Serum concentration of selected adipocytokines in patients with coronary artery disease suitable for surgical revascularization: a preliminary study

Stężenie wybranych adipocytokin u pacjentów z chorobą wieńcową poddanych chirurgicznej rewaskularyzacji: badania wstępne

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Kardiochirurgia i Torakochirurgia Polska 2011; 4: 432–437

Abstract

Introduction: Adipose tissue produces several bioactive mediators - adipocytokines, that influence insulin resistance, diabetes, body-weight homeostasis and inflammation. There has been no precise clinical data about role of adipocytokines in coronary atherosclerosis.

Aim of the study: To estimate serum concentrations of selected adipocytokines in patients with coronary artery disease (CAD) suitable for surgical revascularization.

Material and methods: The study included 17 patients with CAD, who were surgically treatment in the Department of Cardiac Surgery and 16 healthy controls. Serum concentrations of leptin, adiponectin, apelin and resistin were measured by immunoenzymatic ELISA tests. Data were compared with demographical, clinical and laboratory parameters of CAD patients.

Results: Serum leptin concentration was significantly higher in CAD patients when compared to controls ($p < 0.05$), and serum adiponectin and apelin concentrations were significantly lower in CAD patients than in healthy subjects (both, $p < 0.05$). The positive correlation between serum leptin levels and BMI ($p < 0.05$), and the tendency to negative correlation between serum apelin levels and systolic blood pressure ($p = 0.054$) in CAD patients were observed.

Conclusion: Our results suggest that leptin, adiponectin and apelin may play an important role in pathophysiology of CAD. Correlation studies suggest that apelin level can be related to the systolic blood pressure and pathogenesis of hypertension.

Key words: adipocytocines, adipose tissue, surgical revascularization.

Streszczenie

Wstęp: Tkanka tłuszczowa produkuje kilka bioaktywnych mediatorów – adipocytokin, które mogą wpływać na insulinooporność, rozwój cukrzycy, masę ciała i rozwój stanu zapalnego. Nie było dotychczas dokładnych danych klinicznych na temat roli adipocytokin w miażdżycy naczyń wieńcowych.

Cel pracy: Celem pracy była ocena stężenia wybranych adipocytokin u pacjentów z chorobą niedokrwienną mięśnia sercowego (ChNS) zakwalifikowanych do chirurgicznej rewaskularyzacji.

Materiał i metody: Do badania włączono 17 chorych z ChNS, którzy byli leczeni w Klinice Chirurgii Serca, i 16 zdrowych. Stężenie leptyny, adiponektyny, rezystyny i apeliny mierzono za pomocą testów immunoenzymatycznych ELISA. Dane zestawiono z wartościami demograficznymi, klinicznymi i laboratoryjnymi parametrów pacjentów z ChNS.

Wyniki: Stężenie leptyny było istotnie wyższe u chorych z grupy ChNS w porównaniu z grupą kontrolną ($p < 0,05$), a stężenie adiponektyny w surowicy i apelin stężenia były istotnie niższe u chorych z ChNS niż u osób zdrowych (w obu grupach $p < 0,05$). Stwierdzono dodatnią korelację pomiędzy stężeniem leptyny a BMI ($p < 0,05$) oraz negatywną korelację między stężeniem apelin a skurczowym ciśnieniem tętniczym ($p = 0,054$) u chorych z ChNS.

Wnioski: Wyniki uzyskane przez autorów niniejszej pracy sugerują, że osoczowa leptyna, adiponektyna i apelina mogą odgrywać ważną rolę w patofizjologii ChNS w grupie pacjentów leczonych kardiochirurgicznie. Badania korelacji sugerują, że poziom apeliny może być związany z ciśnieniem skurczowym i z patogenezą nadciśnienia tętniczego.

Słowa kluczowe: adipocytokiny, tkanka tłuszczowa, chirurgiczna rewaskularyzacja.

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Introduction

A group of patients with impaired fat metabolism which is reflected by multivessel coronary artery disease, diabetes and obesity is often encountered at the cardiac wards. Demonstration of activity of individual factors active in adipose tissue appears to be an interesting issue to be demonstrated to cardiac surgeons. Atherosclerosis may be characterized by the progressive deposition of cholesterol and other fatty substances in the intima of the arteries. It constitutes a multifactorial process resulting from an excessive inflammatory response to various injurious stimuli to the arterial wall. Formation of plaque in the intima leads to many cardiovascular diseases. Recently, it has become apparent that adipose tissue produces several bioactive mediators – adipocytokines, which influence insulin resistance, diabetes, body-weight homeostasis and inflammation [1, 2]. There have been no precise clinical data about the role of adipocytokines in coronary atherosclerosis. Subjects of CAD with multivessel involvement are the group of the very interest of the cardiac surgeon. In our study we measured the circulating leptin, adiponectin, resistin and apelin concentrations in patients with coronary artery disease (CAD).

Material and methods

Patients

A total of 17 patients with CAD (11 males, 6 females, median age 62 years) – surgical group and 16 healthy controls (9 males, 7 females, median age 61 years) – healthy group, were studied.

Severity of atherosclerotic disease in CAD group was as follows: three vessel disease in 11 patients and in the remaining group - two vessel disease. All surveyed patients had serious (> 50%) stenosis in left anterior descending artery LAD. Patients presented a stable form of coronary disease and were in class CCS II/III.

The CAD patients were surgically treated by myocardial revascularization in the Clinic of Cardiac Surgery in the Wroclaw Medical University. The characteristics of the surgical group and controls are presented in Table I. Both groups were at similar age, also gender distribution among the groups was similar.

Healthy controls were less obese, and had lower values of blood pressure than CAD patients, which reached statistical significance ($p < 0.001$) in all three parameters: BMI, SBP and DBP (body mass index, systolic blood pressure, diastolic blood pressure, respectively). Patients before surgery and blood sampling were weaned/not taking statins, therefore, possible impact of drugs on the levels of adipocytokines has not been analyzed.

Tab. I. Basic characteristics of the CAD patients and the controls

	Patients (n = 17)	Controls (n = 16)	p value
Age (years)	52 (48-72)	51 (43-64)	0.079
Sex (male/female)	11/6	9/7	0.854
BMI (kg/m^2)	28.2 (25.7-31.7)	23.9 (22.0-25.5)	< 0.001*
SBP (mm Hg)	152 (135-161)	110 (101-126)	< 0.001*
DBP (mm Hg)	99 (85-110)	70 (64-82)	< 0.001*
Diabetes type II (yes/no)	7/10	0/16	0.006*

Surgical procedures

The surgery was performed in a typical fashion with the usage of cardiopulmonary bypass and warm blood cardioplegia according to the local protocols. Most surgeries were uneventful and postoperative stay in the intensive care ward was not prolonged. No disturbances in the process of rehabilitation and wound healing were observed. Data related to the surgically treated patients are presented in Table II.

Blood sampling

All blood samples were drawn in the morning after at least 12 h of fasting. Indication of adipocytokines was performed before surgery to exclude the effects of stress of surgery which may affect the level of proinflammatory cytokines. The criterion for inclusion in the study group of surgical patients who were willing to participate in the study, age < 80 years, satisfactory function of left ventricle with ejection fraction > 40%, the incidence of advanced coronary artery disease (with involvement of large arteries 2-3) and the absence of valve pathology. The inclusion criteria for healthy subjects were the desire to participate in the study and no evidence of cardiac disease collected earlier in the interview. Control patients were healthy volunteers, without signs of ischemic heart disease, who were not diagnosed cardiologically.

The samples were collected in sterile test tubes, clotted (15 min, RT) and centrifuged (15 min, 900 xg). The obtained sera were stored at –45°C until assayed. All serum samples were run in the same assay.

Biochemical tests

Concentrations of leptin, adiponectin and resistin were measured by immunosorbent assay ELISA, using commercial kits supplied by RnDSystems (Abingdon, UK). The sensitivities were 7.8 pg/mL for leptin, 0.246 ng/mL for adiponectin and 0.026 ng/mL for resistin. Apelin assay was performed using the ELISA test (Phoenix Pharmaceuticals, California, USA) according to the manufacturer's instructions. Sensitivity of apelin test was 0.09 ng/mL. Glucose and lipids were analyzed enzymatically (BioMaxima, Lublin, Poland).

Tab. II. Characteristics of the CAD patients

No. of patients (n)	17
No. of the grafts mean (n)	3.12 (2-4)
Ejection fraction of the left ventricle (EF) mean (%)	54 (40-68)
Cardiopulmonary bypass time mean (min) (CPB)	104 (65-125)
Crossclamp (CXT) time mean (min)	63 (40-79)
Length of stay in hospital (days)	8.6

Measurement of high-sensitive CRP (hsCRP) was performed using immunoturbidimetric assay with the Quantia-CRP UV (Tulip Diagnostics Ltd., Goa, India). The detection limit was 0.1 mg/mL. Total protein was determined using the Bio-Rad Protein Assay (Bio-Rad, Munich, Germany).

Statistical analysis

Data were expressed as medians and range (25th and 75th percentile). Differences between groups were analyzed using the Mann-Whitney U test. Spearman's rank test was used for correlation analysis. All calculated p values were two-sided and $p < 0.05$ was considered significant.

Results

Laboratory data of healthy controls and surgically treated patients are shown in Table III.

We observed a significantly higher concentration of serum hs-CRP in CAD patients than in control group ($p < 0.001$). No major changes in the concentration of total cholesterol and triglycerides were evaluated. There was a statistically significant change in the concentration of HDL-cholesterol ($p = 0.008$) but we did not find statistically significant changes of LDL in both groups.

Tab. III. Biochemical parameters in CAD patients and the controls. Data are given as median and range (25th percentile and 75th percentile)

	Patients (n = 17)	Controls (n = 16)	p value
Total cholesterol (mg/dL)	145 (141-158)	156.7 (133-183)	0.273
Triglycerides (mg/dL)	127 (112-168)	135 (119-178)	0.652
HDL-cholesterol (mg/dL)	44 (33-52)	49 (41-56)	0.008*
LDL-cholesterol (mg/dL)	98 (78-136)	90 (71-127)	0.089
hs-CRP (mg/L)	38.7 (6.5-156)	0 (0-2.4)	< 0.001*
Total protein (g/L)	50 (45-63)	74 (62-78)	0.004*
Glucose (mg/dL)	128 (103-141)	96 (74-110)	< 0.001*
Leptin (ng/mL)	9.1 (3.8-11.6)	2.8 (1.3-6.3)	0.04*
Adiponectin (µg/mL)	5.6 (5.0-8.4)	11.3 (7.2-13.9)	0.02*
Apelin (ng/mL)	4.9 (3.2-6.2)	7.2 (5.6-10.4)	0.01*
Resistin (ng/mL)	8.4 (6.8-16.0)	6.5 (4.3-8.9)	0.078

**statistically significant (Mann-Whitney U test).*

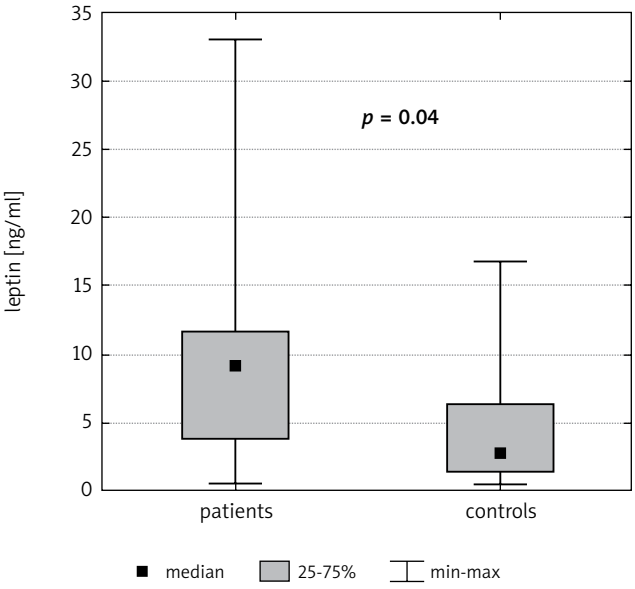

Fig. 1. Leptin concentration in patients and controls

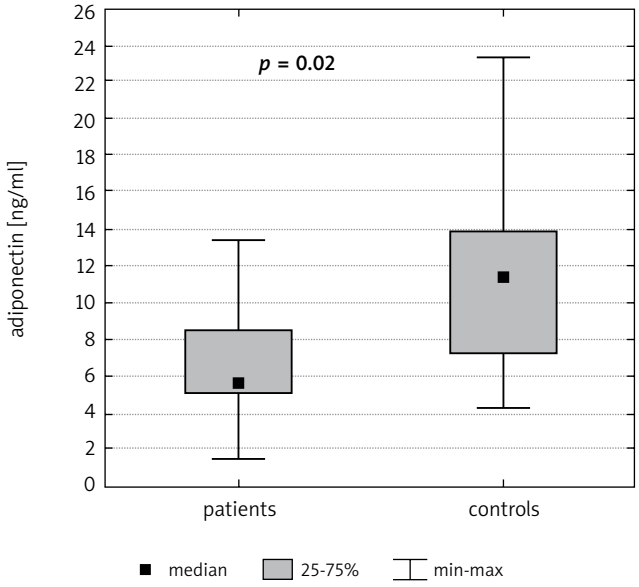

Fig. 2. Adiponectin concentration in patients and controls

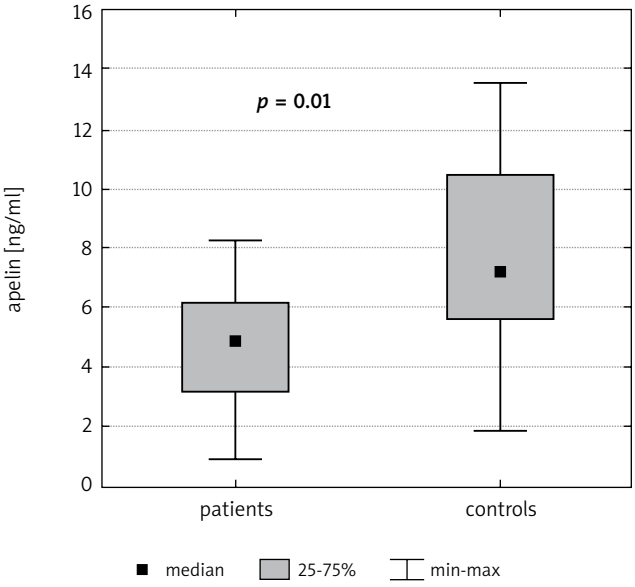

Fig. 3. Apelin concentration in patients and controls

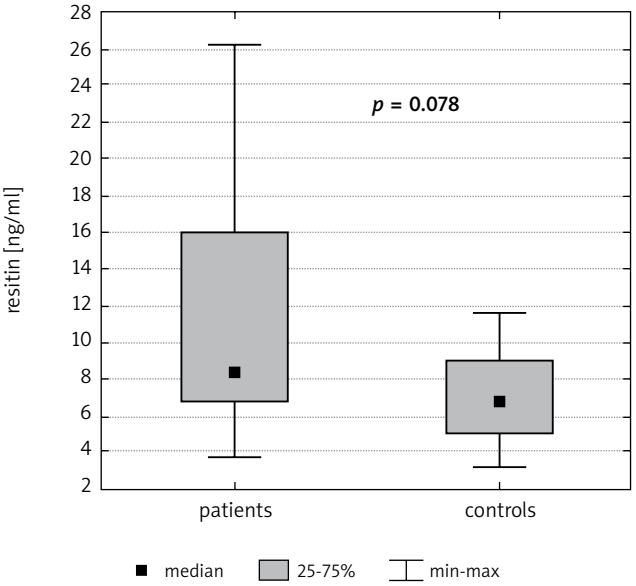

Fig. 4. Resistin concentration in patients and controls

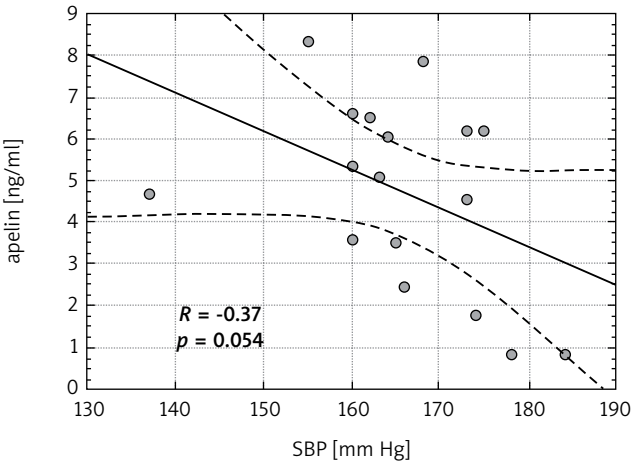

Fig. 6. The tendency to negative correlation between serum apelin levels and SBP in CAD patients

Significant differences were found in the levels of glucose in both groups of patients with p value of < 0.001. The major emphasis in research was put on the adipocytokines. The level of leptin was significantly higher in the surgical group in comparison to the control group (p = 0.04). Serum concentrations of adiponectin and apelin significantly decreased in CAD patients than in healthy subjects (p = 0.02 and p = 0.01, respectively). There were no significant differences in concentrations of serum resistin in the surgical and control group.

Positive correlation between serum leptin levels and BMI in CAD patients was evaluated. We showed the tendency to negative correlation between serum apelin levels and SBP in CAD patients.

Discussion

Disturbed metabolism of fat tissue is the starting point in the discussion on the role of adipocytokines in the

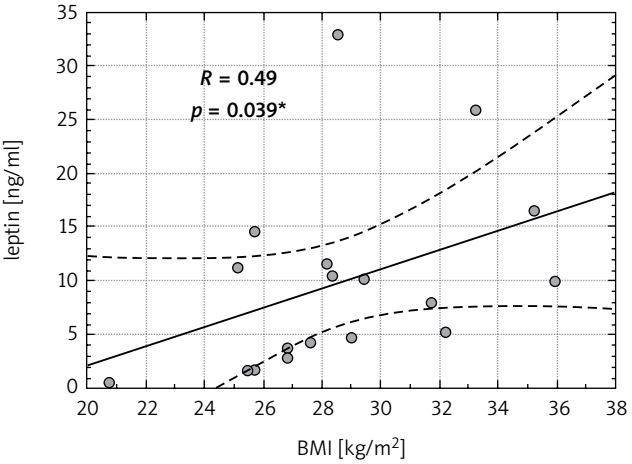

Fig. 5. Positive correlation between serum leptin levels and BMI in CAD patients

pathogenesis of coronary heart disease. Differences in sophisticated biochemical structure of the fat tissue between healthy subjects and patients suffering from CAD are seen when levels of circulating leptin, adiponectin, resistin and apelin are investigated. We demonstrated that concentrations of leptin, adiponectin, resistin and apelin in the group of surgically treated patients with CAD are different from concentrations in healthy subjects.

We believe that differences in the concentrations of adipocytokines may arise not only from CAD but also from concomitant diabetes and obesity. Occurrence of these three conditions is often closely related. Our study confirms that subjects with CAD suitable for surgical revascularization have lower adiponectin levels in comparison to healthy subjects. Smaller concentrations of adiponectin are associated with a risk of the development of CAD [3]. This relation is also described in another group of patients suitable for coronary artery bypass grafting (CABG) [4, 5]. In one-year follow-up [4] significant reduction of adiponectin serum concentration was observed. This observation was made during the investigation in the group of CABG compared to patients treated medically. Fewer studies compare levels of adipocytokines in patients with CAD to a group of healthy subjects. Pala and Monari found a similar relationship of adiponectin concentration in patients with ischemic heart disease (IHD) and healthy subjects. Circulating adiponectin levels were significantly lower in cases of IHD with respect to controls [6].

Further explanation of the different levels of adiponectin dedicated to the healthy and diseased subjects may be given on the inflammatory grounds. The process of inflammation contributes to CAD. In 2001, Blake and Ridker described hs-CRP as a reliable marker of systemic inflammation and adiponectin, which is an adipocyte-derived hormone and presents with anti-inflammatory and antiatherogenic effects [7]. This may explain the advanced stage of CAD and development of multivessel disease with involvement of left anterior descending artery (LAD) in a group with low concentration of adiponectin, which was investigated. The association between the hs-CRP levels and adiponectin

was demonstrated [8]. Statistically significant difference in the concentration of hs-CRP in the surgical arm confirms the similar tendency in other works [9]. Probably proinflammatory capacity of adipose tissue shows important insights in the pathophysiology of formation of the arteriosclerotic plaques. It can be considered that obesity corresponds to a sub-clinical inflammatory condition that promotes the production of pro-inflammatory factors involved in the pathogenesis of ischemic heart disease [10].

Another part of the study focused on the concentration of capelin in CABG patients. This tissue-derived peptide is produced in different parts of the body; especially endothelial cells [11, 12]. Apelin may play a role in the control of the heart rate, angiogenesis and contractility of the left ventricle [13, 14]. Apelin is an important factor in reduction of insulin resistance and it may also improve glucose tolerance in the early phase of development of type two diabetes. More detailed knowledge of its biochemical action may be subject of further research [15]. Apelin is a peptide the concentration of which is reduced in patients with essential hypertension. Our study disclosed the negative correlation between the SBP and level of apelin. The authors concluded that lower concentration was associated with more profound left ventricular systolic and diastolic function impairment [16]. In our study the patient group was collected from those with preserved LV function (EF > 30%, ESD > 55mm) [17] and further investigation may be proposed for such group of surgically treated patients. Resistin concentration was not statistically significantly different between the surgical arm and controls but the tendency in concentration was similar to these obtained as regards the concentration of leptin. Positive correlation between serum leptin levels and BMI in the surgical arm was disclosed. This may suggest that obesity, which in surgical arm is expressed as elevated BMI of the patients, is frequently associated with dyslipidemia and hypertension, all of which can exacerbate atherosclerosis [18]. The advanced form of this process is presented in the surgical arm of the investigation. A negative correlation (as mentioned above) was observed between the serum apelin levels and SBP in the surgical arm. This may suggest the coexistence of protective role of apelin in regulation of blood pressure and, in consequence, the development of the progressive heart failure. Nature of a communication between adipose tissue-derived factors like leptin, adiponectin, resistin, apelin and development of CAD warrants further investigation.

The effect of concentrations of adipocytokines on the survival length of hospitalization, perioperative cardiac ward has not been studied. This type of analysis was of interest to cardiac surgeons and should be analyzed on a larger group of patients.

This work is a part of the bigger project dedicated to evaluation of concentration of adipocytokines contained in fat tissue of patients qualified for CABG surgery. After obtaining the proper permission, further investigation may be carried out with the involvement of the adipose tissue. This requires a larger group of patients, which currently seems to be the main limitation of the presented work. Even such group of patients enabled us to present the following conclusions.

Conclusions

Our preliminary study showed that serum leptin concentration was significantly higher in CAD patients in comparison with controls, and serum adiponectin and apelin concentrations were significantly lower in CAD patients compared to healthy subjects. Correlation studies suggest that apelin can play a potent role in blood pressure regulation and may be an important mediator in the pathological processes of heart failure.

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