VERSITA

Cent. Eur. J. Med. • 9(1) • 2014 • 28-33
DOI: 10.2478/s11536-013-0231-0

Central European Journal of **Medicine**

Intervention for cardiovascular risk factors decreases adipocyte fatty acid-binding protein levels in males – a pilot study

Research Article

Eliska Sovova*[1], Marketa Kaletova[1], David Stejskal[2], Milan Kaminek[3], Marie Budikova[3], Iva Metelkova[3], Dagmar Horakova[4], Milan Sova[5], Marketa Sovova[6], Jindrichv Palcik[7], Jana Zapletalova[8]

1 Department of Internal Medicine I – Cardiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

2 Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

3 Department of Nuclear Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

4 Department of Preventive Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

5 Department of Respiratory Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

6 Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

7 Department of Exercise Medicine and Cardiovascular Rehabilitation, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

8 Department of Medical Biophysics, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic, Olomouc, 779 00, tr. Svobody 8

Received 8 March 2012; Accepted 10 April 2013

Abstract: Cardiovascular disease (CVD) remains the leading cause of mortality in developed countries. According to the 2012 European Guidelines on Cardiovascular Disease Prevention in Clinical Practice, family history is a cornerstone for risk stratification of CVD. First-degree relatives are persons in whom CVD should be assessed and targeted intervention should be performed. The aims of this pilot study were (i) to determine risk factors (RFs) for cardiovascular disease (CVD) in a group of first-degree relatives of patients with CVD at baseline and after 1 year, (ii) to measure adipocyte fatty acid-binding protein (A-FABP) levels as a potential connecting link between metabolic disease and atherosclerosis, and (iii) to determine the impact of targeted intervention on these parameters. The study comprised 62 asymptomatic subjects (41 males; mean age of 53.8±8.3 years). Preventive examinations and interventions were carried out at baseline and at 1-year follow-up to assess RFs and evaluate A-FABP levels. At 1 year, males had significantly lower levels of cholesterol (median 5.18 vs 4.67, p=0.005), HDL (median 1.24 vs 1.14, p=0.021), LDL (median 3.08 vs 2.46, p=0.021), ApoB (median 0.99 vs 0.82, p=0.012) and A-FABP (median 19.84 vs 16.73, p = 0.015). In females after 1 year, only significantly lower levels of fibrinogen (median 3.10 vs 2.79, p=0.043) were found. All subjects were clinically examined or contacted by phone after a mean of 36.7 months (range, 11-55). Over that time, no serious complications were noted. In males, intervention for RFs leads to lower levels of A-FABP as a potential RF linking metabolic syndrome to atherosclerosis.

Keywords: *Adipocyte fatty acid-binding protein • Prevention • Cardiovascular disease • Relatives*

* *E-mail: eliska.sovova@fnol.cz*

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of mortality in developed countries. According to the 2012 European Guidelines on Cardiovascular Disease Prevention in Clinical Practice, family history is a cornerstone for risk stratification of CVD [1]. First-degree relatives are persons in whom CVD should be assessed and targeted intervention should be performed. Routine genetic tests are not recommended in these individuals. The most effective method for improving the prognosis appears to be lifestyle intervention [2].

Recently, options for making the risk estimates even more accurate have been sought. One of those may be determination of the levels of adipocyte fatty acid-binding protein (A-FABP). Also known as FABP4 or aP2, it is a member of a family of 9 fatty acid-binding proteins. A-FABP is produced by adipocytes and macrophages and accounts for about 1.8-8.1% of all proteins produced by these cells [3,4].

A-FABP plays an important role in regulating glucose metabolism. A positive correlation has been found between the level of A-FABP, level of fasting insulin and insulin resistance index [5]. Patients with rare haploinsufficiency of the gene for A-FABP have significantly reduced risk for diabetes and CVD [6]. A-FABP may be a new biomarker predicting the development of type 2 diabetes [7].

A-FABP is involved in regulation of lipid metabolism since it is highly expressed in both murine and human foam cells present in atherosclerotic plaques. There, A-FABP acts through increased expression of anti inflammatory cytokines by macrophages [4]. Several studies confirmed the association between serum A-FABP levels and coronary artery disease [8,9]. Thus, A-FABP may be a connecting link between metabolic syndrome and atherosclerosis [10,11]. It is not clear what effect the treatment of lipid metabolism disorders has on A-FABP levels. Karpisek et al. reported decreased A-FABP in 26 individuals with lipid metabolism disorder treated with statin [12].

The aims of this pilot study were (i) to determine basic risk factors for CVD in a group of first-degree relatives of patients with CVD at baseline and after 1 year, (ii) to measure A-FABP levels, and (iii) to determine the impact of targeted intervention on these parameters.

2. Patients and methods

The study comprised 62 asymptomatic subjects (41 males) with a mean age of 53.8±8.3 years, first-degree relatives of patients with a history of acute myocardial infarction, coronary angioplasty for significant coronary stenosis or aortocoronary bypass. All patients diagnosed with the above conditions in 2006-2007 received a letter offering examination of their relatives in our center. Those relatives who were interested were enrolled in the study. The study was approved by the ethics committee of the Faculty of Medicine and Dentistry and University Hospital Olomouc. Informed consent was obtained from all participants.

The subjects were investigated at the Department of Internal Medicine I – Cardiology in 2006-2009. In January 2011, all subjects were contacted for the assessment of their current health status.

All subjects underwent clinical examination including casual blood pressure, pulse rate, weight and height measurements, body mass index (BMI; weight divided by height squared) calculations, ECG tests, laboratory blood sampling and calcium scoring. These investigations were repeated at a 1-year follow-up. Excluded from the study were those who changed their weight over the year (by more than 5%).

All subjects underwent both pharmacological and non-pharmacological risk factor intervention according to the current recommendations [2]. Non-pharmacological intervention consisted of dietary recommendations, physical activity recommendations, smoking cessation, training in blood pressure measurement and, if applicable, monitoring of diabetes control.

Concentrations of creatinine, uric acid, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, lipoprotein(a), apolipoprotein B (ApoB), apolipoprotein A-I (ApoA), insulin, glucose, C-reactive protein (CRP), fibrinogen, glycated hemoglobin (Hb) and A-FABP were analyzed in fresh serum from morning venous blood samples taken after a 12-hour fasting period according to the manufacturer's instructions. The A-FABP concentration was determined using an enzyme-linked immunosorbent assay (Bio-Vendor Laboratory Medicine, Inc., Brno, Czech Republic) according to the manufacturer's instructions. The intra- and inter-assay variance was calculated from 3 different samples (CV intra-assay <4.8%, CV inter-assay <10%). The laboratory results were blinded.

3. Statistical methods

Data were processed using the SPSS Statistics 15 software (SPSS Inc., Chicago, USA). Normal distribution was assessed by the Shapiro-Wilk test. In the absence of normal distribution, a parameter was evaluated by

the Wilcoxon test for paired samples. In case of normal distribution, Student's paired t-test was used. Data are stated as mean ± SD or median. A p-value of < 0.05 was considered statistically significant.

4. Results

Most frequently, study subjects' relatives with CVD were fathers (29 subjects, 46.8%), mothers (13 subjects, 21.0%) and brothers (10 subjects, 16.1%), with a mean age at time of event of 58.4±10.1 years.

Clinical characteristics of examined subjects, males and females are shown in Table 1 and include fundamental risk factors as well as existing and new medications.

Results of the studied parameters (all subjects, males and females) at baseline and at 1-year follow-up are shown in Table 2. After 1 year, all subjects has significantly lower levels of total cholesterol (median 5.20 vs 4.80, p=0.012), HDL (median 1.33 vs 1.30, p=0.016), ApoB (median 0.96 vs 0.83, p=0.007) and A-FABP (median 22.3 vs 19.2, p=0.005); and significantly higher levels of glucose (5.05 vs 5.20, p=0.018) and glycated Hb (3.70 vs 3.90, p=0.039).

In males after 1 year, the Wilcoxon paired test revealed significantly lower levels of cholesterol (median 5.18 vs 4.67, p=0.005), HDL (median 1.24 vs 1.14, p=0.021), LDL (median 3.08 vs 2.46, p=0.021), ApoB (median 0.99 vs 0.82, p = 0.012) and A-FABP (median 19.84 vs 16.73, p=0.015); and significantly higher levels of glucose (5.20 vs 5.30, p=0.028). With respect to these results, the effect of statin therapy (18 males (43.9%)-atorvastatin 10 mg on individual laboratory parameters was studied – see Table 3. As expected, the levels of total cholesterol, LDL cholesterol and ApoB dropped. There was no statistically significant decrease in A-FABP (p=0.268) in males with new statin therapy.

In females after 1 year, the Wilcoxon test showed significantly lower levels of fibrinogen (median 3.10 vs 2.79, p=0.043). Since significant differences were not found in the other parameters in females, further analyses of the effects of therapy were not carried out.

All subjects were clinically examined or contacted by phone after a mean of 36.7 months (range, 11-55). Over that time, no serious complications were noted, such as acute myocardial infarction, need for PCI or death.

5. Discussion

Recently, there have been efforts to make CVD risk estimates in asymptomatic individuals more accurate. First-degree relatives of patients, especially those with premature atherosclerosis, belong to a group deserving special attention. As seen from our results as well, they have high rates of CVD risk factors such as hypertension, smoking, lipid metabolism disorder and overweight or obesity.

A.FABP is a promising marker linking metabolic syndrome, inflammation and atherosclerosis. Unfortunately, there has been limited clinical study on these associations. They have shown that higher A-FABP levels may play a role in the progression of atherogenesis, mainly through interference with foam cell formation in response to oxidized LDL cholesterol [13]. In a recent study, Peeters et al. determined A-FABP levels in atherosclerotic plaques to prove that higher A-FABP is associated with plaque instability and symptomatic lesions [14]. Doi et al. reported that A-FABP levels in males under 65 years are significantly associated with coronary artery disease, independently of traditional

Table 1. Clinical characteristics of subjects

Parameter	Total n=62 (number, percentage) (mean±SD, median)	Males n=41 (number, percentage) (mean±SD, median)	Females n=21 (number, percentage) (mean±SD, median)
Smoking	19 (30.6%)	15 (36.6%)	4 (19.0%)
BMI (mean±SD, median)	27.8±3.9 27.1	28.4±4.1 28.7	26.5±3.2 26.2
Overweight	29 (46.8%)	18 (43.9%)	11 (52.4%)
Obesity	19 (30.6%)	16 (39.0%)	3 (14.3%)
Diabetes mellitus	5 (8.1%)	3 (7.3%)	2 (9.5%)
Hypertension	34 (54.8%)	23 (56.1%)	11 (52.4%)
BP systolic (mean±SD, median)	135.4±20.3 137.5	137.3±20.6 140.0	131.7±19.5 130.0
BP diastolic (mean±SD, median)	84.9±12.7 80.0	86.7±12.8 85.0	81.4±11.8 80.0

Table 2. Studied laboratory parameters in all subjects, males, females

	All subjects Baseline n=62 (mean±SD, median)	All subjects At 1 year n=62 (mean±SD, median)	Males Baseline n=41 (mean±SD, median)	Males At 1 year n=41 (mean±SD, median)	Females Baseline n=21 (mean±SD, median)	Females At 1 year n=21 (mean±SD, median)
Creatinine μmol/L	78.7±14.1 76.0	77.5±13.4 77.0	83.1±13.3 80.0	81.1±12.4 82.0	70.2±11.7 72.0	70.7±12.8 69.0
Uric acid μmol/L	341.5±95.6 343.0	327.1±96.5 329.0	383.2±75.9 373.0	364.9±71.4 352.0	262.0±77.7 259.0	255.0±98.2 256.0
CRP mg/L	2.31±2.18 1.40	2.13±2.11 1.60	2.30±1.98 1.7	2.29±2.32 1.80	2.34±2.58 1.0	1.85±1.66 1.40
Total cholesterol mmol/L	5.27±0.94 5.20	4.87±0.91 * 4.80	5.21±0.95 5.18	4.68±0.91 * 4.67	5.40±0.91 5.20	5.25±0.78 5.09
HDL cholesterol mmol/L	1.405±0.425 1.33	1.345±0.402* 1.3	1.265±0.328 1.24	1.211±0.345 * 1.140	1.68±0.46 1.54	1.61±0.38 1.59
LDL cholesterol mmol/L	3.053±0.865 3.07	2.733±0.876 2.71	3.072±0.896 3.80	2.248±0.998 * 2.460	3.01±0.82 3.04	2.90±0.56 2.75
Triglycerides mmol/L	1.799±1.139 1.565	1.762±1.403 1.430	1.878±1.046 1.64	1.816±1.198 1.570	1.64±1.32 1.26	1.66±1.76 1.32
ApoA g/L	1.537±0.337 1.480	1.527±0.271 1.470	1.432±0.248 1.390	1.450±0.255 1.380	1.742±0.397 1.710	1.676±0.244 1.630
ApoB g/L	0.953±0.205 0.960	0.868±0.195 * 0.830	0.969±0.219 0.990	0.873±0.214 * 0.820	0.922±0.177 0.890	0.858±0.154 0.840
Lipoprotein(a) g/L	0.341±0.371 0.147	0.367±0.408 0.187	0.400±0.401 0.173	0.430±0.449 0.195	0.205±0.247 0.102	0.222±0.248 0.085
Glucose mmol/L	5.370±1.121 5.05	5.582±1.326 * 5.2	5.432±1.176 5.2	5.720±1.483 * 5.30	5.249±1.022 5.00	5.314±0.925 5.00
Glycated Hb mmol/mol	3.837±0.706 3.7	4.012±1.246 3.9	3.82±0.74 3.7	4.04±1.42 3.90	3.87±0.64 3.95	3.96±0.85 3.95
Insulin mIU/L	11.82±12.72 9.7	10.67±8.37 8.5	11.42±7.47 10.5	11.30±9.15 8.90	12.60±19.39 7.20	9.47±6.65 8.00
Fibrinogen g/L	2.992±0.716 2.99	2.797±0.681 2.805	2.95±0.76 2.73	2.77±0.75 2.83	3.07±0.65 3.10	2.85±0.53 * 2.79
A-FABP g/L	25.83±13.31 22.29	22.84±13.11 * 19.22	22.76±11.44 19.84	19.30±9.03 * 16.73	31.8±14.9 31.1	29.8±16.9 28.3

**a statistically significant change at a 5% level of significance*

Table 3. Changes in laboratory parameters – comparison of males with newly administered statin therapy and males without statin therapy (Mann-Whitney U-test)

	p		p
Creatinine	0.346	LDL cholesterol	0.0004
Uric acid	0.552	Glucose	0.446
CRP	0.656	ApoA	0.692
Cholesterol	0.0004	ApoB	0.002
Triglycerides	0.728	Lipoprotein(a)	0.075
HDL cholesterol	0.311		
Glycated Hb	0.863		
Insulin	0.064		
Fibrinogen	0.387		
A-FABP	0.268		

risk factors [15]. This is probably due to the fact that A-FABP in macrophages and adipocytes has a pathological effect on blood vessels. Our recent study [16] of a group of asymptomatic first-degree relatives of patients with CVD confirmed correlation of A-FABP levels with insulin resistance indices, BMI, age, gender and insulin and creatinine levels. Although A-FABP seems to be a promising marker for making CVD risk estimates more accurate, more clinical studies are needed.

In a group of individuals with manifest atherosclerosis, Myioshi et al. [8] reported an A-FABP cut-off value for CVD risk of 20.1 ng/mL (76% specificity and 65% sensitivity). The mean level of A-FABP in our group was 25.83 g/L, suggesting a higher risk for CVD.

Females have been reported to have higher A-FABP levels than males [8,16]. Similarly, the mean A-FABL levels in our subjects were 22.76±11.44 and 31.8±14.9 for males and females, respectively. The difference has not been clarified as yet but the amount of fat tissue or various hormonal influences are thought to play a role [13].

Few studies have been published on targeted intervention for the main CVD risk factors in asymptomatic individuals including first-degree relatives. Moreover, their results were not encouraging [17,18]. Yet members of families with premature CVD have been confirmed to have as much as 60% higher risk [19]. Much attention has been paid, for example, to possible commercial genetic tests despite the facts that their predictive value is limited, they do not consider external factors and their results do not change adequate preventive recommendations [20].

The preventive measures taken and medication administered had an impact on the presence of traditional risk factors in our study, particularly in males. After 1-year follow-up, females were found to have only significantly lower fibrinogen levels. As expected, males had statistically significantly decreased levels of total cholesterol, LDL cholesterol and apolipoprotein B. Contrary to our expectations, however, glucose levels were slightly raised and HDL cholesterol levels were lower.

Similarly, this pilot study showed a decrease in A-FABP levels in males after the introduction of preventive measures and changes in medication. However, the effect of therapy with statins was not documented, unlike results of the study by Karpisek et al. [12]. The decrease probably resulted from the intervention as a whole with various contributing factors. There was no decrease in A-FABP levels in females. This may be explained by the fact that there were no changes in either lipid metabolism or glucose and insulin levels.

In the pilot study, only a small sample was investigated. In the available literature, there is no study on A-FABP levels in first-degree relatives with respect to risk factor intervention.

Asymptomatic first-degree relatives of CVD patients, in particular males, are a risk group to be carefully examined, their risk for CVD should be determined and targeted intervention should be provided. Such intervention leads to lower levels of A-FABP as a potential risk factor linking metabolic syndrome to atherosclerosis, independently of pharmacological intervention.

6. Acknowledgement

The study was supported by the Czech Ministry of Health grant project IGA MZ CR NT 11098-4. The authors have no financial relationships with commercial interests to disclose.

References

[1] De Backer G., Ambrosionie E., Borch-Johnsen K., Brotons C., Cifkova R., Dallongeville J., et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). http://www.escardio.org/guidelines-surveys/esc-guidelines/GuidelinesDocuments/guidelines-CVD-prevention.pdf. 14.3.2013

[2] Graham I., Atar D., Borch-Johnsen K., Boysen G., Burell G., Cifkova R., et al., European guidelines on cardiovascular disease prevention in clinical practice: executive summary, EJCPR., 2007, 14, 1-113

[3] Kazemi M.R., McDonald C.M., Shigenaga J.K., Grunfeld C., Feingold K.R., Adipocyte fatty acid-binding protein expression and lipid accumulation are increased during activation of murine macrophages by toll-like receptor agonists. Arterioscler. Thromb. Vasc. Biol., 2005, 25, 1220–1224

[4] Erbay E., Cao H., Hotamisligil G.S. Adipocyte/macrophage fatty acid binding proteins in metabolic syndrome, Curr. Atheroscler. Rep., 2007, 9, 222-9.

[5] Lam D.C., Xu A., Lam K.S. Serum adipocyte-fatty acid binding protein level is elevated in severe OSA and correlates with insulin resistance, Eur. Respir. J., 2009, 33, 346-351

[6] Tuncman G., Erbay E., Hom X., De Vivo I., Campos H., Rimm E.B., 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. USA., 2005, 103, 6970–6975

[7] Tso A.W.K., Xu A., Sham P.C., Wat N.M.S, Wang Y, Fong C.H.Y., 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, 2667-2672

[8] Miyoshi T., Onoue G., Hirohata A., Hirohata S., Usui S., Hina K., et al., Serum adipocyte fatty acid-binding protein is independently associated with coronary atherosclerotic burden measured by intravascular ultrasound, Atherosclerosis, 2010, 211, 164-169

[9] Rhee E.J., Lee W.Y., Park CY. The association of serum adipocyte fatty acid-binding protein with coronary artery disease in Korean adults, Eur. J. Endocrinol., 2009, 160, 165-172

[10] Krusinova E., Pelikanova T., Fatty acid binding proteins in adipose tissue: A promising link between metabolic syndrome and atherosclerosis?, Diabetes Research and Clinical Practice, 2008, 82, 127-134

[11] Stejskal D., Karpíšek M., Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome?, Eur. J. Clin. Invest., 2006, 36, 621-625

[12] Karpisek M., Stejskal D., Kotolova H., Kollar P., Janoutova G., Ochmanova R., et al., Treatment with atorvastatin reduces serum adipocyte-fatty acid binding protein value in patients with hyperlipidemia, Eur. J. Clin. Invest., 2007, 37, 637-642

[13] Makowski L., Brittingham K.C., Reynolds J.M., Sutles J., Hotamisligil G.S., The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and IkappaB kinase activities, J. Biol. Chem., 2005, 280, 12888-12895

[14] Peeters W., de Kleijn D.P.V, Vink A., de Weg S., Schoneveld A.H., Ste S.K, 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, 1758-1768

[15] Doi M., Miyoshi T., Hirohata S., Nakamura K., Usui S., Takeda K, et al., Association of increased plasma adipocyte fatty acid-binding protein with coronary artery disease in non-elderly men, Cardiovasc. Diabetol., 2011, 10, 44

[16] Sovova E., Vrbkova J., Stejskal D., Kaletova M., Kaminek M., Metelkova I., Sova M., Benusova I., Doupalova P., Is there dependence between the level of adipocyte fatty acid-binding protein and calcium score in asymptomatic relatives of patients with cardiovascular diseases?, Vnitrni lekarstvi, 2013, 59(1), 31-36

[17] Mosca L., Mochari H., Liao M., Christian A.H., Edelman D.J., Aggarwal B., et al., A novel family-based intervention trial to improve heart health: FIT Heart: results of a randomized controlled trial, Circ. Cardiovasc. Qual. Outcomes, 2008, 1, 98-106

[18] Naslindh-Ylispangar A., Sihvonen M., Sarna S., Vanhanen H., Kekki P., Has brief counseling an impact on the risk factors for cardiovascular disease in 40-year-old me. A three year follow-up study, Cent. Eur. J. Med., 2008, 3, 300-307

[19] Bertuzzi M., Negri E., Tavani A., La Vecchia C., Family history of ischemic heart disease and risk of acute myocardial infarction, Prev. Med., 2003, 37, 183-187

[20] Janssens A.C., Wilde A.A., van Langen I.M., The sense and nonsense of direct-to-consumer genetic testing for cardiovascular disease, Neth. Heart. J., 2011, 19, 85-88