receiving EPA (EPA group) and those not receiving EPA (no EPA group) are shown in Tables 1 and 2. The total cholesterol and TG levels before EPA treatment were significantly higher in the EPA group than in the no EPA group (Table 1), whereas the serum levels of AA were significantly higher in the no EPA group than in the EPA group. However, the overall clinical characteristics and blood chemical variables were similar between the groups, including the etiology of CHF (ICM/DCM ratio), prevalence of the New York Heart Association (NYHA) class at baseline (NYHA class II/III; 62/9 vs. 65/3, p = 0.152, Table 1) and prevalence of statin use before enrollment (Table 1). In addition, the rates of hypertension, diabetes, an active smoking status and an LVEF ≥ of 40% were comparable between the groups (Table 1).

Changes in the Clinical Characteristics from Baseline to 12 Months After Treatment in the Patients Treated with or without EPA

The percent changes in the clinical characteristics from baseline to 12 months in the EPA group and no EPA group are shown in Table 2. After 12 months of treatment, the systolic blood pressure, heart rate and BNP, hsCRP, total cholesterol and TG levels in the EPA group all significantly decreased, by 3.1%, 8.9%, 11.2%, 11.8%, 5.0% and 21.7%, respectively. In addition, the ADMA and AA levels significantly decreased by 6.2% and 18.4%, respectively, while the EPA levels and EPA/AA ratio increased by 42.8% and 173.0%, respectively, in the EPA group after EPA treatment. After 12 months, the heart rate and EPA/AA ratio in the no EPA group significantly decreased by 5.6% and 10.5%, respectively, while the hsCRP, BNP, ADMA and AA levels increased by 40.5%, 4.3%, 5.2% and 9.4%, respectively.

After 12 months, the EPA levels and EPA/AA ratio in the EPA group were significantly higher than those observed in the no EPA group, while the patients in the EPA group exhibited significantly lower systolic blood pressure, heart rate, hsCRP, BNP, AA and ADMA values than those noted in the no EPA group. The BMI, total cholesterol and LDL-cholesterol values were comparable between the groups at 12 months. However, the TG levels remained higher in the EPA group than in the no EPA group at 12 months (p < 0.01).

Changes in the LVEF and NYHA Class from Baseline in the Patients Treated with or without EPA

The percent changes in the LVEF from baseline to 12 months after the initiation of treatment are shown in Table 2 and Fig. 1. The LVEF significantly improved after 12 months of treatment in the EPA group (from 37.3 ± 6.8% to 42.1 ± 7.6%, p < 0.001); however, the LVEF values significantly worsened in the no EPA group (from 37.9 ± 9.1% to 34.6 ± 8.0%, p < 0.001). Furthermore, the LVEF values at 12 months were significantly higher in the EPA group than those observed in the no EPA group (p < 0.001).

As noted above, the NYHA class at baseline was comparable between the patients in the EPA group and the no EPA group (NYHA class I/II/III/IV; 0/62/9/0 vs. 0/65/3/0, p = 0.129, Table 2). At 12 months, 20 of the 68 patients (29.4%) in the no EPA group remained in the same NYHA class, whereas 32 of the 68 patients (47.1%) exhibited an increase in their NYHA class, thus suggesting worsening of their clinical status. In the EPA group, 43 of the 71 patients (60.6%) maintained their initial NYHA class and only 11 patients (15.5%) exhibited a worse NYHA class, whereas 17 patients (23.9%) had a lower NYHA class, thus indicating a functional improvement. These changes between the groups were statistically signifi-
Table 1. Baseline clinical characteristics and blood chemistry parameters in the CHF patients treated with (EPA) and without EPA (No EPA)

<table>
<thead>
<tr>
<th></th>
<th>EPA (n=71)</th>
<th>No EPA (n=68)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.4±7.7</td>
<td>66.2±11.9</td>
<td>0.134</td>
</tr>
<tr>
<td>Gender (male, %)</td>
<td>60 (84.5)</td>
<td>59 (86.8)</td>
<td>0.891</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7±2.4</td>
<td>23.1±2.9</td>
<td>0.167</td>
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<tr>
<td>ICM/DCM</td>
<td>56/15</td>
<td>58/10</td>
<td>0.445</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>60 (84.5)</td>
<td>60 (88.2)</td>
<td>0.624</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>19 (26.8)</td>
<td>27 (39.7)</td>
<td>0.149</td>
</tr>
<tr>
<td>Active smoker (%)</td>
<td>10 (14.1)</td>
<td>11 (16.2)</td>
<td>0.815</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124±10</td>
<td>124±11</td>
<td>0.992</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>73±6</td>
<td>73±5</td>
<td>0.832</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.9±1.8</td>
<td>12.5±1.4</td>
<td>0.104</td>
</tr>
<tr>
<td>High sensitivity-C-reactive protein (mg/L)</td>
<td>1.35 (1.11, 1.62)</td>
<td>1.45 (0.98, 1.77)</td>
<td>0.209</td>
</tr>
<tr>
<td>B-type natriuretic peptide (pg/mL)</td>
<td>195.5±102.5</td>
<td>213.8±114.0</td>
<td>0.309</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>194±13</td>
<td>181±16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>115±10</td>
<td>112±10</td>
<td>0.151</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>46±6</td>
<td>45±10</td>
<td>0.159</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>161 (155, 167)</td>
<td>121 (105, 133)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>100±12</td>
<td>102±18</td>
<td>0.231</td>
</tr>
<tr>
<td>HbAlc (%, JDS)</td>
<td>5.6±0.3</td>
<td>5.7±0.5</td>
<td>0.122</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>58.7±12.5</td>
<td>59.8±14.0</td>
<td>0.633</td>
</tr>
<tr>
<td>Baseline MCP-1 (pg/mL)</td>
<td>313.2±60.9</td>
<td>321.0±92.4</td>
<td>0.560</td>
</tr>
<tr>
<td>Baseline Tumor necrosis factor-α (pg/mL)</td>
<td>2.3±0.4</td>
<td>2.2±0.6</td>
<td>0.580</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (LVEF; %)</td>
<td>37.3±6.8</td>
<td>37.9±9.1</td>
<td>0.658</td>
</tr>
<tr>
<td>LVEF ≤40% (%)</td>
<td>34 (49.3)</td>
<td>35 (50.7)</td>
<td>0.735</td>
</tr>
<tr>
<td>EPA (µg/mL)</td>
<td>37.4 (33.7, 41.0)</td>
<td>36.9 (32.6, 41.3)</td>
<td>0.486</td>
</tr>
<tr>
<td>AA (µg/mL)</td>
<td>127.5 (112.2, 158.8)</td>
<td>151.8 (114.6, 175.1)</td>
<td>0.013</td>
</tr>
<tr>
<td>EPA/AA ratio</td>
<td>0.30 (0.25, 0.33)</td>
<td>0.24 (0.19, 0.36)</td>
<td>0.107</td>
</tr>
<tr>
<td>ADMA (µmol/mL)</td>
<td>0.57±0.06</td>
<td>0.57±0.08</td>
<td>0.940</td>
</tr>
<tr>
<td>Angiotensin converting inhibitors use (%)</td>
<td>41 (57.7)</td>
<td>23 (33.8)</td>
<td>0.006</td>
</tr>
<tr>
<td>Angiotensin receptor blockers use (%)</td>
<td>30 (42.3)</td>
<td>45 (66.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>β-blockers use (%)</td>
<td>61 (85.9)</td>
<td>58 (85.3)</td>
<td>0.917</td>
</tr>
<tr>
<td>Calcium channel blockers use (%)</td>
<td>4 (5.6)</td>
<td>4 (5.9)</td>
<td>0.950</td>
</tr>
<tr>
<td>Aspirin use (%)</td>
<td>61 (85.9)</td>
<td>64 (94.1)</td>
<td>0.158</td>
</tr>
<tr>
<td>Clopidogrel use (%)</td>
<td>15 (21.1)</td>
<td>7 (10.3)</td>
<td>0.105</td>
</tr>
<tr>
<td>Furosemide use (%)</td>
<td>30 (42.3)</td>
<td>40 (58.8)</td>
<td>0.062</td>
</tr>
<tr>
<td>Spironolactone use (%)</td>
<td>19 (26.8)</td>
<td>26 (38.2)</td>
<td>0.204</td>
</tr>
<tr>
<td>Statins use before enrollment (%)</td>
<td>14 (19.7)</td>
<td>9 (13.2)</td>
<td>0.424</td>
</tr>
</tbody>
</table>

Data expressed as the means ± SD or median (interquartile range), ICM; ischemic cardiomyopathy, DCM; dilated cardiomyopathy, LDL; low-density lipoprotein, HDL; high-density lipoprotein, JDS; Japan diabetes society, eGFR; estimated glomerular filtration rate, MCP; monocyte chemoattractant protein, EPA; eicosapentaenoic acid, AA; arachidonic acid, ADMA; asymmetric dimethylarginine

significant (p < 0.01, Table 2).

Relationships between the Serum Levels of TNF-α, MCP-1, EPA and AA, the Plasma Levels of ADMA and the LVEF in the Patients with CHF at Baseline

The serum levels of TNF-α, MCP-1 and AA and the plasma levels of ADMA at baseline were significantly and negatively associated with the baseline LVEF (TNF-α: r = −0.594, p < 0.001, MCP-1: r = −0.562, p < 0.001, AA: r = −0.439, p < 0.001, ADMA: r = −0.641, p < 0.001), while the serum EPA level and EPA/AA ratio were significantly and positively related to the baseline LVEF (EPA: r = 0.351, p < 0.001, EPA/AA ratio: r = 0.538, p < 0.001). In addition, the serum TNF-α and MCP-1 levels and EPA/AA ratio were significantly and positively associated with the plasma
ADMA levels. These data suggest that increased serum EPA and MCP-1 levels and a decreased EPA/AA ratio are significantly associated with endothelial dysfunction and a reduced cardiac function in CHF patients.

Comparison of the Clinical Characteristics of the Patients with and without Cardiac Events

Fifty-five patients experienced cardiac events, including 15 cardiac deaths and 40 readmissions for worsening CHF, during a median follow-up period of 28.0 months (range: 12 to 60 months). Of the 15 patients with cardiac death, all patients died from worsening of CHF due to severe pump failure and none developed sudden cardiac death due to fatal ventricular arrhythmia, atrial fibrillation or myocardial infarction during the follow-up period. In addition, none of the patients received PCI, CABG or implantable cardiovascular defibrillator (ICD) treatment during the follow-up period.

A comparison of the clinical characteristics of the patients with and without cardiac events is shown in Table 3. The patients with cardiac events were significantly older and had lower BMI, Hb, LVEF and eGFR values and serum lipid profiles, including the TG levels, than the patients without cardiac events. Furthermore, the patients with cardiac events had higher plasma hsCRP and BNP levels than those without cardiac events. However, the baseline NYHA class (NYHA class II/III: 40/7 vs. 87/5, p=0.106) and etiology of CHF (DCM or ICM) were comparable between the groups (Table 3).

The serum TNF-α, MCP-1 and AA levels and plasma ADMA levels were significantly higher in the patients with cardiac events than in those without cardiac events (respectively, p<0.001, Table 3), whereas the serum EPA levels and EPA/AA ratios in the patients with cardiac events were significantly lower than those observed in the patients without cardiac events (respectively, p<0.001, Table 3).

Effects of Medications in the CHF Patients with and without Cardiac Events

At baseline, the patients in the EPA group were more likely to receive ACEIs (57.7% vs. 33.8%, p=0.006) and less likely to receive ARBs (42.3% vs.
Eicosapentaenoic Acid in Heart Failure

the patients with ischemic cardiomyopathy during the follow-up period (log-rank: 6.68, \( p = 0.0097 \), Fig. 2B). In addition, the EPA treatment also significantly improved the outcomes of the patients with dilated cardiomyopathy (log-rank: 12.50, \( p = 0.0004 \), Fig. 2C). These data indicate that the EPA treatment significantly reduced cardiac events in the patients with CHF, irrespective of the etiology of CHF.

In the subgroup analysis, after matching the patients for their use of \( \beta \)-blockers, those who did not receive EPA experienced 33 cardiac events (33/58; 56.9%, 13 cardiac deaths and 20 readmissions for worsening of CHF). In comparison, only 16 patients (16/61; 26.2%, six cardiac deaths and 10 readmissions due to worsening of CHF) who received EPA (HR 0.32, 95% CI 0.17-0.58, \( p < 0.001 \)) experienced cardiac events. After matching the patients for their use of ACEIs, the patients who did not receive EPA experienced 11 cardiac events (11/23; 47.8%, two cardiac deaths and nine readmissions for worsening of CHF), whereas only eight patients (8/41; 19.5%, two cardiac deaths and six readmissions due to worsening of CHF) who received EPA experienced cardiac events (HR 0.32, 95% CI 0.17-0.58, \( p < 0.001 \)). In addition, after matching the patients for their use of spironolactone, the patients who did not receive EPA experienced 23 cardiac events (23/26; 88.5%, nine cardiac deaths and 14 readmissions for worsening of CHF), whereas only 66.2%, \( p = 0.006 \) in comparison to those in the no EPA group. However, the use of furosemide, \( \beta \)-blockers, calcium channel blockers (CCBs), aspirin, clopidogrel and spironolactone was comparable between the groups (Table 1). In addition, the use of statins before enrollment was similar between the groups (Table 1).

The patients with cardiac events were less likely to receive EPA (32.7% vs. 44.1%, \( p = 0.001 \)) and ACEIs (34.5% vs. 53.6%, \( p = 0.037 \)) and more likely to receive ARBs (65.5% vs. 46.4%, \( p = 0.037 \)), furosemide (96.4% vs. 22.6%, \( p < 0.001 \)), spironolactone (56.4% vs. 16.7%, \( p < 0.001 \)), aspirin (96.4% vs. 85.7%, \( p = 0.047 \)) and/or clopidogrel (27.3% vs. 8.3%, \( p = 0.004 \)) than those without cardiac events (Table 3). However, the use of \( \beta \)-blockers (89.1% vs. 83.3%, \( p = 0.464 \)) and statins (20.0% vs. 14.3%, \( p = 0.484 \)) was similar between the groups.

During a median follow-up of 28.0 months (range: 12 to 60 months), cardiac events (nine cardiac deaths and 28 readmissions due to worsening of CHF) occurred in 37 of the 68 patients (54.4%) who did not receive EPA, whereas such events (six cardiac deaths and 12 readmissions for worsening of CHF) were observed in only 18 of the 71 patients (25.4%) who received EPA (log-rank: 14.01, \( p < 0.001 \), Fig. 2A).

The Kaplan-Meier curve analysis showed that the EPA treatment significantly improved the outcomes of the patients with ischemic cardiomyopathy during the follow-up period (log-rank: 6.68, \( p = 0.0097 \), Fig. 2B). In addition, the EPA treatment also significantly improved the outcomes of the patients with dilated cardiomyopathy (log-rank: 12.50, \( p = 0.0004 \), Fig. 2C). These data indicate that the EPA treatment significantly reduced cardiac events in the patients with CHF, irrespective of the etiology of CHF.

In the subgroup analysis, after matching the patients for their use of \( \beta \)-blockers, those who did not receive EPA experienced 33 cardiac events (33/58; 56.9%, 13 cardiac deaths and 20 readmissions for worsening of CHF). In comparison, only 16 patients (16/61; 26.2%, six cardiac deaths and 10 readmissions due to worsening of CHF) who received EPA (HR 0.32, 95% CI 0.17-0.58, \( p < 0.001 \)) experienced cardiac events. After matching the patients for their use of ACEIs, the patients who did not receive EPA experienced 11 cardiac events (11/23; 47.8%, two cardiac deaths and nine readmissions for worsening of CHF), whereas only eight patients (8/41; 19.5%, two cardiac deaths and six readmissions due to worsening of CHF) who received EPA experienced cardiac events (HR 0.32, 95% CI 0.17-0.58, \( p < 0.001 \)). In addition, after matching the patients for their use of spironolactone, the patients who did not receive EPA experienced 23 cardiac events (23/26; 88.5%, nine cardiac deaths and 14 readmissions for worsening of CHF), whereas only
eight patients (8/19; 42.1%, two cardiac deaths and six readmissions due to worsening of CHF) who received EPA experienced cardiac events (HR 0.24, 95% CI 0.10−0.57, \( p < 0.001 \)). Furthermore, after matching the patients for their treatment with furosemide, the patients who did not receive EPA experienced 36 cardiac events (36/40; 90.0%, nine cardiac deaths and 27 readmissions for worsening of CHF), whereas only 15 patients (15/30; 50.0%, five cardiac deaths and 10 readmissions due to worsening of CHF) who received EPA (HR 0.28, 95% CI 0.15−0.53, \( p < 0.001 \)) experienced cardiac events. After matching the patients for their treatment with clopidogrel, the patients without EPA treatment experienced seven cardiac events (7/7; 100%, no cardiac deaths and seven readmissions for worsening of CHF). In contrast, only eight patients experienced cardiac events (8/15; 53.3%, four cardiac deaths and four readmissions due to worsening of CHF) (HR 0.05, 95% CI 0.01−0.40, \( p < 0.005 \)).

A total of fourteen patients in the EPA group and nine patients in the no EPA group had received statin therapy before enrollment in the present study. After excluding the patients for their use of statins before enrollment, the patients without EPA treatment experienced 32 cardiac events (32/59; 54.2%, five cardiac deaths and 27 readmissions for worsening of CHF), whereas only 12 patients who received EPA experienced cardiac events (12/57; 21.1%, four car-
Eicosapentaenoic Acid in Heart Failure

Relationships between the Changes in the Serum Levels of TNF-α and MCP-1, the Plasma Levels of ADMA, the EPA/AA Ratio and the LVEF from Baseline to 12 Months After the Initiation of Treatment

At baseline, the serum TNF-α and MCP-1 levels, EPA/AA ratio and plasma ADMA levels were comparable between the patients in the EPA group and those in the no EPA group (Table 2). After 12 months of EPA treatment, the serum TNF-α and MCP-1 levels and plasma ADMA levels significantly decreased (TNF-α: from 2.3 ± 0.4 to 2.0 ± 0.6 pg/mL, p < 0.001, MCP-1: from 313.2 ± 60.8 to 266.2 ± 83.4 pg/mL, p < 0.001, ADMA: from 0.57 ± 0.06 to 0.53 ± 0.06 μmol/mL, p < 0.001, Table 2), while the EPA/AA ratios significantly increased (Table 2), in the patients in the EPA group, whereas the serum TNF-α and MCP-1 levels and plasma ADMA levels significantly increased and the EPA/AA ratios significantly decreased for worsening of CHF (HR 0.31, 95% CI 0.16-0.61, p = 0.001). These data indicate that the EPA treatment significantly improved the long-term prognosis after matching the patients for their use of various medications.

Receiver-Operating Characteristic (ROC) Curve Analysis

The ROC curve analysis revealed the optimal cut-off values for predicting cardiac events for BMI and the serum levels of MCP-1 and LVEF to be 23.0 kg/m², 320.0 pg/ml and 35.0%, respectively. The sensitivity and specificity for BMI and the serum levels of MCP-1 and LVEF in predicting cardiac events were assessed across a range of cut-off values. The optimal cut-off value for each parameter was associated with a sensitivity of 66.3%, 75.0% and 76.6% and a specificity of 61.7%, 66.0% and 73.9%, respectively.
and significantly and positively related to the percent change in the EPA/AA ratio ($r = 0.361$, $p = 0.002$, Fig. 3D). Interestingly, the percent changes in the systolic blood pressure ($r = -0.019$, $p = 0.875$), TG ($r = 0.196$, $p = 0.101$) and HDL-cholesterol ($r = 0.101$, $p = 0.402$) values from baseline were not related to the percent change in the LVEF after 12 months of treatment (data not shown in the Figures). These findings suggest that improvements in the cardiac function may be, at least in part, due to reductions in inflammation and improvements in the endothelial function in CHF patients treated with EPA.

**Fig. 3.** Relationships between the percent change in the LVEF from baseline to 12 months and the percent changes in the tumor necrosis factor (TNF)-α (Fig. 3A), monocyte chemoattractant protein (MCP)-1 (Fig. 3B) and asymmetric dimethylarginine (ADMA, Fig. 3C) levels and the EPA/AA ratio (Fig. 3D) in the patients with CHF treated with EPA. The percent change in the LVEF from baseline was significantly and negatively associated with the percent changes in the TNF-α (Fig. 3A), MCP-1 (Fig. 3B) and ADMA (Fig. 3C) levels. The percent change in the LVEF from baseline was significantly and positively related to the percent change in the EPA/AA ratio. EPA: eicosapentaenoic acid, AA: arachidonic acid.

The percent change in LVEF from baseline to after 12 months of treatment in the patients in the EPA group was significantly and inversely correlated with the percent changes in the serum TNF-α and MCP-1 levels and plasma ADMA levels (TNF-α: $r = -0.693$, $p < 0.001$, Fig. 3A, MCP-1: $r = -0.568$, $p < 0.001$, ADMA: $r = -0.462$, $p < 0.001$, Fig. 3C) and significantly and positively related to the percent change in the EPA/AA ratio ($r = 0.361$, $p = 0.002$, Fig. 3D). Interestingly, the percent changes in the systolic blood pressure ($r = -0.019$, $p = 0.875$), TG ($r = 0.196$, $p = 0.101$) and HDL-cholesterol ($r = 0.101$, $p = 0.402$) values from baseline were not related to the percent change in the LVEF after 12 months of treatment (data not shown in the Figures). These findings suggest that improvements in the cardiac function may be, at least in part, due to reductions in inflammation and improvements in the endothelial function in CHF patients treated with EPA.

**Relationships between the Serum Levels of TNF-α, MCP-1 and ADMA and the Incidence of Cardiac Events**

The ROC curve analysis showed that the optimal
cut-off values for the TNF-α, MCP-1 and ADMA levels in predicting cardiac events were 2.5 pg/mL, 320 pg/mL and 0.56 μmol/mL, respectively. During a median follow-up period of 28.0 months (range: 12 to 60 months), cardiac events (14 cardiac deaths and 29 readmissions due to worsening of CHF) occurred in 43 of the 57 patients (75.4%) with an elevated TNF-α level (≥2.5 pg/mL) and in 12 (one cardiac death and 11 readmissions for worsening of CHF) of the 82 patients (14.6%) without an elevated TNF-α level (<2.5 pg/mL, log-rank: 61.62, p<0.001). In addition, cardiac events (13 cardiac deaths and 27 readmissions for worsening of CHF) occurred in 40 of the 57 patients (70.2%) with an elevated MCP-1 level (≥320 pg/mL) and in only 15 (two cardiac deaths and 13 readmissions due to worsening of CHF) of the 82 patients (18.3%) without an elevated MCP-1 level (<320 pg/mL, log-rank: 45.20, p<0.001).

Furthermore, the patients with an elevated serum level of ADMA (≥0.56 μmol/mL) experienced 40 cardiac events (69.0%, 13 cardiac deaths and 27 readmissions for worsening of CHF), in comparison to the only 15 events (18.5%, two cardiac deaths and 13 readmissions due to worsening of CHF) observed in the patients with an ADMA level of <0.56 μmol/mL (log-rank: 34.38, p<0.001). These data indicate that increased proinflammatory cytokine and chemokine levels and endothelial dysfunction are significantly associated with poor outcomes in patients with CHF.

In order to investigate the effects of the EPA levels on the risk of cardiac events, we compared the incidence of cardiac events between the patients with high and low EPA levels according to the median EPA level at one year (37.4 μg/mL). The Kaplan-Meier curve analysis showed that the patients with a high EPA level (≥37.4 μg/mL) at one year exhibited a significantly lower event-free survival than those with a low EPA level at one year (<37.4 μg/mL) (log-rank: 34.38, p<0.001). These data indicate that increased proinflammatory cytokine and chemokine levels and endothelial dysfunction are significantly associated with poor outcomes in patients with CHF.

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Cox Proportional Hazards Analysis of Cardiac Events

The results of the univariate and multivariate Cox hazard analyses are shown in Table 4. In the univariate analysis, an age ≥70 years, ischemic etiology of CHF, diabetes, treatment with aspirin and the use of statins before enrollment were not found to be predictors of poor outcomes in the patients with CHF (Table 4). The multivariate Cox hazard analysis showed that an elevated triglyceride level (≥154 mg/dL), estimated according to the ROC curve analysis, was not a predictor of cardiac events, whereas a serum MCP-1 level at baseline of ≥320 pg/mL, a BMI of ≤23.0 kg/m² and the use of furosemide were each found to be significantly and independently associated with the risk of cardiac events, and the use of EPA and ACEIs was found to be significantly and independently related to a reduction in the incidence of cardiac events in the patients with CHF.

Discussion

The present study provides evidence that patients with cardiac events exhibit significantly higher plasma levels of TNF-α, MCP-1 and ADMA, as well as lower EPA/AA ratios, than those without cardiac events. Significant positive correlations were observed between the TNF-α, MCP-1 and ADMA levels, and these markers were found to be significantly and negatively correlated with the EPA/AA ratio. In addition, the LVEF was found to be significantly and positively associated with the EPA/AA ratio and negatively correlated with the TNF-α, MCP-1 and ADMA levels. These data suggest that the inflammation associated with endothelial dysfunction plays a significant role in the pathogenesis and exacerbation of CHF. In addition, the EPA/AA ratio was significantly associated with inflammation, endothelial dysfunction and the cardiac function in the patients with CHF.

Finally, the present study provides the first evidence that EPA treatment significantly attenuates the TNF-α, MCP-1, AA and ADMA levels and increases the EPA level and EPA/AA ratio while improving the cardiac function and long-term prognosis in CHF patients with dyslipidemia at baseline (entry).
Metabolism of Omega-3 and Omega-6 Polyunsaturated Fatty Acids

Eicosapentaenoic acid (EPA) is a long-chain omega-3 polyunsaturated fatty acid (PUFA) isolated from fish oil, while arachidonic acid (AA) is the metabolite of linoleic acid, an omega-6 PUFA. EPA and AA are both substrates for cyclooxygenase (COX) and lipoxygenase, which are involved in the production of eicosanoids\textsuperscript{12-14}. Both omega-3 and omega-6 PUFAs and their metabolites influence cellular homeostasis. In humans consuming a typical Western diet, AA is the predominant PUFA in the cell phospholipid membrane, and serves as the precursor to proinflammatory prostaglandins, thromboxanes and leukotrienes\textsuperscript{12-14}.

Mechanisms Underlying the Beneficial Effects of EPA Treatment in CHF Patients with Dyslipidemia

The mechanisms responsible for the beneficial effects of EPA treatment in the present study remain unclear. However, triglyceride-lowering, anti-inflammatory and anti-arrhythmic actions, improvements in the endothelial function and the prevention of myocardial remodeling, as well as the combination of these effects, may play important roles in the treatment of CHF\textsuperscript{13, 14, 18, 19}.

In the present study, the EPA treatment reduced the triglyceride levels by approximately 20%. EPA treatment decreases the assembly and secretion of very low-density lipoproteins, thus resulting in diminished triacylglycerol production via a decreased activity of sterol receptor element-binding protein-1c, the key switch in controlling lipogenesis\textsuperscript{13, 14}. In addition, EPA may promote \(\beta\)-oxidation simultaneously in mitochondria and/or peroxisomes, possibly via the activation of peroxisome proliferator-activated receptor (PPAR)-\(\alpha\), thereby reducing the amount of fatty acid substrates available for triglyceride synthesis\textsuperscript{14}. These triglyceride-lowering effects may play important roles in reducing cardiac events in CHF patients with dyslipidemia.

Recent clinical studies have shown that high AA and low EPA levels, as well as an elevated ratio of AA to EPA (AA/EPA ratio), are independent predictors of...
poor outcomes in patients with coronary artery disease. However, there are scant data regarding the relationship between the AA/EPA ratio and the long-term prognosis in patients with CHF.

The present study showed that EPA treatment increases the EPA level and decreases the AA level, thus resulting in an increased EPA/AA ratio. Recent studies have demonstrated that EPA treatment reduces the content of AA in membrane phospholipids in platelets, endothelial cells and inflammatory cells, with a subsequent decrease in the production of AA-derived proinflammatory mediators, including prostaglandin (PG)-E2, thromboxane (TX)-B2, leukotriene (LT)-B4, hydroxyeicosatetraenoic acid (5-HETE) and LT-E4. In addition, EPA is an alternative substrate for COX and lipoxygenase, thus inducing the production of anti-inflammatory eicosanoids, such as PGE2, TX-B2 and LT-B4. Furthermore, EPA generates resolvins, anti-inflammatory eicosanoids produced via the COX-2 pathway. These findings reflect the beneficial effects of EPA treatment in CHF patients with dyslipidemia.

The present study showed that EPA attenuates the serum TNF-α and MCP-1 levels, and reduces the hsCRP level. These data indicate that EPA treatment reduces inflammation in CHF patients with dyslipidemia. The production of proinflammatory cytokines and chemokines is regulated by nuclear transcription factor kappa B (NF-κB), which is activated in patients with CHF. EPA therapy prevents lipopolysaccharide-induced NF-κB activation.

In addition, the present study showed that the percent change in the LVEF from baseline to 12 months is significantly associated with the percent decrease in the TNF-α and MCP levels in CHF patients treated with EPA. These anti-inflammatory effects of EPA may play significant roles in reducing cardiac events and improving the cardiac function in CHF patients with dyslipidemia.

The present study also demonstrated that EPA reduces the plasma level of ADMA, an endogenous and competitive inhibitor of nitric oxide (NO) synthetase, indicating that treatment with EPA may improve the endothelial function in CHF patients with dyslipidemia.

In support of this hypothesis, recent studies have demonstrated that long-term treatment with fish oil improves the endothelium-dependent relaxation of hypercholesterolemic and atherosclerotic porcine coronary arteries, while EPA therapy augments the endothelium-dependent relaxation induced by NO and endothelin-derver hyperpolarizing factor.

In addition, in the present study, the percent change in the ADMA level from baseline was significantly correlated with the percent changes in the TNF-α and MCP-1 levels, while the percent change in the LVEF from baseline was significantly associated with the percent change in the ADMA level in the CHF patients who received EPA. These data indicate that EPA treatment may improve the cardiac function, at least in part, due to reductions in inflammation and improvements in endothelial dysfunction in CHF patients.

In the present study, EPA treatment reduced the systolic blood pressure (SBP) by 4 mmHg and the heart rate by 9 beats/min. A previous meta-analysis demonstrated that EPA therapy reduced the SBP by 2.1 mmHg and the heart rate by 2.5 beats/min. These blood pressure- and heart rate-lowering effects of EPA may be beneficial for patients with CHF.

Omega-3 fatty acids are incorporated into cell membranes where they affect the ion-channel function of myocytes. Omega-3 fatty acids inhibit the voltage-gated Na channel, prolonging the relative refractory period and increasing the voltage required for membrane depolarization. Omega-3 fatty acids also exert a modulatory effect on L-type calcium channels, thus resulting in decreased rates of cytosolic free Ca and Ca influx and the prevention of cytosolic Ca overload during ischemic insult. Through these mechanisms, EPA treatment may prevent ventricular tachyarrhythmia and hence decrease the risk of sudden cardiac death in patients with CHF. In support of these findings, Nodari et al. showed that treatment with omega-3 PUFAs increases the cardiac function, prevents myocardial remodeling and reduces the rate of hospitalization for heart failure among patients with dilated cardiomyopathy.

Therefore, the triglyceride-lowering, anti-inflammatory and anti-arrhythmic actions, improvements in endothelial dysfunction and prevention of myocardial remodeling, as well as the combination of these effects, may play important roles in the treatment of CHF.

**Effects of Medication on the Outcomes of CHF Patients**

Large-scale, placebo-controlled, randomized clinical trials have demonstrated that β-blockers and ACEIs reduce the mortality and morbidity of patients with CHF. In addition, spironolactone reduces both morbidity and mortality in CHF patients. Therefore, β-blockers, ACEIs and spironolactone, as well as combinations of these medications, are standard treatments in patients with CHF.

Loop diuretics, including furosemide, function
by blocking the sodium-potassium-chloride transporter in the ascending loop of Henle and are often used in the treatment of hospitalized patients with decompensated heart failure and CHF. However, to date, no randomized clinical trials have evaluated the chronic effects of loop diuretics on the long-term outcomes of such patients. Previous subanalyses of randomized clinical trials have demonstrated that the use of diuretics is associated with poor outcomes in patients with CHF. The present study also showed that the use of furosemide is significantly and independently related to poor outcomes in CHF patients. However, after matching patients for their use of furosemide and spironolactone, treatment with EPA continued to significantly reduce the incidence of cardiac events in the patients with CHF, thus indicating that the combined use of EPA with conventional therapeutic agents may improve treatment outcomes. In addition, the use of aspirin and clopidogrel was not found to be significantly and independently related to cardiac events in patients with CHF in this study. Furthermore, after excluding the patients who had received statins before enrollment, EPA treatment was found to significantly reduce the incidence of cardiac events.

Therefore, the present study showed that EPA treatment improves the cardiac function and long-term prognosis in CHF patients with dyslipidemia. After matching the patients for their use of β-blockers, ACEIs, aspirin, clopidogrel, furosemide and spironolactone.

Conflicting Evidence Regarding Cardiovascular Events

The benefits of omega-3 fatty acids in reducing cardiovascular mortality and morbidity have been documented in patients with myocardial infarction. In one study, EPA treatment (1,800 mg/day) significantly reduced the risk of major coronary events, including fatal and non-fatal coronary artery disease, unstable angina, PCI and CABG in Japanese patients with hypercholesterolemia (JELIS: Japan EPA lipid intervention study). In addition, Kromhout et al. showed that low-dose therapy with omega-3 fatty acids (400 mg/day EPA + docosahexaenoic acid) exerts a protective effect against ventricular arrhythmia-related events and fatal myocardial infarction in post-myocardial infarction patients with diabetes. Moreover, a meta-analysis by Casula M. et al. showed that the long-term use of high-dose omega-3 fatty acid supplementation is beneficial in reducing the onset of cardiac death, sudden death and myocardial infarction in patients with a history of cardiovascular disease.

However, recent randomized clinical trials and two meta-analyses have questioned the value of omega-3 fatty acid supplementation for reducing the risk of cardiovascular disease. In addition, a meta-analysis of three clinical trials of fish oil supplementation in patients with ventricular arrhythmia showed that treatment with omega-3 PUFAs does not reduce the incidence of ventricular tachyarrhythmia in patients with an ICD. In the present study, none of the patients developed acute myocardial infarction or sudden cardiac death due to ventricular tachyarrhythmia during the follow-up period. An excellent review was recently published by Harris W.S., which provides very useful information for understanding recent randomized clinical trials.

The potential benefits of omega-3 fatty acids have been extended to the prevention and treatment of CHF. The Cardiovascular Health Study, which involved 4,738 men and women ≥ 65 years of age, found an inverse association between baked or broiled fish intake and the incidence of CHF. A recent analysis by Yamagishi et al. in a prospective study of nearly 60,000 Japanese patients followed for approximately 13 years showed an inverse association between fish and omega-3 PUFA consumption and cardiovascular mortality, especially that due to CHF.

Confirmatory evidence was presented and published in the GISSI-HF trial, a large, factorial, placebo-controlled trial of nearly 7,000 patients with NYHA class II to IV CHF who were randomized to receive either 1 g of omega-3 PUFA containing 850 to 882 mg of EPA + docosahexaenoic acid (DHA), 10 mg of rosvuastatin, both or a dual placebo, clearly showed a statistically significant benefit of the prescription omega-3 PUFA treatment, including a reduction in total mortality (~9%) and total mortality or hospitalization for cardiovascular disease (~8%). In a substudy of the GISSI-HF trial, omega-3 PUFA supplementation was found to provide a small but statistically significant advantage in terms of improving the LVEF in patients with symptomatic CHF of any etiology previously treated with recommended therapies, including ACEIs, ARBs, β-blockers, diuretics and/or any combination of these medications.

In addition, Nordari S. et al. showed that omega-3 PUFA treatment increases the LVEF and functional capacity and may reduce the rate of hospitalization for heart failure. Furthermore, the present study showed that EPA treatment significantly reduces inflammation and may improve the cardiac function and long-term prognosis in patients with CHF.

Why have omega-3 fatty acids been apparently
less effective in more recent studies than in earlier trials or studies involving patients with CHF? These discrepancies may be related to differences in the combination of low-dose 3-omega fatty acids administered, the length (short) of the follow-up period, the presence or absence of a high background 3-omega fatty acid intake, the frequent use of modern pharmacotherapy, the evaluation of relatively low-risk patient populations (with or without coronary artery disease), the prevalence of CHF and/or the use of a small sample size.

The strongest reductions in the cardiovascular endpoints were obtained in the oldest trials. One explanation for this phenomenon is differences in study design. The GISSP-P and JELIS trials used an open-label design \(^{16, 43}\), which may result in different findings. Another explanation is that the patients in the more recent trials were heavily treated with ACEIs, ARBs, statins and/or a combination of these medications. The prevalence of statin treatment in the GISSP-P trial \(^{43}\) was only 29%, compared to the more than 85% observed in most current trials. This difference may account for the high risk of fatal coronary artery disease and sudden death observed in the GISSP-P trial in comparison to other current trials. In the present study, almost all of the patients with CHF received ACEIs, ARBs and β-blockers, while patients with ischemic cardiomyopathy in the acute phase also received revascularization; however, only 15% of the patients had received statins before enrollment into the study. In addition, we showed that the use of statin treatment before enrollment is not associated with cardiac events. However, this study employed a prospective observational design, which may have resulted in different findings.

The OMEGA trial \(^{48}\), a time-nontreatment trial, had a primary outcome of sudden cardiac death within only one year. The events rates in the OMEGA study were much lower than anticipated, presumably due to the use of more aggressive treatment (81% rate of acute revascularization and intensive pharmacological treatment) in 2003-2007 in comparison to that observed in 1993-1995, during which time the GISSI-P study (5% rate of acute revascularization) was conducted.

The alpha OMEGA trial \(^{48}\) aimed to test the single and combined effects of alpha-linolenic acid and classic marine-derived omega-3 fatty acids (EPA + DHA) on cardiovascular disease endpoints in patients with a history of myocardial infarction within 10 years of enrollment. That study was a nutritional, not pharmacologic, intervention in which a relatively low dose of EPA + DHA (376 mg) was given, which is approximately one-third of that typically used in the GISSI-P study and one-fifth of that used in the JELIS study. We used a dose of 1,800 mg/day of EPA in the present study.

Other factors to consider include the long time interval between the index myocardial infarction and enrollment, which indicates that the high-risk period for sudden cardiac death and/or acute reinfarction may have past. These factors may account for the neutral effect of omega-3 fatty acid treatment on the risk of cardiovascular events and sudden death.

The ORIGIN trial \(^{46}\) was the largest and longest placebo-controlled trial and had sufficient power (80% to detect a 16% benefit). The most likely explanation for the inefficacy of omega-3 fatty acids observed in the ORIGIN study lies in the reported background intake of EPA + DHA (210 mg/day). This amount is approximately 10 times higher than the median intake in the USA. As such, many patients were already consuming approximately 250 mg/day, the minimum intake currently recommended in the 2010 Dietary Guidelines for Americans \(^{56}\).

In terms of the meta-analysis, this discrepancy may be due to differences in the inclusion/exclusion criteria (placebo-controlled or open-label, all patients or secondary prevention patients), endpoints (total cardiovascular disease events, sudden cardiac death, stroke, all-cause mortality, etc.), use of EPA + DHA capsules or EPA alone, duration of treatment, background omega-3 fatty acid intake and/or the tissue omega-3 fatty acid levels.

Casula M. et al. \(^{45}\) evaluated 11 randomized, double-blind, placebo-controlled trials of patients with a history of cardiovascular disease who received high doses of fatty acids (1 to 5 g/day). The authors concluded that high-dose treatment with omega-3 fatty acids significantly reduces the incidence of cardiac death (relative risk (RR) 0.68: 95% CI, 0.56-0.83), sudden death (RR 0.67: 95% CI, 0.52-0.87) and myocardial infarction (RR 0.75: 95% CI, 0.63-0.88) but not all-cause mortality or the incidence of stroke.

Kwak et al. \(^{49}\) showed a small reduction in the incidence of cardiovascular death (RR 0.91: 95% CI, 0.84-0.99); however, the statistical significance disappeared after excluding one study from the analysis. Rizos E.C. et al. \(^{50}\) showed that omega-3 PUFA supplementation is not associated with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction or stroke. However, the authors inflated the critical value for significance to 0.0063 instead of the standard 0.05. Without this statistical maneuver, their data support the conclusion that fish oil supple-
m entation significantly reduced the risk of cardiac death by 9% (RR 0.91: 95% CI, 0.85-0.98, p=0.01). These data suggest that the controversy stemming from the use of different databases accounts for the confusion observed in the varying conclusions.

Study Limitations
First, the most significant limitation associated with this study is the fact that the sample size (number of participants) was small. Second, the use of EPA was not assigned in a randomized manner. Only patients with dyslipidemia at baseline (entry), a primary indication for EPA use, were treated with EPA. Third, the patients with CHF had received β-blockers, ACEIs, furosemide or spironolactone for at least three years before enrollment, and patients in the acute decompensated stage of CHF were excluded. Therefore, all of the eligible patients in the present study had been stabilized with standard treatments, including β-blockers, ACEIs, furosemide or spironolactone before enrollment, and the effects of EPA may differ in unstable patients. Fourth, the dietary intake of fish and omega-3 PUFA was not determined in the present study. Fifth, we did not measure the endothelial function using reactive hyperemia peripheral arterial tonometry.

Conclusion
In conclusion, in the present study, the patients with cardiac events exhibited significantly higher plasma levels of TNF-α, MCP-1 and ADMA and lower EPA/AA ratios than those without cardiac events. In addition, the LVEF was found to be significantly and positively associated with the EPA/AA ratio and negatively correlated with the TNF-α, MCP-1 and ADMA levels.

These data suggest that the inflammation associated with endothelial dysfunction plays a significant role in the pathogenesis and exacerbation of CHF. In addition, the EPA/AA ratio was found to be significantly associated with inflammation, endothelial dysfunc tion and a poor cardiac function in the patients with CHF.

Furthermore, our results indicate that EPA treatment may improve the cardiac function and the long-term prognosis of CHF patients with dyslipidemia, at least in part, due to reductions in inflammation and improvements in the endothelial function.

Further studies are needed to elucidate the exact mechanisms involved in this pathophysiological pathway and develop optimal therapeutic strategies for suppressing the deterioration of CHF.

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None.

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