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Plasma Adiponectin Concentration in Relation to Severity of Coronary Atherosclerosis and Cardiovascular Risk Factors in Middle-Aged Men

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Adiponectin, an adipocyte-derived protein, seems to be a link between obesity, insulin resistance, and atherosclerosis. The present study investigated the association between adiponectin and coronary artery disease in middle-aged men. Material and methods: We examined 48 men (aged 40–60) with angiographically confirmed coronary atherosclerosis and 19 healthy men, matched by age, as a control group. Concentrations of glucose and lipids were estimated with enzymatic methods. Plasma level of adiponectin, total and free testosterone, estradiol, estrone, DHEA-S, and insulin were estimated with RIA commercial kits. Results: Men with coronary atherosclerosis had lower plasma adiponectin level than controls (16.2 ± 9.2 vs 20.5 ± 6.7 µg/mL; $p < 0.05$). However, after including BMI and waist as covariate data in ANCOVA, the difference in adiponectin levels between men with CAD and controls lost statistical significance (respectively for BMI and waist: $p = 0.4$ and $p = 0.7$). Moreover, although not significant, adiponectin levels decreased as a function of the number of significantly narrowed coronary arteries. In *a priori* comparison the lowest adiponectin plasma concentration was in men with three-vessel coronary artery disease (14.3 ± 9.8 µg/mL) and the highest in controls (20.5 ± 6.8 µg/mL; $p = 0.09$). Adiponectin plasma level correlated negatively ($p < 0.05$) with BMI, waist, percentage of total fat, fasting-insulin-resistance index (FIRI), total cholesterol and triglycerides, and positively with quantitative insulin sensitivity check index (QUICKI), HDL cholesterol, total testosterone, and total testosterone/estradiol ratio. Conclusions: Our data suggest that low plasma adiponectin level is connected with insulin resistance syndrome and atherogenic lipid profile. It seems that adiponectin plays a role in pathogenesis of coronary atherosclerosis, especially in obese and insulin-resistant subjects.

Key Words: Adiponectin; sex hormones; coronary atherosclerosis; men.

Introduction

Adiponectin (Arcp 30) is a colectine-type protein, which consists of 244 amino acids and is produced by adipocytes after stimulation of PPAR-s gamma ligands *(1)*. It is supposed that adiponectin is one of the factors that influences tissue insulin sensitivity *(2)* and lipid metabolism *(3,4)*. Adiponectin can directly influence muscle and liver to stimulate fatty acid oxidation and can reverse insulin resistance associated with both lipoatrophy and obesity *(5,6)*. However, there are also data showing no association between adiponectin level and the insulin resistance in obese children *(7)*. Adiponectin is also thought to have some anti-atherogenic properties through inhibition of TNF-α secretion and antagonizing its activity *(8,9)* and by inhibiting smooth muscle cell proliferation within the vessel wall *(10,11)*. A significant suppression of adiponectin level was observed in obese subjects with type 2 diabetes *(12–15)*, in subjects with ischemic heart disease *(2,8,11)*, and in young men with high-normal blood pressure *(3)*. Yang et al. *(16)* showed that adiponectin level increases after weight reduction. These findings indicate that adiponectin seems to be an endogenous anti-atherogenic factor, regulated by life style. Visceral obesity and insulin resistance are two of the major components of the metabolic syndrome responsible for increased risk of cardiovascular morbidity and mortality *(17,18)*. For this reason it is crucial to study all possible factors that can influence sensitivity for insulin and cardiovascular risk. We investigated whether adiponectin plasma concentration, a marker of insulin resistance, could be a marker of severity of coronary atherosclerosis. Moreover, we evaluated a relationship between adiponectin plasma level and anthropometrical, metabolic, and hormonal cardiovascular risk factors in middle-aged men.

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Table 1
Anthropometric Parameters in Men with Coronary Artery Disease (CAD) and Controls

	Men with CAD (n = 48)		Controls (n = 19)		
Parameter	Mean	SD	Mean	SD	p^a
Age (yr)	50.5	5.0	45.9	4.0	<0.001
BMI (kg/m^2)	27.2	3.4	24.6	2.9	<0.05
Waist circumference (cm)	98.4	9.4	88.6	7.8	<0.0001
Total fat (%)	26.3	5.7	22.2	5.4	<0.05
Abdominal fat (%)	32.5	5.9	29.1	6.7	<0.05

[a] p in Mann–Whitney U test.

Table 2
Metabolic Characteristic of Men with Coronary Artery Disease (CAD) and Controls

	Men with CAD (n = 48)		Controls (n = 19)		
Parameter	Mean	SD	Mean	SD	p^a
Adiponectin (μg/mL)	16.2	9.2	20.5	6.7	<0.05
Glucose (mmol/L)	5.63	0.83	5.02	0.77	<0.05
Fasting insulin (μU/mL)	17.8	13.7	7.4	3.6	<0.001
Fasting insulin/glucose	0.18	0.14	0.08	0.04	<0.01
FIRI[b]	4.0	3.1	1.5	0.8	<0.001
QUICKI[b]	0.1	0.0	0.2	0.0	<0.001
Total cholesterol (mg/dL)	240.3	46.8	235.4	27.2	ns
Triglicerydes (mg/dL)	221.5	109.5	141.9	69.5	<0.001
HDL-cholesterol (mg/dL)	37.4	11.1	53.2	13.4	<0.00001
Total cholesterol/HDL 6.8		1.7	4.6	0.9	<0.00001
LDL-cholesterol (mg/dL)	148.4	33.9	152.6	26.2	ns

[a] p in Mann–Whitney U test; ns, not significant.
[b] FIRI, fasting insulin resistance index; QUICKI, quantitative insulin sensitivity index.

Results

Anthropometrical data of the studied groups are presented in Table 1. Patients with coronary artery disease (CAD) were more obese, with higher BMI, waist circumference, body fat percentage, and visceral fat deposit than controls. In the CAD group, 42% of men met ATP III criteria *(19)* for metabolic syndrome. On the contrary, there was no one with metabolic syndrome in the control group ($p < 0.01$ in χ^2 test comparing both groups).

Both groups also differed ($p < 0.05$ in χ^2 test) for cigaret smoking: in the CAD group 68% of patients were current smokers, while in controls, 40%. The percentage of past smokers was the same in both groups (30%).

In the CAD group there was significantly lower plasma adiponectin concentration, which was accompanied by lower index of insulin sensitivity (QUICKI), higher values of index of insulin resistance (FIRI), glucose and insulin serum concentration, and insulin/glucose ratio in comparison to the controls (Table 2). Moreover, in the CAD group there was higher serum triglycerides and lower HDL-cholesterol concentrations, and higher total cholesterol/HDL-cholesterol ratio in comparison to the controls (Table 2).

Men with CAD had significantly lower serum levels of total testosterone, free androgens index, and testosterone/estradiol, and higher estrone concentration than controls (Table 3). There was no difference in mean DHEA-S, SHBG, free testosterone, and mean estradiol serum concentration between groups.

Adiponectin plasma concentration (in all men included in the study) correlated negatively with BMI, waist circumference, body fat percentage, abdominal fat deposit percentage, serum glucose, cholesterol, triglycerides and insulin concentration, and FIRI index (Table 4). There was positive correlation between adiponectin plasma concentration and total testosterone level, TT/E2 ratio, SHBG, HDL-cholesterol, and QUICKI index. In CAD group we found similar correlation with respect for BMI, waist circumference, body fat percentage, serum level of cholesterol, triglycerides, SHBG, total testosterone, and TT/E2 ratio (Table 4). In controls we found correlation of adiponectin level

Table 3
Hormonal Parameters in Men with Coronary Artery Disease (CAD) and Controls

	Men with CAD (n = 48)		Controls (n = 19)		
Parameter	Mean	SD	Mean	SD	p^a
Total testosterone (nmol/L)	17.2	6.7	20.8	7.3	0.05
Free testosterone (pmol/L)	51.1	17.3	45.9	13.1	ns
Estradiol (pmol/L)	79.6	21.1	68.7	20.5	ns
Estron (pg/mL)	51.3	28.4	38.0	13.9	<0.05
Estradiol/estrone	3.7	2.6	2.8	1.1	ns
Total testosterone/estradiol	228.2	93.0	316.8	102.6	<0.01
DHEAS (ng/mL)	1932.3	780.6	2159.2	1000.3	ns
SHBG (nmol/L)	29.1	11.4	25.4	12.6	ns
FAI[b]	60.9	14.0	89.3	27.9	<0.0001

[a] p in Mann–Witney U test; ns, no significant difference.
[b] FAI, free androgen index.

Table 4
Spearman's Correlations Between Adiponectin Plasma Level and Estimated Parameters

	All men		CAD group	
Parameter	r Spearman	p	r Spearman	p
Age (yr)	–0.08	ns[a]	0.04	ns
BMI (kg/m^2)	–0.40	<0.001	–0.44	<0.01
Waist circumference (cm)	–0.44	<0.001	–0.40	<0.01
Total fat (%)	–0.37	<0.01	–0.38	<0.05
Abdominal fat (%)	–0.24	<0.05	–0.12	ns
Total testosterone (nmol/L)	0.44	<0.001	0.42	<0.01
Free testosterone (pmol/L)	0.14	ns	0.24	ns
Estradiol (pmol/L)	–0.01	ns	0.07	ns
Estron (pg/mL)	–0.05	ns	0.07	ns
Total testosterone/estradiol	0.38	<0.01	0.36	<0.05
DHEAS (ng/mL)	0.03	ns	0.02	ns
SHBG (nmol/L)	0.37	<0.01	0.51	<0.001
FAI[b]	0.02	ns	–0.09	ns
Total cholesterol (mg/dL)	–0.26	<0.05	–0.28	0.05
Triglicerydes (mg/dL)	–0.42	<0.001	–0.31	<0.05
HDL- cholesterol (mg/dL)	0.36	<0.01	0.23	ns
LDL- cholesterol (mg/dL)	0.02	ns	0.02	ns
Glucose (mmol/L)	–0.31	<0.05	–0.20	ns
Fasting insulin (μU/mL)	–0.24	0.05	–0.06	ns
FIRI[b]	–0.29	<0.05	–0.13	ns
QUICKI[b]	0.29	<0.05	0.13	ns
No. of cigarets per day	–0.04	ns	–0.04	ns
mean stenosis index (MSI) (%)			–0.10	ns

[a] ns, no significant correlation.
[b] FAI, free androgen index; FIRI, fasting insulin resistance index; QUICKI, quantitative insulin sensitivity index.

only with waist circumference (r = –0.43; p = 0.07), serum level of triglycerides (r = –0.48; $p < 0.05$), HDL-cholesterol (r = 0.45; p = 0.05), and abdominal fat deposit percentage (r = –0.46; p = 0.05).

To exclude the influence of obesity on the difference in adiponectin plasma concentration between groups, we excluded extremely obese or extremely lean individuals from the comparison: in the analysis only men with BMI 22–32

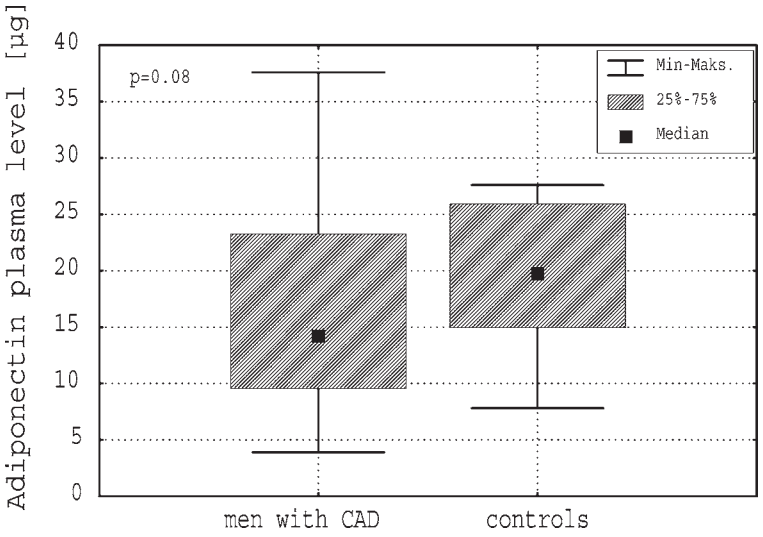

Fig. 1. Comparison of adiponectin plasma level in men with CAD ($n = 44$) and controls ($n = 16$) after controlling of BMI (only men with BMI 22–32) in Mann–Whitney U test.

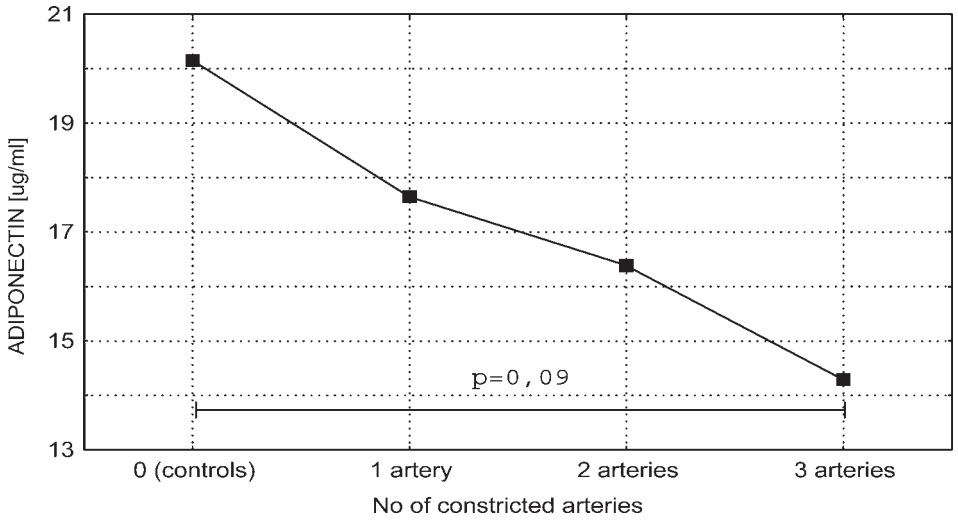

Fig. 2. Adiponectin plasma level in subject with different number of constricted (>70%) arteries and controls (group "0" vs group "3" in ANOVA planned comparison: $p = 0.09$).

were included. The difference in adiponectin plasma concentration between CAD men and controls was no more significant ($p = 0.08$ in Mann–Whitney U test, Fig. 1).

We also performed a covariance analysis in order to adjust data for BMI, age, and waist circumference. After including BMI and waist as covariate data, the difference in adiponectin levels between men with CAD and controls lost statistical significance (respectively for BMI and waist: $p = 0.4$ and $p = 0.7$). After adjusting for age results were just below statistical significance level ($p = 0.06$).

There was no correlation between adiponectin plasma concentration and mean stenosis index (MSI) in men with CAD. However, there was a tendency to lower adiponectin level in patients with more advanced coronary atherosclerosis: although not significant, adiponectin levels decreased as a function of constricted arteries (Fig. 2). In *a priori* comparison adiponectin plasma concentration was the lowest in three-vessel coronary artery disease group (14.3 ± 9.8 µg/mL) and the highest in control group (20.5 ± 6.8 µg/mL), but the difference did not reach statistical significance ($p = 0.09$).

Moreover, estradiol and LDL-cholesterol serum concentrations as well as body fat percentage increased with the number of constricted coronary arteries in ANOVA ($p < 0.05$) (Table 5).

Discussion

We showed that men with coronary atherosclerosis had lower adiponectin plasma level than controls; however, after including BMI and waist as covariate data in ANCOVA,

Table 5
Characteristic of Subjects
with Different Number of Constricted (>70% of Stenosis) Arteries[a]

Parameters	No. of constricted arteries					
	One constricted artery		Two constricted arteries		Three constricted arteries	
No. of individuals	18		19		8	
	Mean	SD	Mean	SD	Mean	SD
LDL-cholesterol (mg/dL)	137.8	30.0	154.3	32.4	162.9	40.4
Estradiol (pmol/L)	21.4	5.4	20.8	3.7	26.9	9.3
Total fat (%)	24.7	4.6	26.0	5.9	31.0	5.7

[a]Only parameters shown have significant difference in ANOVA; $p < 0.05$.

the difference lost the statistical significance. We did not observe significant correlation between adiponectin level and MSI of coronary arteries, although adiponectin levels decreased (not significantly) as a function of the increasing number of constricted arteries (Fig. 2). It did not reach statistical level perhaps because of the limited sample size; nevertheless, it could also be the result of the obesity: percentage of body fat increased as a function of increasing number of constricted arteries (Table 5).

Our results are opposite to other studies which showed that individuals with coronary artery disease had lower adiponectin concentration independent of other cardiovascular risk factors *(11,20–23)*. What is the reason of the different results of our study? Nakamura et al. showed that plasma concentrations of adiponectin in patients with CAD were significantly lower than in the control group, but there were, however, no significant differences between patients with stable CAD and controls *(24)*. We investigated only patients with stable CAD, and thereby they could have similar adiponectin level to the control group. It is possible that in the late stage of atherosclerosis adiponectin has no direct impact on atherosclerotic plaque formation. Although it was proved that adiponectin decreases secretion and antagonizes TNF-α action by influencing the expression of many adhesive molecules and monocyte adhesion to endothelial cells *(8,9)*, and inhibits growth factor–induced human aortic smooth muscle cell proliferation *(10,11)*, this mechanism is mostly important in the early stage of atherosclerosis or in the acute coronary syndrome. It is possible that adiponectin plays a crucial role in development of such conditions, but not in advanced stable angina. Thereby measurement of plasma concentrations of adiponectin seems to be useful, like other inflammatory cytokines, C-reactive protein (CRP), first of all for assessment of the acute coronary incident risk. On the other hand, Shimada et al. demonstrated that adiponectin level could not predict early angiographic restenosis after elective coronary stenting *(25)*. So, this problem requires further investigations.

In accordance to other studies *(4,26)*, we observed significantly lower adiponectin levels in obese compared with non-obese men. Similarly to other authors *(15,27)*, we observed significant negative correlation of plasma adiponectin concentration with anthropometrical parameters such as BMI, waist circumference, and fat tissue content.

Apart from anthropometrical parameters, both groups differed in insulin-resistance features (FIRI, QUICKI, fasting insulin, and glucose): men with CAD were more insulin resistant then controls. Moreover, according to Japanese studies *(15,27)*, in our study differences in adiponectin levels in men with CAD and controls were accompanied by proatherosclerotic lipid profile. Men with coronary atherosclerosis had higher levels of triglycerides, lower levels of HDL-cholesterol, and higher total cholesterol/HDL-cholesterol ratios. We proved the existence of negative correlation of adiponectin concentration with total cholesterol and triglycerides and positive correlation with HDL-cholesterol concentration, which suggests that a relationship of adiponectin with lipid metabolism may be through the hyperinsulinemia and insulin resistance. This fact, in addition to previously described relations of adiponectin to insulin resistance, indicates the important connection of this peptide with disturbances, leading to the metabolic syndrome *(4)*. This mechanism was confirmed recently by Ohashi et al., who showed, that adiponectin gene mutation is associated with the metabolic syndrome and coronary artery disease *(20)*.

According to other studies *(28–32)* we proved that men with CAD had lower levels of total testosterone, total testosterone/estradiol ratio, and higher levels of estrone than controls. The prevalence of atherosclerosis in men increases drastically with aging and age-associated decline in testosterone levels. The inverse correlation between testosterone levels and the severity of coronary artery disease may be related to the fact that andropenia is accompanied by accumulation of visceral fat, which is *per se* a cardiovascular risk factor, because of its metabolic consequences *(5,6, 8)*. We showed that adiponectin level correlated positively

with total testosterone, total testosterone/estradiol ratio, and SHBG. Our observation could indicate that an adiponectin level depends on sex hormone profile. However, these results are opposite to Nishizawa et al., who showed that castration increased and egzogenous testosterone reduced plasma adiponectin level in castrated mice *(33)*, and men had lower level of adiponectin then women. Clarification of this relationship requires further studies.

In summary, our data suggest that low plasma adiponectin level is connected with insulin-resistance syndrome and atherogenic lipid profile. It seems, that adiponectin play a role in pathogenesis of coronary atherosclerosis, especially in obese and insulin resistant subjects.

Materials and Methods

The study included 67 men (aged 40–60 yr): 48 men with coronary artery disease (CAD) and 19 healthy men. Men with CAD have been diagnosed and treated during the period of 3 yr (2000–2002) in the Department of Cardiosurgery, Division of Cardiodiagnostic, in Wroclaw University of Medicine (Poland). Inclusion criteria for patients with ischemic heart disease were as follows: at least 70% of lumen narrowing of at least one of three main coronary arteries—left anterior descending (LAD), circumflex (Cx), or right coronary artery (RCA); no history of diabetes, and no history of lipid lowering or hormonal therapy in the past.

Control group inclusion criteria were as follows: no history of ischemic heart disease and other diseases provoked by atherosclerosis (stroke, peripheral or carotid artery disease), no history of essential hyperlipidemia, arterial hypertension, diabetes, and other systemic diseases, normal presentation on physical examination, normal rest and exercise electrocardiography trace, and normal ultrasonographic examination of carotid arteries. Control group was selected from 56 healthy men who were examined for this scope in the Department of Endocrinology and Diabetology, Wroclaw Medical University; there was no indication for the coronary angiography examination within the control group. To eliminate all men who could have coronary artery disease, including those with "silent" ischemia, all criteria listed above were used.

The study was conducted in accordance and after approval of local Ethic Committee. Written informed consent was obtained from every participant.

Medical History and Physical Examination

In all patients and controls, detailed medical history was collected including family history of atherosclerosis-connected diseases (ischemic heart disease, stroke, peripheral vessel disease) and smoking. For cigaret smoking three groups were created: non-smokers, current smokers, and past smokers.

In all patients and controls detailed physical examination was carried out and included arterial pressure measurement with the use of sphygmomanometer in sitting pos-ition after 10 min of rest and then after 5 min. According to WHO guidelines *(34)* arterial hypertension was diagnosed at arterial pressure 140/90 mmHg and higher or when patient was currently on hypotensive therapy. Anthropometrical measurements, body mass and height, were carried out. Body mass index (BMI) was calculated from equation: body mass (kg)/height (m^2). Body mass and height were measured without top clothing and shoes, waist circumference were measured at half the distance between costal angle and iliac crests. The body fat percentage and visceral fat deposit were assessed using dual-energy X-ray absorptiometry method (DEXA). This measurement was carried out with the use of "Dpx (+) Lunar" device (USA) in Osteoporosis Out-Patient Clinic of the Department of Endocrinology and Diabetology, Wroclaw Medical University. The percentage of abdominal fat (android fat deposit) was calculated with the use of computerized method after measuring fat tissue volume in an area from the upper edge of L_2 disc to the lower edge of L_4 disc. This region is characterized by a large amount of visceral fat *(35)*. Moreover, apart from visceral fat, subcutaneous fat is thought to have an important predictive value for the insulin resistance *(17)*.

Coronary angiography was carried out by means of the Seldinger method using the femoral artery approach. The angiograms were evaluated on the basis of generally accepted criteria. As in other studies *(36)* 70% stenosis of the vessel lumen was considered as significant. Depending on the number of significantly narrowed vessels, patients were divided into three groups: one-, two-, and three-vessel disease *(36)*. The additional calculation of atherosclerotic process advance was done using so-called "mean stenosis index" (MSI): MSI = the sum of all three main coronary arteries stenoses [%]/3.

Treadmill testing was performed using the moving track Burdick T600 system linked with Sicard 460S computer system (Siemens) in accordance with the Bruce protocol. Treadmill methodology and the result interpretation were carried out in accordance with American Heart Association guidelines for treadmill testing *(37)*. In all controls ultrasonographic examination of the left and right common carotid arteries, the carotid bifurcation/bulb area, and the right and left internal and external carotid arteries was performed to confirm absence of atheromatous plaques protruding into the vessel lumen. Ultrasonographic examination was carried out in the Department of Cardiology, Medical University of Wroclaw, with the use of Sonos 100 CF device (Hewlett Packard, USA) equipped with the 7.5 MHz sector array probe.

Biochemical Studies

Blood samples were drawn from the ulnar vein in each subject before breakfast early in the morning (8.00), after an overnight bed-rest, with last meal on the preceding day

at 6 PM. The centrifuged plasma and serum were stored at –20°C until examinations. Plasma adiponectin levels were measured with RIA commercially kits (LINCO, USA). Concentrations of glucose (G), total cholesterol, cholesterol HDL, cholesterol LDL and triglycerides were estimated with routine enzymatic methods (Olympus Au 560; bioMerieux, France). Serum total and free testosterone, estradiol (E_2), estrone (E_1), DHEA-S, and insulin (I) were measured by means of radioimmunological method, while the level of sex hormone binding globulin (SHBG) was measured by means of immunoradiometric method. The hormonal investigations were carried out using commercial kits manufactured by Diagnostic Products Corporation (USA), and, in the case of estrone, by BioSource Europe S.A. (Belgium). All hormonal parameters were measured in Scientific Laboratory of our department (ISO-9).

The following indices were calculated basing on the serum hormone levels: free androgen index, FAI = total testosterone level ([nmol/l] × 100)/SHBG level [nmol/L] *(37)*; T/E_2 ratio; fasting insulin resistance index, FIRI = G [mmol/L] × I [mIU/mL]/25, as well as quantitative insulin sensitivity check index, QUICKI =1/(log I [mIU/mL] + log G [mg/dL]; I = insulin level, G = glucose level) *(18,38)*.

Statistical Analysis

Data were analyzed with Statistic for Windows 5.1 Software. To assess differences between groups obtained means were compared with Mann–Whitney *U* test (because of distributions deviating from normal). Differences between quantitative data were analyzed by means of the χ^2 test with Yates modification (due to a different number of samples). Moreover, analysis of covariance (ANCOVA) with BMI, waist, and age as a covariates data and analysis of variance (ANOVA) with *a priori* (planned) comparisons between three groups, isolated depending on the number of constricted (>70%) vessels, were performed. To assess relationship between adiponectin plasma level and anthropometric, hormonal, and metabolic data, Spearman's correlations were calculated. The level of statistical significance was determined at $p < 0.05$.

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