Introduction

Obesity is a rapidly growing public health problem in industrialized countries and it is associated with cardiovascular complications. Adipose tissue has a high capacity to secrete many biologically active substances (or adipocytokines), including leptin and tumor necrosis factor-α (TNF-α). The serum levels of circulating pro-inflammatory cytokines, such as TNF-α, interleukin (IL)-6, and monocyte chemoattractant protein-1, tend to increase in overweight people with enhanced accumulation of visceral fat. Dysregulation of pro- and anti-inflammatory adipocytokine production is associated with the development of metabolic syndrome. Therefore, abdominal obesity is independently associated with cardiovascular diseases and diabetes (i.e., insulin resistance).

Pericardial fat includes epicardial fat (the epicardial adipose tissue [EAT] depot immediately adjacent to the heart wall) and paracardial fat located on the external surface of the pericardium (i.e., mediastinal fat). Fat depots localized around the heart (i.e., EAT)
are involved in the pathogenesis of coronary artery disease (CAD). EAT produces various bioactive molecules that significantly affect cardiac function. Moreover, EAT and pericardial fat volumes are strongly correlated with each other and are equally associated with the number of atherosclerotic plaques.

n-3 Polyunsaturated fatty acids (PUFAs) and n-6 PUFAs are essential because they are not synthesized by the body and must be obtained through diet or supplementation. n-6 PUFAs, such as arachidonic acid (AA), are pro-thrombotic and pro-inflammatory. In contrast, n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are anti-thrombotic and anti-inflammatory. EPA therapy improves several metabolic problems associated with obesity, including insulin resistance, liver and heart steatosis, and hypertension; it also shows great promise in the secondary prevention of CAD. Moreover, low serum EPA/AA ratios correlate with abdominal visceral adipose tissue (AVAT) accumulation.

The present study aimed to investigate the effects of purified EPA on visceral adipose tissue volume (EAT and AVAT) with multislice computed tomography (CT).

### Methods

#### Patient Population and Protocol

Because the impact of EPA on EAT and AVAT remain to be clarified, we calculated the necessary sample size using the triglyceride (TG) level, which is a known metabolic parameter affected by EPA. Based on a previously reported effect of EPA (31% reduction of TG), the estimated sample size was seven subjects per group. Sample size estimates were based on the following: standard deviation, 19; α-level, 0.05; and power, 80%. Therefore, a total of 30 consecutive patients who received conventional medical therapy for CAD were recruited at the Itoigawa General Hospital from April 2012 to September 2012. Patients were randomized into the control group (n=15, conventional therapy) and the EPA group (n=15, conventional therapy plus purified EPA 1800 mg/day). The envelope method was carried out by an independent administrator for group allocation (Table 1). The present study does not have either a study registration number or a URL.

Patients were excluded if they had atrial fibrillation, iodine-based contrast allergy, renal insufficiency (serum creatinine level ≥1.5 mg/dL), chronic inflammatory disease, coronary artery bypass graft surgery, or an EPA/AA ratio greater than 0.75, which was a cut-off value for the risk of major coronary events. The risk factors included in the analysis were hypertension (oral treatment with antihypertensive drugs or documented history), diabetes mellitus (diet therapy and oral treatment with hypoglycemic drugs), hypercholesterolemia (oral treatment with lipid-lowering drugs or a measurement >220 mg/dL), and cigarette
smoking.

In several studies, 12-24-week-periods have been used to assess decreases in AVAT\textsuperscript{18, 19}. Furthermore, an increase in adiponectine and a decrease in highsensitivity C-reactive protein (CRP) have been induced by a three-month regimen of oral EPA\textsuperscript{20}. Therefore, the EAT and AVAT volume were measured with multislice CT and blood samples were taken at baseline and after six months of conventional therapy with or without oral intake of purified EPA (1800 mg/day). The study protocol was approved by the institutional ethics committee of Itoigawa General Hospital, and written informed consent was obtained from all the patients.

**Definition of CAD**

Documented CAD encompassed one or more of the following: stable angina pectoris diagnosed with stress electocardiography or radioisotope or coronary angiography and previous acute coronary syndrome, including unstable angina and myocardial infarction, which had been diagnosed with coronary angiography and treated with percutaneous coronary intervention.

**Laboratory Testing**

Blood samples were taken from the antecubital vein after a minimum of 12 hours of fasting to determine serum levels of CRP, creatinine, glucose, lipids (total cholesterol, TG, high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]), polynaturated fatty acids (EPA, DHA, AA, and EPA/AA ratio), glycated hemoglobin (HbA1c, National Glycohemoglobin Standardization Program), and insulin. The patients' fatty acid compositions were determined by gas chromatography at SRL, Inc. (Tokyo, Japan). Briefly, total lipids in plasma were extracted according to Folch's procedure, followed by hydrolysis to free fatty acids (FFAs), which were esterified with potassium methoxide/methanol and boron trifluoride-methanol. The methylated fatty acids were analyzed using GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an omegawax250 capillary column (SUPELCO, Sigma-Aldrich Japan, Tokyo, Japan). The homeostatic model assessment insulin resistance (HOMA-IR) score was used as an index of insulin resistance based on the following formula: HOMA-IR = (fasting serum insulin [µU/mL] × fasting blood sugar [mg/dL])/405.

**EAT and AVAT Volume Measurements with Multislice CT**

For the EAT measurements, contrast-enhanced coronary CT angiography images were assessed using electrocardiography-gated cardiac CT scans on 64-slice MDCT (Aquilion, Toshiba Medical Systems, Otawara, Japan). The subjects were examined in a supine position with their arms stretched above their head. Nitroglycerin (0.3 mg) was administered to all subjects immediately before CT imaging, and an oral β-blocker (metoprolol, 20 or 40 mg) was administered one hour before CT imaging to ensure that the heart rates remained at <65 beats/min, if required. The coronary CT angiography protocol applied in the present study was as follows: slice collimation, 64 × 0.5 mm; gantry rotation time, 0.35 s; pitch, 0.175; scan time, 0.4 s; table feed, 10 mm/s; tube voltage, 120 kV; and tube current, 600 mA. At the time of scanning, a bolus of contrast media (Omnipaque, 350 mg iodine/mL or iopamidol, 370 mg iodine/mL) was intravenously injected through an antecubital vein via an 18-gauge catheter, followed by 30 mL of normal saline. A site within the ascending aorta was selected as the region of interest (ROI), and the scan was initiated when the CT density levels reached the target level (120 Hounsfield units [HU] higher than the baseline density). Volumetric measurements were performed on axial views with a 0.5-mm slice thickness. The superior border for EAT volume measurement was set at the lower surface of the left pulmonary artery origin, whereas the inferior border for the measurement was set at the left ventricular apex\textsuperscript{21}. The EAT area was calculated by tracing an ROI, which included the heart and EAT. The ROI was manually placed outside the line of the visceral pericardium on the cross-sectional axial image. The area outside the traced pericardium was excluded (Fig.1).

For determining the abdominal fat volume, all subjects underwent CT at the umbilical level to measure the cross-sectional AVAT area. In addition, we also calculated the subcutaneous adipose tissue (SAT) area. The EAT and abdominal adipose volumes were quantified by calculating the total volume of tissue with a CT density between -150 and -30 HU (Fig.1).

EAT and AVAT volume CT measurements were performed by an experienced cardiologist who was blinded to the quantitative analysis data and the patients’ baseline clinical characteristics.

**Statistical Analysis**

All statistical analyses were performed using Sigma-Plot software program (version 11, Systat Software, Inc., San Jose, CA, USA). The data are presented as means ± standard deviations (SDs). Comparisons among the two groups were performed by χ\textsuperscript{2} or Fisher’s exact test for categorical data. Correlations
Results

The patients’ clinical characteristics are shown in Table 2. There were no significant differences in age, sex, body weight, hypertension prevalence, lipid pro-

Fig. 1. Measurement of epicardial adipose tissue (EAT) and abdominal visceral adipose tissue (AVAT) volumes with multislice computed tomography (CT). See text for details. (a) Three-dimensional image. EAT area was measured on each axial image from the lower surface of the left pulmonary artery origin to the left ventricular apex, and total EAT volume was obtained by multiplying the total EAT areas by the slice thickness. (b) Axial images. A region of interest (ROI) was manually placed along the visceral pericardium (left and middle panels). EAT was extracted on an axial image (green) (right panel). (c) Axial images. A ROI was placed at the umbilical level. AVAT is shown in red, and subcutaneous adipose tissue is shown in blue.
Changes in Laboratory Data

After six months of follow-up, TG level and glycemic markers, including HbA1c and HOMA-IR score, decreased in the EPA group but not in the control group. The CRP level tended to decrease more in the EPA group compared to the control group (Table 3). The changes in EPA and EPA/AA correlated with the changes in HOMA-IR (Fig. 2), and the changes in DHA correlated with the changes in HOMA-IR ($R = -0.02, p = 0.89$).

**Correlations between Metabolic Parameters**

The change in the TG level positively correlated with that in EAT volume ($R = 0.47, p = 0.01$). Furthermore, the change in TG level had a marginal positive correlation with that in the AVAT volume ($R = 0.34, p = 0.07$). Meanwhile, although the change in HOMA-IR positively correlated with the change in AVAT volume ($R = 0.42, p = 0.02$), the change in HOMA-IR did not correlate with the change in EAT volume ($R = 0.25, p = 0.21$).

**Changes in EPA and EPA/AA**

After six months, EPA levels were significantly
Atherogenic Properties of EAT, AVAT, and SAT

Local fatty acids and EAT-derived bioactive molecules, including inflammatory and oxidative stress mediators, are pathophysiological candidates for atherogenesis via their diffusion in the interstitial fluid across the adventitia and the arterial media or their transport through the vasa vasorum to cells in atherosclerotic plaques.22 In addition, epicardial and pericardial fat volumes are both strongly correlated with the number of atherosclerotic plaques.8 The inflammatory status of the EAT in obese patients who were candidates for coronary artery bypass graft was more severe than that of subcutaneous fat located in the legs, indicating that the inflammatory status of EAT increased in the EPA group (p < 0.01), but not in the control group. Similarly, the EPA/AA ratio significantly increased in the EPA group (p < 0.01), but not in the control group (Fig. 3).

Changes in EAT, AVAT, and SAT Volume by EPA

The EAT, AVAT, and SAT volumes decreased in the EPA group but not in the control group (Table 3). The change in AVAT volume negatively correlated with the changes in EPA (Fig. 4a) and EPA/AA (Fig. 4b) but not with the changes in DHA (R = −0.19, p = 0.31). The change in EAT volume also correlated negatively with the change in EPA (Fig. 4a) and in EPA/AA (Fig. 4b) but not the change in DHA (R = −0.18, p = 0.35). However, the SAT volume change did not significantly correlate with the change in EPA (R = −0.24, p = 0.20), EPA/AA(R = −0.20, p = 0.30), or DHA (R = −0.17, p = 0.38).

Discussion

The major findings of the present study are as follows: 1) EPA administration led to decreases in the EAT and AVAT volumes, and these changes negatively correlated with the EPA and EPA/AA values. This effect was not dependent on changes in body weight; 2) EPA administration led to improvements in the metabolic parameters, including TG levels and insulin sensitivity.
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Table 3. Change in parameters after the six-month follow-up

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>EPA</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔBody Weight (kg)</td>
<td>0.47 ± 0.22</td>
<td>0.35 ± 0.83</td>
<td>N.S.</td>
</tr>
<tr>
<td>Glycemic marker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔHbA1c (%)</td>
<td>0.03 ± 0.17</td>
<td>-0.15 ± 0.23</td>
<td>0.03</td>
</tr>
<tr>
<td>ΔHOMA-IR</td>
<td>0.19 ± 0.59</td>
<td>-0.50 ± 0.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔLDL-C (mg/dL)</td>
<td>-0.8 ± 12.6</td>
<td>5.3 ± 15.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>ΔTG (mg/dL)</td>
<td>32.4 ± 34.0</td>
<td>-4.0 ± 39.4</td>
<td>0.01</td>
</tr>
<tr>
<td>ΔHDL-C (mg/dL)</td>
<td>-2.3 ± 8.4</td>
<td>0.3 ± 6.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>ΔCRP (mg/dL)</td>
<td>-0.04 ± 0.35</td>
<td>-0.17 ± 0.24</td>
<td>0.08</td>
</tr>
<tr>
<td>Visceral adipose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔEAT (cm³)</td>
<td>8.8 ± 13.6</td>
<td>-11.6 ± 17.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ΔAVAT (cm²)</td>
<td>8.7 ± 8.8</td>
<td>-7.3 ± 8.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ΔSAT (cm²)</td>
<td>1.5 ± 12.2</td>
<td>-4.6 ± 11.0</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Figures are mean ± SD. HOMA-IR, homeostatic model assessment insulin resistance; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein; EAT, epicardial adipose tissue; AVAT, abdominal visceral adipose fat; SAT, abdominal subcutaneous adipose tissue; N.S., not significant.

could aggravate vascular inflammation, plaque instability, and neovascularization. In general, AVAT has been reported to be a major contributor to metabolic risk, whereas SAT has been suggested to play a protective role.

**Anti-Atherosclerotic Effects of n-3 PUFAs**

EPA is associated with reductions in all-cause mortality, as well as coronary artery disease mortality, which indicates that EPA exerts anti-atherosclerotic effects. As AA and EPA compete for cyclooxygenases (COXs), and both EPA and DHA directly inhibit COX activities, a relatively small increase in plasma n-3 PUFAs levels would significantly inhibit the synthesis of eicosanoids from AA. n-3 PUFAs are a naturally occurring potent regulator of adiponectin secretion, and they exert insulin-sensitizing, anti-atherogenic, and anti-inflammatory effects in vitro.

In fact, lesions in LDL-receptor-deficient, EPA-treated mice contained more collagen and smooth muscle cells and fewer macrophages compared to those that did not receive EPA. In human umbilical vein endothelial cells, EPA pretreatment attenuated the TNF-α-induced up-regulations of vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and monocyte chemoattractant protein-1, as well as the expression levels of matrix metalloproteinase (MMP)-2 and MMP-9 in macrophage-like cells. Furthermore, novel families of lipid mediators derived from n-3 PUFAs, such as resolvins and protectins, are potent locally acting agents involved in the process of acute inflammation and its resolution.

Moreover, n-3 PUFAs induce a shift from the M1 pro-inflammatory macrophage state to the M2-polarized macrophage state. Adipose tissue macrophages of lean mice were found to express many genes that are characteristic of M2 or “alternatively activated” macrophages, such as arginase 1 and IL-10. However, diet-induced obesity decreased the expression of these genes in adipose tissue macrophages but increased the expression of genes encoding TNF-α and inducible nitric oxide synthase (iNOS) that are characteristic of M1 or “classically activated” macrophages. In obese, dyslipidemic patients, n-3 PUFAs administration increased IL-10 expression in peripheral blood monocytes through a peroxisome proliferator-activated receptor (PPAR)-γ-dependent pathway, which indicates a shift from the M1 pro-inflammatory state to the M2-polarized state.

Furthermore, n-3 PUFAs inhibit Toll-like receptor 4 signaling on macrophages, which eventually suppresses adipose tissue inflammation and levels of inflammatory cytokines, including TNF-α, as well as the production of anti-atherosclerotic hormones, especially adiponectin.

The protective effect of n-3 long-chain (LC)-PUFAs on adipose tissue accumulation was stronger for DHA than for EPA and it was partially due to the inhibition of fat cell proliferation. In experiments using cell cultures, DHA inhibited adipocyte differentiation and induced apoptosis in post-confluent preadipocytes. As previously reported, the present study demonstrated that there was an increase in EPA...
followed by oral intake EPA, but there were no changes in DHA or plasma phospholipid levels. Therefore, it is difficult to evaluate the effect of DHA on AVAT and EAT based on our findings.

In the present study, a correlation was found between the metabolic parameters, including the TG levels and the HOMA-IR score and decreases in the EAT and AVAT volumes. As an anti-hypertriglyceridemic agent, EPA might contribute to a decrease in the adipose tissue volume, thus leading to an improved HOMA-IR score. In adipocytes, TG is synthesized from FFA esterified to a glyceride-glycerol backbone. Dysfunctional adipose tissue in obese subjects produces more pro-inflammatory factors, such as FFAs, and fewer anti-inflammatory factors, such as adiponectin. These events exacerbate the risk for developing metabolic diseases. An EPA-mediated decrease in adipose tissue volume might contribute to reduced serum TG levels. Therefore, oral EPA intake could reduce the volumes of EAT and AVAT and TG levels, thereby exerting an anti-atherosclerotic effect.

**Mechanisms of Decreased EAT and AVAT Volumes by EPA**

In the present study, EPA administration led to decreases in EAT and AVAT volumes, which may have occurred through several potential underlying mechanisms. The first involves n-3 PUFA, which is an anti-hypertriglyceridemic and anti-obesity agent, and it inhibits lipogenesis and stimulates fatty acid β-oxidation in the liver. β-oxidation of fatty acids is stimulated via peroxisome PPAR-α, which is induced by EPA. However, lipogenesis is inhibited through down-regulation of the mature form of sterol regulatory element-binding protein-1 in the liver. In rodent models, PPAR-α activation has been found to be related to the anti-obesity effect of fish oil.

Secondly, EPA activates PPAR-γ. PPAR-γ agonists improve insulin resistance by decreasing the expression of molecules that cause insulin resistance, such as TNF-α. PPAR-γ agonists also decrease TG content in muscle/liver tissue and prevent adipocyte hypertrophy, thereby causing the differentiation of adipocytes to small adipocytes. This may be the mechanism through which the combination of telmisartan and a PPAR-γ agent decreases the accumulation of abdominal visceral fat, as reported in a clinical study.

Third, EPA might activate GPR120, a G protein-coupled receptor. GPR120 is highly expressed in enteroendocrine cells in the intestinal tract and in adipose tissue, where it functions as a receptor for n-3 PUFA. A loss-of-function variant of GPR120 was associated with an increased risk for obesity in European populations. However, an adequate dietary intake of n-3 PUFA might compensate for slight defects in GPR120 activity. Therefore, the present study findings indicate that EPA might activate...
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In the present study, the EPA group exhibited a significant improvement in insulin sensitivity compared to the control group, and the changes in HOMA-IR values significantly correlated with the changes in EPA levels. These findings are consistent with previous studies \(49, 50\).

We found that the change in SAT volume did not significantly correlate with the changes in EPA or EPA/AA. AVAT and EAT induce a pro-inflammatory state, but SAT does not. Therefore, the anti-inflammatory effect of EPA might influence AVAT and EAT more strongly than SAT.

**EPA and Diabetes Mellitus**

In wild-type mice, dietary n-3 PUFA but not n-6

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**Fig. 3.** Changes in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), and EPA/AA levels in the two groups.
cause a decrease in TNF-α production, and stimulate PPAR-γ by EPA 43, 32). Although a high n-3 PUFA intake might have moderate unfavorable effects on glucose levels and insulin sensitivity, the daily intake of approximately one gram of purified EPA appears to have negligible unfavorable effects on metabolic control in patients with type two diabetes who are at risk of developing cardiovascular diseases 53).

PUFA administration restored insulin sensitivity 51). The potential mechanisms through which EPA improved insulin sensitivity are as follows: a decrease in diacylglycerol might activate protein kinase C-ε in the liver 51); an increase in adiponectin secretion could activate AMP kinase and PPAR-α in the liver and skeletal muscle 52); and a shift from the M1 pro-inflammatory state to the M2-polarized state, may
Limitations

The present study is associated with some limitations. First, the number of study patients was too small to draw any definite conclusions. The number of women subjects was too small to evaluate gender differences in response to EPA. Second, there might be some EPA response differences in patients with or without metabolic syndrome; this could not be evaluated due to the small sample size. Third, we evaluated EAT and AVAT volumes using multislice CT, but assessments with other methods, including sonography, might be necessary. Fourth, it remains to be determined whether changes in EPA levels exert a cardioprotective effect. Although we estimated the power to detect statistically significant differences in this design, large studies involving multiple centers and long-term follow-up are warranted to confirm the present results.

Clinical Implication and Conclusions

Although this study had several limitations, the results showed that the oral intake of purified EPA appears to be associated with reductions in the EAT and AVAT volumes.

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Conflicts of Interest

There are neither any conflicts of interest nor funding to declare related to this work.

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