ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Proatherogenic adipocytokines levels in metabolic syndrome

MARIA BĂLĂŞOIU[1,2], ANDREI THEODOR BĂLĂŞOIU[2], ALEX EMILIAN STEPAN[1,2], SORIN NICOLAE DINESCU[1,3], CARMEN SILVIA AVRĂMESCU[1,2], DANIELA DUMITRESCU[1,2], DANIELA CERNEA[1,2], DRAGOŞ ALEXANDRU[1]

[1] *University of Medicine and Pharmacy of Craiova, Romania*
[2] *Emergency County Hospital, Craiova, Romania*
[3] *"Dr. Ştefan Odobleja" Emergency Military Hospital, Craiova, Romania*

Abstract

Introduction: Metabolic syndrome was defined by IDF (*International Federation for Diabetes*, 2007) by abdominal obesity plus at least two of the following: high triglycerides, low HDL-cholesterol, hypertension, high levels of glucose or type II diabetes diagnosed. Obesity is associated with a high cardiovascular risk, abdominal obesity being the most aggressive form, because it secretes cytokines and hormones in comparison to subcutaneous adipose tissue. Adipocytokines secreted by adipose tissue are mediators of atherosclerosis and endothelial damage. *Materials and Methods*: We studied a total of 80 subjects aged between 40 and 60 years with metabolic syndrome, in which the following adipocytokines values were determined: hs-CRP (turbidimetric method), IL-6, TNF-alpha, leptin (ELISA method), in comparison to a control group. *Results*: The values of these adipocytokines were significantly higher in the studied group compared with the control group and correlated with increased levels of glucose (patients with type II diabetes or increased tolerance test) and with hyper-triglyceridemia. *Conclusions*: Patients with metabolic syndrome had increased levels of proatherogenic adipocytokines, particularly leptin, leptin-resistance representing the pathogenic link of obesity. The identification as early as possible of the metabolic syndrome patients allows effective monitoring and correction of cardiovascular risk factors, with the opportunity to reduce morbidity and mortality in young ages. In men, proatherogenic cytokines values presented higher values than in women, which prove the role of abdominal obesity in proatherogenic cytokines production. Although women have a higher percentage of adipose tissue, this is not primarily abdominal adipose tissue.

Keywords: adipocytokine, metabolic syndrome, leptin.

Introduction

The metabolic syndrome is defined according to the latest studies carried out by the IDF (*International Federation for Diabetes*) and AHA (*American Heart Association*) in 2005 by abdominal obesity (waist circumference larger than 94 inches for men and larger than 80 inches for women), plus at least two of the following:

- Increased level of triglycerides (over 150 mg/dL) or specific treatment for this type of dyslipidemia;
- Low HDL-cholesterol (less than 40 mg/dL for men and less that 50 mg/dL for women) or specific treatment for this type of dyslipidemia;
- Systolic blood pressure over 130 mmHg or diastolic blood pressure over 85 mmHg or treatment of previously diagnosed hypertension;
- Increased fasting glucose level above 100 mg/dL or previously diagnosed type II diabetes [1, 2].

Epidemiological data, provided by *World Health Organization* (WHO) in recent years, show that in the late XXth century cardiovascular mortality was 28% worldwide, while transmissible disease (infectious, maternal, perinatal and denutrition) achieved a rate of 34%. Twenty first century is characterized by a global transition, cardiovascular mortality is estimated to be the main mortality cause until 2020, when it expects to be responsible for 36% of all deaths, while transmissible diseases are estimated to represent only 15% in 2020 [3].

In some parts of the world (like the USA), the prevalence of obesity (defined as a body mass index over 30 kg/m^2) has doubled in the last decade, reaching values over 30% of the population, with concerning growth among young people, especially female.

Obesity represents a major cardiovascular risk factor (Framingham Heart Study), obese individuals presenting a 1.5–2 times higher risk to develop a coronary artery disease. Obesity affects the proportion of sudden coronary deaths, with increasing weight also increases the percent of sudden deaths from the total coronary disease deaths [4].

Abdominal obesity is the most aggressive obesity form, visceral adipose tissue being metabolically more active, because it produces more cytokines and hormones in comparison to subcutaneous adipose tissue. Thus, the body mass index (BMI) alone cannot be considered a properly risk marker, because it does not correlate strictly to visceral obesity [5]. *WHO* statistics for Romania show a prevalence of obesity (BMI>30 kg/m^2) of 12% for females in 2005. In 2008, *WHO* noted that the prevalence of overweight and obesity in Romania reached an alarming percent of 55.2% [6].

Atherosclerosis is a progressive disease, characterized by the accumulation of the lipids and fibrous elements in the arteries intima. Complications of atherosclerosis (coronary heart disease, stroke, peripheral arterial occlusive disease) cause approx. 50% of deaths in industrialized countries and it is considered the "number one killer" in the world.

According to Ross, atherosclerosis is an inflammatory disease, atherosclerotic lesions occurring because of arterial injury [7–9].

The main pathogenic mechanisms of metabolic syndrome are insulin-resistance and compensatory hyperinsulinemia, sympathetic activation, endothelial dysfunction and systemic inflammation. Undiagnosed and untreated insulin-resistance involves long-term metabolic risks, such as coronary heart disease, dyslipidemia, and diabetes mellitus.

A phenotype of obesity relevant to cardiovascular risk is defined by identifying adipocytokines (biomarkers that quantify metabolic activity of adipose tissue). Regarding their effect, adipocytokines are divided into proatherogenic, proinflammatory adipocytokines, which are mediators of endothelial damage and atherosclerosis: TNF-α (Tumor Necrosis Factor), IL-6, Leptin, Plasminogen Activator Inhibitor (PAI-1), High Sensitive C-Reactive Protein (hs-CRP), angiotensinogen, resistin and antiatherogenic adipocytokines: adiponectin (complement C1 fraction) and Nitric Oxide (NO) [10, 11].

Increased levels of proinflammatory adipocytokines correlate with an additional cardiovascular risk, having poor prognostic value when associated with metabolic syndrome.

Materials and Methods

The study was conducted on a total of 80 subjects, aged between 40 and 60 years, of whom 34 women and 46 men, in 2010–2011, in comparison to a control group of 39 healthy subjects, as part of a research project of University of Medicine and Pharmacy of Craiova, Romania and S.L.I. PETROM Craiova. The parameter on which the patients were chosen was the obesity – the hub of the metabolic syndrome and a major risk factor for cardiovascular complications.

Criteria for the inclusion in the group were obesity, triglycerides over 150 mg/dL, HDL-cholesterol below 40 mg/dL in men and below 50 mg/dL in women, systolic blood pressure over 130 mmHg or diastolic blood pressure over 85 mmHg. Blood glucose concentration did not represent an inclusion criteria because the patients presented normal values or type II diabetes mellitus or decreased glucose oral tolerance test. The exclusion criteria were represented by the normal weight and statin treatment.

The study began with patient's medical history and the results were recorded in a special questionnaire.

Then, a clinical examination was performed and the presence of obesity was determined by measuring abdominal waist circumference. The patients included in the study presented abdominal circumference over 94 inches (men) and over 80 inches (women). The waist-hip ratio was also determined men with this index over 0.95 and women with this index over 0.80 were included in the study. The body mass index (BMI) was not studied, because it cannot differentiate muscular tissue from adipose tissue, individuals with the same BMI presenting different fat quantities. For blood pressure determination, Korotkoff sounds method was used. Blood pressure was determined in supine, at the right arm by three determinations, taking into consideration the lowest value.

The study of carbohydrate metabolism was performed by basal glucose level determination with glucose oxidation method, using an automatic biochemistry analyzer, COBAS C-311 Roche. At the patients with basal glucose values between 100–125 mg/dL, oral glucose tolerance test (OGTT) was performed. These patients received 75 g glucose in 300 mL water and after two hours, blood glucose level was determined. At patients whose blood glucose was below 140 mg/dL, the test was considered normal. Patients whose blood glucose was between 140–199 mg/dL were considered as having impaired glucose tolerance. Patients whose blood glucose was over 200 mg/dL were considered diabetic patients.

The study of lipid metabolism was performed using a biochemistry analyzer (COBAS C-311 Roche) for the determination of total cholesterol, triglycerides, HDL-cholesterol. LDL-cholesterol was calculated using Friedewald formula.

The adipocytokines studied were: hs-CRP (determined with turbidimetric method on COBAS C-311 analyzer; normal values <3 mg/L), IL-6 (determined with ELISA method using PeliKine Human IL-6 kit, Netherlands; normal values <10 pg/mL), TNF-α (using PeliKine Human TNF-α kit, Netherlands; normal values <10 pg/dL) and leptin (determined with ELISA, using Leptin Sandwich Diagnostic Automation Inc., CA, USA; normal values: for women, 7.36±3.73 ng/mL; for men, 3.84±1.79 ng/mL).

Statistical analysis

We used Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA) for processing the data.

To test the normality of the data, we used the Anderson–Darling test. None of the four numerical variables investigated had a normal distribution of data, globally or inside each studied group (metabolic syndrome or control group, males or females).

Because the data did not follow a normal distribution, non-parametric statistical tests had to be used and the results were summarized as median value and inter quartile interval.

Because the study involved a numerical comparison between two groups of patients that did not have a normal (Gaussian) distribution, the non-parametric Mann–Whitney test was primarily used, instead of the Student's *t*-test, to detect significant differences between the values in the compared data series. This test mainly assesses if there is a difference between the median values of the compared data series (or between the ranks or locations of the data in the compared series), and not between the mean/average values, as Student's *t*-test does.

We also used the non-parametric Spearman's rank correlation test to measure the strength of association between two ranked variables.

Results

High Sensitive C-Reactive Protein (hs-CRP)

In the studied group, we found elevated hs-CRP values with a median value (25–75% interval) of 4.25 (2.3–

9.525) mg/L. We noticed a median value for women of 5.2 (2.675–11.05) mg/L, higher than the median value for men 3.9 (2.3–7.975) mg/L, but this difference was not statistically significant (Mann–Whitney test, p=0.195). The values in the study were significantly higher than the values in the control group 1.6 (0.7–2.5) mg/L (Figure 1).

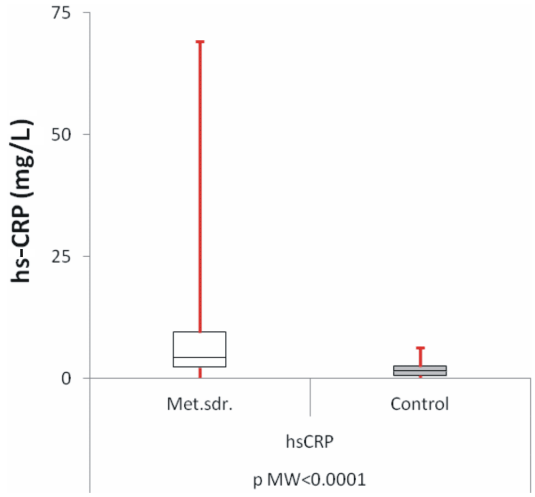

Figure 1 – ***Comparison between the hs-CRP values for the study and control groups.***

TNF-α

Fifty-two (65%) patients presented elevated TNF-α values, with a median value for the entire study group of 13.4 (7.025–21.525) pg/mL. TNF-α in men, 15.25 (10.225–24.75) pg/ml expressed higher values compared to women – 10 (6.2–14.3) pg/mL, but the difference was not statistically significant (p=0.54) for our group, yet it was very close to the threshold that shows a statistical significant difference (p<0.05). The values for the patients included in our study were significantly higher than the values found in the control group 5.5 (2.75–7.15) pg/mL (Figure 2).

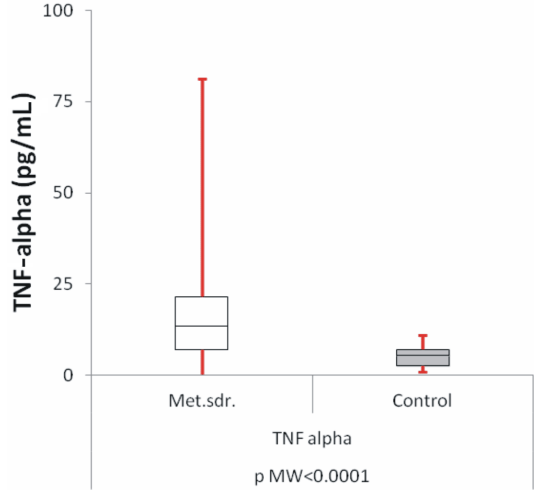

Figure 2 – ***Comparison between the TNF-α values for the study and control groups.***

IL-6

In the studied group, the median value for IL-6 was 15.6 (8.725–21.55) pg/mL, in comparison to the control group, which presented much lower values, with a median of 4.8 (1.9–7.3) pg/mL (Figure 3).

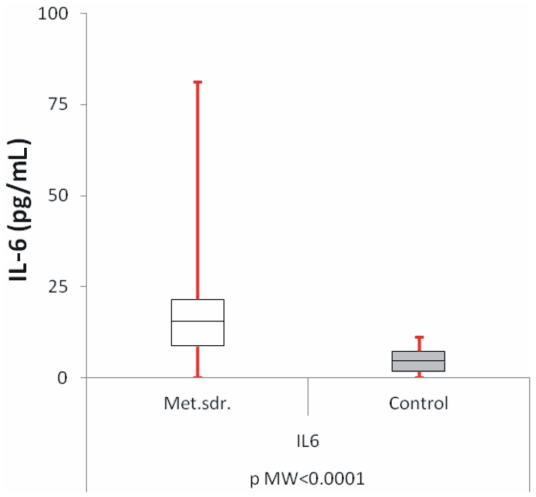

Figure 3 – ***Comparison between the IL-6 values for the study and control groups.***

The statistical analysis test showed statistically significant differences between the two groups (Mann–Whitney test, p<0.001). Although IL-6 values were superior for male patients, the Mann–Whitney test shows non-significant differences of IL-6 values between female patients and male patients, F=13.25 (6.75–18.8), M= 17.2 (11.3–22.725), p=0.140.

Leptin

In 53 (62.2%) patients, serum leptin levels were outside the normal values range. More than half (58.7%, N=47) presented increased leptin values and only six cases presented leptin levels under the inferior limit (7.5%). Leptin increased values were up to three times more frequent in males than females: 41/46 males – 89.13% *versus* 6/34 females – 17.65%. Male patients odds ratio to present increased leptin values was almost 40 times higher than female patients risk (OR=38.267; 95% CI 10.635–136.690; p<0.001).

Even if the normal values for males are lower than for females (3.84±1.79 ng/mL for men *versus* 7.36±3.73 ng/mL for women), in our study, for patients with metabolic syndrome, the values for males 9.7 (7.225–14.5) were much higher than the values for females 5.6 (3.675–9.725), the Mann–Whitney test showing a highly significant difference between the sexes, with a p-value=0.00029 <0.001 (Figure 4).

In the studied group, the median value for leptin was 8.25 (5.275–12.4) ng/mL, in comparison to the control group, which presented much lower values, with a median of 4.9 (2.3–6.2) pg/mL (Figure 5).

The correlation between leptin and the other pro-inflammatory, proatherogenic markers did not present significant values, with one exception: the significant correlation between plasmatic leptin levels and plasmatic hs-CRP levels at female cases (r=0.41, 95% CI 0.017–0.69; p=0.042).

The statistical analysis of correlations between the values of the studied biological parameters (IL-6, hs-CRP, TNF-α, leptin) found a positive linear relationship only between IL-6 and TNF-α (Spearman's test, ρ=0.276, p=0.014) (Figure 6).

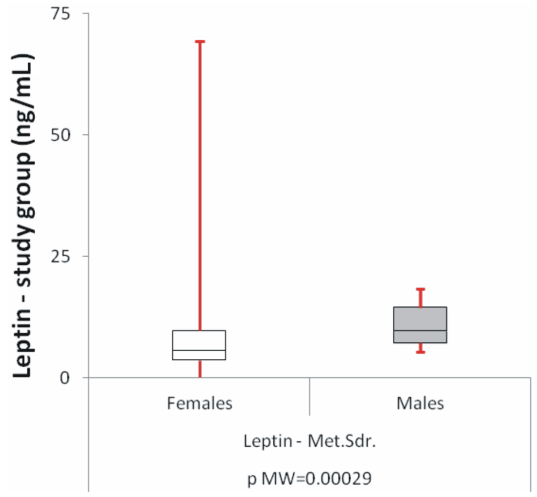

Figure 4 – ***Comparison between the leptin values for females and males in the study groups.***

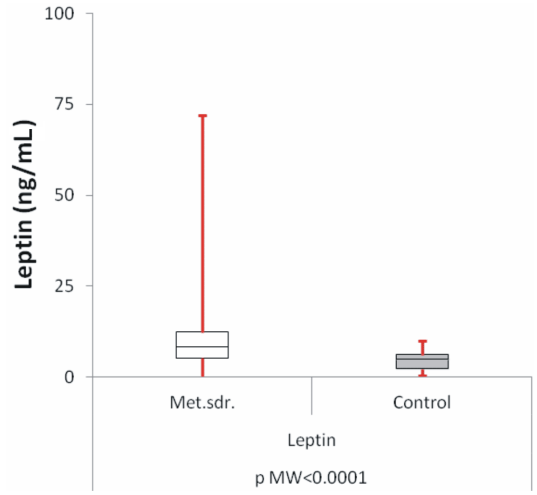

Figure 5 – ***Comparison between the leptin values for the study and control groups.***

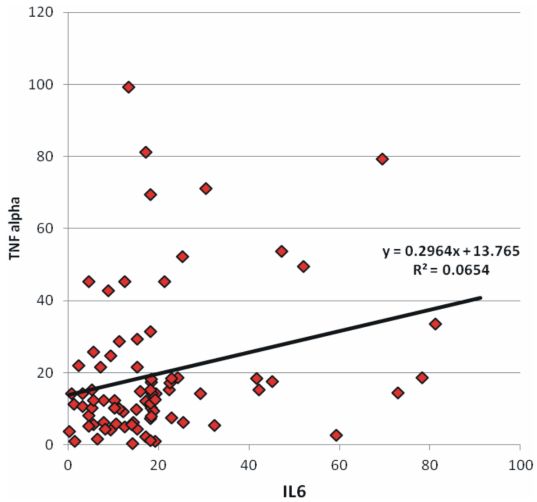

Figure 6 – ***Statistical correlation between values of IL-6 and TNF-α.***

Discussion

Studied proatherogenic adipocytokines represent biomarkers that quantify the metabolic activity of adipose tissue, being also mediators of atherosclerosis and endothelial damage [11].

Hs-CRP is considered as a new proinflammatory, proatherogenic adipocytokine and an acute phase reactant. It is synthesized mainly in the liver and it is regulated by circulating levels of IL-6, IL-1 and TNF-α. Plasma levels of hs-CRP have become significant and independent predictors of coronary heart disease; increased levels of hs-CRP were found at obese patients. Hs-CRP directly participates in the process of atherogenesis by modulating endothelial function by expression of adhesion molecules, selectins and MCP-1. Hs-CRP may also play a role in amplifying the proinflammatory activity of other adipocytokines such as PAI-1. Increased plasma levels at the studied group can be considered as important predictors for myocardial infarction or stroke. Hs-CRP levels over 3 mg/L signify increased cardiovascular risk, according to the *American Heart Association* [12].

IL-6 is another proatherogenic marker associated to insulin resistance. IL-6 reduces hepatic glycogen synthesis induced by insulin and glucose uptake by adipocytes. This adipocytokine causes insulin resistance *via* induction of nuclear factor NF-κB [12].

TNF-α is an inflammatory cytokine released in large quantities in obese patients and in patients with increased insulin resistance, which contributes to the initiation and formation of atherosclerotic lesions. It activates NF-κB transcription, which accelerates atherogenesis by inducing the expression of adhesion molecules and E-selectin in vascular smooth muscle cells and aortic endothelium. TNF-α reduces the bioavailability of nitric oxide in endothelial cells and alters endothelium-dependent vasodilatation, promoting endothelial dysfunction [12].

Elevated levels of IL-6 and TNF-α higher in men than in women (although women have a higher percentage of adipose tissue) proves that abdominal obesity, which is found predominantly in men, produces more proatherogenic cytokines, so men have a higher risk for cardiovascular events than women.

Leptin is a proinflammatory, proatherogenic new marker at which plasma levels increase proportionally with the increase of adipose tissue, although there are significant differences for leptin values for the same body weight. Expression and production of leptin from adipose tissue are induced by IL-6 and TNF-α, which proves the role of adipocytokine interaction regarding their release from adipose tissue [13–15]. *In vitro*, leptin produces proatherogenic effects such as stimulation, migration and proliferation of smooth muscular cells, accelerating the production of cellular calcification and increasing the production of oxygen radicals [16–18]. Our study shows a significant increase of leptin levels in men and a correlation with hs-CRP levels. The correlation between increased leptin and hs-CRP values can be explained through the fact that both are stimulated by high IL-6 levels and through the fact that plasma levels of leptin and hs-CRP increase proportionally with the adipose tissue, women having a higher percentage of adipose tissue in comparison to men.

Statistical correlation between IL-6 and TNF-α levels proves the adipocytokines interaction regarding their release from adipose tissue and their proatherogenic cumulative effect. These facts can also be interpreted as predictors of a higher cardiovascular risk.

Conclusions

Proatherogenic cytokines values in the studied group were significantly increased, particularly leptin, leptin resistance representing the pathogenic link of obesity, directly correlated to insulin resistance. Leptin resistance and insulin resistance determine lipid metabolism and protein metabolism alterations, and also endothelial dysfunction, increasing the cardiovascular risk. Identification of proatherogenic adipocytokines must be investigated in people with metabolic syndrome because it represents the best way to define a phenotype of obesity relevant for cardiovascular risk. Knowing the levels of these adipocytokines as fast as possible is necessary because it allows effective therapeutic correction of risk factors and it increases the chance of reducing mortality.

Contribution Note

All authors equally contributed to this paper.

References

[1] ***, *The IDF Consensus Worldwide Definition of the Metabolic Syndrome*, International Diabetes Federation (IDF), IDF Communications, Brussels, Belgium, 2006, http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf.

[2] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F; American Heart Association; National Heart, Lung, and Blood Institute, *Diagnosis and management of the metabolic syndrome: an American Heart Association/ National Heart, Lung, and Blood Institute Scientific Statement*, Circulation, 2005, 112(17):2735–2752.

[3] ***, World Health Report 2002 – Reducing risk, promoting healthy life. Mentioned in: Zipes DP, Libby P, Bonow RO, Braunwald E (eds), *Braunwald's heart disease: a textbook of cardiovascular medicine*, 7th edition, Elsevier Saunders, 2005, 1–19.

[4] Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB, *Overweight and obesity as determinants of cardiovascular risk: the Framingham experience*, Arch Intern Med, 2002, 162(16):1867–1872.

[5] Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA, Stampfer MJ, Willett WC, Manson JE, *Abdominal adiposity and coronary heart disease in women*, JAMA, 1998, 280(21): 1843–1848.

[6] ***, *Diabetes and obesity: time to act*, International Association for the Study of Obesity, 2007.

[7] Ross R, *Atherosclerosis – an inflammatory disease*, N Engl J Med, 1999, 340(2):115–126.

[8] Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group, *The metabolic syndrome – a new worldwide definition*, Lancet, 2005, 366(9491):1059–1062.

[9] Berg AH, Scherer PE, *Adipose tissue, inflammation, and cardiovascular disease*, Circ Res, 2005, 96(9):939–949.

[10] Poiac M, Brega D, Popa I, *Sindromul metabolic la copil şi adolescent – factor de risc cardiovascular la adult (The metabolic syndrome in children and adolescents – risk factor for adult cardiovascular diseases)*, Revista Română de Pediatrie, 2009, LVIII(3):234–239.

[11] Pischon T, Rimm EB, *Adiponectin and risk of acute coronary syndromes: defining the obesity phenotype*, Eur Heart J, 2007, 28(3):274–275.

[12] Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S, *Adipokines: molecular links between obesity and atherosclerosis*, Am J Physiol Heart Circ Physiol, 2005, 288(5):H2031–H2041.

[13] Popa A, Adam F, Câltea C, Câltea A, Buzatu I, Chiurcu CI, Sinescu C, *Aspecte ale interrelaţiei obezităţii cu apariţia şi evoluţia afecţiunilor vasculare*, Medicina Modernă, 2009, XVI(11):130–145.

[14] Konstantinides S, Schafer K, Loskutoff DJ, *The prothrombotic effects of leptin possible implications for the risk of cardio-vascular disease in obesity*, Ann N Y Acad Sci, 2001, 947: 134–141; discussion 141–142.

[15] Cooke JP, Oka RK, *Does leptin cause vascular disease?* Circulation, 2002, 106(15):1904–1905.

[16] Matsuda K, Teragawa H, Fukuda Y, Nakagawa K, Higashi Y, Chayama K, *Leptin causes nitric-oxide independent coronary artery vasodilation in humans*, Hypertens Res, 2003, 26(2): 147–152.

[17] Friedman JM, *Effects of leptin treatment on weight loss*, ClinicalTrials.gov Identifier: NCT00050791; Rockefeller University, Last updated: October 18, 2010.

[18] Erdmann J, Lippl F, Wagenpfeil S, Schusdziarra V, *Differential association of basal and postprandial plasma ghrelin with leptin, insulin, and type 2 diabetes*, Diabetes, 2005, 54(5): 1371–1378.

Corresponding author

Maria Bălăşoiu, Associate Professor, MD, PhD, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40723–319 879, e-mail: balasoiu.maria@yahoo.com

Received: September 5, 2013

Accepted: January 28, 2014