

Hyperleptinemia May Protect From Cardio-Vascular Complications: A Small Georgian Study

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Abstract

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Key words: Leptin; Anthropometric parameters; Body composition; Cardio-vascular risk factors; Lipid panel.

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List of Abbreviations: BMC – Bone Mineral Component; BMI – Body Mass Index; DXE – Dual Energy X-ray absorptiometry; HDL-C – High Density Lipoprotein Cholesterol; HOMA-IR – Homeostatic Model Assessment of Insulin Resistance; LDL-C – Low Density Lipoprotein Cholesterol; TG – Triglycerides; Total Chol – Total Cholesterol; WC – Waist Circumference.

BACKGROUND AND AIM: Leptin was assessed to play a coordinating role in obesity and its cardio-vascular complications, however the findings are conflicting and further clinical investigation is required. The aim of the present study was to evaluate the association of serum leptin with cardio-vascular risk factors in different body mass index and age groups.

MATERIALS AND METHODS: One hundred and forty nine female patients were enrolled in the study and divided into groups according to body mass index (BMI) and age. Following measurements were carried out: height, weight, BMI, waist circumference, blood pressure. Venous blood sample was obtained for plasma leptin, insulin, glucose and lipid profile analysis. Insulin resistance index was calculated for each patient. Body fat distribution was measured using Dual energy X-ray Absorptiometry.

RESULTS: The lowest leptin concentration was observed in overweight patients, the highest concentration was seen in obese patients. The difference between leptin levels were not observed in age groups. Leptin positively correlated with high density lipoprotein cholesterol levels in obese and elder patients.

CONCLUSION: Leptin might act as a preventive measure for cardiovascular complications only in the presence of sufficient amount of fat mass. Further studies are warranted in order to support these results.

Introduction

Obesity is an increasingly prevalent metabolic disorder affecting developed and developing countries. It contributes to abnormality in glucose metabolism, lipid profile and blood pressure [1].

The etiology of obesity is multifactorial, with the underlying causes including both lifestyle and genetic factors [2]. Obesity has long been considered as a behavioral and social disorder. The discovery of leptin in 1994 brought a complete revolution to the concept of obesity [3]. Leptin is a 16 kDa protein hormone and a cytokine, which is mainly synthesized and secreted by subcutaneous white adipose cells; its serum levels are directly proportional to the adipocyte mass [4]. Leptin stimulates energy expenditure and

inhibits appetite via the hypothalamus [5]. The importance of leptin was clearly demonstrated by individuals with congenital leptin deficiency. However, congenital leptin deficiency is a rare human genetic syndrome [6]. At present, the obese population is not characterized by leptin deficiency, but by hyperleptinemia, which is indicative of a leptin-resistant state [7].

Several experimental studies have shown that increased leptin may directly or indirectly exert multiple actions at the cardio-vascular level. Number of investigations has detected a significant association between circulating plasma leptin with insulin resistance and inflammatory markers, suggesting a possible role of leptin in development of cardiovascular disease. Some animal studies have revealed that specific high density lipoprotein

cholesterol (HDL-C) catabolic pathways are likely regulated by obesity and/or by leptin signaling; furthermore, serum leptin production in humans has been found to be related to arterial hypertension. These observations led to the hypothesis that leptin may play a coordinating role in obesity and may lead to cardio-vascular complications [8 – 12]. In a study conducted by Adami et al., the higher leptin concentration was associated with the higher serum HDL-C levels and lower cardiovascular risk [13]. Based on these conflicting findings, further clinical investigation is required to resolve this issue. Statistical data demonstrate a remarkable growth in the obesity rates among Georgian population. The aim of the present study is to investigate the role of leptin in pathophysiological consequences of human obesity, including cardiovascular disease. Specifically, we assessed the relationship between serum leptin concentration and cardiovascular risk parameters in the overweighted and obese patients.

Materials and Methods

Subject characteristics

Subject enrolment was conducted during one year (2010), at the National Institute of Endocrinology Clinic (Tbilisi, Georgia). The study was approved by the ethical committee of the National Institute of Endocrinology according to the declaration of Helsinki.

Inclusion criteria were: females, age from 20 to 70, informed written consent for participation in the study. Exclusion criteria were: alcohol or drug abuse, pregnancy, nursing (lactation), cardiac, respiratory, inflammatory or other endocrine diseases. Overall 149 female subjects were enrolled in the study.

In order to assess the relationship between serum leptin concentration and cardiovascular risk parameters, the leptin levels were determined in whole targeted population. Leptin levels were separately assessed in three different groups of weight and two different groups of age categories. The groups were defined as follows:

Weight category (according to body mass index – BMI): Group I – normal weight; Group II – overweight patients; and Group III – obese patients.

Age category: Group I – 20-40 year old patients; and Group II – 40-70 year old patients.

Following measurements were carried out for all participants: assessment of height, weight, body mass index (BMI), waist circumference and blood pressure. Venous blood samples were obtained to measure plasma levels of leptin, insulin and glucose, as well as for lipid profile analysis (including total cholesterol, triglycerides, high density lipoprotein

cholesterol and low density lipoprotein cholesterol). Insulin resistance index (HOMA IR) was calculated for each patient. Body fat distribution was measured using Dual energy X-ray Absorptiometry (DXA; GE Healthcare; Lunar Prodigy Primo).

Anthropometric measurements

Height and weight were evaluated in all subjects. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by square of height in meters (m²) and was expressed in kg/m². According to the world health organisation classification normal weight was defined as the BMI from 18.5 to 24.9 kg/m², overweight was specified as BMI from 25 to 29.9 kg/m² and obesity was defined as BMI > 30 kg/m² [14].

Waist circumference (WC) was measured as a midway between the iliac crest and the lower margin of the 12th rib, and was expressed in centimetres.

Blood Pressure

Unit of pressure was expressed in millimetres of mercury (mmHg). Blood pressure was measured after 5 - minute rest on the right arm, using a manual sphygmomanometer. After 2 minute interval the pressure was measured on the left arm. The arm with the highest blood pressure was used for another two measurements with 2 - minute intervals. The average value after 3 measurements on the same arm was used in analysis. All the measurements were done by a single operator (T. Z).

Biochemical Measurements

After a 10 – 12 - hour fasting period, venous blood samples were obtained between 09:00 a.m. and 11:00 a.m., and stored at 4°C. Blood plasma glucose and serum lipid profile [total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)] were estimated using a glucose oxidase-peroxidase and enzymatic methods, respectively. Leptin and insulin levels were determined by sandwich enzyme-linked immunosorbent assay (ELISA). Leptin was measured in nanograms per milliliter (ng/mL), and insulin was measured in milliunits per liter (mU/L). Insulin resistance index – HOMA-IR was calculated according to the formula: fasting insulin (mU/L) X fasting glucose (mmol/l) / 22.5 [15].

Body Composition

Body composition was assessed by dual-energy X-ray absorptiometry (DXA; GE Healthcare; Lunar Prodigy Primo). Measurements were conducted in the morning, while the subjects were still in fasting

regiment. We performed a whole-body DXA scan and analysis, which generates estimates of fat-free mass, fat mass and bone mineral content in each separate body regions – arms, legs, trunk, android and gynoid.

Statistical analysis

The data were presented as mean \pm standard deviation (SD). Differences between groups were measured using student's t-test. Pearson's correlation coefficient (r) was calculated to determine the relationship between study parameters. P-value < 0.05 was considered statistically significant for all analyses. Statistical analyses were performed using the SPSS 15.0 software package (SPSS, Inc., Chicago, IL).

Results

One hundred and forty nine female patients had been involved in research. Patients were subdivided according to BMI.

Group I consisted of subjects with normal weight (BMI 18.5 - 24.9) – 19 individuals (12.7%); group II was formed by 41 overweight (BMI 25 - 29.9) individuals (27.52%); group III included 89 obese (BMI 30 - 44.9) patients (59.7%).

Correlations between leptin and assessed parameters (weight, height, BMI, waist circumference, systolic and diastolic blood pressure, lipid profile, fasting glucose, insulin and insulin resistance index, total fat distribution and fat distribution in different regions of the body) were evaluated in the whole examined population, as well as among the groups divided according to BMI and age. The mean values

and standard deviation of the evaluated parameters are given in Table 1.

In the whole population group, leptin concentrations correlated with weight, BMI, waist circumference, systolic, diastolic arterial blood pressure, HDL-C, LDL-C, fasting glucose, HOMA-IR; Body composition parameters (fat tissue %, and distribution). Statistically significant correlations were estimated between leptin concentrations and weight, BMI, waist circumference, blood pressure, fat tissue mass%, fat tissue distribution: arms, legs, trunk, abdomen, android, gynoid (Fig. 1).

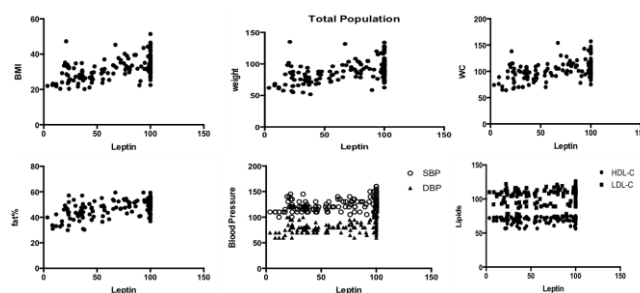


Figure 1: Positive correlations of leptin in total population. The figure shows correlation of leptin in total study population with BMI ($r = 0.53$, $p < 0.001$), weight ($r = 0.49$, $p < 0.001$), WC ($r = 0.47$, $p < 0.001$), total body fat% ($r = 0.55$, $p < 0.001$), systolic and diastolic blood pressure ($r = 0.36$, $p < 0.001$ and $r = 0.3$, $p < 0.001$, respectively), HDL-C and LDL-C ($r = 0.18$, $p < 0.05$ and $r = 0.23$, $p < 0.01$, respectively).

Correlation was changed in the examined sub-groups: in normal weight (I group) individuals leptin levels positively correlated with systolic blood pressure, fasting glucose level, and fat mass % (Fig. 2).

In group II individuals leptin levels positively correlated with BMI and LDL-C (Fig. 3).

Table 1: Mean values and standard deviation of the evaluated parameters.

Study Components	Mean \pm SD					
	Total Study Population n = 149	Groups Divided by BMI			Groups Divided by Age	
		Normal-Weight n = 19	Overweight n = 41	Obese n = 89	20 - 40 Year n = 119	40 - 70 Year n = 30
Age (year)	31.58 \pm 10.37	25.32 \pm 5.49	29 \pm 7.48	34.11 \pm 11.45	27.34 \pm 6	48.43 \pm 5.86
Weight (kg)	87.78 \pm 17.2	63.51 \pm 6.84	77.62 \pm 6.15	97.65 \pm 14.04	85.59 \pm 16.99	96.48 \pm 15.44
Height (cm)	165.9 \pm 5.7	166.21 \pm 5.93	167.68 \pm 5.18	165.01 \pm 5.74	166.11 \pm 5.58	165.07 \pm 6.19
BMI (kg/m ²)	31.86 \pm 6.39	22.57 \pm 1.39	27.5 \pm 1.52	35.86 \pm 4.86	30.98 \pm 6.25	35.37 \pm 5.80
WC (cm)	102.52 \pm 18.31	75.74 \pm 8.25	92.78 \pm 8.65	112.72 \pm 14.54	100.13 \pm 18.15	111.97 \pm 15.97
SBP (mmHg)	124.39 \pm 12.67	113.68 \pm 5.74	117.93 \pm 8.73	129.65 \pm 12.5	121.59 \pm 10.61	135.5 \pm 14.16
DBP (mmHg)	81.48 \pm 10.78	71.84 \pm 6.5	76.32 \pm 8.28	85.91 \pm 10.16	79.33 \pm 9.61	90 \pm 11.06
Leptin (ng/mL)	67.76 \pm 31.71	67.74 \pm 29.35	54.38 \pm 27.89	80.84 \pm 26.05	65.83 \pm 31.49	75.43 \pm 31.95
Insulin (mU/L)	13.87 \pm 7.83	9.51 \pm 4.54	12.48 \pm 4.67	15.09 \pm 8.91	13.39 \pm 7.45	15.72 \pm 9.14
Glucose (mg/dl)	93.47 \pm 10.10	89 \pm 7.22	93.80 \pm 8.82	94.27 \pm 10.97	92.34 \pm 9.04	97.93 \pm 12.73
HOMA-IR	3.27 \pm 2.2	2.07 \pm 0.99	2.9 \pm 1.13	3.6 \pm 2.56	3.05 \pm 1.76	4.1 \pm 3.34
Total Chol. (mg/dl)	206.14 \pm 20.6	199.57 \pm 15.29	200.88 \pm 14.98	209.97 \pm 22.95	203.04 \pm 18.18	218.47 \pm 24.98
TG (mg/dl)	195.42 \pm 22.75	188.13 \pm 18.68	190.47 \pm 25.31	199.25 \pm 21.68	192.73 \pm 21.77	206.08 \pm 23.80
HDL-C (mg/dl)	69.89 \pm 4.59	70.52 \pm 4.49	69.49 \pm 4.55	69.93 \pm 4.66	69.92 \pm 4.69	69.75 \pm 4.2
LDL-C (mg/dl)	105.17 \pm 10.64	101.74 \pm 10.76	102.67 \pm 11.92	107.08 \pm 9.64	103.66 \pm 10.79	111.1 \pm 7.66
Fat %	47.34 \pm 6.62	37.95 \pm 5.86	43.57 \pm 4.58	51.12 \pm 4.12	47 \pm 6.9	48.65 \pm 5.3
Fat (kg)	39.97 \pm 11.93	22.90 \pm 4.8	32.01 \pm 5.09	47.37 \pm 8.66	38.78 \pm 12.07	44.65 \pm 10.25
Lean (kg)	42.97 \pm 6.35	37.15 \pm 4.47	41.18 \pm 3.43	45.06 \pm 6.73	42.16 \pm 6.17	46.17 \pm 6.13
BMC (kg)	2.91 \pm 0.41	2.46 \pm 0.44	2.90 \pm 0.34	3.02 \pm 0.37	2.90 \pm 0.42	2.95 \pm 0.36
Arms (kg)	43.85 \pm 7.38	34.51 \pm 6.97	39.9 \pm 5.27	47.71 \pm 5.27	43.17 \pm 7.45	46.54 \pm 6.57
Legs (kg)	50.12 \pm 7.41	41.52 \pm 7.55	46.15 \pm 5.41	53.83 \pm 5.48	50.02 \pm 7.76	50.54 \pm 5.94
Trunk (kg)	48.24 \pm 6.88	38.29 \pm 5.88	44.74 \pm 5.33	52.01 \pm 4.25	47.83 \pm 7.08	49.85 \pm 5.87
Android (kg)	52.21 \pm 7.62	41.43 \pm 7.35	48.33 \pm 6.11	56.35 \pm 4.48	51.61 \pm 7.67	54.61 \pm 7.06
Gynoid (kg)	53.11 \pm 5.86	46.63 \pm 5.46	50.14 \pm 4.37	55.90 \pm 4.69	53.21 \pm 6.09	52.73 \pm 4.97

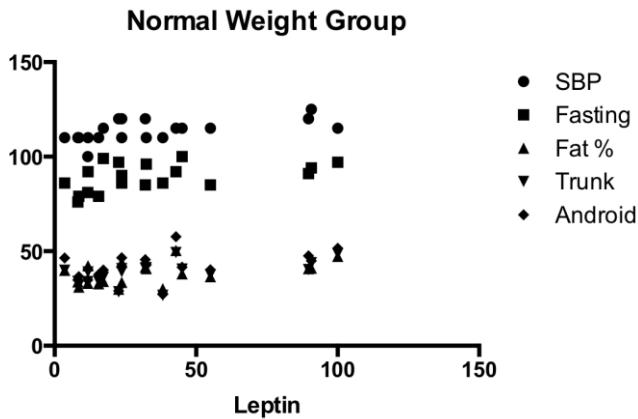


Figure 2: Positive correlations of leptin in a group of patients with normal weight. In normal weight group leptin positively correlation with systolic blood pressure ($r = 0.34, p < 0.01$), fasting plasma glucose ($r = 0.2, p = 0.005$), total body fat% ($r = 0.22, p = 0.004$), fat amount in trunk ($r = 0.28, p = 0.02$) and android fat ($r = 0.2, p = 0.05$).

In group III leptin levels positively correlated with BMI and HDL-C, fat mass %, fat distribution in arms, trunk and android regions (Fig. 4).

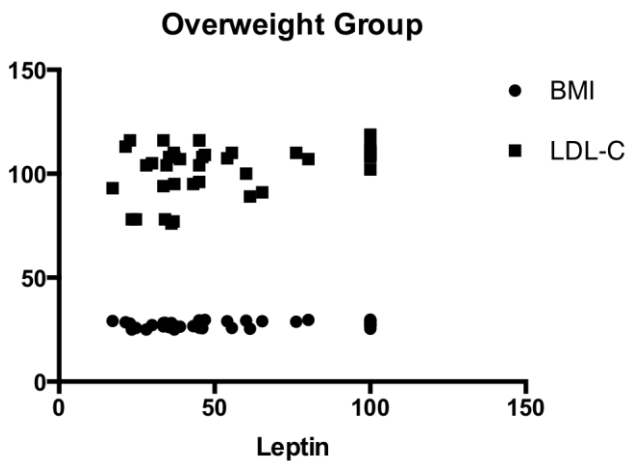


Figure 3: Positive correlations of leptin in overweight patients. In overweight study group leptin positively correlated with BMI ($r = 0.28, p = 0.05$) and LDL-C ($r = 0.37, p = 0.02$).

Analysis revealed lowest leptin concentrations in group II, and highest leptin concentrations in group III. The leptin concentration diversity had the highest statistical significance among the groups.

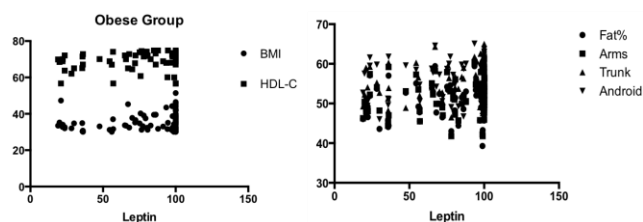


Figure 4: Positive correlations of leptin in obese patients. The correlation of leptin was positive with BMI ($r = 0.22, p = 0.04$), HDL-C ($r = 0.27, p = 0.01$), total body fat% ($r = 0.25, p = 0.01$), fat amount in arms ($r = 0.33, p < 0.001$), trunk ($r = 0.22, p = 0.03$) and android fat ($r = 0.31, p = 0.003$) in the study group consisted of obese patients.

According to the age division the group of subjects from 20 to 40 years consisted of 119 women and the group of subjects from 40 to 70 years consisted of 30 women.

Comparison of leptin levels in two different age groups revealed that older age was associated with higher leptin levels. However, according to the regression analysis, age is not an important determinant of leptin, when adjusted with the weight. Analysis also demonstrated that age positively correlated with weight.

Among the 20 - 40 year old age group, leptin concentration positively correlated with weight, BMI, waist circumference, systolic and diastolic blood pressure, fasting glucose level, LDL-C level, total fat mass, BMD, fat tissue distribution: arms, legs, trunk, android and gynoid (Fig. 5).

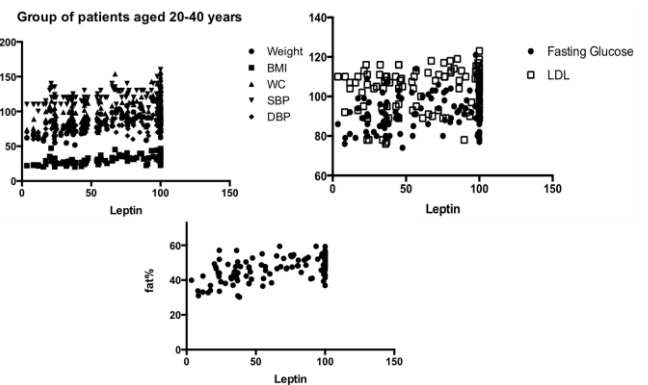


Figure 5: Positive correlations of leptin in a group of patients aged 20-40 years. In the study group consisted of patients aged 20-40 years leptin showed positive correlation with weight ($r = 0.48, p < 0.001$), BMI ($r = 0.53, p < 0.001$), WC ($r = 0.45, p < 0.001$), systolic and diastolic blood pressure ($r = 0.37, p < 0.001$ and $r = 0.33, p < 0.001$, respectively), fasting plasma glucose ($r = 0.24, p = 0.01$), LDL-C ($r = 0.21, p = 0.02$) and total body fat% ($r = 0.56, p < 0.001$).

In 40 - 70 year old patents, leptin positively correlated with weight, B.M.I., waist circumference, HDL-C, Total fat mass and fat tissue distribution: arms, legs, trunk, android, and gynoid (Fig. 6).

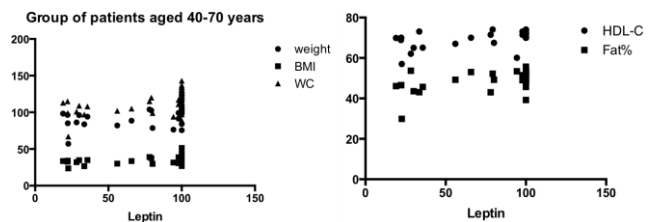


Figure 6: Positive correlations of leptin in a group of patients aged 40-70 years. In the group of patients aged 40-70 leptin positively correlated with weight ($r = 0.47, p = 0.01$), BMI ($r = 0.48, p = 0.01$), WC ($r = 0.49, p = 0.01$), HDL-C ($r = 0.53, p < 0.001$) and total body fat % ($r = 0.48, p = 0.01$).

Discussion

The aim of the present study was to assess the association of serum leptin with cardio-vascular risk parameters and to compare the levels of the investigated factors among the patient groups divided according to BMI and age.

Based on the obtained data, the main conclusions are the following: 1. Leptin concentration was highest in the obese group; 2. Leptin levels increase with the age; 3. Leptin expression positively correlates with HDL-C, in total population and in the groups of obese and 40 - 70 year old subjects; 4. The strongest positive correlation was detected between leptin and body fat mass in every region of the body, mostly in the total study population and in the group of obese subjects.

Levels of leptin increased with the age, which is consistent with previous reports by Andreasson et al. and others, and can be explained by the increase in BMI [16].

Our study revealed a correlation between serum leptin and anthropometric characteristics in the total population, but when the population was divided into groups (according to BMI), the correlation was changed and positive correlation was seen only in the groups of overweight and obese subjects. Our results are in line with Liuzzi A *et al.* and Adami GF *et al.*, who also showed that leptin appeared to positively correlate with BMI in all age groups [13, 17]. In our study the correlation of leptin with BMI was observed in both age groups. Similar results, regarding highly significant correlation between serum leptin and BMI, were demonstrated in the study performed by Al-Shoumer KA et al [18]. Concerning the correlation of leptin with WC had been observed only in total study population and also in both age groups (Fig-s. 1, 5, 6).

Along with the assessment of body weight and BMI characterizing the state of obesity of an individual, we also evaluated fatty tissue, fat free mass and BMC. A detailed evaluation of these body composition components is important.

In the present study, leptin showed most significant and positive correlation with body fat distribution. Correlation of serum leptin and lean mass, as well as BMC, was not detected (Fig. 2). Correlation of leptin with fat mass and fat distribution in regions was positive in total study population. In overweight patients the correlation was not observed. In patients with normal weight and obesity leptin correlated with total fat mass and fat mass in trunk and abdomen (Fig. 3). Our data are in line with the study conducted by Garcia-Lorda P *et al.*, although their study was conducted only on females [19].

The present study confirms the strong association of total adiposity with leptin. Our results indicate that total percentage of fat had the same

correlation with leptin concentration as total body fat mass.

The association of leptin with blood pressure was seen in the total population and 20 - 40 year old subjects. Obesity is associated with risk of high blood pressure (Fig. 4). Several studies confirm that leptin increases the activity of sympathetic nervous system, though contribution to elevated blood pressure. Differences in leptin transport across the blood-brain barrier may exist between men and women [20]. As a result, women's sympathetic nervous system may have a weaker response to leptin than men's. If this is the case, women could be more protected against leptin-induced high blood pressure, as compared to men [21]. In the study, conducted by Adreasson et al., women showed lower associations between high levels of leptin and CVD risk factors [16].

Our results are in agreement with the results obtained in the study conducted by Alvarez-Aguilar C et al. They suggest that hyperleptinemia has a direct role in the physiopathologic mechanism of obesity-associated high blood pressure [22].

Our study shows that leptin levels correlate with fasting glucose and HOMA-IR, only in the total study population. Leptin positively correlated with fasting glucose in 20 - 40 year old patients.

Low and high-density lipoproteins are major determinants of cholesterol transporter in human plasma. The pre-atherogenic LDL-C and HDL-C are high biological predictors of cardiovascular disease. In our study leptin positively correlated with HDL-C in total study population and in the group of patients with obesity (Fig. 5). Similar results are shown in Adami GF *et al.*, where leptin appeared to positively correlate with HDL-C. In line with the Adami et al. results, our data showed that higher the leptin concentration associated with higher serum HDL-C level [13]. HDL-C is considered as the single major factor for predicting the risk of atherosclerosis and coronary artery disease [23]. A rise in HDL-C reduces the incidence of coronary artery disease. The positive relationship between leptin and HDL-C in obese and 40 - 70 year old patients shows that increased leptin in obese and elder patients increase HDL-C, thus decreasing the incidence of coronary disease in these patients. Leptin showed positive correlation with both, LDL-C and HDL-C, in total study population. In patients with normal weight leptin had no correlation with either of the cholesterol transporters. In overweight and 20 - 40 year old patients the positive correlation was seen only with LDL-C (Fig. 5). This phenomenon could be explained by theory that increased levels of leptin might act as protective mechanism against cardio-vascular disease in obese and aged patients. Our results are in conflict with major studies done in these filed. According to the results of some studies, leptin may protect from cardiovascular complications, most likely due to its association with peripheral, but not central body fat

distribution [12, 24]. Furthermore, several studies detected a complete lack of relationship between serum leptin and HDL-cholesterol concentration in normal weight individuals [25 – 27]. We hypothesize that leptin might act as a preventive measure for cardiovascular complications only in the presence of sufficient amount of fat mass. However, further investigation is needed in order to support these results.

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