Reduction of circulating FABP4 level by treatment with omega-3 fatty acid ethyl esters

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Abstract

Background: Fatty acid-binding protein 4 (FABP4/A-FABP/aP2) mainly expressed in adipocytes is secreted and acts as an adipokine. Increased circulating FABP4 level is associated with obesity, insulin resistance and atherosclerosis. However, little is known about the modulation of serum FABP4 level by drugs including anti-dyslipidemic agents.

Methods: Patients with dyslipidemia were treated with omega-3 fatty acid ethyl esters (4 g/day; n = 14) containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for 4 weeks. Serum FABP4 level was measured before and after treatment. Expression and secretion of FABP4 were also examined in mouse 3T3-L1 adipocytes treated with EPA or DHA.

Results: Treatment with omega-3 fatty acid ethyl esters significantly decreased triglycerides and serum FABP4 level (13.5 ± 1.5 vs. 11.5 ± 1.1 ng/ml, P = 0.017). Change in FABP4 level by omega-3 fatty acids was negatively correlated with change in levels of EPA + DHA (r = −0.643, P = 0.013), EPA (r = −0.540, P = 0.046) and DHA (r = −0.650, P = 0.011) but not change in the level of triglycerides or other fatty acid composition. Treatment of 3T3-L1 adipocytes with EPA or DHA had no effect on short-term (2 h) secretion of FABP4. However, gene expression and long-term (24 h) secretion of FABP4 were significantly reduced by treatment with EPA or DHA.

Conclusions: Omega-3 fatty acids decrease circulating FABP4 level, possibly by reducing expression and consecutive secretion of FABP4 in adipocytes. Reducing FABP4 level might be involved in suppression of cardiovascular events by omega-3 fatty acids.

Keywords: Adipokine, Fatty acid-binding protein 4, Adipocyte, Eicosapentaenoic acid, Docosahexaenoic acid

Background

Fatty acid-binding proteins (FABPs) are predominantly cytosolic proteins that bind hydrophobic ligands, such as long chain fatty acids [1–3]. It has been proposed that FABPs promote the transport of fatty acids to several organelles in the cell [1]. Fatty acid-binding protein 4 (FABP4), also referred to as adipocyte FABP (A-FABP) or aP2, is expressed in adipocytes and macrophages. Previous studies using FABP4-deficient mice have demonstrated that FABP4 plays important roles in the development of insulin resistance, diabetes mellitus and atherosclerosis [4–7], and chemical inhibition of FABP4 might be a novel therapeutic agent against insulin resistance, diabetes mellitus and atherosclerosis [8].

It has recently been shown that FABP4 is secreted from adipocytes via a non-classical secretion pathway during lipolysis [9–12], though the sequence of FABP4 lacks signal peptides [1]. Furthermore, FABP4 has been demonstrated to act as an adipokine for the development of insulin resistance in liver [10], suppression of cardiomyocyte contraction [13], inhibition of endothelial nitric oxide synthase in endothelial cells [14] and
proliferation and migration of vascular smooth muscle cells [15]. It has also been shown that an elevated serum level of FABP4 is associated with obesity, insulin resistance, hypertension, cardiac dysfunction, atherosclerosis and cardiovascular events [9, 16-23]. However, little is known about the alteration of FABP4 level by drugs including anti-dyslipidemic agents.

It has been reported that atorvastatin, a cholesterol-lowering statin, decreases FABP4 level [24]. Furthermore, omega-3 fatty acids have been reported to inhibit adipocyte differentiation and lipid accumulation in vitro, possibly leading to a decrease in the expression of FABP4, also known as a differentiation marker of adipocytes [25-27]. We hypothesized that modulation of lipid levels by drugs can decrease the circulating FABP4 level. In this study, we investigated the effect of omega-3 fatty acid ethyl esters containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on serum FABP4 level in patients with dyslipidemia. We also examined drug-induced regulation of the expression and secretion of FABP4 in adipocytes in the presence or absence of lipolytic stimulation.

**Methods**

Human study (Study 1) conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the Ethical Committee of Fujita Health University. Written informed consent was received from all of the subjects. Experimental procedures for in vitro study using mouse 3T3-L1 adipocytes (Study 2) were performed with approval from the Animal Care and Experiment Committee of Sapporo Medical University.

**Study 1: Effects of omega-3 fatty acids on serum FABP4 level**

Male patients with dyslipidemia (n = 14; mean age: 40.2 ± 1.7 years) were enrolled from outpatient clinics affiliated with Fujita Health University. Written informed consent was received from all of the subjects. Experimental procedures for in vitro study using mouse 3T3-L1 adipocytes (Study 2) were performed with approval from the Animal Care and Experiment Committee of Sapporo Medical University.

**Measurements**

Body mass index (BMI, kg/m²) was calculated as body weight divided by the square of body height. Before and after the 4-week treatment with omega-3 fatty acid ethyl esters, blood samples were collected after an overnight fast, and serum and plasma samples were analyzed immediately or stored at −80 °C until biochemical analyses. Serum FABP4 concentration was measured using an enzyme-linked immunosorbent assay kit (Biovendor R&D, Modrice, Czech Republic). Precision, accuracy and reproducibility of the kit have been described previously [9]. Hemoglobin A1c (HbA1c) was determined by a latex coagulation method and was expressed in NGSP scale. Plasma glucose and fasting plasma insulin was measured by the glucose oxidase method and a radioimmunoassay method, respectively. Levels of creatinine (Cr), triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol were determined by enzymatic methods. Level of low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation. The compositions of 24 fatty acids, including lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1ω5), palmitic acid (C16:0), palmitoleic acid (C16:1ω7), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2ω6), γ-linolenic acid (C18:3ω6), α-linolenic acid (C18:3ω3), arachidic acid (C20:0), eicosanoic acid (C20:1ω9), eicosadienoic acid (C20:2ω6), eicosatrienoic acid (C20:3ω9), dihomo-γ-linolenic acid (C20:3ω6), arachidonic acid (C20:4ω6), EPA (C20:5ω3), behenic acid (C22:0), erucic acid (C22:1ω9), docosatetraenoic acid (C22:4ω6), docosapentaenoic acid (C22:5ω3), lignoceric acid (C24:0), DHA (C22:6ω3) and nervonic acid (C24:1ω9) and were determined by using capillary gas chromatography. High-sensitivity C-reactive protein (hsCRP) was measured by a nephelometry method. Serum level of high-molecular weight (HMW)-adiponectin was measured using a commercially available chemiluminescent enzyme immunoassay kit (Fujirebio, Tokyo, Japan). Estimated glomerular filtration rate (eGFR), an index of renal function, was calculated by an equation for Japanese [28]: eGFR(ml/min/1.73m²) = 194 × Cr(-1.094) × age(-0.287) × 0.739 (if female). HOMA-IR, an index of insulin resistance, was calculated by the previously reported formula: insulin (µU/ml) × glucose (mg/dl) / 405.

**Study 2: Effects of omega-3 fatty acids on expression and secretion of FABP4 in mouse 3T3-L1 adipocytes**

All biochemical reagents were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Pre-adipocyte 3T3-L1 cells were purchased from Health Science Research Resources Bank (Osaka, Japan). Differentiation of 3T3-L1 cells into adipocytes was induced as previously described [11]. Differentiated 3T3-L1 adipocytes were stimulated for 2 h (short-term secretion analysis) in the presence and absence of 10 µM isoproterenol or 24 h (long-term secretion and gene expression analyses) with 0–100 µM EPA or 0–100 µM DHA in Dulbecco’s Modified Eagle’s Medium (DMEM)
Quantitative real-time PCR
Total RNA was isolated using Trizol Reagent (Invitrogen), and cDNA was synthesized using a kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR analysis using SYBR Green was performed (Applied Biosystems). The thermal cycling program was 10 min at 95°C for enzyme activation and 40 cycles of denaturation for 15 s at 95°C, 30-s annealing at 58°C and 35-s extension at 72°C. Two pairs of specific primers used are as follows: 5’-AAG GTG AAG AGC ATC ATA ACC CT -3’ and 5’- TCA CGC CTT TCA TAA CAC ATT CC -3’ for FABP4, 5’- TCG CTG ATG CAC TGC CTA TG -3’ and 5’- GAG AGG TCC ACA GAG CTG ATT -3’ for peroxisome proliferator-activated receptor (PPAR)-γ2, 5’- CAA GAA CAG CAA CGA GTA CCG -3’ and 5’- GTC ACT GGT CAA CTC CAG CAC -3’ for CCAAT/enhancer binding protein α (C/EBPα) and 5’- AGT CCC TGC CCT TTG TAC ACA -3’ and 5’- CGA TCC GAG GGC CTC ACT A -3’ for 18s rRNA as an internal control gene.

Results
Study 1
Characteristics of the patients are shown in Table 1. No one dropped out from the protocol in Study 1. Treatment with omega-3 fatty acid ethyl esters for 4 weeks significantly decreased triglycerides (163.7 ± 20.6 vs. 98.1 ± 11.4 mg/dl, P = 0.003), but no significant

| Variables       | Pre          | Post         | P     |
|-----------------|--------------|--------------|-------|
| n               | 14           |              |       |
| Age (years)     | 40.2 ± 1.7   | 26.1 ± 0.7   |       |
| Body mass index | 25.9 ± 0.7   | 26.1 ± 0.7   |       |
| Waist circumference (cm) | 89.5 ± 1.8  | 89.0 ± 2.0   |       |
| Biochemical data |              |              |       |
| Total cholesterol (mg/dl) | 217.2 ± 7.1 | 207.6 ± 8.5 |       |
| HDL cholesterol (mg/dl) | 57.6 ± 3.7  | 57.6 ± 4.3   |       |
| LDL cholesterol (mg/dl) | 132.6 ± 6.5 | 125.8 ± 8.3  |       |
| Triglycerides (mg/dl) | 163.7 ± 20.6 | 98.1 ± 11.4* |       |
| Glucose (mg/dl) | 97.6 ± 2.2   | 98.0 ± 1.8   |       |
| Insulin (μU/ml) | 7.9 ± 1.9    | 6.5 ± 1.2    |       |
| HOMA-IR         | 1.99 ± 0.56  | 1.57 ± 0.28  |       |
| HbA1c (%)       | 5.5 ± 0.1    | 5.5 ± 0.1    |       |
| hsCRP (mg/dl)   | 0.60 ± 0.17  | 0.75 ± 0.20  |       |
| HMW-adiponectin (μg/ml) | 2.42 ± 0.23 | 2.31 ± 0.23  |       |
| FABP4 (ng/ml)   | 13.5 ± 1.5   | 11.5 ± 1.1*  |       |

Variables are expressed as n or means ± SEM. hsCRP, high-sensitivity C-reactive protein; HMW, high-molecular weight. *P <0.05 vs. Pre
differences were found before and after treatment in waist circumference, BMI or levels of glucose, insulin, HOMA-IR, total cholesterol, LDL cholesterol, HDL cholesterol, hsCRP or HMW-adiponectin. Regarding fatty acid composition, treatment with omega-3 fatty acid ethyl esters significantly increased EPA (48.6 ± 5.3 vs. 144.6 ± 12.4 μg/ml, \( P < 0.001 \)), DHA (139.8 ± 8.2 vs. 179.5 ± 7.6 μg/ml, \( P < 0.001 \)), behenic acid and docosapentaenoic acid and conversely decreased palmitic acid, palmitoleic acid, linoleic acid, \( \gamma \)-linolenic acid, eicosadienoic acid, eicosatetraenoic acid, arachidonic acid, docosatetraenoic acid and total fatty acids (Table 2). Serum FABP4 level was significantly decreased by 14.8 % after treatment with

### Table 2 Fatty acid composition (Omega-3 FAs, 4w)

| Saturated fatty acids | \( \mu g/ml \) | %vol | \( \mu g/ml \) | %vol |
|-----------------------|----------------|-----|----------------|-----|
| Lauric acid C12:0     | 5.4 ± 0.9      | 0.16 ± 0.02 | 3.4 ± 0.6      | 0.12 ± 0.02 |
| Myristic acid C14:0   | 31.0 ± 4.2     | 0.89 ± 0.09 | 20.7 ± 2.2     | 0.71 ± 0.06 |
| Palmitic acid C16:0   | 607.5 ± 40.8   | 17.7 ± 0.4  | 491.0 ± 18.3*  | 17.0 ± 0.3  |
| Stearic acid C18:0    | 3046.5 ± 20.0  | 89.0 ± 0.3  | 2652.2 ± 11.0  | 92.2 ± 0.2 |
| Arachidic acid C20:0  | 20.0 ± 0.2     | 0.058 ± 0.004 | 1.6 ± 0.1      | 0.055 ± 0.004 |
| Behenic acid C22:0    | 1.1 ± 0.1      | 0.0077 ± 0.0077 | 1.9 ± 0.2*  | 0.092 ± 0.014* |
| Lignoceric acid C24:0 | 0.81 ± 0.006   | 0.025 ± 0.001 | 0.75 ± 0.03    | 0.027 ± 0.002 |

| Monounsaturated fatty acids | \( \mu g/ml \) | %vol | \( \mu g/ml \) | %vol |
|-----------------------------|----------------|-----|----------------|-----|
| Myristoleic acid C14:1\( \omega \)5 | 3.0 ± 0.6 | 0.083 ± 0.016 | 1.4 ± 0.2* | 0.048 ± 0.006 |
| Palmitoleic acid C16:1\( \omega \)7 | 66.2 ± 6.8 | 1.9 ± 0.1 | 42.2 ± 3.9* | 1.4 ± 0.1* |
| Oleic acid C18:1\( \omega \)9 | 749.7 ± 66.9 | 21.6 ± 0.9 | 526.7 ± 36.5* | 18.0 ± 0.6* |
| Eicosenoic acid C20:1\( \omega \)9 | 6.0 ± 0.7 | 0.17 ± 0.001 | 5.3 ± 0.6 | 0.18 ± 0.02 |
| Erucic acid C22:1\( \omega \)9 | 34.0 ± 0.2 | 0.10 ± 0.001 | 3.3 ± 0.1 | 0.11 ± 0.01 |
| Nervonic acid C24:1\( \omega \)9 | 1.9 ± 0.1 | 0.060 ± 0.0048 | 1.9 ± 0.1 | 0.068 ± 0.005 |

| Polyunsaturated fatty acids | \( \mu g/ml \) | %vol | \( \mu g/ml \) | %vol |
|-----------------------------|----------------|-----|----------------|-----|
| \( \alpha \)-Linolenic acid C18:3\( \omega \)3 | 26.9 ± 2.7 | 0.78 ± 0.05 | 22.3 ± 2.8 | 0.75 ± 0.07 |
| Eicosapentaenoic acid (EPA) C20:5\( \omega \)3 | 486.5 ± 53 | 1.5 ± 0.2 | 144.6 ± 12.4* | 5.0 ± 0.6* |
| Docosapentaenoic acid C22:5\( \omega \)3 | 198.8 ± 1.2 | 0.59 ± 0.04 | 25.7 ± 1.4* | 0.90 ± 0.05* |
| Docosahexaenoic acid (DHA) C22:6\( \omega \)3 | 139.8 ± 82 | 4.2 ± 0.3 | 1795.7 ± 6.4* | 6.4 ± 0.4* |

| Omega-6 fatty acids | \( \mu g/ml \) | %vol | \( \mu g/ml \) | %vol |
|---------------------|----------------|-----|----------------|-----|
| Linoleic acid C18:2\( \omega \)6 | 1064.3 ± 46.2 | 31.6 ± 0.9 | 894.0 ± 54.3* | 30.6 ± 1.1 |
| \( \gamma \)-Linolenic acid C18:3\( \omega \)6 | 110.0 ± 1.5 | 0.32 ± 0.04 | 64.0 ± 1.0* | 0.22 ± 0.03* |
| Eicosadienoic acid C20:2\( \omega \)6 | 69.0 ± 0.5 | 0.20 ± 0.01 | 4.7 ± 0.3* | 0.16 ± 0.01* |
| Dihomo-\( \gamma \)-Linolenic acid C20:3\( \omega \)6 | 472.3 ± 34 | 1.4 ± 0.1 | 27.4 ± 3.1* | 1.0 ± 0.1* |
| Arachidonic acid (AA) C20:4\( \omega \)6 | 2480.0 ± 148 | 7.5 ± 0.4 | 2224.0 ± 12.1* | 7.8 ± 0.3 |
| Docosatetraenoic acid C22:4\( \omega \)6 | 47.0 ± 3 | 0.14 ± 0.01 | 2.8 ± 0.2* | 0.10 ± 0.01* |

| Omega-9 fatty acids | \( \mu g/ml \) | %vol | \( \mu g/ml \) | %vol |
|---------------------|----------------|-----|----------------|-----|
| Eicosatetraenoic acid C20:3\( \omega \)9 | 1.5 ± 0.1 | 0.045 ± 0.002 | 1.1 ± 0.1* | 0.035 ± 0.002* |

| Total fatty acids | \( \mu g/ml \) | %vol | \( \mu g/ml \) | %vol |
|------------------|----------------|-----|----------------|-----|
| 3402.8 ± 181.2 | 100 | 2899.1 ± 1200 | 100 |

| Calculation | \( \mu g/ml \) | %vol |
|-------------|----------------|-----|
| EPA/AA | 0.20 ± 0.02 | 0.68 ± 0.07* |
| DHA/AA | 0.57 ± 0.03 | 0.83 ± 0.04* |
| EPA + DHA | 1884.4 ± 12.2 | 3242.4 ± 18.6* | 5.7 ± 0.5 | 11.4 ± 1.0* |
| (EPA + DHA)/AA | 0.78 ± 0.05 | 1.50 ± 0.11* |

Variables are expressed as n or means ± SEM. *P <0.05 vs. Pre.
omega-3 fatty acid ethyl esters (13.5 ± 1.5 vs. 11.5 ± 1.1 ng/ml, \( P = 0.017 \)) (Fig. 1a). Change (Post - Pre) in FABP4 level was negatively correlated with change in levels of EPA + DHA (\( r = -0.643, P = 0.013 \)) (Fig. 1b), EPA (\( r = -0.540, P = 0.046 \)) (Fig. 1c) and DHA (\( r = -0.650, P = 0.011 \)) (Fig. 1d). However, change in FABP4 level was not correlated with change in levels of total fatty acids (\( r = -0.003, P = 0.911 \)) (Fig. 1e), triglycerides (\( r = -0.019, P = 0.948 \)) (Fig. 1f) or other fatty acid composition.

**Study 2**
Treatment with both EPA and DHA for 24 h significantly decreased gene expression of FABP4 in 3T3-L1 adipocytes in a dose-dependent manner, and the effect tended to be more augmented with DHA treatment than with EPA treatment (Fig. 2a, b). Furthermore, both EPA and DHA significantly decreased gene expression of PPAR\( \gamma \)2 and C/EBP\( \alpha \) in 3T3-L1 adipocytes in a dose-dependent manner (Fig. 2c-f). Western blot analysis showed that FABP4, but not a non-secretory protein GAPDH, was present in the CM of 3T3-L1 (Fig. 2g, h), indicating that FABP4 is secreted from adipocytes without cell destruction. FABP4 secretion was induced by lipolytic stimulation with 10 \( \mu \)M isoproterenol, a pan-\( \beta \)-adrenergic agonist, for 2 h (Fig. 2g) as previously reported [11]. Treatment with 50 \( \mu \)M EPA or 50 \( \mu \)M DHA for 2 h (short-term) did not significantly change FABP4 secretion from adipocytes in the absence or presence of 10 \( \mu \)M isoproterenol (Fig. 2g). However, a
significant decrease of FABP4 secretion from adipocytes was observed at 24 h (long-term) after treatment with 50 μM EPA or 50 μM DHA (Fig. 2h).

**Discussion**

The present study demonstrated for the first time that treatment with omega-3 fatty acid ethyl esters containing EPA and DHA for 4 weeks significantly decreased serum FABP4 concentration in patients with dyslipidemia. Furthermore, *in vitro* experiments showed that treatment with EPA or DHA dose-dependently (up to 100 μM) decreased the expression and consecutive long-term secretion, but not short-term secretion, of FABP4 in 3T3-L1 adipocytes. Average changes in the levels of EPA and DHA by treatment with omega-3 fatty acid ethyl esters were 96.0 μg/ml (317 μM) and 39.7 μg/ml (121 μM), respectively, in the present study, indicating that the doses of EPA and DHA in *in vitro* experiments
were physiological but not pharmacological. These findings suggest that a direct suppressive effect of omega-3 fatty acids on FABP4 expression and its consequent secretion in adipocytes plays a role in the decrease in serum FABP4 level.

In the present study, treatment with omega-3 fatty acid ethyl esters significantly decreased triglycerides and total fatty acids and modulated levels of fatty acid composition. However, changes in the levels of triglycerides, total fatty acids and each fatty acid composition except for EPA and DHA were not correlated with reduction of FABP4 level, suggesting that qualitative, but not quantitative, change in fatty acids contributes to the reduction in FABP4 concentration. It has been reported that expression of FABP4 in adipocytes is up-regulated by PPARγ agonists and saturated and monounsaturated fatty acids [1, 2, 30–32]. On the other hand, polyunsaturated fatty acids, such as omega-3 fatty acids, have been reported to inhibit adipocyte differentiation and lipid accumulation, leading to a decrease in the expression of FABP4, also known as an adipocyte differentiation marker [25–27]. Furthermore, it has been shown that unsaturated fatty acids, including EPA, repress expression of FABP4 in RAW264.7 macrophages [33], though the predominant contributors of circulating FABP4 are adipocytes rather than macrophages [10]. In the present study, we showed that both EPA and DHA decreased FABP4 expression even in differentiated 3T3-L1 adipocytes, at least in part, via reduction in gene expression of PPARγ2 and C/EBPα, which are critical transcription factors for regulation of adipocyte differentiation [34].

There have been some reports about modulation of FABP4 concentration by drugs. Atorvastatin, a cholesterol-lowering statin, has been reported to decrease FABP4 level, but the mechanism is totally unknown [24]. It has also been shown that several angiotensin II receptor blockers (ARBs) decrease circulating FABP4 concentration [29, 35], and reduction of sympathetic nerve activation due to a class effect of ARBs, but not a direct effect of angiotensin II receptor blockade, has been postulated as a possible mechanism of decreased FABP4 level by ARBs [29], since FABP4 secretion from adipocytes is associated with β-adrenergic receptor-mediated lipolysis [10, 11]. Previous studies showed that dietary fish and omega-3 fatty acid consumption decreased sympathetic nerve activity [36, 37]. Other than direct suppressive effects of EPA and DHA on FABP4 expression in adipocytes, treatment with omega-3 fatty acid ethyl esters containing EPA and DHA may decrease FABP4 concentration by inhibiting FABP4 secretion from adipocytes associated with sympathetic tone-mediated lipolysis.

Previous studies including a recent meta-analysis have shown that both dietary and circulating EPA and DHA are associated with the low incidence of cardiovascular disease in a multiethnic population [38, 39]. Furthermore, recent clinical trials have demonstrated that omega-3 fatty acids, including EPA alone, EPA + DHA and fish oil, substantially reduce cardiovascular events [40–43]. Since there is accumulating evidence demonstrating significant roles of circulating FABP4 in insulin resistance, atherosclerosis and cardiovascular events [10, 14, 15, 17, 21–23], the results of the present study support the notion that reduction of FABP4 concentration is one of the important mechanisms by which omega-3 fatty acids prevent the development of cardiovascular disease.

The present study has several limitations. The number of patients recruited in Study 1 was small, and the possibility of a type 1 error cannot be excluded. Since it has been reported that there is a gender difference in serum FABP4 levels [9, 18], we recruited only male patients to reduce confounding factors. The present study also lacked a placebo control group. Interventional studies of placebo-control design using larger numbers of both male and female patients are necessary for evaluating the impact of omega-3 fatty acids on circulating FABP4 level. Furthermore, mouse 3T3-L1 adipocytes, a well-used cell line of adipocytes, were used in vitro experiments, but there might be a difference between mice and humans in regulation of the expression and secretion of FABP4 by omega-3 fatty acids. Lastly, there has been accumulating evidence indicating FABP4 is expressed in several types of cells, in addition to adipocytes and macrophages, under both physiological and pathological conditions [2, 3, 44–48]. Omega-3 fatty acids may affect the expression and secretion of FABP4 in cells other than adipocytes, though the predominant contributors of circulating FABP4 level are adipocytes rather than macrophages and other cells [3, 10].

Conclusions
Treatment with omega-3 fatty acid ethyl esters containing EPA and DHA decreases serum FABP4 concentration in patients with dyslipidemia, at least in part, via a direct reduction in expression and consecutive secretion of FABP4 in adipocytes. Suppression of FABP4 levels may lead to the reduction of cardiovascular events as a pleiotropic effect of supplements of omega-3 fatty acids.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Design of the study: MF, SH, JI. Management of acquisition of data: SH, JI. Analysis of data: MF, TMita, AO, TF, SJ, YW, KH, MM, MT, NM, HY. Draft of the manuscript: MF, TMura. All authors read and approved the final manuscript.
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