

## BETTER PROGNOSIS IN OVERWEIGHT/OBESE CORONARY HEART DISEASE PATIENTS WITH HIGH PLASMA LEVELS OF LEPTIN

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### Abstract

**Background and aim.** The involvement of leptin in atherosclerosis is very complex, including inflammation, the oxidative stress and thrombosis. Leptin has atherogenic and also antiatherogenic actions. In obesity elevated leptin levels are not sufficient to prevent disturbances of energy balance, suggesting that obese people are leptin resistant. The aim of the study was to investigate the relationship between baseline plasma levels of leptin and the incidence of new ischemic events in patients with CHD.

**Methods.** Plasma levels of leptin in fifty nine consecutive patients (29 men and 30 women) with CHD hospitalized in the County Emergency Clinical Hospital of Cluj-Napoca were measured using commercially available ELISA at admission. Patients with active infectious disease, neoplasia, acute coronary syndrome, stroke, hepatic or renal failure and severe heart failure were excluded. The relationship between leptin levels and incident cardiovascular events (angina, nonfatal myocardial infarction or heart failure) over two years follow-up was studied using MEDCALC version 9.6.

**Results.** 73.6% patients with CHD were overweight or suffered of obesity. There were no significant differences between women and men regarding the plasma levels of leptin, the body mass index (BMI), the number of rehospitalizations, rehospitalizations/patient, diabetes mellitus, hypertension or dyslipidemia. Only in women plasma levels of leptin are correlated with BMI. As compared with men with overweight and obesity ( $BMI \geq 25 \text{ kg/m}^2$ ), plasma levels of leptin were significantly higher in women with overweight and obesity ( $3905.97 \pm 463.91 \text{ pg/ml}$  vs  $1835.17 \pm 533.9 \text{ pg/ml}$ ) ( $p < 0.002$ ). Patient gender could not be demonstrated to influence prognosis. During the two years we recorded one or more readmissions in 26 patients (44%). The analysis of time till readmission using Kaplan-Meier curves, showed that leptin level (cut-off 2000 pg/ml, HR 0.38, 95% CI 0.17-0.83;  $p = 0.01$ ) and BMI (cut-off 28 kg/m<sup>2</sup>, HR 0.3164, 95% CI 0.145-0.0689;  $p < 0.01$ ) were significantly associated with prognosis.

**Conclusion.** Patients with plasma levels of leptin  $> 2000 \text{ pg/ml}$  and BMI  $> 28 \text{ kg/m}^2$  had a better prognosis, suggesting a protective role of leptin in overweight/mild obesity.

**Keywords:** leptin, coronary heart disease, overweight, obesity, prognosis

### Introduction

The adipocyte releases free fatty acids, cytokines (leptin, adiponectin, TNF- $\alpha$ ) and hormones (angiotensin II) [1]. Leptin is primarily involved in the central regulation of food intake and energy expenditure [2]. Leptin receptors are present in endothelial cells. It may exert atherogenic [2,3-7], thrombotic [8,9] and angiogenic effects [3-5]. Hyperleptinemia predicts restenosis after coronary angioplasty, coronary events and cerebral stroke independent of the traditional risk factors [10-13].

As compared with healthy men, plasma levels of leptin in healthy women are higher [14].

In obesity, elevated leptin levels are not sufficient to prevent disturbances of energy balance, suggesting that obese people are leptin resistant [6,15]. According to the concept of selective leptin resistance [15] only the anorectic effect of leptin is impaired in obese subjects. Leptin resistance in obese individuals may be acquired or determined by genetic factors.

Leptin can up-regulate iNO synthesis to generate large amounts of NO that promote atherogenesis by inducing oxidative stress through generation of peroxynitrite [16,17,18,19]. By contrast, some data indicate that leptin may protect against atherosclerosis in specific animal models [2]. Leptin correlates with plasma levels of soluble thrombomodulin and stimulates synthesis and secretion of endothelin-1 in endothelial cells [20,21]. It stimulates lipoprotein lipase secretion by cultured human macrophages [22], promotes hepatic HDL clearance and decreases plasma HDL level [23]. In macrophages leptin stimulates the secretion of cytokines (TNF- $\alpha$ , IL-2 and IL-6) [22], increases generation of reactive oxygen species, and enhances expression of cell adhesion molecules (MCP-1) [18] and TGF- $\beta$  synthesis by endothelial cells [19]. Leptin stimulates the expression of CRP in hepatocytes [25], the migration and proliferation of VSMC and the expression of metalloproteinase by VSMC [26].

In patients with heart failure an inverse correlation between leptin and mortality was found, suggesting a protective role of leptin [27]. In severe and end stage cachectic heart failure the plasma levels of leptin have been shown to be lower as compared with non-cachectic heart failure patients [28,29].

In a previous study we found a different pattern of plasma levels of leptin and resistin in patients with coronary artery diseases and peripheral occlusive artery diseases [30].

The aim of the present study was to investigate in patients with CHD the relationship between plasma levels of leptin and the cardiovascular events in two years follow-up.

### Material and method

**Subjects.** Fifty nine subjects with CHD hospitalized consecutively (2008-2009) in the County Emergency Clinical Hospital of Cluj-Napoca were included in study.

We followed the readmissions for cardiovascular events (angina, nonfatal myocardial infarction or heart failure) for a period of two years (2009-2011). The clinical variables were analyzed.

**Methods.** Plasma levels of leptin were measured by commercially available ELISA, quantikine reagents (R&D System). For the leptin assay the analytical limit of detection was 7.8 pg/ml; intra-assay coefficients of variation (%) were 3.2-3.3 and inter assay coefficients of variation (%) were 3.5-5.4.

All patients participated voluntarily and each subject included in the study signed a written informed consent for the participation in the study approved by the Local Ethics Committee.

**Exclusion criteria** were active infectious disease, neoplasia, acute coronary syndrome, strokes, hepatic or renal failure, severe heart failure.

**Statistical analysis.** The primary endpoint of the study was the prediction of readmission which was assessed after the last discharge until a new readmission. Probability to survive with no readmission was evaluated by Kaplan-Meier method and differences between Kaplan-Meier curves were evaluated by log rank test [30].

By correlation we mean the Fisher correlation method. Comparison of means was done by the Student or Mann-Whitney test. Chi squared test was used in statistical comparison of percentages of different categories. Yates corrections was applied if necessary.

For all used tests the p-value under 0.05 was considered significant. Confidence interval values were also calculated with the threshold of 0.05.

As a statistical software we used Excel 1997-2013 and MEDCALC version 9.6.

### Results

Fifty nine patients, 29 men and 30 women were included in the study. The clinical characteristics of the study groups are shown in the Table I.

No significant differences between the age (64.9 $\pm$ 1.99 years vs 68.17 $\pm$ 1.98 years), the plasma levels of leptin (2875.1 $\pm$ 490.5 pg/ml vs 2888.7 $\pm$ 573.8 pg/ml) and the BMI (27.08 $\pm$ 0.98 kg/ m<sup>2</sup> vs 28.94 $\pm$ 1.2 kg/ m<sup>2</sup>) of men and women were found.

As compared with men with overweight and obesity (BMI $\geq$ 25kg/m<sup>2</sup>) plasma levels of leptin were significantly higher in women with overweight and obesity (3905.97 $\pm$ 463.91 pg/ml vs 1835.17 $\pm$ 533.9 pg/ml) (p<0.002).

Plasma levels of leptin were correlated with BMI in the whole group (r=0.39; p<0.01) (Figure 1) and in the subgroup of women (r=0.55; p<0.01) (Figure 2). In patients with BMI $\geq$ 25 kg/m<sup>2</sup> the correlation was present also only in women (r=0.59 p<0.01) (Figure 3) and absent in men (Figure 4).

During the two years we recorded one or more

readmissions in 26 patients (44%), 12 women and 14 men. No significant difference between the number of readmissions in women and men ( $3.33 \pm 0.61$  vs  $2.79 \pm 0.49$  readmissions/patient) was found.

For numeric variables we chose for cut-off points the corresponding values that provided the lowest p. Under the threshold of significance 0.05 we have p-values only for leptin (2000 pg/ml) and BMI (28 kg/m<sup>2</sup>).

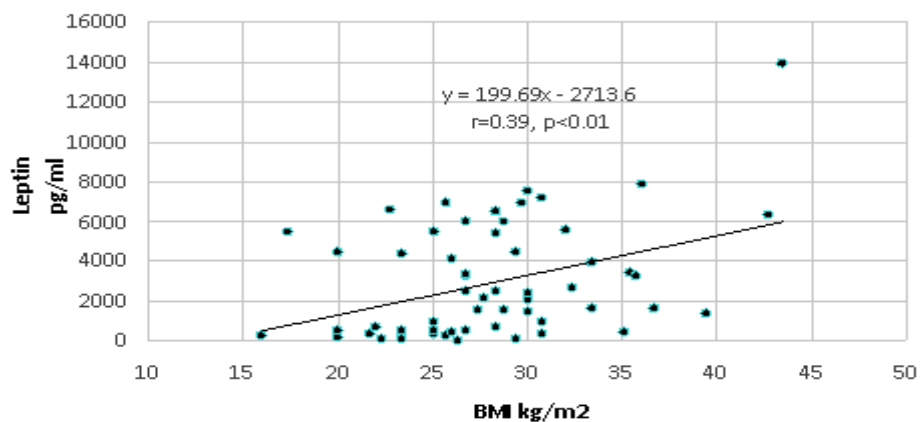
In the simple analysis of times till readmission on Kaplan-Meier curves, leptin (cut-off 2000 pg/ml, HR 0.38,

95% CI 0.17-0.83;  $p=0.01$ ) and BMI (cut-off 28 kg/m<sup>2</sup>, HR 0.3164, 95% CI 0.145-0.0689;  $p<0.01$ ) were significantly associated with prognosis. Among the patients with low (< 2000 pg/ml) leptin only 41% had free survival rate compared with 70% in those with leptin > 2000pg/ml. In patients with BMI < 28kg/m<sup>2</sup> only 39% had a free survival rate as compared with 75% of those with BMI  $\geq$ 28kg/m<sup>2</sup>.

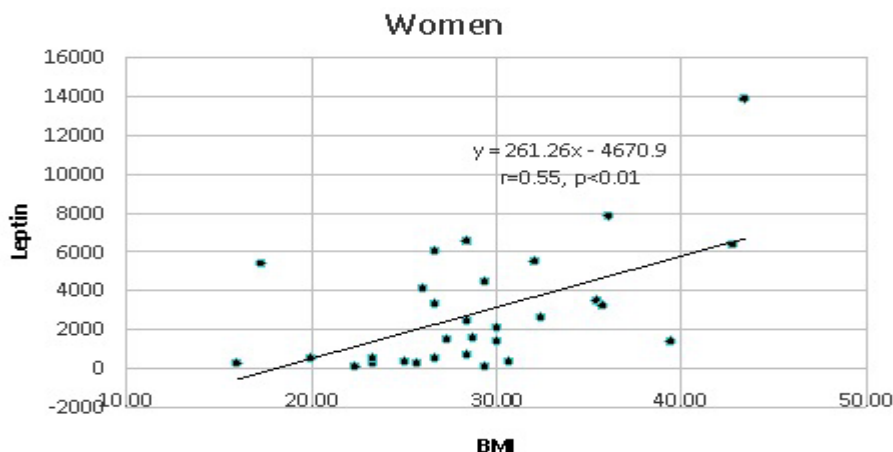
Figures 4 and 5 shows the Kaplan-Meier curves for these cut-off values.

**Table I.** Clinical characteristics of patients (p).

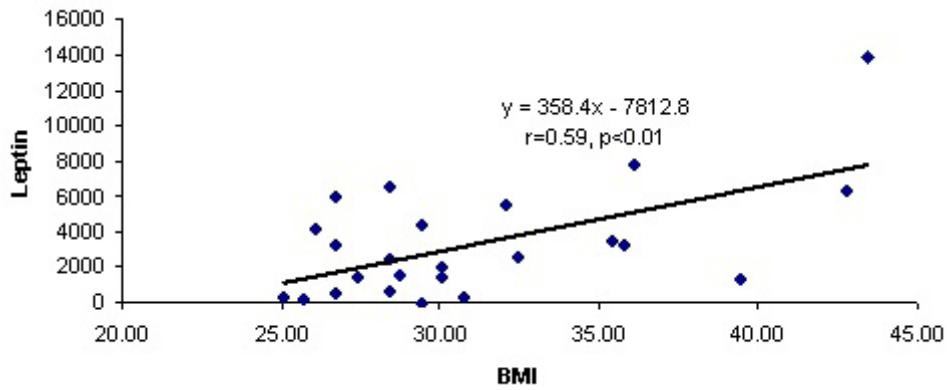
59p with CHD	Overweight or obesity (BMI $\geq$ 25kg/m <sup>2</sup> )	Diabetes mellitus	Hypertesion	Dyslipidemia	Smokers
	45p (76.3%)	24p (40.7%)	11p (18.6%)	29p (49.2%)	5p (8.5%)
Women 30p	24p (80%)	15p (59%)	5p (16.66%)	18 p(60%)	2p (6.7%)
Men 29p	21p (72.4%)	9p (31%)	6p(20.68%)	11 p(37%)	3p (10.3%)
Statistical significance	0.49 (NS)	0.14 (NS)	0.69 (NS)	0.09 (NS)	0.97 (NS)



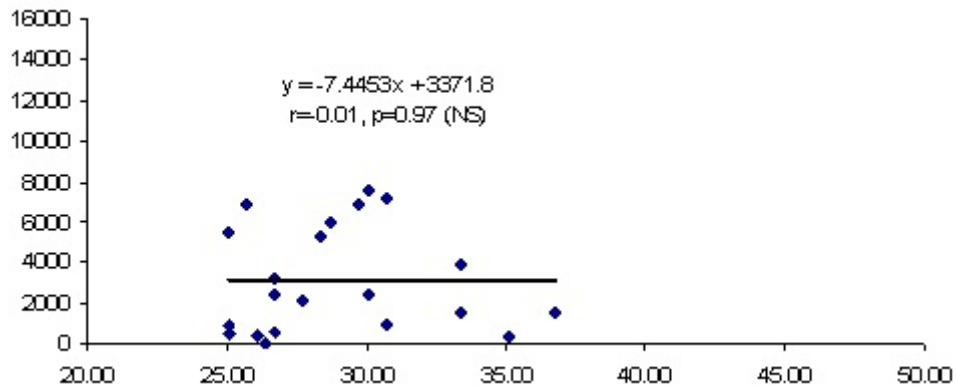
**Figure 1.** In patients with CHD correlation between plasma levels of leptin and BMI.



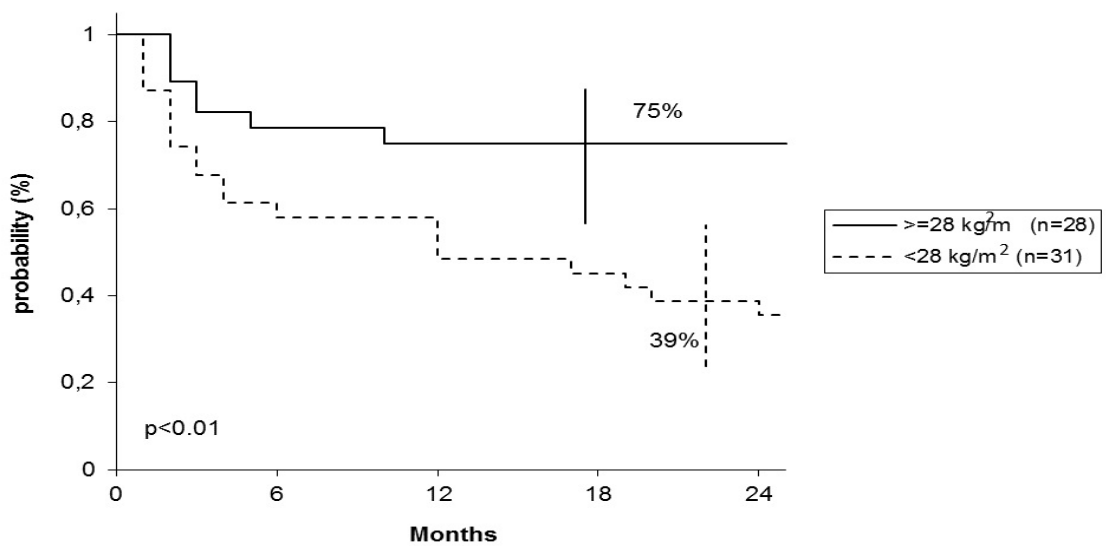
**Figure 2.** In women with CHD correlation between plasma levels of leptin (pg/ml) and BMI (kg/m<sup>2</sup>).



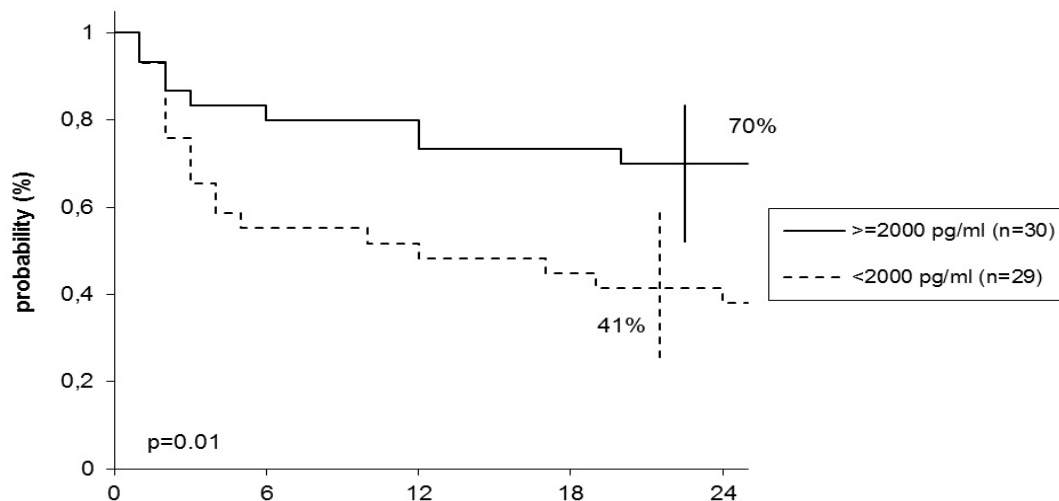
**Figure 3.** In women with CHD and BMI  $\geq 25$  kg/m<sup>2</sup> correlation between plasma levels of leptin (pg/ml) and BMI (kg/m<sup>2</sup>).



**Figure 4.** In men with CHD and BMI  $\geq 25$  kg/m<sup>2</sup> no correlation between plasma levels of leptin (pg/ml) and BMI (kg/m<sup>2</sup>).



**Figure 5.** Free time to re-hospitalization in relation to BMI.



**Figure 6.** Free time to re-hospitalization in relation to plasma levels of leptin.

### Discussion

In our study 73.6% patients with CHD were overweight or suffered of obesity. Only the plasma levels of leptin and BMI were related to prognosis in CHD patients. There were no significant differences between women and men regarding the number of rehospitalizations, rehospitalizations/patient, BMI, diabetes mellitus, hypertension or dyslipidemia.

Many authors have been reported on plasma levels of leptin in patients with atherosclerosis and obesity. Our study identified cut off values for plasma levels of leptin and BMI in relation to readmissions. Only 39% of patients with CHD with normal or overweight  $<28$  kg/m<sup>2</sup> had free survival rate as compared with 75% in those with overweight  $\geq 28$ kg/m<sup>2</sup> or mild obesity.

There were controversial data concerning the relationship between BMI and the prognosis of cardiovascular patients. Many studies reported an obesity paradox in CHD, suggesting that despite the fact that obesity increases the risk for developing CHD, just overweight and mild obesity do not seem to affect prognosis in patients with established CHD. Kenchaiah et al. [31] showed that during a 14-year follow-up, for every 1 kg/m<sup>2</sup> increment in BMI, the risk of HF increased by 5%-7%. Despite the known adverse effects of obesity on the CV functions and the epidemiologic data, many studies have suggested that the obese HF patients had a better prognosis.

An obesity paradox has been reported not only in CHD but also in hypertension, HF, cardiac surgery patients, PCI patients, heart valve patients, coronary artery bypass patients, and in atrial fibrillation (26,32-37). In a meta-analysis of 9 observational HF studies (an average of 2.7 years follow-up) Oreopoulos [36] showed that compared with individuals without elevated BMI, overweight and obese HF patients had reductions in CV and all-cause mortality.

The same observation was reported by Romero-Corral in a review of over 250,000 patients (3.8 years follow-up). The overweight and mild/moderate obese coronary artery disease patients have a lower risk for total and CV mortality compared with underweight and normal-weight patients. However, in patients with a BMI  $\geq 35$  kg/m<sup>2</sup> there was an excess risk for CV mortality without any increase in total mortality [37].

These investigators explained the better outcomes for CV and total mortality in overweight and mildly obese CHD patients by implicating the lack of discriminatory power of BMI to differentiate between body fat and lean mass [36]. Another explanation is the pre-existing illness with unintended weight loss and higher mortality in the groups with lower BMI [34].

Our data confirm the Oreopoulos and Romero-Corral meta-analysis. We found a cutoff value for BMI related to readmissions. Patients with overweight  $\geq 28$ kg/m<sup>2</sup> and mild obesity have a better short time prognosis estimated by readmission for cardiovascular events.

We found no significant differences between plasma levels of leptin in women and men. Although there were no differences between plasma levels of leptin and BMI in women and men, only in women plasma levels of leptin are correlated with BMI.

As compared with men with overweight/obesity, in women with overweight/obesity we found higher plasma levels of leptin and these levels were correlated with BMI. Despite these differences, patient gender could not be demonstrated to influence prognosis.

The gender-based differences in plasma levels of leptin were revealed by some studies. Leptin levels in healthy women are higher than in healthy men [11], but in men elevated plasma levels predict myocardial infarction [10].

We found that 2000 pg/ml is a cut-off level of plasma leptin correlated with prognosis. Among CHD patients with plasma levels of leptin >2000 pg/ml, 70% had free survival rate as compared with 41% in those with low levels (< 2000 pg/ml) suggesting a protective role of leptin. This effect was also observed in animal models [2]. The protective effect of leptin was explained by the fact that leptin is a regulator of non-esterified FFAs oxidation by peripheral tissues like heart and skeletal muscles, preventing the accumulation of the cytotoxic lipids, ceramides. The ceramides cause apoptosis on the cardiomyocytes [35].

In obesity, many studies have suggested that obese people are leptin resistant [6]. There are few data about the peripheral effects of leptin in obese individuals. It is possible that hyperleptinemia is not causally linked to atherogenesis but only reflects the state of leptin resistance [2]. Higher levels of leptin may represent a counter – regulatory response.

A similar “resistance” was described for adiponectin. Though adiponectin has direct protective effects on cardiomyocytes, in chronic HF increased levels of adiponectin were associated with a severe prognosis. In these circumstances the higher levels of adiponectin represent a regulatory response to compensate the heart [35,38,39].

### Conclusions

1. In our study 73.6% patients with CHD were overweight or suffered of obesity.
2. As compared with men plasma levels of leptin are significantly higher only in women with overweight and mild obesity.
3. Only in women plasma levels of leptin were correlated with BMI.
4. Despite these differences, patient gender could not be demonstrated to influence prognosis.
5. Patients with plasma levels of leptin >2000 pg/ml and BM  $\geq 28\text{kg/m}^2$  had a better prognosis, suggesting a protective role of leptin in overweight and mild obesity.

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