Hyperleptinemia is associated with hypertension, systemic inflammation and insulin resistance in overweight but not in normal weight men

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Abstract *Background and Aim:* High leptin (LPT) is associated with high blood pressure (BP), insulin resistance and systemic inflammation but also excess body weight and adiposity. To disentangle these multiple relations, we analyzed BP, HOMA and circulating C-reactive protein concentration (hs-CRP) in white male adults with different LPT levels but similar age, body mass index (BMI) and body fat distribution. The novel aspect is the different statistical approach used to investigate the relation between LPT and the other alterations present in obesity.
Methods and Results: 972 Olivetti Heart Study participants were stratified according to the median LPT distribution (2.97 ng/ml) into low LPT (l-LPT) and high LPT (h-LPT). The two groups were then carefully matched for age and BMI. We identified two groups of 207 h-LPT and 207 l-LPT individuals with overlapping age, BMI and waist/hip ratio. The two groups had different BP (132.9 ± 16.2/85.7 ± 9.0 vs 128.7 ± 18.2/82.8 ± 9.8 mmHg, $p = 0.014$ for SBP and $p = 0.002$ for DBP) and prevalence of hypertension (57% vs 43%, $p = 0.027$). Upon separate evaluation of untreated individuals with BMI < 25 or BMI ≥ 25, within the latter subgroup h-LPT compared with l-LPT participants ($n = 133$ each group) had higher BP ($p = 0.0001$), HOMA index ($p = 0.013$), hs-CRP ($p = 0.002$) and heart rate ($p = 0.008$) despite similar age and BMI. By contrast, within the normal weight subgroup, h-LPT individuals did not differ from l-LPT ($n = 37$ each) for any of these variables.
Conclusions: High LPT is associated with higher BP, HR, hs-CRP and HOMA index independently of BMI and fat distribution but only among overweight individuals.
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Introduction

Leptin (LPT) is produced by differentiated adipocytes in white adipose tissue, but also in other organs with a large contribution of brain and with greater LPT release in obese than in lean men [1]. Its main and better known physiological effect, mediated by specific structures in the hypothalamus, is the suppression of the desire for food and the increment of energy expenditure by a sympathetically mediated rise of thermogenesis in brown adipose tissue [2]. In addition, leptin exerts a beneficial effect on insulin sensitivity [3] but also behaves as a proinflammatory substance [4] together with other molecules produced within the adipose tissue [5]. Obesity is associated with elevated plasma LPT levels, the higher the BMI or the waist circumference the higher the serum LPT level [6]. However, the main metabolic actions of LPT are largely ineffective in this disorder, a condition referred to as leptin resistance and resembling the condition of insulin resistance observed in the same individuals [2].

We have previously reported that circulating plasma LPT levels were directly associated with BP values in a cross-sectional setting [7] and these data are been also confirmed [8]. In addition were able to predict the risk to develop arterial hypertension [9] and metabolic syndrome [10] in a prospective cohort investigation. These findings supported the hypothesis raised by the results of previous studies in animal models of a pathogenic role of hyperleptinemia and/or leptin resistance in hypertension.

Given the strong relationship of plasma leptin with excess body weight and adiposity, we decided to disentangle these relations, analyzing BP, HOMA and circulating C-reactive protein concentration (hs-CRP) in white male adults with different LPT levels but with overlapping age, body mass index (BMI) and body fat distribution. The novel aspect is the different statistical approach used to investigate the relation between LPT and the other alterations present in obesity. The opportunity was also taken to evaluate the associations of plasma LPT levels with known indicators of insulin resistance (HOMA index) and low grade systemic inflammation (hs-CRP) in the same setting.

Methods

Population

Similarly to previous reports by our group [11], we used the database of the Olivetti Heart Study, an occupational based investigation of the genetic, nutritional and metabolic precursors of cardiovascular disease involving the whole male workforce of the Olivetti factories of Pozzuoli (Naples) and Marcianise (Caserta). The local Ethics Committee approved the study protocol and the participants gave their informed consent to participate. A total of 972 individuals in the age range 25–74 years (51.5 ± 7.2 years), examined in 1994–5, for whom LPT plasma levels were available, were considered for the present analysis. The relevant demographic and anthropometric features of this group were similar to those of the original OHS population.

Examination procedures

Body weight and height were measured on a standard beam balance scale with an attached ruler. Body weight was measured to the nearest 0.1 kg and body height was measured to the nearest 1.0 cm, with subjects wearing light indoor clothing without shoes. BMI was calculated according to the standard formula. Overweight was defined as a BMI $\geq$ 25. The waist circumference was measured at the umbilicus level with the subject standing erect with abdomen relaxed, arms at the sides, and feet together; hip circumference was measured at the point where the buttocks extended the maximum, when viewed from the side. Measurements were performed at the nearest 0.1 cm with a flexible inextensible plastic tape.

Systolic (SBP) and diastolic (DBP; phase V) blood pressure were taken three times 2 min apart with a random zero sphygmomanometer (Gelman Hawksley Ltd., Sussex, UK) after the subject had been sitting for at least 10 min. The average of the second and third reading was recorded. A subject was classified as hypertensive if his BP was $\geq$140 and/or 90 mm Hg or if he was on current antihypertensive treatment, according to ESH/ISH guidelines. Resting supine heart rate was measured by standard 12-lead ECG, recorded in all subjects at 25 mm/s and 1 mV/cm calibration.

Biochemical measurements

A fasting venous blood sample was taken in the seated position between 8:00 AM and 10:00 AM, after the BP measurements, for determination of serum LPT, adiponectin, serum insulin, glucose and lipids.

The blood specimens were immediately centrifuged and stored at -70 °C until analyzed. Serum LPT was measured by an enzyme-linked immunosorbent assay (R&D System GmbH, Wiesbaden-Nordenstadt, Germany). Intra- and inter-assay coefficients of variation were 3.0% and 5.4%, respectively [12]. Serum adiponectin was measured by an enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic). Intra- and inter-assay coefficients of variation were 4.2% and 5.1%, respectively. High-Sensitivity C-Reactive Protein (hs-CRP) was measured by an immunoturbidimetric method using a Roche Diagnostics, Milan, Italy, automated analyzer. Serum glucose levels were measured with automated methods (Cobas-Mira, Roche, Italy). Serum insulin was determined by radioimmunoassay (Insulin Lisophase; Technogenetics, Milan, Italy). The homeostatic assessment model (HOMA) index was used to estimate insulin resistance and calculated as fasting serum insulin (μU/mL) $\times$ fasting serum glucose (mM)/22.5 [13].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS-PC, version 16; SPSS, Inc., Chicago, IL). The participants were stratified according to the median of plasma LPT distribution of whole OHS population (2.97 ng/ml) into subjects with low (l-LPT) and subjects with high LPT (h-LPT) levels. Thereafter, the two groups of subjects underwent a careful one-by-one

matching for age and BMI, with a first decimal digit precision. We thus identified 207 subjects with l-LPT and 207 with h-LPT, who had the same age and BMI. As the distributions of plasma LPT levels deviated significantly from normality, they were normalized by log transformation; log-transformed values were used in the analysis, as appropriate. Analysis of variance (ANOVA) was used to assess differences between group means. Cross-tabulation analysis was used to analyze the frequency of a given condition across different study groups. The general linear model was adopted to evaluate the interrelations between selected variables adjusting for confounders. Logistic regression analysis was carried out to estimate the role of LPT on hypertension in overweight and normal weight participants, separately. Results are expressed as means and standard deviation unless otherwise indicated. Two sided P values less than 0.05 were considered statistically significant.

Results

The two groups ($n = 207$ each) identified by the one-to-one matching procedure had, by selection, similar age and BMI but significantly different plasma LPT levels (Table 1). The high- and low-leptin groups had also similar body fat distribution (waist/hip: 0.98 ± 0.04 vs 0.98 ± 0.04) but were found to differ with regard to prevalence of hypertension (l-LPT = 43%, h-LPT = 57%; $\chi^2 = 5.31$; $p = 0.027$) and BP levels (Table 1). In addition logistic regression analysis showed that h-LPT was associated with hypertension, also adjusting for smoking status, physical activity levels and alcohol intake, but only in overweigh participants (O.R. [95% CI]: 3.01 [1.70–5.34]; $p = 0.0001$) (Table 2).

In the h-LPT compared with the l-LPT group trends were observed toward higher HR, hs-CRP and HOMA index levels as well as toward lower plasma adiponectin concentration: however, none of these difference attained statistical significance (Table 1).

With respect to the relationship of plasma LPT with BP we detected a significant interaction between LPT and BMI, both in the l-LPT ($p = 0.031$) and the h-LPT group ($p = 0.039$). Therefore, we carried out separate evaluations

of participants having normal BMI and of those with overweight or obesity. To rule out the possible influence of antihypertensive drug treatment, in this analysis we excluded subjects on pharmacological therapy. We identified 133 pairs of h-LPT and l-LPT untreated subjects with BMI ≥ 25 and 37 pairs of subjects with BMI < 25. Tables 2 and 3 report the relevant features of the two subgroups. Within both subgroups, the average age and BMI of h-LPT and l-LPT individuals were again exactly comparable. Among overweight participants, the difference in plasma LPT concentration was again associated with significant differences in BP. Further more, significant differences were detected in hs-CRP, HOMA index and heart rate (Table 3). Conversely, among normal weight individuals, no difference between h-LPT and l-LPT participants was detected in any of these variables (Table 4).

GFR and plasma adiponectin levels of h-LPT and l-LPT individuals did not differ either in the normal weight or in the overweight subgroups.

Discussion

This is to our knowledge the first case-control study of individuals with high versus others with low plasma LPT concentration, derived from an unselected sample of adult general population and carefully matched for sex, age, body mass and fat distribution. Its main findings are threefold: i) for a comparable body mass and adiposity index, high

Table 2 Logistic regression analysis: Role of LPT on Hypertension.

Overweight subjects			P
Iindependent variable	$\beta \pm SE$	OR (95% CI)	
h-LPT	1.10 ± 0.29	3.01 (1.70–5.34)	0.0001
Normalweight subjects			
h-LPT	-0.35 ± 0.59	0.70 (0.22–2.24)	0.55

Models adjusted for smoking, physical activity levels, alcohol intake. h-LPT: High Leptin.

Table 1 Characteristics of low LPT and high LPT participants matched for age and BMI.

	l-LPT ($n = 207$)	h-LPT ($n = 207$)	P
Age (years)	51.37 ± 7.44	51.82 ± 8.33	0.557
BMI (kg/m^2)	27.09 ± 2.44	27.09 ± 2.43	0.983
LPT (ng/mL)	1.80 ± 0.83	5.97 ± 2.10	0.000
SBP (mm Hg)	128.7 ± 18.2	132.9 ± 16.2	0.014
DBP (mm Hg)	82.8 ± 9.8	85.7 ± 9.0	0.002
ECG-HR (b/min)	61.40 ± 9.73	63.02 ± 8.59	0.072
hs-CRP (mg/L)	1.57 ± 2.13	1.94 ± 1.93	0.067
HOMA index	2.32 ± 1.50	2.59 ± 1.54	0.082
Adiponectin (μg/mL)	4.83 ± 2.53	4.58 ± 2.44	0.309
GFR (mL/min)	88.7 ± 16.2	90.8 ± 17.5	0.190

M $\pm$ SD; LPT: leptin, SBP: systolic blood pressure, DBP: diastolic blood pressure, ECG-HR: heart rate by ECG, GFR: glomerular filtration rate, l-LPT: Low Leptin, h-LPT: High Leptin.

Table 3 Characteristics of untreated subjects matched for age and BMI in overweight subjects.

	l-LPT ($n = 133$)	h-LPT ($n = 133$)	P
Age(years)	50.24 ± 6.51	51.57 ± 8.11	0.141
BMI (kg/m^2)	27.69 ± 1.77	27.73 ± 1.87	0.869
LPT (ng/mL)	1.85 ± 0.81	5.84 ± 2.46	0.001
SBP (mm Hg)	124.5 ± 13.9	132.9 ± 15.1	0.001
DBP (mm Hg)	80.8 ± 8.4	85.9 ± 7.8	0.001
ECG-HR (b/min)	60.13 ± 9.32	63.02 ± 8.26	0.008
hs-CRP (mg/L)	1.33 ± 1.42	2.05 ± 2.15	0.002
HOMA index	2.19 ± 1.22	2.64 ± 1.65	0.013
Adiponectin (μg/mL)	4.59 ± 2.16	4.40 ± 2.40	0.499
GFR (mL/min)	91.9 ± 15.6	92.1 ± 15.4	0.940

M ± SD; LPT: leptin, SBP: systolic blood pressure, DBP: diastolic blood pressure, ECG-HR: heart rate by ECG, GFR: glomerular filtration rate, l-LPT: Low Leptin, h-LPT: High Leptin.

plasma LPT is associated with higher blood pressure and greater prevalence of hypertension; ii) besides blood pressure, also hs-CRP, insulin resistance and heart rate tend to be elevated in individuals with higher LPT levels but similar BMI and fat distribution; iii) excess body weight is a condition necessary for the detection of these multiple associations. Altogether, these results are in keeping with previous cross-sectional reports by our group [10] and others [14—18] of a significant association of plasma LPT concentration with BP and metabolic syndrome. Particularly impressive in this respect is the paper by Itoh and co-workers in which LPT was correlated with BP also after weight loss, in obese hypertensive women [17]. Finally, cross-sectional results are in line with our further reports [9,10] that plasma LPT is a predictor of the development of HPT and metabolic syndrome.

Leptin and BP

Aside to the epidemiological evidence, the results of several experimental studies support the contention that leptin plays a significant role in BP modulation [19—22]. A number of clinical studies shed light on the possible mechanisms whereby hyperleptinemia and the attendant LPT resistance may contribute to development and maintenance of hypertension and eventually of cardiovascular disease [23—25]. Thus, Eikelis et al. demonstrated that in

men with various body size, plasma LPT concentration correlated with the degree of adiposity and with renal sympathetic nerve activity determined through the renal spillover of norepinephrine [26]. Alpha-1-receptor blockade by bunazosin hydrochloride improves insulin resistance and decreases plasma LPT levels in hypertensive patients with hyperleptinemia. These findings suggest that excess LPT causes sympathetic activation in human obesity [27,28]. The small but significant difference in heart rate found in our study between subjects with different plasma LPT levels is in accordance with this hypothesis. Central sympathetic activation by high LPT levels is translated into increased sympathetic discharge to brown adipose tissue but also to other organs and, in particular, to the kidney [29]. The leptin-mediated renal sympathetic stimulation may be followed by increased sodium and water retention and by stimulation of renin secretion.

Leptin and CRP

The relationship between plasma leptin and CRP has been previously reported. Leptin induces CRP expression in vitro in human primary hepatocytes [30] as well as in human coronary artery endothelial cells [31]. Accordingly, studies in healthy humans show a direct independent association between leptin and plasma CRP concentrations [32]. Leptin administration in vivo increases CRP concentration in non-obese

Table 4 Characteristics of untreated subjects matched for age and BMI in normal weight subjects.

	l-LPT ($n = 37$)	h-LPT ($n = 37$)	P
Age(years)	51.10 ± 8.72	49.59 ± 9.27	0.474
BMI (kg/m^2)	23.93 ± 0.89	23.97 ± 0.84	0.884
LPT (ng/mL)	1.87 ± 0.74	5.01 ± 3.22	0.001
SBP (mm Hg)	127.0 ± 16.5	124.1 ± 17.4	0.458
DBP (mm Hg)	81.9 ± 9.2	80.1 ± 9.0	0.399
ECG-HR (b/min)	62.22 ± 9.42	61.11 ± 8.49	0.597
hs-CRP (mg/L)	1.38 ± 1.99	1.21 ± 0.97	0.644
HOMA index	2.09 ± 1.59	1.89 ± 0.72	0.500
Adiponectin (μg/mL)	4.57 ± 2.42	4.35 ± 1.79	0.659
GFR (mL/min)	80.3 ± 15.3	88.6 ± 23.4	0.07

M ± SD; LPT: leptin, SBP: systolic blood pressure, DBP: diastolic blood pressure, ECG-HR: heart rate by ECG, GFR: glomerular filtration rate, l-LPT: Low Leptin, h-LPT: High Leptin.

individuals [33] although it does not so in obese subjects [34], perhaps as a consequence of leptin resistance. Besides to raise CRP production, leptin also promotes intimal monocyte recruitment [35], elicits macrophage foam cell formation [36] and induces secretion of proinflammatory cytokines [37]. Conversely, a role of CRP in the induction of leptin resistance has been proposed. CRP was identified as SLIP-1, one of the circulating factors known to bind plasma leptin and to reduce its circulating active form [38]. Moreover, the ability of human CRP to inhibit leptin binding to its receptor and the ensuing cell signaling process was shown in HEK293 cells and hypothalamic neurons in vitro [39].

Leptin and insulin sensitivity

Leptin decreases insulin secretion via direct action on leptin receptors in pancreatic beta-cells [40], enhances skeletal muscle glucose uptake and oxidation [41] and suppresses gluconeogenesis [42]. Conversely, insulin and glucose appear to stimulate leptin secretion by adipocytes [43]. Taken together, these results suggest that leptin resistance may favour insulin resistance and diabetes. Our data confirm previous reports that increased leptin is associated with hyperinsulinemia and insulin resistance independently of BMI [3].

Leptin and related variables in overweight and normal weight subjects

The most intriguing result of our study was the finding of a significant interaction between plasma LPT and BMI in their association with BP and the consequent observation that the statistical association of plasma LPT with BP was undetectable in the absence of overweight. A similar pattern was also observed for the relationship of plasma LPT to hs-CRP, HOMA index and heart rate. This implies that the relationship between leptin and related variables is different in the presence or absence of excess body weight. The explanation(s) of this finding is (are) not at hand. However, this finding might be related to the parallel questions of why plasma leptin concentration was increased in a substantial group of normal weight individuals in our study population and where the excess LPT was produced in these subjects. The answer to these questions could help understand why in these subjects an elevated LPT concentration was not associated with the unfavourable effects otherwise observed in overweight individuals.

LPT is physiologically expressed in several other cells besides the adipocyte and one possibility is that the *surplus* of LPT production occurring in subjects without excess adiposity takes place in one or more sites different from the adipose tissue. According to Eikelis et al., the brain is a substantial source of circulating leptin in humans but probably more so in the obese than in the lean subject [1]. Hyperleptinemia is generally expression of leptin resistance, albeit not a very accurate one: thus, for similarly elevated plasma LPT levels different degrees of LPT resistance may occur, possibly explaining differences in the pleiotropic effects of the molecule. A different explanation could be related to GFR. It is well known that high LPT levels are associated with a impaired renal function [44].

Nevertheless this relationship there was not evident in "normal" renal function [45], as well as for our results where GFR was not different between l-LPT and h-LPT groups, also after stratification for overweight and normal weight participants.

In this respect, a limitation of our study was the lack of measurement of the Serum Leptin Interacting Proteins (SLIPs) and of the circulating leptin-receptor, all factors able to bind circulating LPT and to alter the active free LPT concentration in the blood.

Another limitation is the recruitment of white male participants only, so that its results may be generalized only to a comparable white male population.

Conclusions and perspectives

In conclusion, our study provides the direct demonstration, in a case-control study of high LPT vs low LPT adult men carefully matched for age, BMI and body fat distribution, that elevated plasma LPT is independently associated with high BP and higher levels of heart rate, hs-CRP and insulin resistance. Moreover, it showed that these associations can be observed in overweight and obese subjects but not in subjects with normal weight. Although the mechanistic interpretation of our findings is only speculative, these results support the hypothesis of a pathogenic role of hyperleptinemia and leptin resistance in the development of obesity hypertension and, as a consequence, also strengthen the potential benefits deriving from correction of overweight and obesity in the prevention of hypertension and cardiovascular disease.

Disclosure statement

Acknowledgments

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