Circulating Fatty Acid-Binding Protein 4 Concentration Predicts the Progression of Carotid Atherosclerosis in a General Population Without Medication

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Background: Fatty acid-binding protein 4 (FABP4), which is expressed in both adipocytes and macrophages, is secreted from the cells and acts as an adipokine. An elevated circulating FABP4 level is associated with insulin resistance and atherosclerosis.

Methods and Results: We investigated the causative association between FABP4 level and progression of atherosclerosis in subjects of the Tanno-Sobetsu Study, a population-based cohort. In 281 subjects without medication (male/female: 109/172) in the year 2010 or 2013, the carotid intima-media thickness (CIMT) assessed using carotid ultrasonography was significantly correlated with age, adiposity, blood pressure, renal dysfunction and levels of cholesterol, triglycerides, fasting glucose, HbA1c and FABP4 (r=0.331, P<0.001). Multiple regression analysis demonstrated that age, sex and FABP4 concentration were independent predictors of CIMT. A total of 78 (male/female: 29/49) of the 156 subjects in 2010 underwent carotid ultrasonography again in 2013. The change in CIMT each year during that 3-year period (mean±SD: 3.8±22.3 µm/year) was positively correlated with basal levels of high-sensitivity C-reactive protein (hsCRP) (r=0.231, P=0.046) and FABP4 (r=0.267, P=0.018) in 2010. After adjustment for age, sex and hsCRP level, the basal FABP4 level was independently associated with the change in CIMT per year.

Conclusions: FABP4 concentration is an independent predictor of the progression of carotid atherosclerosis.

Key Words: Adipokines; Atherosclerosis; Fatty acid-binding protein 4; Intima-media thickness

Fat cell adipokines (FABPs) are a family of intracellular lipid chaperones, approximately 14–15 kDa predominantly cytosolic proteins that can reversibly bind hydrophobic ligands such as saturated and unsaturated long-chain fatty acids. FABPs have been proposed to facilitate the transport of lipids to specific compartments in the cell. Of them, FABP4, also known as adipocyte FABP (A-FABP) or aP2, is mainly expressed in adipocytes and macrophages and plays an important role in the development of obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM) and atherosclerosis. We previously demonstrated that inhibition of FABP4 by a small molecule might be a novel therapeutic strategy against IR, T2DM and atherosclerosis.

FABP4 is secreted from adipocytes in association with lipolysis via a non-classical secretion pathway though there are no typical secretory signal peptides in the sequence of FABP4. It has recently been shown that FABP4 is also secreted from macrophages, but the mechanism remains unknown. Circulating FABP4 acts as an adipokine for the development of IR and atherosclerosis in experimental models. Furthermore, a recent study demonstrated the possibility of a new strategy to treat metabolic disease by targeting serum FABP4 with a monoclonal antibody to FABP4.

Elevated circulating FABP4 levels are associated with obesity, IR, T2DM, hypertension, dyslipidemia, cardiac dysfunction, renal dysfunction, atherosclerosis and cardiovascular events. Notably, an increased plasma level of FABP4 was shown to be independently associated with the presence of coronary artery disease. Moreover, FABP4 has been found in human atherosclerotic plaques, and its presence was associated with the features of inflammatory and vulnerable plaques as high-risk phenotypes.
However, little is known about the causal link between circulating FABP4 and progression of atherosclerosis in humans. In the present study, we investigated the association of FABP4 level with the extent of atherosclerosis assessed by carotid intima-media thickness (CIMT) measured by carotid ultrasonography and its association with the progression of carotid atherosclerosis during a 3-year period in a general population of subjects who had not regularly taken any medications.

Methods

The present study consisted of 2 studies (Study 1 and Study 2) in the Tanno-Sobetsu Study, which had a population-based cohort design and was conducted in 2 rural towns, Tanno and Sobetsu, in Hokkaido, the northernmost island of Japan. All studies conformed to the principles outlined in the Declaration of Helsinki and were performed with the approval of the Ethical Committee of Sapporo Medical University. Written informed consent was received from all of the study subjects.

Study 1: FABP4 Level and CIMT

A total of 818 Japanese subjects (male/female: 353/465, mean age: 64±15 years) underwent annual examinations in Sobetsu Town in 2010 and/or 2013. All participants were invited to have a carotid ultrasonographic examination for assessment of CIMT, a marker of carotid atherosclerosis. Subjects who were being treated with any medications were excluded. A total of 281 subjects (male/female: 109/172) without medication who underwent carotid ultrasonography were enrolled in Study 1. The numbers of recruited subjects were 156 (male/female: 52/104, mean age: 61±14 years) in 2010 and 125 (male/female: 57/68, mean age: 63±17 years) in 2013. Among the 281 subjects without medication, past histories of heart disease and stroke in 8 and 3 subjects, respectively, were ascertained by a self-reported questionnaire.

Study 2: FABP4 Level and Change in CIMT

Of the 156 subjects without medication in 2010 in Study 1, 78 (male/female: 29/49) underwent carotid ultrasonography again in 2013. They were enrolled in Study 2. During that 3-year period, 15 of the 78 subjects started to take medications, including antihypertensive drugs (n=9), antidysslipidemic drugs (n=3), antiplatelet drugs (n=2) and antiarrhythmic drugs (n=1). The change in CIMT per year (µm/year) was calculated using the CIMT values from 2010 and 2013.

Table 1. Characteristics of the Study 1 Subjects Without Medication (n=281)

| Variable                        | Total   | Male     | Female    | P value |
|---------------------------------|---------|----------|-----------|---------|
| n                               | 281     | 109      | 172       |         |
| Age (years)                     | 62±15   | 63±16    | 61±15     | 0.399   |
| BMI (kg/m²)                     | 22.8±3.2| 23.9±2.9 | 22.1±3.2  | <0.001  |
| WC (cm)                         | 82.2±10.6| 86.3±8.9 | 79.6±10.8 | <0.001  |
| Systolic BP (mmHg)              | 129±20  | 135±18   | 126±21    | 0.015   |
| Diastolic BP (mmHg)             | 74±12   | 79±11    | 72±11     | 0.001   |
| Pulse rate (beats/min)          | 71±11   | 70±13    | 72±10     | 0.512   |
| Habitual smoking                | 49 (17.4) | 26 (23.9) | 23 (13.4) | 0.024   |
| Biochemical data                |         |          |           |         |
| TC (mg/dL)                      | 204±34  | 196±32   | 209±34    | 0.002   |
| LDL-C (mg/dL)                   | 122±29  | 120±27   | 123±30    | 0.291   |
| HDL-C (mg/dL)                   | 66±18   | 58±17    | 70±16     | <0.001  |
| Triglycerides (mg/dL)           | 92 (65–125) | 103 (74–145) | 85 (61–116) | <0.001  |
| Fasting glucose (mg/dL)         | 95 (90–103) | 97 (90–105) | 91 (86–98) | <0.001  |
| Insulin (µU/mL)                 | 5.3 (4.4–5.9) | 5.4 (4.9–5.9) | 5.2 (4.2–5.9) | 0.189   |
| HOMA-R                          | 1.22 (0.97–1.48) | 1.26 (1.07–1.58) | 1.16 (0.92–1.41) | 0.024   |
| HbA1c (%)                       | 5.2±0.4 | 5.3±0.5  | 5.2±0.4   | 0.002   |
| BUN (mg/dL)                     | 15±5    | 16±6     | 14±5      | 0.003   |
| Creatinine (mg/dL)              | 0.8±0.2 | 0.9±0.3  | 0.7±0.1   | <0.001  |
| eGFR (mL/min/1.73m²)            | 72.6±15.8 | 73.3±16.6 | 72.1±15.3 | 0.524   |
| Uric acid (mg/dL)               | 5.1±1.3 | 6.0±1.2  | 4.8±1.0   | <0.001  |
| AST (IU/L)                      | 22 (19–26) | 23 (20–27) | 21 (18–25) | 0.049   |
| ALT (IU/L)                      | 18 (14–23) | 21 (16–30) | 16 (13–21) | <0.001  |
| γGTP (IU/L)                     | 20 (15–31) | 27 (19–44) | 18 (14–24) | <0.001  |
| BNP (pg/mL)                     | 17 (11–31) | 15 (9–30) | 19 (12–31) | 0.024   |
| hsCRP (mg/dL)                   | 0.03 (0.01–0.06) | 0.03 (0.02–0.06) | 0.03 (0.01–0.06) | 0.435   |
| FABP4 (ng/ml)                   | 10.8 (7.8–14.3) | 9.3 (7.3–13.0) | 11.5 (8.4–15.4) | 0.001   |

Carotid ultrasonographic data

| Variable | Total | Male     | Female    | P value |
|----------|-------|----------|-----------|---------|
| CIMT (mm)| 0.69±0.13 | 0.71±0.14 | 0.67±0.13 | 0.011   |

Variables are expressed as number (%), mean±SD or median (interquartile range). AST, aspartate transaminase; ALT, alanine transaminase; BMI, body mass index; BNP, B-type natriuretic peptide; BP, blood pressure; BUN, blood urea nitrogen; CIMT, carotid intima-media thickness; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; γGTP, γ-glutamyl transpeptidase; hsCRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; WC, waist circumference.
FABP4 Level and Progression of Atherosclerosis

**Table 2. Correlation and Multiple Regression Analyses for FABP4 and CIMT (Study 1: n=281)**

|                      | Log FABP4 |                      |                      |
|----------------------|-----------|----------------------|----------------------|
|                      | r         | P value              | β                    | P value              |
| Age                  | 0.405     | <0.001               | 0.059                | <0.001               |
| Sex (male)           | –         | –0.377               | <0.001               | –0.627               |
| BMI                  | 0.459     | <0.001               | –                    | –0.445               |
| WC                   | 0.462     | <0.001               | 0.481                | <0.001               |
| Systolic BP          | 0.261     | 0.001                | –                    | –0.412               |
| Diastolic BP         | 0.124     | 0.124                | –                    | 0.126                |
| Pulse rate           | 0.144     | 0.160                | –                    | 0.010                |
| Smoking habit        | –         | –0.141               | <0.001               | 0.141                |
| TC                   | 0.033     | 0.578                | –                    | 0.051                |
| LDL-C                | 0.098     | 0.010                | –                    | 0.204                |
| HDL-C                | –0.140    | 0.019                | –                    | 0.232                |
| Log triglycerides    | 0.087     | 0.014                | –                    | 0.017                |
| Log fasting glucose  | 0.143     | 0.017                | –                    | 0.018                |
| Log insulin          | 0.192     | 0.001                | –                    | 0.299                |
| Log HOMA-R           | 0.210     | <0.001               | –                    | 0.078                |
| HbA1c                | 0.258     | <0.001               | –                    | 0.259                |
| BUN                  | 0.236     | <0.001               | –                    | 0.251                |
| Creatinine           | 0.204     | 0.001                | –                    | 0.279                |
| eGFR                 | –0.417    | <0.001               | –0.212               | <0.001               |
| Uric acid            | 0.084     | 0.160                | –                    | 0.168                |
| Log AST              | 0.204     | 0.001                | 0.131                | 0.004                |
| Log ALT              | 0.142     | 0.017                | NS                   | 0.151                |
| Log γGTP             | –0.001    | 0.990                | –                    | –0.111               |
| Log BNP              | 0.240     | <0.001               | –                    | 0.354                |
| Log hsCRP            | 0.163     | 0.007                | NS                   | 0.080                |
| Log FABP4            | –         | –                    | –                    | 0.331                |
| CIMT                 | 0.331     | <0.001               | 0.130                | <0.001               |
|                      | R²=0.478  |                      |                      |

NS, not selected. Other abbreviations as in Table 1.

**Measurements**

Medical check-ups were performed between 06:00 hours and 09:00 hours after an overnight fast. After measuring anthropometric parameters, blood pressure (BP) was measured twice consecutively in the upper arm using an automated sphygmomanometer (HEM-907, Omron Co., Kyoto, Japan) with subjects in a seated resting position, and the average BP was used for analysis. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters). Peripheral venous blood samples were obtained from study subjects after the physical examination for complete blood count and biochemical analyses. Samples of serum and plasma were analyzed immediately or stored at −80°C until biochemical analyses.

The concentration of FABP4 was measured using a commercially available enzyme-linked immunosorbent assay kit for FABP4 (Biovendor R&D, Modrice, Czech Republic). The accuracy, precision and reproducibility of the kit have been described previously. The intra- and interassay coefficients of variation in the kit were <5%. According to the manufacturer's protocol, no cross-reactivity of FABP4 with other FABP types occurs. Plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin was measured by a chemiluminescent enzyme immunoassay method. Hemoglobin A1c (HbA1c) was determined by a latex coagulation method and expressed on the National Glycohemoglobin Standardization Program scale. Creatinine, blood urea nitrogen (BUN), uric acid, aspartate transaminase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γGTP) and lipid profiles, including total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides, were determined by enzymatic methods. The low-density lipoprotein cholesterol (LDL-C) level was calculated by the Friedewald equation. B-type natriuretic peptide (BNP) was measured using an assay kit (Shionogi & Co., Osaka, Japan). High-sensitivity C-reactive protein (hsCRP) was measured by a nephelometry method. The homeostasis model assessment of IR (HOMA-R), an index of IR, was calculated by the previously reported formula: HOMA-R = insulin (μU/ml) × glucose (mg/dL)/405. As an index of renal function, estimated glomerular filtration rate (eGFR) was calculated by an equation for Japanese: eGFR (mL/min/1.73 m²) = 194 × creatinine⁻¹.⁰⁹⁴ × age⁻¹.₃₂⁸ × 1.₅³⁴ (if male, 1.₃₈⁵ (if female).

**Carotid Ultrasonography**

Carotid ultrasonographic examinations were performed by 3 experienced examiners certified by the Japan Society of Ultrasonics in Medicine, who were blinded to the clinical data, using a Vivid 9 (GE Health Care, Tokyo, Japan).
equipped with a multifrequency 4–10-MHz linear-array transducer. Mean CIMT of the far wall of both of the common carotid arteries was measured using commercially available semi-automated edge-detection software (IMT Option, GE Medical System, Milwaukee, WI, USA). The region of interest was placed from the beginning of the carotid bulbs to a 2-cm proximal site in each common carotid artery. In our laboratory, coefficients of variance of intra- and interoperator variability for semi-automated CIMT measurement (3.5% and 8.0%, respectively) were significantly lower than those for manual CIMT measurement (14.1% and 16.7%, respectively).22 The average value of CIMT in both of the common carotid arteries was used for analysis because of a significant positive correlation between the left and right CIMT values (n=281, r=0.670, P<0.001).

Statistical Analysis
Numeric variables are expressed as mean±SD for normal distributions or median (interquartile range) for skewed variables. The distribution of each parameter was tested for its normality using the Shapiro-Wilk W test, and non-normally distributed parameters were logarithmically transformed for correlation and regression analyses. Comparison between groups was done with the chi-square test, Wilcoxon signed-rank test for paired samples and the Mann-Whitney U test for unpaired samples. The correla-

Table 3. Characteristics of the Recruited and Non-Recruited Subjects (Study 2)

|                         | Recruited     | Non-recruited | P value  |
|-------------------------|---------------|---------------|----------|
| n (male/female)         | 78 (29/49)    | 78 (23/55)    | 0.308    |
| Age (years)             | 62±11         | 60±17         | 0.380    |
| BMI (kg/m²)             | 22.7±3.2      | 22.3±2.8      | 0.438    |
| WC (cm)                 | 80.9±10.1     | 79.8±10.3     | 0.497    |
| Systolic BP (mmHg)      | 128±18        | 130±22        | 0.443    |
| Diastolic BP (mmHg)     | 74±11         | 75±12         | 0.511    |
| Pulse rate (beats/min)  | 70±11         | 71±10         | 0.845    |
| Habitual smoking        | 11 (14.1)     | 14 (17.9)     | 0.513    |
| Biochemical data        |               |               |          |
| TC (mg/dL)              | 215±30        | 202±29        | 0.006    |
| LDL-C (mg/dL)           | 126±28        | 115±26        | 0.012    |
| HDL-C (mg/dL)           | 67±19         | 66±18         | 0.639    |
| Triglycerides (mg/dL)   | 101 (69–127)  | 90 (63–123)   | 0.166    |
| Fasting glucose (mg/dL) | 93 (89–99)    | 92 (87–100)   | 0.859    |
| Insulin (µU/mL)         | 5.4 (3.5–7.6) | 4.6 (3.0–6.3) | 0.117    |
| HOMA-R                  | 1.29 (0.78–1.85) | 1.02 (0.68–1.55) | 0.218    |
| HbA1c (%)               | 5.1±0.2       | 5.1±0.3       | 0.979    |
| BUN (mg/dL)             | 14±4          | 14±4          | 0.682    |
| Creatinine (mg/dL)      | 0.7±0.1       | 0.7±0.1       | 0.202    |
| eGFR (mL/min/1.73m²)    | 73.0±12.7     | 76.8±16.3     | 0.102    |
| Uric acid (mg/dL)       | 4.9±1.2       | 5.0±1.2       | 0.814    |
| AST (IU/L)              | 22 (19–26)    | 22 (19–26)    | 0.798    |
| ALT (IU/L)              | 19 (15–23)    | 17 (13–24)    | 0.386    |
| YGTP (IU/L)             | 20 (16–32)    | 19 (15–28)    | 0.322    |
| BNP (pg/mL)             | 15 (11–28)    | 20 (12–33)    | 0.128    |
| hsCRP (mg/dL)           | 0.02 (0.02–0.05) | 0.03 (0.02–0.05) | 0.773    |
| FABP4 (ng/mL)           | 10.7 (7.8–13.2) | 10.1 (7.6–14.0) | 0.836    |
| Carotid ultrasonographic data |       |               |          |
| CIMT (mm)               | 0.70±0.13     | 0.68±0.12     | 0.539    |

Variables are expressed as number (%), mean±SD or median (interquartile range). Abbreviations as in Table 1.
tion between 2 variables was evaluated using Pearson’s correlation coefficient. Stepwise and subsequent multivariate regression analyses were performed to identify independent determinants of FABP4, CIMT and change in CIMT using the variables with a significant correlation as independent determinants of FABP4, CIMT and change in CIMT. Among the candidate models, the best-fit model using AIC explained (R^2 =0.478) (Figure 2) of the variance in this measure (R^2 =0.478) (Figure 2). A significantly positive correlation was found between FABP4 level and CIMT (r=0.331, P<0.001) (Table 1). Multiple regression analysis showed that the FABP4 level was independently associated with age, WC and levels of eGFR, AST and CIMT, explaining a total of 47.8% of the variance in this measure (R^2 =0.478) (Table 2).

**Results**

**Study 1**

The characteristics of the 281 recruited subjects (male/female: 109/172) are shown in Table 1. Mean age, BMI and waist circumference (WC) of the recruited subjects were 62±15 years, 22.8±3.2 kg/m^2^ and 82.2±10.6 cm, respectively. Male subjects had significantly larger BMI and WC, significantly higher frequency of habitual smoking, and significantly higher levels of systolic and diastolic BPs, triglycerides, fasting glucose, HOMA-R, HbA1c, BUN, creatinine, uric acid, AST, ALT, GTP and CIMT and lower levels of total cholesterol, HDL-C, BNP and FABP4 than did female subjects. No significant difference in age, pulse rate or level of LDL-C, eGFR, or hsCRP was found between the male and female subjects. Hypertension (systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg), hyperlipidemia (LDL-C ≥140 mg/dL, HDL-C <40 mg/dL), or triglycerides ≥150 mg/dL and DM (HbA1c ≥6.5% and fasting glucose ≥126 mg/dL) were found in 91, 110, and 4 subjects, respectively.

There was no significant difference between the level of FABP4 or CIMT in male subjects with and without a smoking habit. Levels of FABP4 and CIMT were significantly lower in female subjects with a smoking habit than in those without a smoking habit.

The logarithmically transformed serum FABP4 level was positively correlated with age, BMI, WC, systolic BP, and levels of fasting glucose, insulin, HOMA-R, HbA1c, BUN, creatinine, AST, ALT, GTP and CIMT and lower levels of total cholesterol, HDL-C, BNP and FABP4 than did female subjects. No significant difference in age, pulse rate or level of LDL-C, eGFR, or hsCRP was found between the male and female subjects. Hypertension (systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg), hyperlipidemia (LDL-C ≥140 mg/dL, HDL-C <40 mg/dL, or triglycerides ≥150 mg/dL) and DM (HbA1c ≥6.5% and fasting glucose ≥126 mg/dL) were found in 91, 110, and 4 subjects, respectively.

| Variables | Total | Male | Female | P value |
|-----------|-------|------|--------|---------|
| n         | 78    | 29   | 49     | 0.990   |
| Age (years) | 62±11 | 62±10 | 62±11 | 0.800   |
| BMI (kg/m^2^) | 22.7±3.2 | 23.5±3.2 | 22.2±3.1 | 0.003   |
| WC (cm) | 80.9±10.1 | 85.4±8.6 | 78.3±10.1 | 0.003   |
| Systolic BP (mmHg) | 128±18 | 132±17 | 125±19 | 0.110   |
| Diastolic BP (mmHg) | 74±11 | 78±11 | 72±11 | 0.025   |
| Pulse rate (beats/min) | 70±11 | 69±14 | 72±10 | 0.404   |
| Habitual smoking | 11 (14.1) | 6 (20.7) | 5 (10.2) | 0.199   |

**Biochemical data**

| Variables | Total | Male | Female | P value |
|-----------|-------|------|--------|---------|
| TC (mg/dL) | 215±30 | 204±27 | 221±30 | 0.019   |
| LDL-C (mg/dL) | 126±28 | 122±28 | 129±27 | 0.331   |
| HDL-C (mg/dL) | 67±19 | 59±21 | 73±16 | 0.001   |
| Triglycerides (mg/dL) | 101 (69–127) | 117 (84–163) | 92 (65–113) | 0.001   |
| Fasting glucose (mg/dL) | 93 (89–99) | 94 (91–104) | 92 (88–97) | 0.033   |
| Insulin (μU/mL) | 5.4 (3.5–7.6) | 5.4 (3.5–7.5) | 5.8 (3.4–7.6) | 0.871   |
| HOMA-R | 1.29 (0.78–1.85) | 1.24 (0.79–1.90) | 1.34 (0.75–1.83) | 0.871   |
| HbA1c (%) | 5.1±0.2 | 5.1±0.3 | 5.0±0.2 | 0.150   |
| BUN (mg/dL) | 14±4 | 14±3 | 15±4 | 0.636   |
| Creatinine (mg/dL) | 0.7±0.1 | 0.8±0.1 | 0.7±0.1 | <0.001   |
| eGFR (mL/min/1.73m^2^) | 73.0±12.7 | 77.9±11.6 | 70.1±12.6 | 0.008   |
| Uric acid (mg/dL) | 4.9±1.2 | 5.7±1.1 | 4.4±1.0 | <0.001   |
| AST (IU/L) | 22 (19–26) | 24 (20–26) | 22 (19–25) | 0.597   |
| ALT (IU/L) | 19 (15–23) | 20 (17–25) | 18 (14–22) | 0.201   |
| GTP (IU/L) | 20 (16–32) | 24 (19–36) | 18 (14–28) | 0.007   |
| BNP (pg/mL) | 15 (11–28) | 12 (6–22) | 18 (12–30) | 0.413   |
| hsCRP (mg/dL) | 0.02 (0.02–0.05) | 0.02 (0.02–0.05) | 0.02 (0.02–0.06) | 0.818   |
| FABP4 (ng/mL) | 10.7 (7.8–13.2) | 9.2 (7.7–11.4) | 11.2 (8.1–13.8) | 0.064   |

**Carotid ultrasonographic data**

| Variables | Total | Male | Female | P value |
|-----------|-------|------|--------|---------|
| CIMT (mm) | 0.70±0.13 | 0.72±0.12 | 0.69±0.13 | 0.323   |

Variables are expressed as number (%), mean±SD or medians (interquartile range). Abbreviations as in Table 1.
On the other hand, CIMT was positively correlated with age (r=0.677, P<0.001), BMI, WC, systolic BP, and levels of LDL-C, triglycerides, fasting glucose, HbA1c, BUN, creatinine, uric acid, AST, BNP and FABP4, and was negatively correlated with levels of HDL-C and eGFR (Table 2). After adjustment for age and sex, CIMT was independently associated with the FABP4 level (β=0.105, P=0.032), explaining a total of 48.1% of the variance in this measure (R²=0.481) (Table 2).

**Study 2**

Among the 156 subjects without medication enrolled in Study 1 in 2010, 78 subjects were recruited for Study 2. There was no significant difference in basal parameters except for total cholesterol and LDL-C, between the 78 recruited subjects and 78 non-recruited subjects (Table 3). The characteristics of the 78 recruited subjects who underwent carotid ultrasonography in both 2010 and 2013 are shown in Table 4. Mean age, BMI and WC of the recruited subjects were 62±11 years, 22.7±3.2 kg/m² and 80.9±10.1 cm, respectively. As in Study 1, male subjects had significantly larger WC and had higher levels of diastolic BP, triglycerides, fasting glucose, creatinine, eGFR, uric acid and γGTP and lower levels of total cholesterol and HDL-C than did female subjects.

The distribution of changes in CIMT per year during the 3-year period is shown in Figure 2A, and the mean value was 3.8±22.3 µm/year. Progression and regression of CIMT were found in 43 (55.1%) and 35 (44.9%) of the subjects, respectively. There was no significant difference in CIMT per year between the male and female subjects (3.8±25.5 vs. 3.7±20.5 µm/year, P=0.992). No significant difference was found between changes in CIMT in subjects with and without a smoking habit (9.5±16.5 vs. 2.8±23.1 µm/year, P=0.992). The change in CIMT per year was positively correlated with logarithmically transformed levels of hsCRP (r=0.299, P=0.009) (Figure 2B) and FABP4 (r=0.267, P=0.018) (Figure 2C). No significant correlation was found between the change in CIMT per year and other basal parameters (Table 5).

No significant change between the years 2010 and 2013 was found in the level of hsCRP (0.02 [0.02–0.05] vs. 0.02 [0.01–0.05] mg/dL, P=0.148) or FABP4 (median [IQR]: 10.7 [7.8–13.2] vs. 11.0 [7.3–15.3] ng/mL, P=0.112). The change in the level of FABP4 (r=−0.062, P=0.589) or hsCRP (r=−0.168, P=0.150) during the 3-year period was...
not significantly correlated with the change in CIMT.

Multiple regression analysis showed that the levels of FABP4 and hsCRP were independently associated with CIMT after adjustment for age and sex, explaining a total of 14.7% of the variance in this measure (R²=0.147) (Table 6).

**Discussion**

The present study showed for the first time that the serum FABP4 concentration was an independent predictor of the progression of carotid atherosclerosis assessed by the change in CIMT per year during a 3-year period in a general population of subjects who had not taken any relevant medications. FABP4 is secreted from adipocytes and macrophages despite a lack of secretory signal peptides in the sequence of FABP4.7,8 Previous studies using in vitro and in vivo experiments showed that FABP4 acts as a adipokine for the development of hepatic IR through increased hepatic glucose production,9 suppression of cardiomyocyte contraction,21 and development of atherosclerosis through induction of inflammatory responses, inhibition of endothelial nitric oxide synthase activity in endothelial cells and proliferation and migration of vascular smooth muscle cells.9 Furthermore, a recent study demonstrated the possibility of a new strategy to treat metabolic disease by targeting serum FABP4 with a monoclonal antibody.10 Circulating FABP4 may directly promote the progression of atherosclerosis in humans.

Elevated serum FABP4 level is associated with atherosclerosis as assessed by several examinations, including cardio-ankle vascular index,12 carotid-femoral pulse wave velocity,13 CIMT15,26,27 and plaque volume in the coronary artery measured by intravascular ultrasound.28 In the present study, we confirmed that the serum FABP4 level was positively and independently correlated with CIMT, a morphological index of atherosclerosis. Several drugs, including angiotensin II receptor blockers,24,29 a statin,30 omega-3 fatty acid ethyl esters,31 a dipeptidyl peptide-4 inhibitor,32 a sodium glucose cotransporter 2 inhibitor33 and a thiazolidinedione,34 have been reported to modulate the circulating FABP4 level. The serum FABP4 level has also been reported to predict long-term cardiovascular events.16–18 Reduction of the FABP4 level would be a novel therapeutic strategy for preventing the development of atherosclerosis and reducing the incidence of atherosclerotic cardiovascular disease. However, it is unclear whether FABP4 acts by an intracellular signaling mechanism via unidentified receptors, though it is a potential adipokine, an adipocyte-derived bioactive molecule.7,9,35,36 It has also been reported that extracellular FABP4 is partially internalized into cells,36 though the mechanism and significance of internalization remain unclear. Further understanding of the mechanism of FABP4’s action may enable the development of new therapeutic strategies for atherosclerotic cardiovascular disease, such as neutralization of FABP4 and/or blockade of the FABP4 receptor, if any.

Treatment with several drugs for dyslipidemia, hypertension and DM has been shown to prevent the progression of atherosclerosis, indicated by regression of the CIMT.37 Conversely, it has been reported that the average increase in CIMT per year is 30–40 µm in patients with T2DM.38,39 However, little is known about the average change in CIMT in a general population without medications. A predictive increase in CIMT per year in healthy subjects has been reported to be approximately 8–9 µm according to the regression coefficient with age in cross-sectional analyses,40,41 because there was a strong positive correlation between age and CIMT. In the present study,

| Table 5. Correlation Between Change in CIMT and Basal Clinical Variables (Study 2: n=78) |
|-----------------|-----|-----|
|                 | r   | P value |
| Age             | −0.018 | 0.088 |
| BMI             | 0.068 | 0.554 |
| WC              | 0.057 | 0.621 |
| Systolic BP     | 0.049 | 0.672 |
| Diastolic BP    | 0.064 | 0.576 |
| Pulse rate      | 0.060 | 0.691 |
| TC              | 0.123 | 0.284 |
| LDL-C           | 0.137 | 0.254 |
| HDL-C           | 0.001 | 0.991 |
| Log triglycerides | 0.003 | 0.978 |
| Log fasting glucose | 0.181 | 0.113 |
| Log insulin     | 0.055 | 0.634 |
| Log HOMA-R      | 0.085 | 0.462 |
| HbA1c           | 0.198 | 0.082 |
| BUN             | 0.203 | 0.075 |
| Creatinine      | −0.067 | 0.562 |
| eGFR            | 0.082 | 0.478 |
| Uric acid       | 0.099 | 0.821 |
| Log AST         | −0.026 | 0.821 |
| Log ALT         | 0.018 | 0.877 |
| Log YGTP        | 0.017 | 0.886 |
| Log BNP         | −0.220 | 0.055 |
| Log hsCRP       | 0.299 | 0.009 |
| Log FABP4       | 0.267 | 0.018 |
| CIMT            | 0.014 | 0.904 |

Abbreviations as in Table 1.

| Table 6. Multiple Regression Analysis for Change in CIMT (µm/year) (Study 2: n=78) |
|-----------------|-----|-----|-----|-----|-----|-----|
|                 | Regression coefficient | SE | Standardized regression coefficient (γ) | t | P value |
| Age             | −0.306 | 0.242 | −0.691 | −0.20 | 0.208 |
| Sex (male)      | 2.095 | 5.166 | 0.046 | 0.41 | 0.686 |
| Log hsCRP       | 6.242 | 2.743 | 0.261 | 2.28 | 0.026 |
| Log FABP4       | 15.713 | 7.508 | 0.250 | 2.09 | 0.040 |

R²=0.147. Abbreviations as in Table 1.
the distribution of the change in CIMT during the 3-year period was relatively large, and progression and regression of CIMT were found in 43 (55.1%) and 35 (44.9%), respectively, of the 78 studied subjects (Figure 2A). The average value of the change in CIMT was 3.8±22.3 (median: 1.7) µm/year and was smaller than the predictive value, suggesting a variety of alterations in CIMT caused by several factors, including modification of lifestyle, foods, salt intake, smoking habit and other factors, over a certain period of time in a general population without medications. The present study revealed that the basal levels of hsCRP and FABP4, but not the change in hsCRP or FABP4 during the 3-year period, were independent predictors of the change in CIMT during the 3-year period after adjustment for age and sex.

Study Limitations
First, the number of patients enrolled, especially in Study 2, was small, and the possibility of type 1 or type 2 errors in statistical tests cannot be excluded. Second, only CIMT including plaque in the common carotid arteries was evaluated in the present study, and the use of different methods to measure CIMT or different parameters may yield different results. Third, errors of measurement of CIMT at 2 time points may affect the change in CIMT per year, though the coefficients of variance of intra- and inter-operator variability for semi-automated CIMT measurement (3.5% and 8.0%, respectively) in our laboratory were better than those for manual CIMT measurement (14.1% and 16.7%, respectively). Fourth, some medications were started in 15 of the 78 subjects in Study 2 and might have modulated the progression and regression of atherosclerosis, though the results of analyses were similar when the 15 subjects were excluded (data not shown). Lastly, because the recruited subjects were only Japanese, it is unclear whether the present findings can be generalized to other ethnicities.

Conclusions
FABP4 concentration is an independent predictor of atherosclerosis assessed by CIMT and the progression of carotid atherosclerosis. A further understanding of the mechanism underlying the link between circulating FABP4 and progression of atherosclerosis may enable the development of new therapeutic strategies for cardiovascular and metabolic diseases.

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Conflict of Interest
The authors declare no conflict of interest.

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