Purified Eicosapentaenoic Acid Reduces Small Dense LDL, Remnant Lipoprotein Particles, and C-Reactive Protein in Metabolic Syndrome

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Eicosapentaenoic acid (EPA), one representative of n-3 unsaturated fatty acids (n-3 PUFAs), is clinically used for its lipid-lowering effects (1). n-3 PUFAs were shown to exert various physiological functions such as antiplatelet actions (by antagonizing effects of arachidonic acid) and plaque stabilization (2,3). Several epidemiological studies have explored antiatherogenic and cardioprotective effects of n-3 PUFA that are abundantly contained in fish oil (4). Dyslipidemia accompanying the metabolic syndrome is often associated with elevated levels of remnant lipoprotein particles and small dense LDL (sdLDL), which are newly recognized risk factors for cardiovascular disease (CVD) (5). It was reported that fish oil improved lipoprotein subclass profiles in subjects with an atherogenic lipoprotein phenotype (6). Besides EPA, docosahexaenoic acid and cholesterol are present in fish oil (7), but it is not clear whether purified EPA independently affects lipoprotein subclass profiles. Therefore, we used purified EPA ethyl ester and examined effects of EPA on atherogenic sdLDL particles and remnant lipoprotein particles in the metabolic syndrome, a precursor of CVD. Furthermore, sdLDL has been reported to synergistically interact with inflammation in pathophysiologic processes leading to CVD (8). Therefore, we simultaneously measured effects of EPA on C-reactive protein (CRP), a marker of inflammation, and examined how alteration of lipoprotein profiles by EPA affects systemic inflammation.

RESEARCH DESIGN AND METHODS

A total of 44 Japanese obese type 2 diabetic patients were recruited in our clinics (Table 1). All patients satisfied the definition and diagnostic criteria of the metabolic syndrome proposed by the National Metabolic Syndrome Criteria Study Group of Japan in 2005 (9). Accordingly, an individual is diagnosed with metabolic syndrome if he or she has central adiposity plus two or more of the following three factors: 1) raised concentration of triglycerides ($\geq$150 mg/dl) or reduced concentration of HDL cholesterol ($<$40 mg/dl); 2) raised blood pressure: systolic blood pressure ($\geq$130 mmHg) or diastolic blood pressure ($\geq$85 mmHg) or treatment of previously diagnosed hypertension; and 3) raised fasting plasma glucose concentration ($\geq$110 mg/dl). The thresholds for waist circumference to define central adiposity were $\geq$85 cm for men and $\geq$90 cm for women. The study protocol was approved by the ethical committee on human research of the Kyoto Medical Center, and all participants gave written informed consent. Patients were assigned to one of the following treatment groups (a single-blind and a run-in period randomization, which patients received): 1) treated for 3 months with either diet alone (the control group) (8 men and 14 women; mean $\pm$ SE age 51.6 $\pm$ 3.2 years) or 2) diet plus EPA (1.8 g daily) (the EPA group) (8 men and 14 women; mean age 51.6 $\pm$ 2.8 years). The subjects in the EPA group received an EPA capsule containing highly purified ($>$98%) EPA ethyl ester. Patient's diets are based on the Japan Atherosclerosis Society Guidelines for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases, consisting of 25 kcal/kg of ideal body weight per day (60% of total energy as carbohydrates, 15–20% as protein, and 20–25% as fat with the ratio of polyunsaturated, monounsaturated, and saturated fatty acids being 3:4:3). Lipid-lowering medications such as statins and fibrates were excluded.

At the beginning and at the end of the study, we measured BMI, serum levels of EPA and arachidonic acid, and glycolipid parameters according to standard procedures. Remnant lipoprotein particle (RLP) cholesterol and RLP triglycerides were measured using an assay kit (Japan Immunoresearch Laboratories, Takasaki, Japan) (10). Plasma cholesteryl ester transfer protein (CETP) activity was measured using an assay kit (BioVision, Mountain View, CA) (11). LDL cholesterol subfractions were separated using the Quantimetrix Lipoprotein LDL system (12). According to the specific subfractions of LDL cholesterol obtained by this system (LDL3–7:sdLDL), the sdLDL proportion was defined as the percentage of sdLDL over the whole amount of LDL (13). Plasma level of CRP was measured

Table 1—*Baseline characteristics and effects of EPA on metabolic parameters, lipoprotein profiles, and CRP*

	Control		EPA	
	Before	3 months	Before	3 months
BMI (kg/m^2)	29.2 ± 0.91	29.1 ± 0.92	31.0 ± 1.22	30.8 ± 1.26
EPA (μg/ml)	79.2 ± 17.8	64.6 ± 11.6*	77.2 ± 11.5	125 ± 12.3†‡
Arachidonic acid (μg/ml)	145 ± 12.1	148 ± 8.08	165 ± 7.34	152 ± 9.72†
EPA/arachidonic acid	0.52 ± 0.10	0.44 ± 0.08*	0.48 ± 0.07	0.88 ± 0.11†‡
FPG (mmol/l)	6.88 ± 0.47	7.16 ± 0.62	6.16 ± 0.57	6.00 ± 0.52
A1C (%)	6.57 ± 0.25	6.47 ± 0.26	6.11 ± 0.20	6.02 ± 0.24
Insulin concentration (pmol/l)	101 ± 14.5	100 ± 12.0	104 ± 23.1	95.4 ± 15.1
Total cholesterol (mmol/l)	5.38 ± 0.21	5.30 ± 0.24	5.46 ± 0.19	5.15 ± 0.16
LDL cholesterol (mmol/l)	3.18 ± 0.16	2.97 ± 0.19	3.44 ± 0.15	3.18 ± 0.15
HDL cholesterol (mmol/l)	1.46 ± 0.07	1.38 ± 0.11	1.42 ± 0.08	1.39 ± 0.07
Triglyceride (mmol/l)	1.89 ± 0.19	1.62 ± 0.21	1.93 ± 0.20	1.57 ± 0.11
RLP cholesterol (mmol/l)	0.19 ± 0.02	0.16 ± 0.03	0.19 ± 0.02	0.15 ± 0.01
RLP triglyceride (mmol/l)	0.44 ± 0.06	0.58 ± 0.16	0.43 ± 0.09	0.26 ± 0.02*§
CETP activity (nmol · ml^{-1} · h^{-1})	203 ± 1.47	202 ± 2.26	205 ± 3.05	200 ± 1.75*
sdLDL (mmol/l)	0.26 ± 0.08	0.23 ± 0.08	0.23 ± 0.04	0.18 ± 0.04†
sdLDL proportion (%)	7.33 ± 2.18	6.82 ± 2.36	6.78 ± 1.28	5.51 ± 1.09†
CRP (mg/dl)	0.11 ± 0.03	0.10 ± 0.03	0.22 ± 0.08	0.08 ± 0.02*

Data are means ± SE. *$P < 0.05$, †$P < 0.01$ vs. before determined by two-tailed, paired t test. ‡$P < 0.01$, §$P < 0.05$ vs. control determined by Student's t test. FPG, fasting plasma glucose.

by the latex-enhanced assay using the particle-enhanced technology performed on the Behring BN nephelometer (Dade Behring, Marburg, Germany) (14). Data are presented as mean ± SE, and $P < 0.05$ was considered statistically significant. Repeated-measures ANOVA (control and EPA groups × before and after treatment) was used to access the comparative effects of EPA treatment on the measured variables. A two-tailed, paired t test was applied for the evaluation of changes from baseline conditions to those at 3 months. Comparisons of the means between the two groups at baseline or posttreatment were performed by Student's t test. All statistical analyses were performed using the Stat View program version 5.0 for Windows (SAS Institute, Cary, NC).

RESULTS — There were no significant differences between the control and EPA groups for all measured variables at baseline (Table 1). Treatment with EPA significantly increased EPA and EPA/arachidonic acid levels, while it decreased arachidonic acid levels compared with baseline levels ($P < 0.01$). Differences of EPA and EPA/arachidonic acid levels at 3 month were observed between the control and EPA groups ($P < 0.01$). EPA also caused significant overall effects on RLP triglyceride, CETP activity, sdLDL, and the proportion of sdLDL and CRP by repeated-measures ANOVA. There were also significant reductions in values compared with baseline by paired t test, despite no changes in BMI, fasting plasma glucose, A1C, insulin concentration, and HDL cholesterol in both groups. Significant reductions of total cholesterol, LDL cholesterol, triglycerides, and RLP cholesterol in the EPA group was observed ($P = 0.035, 0.004, 0.047,$ and 0.035, respectively) by two-tailed, paired t test, although there were no significant overall effects on those parameters by repeated-measures ANOVA. Increases in EPA/arachidonic acid for 3 months inversely correlated with decreases in RLP cholesterol, sdLDL, and CRP for 3 months ($P = 0.0379, 0.0479,$ and 0.0467, respectively). Furthermore, reduction in CRP with EPA treatment for 3 months showed a significant positive correlation with reductions in RLP cholesterol and sdLDL for 3 months ($P = 0.0075$ and 0.0142, respectively).

CONCLUSIONS — This study is the first to demonstrate that EPA significantly reduces serum sdLDL and CRP in the metabolic syndrome. Reduction of sdLDL by EPA treatment in this study is believed to be due to a suppression of triglycerides production in the liver by EPA. In addition, since CETP is an important enzyme in cholesterol metabolism—responsible for the transfer of cholesteryl esters from HDL to LDLs (11)—degradation of CETP activity by EPA treatment may also have contributed to the decrease in the generation of remnants and sdLDL. Furthermore, we detected that reductions in RLP cholesterol and sdLDL also correlated with a decrease in CRP by EPA, which was consistent with a previous report (8) showing that LDL particle size had a strong inverse association with CRP. Atherogenic sdLDL particles are susceptible to oxidative modifications; then, oxidized LDL is easily taken into macrophages through damaged endothelial cells, thereby inducing inflammation and early atherosclerotic lesions (5,15). On the other hand, CRP has also been shown to accelerate LDL modifications during inflammatory processes (8). These findings suggest that EPA may be capable of preventing the progression of atherosclerosis in the metabolic syndrome by suppressing reciprocal interactions of atherogenic lipoproteins and inflammation. There are several reports demonstrating that n-3 PUFA does not decrease CETP protein mass and CRP (16,17). This may be caused by the differences outlined in the research designs and methods of each report.

Recently, the Japan EPA Lipid Intervention Study reported that EPA provided further benefits in preventing major coronary events without changing reductions in LDL cholesterol levels (18). Considering the improvements in lipoprotein profiles by EPA in this study, EPA may exert cardioprotective effects not by changing the quantity but by improving the quality of LDL cholesterol.

Collectively, the present study is the first to demonstrate that purified EPA re-

duces sdLDL, remnants, and CRP, thereby potentially leading to the reduction in development of atherosclerosis and CVD in the metabolic syndrome.

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