Evaluation of total adiponectin, adipocyte fatty acid binding protein and fibroblast growth factor 21 levels in individuals with metabolic syndrome

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Short title: Adiponectin, A-FABP and FGF 21 in individuals with MetS

Summary:

Objectives: Although many studies have investigated the relationships of several adipokines to metabolic syndrome (MetS), the interrelationships of adiponectin (ADP), adipocyte fatty acid binding protein (A-FABP) and fibroblast growth factor 21 (FGF 21) have not been described in detail.

We examined 209 asymptomatic dyslipidemic patients divided into MetS+ (n = 73) and MetS- (n = 136) groups. The aim of study was to evaluate the relationships between observed adipokines, to compare the levels of total ADP, A-FABP and FGF 21 in individuals with and without MetS, and to elucidate the relationships of individual adipokines to lipid parameters, markers of insulin resistance and endothelial haemostatic markers in these groups.

Results: In MetS+ group, we found the independent positive association ADP with A-FABP (beta= 0.4888, p= 0.0382), A-FABP with FGF 21 (beta= 0.3811, p= 0.0002) and von Willebrand factor (beta= 0.4502, p= 0.0013), and FGF 21 with A-FABP (beta= 0.4422, p= 0.0002). Our study has also confirmed the well-established risk profile of subjects with MetS, although clinically asymptomatic. MetS+ patients had also lower levels of ADP and higher levels of A-FABP and FGF 21.

Conclusion: Our study evaluated the interrelationships of ADP, A-FABP and FGF 21 in asymptomatic dyslipidemic subjects with diagnosis of MetS. Especially strong association between A-FABP and FGF 21 needs to be clarified in further studies.

Key words: metabolic syndrome, adiponectin, adipocyte fatty acid binding protein, fibroblast growth factor 21, dyslipidemia

Introduction

The metabolic syndrome is a common metabolic disorder associated with increased risk of type 2 diabetes mellitus and cardiovascular diseases (Eckel *et al.* 2005). Although the mechanisms underlying MetS have not been well understood, recent research studies support the idea that visceral obesity plays an important role (Han *et al.* 2002).

Adipose tissue serves both as reservoir of the energy storage and the active endocrine tissue producing many proactive substances including adipokines. These molecules have many important metabolic effects (Funahashi *et al.* 1999). Inflammation of adipose tissue is characterized by infiltration of the macrophages and other types of inflammatory cells. Proinflammatory adipokines exert adverse effects on the vasculature by promoting of insulin resistance and monocyte infiltration into the vessel wall (Libby *et al.* 2011).

Adiponectin is an adipose tissue-derived adipokine with a protective role in initiation and progression of atherosclerosis through its antiinflammatory and antiatherogenic effects. Serum adiponectin levels are decreased in obesity, type 2 diabetes and patients with coronary artery disease, etc (Haluzik *et al*. 2004, Shimada *et al.* 2004). Low adiponectin concentrations were found to independently associate with both MetS and coronary atherosclerosis (Saely *et al.* 2007).

Adipocyte fatty acid binding protein is an „unfavourable“ adipokine, probably a new marker and/or predictor of metabolic syndrome. A-FABP is a dominant cytoplasmic protein of mature adipocytes and a regulator of lipid and glucose metabolism, present also in macrophages of fat tissue. Oxidized LDL induces its expression. Higher levels of A-FABP were associated with increased fasting glucose, triglycerides, insulin, body mass index (BMI) and waist circumference, and decreased HDL cholesterol (HDLc) in patients with metabolic syndrome in the study of Xu *et al.* (2006). A-FABP is also considered a valuable marker of metabolic disturbances in patients with type 2 diabetes mellitus (Haluzik *et al.* 2009).

Fibroblast growth factor 21 is a “favourable” cytokine considered as a new metabolic regulator of non-insulin dependent glucose transport in cells. Systematic administration of FGF 21 decreases plasma levels both of glucose and triglycerides and leads to improving of lipoprotein profiles in genetic compromised FGF transgenic mice and primates (Kharitonenkov *et al.* 2007). Nevertheless, increased levels of FGF 21 and a negative correlation with HDL and adiponectin were found in patients with MetS (Zhang *et al.* 2008). In general, high levels of FGF 21 are found in cardiometabolic disorders, such as obesity, MetS, type 2 diabetes, non-alcoholic fatty liver disease and coronary artery disease in human studies (Woo *et al.* 2013). These findings may indicate a compensatory response to metabolic stress or resistance to FGF 21. Nevertheless, serum FGF 21 has been implicated as a potential biomarker for early detection of these syndromes (Woo *et al.* 2013).

Although many studies investigated the relationships of adipokines to MetS, there is only limited information about all three parameters and their interrelationships in asymptomatic subjects with signs of MetS.

Therefore, the aim of our study was to compare the levels of ADP, A-FABP and FGF 21 in individuals with and without MetS, and to elucidate the relationships of individual adipokines to lipid parameters, markers of insulin resistance and endothelial haemostatic markers in these groups.

Materials and methods

Study design and subjects

The study was performed with asymptomatic dyslipidemic subjects (i.e. individuals without history of clinically manifest atherosclerosis- coronary artery disease, heart failure, cerebrovascular ischemic disease and peripheral vascular disease, with altered plasma lipids), their relatives and spouses, without lipid-modifying therapy. They had been examined for

the first time in the Lipid Centre of the 3rd Department of Internal Medicine, University Hospital Olomouc, Czech Republic, during the period from January 2009 to March 2012. All subjects were tested for the signs of secondary hyperlipidemia: diabetes mellitus, hypothyroidism, renal or hepatic diseases and nephrotic syndrome. Other exclusion criteria were as follows: history of clinically manifested atherosclerosis presented by coronary artery disease, cerebrovascular disease and peripheral arterial disease, hypolipidemic therapy in previous 8 weeks, hormone therapy and clinical presence of acute infections. All individuals filled out a questionnaire on their previous medical history, especially cardiovascular status, medication and smoking habits. Body mass index and systolic and diastolic blood pressure (SBP, DBP) were also determinated. The study was reviewed and approved by Ethics Committee of Medical Faculty and University Hospital Olomouc and informed consent was obtained from all participants.

Individuals who met criteria mentioned above (n = 209) were divided into two groups: patients with presence of metabolic syndrome (MetS+, n = 73, 31 males, 42 females), and individuals with absence of metabolic syndrome (MetS-, n = 136, 74 males, 62 females). Following criteria were used for identification of MetS according to NCEP- ATPIII Panel 2001: waist circumference (men >102 cm, women > 88 cm), triglycerides (TG) ≥ 1.7 mmol/l, HDL cholesterol (men < 1.04 mmol/l, women < 1.30 mmol/l), blood pressure ≥130 / ≥85 mm Hg and fasting glucose ≥ 6.1 mmol/l. The presence of minimally three of following factors was sufficient for diagnosis of MetS.

Laboratory analysis

Venous blood samples were drawn in the morning after a 12-h fast. After centrifugation, the serum was used for other analyses. For assessment of prothrombotic markers, venous blood was collected in 3.8% sodium citrate tubes and plasma was obtained after centrifugation.

Routine serum biochemical parameters were analyzed on Modular SWA (Roche, Basel, Switzerland) in the day of blood collection. Concentrations of adipokines and other special analytes were measured in the sample aliquotes stored at -80 (-20) °C, no longer than 6 months- see below in text.

Total cholesterol (TC), TG and HDLc were determined enzymatically on a Modular SWA system (Roche, Basel, Switzerland). Determination of HDLc was realized by a direct method without precipitation of apoB containing lipoproteins. Low density lipoprotein cholesterol (LDLc) levels were calculated using Friedewald formula (for TG less than 4.5 mmol/l). Another calculated parameters were as follows: non-HDL-cholesterol (nonHDLc =TC-HDLc) and atherogenic index of plasma (AI) (logTG/HDLc). Concentration of Apo B and Apo A1were determined immunoturbidimetrically using Tina-Quant ApoB and ApoA-1 kits (Roche, Basel, Switzerland). Lipoprotein (a) [Lp(a)] was determined immunoturbidimetrically using Tina-Quant Lipoprotein(a) TQ kit (Roche, Basel, Switzerland). C-reactive protein (CRP) was assessed by an ultrasensitive immunoturbidimetric method using the kit Tina-Quant (Roche, Basel, Switzerland). Glucose was determined using GOD-PAP method (Roche, Basel, Switzerland). N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was determined by ECLIA method (Elecsys pro BNP reagent kit, Roche, Basel, Switzerland). All tests were measured from fresh sera in the day of blood collection.

Insulin was determined by the commercially available kit (Immunotech, Marseille, France) using specific antibodies by the IRMA method. C-peptide and proinsulin (PINS) were determined using the commercially available kits: C-peptide (Immunotech, Marseille, France), and Proinsulin (DRG Instruments GmbH, Marburg, Germany), by the IRMA method, and RIA method, respectively. The sample aliquotes were stored at - 20 °C, no longer than 6 months.

The following thrombotic markers were examined from human plasma stored at - 20 °C: von Willebrand factor (immunoturbidimetric assay, Instrumentation Laboratory Spa, Milan, Italy), plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) (ELISA, Technoclone, Vienna, Austria).

Total adiponectin, A-FABP and FGF 21 were measured in serum (one separate aliquot stored at -80 °C until the day of analysis) by following imunochemical kits: Human Adiponectin ELISA, Human A-FABP ELISA and Human FGF 21 ELISA (all Biovendor Laboratory Medicine Inc., Brno, Czech Republic), according to the manufacturer's instructions and after verification of all three methods. Both the intra- and inter-assay coefficients of variation were below 10 % for all parameters.

Serum levels of the soluble adhesion molecules s-ICAM-1 and sVCAM-1 were determined by immunoenzymatic assay using commercially available kits s-ICAM-1 and sVCAM-1 (both Immunotech, Marseille, France) from one separate aliquot stored at - 20 °C.

Statictical analysis

All values are expressed as means ± standard deviation (SD) and parameters with skewed distribution also as medians. The Kolmogorov-Smirnov test was used to test for normal distribution. Variables with skewed distribution (CRP, TG, Lp(a), fibrinogen, vWF, tPA, PAI-1, insulin, C-peptide, PINS, ADP, FGF 21, AFABP, NT-proBNP) were log transformed in order to normalize their distribution before statistical analysis. Differences in variables between individual groups were analyzed with ANCOVA after adjustment for age and sex. For statistical evaluation of a correlation between individual parameters we used a Pearson correlation analysis for analytes with normal distribution, and a univariate Spearman correlation analysis for variables with skewed distribution. Multiple regression analysis was performed for testing of an independent association between dependent and independent

variables. Statistical analysis was performed using SPSS for Windows version 12.0 (Chicago, IL, USA). Probability values of $p<0.05$ were considered as statistically significant.

Results

The basic clinical and laboratory characteristics of all subjects and divided into two groups according to absence/presence of MetS are summarized in Table 1. Besides of expected unfavourable lipid and lipoprotein profiles (elevated TC, TG, nonHDLc, AI, Apo B, and decreased levels of HDLc and Apo A1) and pronounced insulin resistance (increased levels of glucose, insulin, proinsulin and C-peptide), individuals with MetS had significantly elevated concentrations of endothelial and haemostatic markers tPA, PAI-1 and adhesive molecules sVCAM-1 and sICAM-1. Adiponectin was significantly lower in MetS + ($p<0.001$), whilst A-FABP and FGF 21concentrations were elevated in comparison with MetS- group ($p<0.001$).
In Table 2, significant correlations of adipokines with other parameters in MetS+ and MetS- groups are introduced. In MetS+ group, ADP positively correlated with age, HDLc, Apo A1, A-FABP and NT-proBNP, and negatively with sex and waist circumference. A-FABP positively correlated with vWF, ADP, FGF 21 and NT-proBNP, whereas FGF 21 positively correlated with TG, AI, vWF, PAI-1, A-FABP and waist circumference.
In order to evaluate the independent association of followed up parameters with ADP, A-FABP and FGF 21, the multiple regression analysis with adipokines as dependent variables and correlated parameters as independent predictors was performed (see Table 3). In MetS+ group, ADP was independently positively associated with A-FABP (beta = 0.4888, $p<0.05$), A-FABP was positively associated with FGF 21 (beta = 0.3811, $p<0.001$) and vWF (beta = 0.4502, $p<0.01$), whilst FGF 21 was positively associated only with A-FABP (beta = 0.4422, $p<0.001$). In MetS- group, ADP was positively associated with vWF and negatively with sex,

A-FABP was positively associated with FGF 21, vWF and BMI, and FGF 21 was positively associated with A-FABP, TG, and negatively with AI.

Discussion

Our study confirms the well-established risk profile of subjects with MetS, although clinically asymptomatic. They had unfavourable lipid and lipoprotein profiles, increased parameters insulin resistance, and significantly elevated concentrations of endothelial haemostatic markers represented by tPA, PAI-1 and adhesive molecules, in comparison with MetS- individuals. Decreased levels of ADP in MetS+ individuals are not surprising and they are consistent with recent literature (Saely *et al.* 2007, Ryo *et al*. 2004), as well as the increase of A-FABP levels (Horakova *et al.* 2011, Park *et al.* 2012), and FGF 21 concentrations (Zhang *et al.* 2008, Chen *et al.* 2011, Reinehr *et al*. 2012).

Significant correlations of adipokines with other selected parameters in MetS + and MetS – groups as a result of univariate correlation analysis are described in Table 2. There are minimally three interesting points in this summary: 1. positive correlation of ADP and A-FABP with NT-proBNP in both groups, which was amplified especially in MetS+ group (although no differences were seen in NT-proBNP levels), 2. only weak or no correlation of A-FABP with metabolic and anthropometric parameters of insulin resistance, and 3. strong correlation of A-FABP and FGF 21 in both observed groups.

Adiponectin is one of the few adipokines that has multiple favourable effects on the prevention of cardiovascular disease through its pleiotropic actions on the blood vessels and the heart. In our study, we have verified correlations with many clinical, anthropometrical and laboratory parameters (age, sex, waist circumference, HDLc, Apo A1), mainly well described in previous studies. But positive relationship of ADP to A-FABP as an independent predictor was seen only in MetS + individuals and was confirmed by multiple regression analysis

(however, A-FABP as dependent variable did not correlate with ADP in this group). We can speculate about the reason of this relationship. One of the main beneficial functions of adiponectin is cardioprotective action (Xu *et al.* 2012). The antiapoptotic activity represents a key mechanism, whereby ADP protects against cardiac injury (Tao *et al.* 2007). In cardiomyocytes, adiponectin promotes decrease of oxidative/nitrosative stress, apoptosis, fibrosis and inflammation and increase of fatty acid and glucose uptake. On the other side, despite its atheroprotective properties, ADP has been associated with both decreased (Pischon *et al.* 2004, Frystyk *et al.* 2007) and increased (Lindsay *et al.* 2005) risk of cardiovascular disease and/or mortality. In our recent study, the positive association of ADP was found with thrombomodulin, vWF and sVCAM-1 in dyslipidemic subjects, which might contribute to increased risk of cardiovascular disease associated with higher plasma ADP levels (Vaverkova *et al.* 2013).

Data from animal studies support an etiological role of A-FABP in cardiovascular disease (Furuhashi *et al.* 2008). A-FABP has been identified as a major cardiodepressant factor that confers the suppressive effect of adipocytes on cardiac contractile functions (Lamounier-Zepter *et al.* 2009). Thus, independent association of ADP with A-FABP in MetS + group may be connected with the relationship of these adipokines to cardiac and/or vascular functions, and may reflect compensatory response of ADP to higher risk of cardiovascular disease. In addition, both parameters have correlated positively with NT-proBNP (see Table 2), which has been shown to be an accurate and sensitive diagnostic marker in patients with heart failure, although, after multiple regression analysis, the association has lost significance in our study. Von Eynatten *et al.* (2006) have investigated the relationship of adiponectin to markers of inflammation, atherogenic dyslipidemia and heart disease in patients with coronary artery disease. After adjusting for age and sex, adiponectin was associated positively with HDL cholesterol and NT-proBNP. Moreover, several studies found the relationship

between A-FABP and coronary atherosclerosis (Miyoshi *et al.* 2010, Rhee EJ *et al.* 2009) and its possible role in the development of cardiac dysfunction have been suggested, including positive correlation with NT-proBNP (Zhou *et al.* 2011).

In recent cross-sectional studies, serum A-FABP correlated with parameters of IR (glucose, insulin, waist circumference, BMI) in obese individuals, patients with type 2 diabetes mellitus, patients with familial combined hyperlipidemia and patients with metabolic syndrome (Kralisch *et al.* 2013). But we have found only weak or no correlation of A-FABP with these parameters in MetS+ group, although significant differences were observed between MetS+ and MetS- groups, as shown in Table 1. We speculate about relatively small number of individuals in MetS+ group. In any case, this phenomenon is surprising and we have no other relevant explanation for it.

A-FABP is a key proinflammatory mediator that links obesity with cardiovascular disease in humans (Xu *et al.* 2012). The proatherogenic activity is mediated by its direct action on macrophages, independently of lipid metabolism and insulin sensitivity (Hoo *et al.* 2008). In MetS+ group, the positive correlation between A-FABP with vWF, ADP, FGF21 and NT-proBNP was observed, but independent relationship with only vWF and FGF 21 was revealed by the multiple regression analysis (see Table 3). Positive association with vWF is in accordance with our previous study (Karasek *et al.* 2012) and supports the role of A-FABP in development of endothelial dysfunction. The independent association with FGF 21 is discussed below.

Fibroblast growth factor 21 is a member of the FGF superfamily, with relevant metabolic actions (Iglesias *et al.* 2012). FGF 21 has been recently considered as a metabolic hormone regulated by nutritional status, with multiple beneficial effects on glucose homeostasis and lipid metabolism in animal models. Indeed, FGF 21 improves insulin sensitivity, glucose, and lipid homeostasis and preserves beta-cell functions in diabetic animal models (Kharitonenkov

et al. 2007, Coskun *et al.* 2008, Kralisch *et al.* 2011). However, increased levels of FGF 21 and negative correlation with HDLc and adiponectin were found in patients with MetS (Zhang *et al.* 2008). In general, high levels of FGF 21 are found in cardiometabolic disorders, such as obesity, MetS, type 2 diabetes, non-alcoholic fatty liver disease and coronary artery disease in human studies (Woo *et al.* 2013).

As shown in Table 2, FGF 21 has positively correlated with waist, TG, AI, vWF, PAI-1 and A-FABP in MetS+ group. Some of these relationships have been described in previous studies: higher levels of FGF 21 and positive correlation with TG in patients with coronary artery disease and dyslipidemia (Lin *et al.* 2010), independent association with TG and LDLc in patients with impaired glucose tolerance and/or type 2 diabetes (Chen *et al*. 2011) or above mentioned study of Zhang *et al.* (2008) revealing positive correlation with adiposity and TG in obese individuals with metabolic syndrome.

There are only limited information about the relationships of FGF 21 and endothelial haemostatic markers in recent literature. PAI-1 is probably less specific marker of endothelial damage (Karasek *et al.* 2012). It originates from several sites, including endothelium, liver and adipose tissue and higher levels may not reflect endothelial dysfunction. In our recent study, positive associations of ADP and vWF were found in dyslipidemic patients (Vaverkova *et al.* 2013). Correlation of FGF 21 with vWF is surprising and may reflect higher risk of atherothrombosis in MetS+ group in general. However, both relationships have lost significance after the multiple regression analysis.

The favourable effects in animal studies would support the potential role of FGF 21 as a therapeutic agent for diabetes and obesity (Dostalova *et al.* 2009, Woo *et al.* 2013). However, high serum FGF 21 levels were observed in obese individuals, and patients with obesity-related disorders and insulin resistance (Zhang *et al.* 2008, Chen *et al.* 2011). The causes of

this phenomenon need to be clarified. FGF 21 resistance has been proposed as one of the causes in animal study (Fisher *et al*. 2010).

The most important finding of our study was the strong reciprocal positive association of FGF 21 with A-FABP levels in both investigated groups, with pronounced relationship in MetS+ group (p=0.0002). One of the reasons of elevated FGF 21 could be the presence of compensatory response to higher metabolic stress presented by high levels of A-FABP. As mentioned above, high levels of FGF 21 were found in cardiometabolic disorders, such as obesity, MetS, type 2 diabetes, non-alcoholic fatty liver disease and coronary artery disease in human studies (Woo *et al.* 2013). It seems likely that this circulating FGF 21 is derived from the liver, perhaps due to the induction of FGF 21 by elevated hepatic lipid and carbohydrate levels (Huating *et al.* 2013). But it is not clear if circulating A-FABP is only a strong marker of metabolic disturbances or one of its primary causes (Haluzik *et al.* 2009).

FGF 21 resistance has been proposed as one of the causes for the raised circulating levels in obese mice (Fischer *et al.* 2010). For activation of FGF receptor mediated signalling, FGF21 has to bind a FGF receptor: beta-Klotho complex (Kharitonenkov *et al.* 2008). Beta-Klotho is highly expressed in metabolically active tissues including adipose tissue, liver and pancreas. A recent study suggests that adipose tissue inflammation in obesity can lead to the repression of beta-Klotho expression by TNF alpha and impaired FGF 21 in adipocytes (Diaz-Delfin *et al.* 2012). Similar actions may also lead to FGF 21 resistance in subclinical inflammation such as metabolic syndrome, type 2 diabetes and coronary artery disease (Woo *et al.* 2013). Therefore, it is possible that A-FABP (as a key proinflammatory mediator that links obesity with cardiovascular disease) may participate in the process of FGF 21 resistance, for example by its proinflammatory effect. Further studies are needed to address the mechanisms underlying the observed relationship.

Conclusion

Although many studies investigated the relationships of several adipokines to metabolic syndrome, we have first evaluated the interrelationships of ADP, A-FABP and FGF 21 in dyslipidemic subjects with signs of MetS. We have found independent positive association of ADP with A-FABP, and, to our best knowledge, we have first documented strong positive reciprocal association between FGF 21 and A-FABP in dyslipidemic subjects highlighted in MetS+ group. In addition, we have confirmed previously described independent relationship of A-FABP and vWF.

Conflict of interest statement

None.

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Table 1. Basic characteristics of all individuals and in subjects without MetS and with MetS

	All individuals	**MetS -**	**Met S +**
	n = 209	**n = 136**	**n = 73**
Age years	46.7 ± 14.5	44.7 ± 15.9	50.1 ± 11.0
CRP mg/l	3.8 ± 10.1 (1.6)	2.9 ± 4.2 (1.4)	3.8 ± 4.5 (2.3) *
TC mmol/l	6.69 ± 1.84	6.42 ± 1.48	7.12 ± 2.25 **
TG mmol/l	3.01 ± 4.21 (1.77)	1.76 ± 1.74 (1.38)	4.34 ± 6.18 (3.31) ***
AI	0.1931 ± 0.4373	- 0.0223 ± 0.3207	0.5827 ± 0.3563 ***
nonHDL mmol/l	5.33 ± 1.92	4.87 ± 1.51	6.10 ± 2.27 ***
HDLc mmol/l	1.37 ± 0.46	1.55 ± 0.45	1.03 ± 0.23 ***
LDLc mmol/l	4.06 ± 1.57	4.06 ± 1.33	3.97 ± 1.91
Apo A1 g/l	1.54 ± 0.37	1.65 ± 0.38	1.34 ± 0.25 ***
Apo B g/l	1.21 ± 0.38	1.13 ± 0.34	1.33 ± 0.43 ***
Lp(a) g/l	0.399 ± 0.465 (0.201)	0.409 ± 0.455 (0.221)	0.381 ± 0.489 (0.189)
Fibrinogen g/l	2.98 ± 0.76 (2.80)	2.90 ± 0.70 (2.73)	3.04 ± 0.64 (2.91)
vWF %	130 ± 55 (118)	128 ± 50 (118)	132 ± 60 (118)
tPA ng/ml	3.35 ± 2.43 (3.0)	2.79 ± 0.88 (2.8)	4.11 ± 2.82 (3.0) ***
PAI-1 ng/ml	65 ± 42 (58)	56 ± 36 (44)	83 ± 48 (78) **
sICAM-1 ng/ml	358 ± 148	335 ± 128	389 ± 173 *
sVCAM-1 ng/ml	743 ± 360	709 ± 327	819 ± 411 *
Glucose mmol/l	5.38 ± 1.04	5.10 ± 0.56	5.92 ± 1.46 ***
Insulin mIU/l	10.3 ± 6.6 (8.5)	8.4 ± 4.5 (7.5)	13.7 ± 8.2 (11.7) ***
C-peptide mg/l	2.7± 1.4 (2.5)	2.3 ± 1.0 (2.2)	3.6 ± 1.7 (3.4) ***
BMI kg/m^2	26.99 ± 4.75	25.19 ± 4.17	30.36 ± 4.05 ***

Waist cm	91.1 ± 14.2	85.9 ± 11.9	101.0 ± 12.7 ***
PINS mIU/l	14.8 ± 10.5 (11.4)	12.0 ± 8.3 (9.6)	20.3 ± 12.2 (16.6) ***
ADP mg/l	8.6 ± 5.2 (7.4)	9.4 ± 5.0 (8.0)	7.0 ± 5.3 (5.4) ***
FGF 21 ng/l	317.7 ± 440.4 (197.6)	222.3 ± 299.9 (156.5)	471.4 ± 531.7 (305.6) ***
A-FABP µg/l	26.2 ± 17.9 (21.9)	22.2 ± 12.7 (19.9)	33.8 ± 23.5 (29.1) ***
NT-proBNP ng/l	65.7 ± 82.1 (40.7)	65.4 ± 84.2 (44.5)	67.7 ± 81.0 (41.4)
SBP mm Hg	128.7 ± 15.2	125.1 ± 13.6	136.1 ± 15.6 ***
DBP mmHg	77.8 ± 8.7	75.9 ± 8.3	81.7 ± 8.0 ***

Data are expressed as means ± standard deviations, in parameters with skewed distribution also as medians (in parentheses). Differences in variables between groups were analyzed with ANCOVA after adjustment for age and sex. Variables with skewed distribution (CRP, TG, Lp(a), fibrinogen, vWF, tPA, PAI-1, insulin, C-peptide, PINS, ADP, FGF 21, A-FABP, NT-proBNP) were log transformed to normalize their distribution before statistical analyses.

Significant differences between MetS- and MetS+ groups:

* $p<0.05$

** $p<0.01$

*** $p<0.001$

Abbreviations: CRP, C-reactive protein; TC, total cholesterol; TG, triglycerides; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; nonHDL= TC – HDLc; AI, atherogenic index of plasma (logTG/ HDLc); Apo, apolipoprotein; Lp(a), lipoprotein (a); vWF, von Willebrand factor; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; BMI, body mass index; PINS, proinsulin; ADP, adiponectin; FGF 21, fibroblast growth factor 21; A-FABP, adipocyte fatty

acid binding protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Significant correlations of adipokines with other parameters in MetS+ and MetS- groups (r and *p* values).

a) ADP

	age	sex	waist	HDLc	Apo A1	vWF	A-FABP	NT-proBNP
ADP MetS +	**0.386** ***0.001***	**-0.520** ***0.000***	**-0.414** ***0.001***	**0.499** ***0.000***	**0.355** ***0.002***	0.139 *0.254*	**0.267** ***0.022***	**0.411** ***0.000***
ADP MetS -	**0.261** ***0.002***	**-0.399** ***0.000***	**-0.253** ***0.008***	**0.476** ***0.000***	**0.507** ***0.000***	**0.310** ***0.000***	0.124 *0.151*	**0.442** ***0.000***

b) A-FABP

	age	BMI	waist	vWF	ADP	FGF 21	NT-proBNP
A-FABP MetS +	0.117 *0.326*	0.050 *0.678*	0.046 *0.729*	**0.404** ***0.001***	**0.267** ***0.022***	**0.521** ***0.000***	**0.399** ***0.001***
A-FABP MetS -	**0.327** ***0.001***	**0.379** ***0.000***	**0.288** ***0.002***	**0.266** ***0.002***	0.124 *0.151*	**0.282** ***0.001***	**0.198** ***0.023***

c) FGF 21

	TG	AI	vWF	PAI-1	A-FABP	waist
FGF 21 MetS +	**0.374** ***0.001***	**0.340** ***0.001***	**0.362** ***0.002***	**0.223** ***0.050***	**0.521** ***0.000***	**0.256** ***0.050***
FGF 21 MetS -	**0.240** ***0.005***	**0.172** ***0.046***	0.079 *0.371*	**0.201** ***0.022***	**0.282** ***0.001***	0.050 *0.606*

Pearson correlation analysis for parameters with normal distribution. Spearman correlation analysis for parameters with skewed distribution (TG, vWF, tPA, PAI-1, ADP, FGF 21, A-FABP, NT-proBNP). Bold values indicate significance at $p<0.05$.

Table 3. Independent associations of ADP, A-FABP and FGF 21 as result of the multiple regression analysis (bold values).

a) ADP

	A-FABP	vWF	sex
ADP MetS +	**beta = 0.4888** ***p = 0.0382***	NS	NS
ADP MetS -	NS	**beta = 0.4332** ***p = 0.0245***	**beta = -0.0609** ***p = 0.0447***

b) A-FABP

	FGF 21	vWF	BMI
A-FABP MetS +	**beta = 0.3811** ***p = 0.0002***	**beta = 0.4502** ***p = 0.0013***	NS
A-FABP MetS -	**beta = 0.1491** ***p = 0.0392***	**beta = 0.3524** ***p = 0.0352***	**beta = 0.3785** ***p = 0.0026***

c) FGF 21

	A-FABP	TG	AI
FGF 21 MetS +	**beta = 0.4422** ***p = 0.0002***	NS	NS
FGF 21 MetS -	**beta = 0.2984** ***p = 0.0026***	**beta = 0.1150** ***p = 0.0358***	**beta = -0.1105** ***p = 0.0178***

Bold values indicate significance at $p<0.05$. NS- no significance.