Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2011 Dec; 155(4):355–360. DOI 10.5507/bp.2011.042

ADIPOCYTE FATTY ACID BINDING PROTEIN AND C-REACTIVE PROTEIN LEVELS AS INDICATORS OF INSULIN RESISTANCE DEVELOPMENT

Dagmar Horakova[a]*, Dalibor Pastucha[b], David Stejskal[c], Helena Kollarova[a], Katerina Azeem[a], Vladimir Janout[a]

[a] *Department of Preventive Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic*
[b] *Department of Exercise Medicine and Cardiovascular Rehabilitation, Faculty of Medicine and Dentistry, Palacky University Olomouc*
[c] *Department of Laboratory Medicine and Metabolic Out Patient Centre, Central Moravian Hospital, Prostejov*
E-mail: dagmar.horakova@upol.cz

Received: April 20, 2011; Accepted with revision: July 12, 2011; Available online: September 16, 2011

Key words: aFABP/CRP/Insulin resistance/Metabolic syndrome/Inflammation/Adiponectin/Obesity

Background. Adipocyte fatty acid-binding protein (aFABP) has recently been identified as a potential circulating marker for metabolic syndrome. C-reactive protein (CRP), a sensitive marker of inflammation, is increased in individuals with diabetes and metabolic syndrome due to the development of subclinical inflammation. The study uses logistic regression models to analyze the relations between aFABP and CRP along with other parameters of insulin resistance. The objective was to investigate the potential use of aFABP and CRP levels as tools in the diagnosis of the metabolic syndrome.

Methods and results. The following groups were studied: healthy individuals (A, n=122), obese ind1ividuals (B, n=213) and patients diagnosed with metabolic syndrome (C, n=79). Obese persons in Group B had parameters suggestive of early insulin resistance: hypertension, hyperglycaemia, QUICKI (0.305) and higher aFABP levels as compared with the healthy subjects. Group C individuals were diagnosed with metabolic syndrome, as evidenced by the QUICKI markers for insulin resistance (0.293), high aFABP levels (35.3 mg/l). CRP concentrations were lowest in Group A healthy individuals (0.67 mg/l), higher in Group B obese subjects (2.65 mg/l) and highest in Group C patients with metabolic syndrome (3.62 mg/l). Logistic regression models showed an association of aFABP and CRP with BMI (OR 1.12 and 1.39, compared Group A vs C). An association of aFABP and CRP with the QUICKI index showed OR 1.48 and 1.37 (Group A vs C); with triglyceride levels showed OR 1.68 and 1.52 (Group A vs C). An association of aFABP and CRP with glycaemia showed OR 1.48 and 1.51 (Group A vs C), with insulinaemia showed OR 1.44 and 1.38 (Group A vs C) respectively.

Conclusions. AFABP levels were higher in obese individuals and highest in those with metabolic syndrome. CRP concentrations were increased in obese persons whereas individuals with metabolic syndrome were found to have high-risk CRP levels. Logistic regression models showed an association of aFABP and CRP with BMI as well as an association of aFABP and CRP with parameters of insulin resistance, namely the QUICKI index, triglyceride levels, glycaemia and insulinaemia. Both methods are of diagnostic benefit for predicting metabolic syndrome, especially in previously untreated patients.

INTRODUCTION

Fat accumulation and adipocyte hypertrophy, accompanying the development of obesity, lead to impaired fat and glucose metabolism and thus the development of insulin resistance. As obesity develops, there is an increase in the discrepancy between adipocyte mass and capacity of the bloodstream in adipose tissue, resulting in adipose tissue hypoxia. In adipose tissue, production of certain adipokines rises, such as that of aFABP whose concentration increases, the development of insulin resistance becomes more pronounced and so is endothelial dysfunction of adipose tissue[1]. AFABP, highly expressed in adipose tissue, has recently been identified as a biomarker for the development of metabolic syndrome based on insulin resistance. The published results suggest that aFABP might be the central regulator of insulin resistance, lipid metabolism and inflammation[2,3]. CRP is a sensitive marker of inflammation. The development of insulin resistance is accompanied by a complex inflammatory reaction with no clinical manifestations. CRP, easily measured in clinical practice, is considered for inclusion in the diagnosis of metabolic syndrome[4].

PATIENTS AND METHODS

Three groups of subjects were enrolled in the study. Group A comprised 122 healthy individuals (59 males and 63 females) with no signs of insulin resistance. Group B consisted of 213 obese individuals with a BMI ≥ 30 (103 males and 110 females) who were not treated for metabolic disorders. Group C comprised of 79 treated individuals (40 males and 39 females) diagnosed with metabolic

syndrome as defined by the NCEP ATP III guidelines[5]. The subjects were enrolled in the study at the Department of Exercise Medicine and Cardiovascular Rehabilitation, University Hospital Olomouc. In all patients, blood pressure, height and weight were measured and blood samples were collected by venipuncture in the morning after 12 h fasting. Basic characteristics of all individuals from the three groups and results of their examination are shown in (Table 1). Height and weight were used to calculate body mass index (BMI). The blood samples were analyzed for glucose, insulin, total cholesterol, triglyceride, HDL cholesterol, CRP, adiponectin and aFABP levels. Serum concentrations of insulin (DPC, USA), glucose, total cholesterol, HDL cholesterol, triglycerides, CRP, adiponectin and aFABP (BioVendor Laboratoty Medicine, Inc., Brno, Czech Republic) were analyzed in fresh serum from morning venous samples taken after 12-hour fasting period. The blood samples were drawn under aseptic conditions from the cubital vein and the serum samples were then obtained in a cooling centrifuge at 3000 *g* at 4 ˚C and subsequently frozen at -80 ˚C. Concentrations of aFABP and adiponectin were determined after defrosting of individual samples throughout the course of one day. Good Laboratory Practice principles were followed. The study was approved by the Faculty of Medicine and Dentistry, Palacky University Olomouc ethics committee.

The quantitative insulin sensitivity check index (QUICKI) was calculated as follows[6].

QUICKI = 1 / log fasting insulin (μIU/ml) + log fasting glucose (mg/100ml)

Statistical analysis was performed using the Statistica 6 software. To test distribution, the Kolmogorov-Smirnov test was used. The statistical significance of differences between values of the control and study groups was assessed by the Mann-Whitney U test. Data in (Table 1) were expressed as means (±standard error SE; Gaussian distribution) or medians (25th and 75th percentiles; non-Gaussian distribution). Spearman's rank correlation coefficient was used to express the relationship of two variables. The significance of CRP and aFABP coefficinents in the logistic models was tested by R-test (www.r-project.org) (ref.[7]).

RESULTS

Table 1 shows the results of analyses in all three groups. Variables in the table are presented as the mean or median. All the studied parameters in Groups B and C were different from Group A comprising healthy individuals. The values shown for Group A indicate that all the studied parameters were in normal ranges. Subjects in Group A had high adiponectin levels, low aFABP levels typical for healthy individuals, and very low CRP levels (0.67 mg/l). Obese individuals in Group B had values typical for persons who begin to develop insulin resistance, i.e. hypertension (141/85 mm Hg) and hyperglycaemia (7.0 mmol/l). The QUICKI, which correlates with the clamp techniques for detecting insulin resistance, showed values typical for individuals developing insulin resistance (0.305). As compared with healthy Group A, adiponectin levels were lower (8.6 mg/l) while aFABP levels were higher (29.2 mg/l). CRP levels were slightly increased (2.65 mg/l). Individuals with metabolic syndrome in Group C had levels suggestive of the diagnosis – obesity (BMI 34), hypertension (145/87 mm Hg), hyperglycaemia or type 2 diabetes (8.1 mmol/l) and hypertriglyceridaemia (2.43 mmol/l). The QUICKI levels were typical for individuals with insulin resistance (0.293). aFABP levels were the highest (35.3 mg/l) of all three groups and adiponectin levels were the lowest (7.3 mg/l). CRP concentrations were in the risk range (3.62 mg/l). Table 2 shows statistically significant correlation coefficients between CRP and aFABP and parameters associated with insulin resistance. The highest correlations were between CRP and aFABP (0.46) and between aFABP and BMI (0.57).

Tables 3a and 3b compare aFABP and CRP parameters in four logistic regression models. Healthy subjects in Group A are compared with obese Group B individuals in Table 3a and with those with metabolic syndrome (Group C) in Table 3b. Model 1 was adjusted for age and gender, Model 2 for age, gender and BMI, Model 3 for age, gender and the QUICKI, and Model 4 for age, gender and triglycerides, Model 5 for age, gender and glucose, Model 6 for age, gender and insulin. After aFABP and CRP were adjusted for age and gender (Model 1), there was a slight association of aFABP and CRP with gender in both groups (OR 1.19 and 1.34 and 1.47 and 1.43). After adjustment for age, gender and BMI (Model 2), there was a slight association of aFABP and CRP with BMI in both groups (OR 1.06 and 1.25 and 1.12 and 1.39). After adjustment for age, gender and the QUICKI, Model 3 showed a slight association of aFABP and CRP with the QUICKI in both groups (1.13 and 1.32 and 1.48 and 1.37), with the exception of CRP and the QUICKI between the healthy and obese groups (95% CI 0.93-1.37). Model 4, after adjustment for age, gender and triglycerides, showed a slight association of aFABP and CRP with triglycerides in both groups (OR 1.19 and 1.32 and 1.68 and 1.52). Model 5 showed a slight association of aFABP and CRP with glucose in both groups (OR 1.19 and 1.44 and 1.48 and 1.51). Model 6 showed a slight association of aFABP and CRP with insulin in both groups (OR 1.17 and 1.33 and 1.44 and 1.38).

DISCUSSION

The time from the development of insulin resistance to clinical manifestation of components of metabolic syndrome is relatively long (in the order of years). Early detection of potential patients and initiation of intervention are very important for the health of the entire population[6]. The presence of obesity in association with inflammatory reaction accompanied by increased CRP levels is considered a significant predictor of the gradual development of metabolic syndrome. Increased CRP levels are detected in individuals with metabolic syndrome[8]. In Group B obese persons, CRP levels were increased (2.65 mmol/l). The highest CRP levels were in Group C subjects with meta-

Table 1. Basic metabolic and clinical characteristics of subjects.

	Group A Healthy (n = 122) ♂59, ♀63	Group B Obese (n = 213) ♂103, ♀110	Group C Metabolic syndrome (n = 79) ♂40, ♀39
Age *(yers)*	57.8 ± 11.6	61.8 ± 10.8 ***	61.0 ± 10.2 ***
Systolic pressure (*mmHg)*	127 ± 14	141 ± 16 ***	145 ± 15 ***
Diastolic pressure (*mmHg)*	77 ± 10	85 ± 10 ***	87 ± 10 ***
Body Mass Index (*kg/m²*	24.3 ± 1.2	32.4 ± 2.0 ***	34.0 ± 3.5 ***
Glucose (*mmol/l)*	5.1 (4.9 – 5.4)	7.0 (6.3 – 8.6) ***	8.1 (6.7 – 9.8) ***
Insulin (*mIU/l)*	7.5 (5.6 – 9.2)	14.1 (9.8 – 20.3) ***	16.0 (12.8 – 23.3) ***
Cholesterol (*mmol/l)*	5.2 (4.8 – 5.6)	4.9 (4.3 – 5.5) ***	4.8 (4.1 – 5.5) ***
Triglyceride (*mmol/l)*	1.4 (0.96 – 1.51)	1.88 (1.39 – 2.63) ***	2.43 (1.79 – 3.22) ***
HDL- cholesterol (*mmol/l)*	1.7 (1.43 – 1.91)	1.42 (1.24 – 1.63) ***	1.37 (1.12 – 1.52) ***
QUICKI	0.350 (0.334 – 0.376)	0.305 (0.290 – 0.324) ***	0.293 (0.277 – 0.307) ***
Adiponectin (*mg/l)*	14.5 (10.1-18.5)	8.6 (6.1 – 11.0) ***	7.3 (5.6 – 10.3) ***
aFABP (*mg/l)*	17.9 (15.9 – 20.2)	29.2 (20.5 – 40.0) ***	35.3 (24.9 – 51.2) ***
CRP (*mg/l)*	0.67 (0.34 – 1.22)	2.65 (1.82 – 5.13) ***	3.62 (2.75 – 6.61) ***

* p < 0.05, ** p < 0.01, *** p < 0.001, (comparison with healthy subject)
The variables are presented as median (upper and lower quartile)
QUICKI = 1 / (log fasting insulin (μIU/ml) + log fasting glucose (mg/100ml))

Table 2. Statistically significant correlation coefficients among the markers studied (aFABP, CRP) as well as selected parameters of insulin resistance.

	BMI	Glucose	Insulin	Triglycerides	aFABP	QUICKI
CRP	0.39***	0.24***	0.27***	0.23***	0.46***	-0.29***
aFABP	0.57***	0.21**	0.33***	0.40***	1***	-0.31***

* p < 0.05, ** p < 0.01, *** p < 0.001

bolic syndrome (3.62 mmol/l). Similarly increased CRP levels (2.54 mmol/l) (ref.[9]) in females with metabolic syndrome were reported by Stefanska[9]. Females should have higher CRP and aFABP levels than males[8]. Logistic regression Model 1 suggested an association between the two parameters and gender. Individuals with fully developed metabolic syndrome may have CRP concentrations of up to 10 mg/l (ref.[10]), which was not confirmed by CRP measurements in Group C. Group C subjects have already been treated for metabolic syndrome, most of them with atorvastatin. Statins are known to lower both CRP and aFABP levels[11]. In untreated individuals, the levels of both parameters could be even higher. Therefore, determination of aFABP and CRP levels is useful for early detection of the development of insulin resistance in previously untreated patients. Adipocyte FABP produced by the adipose tissue is considered a potential marker for the development of metabolic syndrome[2] as well as type 2 diabetes[3]. aFABP is considered a predictor of the development of metabolic syndrome based on insulin resistance and overweight or obese individuals are found to have increased levels[12] and so were our groups with obesity and metabolic syndrome. In a group of overweight Korean boys, aFABP levels (23.6 mg/l) (ref.[12]) were significantly increased as compared with healthy controls[12]. Relationship between CRP and aFABP has also been suggested. The logistic regression models assessed the potential use of these markers as tools for estimating the probability of the development of metabolic syndrome. Concentration of aFABP is equivalent with CRP and both have a reasonable diagnostic potential[9]. In accordance with these data, our logistic regression models also showed a slight association of aFABP and CRP with BMI, the QUICKI and triglycerides, related to the development of insulin resistance. Increasing serum aFABP levels are closely associated with impaired glucose metabolism and

Table 3a. Comparison of CRP and aFABP coefficients significance at healthy subjects in Group A with obese Group B individuals in logistic regression models.

	Model 1 (sex, age)		Model 2 (sex, age, BMI)		Model 3 (sex, age, Quicki)		Model 4 (sex, age, TAG)		Model 5 (sex, age, glucose)		Model 6 (sex, age, insulin)	
	CRP	aFABP	CRP	aFABP	CRP	aFABP	CRP	aFABP	CRP	aFABP	CRP	aFABP
p-value (Wald´s test)	0.021	<0.001	0.05	<0.001	0.211	<0.001	0.03	<0.001	0.143	<0.001	0.053	<0.001
p-value (LR test)	0.014	<0.001	0.048	<0.001	0.197	<0.001	0.023	<0.001	0.146	<0.001	0.043	<0.001
Odds ratio (OR)	1.19	1.34	1.06	1.25	1.13	1.32	1.19	1.32	1.19	1.44	1.17	1.33
95% CI for OR	1.03–1.38	1.25–1.43	1.01–1.27	1.16–1.34	0.93–1.37	1.22–1.43	1.02–1.4	1.23–1.42	0.94–1.52	1.28–1.62	0.99–1.38	1.23–1.43
Other signifficant variables in the model	sex		BMI		QUICKI		TAG		glucose		insulin	

Model 1 adjusted for sex, age; Model 2 adjusted for sex, age, BMI; Model 3 adjusted for sex, age, Quicki; Model 4 adjusted for sex, age, TAG, Model 5 adjusted for sex, age, glucose; Model 6 adjusted for sex, age, insulin.

Table 3b. Comparison of CRP and aFABP coefficients significance at healthy subjects in Group A with metabolic syndrome Group C individuals in logistic regression models.

	Model 1 (sex, age)		Model 2 (sex, age, BMI)		Model 3 (sex, age, Quicki)		Model 4 (sex, age, TAG)		Model 5 (sex, age, glucose)		Model 6 (sex, age, insulin)	
	CRP	aFABP	CRP	aFABP	CRP	CRP	CRP	aFABP	CRP	aFABP	CRP	aFABP
p-value (Wald´s test)	0.003	<0.001	0.033	<0.001	0.02	0.02	0.02	<0.001	0.02	<0.001	0.012	<0.001
p-value (LR test)	<0.001	<0.001	0.28	<0.001	0.003	0.003	0.003	<0.001	0.003	<0.001	0.004	<0.001
Odds ratio (OR)	1.47	1.43	1.12	1.39	1.68	1.68	1.68	1.37	1.48	1.51	1.44	1.38
95% CI for OR	1.14-1.89	1.26-1.62	1.02-1.27	1.18-1.46	1.08-2.59	1.08-2.59	1.08-2.59	1.18-1.58	1.11-1.72	1.26-1.79	1.08-1.92	1.21-1.57
Other signifficant variables in the model	sex		BMI		QUICKI		TAG		glucose		insulin	

Model 1 adjusted for sex, age; Model 2 adjusted for sex, age, BMI; Model 3 adjusted for sex, age, Quicki; Model 4 adjusted for sex, age, TAG; Model 5 adjusted for sex, age, glucose; Model 6 adjusted for sex, age, insulin.

may also be considered a useful indicator of the development of type 2 diabetes[13]. Increasing aFABP levels have been shown to be associated with inflammatory markers such as CRP and interleukin-6 in obese individuals and those with metabolic syndrome[14]. The association of AFABP and CRP with metabolic syndrome is often explained by the association with obesity. Some studies reported obesity to be the main factor associating aFABP and CRP (ref.[15,16]). Other authors pointed to an association of aFABP and CRP with metabolic syndrome independent of obesity[8,17]. Our analyzed groups adjusted for obesity showed an association of aFABP and CRP with BMI in individuals with both obesity and metabolic syndrome. When assessing aFABP and CRP as diagnostic tools, the sensitivity and specificity of the two methods was evaluated. The results showed similar sensitivity (76%) (ref.[3]) and specificity (64%) (ref.[3]) levels for both diagnostic methods[3]. Measuring aFABP levels corresponds with the value of CRP in diagnosing metabolic syndrome and assessment of both parameters is of satisfactory (but not excellent) diagnostic potential[9].

CONCLUSION

Early diagnosis of metabolic syndrome is crucial for decreasing morbidity of populations. Increased aFABP levels accompanied by a rise in CRP levels together with obesity may suggest the development of insulin resistance. Logistic regression models showed an association of aFABP and CRP with BMI as well as an association of aFABP and CRP with parameters of insulin resistance, namely the QUICKI index, triglyceride levels, glycaemia and insulinaemia. Both methods are of diagnostic benefit for predicting metabolic syndrome, especially in previously untreated patients.

ACKNOWLEDGEMENT

The study was Supported by the Czech Ministry of Health grant project IGA MZ ČR NT 11098-4.

The authors have no financial relationship(s) with commercial interests to disclose.

REFERENCES

1. Jansson PA. Endothelial dysfunction in insulin resistence and type 2 diabetes. J Intern Med 2007;262(2):173-83.
2. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem 2006;52:405-13.
3. Tso AW, Xu PC, Sham MN, Wat NM, Wang Y, Fong CH, Cheung BM, Janus ED, Lam KS. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes. Diab Care 2007;30:2667-72.
4. Ridker PM, Wilson PW, Grundy SM. Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? Circulation 2004;109(23):2818-25.
5. National Institutes of Health. 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). Washington DC: US Govt. Printing Office 2001, NIH publ. no. 01–3670.
6. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.
7. R Development Core Team (2005). R: A language and environment for statistical computing, reference index version 2.13.0. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
8. Bo S, Gentile L, Ciccone G, Baldi C, Benini L, Dusio F, Lucia C, Forastiere G, Nuti C, Cassader M, Franco Pagano G. The metabolic syndrome and high C-reactive protein: prevalence and differences by sex in a southern-European population-based cohort. Diabetes Metab Res Rev 2005;21(6):515-24.
9. Stefanska A, Sypniewska G, Blaszkiewicz N, Ponikowska I, Cwiklinska-Jurkowska M. Comparison between C-reactive protein and adipocyte fatty acid-binding protein as a component of metabolic syndrome in middle-aged women. Clin Biochem 2011;44(4):304-6.
10. Devaraj S, Singh U, Jialal I. Human C-reactive protein and the metabolic syndrome. Curr Opin Lipidol 2009;20(3):182-9.
11. Karpisek M, Stejskal D, Kotolova H, Kollar P, Janoutova G, Ochmanova R, Cizek L, Horakova D, Yahia RB, Janout V. Treatment with atorvastatin reduces serum adipocyte-fatty acid binding protein value in patients with hyperlipidaemia. Eur J Clin Invest 2007;37(8):637-42.
12. Choi KM, Yannakoulia M, Park MS, Cho GJ, Kim JH, Lee SH, Hwang TG, Yang SJ, Kim TN, Yoo HJ, Baik SH, Kim SM, Mantzoros CS. Serum adipocyte fatty acid-binding protein, retinol-binding protein 4, and adiponectin concentrations in relation to the development of the metabolic syndrome in Korean boys: a 3-y prospective cohort study. Am J Clin Nutr 2011;93(1):19-26.
13. Ye WH, Peng X H, Ai YQ, Chen H. Relations between serum adipocyte fatty acid-binding protein and type 2 diabetes in a community population. Nan Fang Yi Ke Da Xue Xue Bao, 2011;31(3):508-11.
14. Terra X, Quintero Y, Auguet T, Porras JA, Hernandez M. FABP is associated with inflammatory markers and metabolit syndrome in morbidly obese women. Eur J Endocrinol 2011;164(4):539-47.
15. Kahn SE, Haffner SM, Viberti G, Herman WH, Lachin JM, Kravitz BG, Yu D, Paul G, Holman RR, Zinman B; Diabetes Outcome Progression Trial (ADOPT) Study Group. Rosiglitazone decreases C-reactive protein to a greater extent relative to glyburide and metformin over 4 years despite greater weight gain: observations from A Diabetes Outcome Progression Trial (ADOPT). Diab Care 2010;33(1):177-83.
16. Aronson D, Bartha P, Zinder O, Kerner A, Markiewicz W, Avizohar O, Brook GJ, Levy Y. Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. Int J Obes Relat Metab Disord 2004;28(5):674-9.
17. Stejskal D, Karpisek M. Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? Eur J Clin Invest 2006;36(9):621-5.