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Relationship of endogenous hyperleptinemia to serum paraoxonase 1, cholesteryl ester transfer protein, and lecithin cholesterol acyltransferase in obese individuals

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Abstract

Altered activities of high-density lipoprotein (HDL)–associated antioxidant enzyme paraoxonase 1 (PON1) and lipid transfer proteins, for example, cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyltransferase (LCAT), participating in lipoprotein remodeling seem to play important roles in obesity-related accelerated atherosclerosis. Inverse associations of PON1 with obesity and serum leptin levels have been demonstrated. However, the relationship of leptin with CETP and LCAT in humans is less clear. Our aims were to investigate whether the elevated leptin level is (*a*) an independent predictor of low PON1 and (*b*) associated with alterations of CETP and LCAT activities. Seventy-four white subjects forming 3 age- and sex-matched groups were included into the study (groups 1 and 2: nondiabetic obese patients, n = 25 with body mass index [BMI] 28-39.9 kg/m^2 and n = 25 with BMI ≥40 kg/m^2, respectively; and group 3: 24 healthy, normal-weight control subjects). Paraoxonase 1 correlated inversely with BMI ($r = -0.39$, $P < .01$), waist circumferences ($r = -0.42$, $P < .001$), and leptin concentrations ($r = -0.38$, $P < .001$). However, in a multiple regression model, neither these variables nor others, for example, age, sex, blood pressure, insulin resistance (in homeostasis model assessment of insulin resistance [HOMA-IR]), HDL cholesterol, low-density lipoprotein cholesterol, or lipid peroxidation (measured as thiobarbituric acid reactive substances), proved to be independent predictors of PON1. Lecithin cholesterol acyltransferase correlated negatively with BMI ($r = -0.40$, $P < .01$), waist circumferences ($r = -0.42$, $P < .001$), and leptin levels ($r = -0.40$, $P < .01$). During multiple regression analyses, BMI was an independent predictor of LCAT after adjustments for age, sex, HOMA-IR, and HDL cholesterol. However, this was replaced by leptin and HOMA-IR when leptin was also included into the model. The CETP activities correlated with HOMA-IR ($r = 0.33$, $P < .01$), thiobarbituric acid reactive substances ($r = 0.45$, $P < .001$), and leptin ($r = 0.36$, $P < .01$) levels in univariate but not in multivariate models. Elevated leptin level is an independent predictor of low LCAT, but not PON1, activity. In a population with a wide range of BMI, LCAT correlates inversely with obesity and CETP directly with insulin resistance.

1. Introduction

Leptin, a hormone secreted by adipose tissue, decreases food intake via the neuroendocrine system in the hypothalamus. Serum leptin concentration is proportional to adipose tissue mass, is increased in obesity, and correlates well with other constituents of the metabolic syndrome, namely, insulin resistance, dyslipidemia, and hypertension [1]. Hyperleptinemia itself has also been suggested to be involved in the pathogenesis of obesity-related disorders, such as arterial hypertension and atherosclerosis; but its exact role in the pathogenesis of these processes is currently still far from unequivocal. The potential proatherosclerotic effect of the leptin may involve stimulation of sympathetic

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activity, endothelial production of endothelin-1 and reactive oxygen species, proinflammatory immune response, migration, and proliferation of vascular smooth muscle cells, and calcification of vascular cells [2]. In some large human studies, the high plasma leptin level correlated with the risk of coronary artery disease [3], but not in the Quebec Cardiovascular Study [4].

High-density lipoprotein (HDL) possesses antioxidative characteristics in which one of its associated enzymes, the paraoxonase 1 (PON1), plays an outstanding role [5] by protecting low-density lipoprotein (LDL) from oxidative modifications [6,7]. The PON1 activities are reduced, among others, in diabetes mellitus [8,9] and ischemic cardiac disease [10]. Inverse associations of PON1 with obesity and serum leptin levels have been demonstrated [11-13]. Beltowski et al [11] found that exogenous leptin administration in rats markedly decreased plasma PON activity and induced oxidative stress, suggesting that these mechanisms may be involved in atherogenesis in hyperleptinemic obese individuals. According to this, Ferretti et al [12] have recently demonstrated that PON1 activity was significantly lower in obese subjects as compared with controls and that plasma levels of leptin correlated negatively with PON1 activity. Uzun et al [13] have also shown an inverse association between serum leptin levels and PON1 activity in morbidly obese patients after surgical intervention. Leptin level is also elevated in chronic renal failure [14], where we earlier found reduced PON1 activity [15]. Therefore, it seemed plausible that elevated leptin level is responsible for low PON1 activity among these patients. However, in our recently published study [16] carried out in hemodialysis patients, no negative correlation could be demonstrated between serum leptin concentrations and PON1 activities, suggesting that other mechanisms might lead to decreased PON1 activity in chronic renal failure as in obesity-related cases.

Both cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyltransferase (LCAT) participate in HDL remodeling and reverse cholesterol transport; therefore, they may play important roles in atherosclerosis [17]. Disturbed activities of these enzymes may contribute to alterations in lipoprotein metabolism typically seen in insulin resistance and type 2 diabetes mellitus [18]. The enhanced CETP-mediated exchange of very low-density lipoprotein–triacylglycerols for LDL and HDL cholesteryl esters in insulin resistance and type 2 diabetes mellitus increases the formation of highly atherogenic small dense LDL, as well as the catabolism of HDL [19]. However, the relationship of leptin with CETP and LCAT in humans is not properly clarified.

Our aims in this present study were to test whether the elevated leptin level in obese subjects is (*a*) an independent predictor of low PON1 and (*b*) associated with alterations of CETP and LCAT activities.

2. Subjects and methods

2.1. Participants

The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and approved by the ethics committee at the Medical and Health Science Center, University of Debrecen. Subjects participated in the study after their written informed consent was obtained, and their basic characteristics are shown in Table 1. The study was performed according to the requirements of the ethics committee of the Medical and Health Science Center, University of Debrecen.

This is a cross-sectional study for which 50 (30 female and 20 male) ambulatory overweight-obese participants (body mass index [BMI] exceeding 28 kg/m^2) aged 35.7 ±

Table 1
Anthropometric and selected laboratory characteristics in studied subjects

BMI	≥40	28-39.9	20-24.9
n	25	25	24
Age (y)	37.0 ± 10.4	37.1 ± 11.4	38.4 ± 9.9
Female/Male	15/10	15/10	14/10
BMI (kg/m^2)	45.8 ± 5.3[b,c]	34.0 ± 2.8[b,c]	22.4 ± 1.7
Waist (cm)	128.5 ± 17.9[b,c]	108.3 ± 11.6[b,c]	79 ± 7.5
Systolic blood pressure (mm Hg)	137 ± 10.6[b]	137 ± 14.8[b]	112 ± 10.8
Diastolic blood pressure (mm Hg)	86 ± 10.0[b]	85 ± 7.8[b]	71 ± 8.0
Uric acid (μmol/L)	338 ± 104[b]	328 ± 72[b]	250 ± 64
Fasting plasma glucose (mmol/L)[a]	5.5 (4.7/5.6)[b]	5.1 (4.8/5.3)[b]	4.4 (4/4.7)
HbA_{1C} (%)[a]	5.6 (5.4/6.2)	5.6 (5.4/5.9)	5.45 (5.3/5.7)
Fasting plasma insulin (mU/L)[a]	23.7 (19.0/27.7)	17.3 (14.5/30.6)	16.4 (14.9/20.0)
HOMA-IR[a]	5.16 (3.9/6.7)[b]	3.92 (3.1/7.2)	3.22 (2.64/4.14)
hsCRP (mg/L)	9.6 ± 6.4	5.25 ± 6.2	1.4 ± 1.5
TG (mmol/L)[a]	1.70 (1.1/2.3)[b]	1.47 (1.2/1.9)[b]	0.89 (0.72/1.26)
Total cholesterol (mmol/L)[a]	5.38 (4.6/5.7)[b]	5.40 (5.0/5.75)[b]	4.86 (4.3/4.5)
LDL-C (mmol/L)[a]	3.30 (2.9/3.7)[b]	3.40 (2.9/3.5)[b]	2.74 (2.2/3.35)
HDL-C (mmol/L)	1.17 ± 0.24[b]	1.23 ± 0.28[b]	1.7 ± 0.53
NEFA (mmol/L)	0.48 ± 0.19	0.53 ± 0.22	0.26 ± 0.14
PON1 activity (U/L)[a]	62.9 (57/72)[b]	63.5 (54/91)[b]	100.3 (77/113)
CETP activity (U/L)	170.0 ± 28.5[b]	174.6 ± 33.0[b]	150 ± 31.5
LCAT activity (U/L)	47.1 ± 8.2[b]	48.6 ± 10.0[b]	59.6 ± 11.3
TBARS (μmol/L)[a]	1.23 (1.2/1.5)[b]	1.18 (1.1/1.3)[b]	0.27 (0.21/0.36)
sE-Selectin (ng/mL)[a]	47.2 (36.2/58.3)	46.2 (22.0/59.2)	35.4 (26.0/51.0)
Leptin (ng/mL)[a]	40.4 (20.6/64.3)[b]	37.15 (19.8/55.8)[b]	2.55 (1.38/3.58)

In case of normal distribution, data are means ± SD.

[a] In case of non-normal distribution, data are median (lower/upper quartile).

[b] Significant differences between normal and obese BMI groups.

[c] Significant differences between the 2 obese groups.

10.9 years (range, 18-55 years) were selected from 18- to 65-year-old white people attending our obesity clinic. All subjects were in good general condition. The recruitment of participants was performed so that 2 BMI groups with similar age and sex distributions were formed, that is, BMI $\geq$40 kg/m^2 and BMI between 28 and 39.9 kg/m^2, respectively. Exclusion criteria were the presence of a significant endocrine disorder, active liver disease, or hepatic dysfunction defined as alanine aminotransferase or aspartate aminotransferase >1.5 times the upper limit of the reference range; renal disease; malignancy; diabetes mellitus; coronary artery disease; blood pressure >160/100 mm Hg; cerebral vascular disease; smoking; triglyceride (TG) level >4.5 mmol/L; alcoholism; drug dependence; infectious disease; significant inflammation; pregnancy or lactation; or anticoagulant, lipid-lowering, glucocorticoid, oral contraceptive, or sex hormone replacement medication. Twenty-three patients had hypertension. One of the women was in postmenopausal state (classified by the combination of age and lack of periods for more than 6 months).

Twenty-four age- and sex-matched healthy volunteers (14 and 10 women and men, respectively) with BMI of 20 to 24.9 kg/m^2 (mean, 22.4 ± 1.7 kg/m^2) were recruited to serve as a control group. Their mean age was 38.4 ± 9.9 years (range, 26-58 years). *Healthy subjects* were defined as individuals without known major disease and/or complaints, with a blood pressure less than 140/90 mm Hg, and with normal laboratory test results, including renal and liver function and fasting plasma glucose levels. None of them (*a*) had hyperlipidemia or (*b*) took medication at the time of the study.

Height and weight were measured while the subjects wore indoor clothes and shoes. The BMI was calculated as weight (in kilograms)/[height (in meters)]^2. The waist measurement was taken in standing position as the narrowest circumference midway between the lower border of the ribs and upper border of the iliac crest. Systolic and diastolic blood pressures (BPs) were measured twice with the subject in the sitting position after resting for at least 5 minutes using a quality-approved automatic electronic sphygmomanometer.

2.2. Biochemical analyses

Blood specimens were obtained after overnight fast. For routine automated laboratory analyses, Cobas Integra 700 Autoanalyzer was used (Roche, Basel, Switzerland), applying hexokinase method for serum glucose, uricase-PAP (peroxidase–antiperoxidase complex) method for uric acid, cholesterol esterase/oxidase-PAP method for total cholesterol, and glycerol-phosphate-oxidase-PAP method for TG determination. High-sensitivity C-reactive protein (hsCRP) was measured by turbidimetric immunoassay (Roche), whereas HDL cholesterol (HDL-C) was measured by direct method. Low-density lipoprotein cholesterol (LDL-C) level was estimated using the Friedewald formula. Hemoglobin A_{1C} (HbA_{1C}) (percentage) was measured by high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA). The serum concentration of insulin was measured by a commercially available radioimmunoassay kit (MP Biomedicas, Orangeburg, NY) with intra- and interassay coefficients of variance (CVs) ranging from 4.2% to 8.2% and from 6.4% to 8.8%, respectively. The insulin resistance index calculation was based on the homeostasis model assessment of insulin resistance (HOMA-IR) [20]. The plasma concentration of nonesterified fatty acid (NEFA) was measured by optimized enzymatic colorimetric assay (Roche Diagnostics, Penzberg, Germany). Serum concentration of leptin was measured by competitive enzyme immunoassay (WAK-Chemie Medical, Bad Soden, Germany) that had intra- and interassay CVs ranging from 4.2% to 5.4% and from 3.6% to 8.6%, respectively. Serum concentrations of soluble E-selectin (sE-selectin) were measured by sandwich enzyme immunoassays (Quantikine; R&D Systems, Minneapolis, MN) with intraassay CVs ranging from 4.7% to 5.0% and interassay CVs ranging from 5.7% to 8.8%.

Paraoxonase 1 activity was measured in an assay that contained 1 mmol/L phenylacetate in 20 mmol/L Tris-HCl (pH 8.0). The reaction was started by the addition of the serum, and the increase in absorbance was read at 270 nm using a Hewlett-Packard 8453 UV-Visible Spectrophotometer (Hewlett-Packard GmbH, Waldbronn, Germany). Blanks were included to correct the spontaneous hydrolysis of substrate. Enzyme activity was calculated using a molar extinction coefficient of 1310 $(mol/L)^{-1}$ cm^{-1}. *One unit* (U) is defined as 1 μmol substrate hydrolyzed per minute.

The CETP and LCAT activities were determined by commercially available kits from Roar Biomedical (New York, NY). The CETP assay included donor (synthetic phospholipid and cholesteryl ester) and acceptor (very low-density lipoprotein) particles. Ten microliters of plasma was diluted in 90 μL of sample buffer (10 mmol/L Tris, 150 mmol/L NaCl, and 2 mmol/L EDTA [pH 7.4]). In a low-fluorescence microtiter plate (black), 20 μL of the plasma dilution was combined with 4 μL of donor and 4 μL of acceptor in a total volume of 200 μL and incubated for 3 hours at 37°C. The assay was read in a fluorescence microtiter plate reader (Victor 1420 Multilabel Counter, excitation 465 nm/emission 535 nm; Perkin Elmer Life & Analytical Sciences, Shelton, CT). Activity of LCAT that mediates the formation of cholesteryl esters in human plasma by transferring an acyl chain from the sn-2 position of phosphatidylcholine to cholesterol was measured with incubation of plasma and fluorescently labeled phosphatidylcholine. The intact substrate fluoresces at 470 nm. During hydrolysis by LCAT, the monomer emission at 390 nm increases. The LCAT activity was assessed as a change in 470/390 nm emission intensity measured in spectrofluorometer (Hitachi F-4500; Hitachi, Tokyo, Japan).The intraassay and interassay CVs were <3% in all the 3 tests.

Lipid peroxidation was evaluated by the measurement of plasma levels of thiobarbituric acid reactive substances

Table 2
Pearson correlation coefficients between selected variables in the whole investigated population

	BMI	BPS	HOMA	TG	HDL-C	LDL-C	PON	CETP	LCAT	TBARS
BPS	0.64 **									
HOMA [a]	0.30 *	0.29 *								
TG [a]	0.40 ***	0.51 ***	0.32 **							
HDL-C	−0.53 **	−0.43 ***	−0.10	−0.51 ***						
LDL-C [a]	0.29 *	0.31 *	0.13	0.31 **	−0.37 **					
PON [a]	−0.39 **	−0.32 **	−0.25 *	−0.22	0.28 *	−0.10				
CETP	0.22	0.18	0.33 **	0.21	−0.04	0.15	−0.21			
LCAT	−0.40 **	−0.32 **	0.01	−0.22	0.10	−0.26 *	0.26 *	−0.11		
TBARS [a]	0.73 ***	0.74 ***	0.53 ***	0.41 **	−0.44 ***	0.41 **	−0.40 **	0.45 ***	−0.44 ***	
Leptin	0.76 ****	0.66 ***	0.53 ***	0.51 ***	−0.39 ***	0.28 *	−0.38 ***	0.36 **	−0.43 ***	0.85 ****

BPS indicates systolic blood pressure.
[a] Log-transformed statistics.
* $P < .05$.
** $P < .01$.
*** $P < .001$.
**** $P < .0001$.

(TBARSs). The latter was measured as we described previously [21]. Briefly, assay was done shortly after blood taking; 300 μL of plasma was added to 600 μL of acidic thiobarbituric acid reagent. The samples were heated at 100°C for 15 minutes; and after cooling, the TBARS was extracted by 3 mL of butanol. The absorbance of extract was recorded at 532 nm, and the results were calculated using the extinction coefficient of 1.56×10^5 $(mol/L)^{-1}$ cm^{-1}.

2.3. Statistical analysis

Statistical analyses were performed using the 11.0 software of the SPSS (SPSS, Chicago, IL). Normality of

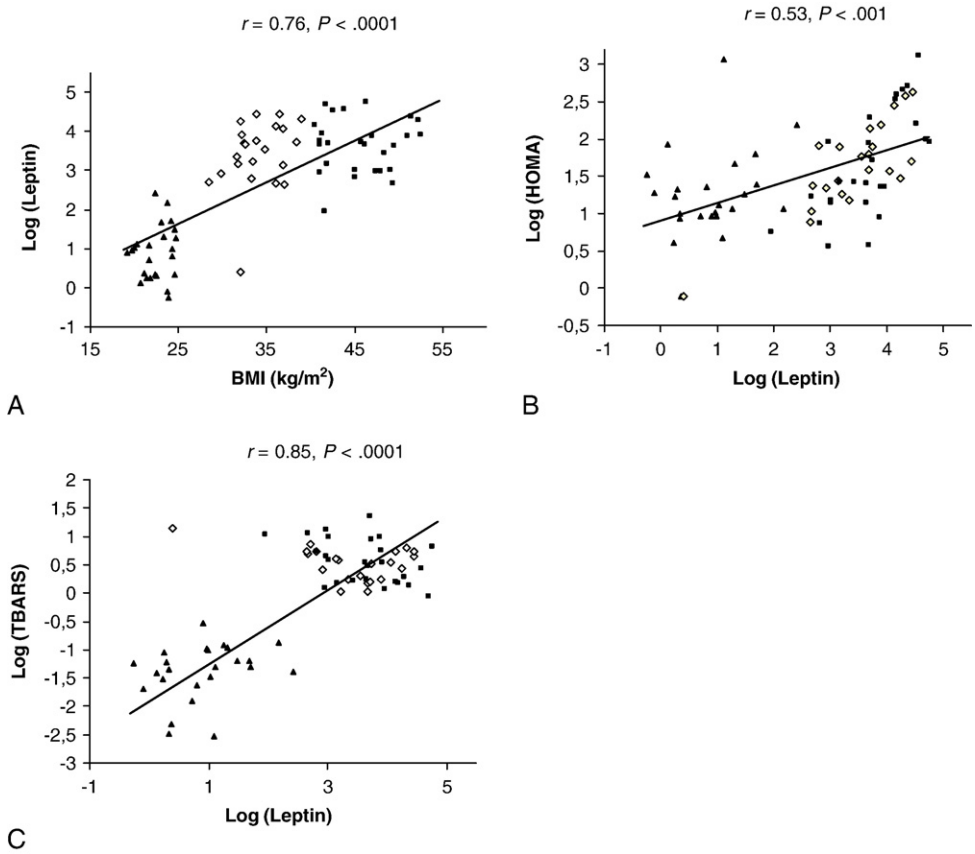

Fig. 1. Relationships between leptin levels and selected other relevant variables. Log-transformed leptin concentrations are correlated with BMI (A), log-transformed HOMA-IR (B), and log-transformed TBARS (C).

distribution of data was tested by Kolmogorov-Smirnov test. Non-normally distributed parameters were transformed logarithmically to correct their skewed distributions. Differences between anthropometry and laboratory characteristics across various BMI groups were tested with 1-way analysis of variance, and Student *t* test was used to compare male and female subpopulations. Correlations between continuous variables were assessed by calculation of linear regression using Pearson test. Multiple regression analyses were performed to determine the variables that best predicted PON1, CETP, and LCAT activities. If BMI turned out to be an independent predictor of an enzyme activity, we also tested this relationship in additional models either containing leptin level or not. The reason for this was that a dependent variable (eg, leptin) closely related to another (eg, BMI) may neutralize the latter's effect in the model. Data were expressed as means ± SD in case of normal distribution and median (lower/upper quartile) in case of non-normal distribution.

3. Results

Anthropometric and laboratory characteristics of subjects with BMI ≥40 kg/m^2 and BMI of 28 to 39.9 kg/m^2, respectively, as well as of lean controls are shown in Table 1.

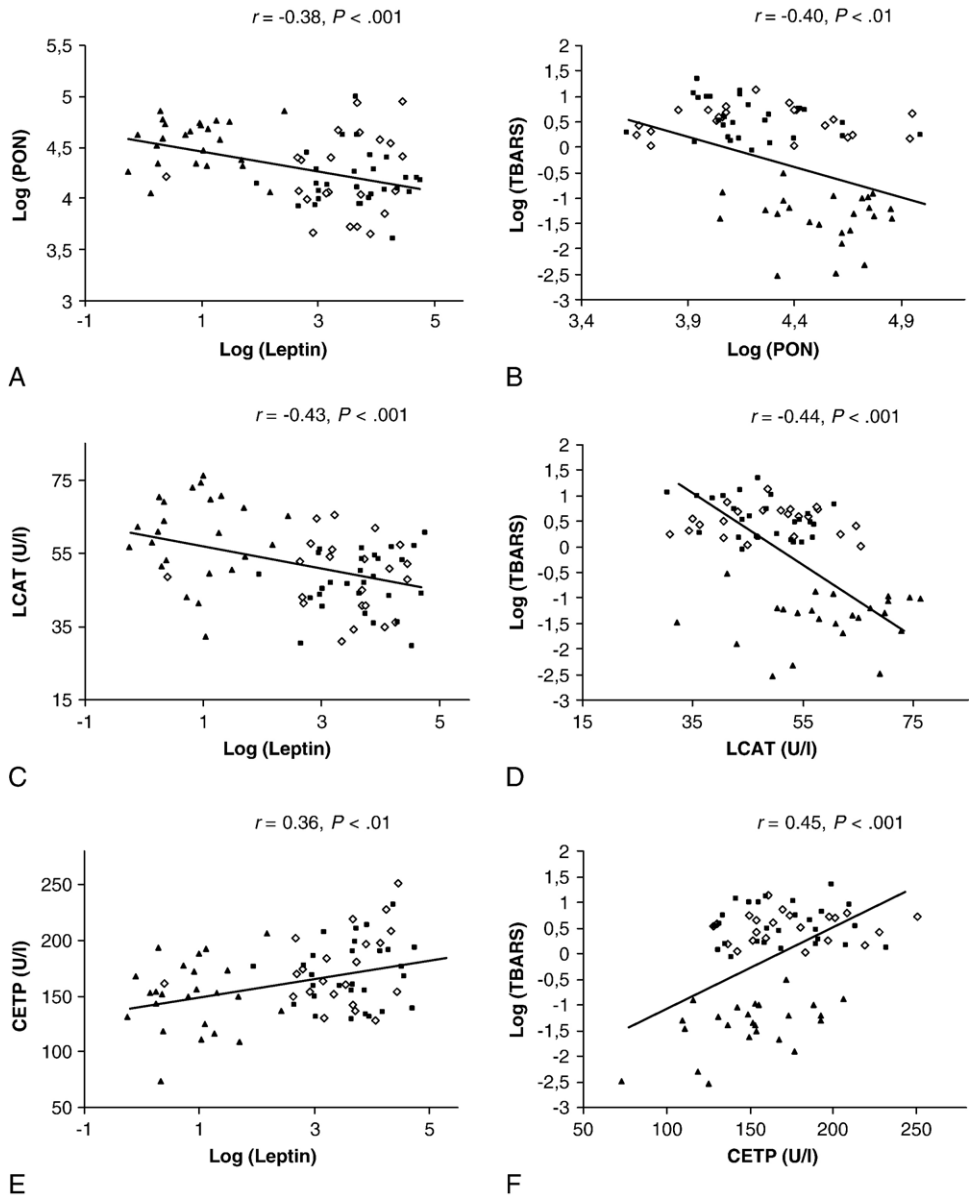

Fig. 2. Relationships between leptin and TBARS levels, and PON1, LCAT, and CETP activities. Log-transformed leptin is inversely related to log-transformed PON1 (A) and LCAT (C) and directly related to CETP (E). Log-transformed TBARS is inversely related to log-transformed PON1 (B) and LCAT (D) and directly related to CETP (F).

Among overweight-obese subjects, the BMI had a wide range, from 28 to 62 kg/m^2, with a mean of 40.6 ± 7.35 kg/m^2. The following parameters were significantly higher in the obese groups as compared with normal controls: systolic and diastolic BPs, uric acid, blood glucose, insulin, HOMA-IR, TG, total and LDL-C, TBARS, leptin levels, and CETP activities, whereas HDL-C levels and PON1 and LCAT activities were significantly lower.

3.1. Univariate correlations

The following parameters had to be transformed logarithmically to approximate normal distributions: blood glucose, HbA_{1C}, insulin, HOMA-IR, TG, total and LDL-C, PON1 activity, TBARS, sE-selectin, and leptin. Values of $P < .05$ were considered statistically significant, but only those associations that have $P < .01$ were regarded as having major importance because multiple comparisons were carried out.

Results of Pearson correlation analysis between selected variables are shown in Table 2, with scatter plots in Figs. 1 and 2, respectively. Besides these associations, the following highly significant correlations merit highlighting: in case of leptin level, waist circumferences ($r = 0.67$, $P < .0001$), diastolic BP ($r = 0.55$, $P < .0001$), glucose ($r = 0.54$, $P < .0001$), and insulin ($r = 0.46$, $P < .0001$); in case of PON1 activities, waist circumferences ($r = -0.42$, $P < .001$) and hsCRP ($r = -0.33$, $P < .01$). The relationship between leptin and PON1 was also evaluated in male and female subjects separately, and stronger correlation was found among men ($r = -0.50$, $P < .01$) than among women ($r = -0.28$, $P = .06$). The LCAT activities were negatively correlated with BMI, waist circumferences ($r = -0.34$, $P < .01$), systolic BP, uric acid ($r = -0.23$, $P < .05$), hsCRP ($r = -0.27$, $P < .05$), LDL-C ($r = -0.26$, $P < .05$), NEFA ($r = -0.46$, $P < .0001$), and leptin, whereas a negative trend with glucose ($r = -0.19$, $P = .10$) was observed.

The CETP activities were correlated significantly with diastolic BP ($r = 0.24$, $P < .05$), levels of glucose ($r = 0.34$, $P < .01$), insulin ($r = 0.24$, $P < .05$), HOMA-IR, NEFA ($r = 0.28$, $P < .05$), TBARS, and leptin, and displayed a trend to higher BMI, too, but did not reach significant level ($r = 0.22$, $P = .06$).

Soluble E-selectin was correlated negatively with HDL-C ($r = -0.39$, $P < .001$) and positively with diastolic BP ($r = 0.29$, $P < .05$), uric acid ($r = 0.37$, $P < .01$), and TG ($r = 0.25$, $P < .05$). Soluble E-selectin also displayed a trend to higher waist circumferences ($r = 0.20$, $P = .10$) and systolic BP (0.21, $P = .09$), but did not reach significant levels. Soluble E-selectin had no associations with the other investigated parameters.

3.2. Multivariate correlations

To test whether the associations of leptin with PON1, CETP, and LCAT existing in the univariate analysis were independent of anthropometric and other laboratory parameters, we carried out multiple regression analyses. During those 2 tests, where PON1 and CETP were the dependent variables, none of the other tested ones turned out to be independent predictors (Tables 3, 4). In contrast, BMI was an independent predictor of LCAT activity after adjusting for age, sex, HOMA-IR, and HDL-C (Table 5, model 1). However, when leptin was included in the model, leptin together with HOMA-IR replaced BMI as an independent predictor (Table 5, model 2), showing that these variables are more strongly related to LCAT activity than merely the BMI.

Table 3
Multiple regression analysis for PON-1 as a dependent variable (model $R^2 = 0.227$)

Independent variable	Regression coefficient	SE of regression coefficient	t	P
Intercept	5.18	0.79	**6.52**	**<.001**
Age	0.0005	0.005	0.10	.992
Sex	−0.056	0.125	−0.452	.654
BMI	0.001	0.009	−0.107	.915
Systolic BP	0.001	0.005	−0.359	.722
HOMA-IR	−0.192	0.105	−1.823	.75
LDL-C	−0.033	0.144	−0.229	.820
HDL-C	−0.007	0.144	0.54	.958
TBARS	−0.123	0.099	−1.24	.223
Leptin	0.052	0.075	0.686	.497

Significant value indicated in bold.

Table 4
Multiple regression analysis for CETP as a dependent variable (model $R^2 = 0.194$)

Independent variable	Regression coefficient	SE of regression coefficient	t	P
Intercept	131.60	27.45	**4.79**	**<.001**
Age	−0.43	0.37	−1.18	.24
Sex	9.34	8.75	−1.07	.29
BMI	−0.11	0.60	−0.18	.86
HOMA-IR	9.51	6.66	1.43	.16
HDL-C	5.42	10.75	0.51	.62
Leptin	5.9	4.77	1.24	.22

Significant value indicated in bold.

Table 5
Multiple regression analysis for LCAT as a dependent variable

Independent variable	Model 1 ($R^2 = 0.229$)		Model 2 ($R^2 = 0.293$)	
	t	P	t	P
Intercept	8.70	**<.001**	**7.50**	**<.001**
Age	−0.033	.97	−0.25	.80
Sex	−1.08	.28	0.016	.99
BMI	**−3.97**	**<.001**	−1.19	.24
HOMA-IR	1.30	.20	**2.41**	**.019**
HDL-C	−0.745	.46	−1.09	.28
Leptin	–	–	**−2.41**	**.019**

Significant values indicated in bold. Body mass index is an independent predictor of LCAT in model 1; but when leptin is also included into the regression analysis, leptin and HOMA-IR replace it in this role.

4. Discussion

In our present study, we investigated the possible impact of elevated leptin level on alterations in activities of HDL-associated antioxidant enzyme PON and 2 of the lipid transfer proteins participating in remodeling of HDL, namely, CETP and LCAT. We found that leptin concentrations were correlated inversely with PON1 and LCAT and directly with CETP activities. To the best of our knowledge, this is the first published work that investigated the relationship of leptin with LCAT and CETP. In respect to PON1, these results are concordant with previous investigations [12,13]. However, the correlation between PON1 and leptin levels was less strong than those in the earlier studies ($r = -0.38$ in our study vs -0.63 and -0.90, findings of Uzun et al [13] and Ferretti et al [12], respectively). This discrepancy may originate from the difference of studied patient population in respect to BMI and sex. Moreover, when we tested in multiple regression analysis the association between leptin and PON1, we found that it was independent of anthropometric and other laboratory parameters and that leptin was not an independent predictor of PON1. Actually, neither the BMI nor the other included variables, that is, age, sex, systolic BP, HOMA-IR, LDL-C, HDL-C, and TBARS, were significant predictors of PON1 activity. Nevertheless, adverse effect of leptin on PON1 activity has been previously demonstrated in animal model [11]. Putative mechanisms leading to decreased PON1 activity can be the inactivation of enzyme by increased oxidative stress [22] or accompanying acute phase response that inhibits hepatic synthesis of PON1 [23]. The role of oxidative stress in decreased PON1 activity might be confirmed by the inverse association between lipid peroxidation and PON1 activity. In induction of oxidative stress, leptin might also play a role because a direct relationship between lipid peroxidation and serum leptin levels was found. Lipid peroxidation also correlated negatively with LCAT and positively with CETP. Lecithin cholesterol acyltransferase is also a member of defense mechanisms protecting against lipid peroxidation by increasing the HDL size and contributing to the safe reverse transport of lysolecithin in HDL to the liver. Earlier studies with small groups of obese persons were conflicting in respect to association of obesity and LCAT activity [24,25]. In our present, larger study, LCAT correlated negatively with the degree of adiposity as well as with leptin levels. Moreover, both parameters were independent predictors of LCAT activity. However, the latter had a closer association to a combination of leptin level and degree of insulin resistance than the BMI itself.

Although increased CETP activity was demonstrated in obesity [24,26], in nondiabetic subjects, plasma CETP activity was not linked to insulin or insulin sensitivity [27,28]. However, in our subjects, CETP activities showed positive correlations with insulin levels and HOMA-IR. These contradictory results may originate from the fact that (*a*) the study of MacLean et al [27] was carried out only in women who had a narrower range of BMI (22-37 vs 20-62 kg/m^2 among our subjects) and (*b*) in the work of Riemens et al [28], the BMI was only 26.1 ± 1.8 kg/m^2 even in the most insulin-resistant quartile of investigated men. However, in our subjects, the correlations between CETP activity and insulin levels or HOMA-IR were significant only in simple regression analysis and not in a multiple regression one. Actually, none of the investigated variable was a significant predictor of CETP variance, indicating the relevance of other determinants of CETP activity [29].

In general, LCAT showed negative and CETP positive associations or trends with typical signs of metabolic syndrome (such as adiposity, blood pressure, uric acid, glucose, hsCRP, TG, NEFA, and leptin). However, levels of HDL-C and sE-selectin, as a marker of inflammatory activation of endothelium, were associated with neither LCAT nor CETP.

Limitations of our study are that its population included both sexes, other transfer proteins (eg, phospholipid transfer protein) and enzymes (ie, lipoprotein lipase, hepatic lipase, platelet-activating factor acetylhydrolase) participating in the HDL metabolism were not investigated, and insulin resistance was assessed by a surrogate marker, HOMA-IR.

5. Conclusions

The negative association between serum leptin level and activity of the antiatherogenic LCAT might contribute to the proatherogenic properties of obesity-related hyperleptinemia that is independent of BMI or other anthropometric parameters, as well as insulin resistance or dyslipidemia. However, the role of high leptin level in the decreased activity of the antiatherogenic PON1 enzyme remains unclear.

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