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Factors associated with longitudinal changes in serum concentrations of Mac-2 binding protein: A prospective 3-year observational study

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Abstract *Background and aims:* Mac-2 binding protein (M2BP) plays an important role in cell adhesion. In a recent cross-sectional study we reported that serum M2BP concentrations may reflect silent atherosclerosis. The aim of the present prospective follow-up study was to investigate possible relationships between changes in concentrations of M2BP and other factors over a >3-year period.
Methods and results: The present study enrolled subjects who visited Enshu hospital from 2014 to 2015 for a periodic physical check-up and then attended for another physical check-up after >3 years (n = 174). Factors affecting changes in M2BP concentrations were investigated at both baseline and follow-up. Subjects with liver dysfunction, a history of hepatic disease, malignant neoplasm, or cardiovascular events at baseline were excluded. Univariate and multivariate regression analyses showed that changes in serum M2BP concentrations during the follow-up period were significantly associated with changes in low-density lipoprotein cholesterol (LDL-C), triglyceride, and oxidative stress marker derivatives of reactive oxygen metabolites (d-ROM) concentrations. Moreover, the increase in LDL-C was significantly greater in subjects in whom M2BP concentrations increased during the follow-up period. Logistic regression analysis with an endpoint of increased M2BP revealed that increased LDL-C was an independent determinant of an increase in M2BP during the follow-up period.
Conclusion: During the observation period of >3 years, serum M2BP concentrations were increased in subjects who also exhibited increases in levels of metabolic parameters, especially LDL-C, and the oxidative stress marker d-ROM. These results support that serum M2BP reflects one of the contributors to the progression of silent atherosclerosis.

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Introduction

Mac-2 binding protein (M2BP) is a ligand for galectin-3 and E-selectin that plays an important role in cell adhesion [1]. M2BP is ubiquitously expressed in the body and therefore, under stable conditions, there is not much variation in circulating M2BP concentrations within individuals [2]. Conversely, serum M2BP concentrations have been shown to be a diagnostic biomarker for hepatic fibrosis and several types of malignant tumors [3–6]. However, in a preliminary investigation we showed that serum M2BP concentrations varied in individuals without hepatic or malignant disease, although the concentrations in the serum were relatively low [7].

Recently, we investigated the hypothesis that serum M2BP concentrations may change during the atherosclerotic process, demonstrating that concentrations of the fibrosis-specific isoform of glycosylated M2BP (M2BPGi) were associated with increased arterial stiffness and subclinical atherosclerosis [7]. Moreover, several studies have reported a relationship between M2BP concentrations and coronary artery disease [8,9]. Although a close relationship between M2BP concentrations and atherosclerotic disease has been implied, longitudinal changes in M2BP concentrations in subjects without hepatic or malignant disease have not been investigated. Thus, we hypothesized that changes in serum M2BP concentrations may be associated with other atherosclerotic parameters. The aim of the present study was to investigate the possible relationship between changes in M2BP concentrations and changes in other atherosclerotic parameters in a prospective follow-up study over a >3-year period.

Methods

Study subjects

Enshu hospital provides an annual physical check-up program as recommended by the Japanese Government. Participants in this program are encouraged to have a comprehensive medical examination once a year to check their overall health; however, some fail to have such a check-up every year. The present study included subjects who visited our hospital during 2014 and 2015 for an annual physical check-up, and thereafter, had another physical check-up with more than a 3-year interval. All subjects included in the present study agreed to serum M2BP testing at both baseline and follow-up visits. Subjects agreed to measure serum M2BP concentrations at both baseline and follow-up were enrolled in the present study. Factors affecting changes in M2BP concentrations were investigated. At baseline, subjects with liver dysfunction (aspartate transaminase [AST] $>$ 35 U/L, alanine transaminase [ALT] $>$ 35 U/L, and/or γ-glutamyl transpeptidase [γ-GTP] $>$ 45 U/L), fatty liver diagnosed by ultrasonography, renal insufficiency (creatinine $\geq$ 1.5 mg/dL), malignant neoplasm, active inflammatory disease, a history of obvious hepatic disease, a history of cardiovascular events (stroke, myocardial infarction, and peripheral artery disease), and current smokers were excluded. Subjects with a history of heart failure were also excluded, because they might develop liver congestion, leading to liver fibrosis with M2BP elevation. Since medications could potentially influence the M2BP level, subjects under medications at baseline were similarly excluded [10–12].

Blood pressure was measured using a standard mercury sphygmomanometer with the participant in the sitting position. The mean blood pressure of the two measurements was recorded as the blood pressure. Subjects with systolic blood pressure $\geq$ 140 mmHg and diastolic blood pressure $\geq$ 90 mmHg, or under medications were defined as having hypertension [13]. Subjects with high-density lipoprotein cholesterol (HDL-C) $<$ 40 mg/dL, low-density lipoprotein cholesterol (LDL-C) $\geq$ 140 mg/dL, or triglycerides $\geq$ 150 mg/dL, or under medications were defined as having dyslipidemia [14]. Subjects with a fasting plasma glucose (FPG) $\geq$ 126 mg/dL or under medications were defined as having diabetes mellitus. For assessment of cardiovascular risk severity, patients were categorized into none, low-, moderate-, and high-risk groups according to the cardiovascular risk stratification [13].

The study protocol was approved by the Ethics Committee of Nagoya City University Graduate School of Medical Sciences and Enshu Hospital. The study was performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each subject.

Biochemical analysis

Biochemical tests, including determination of serum concentrations of LDL-C, HDL-C, triglyceride, AST, ALT, and γ-GTP, were performed using standard laboratory assays. To assess oxidative stress, serum concentrations of derivatives of reactive oxygen metabolites (d-ROM) were measured [15]. The estimated glomerular filtration rate (eGFR) was calculated using a modified formula from the Modification of Diet in Renal Disease study for the Japanese population [16].

Measurement of M2BP

Serum concentrations of M2BP were measured using M2BPGi reagent, with levels expressed using the cut-off index (COI), as described previously [17]. Briefly, serum M2BP concentrations were determined as follows. All assays were performed using an automated chemiluminescence enzyme immunoassay system (HISCL-5000; Sysmex, Kobe, Japan). Levels of M2BPGi were measured based on a sandwich immunoassay. M2BPGi was captured by *Wisteria floribunda* agglutinin (WFA) immobilized on magnetic beads, and the bound product was assayed with an anti-human M2BP monoclonal antibody linked to alkaline phosphatase (ALP-αM2BP). Two reagent packs (M2BP-WFA detection pack and a chemiluminescence substrate pack) were loaded in the HISCL-5000. The detection pack comprised three reagents: a

reaction buffer solution (R1), a solution of WFA-coated magnetic beads (R2), and an ALP-αM2BP solution (R3). The chemiluminescence substrate reagent pack contained a buffer solution (R4) and a CDP-Star substrate solution (R5). Serum (10 μL) was diluted to 60 μL with R1 and then mixed with R2 (30 μL). After the binding reaction, R3 (100 μL) was added to the reaction solution. The resulting conjugates were magnetically separated from unbound components and mixed well with R4 (50 μL) and R5 (100 μL) before fluorescence readings were performed. The chemiluminescent intensity was acquired ≤17 min after preparation of the assay. The reaction chamber was kept at 42 °C throughout.

Measurement of radial augmentation index

Measurement of radial artery pressure waveforms and assessment of radial augmentation index (AI) was performed using a fully automated device (HEM-9000AI) at baseline [7]. The radial AI, which was reported to be a marker of arterial stiffness and subclinical atherosclerosis, was calculated as using a following equation:

$$\mathrm{AI}\,(\%) = (\mathrm{P2}\,/\,\mathrm{PP}) \times 100$$

where P2 and PP are the height of the late systolic shoulder/peak pressure and the pulse pressure of the radial arterial pressure contour, respectively.

Statistical analysis

The dichotomous variable (sex) was assigned values of 0 (female) and 1 (male). Univariate and multivariate linear regression analyses associated with M2BP were performed. In another series of analyses, logistic regression analysis was performed to investigate possible factors associated with increasing M2BP concentrations. Comparisons between two groups were made using paired and unpaired *t*-tests. Statistical significance was set at $P < 0.05$. Data were analyzed using IBM SPSS Statistics 19 (IBM Corp., Chicago, IL, USA) and are expressed as the mean ± SD or 95% confidential intervals (CI).

Results

During the period from 2014 to 2015, 4010 individuals participated in our physical check-up program and were screened for eligibility in the present study. We excluded 3362 participants as per the stated criteria, leaving 648 eligible subjects at baseline. Among them, 174 subjects who re-visited our hospital for a physical check-up after more than 3 years comprised the subjects for analysis (Table 1). During the follow-up period, 48 (27.6%), 37 (21.3%), and 11 (6.3%) subjects were started on antihypertensive, lipid-lowering, and hypoglycemic medications, respectively. Of these enrolled 174 subjects, 46 (26.4%), 56 (32.2%), and 13 (7.5%) had hypertension, dyslipidemia, and diabetes mellitus at baseline, respectively. Obvious new onset malignant tumor and hepatic disease were not confirmed.

Table 1 Characteristics of subjects at baseline and at follow-up (after >3 years; $n = 174$).

	Baseline	Follow-up	*P*-value
Age (years)	57.3 ± 9.6	61.1 ± 9.6	<0.0001
No. males (%)	101 (58.0)	–	–
BMI (kg/m^2)	21.2 ± 2.4	21.4 ± 2.6	0.11
SBP (mmHg)	122.9 ± 16.2	123.2 ± 15.5	0.77
DBP (mmHg)	74.3 ± 9.6	72.0 ± 9.3	<0.001
Hemoglobin (g/dL)	13.3 ± 1.3	13.5 ± 1.1	<0.05
Creatinine (mg/dL)	0.73 ± 0.15	0.75 ± 0.16	<0.0001
FPG (mg/dL)	96 ± 14	96 ± 15	0.54
HDL-C (mg/dL)	64 ± 14	65 ± 16	<0.05
LDL-C (mg/dL)	119 ± 25	125 ± 24	<0.01
Triglyceride (mg/dL)	89 ± 38	92 ± 39	0.30
AST (U/L)	20.3 ± 4.6	21.0 ± 4.4	0.52
ALT (U/L)	16.9 ± 6.2	17.4 ± 5.7	0.29
γ-GTP (U/L)	24.5 ± 12.5	24.1 ± 11.2	0.61
Uric acid (mg/dL)	5.0 ± 1.2	5.1 ± 1.2	<0.01
eGFR (mL/min per 1.73 m^2)	75.5 ± 13.5	71.7 ± 12.6	<0.0001
d-ROM (Carratelli units)	318.3 ± 53.9	370.9 ± 61.0	<0.0001
M2BP (COI)	0.45 ± 0.20	0.53 ± 0.25	<0.0001

Unless indicated otherwise, data are given as the mean ± SD.
ALT, alanine transaminase; AST, aspartate transaminase; COI, cut-off index; d-ROM, derivatives of reactive oxygen metabolites; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M2BP, Mac-2 binding protein; SBP, systolic blood pressure; BMI, body mass index.

Consistent with our previous reports, the severity of cardiovascular risk in individuals assessed at baseline was significantly associated with M2BP concentrations at both baseline and follow-up (Fig. 1a and b), but not with changes in M2BP concentrations during the follow-up period (data not shown). During the follow-up period, the elderly (>65 years) or subjects with diabetes mellitus at baseline showed greater increase in the M2BP concentrations than non-elderly or subjects without diabetes mellitus (Fig. 2a and d). On the other hand, subjects who had indicated higher radial AI than the mean value (83.1 ± 12.7%) at baseline showed a greater increase in the M2BP concentrations during the follow-up period than those who showed lower radial AI (Fig. 2e). Univariate regression analyses showed that a change in serum M2BP concentrations during the follow-up period was significantly correlated with changes in body mass index (BMI), as well as HDL-C, LDL-C, triglyceride, AST, ALT, γ-GTP, and d-ROM concentrations during the follow-up period (Table 2). Multivariate regression analyses showed that changes in serum M2BP concentrations were significantly associated with changes in LDL-C and triglyceride concentrations during the follow-up period (Table 3, Model 1). Similarly, multivariate regression analysis revealed that changes in M2BP concentrations were significantly associated with changes in d-ROM concentrations (Table 3, Model 2). Logistic regression analysis with the endpoint of an increase in M2BP at the second versus first measurement revealed that an increase in LDL-C concentrations was an independent determinant of increases in M2BP during the follow-up period (Table 4). Actually, subjects in whom

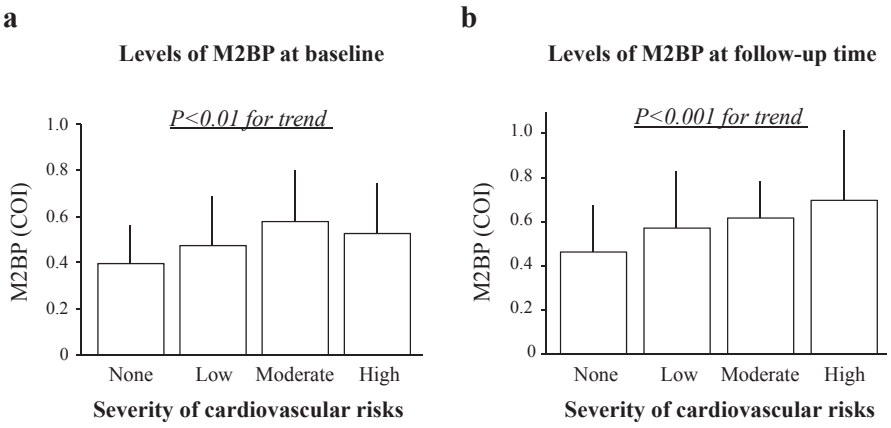

Figure 1 Relationship between the severity of cardiovascular risk in individuals assessed at baseline and Mac-2 binding protein (M2BP) concentrations at (a) baseline and (b) follow-up. Data are shown as the mean ± SD. COI, cut-off index.

M2BP concentrations increased during the study period exhibited significantly greater increase in LDL-C concentration than subjects in whom M2BP concentrations did not increase (data not shown). These results were not significantly affected by the adjustment for medications started during the follow-up period (Table 3, Model 3 and Table 4, Model 3).

Discussion

The main findings of the present study are that: (i) the severity of cardiovascular risk in individuals assessed at baseline was significantly associated with M2BP concentrations at both baseline and follow-up; (ii) the elderly, subjects with diabetes mellitus, or subjects with higher radial AI than the mean value at baseline showed a greater increase in the M2BP concentrations during the follow-up period than the non-elderly, those without diabetes mellitus, or those with lower radial AI; (iii) a change in serum M2BP concentrations was significantly associated with changes in LDL-C, triglyceride, and d-ROM concentrations during the follow-up period in both univariate and multivariate regression analyses; and (iv) an increase in LDL-C concentrations was an independent determinant of increases in M2BP concentrations during the follow-up

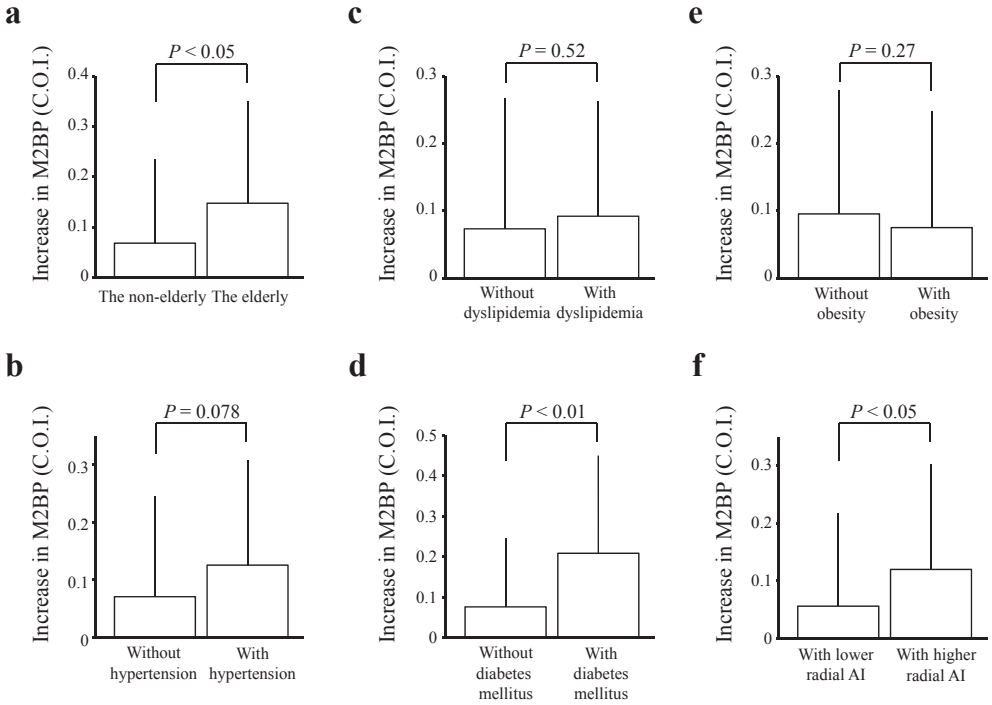

Figure 2 Increases in Mac-2 binding protein (M2BP) concentrations in (a) the elderly and subjects with (b) hypertension, (c) dyslipidemia, (d) diabetes mellitus, (e) obesity, and (f) subjects with higher radial augmentation index (AI) than the mean value (83.1 ± 12.7%) at baseline than in the non-elderly (a) and subjects without (b) hypertension, (c) dyslipidemia, (d) diabetes mellitus, (e) obesity (body mass index ≥ 25 kg/m^2), and (f) subjects with lower radial AI than the mean value at baseline. Data are shown as the mean ± SD.

Table 2 Univariate regression analysis demonstrating possible relationships between percentage changes in Mac-2 binding protein and other factors during the follow-up period ($n = 174$).

Variable	% Change from baseline	r	P-value
Body mass index	0.61 ± 5.0	0.20	<0.01
SBP	0.98 ± 11.5	0.081	0.29
DBP	−2.4 ± 12.2	0.11	0.15
Hemoglobin	1.9 ± 8.7	0.12	0.10
Creatinine	3.8 ± 10.1	0.045	0.55
FPG	−0.23 ± 7.65	0.11	0.17
HDL-C	2.3 ± 12.7	−0.20	<0.01
LDL-C	6.7 ± 18.5	0.25	<0.01
Triglyceride	10.5 ± 42.9	0.25	<0.001
AST	5.7 ± 21.2	0.21	<0.01
ALT	9.7 ± 37.8	0.25	<0.01
γ-GTP	−5.3 ± 32.8	0.15	<0.05
Uric acid	4.1 ± 13.2	−0.69	0.37
eGFR	−5.0 ± 9.6	−0.016	0.83
d-ROM	19.5 ± 27.4	0.19	<0.05
M2BP	25.1 ± 40.2	–	–

ALT, alanine transaminase; AST, aspartate transaminase; d-ROM, derivatives of reactive oxygen metabolites; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M2BP, Mac-2 binding protein; SBP, systolic blood pressure.

period in logistic regression analysis with the endpoint of increased M2BP concentrations at the second measurement. These results support the notion that serum M2BP may be a novel biomarker that increases with increasing levels of LDL-C, triglyceride, and the oxidative stress marker d-ROM.

Cardiovascular risk factors, such as hypertension, dyslipidemia, and diabetes mellitus, promote atherosclerosis, which is a slowly progressive disease of the vascular system initiated by functional alterations and followed by structural changes to the vascular wall [18–21]. A close association was observed in the present study between M2BP concentrations and the severity of cardiovascular risk, confirming our hypothesis based on our previous report [7]. There have been no previous reports regarding specific medications that influence M2BP levels. However, several drugs, such as antimicrobial, gastrointestinal, cardiovascular, lipid-lowering, and anti-thrombotic drugs, may induce liver damage [10,11]. Such liver damage in addition to liver fibrosis could cause an increase of M2BP levels [12]. Subjects under medication at baseline were excluded in the present study in order to minimize the possibility that latent liver damage (which cannot be identified by screening examinations) might influence M2BP levels. Moreover, adjustment for medication started during the follow-up period did not influence the results in the present study, although it should be stated that the assessment of medications was based on questionnaire data. Among the parameters examined, changes in LDL-C and triglyceride, relevant to both cardiovascular risk and metabolic parameters, were significantly associated with changes in M2BP concentrations during the follow-up period. Both dyslipidemia and metabolic syndrome have been shown to exert considerable effects on the progression of atherosclerosis and cardiovascular disease [22–25]. In particular, LDL-C, one of the main causes of the accumulation of atherosclerotic plaques in coronary artery disease [25–27], was an independent determinant of increases in M2BP concentrations. These results are consistent with recent reports and indicate that M2BP may have a role as one of the contributors to the development of atherosclerotic disease [8,9]. Xie et al. [8] reported elevated circulating M2BP levels in association with plaque instability and acute coronary syndrome, and further showed recently the expression of M2BP with plaque instability in human carotid atheroma sampled during carotid endarterectomy [28]. These reports suggest a close association between M2BP levels and progression of atherosclerosis that is independent of hepatic fibrosis and malignant tumor, strongly supporting our present findings. On the other hand, heart failure was recently associated with serum M2BP levels [29], in that subjects with heart failure complicated with liver dysfunction showed increased serum M2BP level, which decreased with improvements in the heart failure symptoms. Although these previous findings do not necessarily support our hypothesis, subjects with heart failure might often have advanced atherosclerosis leading to elevated M2BP when heart failure is a consequence of coronary artery disease.

The onset of cardiovascular disease is based largely on the development of atherosclerosis, which brings about decreased blood flow in the peripheral circulation and subsequent ischemic changes that lead to systemic organ damage [21,30,31]. Increased oxidative stress is one of the common pathways underlying the initiation and progression of atherosclerosis [15,32,33]. In a previous cross-sectional study we reported a positive correlation between d-ROM and M2BP concentrations [7]. The longitudinal association between changes in d-ROM and M2BP concentrations observed in the present study further reinforces the concept that oxidative stress plays an important role in the atherosclerotic process. However, changes in d-ROM concentrations were not an independent determinant for increases in M2BP during the follow-up period in logistic regression analysis. The detailed mechanism is not clear, but the relatively small progression of atherosclerosis during the short observational period may have affected the results. In fact, obvious cardiovascular events, which may be relevant to the increase in M2BP concentrations, were not been observed during the study period. Extension of the observation period may be needed to confirm significant progression of atherosclerotic disease in subjects without previous cardiovascular events.

The present study has several limitations. First, the sample size was small and the background of the subjects was heterogeneous. Moreover, a selection bias cannot be completely excluded, because subjects in the present study were participants in our physical check-up program and thus might have had underlying concerns about their health condition. In addition, the dropout rate was higher than expected due to the study design. Second, the

Table 3 Multivariate regression analysis investigating possible relationships between percentage changes in Mac-2 binding protein and other factors during the follow-up period ($n = 174$).

Variable	SE	β	*P*-value
Model 1			
BMI	0.63	0.050	0.48
SBP	0.25	0.11	0.14
Creatinine	0.29	0.074	0.31
FPG	0.39	0.040	0.59
HDL-C	0.25	−0.14	0.07
LDL-C	0.16	0.26	<0.001
Triglyceride	0.075	0.18	<0.05
AST	0.23	0.19	0.12
ALT	0.15	0.12	0.37
γ-GTP	0.11	−0.061	0.51
Model 2			
BMI	0.62	0.050	0.52
SBP	0.25	0.11	0.14
Creatinine	0.29	0.074	0.31
FPG	0.39	0.032	0.66
HDL-C	0.24	−0.15	0.06
LDL-C	0.16	0.24	<0.01
Triglyceride	0.075	0.17	<0.05
AST	0.23	0.16	0.18
ALT	0.14	0.15	0.26
γ-GTP	0.11	−0.081	0.38
d-ROM	0.10	0.15	<0.05
Model 3			
BMI	0.62	0.051	0.51
SBP	0.26	0.13	0.079
Creatinine	0.29	0.033	0.65
FPG	0.39	0.007	0.92
HDL-C	0.24	−0.14	0.075
LDL-C	0.17	0.27	<0.001
Triglyceride	0.076	0.15	0.072
AST	0.23	0.13	0.30
ALT	0.14	0.18	0.19
γ-GTP	0.11	−0.061	0.51
d-ROM	0.11	0.14	<0.05
Antihypertensive medications	6.92	0.13	0.10
Lipid-lowering medications	7.73	0.034	0.67
Hypoglycemic medications	12.0	0.089	0.22

Model 1 included major risk factors and biochemical parameters showing significant association with Mac-2 binding protein (M2BP) in Table 2 after adjustment for age and sex.
Model 2 was adjusted for derivatives of reactive oxygen metabolites (d-ROM) in addition to the factors included in Model 1 after adjustment for age and sex.
Model 3 was adjusted for medications in addition to the factors included in Model 2 after adjustment for age and sex.
ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.

Table 4 Results of logistic regression analysis with the endpoint of an increase in Mac-2 binding protein during the follow-up period associated with percentage changes in factors ($n = 174$).

Variable	OR (95% CI)	*P*-value
Model 1		
BMI	1.06 (0.97–1.15)	0.20
SBP	1.01 (0.98–1.05)	0.47
Creatinine	0.99 (0.96–1.04)	0.92
FPG	0.98 (0.92–1.03)	0.42
HDL-C	0.99 (0.95–1.02)	0.43
LDL-C	1.03 (1.01–1.06)	<0.01
Triglyceride	1.01 (0.99–1.02)	0.44
AST	1.00 (0.97–1.03)	0.99
ALT	1.00 (0.98–1.02)	0.72
γ-GTP	0.99 (0.98–1.01)	0.52
Model 2		
BMI	1.06 (0.97–1.15)	0.20
SBP	1.01 (0.98–1.05)	0.47
Creatinine	0.99 (0.96–1.04)	0.92
FPG	0.98 (0.92–1.03)	0.42
HDL-C	0.99 (0.95–1.02)	0.40
LDL-C	1.03 (1.01–1.06)	<0.01
Triglyceride	1.00 (0.99–1.02)	0.47
AST	1.00 (0.97–1.03)	0.96
ALT	1.00 (0.99–1.02)	0.68
γ-GTP	0.99 (0.98–1.01)	0.48
d-ROM	1.00 (0.99–1.02)	0.58
Model 3		
BMI	1.07 (0.99–1.17)	0.13
SBP	1.03 (0.99–1.07)	0.17
Creatinine	1.00 (0.96–1.05)	0.85
FPG	0.96 (0.91–1.02)	0.21
HDL-C	0.99 (0.96–1.03)	0.84
LDL-C	1.04 (1.02–1.07)	<0.01
Triglyceride	1.01 (0.99–1.02)	0.44
AST	0.99 (0.97–1.03)	0.98
ALT	1.01 (0.99–1.03)	0.42
γ-GTP	0.99 (0.98–1.01)	0.41
d-ROM	1.00 (0.99–1.02)	0.81
Antihypertensive medications	1.42 (0.53–3.84)	0.49
Lipid-lowering medications	2.52 (0.81–7.83)	0.11
Hypoglycemic medications	4.02 (0.38–42.2)	0.25

Model 1 included major risk factors and biochemical parameters showing significant association with Mac-2 Binding Protein (M2BP) in Table 2 after adjustment for age and sex.
Model 2 was adjusted for derivatives of reactive oxygen metabolites (d-ROM) in addition to the factors included in Model 1 after adjustment for age and sex.
Model 3 was adjusted for medications in addition to the factors included in Model 2 after adjustment for age and sex.
ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; FPG, fasting plasma glucose; γ-GTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; SBP, systolic blood pressure.

observation interval of 3 years may not be enough to investigate a possible role of M2BP in the process of atherosclerosis. Moreover, we could not measure arterial stiffness at the end of the follow-up period and therefore the progression of atherosclerosis was not examined directly. Third, the present study is an observational study and, as such, could not examine the causal relationship between M2BP and the other parameters. An interventional or biological study is needed to elucidate the molecular basis for the observations in the present study. Fourth, the influence of medications could not be completely excluded because although subjects under medication at baseline were not included and an adjustment for medication started during the follow-up did not alter the results, the assessment of medications was based on questionnaire. Fifth, new onset cardiovascular events or heart failure were not investigated during the follow-up period in the present study. Similarly, the presence of a new malignant tumor or hepatic disease could not be completely excluded during the follow-up period.

Regardless of these limitations, the present study is the first prospective observational study to demonstrate the relationship between M2BP and the other parameters. Although the spectrum was narrow, the slight increase in serum M2BP concentrations may be associated with increasing levels of LDL-C, triglyceride, and the level of oxidative stress. Serum M2BP concentrations may be a novel way to detect the progression of subclinical atherosclerosis.

Conclusions

During the observation period of >3 years, serum M2BP concentrations were increased in subjects who also exhibited increased levels of LDL-C, triglyceride, or the oxidative stress marker d-ROM. Of note, increased LDL-C concentrations were significantly associated with increases in M2BP during the follow-up period. These results support that serum concentrations of M2BP reflects one of the contributors to the progression of silent atherosclerosis.

Conflicts of interest

There are no conflicts of interest to declare.

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