Journal of Diabetes and Its Complications 32 (2018) 1100–1104

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Circulating adipocyte fatty acid-binding protein levels predict the development of subclinical atherosclerosis in type 2 diabetes

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ARTICLE INFO

Article history:
Received 16 April 2018
Received in revised form 20 August 2018
Accepted 1 September 2018
Available online 7 September 2018

Keywords:
A-FABP
Atherosclerosis
Biomarker
Diabetes
Prediction

ABSTRACT

Objective: The aim of this study was to investigate the prospective association of circulating adipocyte fatty acid-binding protein (A-FABP) levels with the development of subclinical atherosclerosis in patients with type 2 diabetes in an 8-year prospective study.
Methods: A total of 170 patients with newly diagnosed type 2 diabetes were recruited in the study and 133 patients completed the follow-up of 8 years. Baseline plasma A-FABP levels were measured with enzyme-linked immunosorbent assays. The role of A-FABP in predicting the development of subclinical atherosclerosis over 8 years was analyzed using multiple logistic regression.
Results: Of the 133 patients without subclinical atherosclerosis at baseline, a total of 100 had progressed to subclinical atherosclerosis over 8 years. Baseline A-FABP level was significantly higher in patients who had progressed to subclinical atherosclerosis at year 8 compared with ones who had not developed subclinical atherosclerosis after adjustment for sex (15.3 [12.1–23.2] versus 13.3 [10.0–18.9] ng/ml, $P = 0.021$). High baseline A-FABP level was an independent predictor for the development of subclinical atherosclerosis in patients with type 2 diabetes (odds ratio: 16.24, $P = 0.022$).
Conclusions: Circulating A-FABP levels predict the development of subclinical atherosclerosis in type 2 diabetes patients.

1. Introduction

Atherosclerosis is a chronic, progressive, inflammatory disease with subclinical stage and clinical stage. Subclinical atherosclerosis, characterized by thicken of artery wall and vulnerable plaque, is regarded as the early and asymptomatic phase of atherosclerosis; while clinical atherosclerosis is defined with overt manifestations such as angina, myocardial infarction, stroke and vascular death.[1] However, if subclinical atherosclerosis is left untreated, it can lead to clinical atherosclerosis which continue to be the leading causes of morbidity and mortality worldwide.[2]

Patients with diabetes are at a two-fold higher risk of developing atherosclerosis compared with subjects without diabetes.[3] Therefore, identification of reliable biomarkers for prediction and stratification of subclinical atherosclerosis is crucial for the prevention in diabetes patients. Previous studies have identified a number of potential biomarkers for atherosclerosis,[4] including high sensitivity C-reactive protein (hsCRP), interleukin-1 (IL-1), IL-6, apolipoproteins B and A-I. However, the clinical relevance and predictive value of these biomarkers in macrovascular complications of type 2 diabetes shows limitations and remains controversial, therefore further investigations are needed.

Adipocyte fatty acid-binding protein (A-FABP) is a 15-kD protein which is highly expressed in mature adipocytes and macrophages while also detectable in serum. It has been reported that increased circulating A-FABP is associated with atherosclerosis.[5,6] A-FABP concentration was found to be an independent predictor of atherosclerosis and the progression of carotid atherosclerosis in a population-based cohort design.[7] In both Korean and Chinese patients with coronary artery disease, the serum A-FABP levels increased as the number of stenotic

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https://doi.org/10.1016/j.jdiacomp.2018.09.001

coronary arteries increased.[8,9] Furthermore, observational long term follow-up studies suggested a pathogenetic role of A-FABP in atherosclerotic diseases in human. Peeters et al. showed that A-FABP levels in carotid atherosclerotic lesions were associated with unstable plaque phenotype and an increased risk for cardiovascular events during a 3-year follow-up in patients after carotid endarteryectomy.[10] Furuhashi et al. reported that serum A-FABP was a predictor of 7-year cardiovascular mortality in 61 patients with end-stage renal disease.[11] Higher A-FABP level was associated with 10-year cardiovascular morbidity and mortality in patients with coronary heart disease.[12] Patients with type 2 diabetes not only had a higher risk for developing atherosclerosis, but also had elevated A-FABP levels compared to control groups,[13,14] One report showed that A-FABP was positively associated with higher CVD mortality among men with type 2 diabetes in a 22-year prospective study,[15] but until now there have been no clinical studies to determine whether circulating A-FABP could be a predictor for subclinical atherosclerosis in type 2 diabetes.

In the current study, we performed an 8-year prospective study to evaluate the predictive effect of circulating A-FABP levels on subclinical atherosclerosis in patients with newly diagnosed type 2 diabetes under an intensified, targeted and multifactorial intervention comprising behavior modification and polypharmacologic therapy aimed at several risk factors.

2. Subjects and methods

2.1. Patients and study design

The patients were enrolled from the Chinese National Tenth Five Tackling Key Project which was a multicenter study.[16,17] We recruited 170 patients (87 male, 83 female, average age 53.9 ± 8.6 years) with newly diagnosed type 2 diabetes from the diabetes outpatient department of The Second Xiangya Hospital conducted from January to October 2002. They were further followed up for 8 years by Diabetes Center of The Second Xiangya Hospital, Central South University. Type 2 diabetes was diagnosed according to the American Diabetes Association criteria.[18] Inclusion criteria were: (1) disease duration <1 year; (2) age between 35 and 70 years; (3) body mass index (BMI) between 19 and 35 kg/m^2; (4) without subclinical atherosclerosis; (5) no ketosis within the first 6 months after diagnosis; (6) 4-week wash out if treated with insulin sensitizers; (7) no treatment of antihypertensive drugs or lipid-lowering drugs within 2 months; (8) without subclinical atherosclerosis by vascular ultrasound measurement. The patients were surveyed on lifestyle information (smoking, physical activity, and dietary habits), disease history, and medications by questionnaires.

The patients were followed up for 8 years and from the first beginning they were assigned to undergo intensive multifactorial intervention involving strict treatment goals, to be achieved through behavior modification and a stepwise introduction of pharmacologic therapy overseen by doctors at our center. On average, the patients were offered individual consultations every three months during the 8-year follow-up. The study has been carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association, and has been approved by the Ethics Committee of The Second Xiangya Hospital, Central South University with the approval number 2001-Research-09. All study subjects provided written informed consent.

2.2. Intervention methods

Diabetic diet and light-to-moderate exercise were recommended. Patients with a BMI of 25 or more were started on metformin; those with a BMI of <25 were started on glipizide. All agents were started on half the maximal doses. Subsequently, the dosages were adjusted to meet the goal of glycated hemoglobin A1c (HbA1c) levels <7.0%. Dyslipidemia was diagnosed as having one or more of the following criteria: (1) fasting triglyceride (TG) ≥1.7 mmol/L; (2) high-density lipoprotein-cholesterol (HDL-c) <1.30 mmol/L in female and <1.0 mmol/L in male; (3) low-density lipoprotein-cholesterol (LDL-c) ≥3.4 mmol/L; (4) already on lipid-lowering drugs according to the United States Adult Treatment Panel III.[19] Antihypertensive and lipid-lowering medication and dosages were determined according to protocol guidelines. The goal of blood pressure control is lower than 130/80 mm Hg. The goal of lipid control is based on recommendations of the United States Adult Treatment Panel III.[19] Dietary, exercise, and diabetes education were provided to all patients following the recommendations from the American Diabetes Association.[20] All patients were prescribed aspirin unless contraindicated.

2.3. Clinical and biochemical assessment

The study started from 2002. Biochemical[21] and clinical[22] data were obtained every three months. After at least 10 h of overnight fasting, a venous blood specimen was collected in the morning (around 8:00), for the assay of biochemical parameters: plasma glucose, insulin, TG, LDL-c, HDL-c and hsCRP.[23] The anthropometric measurements (body weight, height, waist circumference, and resting blood pressure) were carried out. Plasma glucose was measured by hexokinase method on a Hitachi 7170 analyzer (Boehringer Mannheim, Mannheim, Germany). Serum cholesterol, and TG levels were determined enzymatically on the Hitachi 7170 analyzer (Boehringer Mannheim). Serum hsCRP was measured with an immunoturbidimetric assay on the Hitachi 7170 analyzer (Boehringer Mannheim). Serum insulin was assessed by chemiluminescence on a Bayer 180SE Automated Chemiluminescence Systems (Bayer AG, Leverkusen, Germany). Insulin resistance (IR) was estimated using homeostasis model assessment (HOMA) index. Serum lipocalin-2, RBP4 and adiponectin levels were detected with ELISA kits (Antibody and Immunoassay Services, The University of Hong Kong, Hong Kong). Human A-FABP was measured with an enzyme-linked immunosorbent assays (ELISA) kit (BioVendor Laboratory Medicine, Inc., Modrice, Czech Republic) as reported.[24,25]

2.4. Vascular ultrasound measurement

Intima-media thickness is a marker of subclinical atherosclerosis at the level of carotid arteries. The intima-media thickness (IMT) of common carotid, femoral and common iliac arteries on the right side were evaluated by high-resolution B-mode ultrasound (128XP/10 system; Acuson, Mountain View, California, USA). The measurements of IMT were made at the site of greatest thickness. Plaque was defined as having an IMT ≥1.3 mm or a focal protrusion into the lumen with a thickness of at least 50% more than the adjacent intima-media complex. Subclinical atherosclerosis was defined as having an IMT >1.0 mm and/or plaque on one or more of the three arteries without any clinical manifestations.[26–29]

2.5. Statistical analysis

All statistical analyses were performed with Statistical Package for Social Science Version 16.0 (SPSS 16.0, Inc., Chicago, IL). Data were expressed as mean ± SD or median with interquartile range as appropriate. Data that were not normally distributed, as determined using Kolmogorov-Smirnov test, were naturally logarithmically transformed before analysis. Correlations between A-FABP and biochemical variables were analyzed with Pearson correlation or partial correlation as appropriate. Data comparisons between groups were performed using χ^2 tests for categorical variables and independent-samples *t*-test or univariate general linear model as indicated for continuous variables. Multiple logistic regression analysis was done to determine independent predictors of the development of subclinical atherosclerosis, and included baseline variables those were significantly different between patients with and without subclinical atherosclerosis and were biologically likely

to be related with atherosclerotic status. Two-sided P values < 0.05 were considered significant.

3. Results

3.1. Plasma A-FABP and clinical parameters at baseline

Pearson correlation analysis was performed to identify the association of plasma A-FABP levels with various clinical and biochemical parameters in type 2 diabetes subjects (n = 133). At baseline, plasma A-FABP correlated positively with BMI, fasting insulin, HOMA-IR and hsCRP. After adjustment for sex and age, plasma A-FABP correlated positively with BMI, waist circumference, waist hip ratio, fasting insulin, HOMA-IR and hsCRP. Consistent with our previous reports,[24,25] women had higher plasma A-FABP levels: 19.9 [13.9–31.8] versus 12.9 [9.8–16.5] ng/ml in men; $P < 0.001$ (Table 1).

3.2. Plasma A-FABP was a predictor for the development of subclinical atherosclerosis in type 2 diabetes over 8 years

None of the 170 newly-diagnosed patients with diabetes had subclinical atherosclerosis at baseline. Among them, 133 patients completed the follow-up assessment at year 8. The main reasons for being lost to follow-up included withdrawal of consent, emigration, changes of address, disability and death. There were no significant differences in any baseline parameters between the patients who finished final visit and those who did not.

During the 8-year follow-up, a total of 100 type 2 diabetes patients (75.2%) had developed subclinical atherosclerosis. Unexpectedly, only baseline age, sex and plasma A-FABP were found to be different between the group with subclinical atherosclerosis and the one without at year 8. Baseline A-FABP concentration was significantly higher in type 2 diabetes patients who developed subclinical atherosclerosis than those who did not develop subclinical atherosclerosis at year 8 after adjustment for sex (15.3 [12.1–23.2] versus 13.3 [10.0–18.9] ng/ml, $P = 0.021$, Table 2).

Independent predictors for the development of subclinical atherosclerosis were identified using a multiple logistic regression model including age, sex, BMI, smoking status, fasting insulin, glucose level, presence of hypertension, presence of dyslipidemia and A-FABP (Model 1, Table 3). As shown in Table 3, after adjustment for sex, age and other factors, baseline A-FABP was a significant independent predictor for the development of subclinical atherosclerosis (OR: 16.29; 95% confidence interval (CI): 1.50–176.68; $P = 0.022$), together with age ($P = 0.012$) and sex ($P < 0.001$). If baseline fasting glucose was replaced by 2-h glucose (Model 2) or HbA1c (Model 3) in the model, A-FABP entered the models as well, serving as independent predictors for the development of subclinical atherosclerosis ($P = 0.035$ and $P = 0.021$, respectively).

Table 1
Pearson correlations of baseline plasma A-FABP with various clinical and biochemical parameters in Type 2 diabetes patients (n = 133).

Parameters	A-FABP			
	r	P	r[b]	P[b]
Age	0.078	NS		
Sex	0.441	<0.001		
BMI	0.299	<0.001	0.418	<0.001
Waist circumference	0.170	NS	0.414	<0.001
Waist hip ratio	0.020	NS	0.284	0.001
Systolic blood pressure	0.132	NS	0.106	NS
Diastolic blood pressure	0.052	NS	0.095	NS
Fasting glucose	0.005	NS	−0.006	NS
Triglycerides[a]	0.051	NS	0.162	NS
HDL cholesterol	0.089	NS	−0.095	NS
LDL cholesterol	0.129	NS	0.121	NS
Fasting insulin[a]	0.197	0.023	0.211	0.016
HOMA-IR[a]	0.171	0.048	0.175	0.047
hsCRP[a]	0.331	<0.001	0.243	0.005

r indicates Pearson correlation coefficient; A-FABP, adipocyte fatty acid-binding protein; BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein.
[a] Log transformed before analysis.
[b] Adjusted for sex and age.

Table 2
Baseline clinical parameters of type 2 diabetes patients who had or had not developed subclinical atherosclerosis at year 8.

Parameters	subAS (n = 100)	Non-subAS (n = 33)	P
Age (yr)	54.9 ± 8.5	50.3 ± 7.7	0.012
Sex (M/F)	61/39	12/21	0.014
Smoking status (Ever/Never)	42/58	8/25	NS
BMI (kg/m^2)	24.2 ± 2.6	24.4 ± 2.9	NS
Waist circumference (cm)			
Men	89.6 ± 8.5	88.3 ± 5.2	NS[b]
Women	83.2 ± 6.9	82.8 ± 8.4	
Systolic blood pressure (mm Hg)	119 ± 16.4	115 ± 17.1	NS
Diastolic blood pressure (mm Hg)	77 ± 9.6	76 ± 11.1	NS
Fasting glucose (mmol/L)	7.1 ± 2.2	7.4 ± 1.7	NS
Fasting Insulin (mIU/L)[a]	14.0 (9.0–19.0)	13.0 (9.0–18.0)	NS
HOMA-IR[a]	4.1 (2.7–7.3)	4.0 (2.7–5.6)	NS
Triglycerides (mmol/L)[a]	1.7 (1.1–2.9)	1.7 (1.2–2.6)	NS
LDL cholesterol (mmol/L)	3.0 ± 0.8	3.0 ± 1.2	NS
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.4 ± 0.3	NS
hsCRP (mg/L)[a]	1.7 (0.5–3.9)	1.1 (0.5–2.3)	NS
A-FABP (μg/L)[a]			
Men	15.1 (10.8–31.2)	11.2 (8.9–13.9)	0.021[b]
Women	21.1 (17.0–32.4)	13.2(10.0–17.5)	
Adiponection (mg/L)[a]			
Men	3.7 (2.6–5.7)	2.6 (2.0–4.7)	NS
Women	5.3 (3.8–9.3)	5.0 (4.3–6.7)	
RBP4 (mg/L)[a]	22.6 (19.9–25.9)	21.9 (20.1–24.8)	NS
Lipocalin-2 (μg/L)[a]	75.9 (48.9–165.5)	90.2 (61.4–141.1)	NS

subAS, subclinical atherosclerosis; M, male; F, female; BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; A-FABP, adipocyte fatty acid-binding protein; RBP4, retinol-binding protein 4.
[a] Log transformed before analysis.
[b] Sex-adjusted.

4. Discussion

In this long-term prospective study, we provided the first clinical evidence demonstrating that plasma A-FABP levels were independently related to the development of subclinical atherosclerosis in type 2 diabetes. We performed a longitudinal study to assess the predictive value of plasma A-FABP for future subclinical atherosclerosis during an 8-year follow-up. Baseline plasma A-FABP levels were significantly increased in patients who suffered from subclinical atherosclerosis 8 years later. Multiple logistic regression analysis revealed that baseline A-FABP was a predictor for the development of subclinical atherosclerosis in type 2 diabetes, and the predictive value of A-FABP was independent of age, sex, BMI, and other classical cardiovascular risk factors. It should be noted that our patients were given intensive intervention for 8 years, which provided a relatively stable metabolic environment that the effects of many other risk factors were excluded. More importantly, the presence of such a stable metabolic environment further underlined the strong capacity of A-FABP for prediction of subclinical atherosclerosis, even in treatment populations.

A-FABP is now established as a key pro-inflammatory molecule that links obesity, diabetes and atherosclerosis.[30–33] A-FABP was initially identified as an intracellular lipid-binding protein abundantly present in adipocytes.[34] The expression of A-FABP is also detectable in macrophages.[35] A-FABP can bind to various saturated and unsaturated fatty acids to facilitate their intracellular transport. Earlier studies on A-FABP have been focused on its role in regulating glucose and lipid

Table 3
Baseline predictors of the development of subclinical atherosclerosis in type 2 diabetes over 8 years, examined using multiple logistic regression.

Parameters	Model 1		Model 2		Model 3	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Age	1.08 (1.02–1.14)	0.012	1.08 (1.02–1.15)	0.008	1.08 (1.02–1.14)	0.011
Sex (female)	0.15 (0.04–0.66)	0.012	0.18 (0.04–0.79)	0.023	0.16 (0.04–0.68)	0.014
BMI	0.88 (0.72–1.07)	0.198	0.87 (0.71–1.07)	0.175	0.87 (0.71–1.07)	0.194
Smoking status	1.07 (0.25–4.54)	0.926	1.27 (0.29–5.58)	0.756	1.09 (0.26–4.63)	0.908
Fasting insulin[a]	1.00 (0.14–7.33)	0.997	1.03 (0.14–7.58)	0.979	0.94 (0.13–6.92)	0.955
Fasting glucose	0.93 (0.76–1.14)	0.491	–	–	–	–
2-h glucose	–	–	0.99 (0.79–1.24)	0.923	–	–
HbA1c	–	–	–	–	1.07 (0.88–1.30)	0.487
Presence of hypertension	0.93 (0.29–2.99)	0.908	0.95 (0.30–3.06)	0.934	0.90 (0.28–2.89)	0.865
Presence of dyslipidemia	1.23 (0.43–3.55)	0.701	1.23 (0.42–3.61)	0.710	1.17 (0.40–3.42)	0.781
A-FABP[a]	16.24 (1.50–176.06)	0.022	12.88 (1.20–137.92)	0.035	16.21 (1.53–172.20)	0.021

Multiple logistic regression models: 1. Model 1 included age, sex, BMI, smoking status, fasting insulin, fasting glucose, presence of hypertension, presence of dyslipidemia and A-FABP. 2. Model 2 replaced fasting glucose by 2-h glucose. 3. Model 3 replaced fasting glucose by HbA1c. A-FABP remained a significant independent predictor for the development of subclinical atherosclerosis in all three models.
[a] Log transformed before analysis.

metabolism. Nevertheless, the most striking phenotypic change observed was the remarkable resistance to atherosclerosis in the animal model of apolipoprotein E (apoE)-deficient mice with A-FABP deficiency.[36] Targeted disruption of the A-FABP gene caused approximately 88% reduction of atherosclerotic lesion throughout aorta in apolipoprotein E deficient (apoE−/−) mice.[35] This A-FABP deficiency-mediated protection against atherogenesis persisted even when the animals were kept on a hypercholesterolemic Western diet.[37] Furthermore, when mice were challenged with a high-fat atherogenic diet for 1 year, the survival rates of apoE−/− mice null for A-FABP were 67% higher than those of apoE−/− control mice, primarily due to the increased stability of atherosclerotic plaques.[36] Treatment of apoE−/− mice with A-FABP selective inhibitor BMS309403 led to approximately 50% less atherosclerotic lesions in the aorta compared to the vehicle-treated group,[38] suggesting that the pharmacological inhibition of A-FABP might represent an effective therapeutic strategy for treatment of atherosclerosis. These results suggest that A-FABP is a major mediator of atherosclerotic in mice. In humans, A-FABP is expressed in monocytes on PPARgamma activation,[39] and oxidized LDL has been shown to induce A-FABP expression in human THP-1 macrophage cell lines.[40] Furthermore, a genetic variant at A-FABP locus with reduction in A-FABP expression reduced the risk for cardiovascular disease and type 2 diabetes in a population genetic study.[41] Circulating A-FABP was found to be significantly associated with a greater risk of atherosclerotic diseases in several clinical studies. Agardh et al. collected endarterectomy samples from patients undergoing surgery for symptomatic and asymptomatic carotid stenosis prospectively and found out expression of A-FABP was increased at the mRNA level in unstable plaques.[42] Further investigations carried out by Furuhashi et al. showed localization of A-FABP to macrophage population via immunohistochemical analyses of endarterectomy samples; subsequently they proved that A-FABP locally produced by epicardial/perivascular fat and macrophages in vascular plaques contributed to the development of coronary athersosclerosis.[43] These results suggest that A-FABP may play a causative role in the development of atherosclerotic diseases in humans, serving as a key factor connecting vascular and cellular lipid accumulation to inflammation.

The relationship of A-FABP with metabolic disease has been well demonstrated in preclinical[44] and several clinical studies, which might partly explain the impact of A-FABP on subclinical atherosclerosis. Xu et al. demonstrated that A-FABP levels were not only associated with insulin resistance, adiposity, dyslipidemia and glucose tolerance parameters, but also predicted the development of the metabolic syndrome and type 2 diabetes in 5-year and 10-year prospective studies.[13,24,25] In agreement with these findings, we found that BMI, waist circumference, waist hip ratio, fasting insulin, HOMA-IR and hsCRP were associated with A-FABP after adjustment for sex and age.

However, our study has some limitations. Firstly, this study only included subjects with type 2 diabetes. Even though we evaluated the predictive effect of plasma A-FABP for subclinical atherosclerosis, the predictive effect in the general population should be further studied in population-based studies. Secondly, due to the relatively low number of the specimens, the cut-off point of plasma A-FABP as a predictor is still unclear in the present study, which should be determined in a larger cohort. Thirdly, ultrasound can assess only the structural changes in arteries, but not the vascular inflammatory status or qualitative assessment. Therefore, fluorodeoxyglucose positron emission tomography and virtual histology-intravascular ultrasound may provide more precise data on the vascular inflammation and plaque components.

5. Conclusion

In summary, our study found that type 2 diabetes patients who developed subclinical atherosclerosis 8 years later had higher baseline circulating A-FABP levels, and demonstrated that circulating A-FABP could serve as an independent predictor for the development of subclinical atherosclerosis in type 2 diabetes patients. Our data, in conjunction with previous animal and clinical studies, supports an important role of A-FABP in the development of atherosclerosis in the context of type 2 diabetes.

Abbreviations

A-FABP	adipocyte fatty acid-binding protein
BMI	body mass index
HbA1c	hemoglobin A1c
TG	triglyceride
HDL-c	high-density lipoprotein-cholesterol
LDL-c	low-density lipoprotein-cholesterol
hsCRP	high sensitivity C-reactive protein
IR	insulin resistance
HOMA	homeostasis model assessment
IMT	intima-media thickness
apoE	apolipoprotein E

Acknowledgements

We thank all investigators from the Chinese National Tenth Five and Eleventh Five Tackling Key Project for collecting blood samples and data, and we also thank the patients.

Funding

This work was supported by the National Natural Science Foundation of China (NSFC)-Research Grants Council (RGC) grant [81461168031, to Z.Z.] and NSFC grant [81670772, to Y.X.].

Authors' contribution

Y Xiao and X Xiao together analyzed and interpreted the data, and drafted the article. A Xu interpreted the data, and revised the article. XY Chen and WL Tang collected and interpreted data. ZG Zhou supervised the studies, contributed to the discussion and edited the article. All authors approved the final version of the article.

Ethics approval and consent to participate

The study has been approved by the Ethics Committee of The Second Xiangya Hospital, Central South University. All study subjects provided written informed consent.

Declarations of interest

None.

References

1. Fernandez-Friera L, Penalvo JL, Fernandez-Ortiz A, Ibanez B, Lopez-Melgar B, Laclaustra M, et al. Prevalence, vascular distribution, and multiterritorial extent of subclinical atherosclerosis in a middle-aged cohort: the PESA (Progression of Early Subclinical Atherosclerosis) study. Circulation 2015;131:2104-13.
2. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 2011;17:1410-22.
3. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 2010;375:2215-22.
4. Kampoli AM, Tousoulis D, Antoniades C, Siasos G, Stefanadis C. Biomarkers of premature atherosclerosis. Trends Mol Med 2009;15:323-32.
5. Yeung DC, Xu A, Cheung CW, Wat NM, Yau MH, Fong CH, et al. Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis. Arterioscler Thromb Vasc Biol 2007;27:1796-802.
6. Jin J, Peng DQ, Yuan SG, Zhao SP, Ning XH, Wang SH, et al. Serum adipocyte fatty acid binding proteins and adiponectin in patients with coronary artery disease: the significance of A-FABP/adiponectin ratio. Clin Chim Acta 2010;411:1761-5.
7. Furuhashi M, Yuda S, Muranaka A, Kawamukai M, Matsumoto M, Tanaka M, et al. Circulating fatty acid-binding protein 4 concentration predicts the progression of carotid atherosclerosis in a general population without medication. Circ J 2018;82:1121-9.
8. Rhee EJ, Lee WY, Park CY, Oh KW, Kim BJ, Sung KC, et al. The association of serum adipocyte fatty acid-binding protein with coronary artery disease in Korean adults. Eur J Endocrinol 2009;160:165-72.
9. Bao Y, Lu Z, Zhou M, Li H, Wang Y, Gao M, et al. Serum levels of adipocyte fatty acid-binding protein are associated with the severity of coronary artery disease in Chinese women. PLoS One 2011;6:e19115.
10. Peeters W, de Kleijn DP, Vink A, van de Weg S, Schoneveld AH, Sze SK, et al. Adipocyte fatty acid binding protein in atherosclerotic plaques is associated with local vulnerability and is predictive for the occurrence of adverse cardiovascular events. Eur Heart J 2011;32:1758-68.
11. Furuhashi M, Ishimura S, Ota H, Hayashi M, Nishitani T, Tanaka M, et al. Serum fatty acid-binding protein 4 is a predictor of cardiovascular events in end-stage renal disease. PLoS One 2011;6:e27356.
12. von Eynatten M, Breitling LP, Roos M, Baumann M, Rothenbacher D, Brenner H. Circulating adipocyte fatty acid-binding protein levels and cardiovascular morbidity and mortality in patients with coronary heart disease: a 10-year prospective study. Arterioscler Thromb Vasc Biol 2012;32:2327-35.
13. Tso AW, Xu A, Sham PC, Wat NM, Wang Y, Fong CH, et al. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care 2007;30:2667-72.
14. Haluzik MM, Anderlova K, Dolezalova R, Adamikova A, Haluzikova D, Housova J, et al. Serum adipocyte fatty acid binding protein levels in patients with type 2 diabetes mellitus and obesity: the influence of fenofibrate treatment. Physiol Res 2009;58:93-9.
15. Liu G, Ding M, Chiuve SE, Rimm EB, Franks PW, Meigs JB, et al. Plasma levels of fatty acid-binding protein 4, retinol-binding protein 4, high-molecular-weight adiponectin, and cardiovascular mortality among men with type 2 diabetes: a 22-year prospective study. Arterioscler Thromb Vasc Biol 2016;36:2259-67.
16. Tian J, Wen Y, Yan L, Cheng H, Yang H, Wang J, et al. Vascular endothelial dysfunction in patients with newly diagnosed type 2 diabetes and effects of 2-year and 5-year multifactorial intervention. Echocardiography 2011;28:1133-40.
17. Li H, Xiao Y, Liu H, Chen XY, Li XY, Tang WL, et al. Hypoadiponectinemia predicts impaired endothelium-independent vasodilation in newly diagnosed type 2 diabetic patients: an 8-year prospective study. Chin Med J (Engl) 2011;124:3607-12.
18. Diagnosis and classification of diabetes mellitusDiabetes Care 2007;30:S42-7.
19. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
20. American Diabetes A. Standards of medical care for patients with diabetes mellitus. Diabetes Care 2002;25:213-29.
21. Xiao Y, Xu A, Law LS, Chen C, Li H, Li X, et al. Distinct changes in serum fibroblast growth factor 21 levels in different subtypes of diabetes. J Clin Endocrinol Metab 2012;97:E54-8.
22. Xiao Y, Liu L, Xu A, Zhou P, Long Z, Tu Y, et al. Serum fibroblast growth factor 21 levels are related to subclinical atherosclerosis in patients with type 2 diabetes. Cardiovasc Diabetol 2015;14:72.
23. Xiang Y, Zhou P, Li X, Huang G, Liu Z, Xu A, et al. Heterogeneity of altered cytokine levels across the clinical spectrum of diabetes in China. Diabetes Care 2011;34:1639-41.
24. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem 2006;52:405-13.
25. Xu A, Tso AW, Cheung BM, Wang Y, Wat NM, Fong CH, et al. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. Circulation 2007;115:1537-43.
26. Kardys I, Oei HH, van der Meer IM, Hofman A, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A2 and measures of extracoronary atherosclerosis: the Rotterdam Study. Arterioscler Thromb Vasc Biol 2006;26:631-6.
27. Balbarini A, Buttitta F, Limbruno U, Petronio AS, Baglini R, Strata G, et al. Usefulness of carotid intima-media thickness measurement and peripheral B-mode ultrasound scan in the clinical screening of patients with coronary artery disease. Angiology 2000;51:269-79.
28. Wang M, Long W, Li D, Wang D, Zhong Y, Mu D, et al. Plasma 7-ketocholesterol levels and the risk of incident cardiovascular events. Heart 2017;103:1788-94.
29. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 2011;364:127-35.
30. Kralisch S, Fasshauer M. Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease? Diabetologia 2013;56:10-21.
31. Rodriguez-Calvo R, Girona J, Alegret JM, Bosquet A, Ibarretxe D, Masana L. Role of the fatty acid-binding protein 4 in heart failure and cardiovascular disease. J Endocrinol 2017;233:R173-84.
32. Erbay E, Cao H, Hotamisligil GS. Adipocyte/macrophage fatty acid binding proteins in metabolic syndrome. Curr Atheroscler Rep 2007;9:222-9.
33. Hoo RC, Yeung CY, Lam KS, Xu A. Inflammatory biomarkers associated with obesity and insulin resistance: a focus on lipocalin-2 and adipocyte fatty acid-binding protein. Expert Rev Endocrinol Metab 2008;3:29-41.
34. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 1996;274:1377-9.
35. Makowski L, Boord JB, Maeda K, Babaev VR, Uysal KT, Morgan MA, et al. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. Nat Med 2001;7:699-705.
36. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, et al. Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. Circulation 2004;110:1492-8.
37. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, et al. Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. Arterioscler Thromb Vasc Biol 2002;22:1686-91.
38. Furuhashi M, Tuncman G, Gorgun CZ, Makowski L, Atsumi G, Vaillancourt E, et al. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. Nature 2007;447:959-65.
39. Pelton PD, Zhou L, Demarest KT, Burris TP. PPARgamma activation induces the expression of the adipocyte fatty acid binding protein gene in human monocytes. Biochem Biophys Res Commun 1999;261:456-8.
40. Fu Y, Luo N, Lopes-Virella MF. Oxidized LDL induces the expression of ALBP/aP2 mRNA and protein in human THP-1 macrophages. J Lipid Res 2000;41:2017-23.
41. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, et al. A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. Proc Natl Acad Sci U S A 2006;103:6970-5.
42. Agardh HE, Folkersen L, Ekstrand J, Marcus D, Swedenborg J, Hedin U, et al. Expression of fatty acid-binding protein 4/aP2 is correlated with plaque instability in carotid atherosclerosis. J Intern Med 2011;269:200-10.
43. Furuhashi M, Fuseya T, Murata M, Hoshina K, Ishimura S, Mita T, et al. Local production of fatty acid-binding protein 4 in epicardial/perivascular fat and macrophages is linked to coronary atherosclerosis. Arterioscler Thromb Vasc Biol 2016;36:825-34.
44. Burak MF, Inouye KE, White A, Lee A, Tuncman G, Calay ES, et al. Development of a therapeutic monoclonal antibody that targets secreted fatty acid-binding protein aP2 to treat type 2 diabetes. Sci Transl Med 2015;7, 319ra205.