

Leptin levels predict the development of insulin resistance in a sample of adult men—The Olivetti Heart Study

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Abstract *Background and aims:* Leptin (LPT) is associated with unfavourable cardio-metabolic risk profile. Although a number of studies have found a positive association between LPT and insulin resistance (IR), no observational study has evaluated a prospective association to detect a predictive role of LPT in IR. Therefore, the aim of this study was to estimate the role of LPT on the incidence of IR in an 8-year follow-up of a sample of adult men (The Olivetti Heart Study). *Methods and results:* The study included 527 not diabetic men without IR (homeostasis model assessment - HOMA index < 2.77 UI) at baseline. Baseline LPT was significantly and positively associated with HOMA index, body mass index (BMI), waist circumference and blood pressure. At the end of the 8-year follow-up period, a positive and significant association was detected between baseline LPT and changes in HOMA index ($r = 0.25$, $p < 0.01$) and incidence of IR (OR: 2.6, 95%CI: 1.9–3.4). This trend was also confirmed after adjustment for potential confounders. In addition, the predictive value of LPT was found in subjects who had not experienced any weight increase over the years, and for normal weight and excess body weight participants, separately. *Conclusions:* The results of this prospective study suggest a predictive role of circulating LPT levels on a reduction of insulin sensitivity over time, independently of main potential confounders, in non-diabetic men without IR at baseline. In addition, in normal weight individuals, LPT levels were associated with development of IR.

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Introduction

Leptin (LPT) is a hormone with *pleiotropic* actions, mainly produced by adipocytes. In particular, LPT helps regulate body weight by inhibiting the feeling of appetite and by stimulating energy expenditure through the increase in sympathetic-mediated thermogenesis in brown adipose tissue [1]. On the other hand, excess body weight has been

associated with high-circulating LPT levels [2], suggesting that, in the presence of hyperleptinemia, the main metabolic actions of LPT are largely ineffective (namely LPT resistance), as observed during insulin resistance (IR) [3]. This condition is supported by studies on genetic variations of the hypothalamic LPT receptors gene [4]. Indeed, the inverse relationship between LPT and insulin secretion, mediated by LPT receptors [5,6], is not preserved in the presence of LPT resistance [7–10].

Our previous investigations have shown that circulating LPT levels are associated with unfavourable cardio-metabolic profile in both cross-sectional and prospective evaluations [11–14]. Conversely, scanty data are available on a prospective association between LPT and insulin

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sensitivity to detect a predictive role of LPT. One observational study found an unfavourable association between baseline LPT and changes in insulin levels over the years in both men and women, but the study did not exclude participants with IR at baseline [15].

Considering the well-known strong association between IR and cardiovascular risk, the aim of our study was to prospectively analyse baseline LPT levels in relation to the development of IR, in a sample of euglycemic men without diabetes and IR at baseline, participating in the Olivetti Heart Study (OHS).

Methods

Study population

The OHS was an occupational investigation of the male workforce of the Olivetti factories in Southern Italy (Pozzuoli-Naples and Marcianise-Caserta), as previously described [11]. A total of 1085 individuals (95% of the total male workforce) aged 25–75 years (49.8 years) was examined in 1994–95. For the purposes of the present analysis, we excluded participants with diabetes ($n = 83$) (fasting blood glucose level ≥ 126 mg/dL or current anti-diabetic therapy) and IR (homeostasis model assessment - HOMA index > 2.77 UI) ($n = 264$). We also excluded participants whose demographic and anthropometric characteristics and/or cardio-metabolic risk factors were not available at baseline ($n = 80$). Finally, from the initial sample of 658 men without diabetes and IR, 527 (80%) were seen again in 2002–04 and were considered eligible for the present analysis.

The Ethics Committee of “Federico II” University in Naples approved the Olivetti study protocol and the participants provided their informed written consent to participate.

Examination procedures

The OHS study procedures have been described previously [11]. Briefly, physical examinations for both baseline and follow-up visit were performed between 08:00 and 11:00 h, in a quiet and comfortable room, with the participants having fasted for at least 13 h. Baseline and 8-year follow-up visits included a physical examination and anthropometric measurements, a blood test, a fasting timed urine collection and the administration of a questionnaire.

A fasting venous blood sample was taken in the seated position between 8:00 AM and 10:00 AM. Blood specimens were immediately centrifuged and stored at -70 °C until analysis. Serum LPT was measured by an enzyme-linked immunosorbent assay [R&D System GmbH, Wiesbaden-Nordenstadt, Germany] [16]. Serum glucose levels were measured with automated methods (Cobas-Mira, Roche, Italy). Serum insulin was determined by radioimmunoassay (Insulin Lisophase; Technogenetics, Milan, Italy). Insulin sensitivity was estimated by the HOMA index using the formula: fasting plasma insulin

($\mu\text{U/mL}$) \times fasting plasma glucose (mmol/L)/22.5. A HOMA index > 2.77 UI was considered as a cut-off value for IR [17]. Serum creatinine was measured by the picric acid colourimetric method.

Systolic and diastolic blood pressure (BP) (phase V) were measured three times at 2 min intervals, 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. The diagnosis of hypertension was defined as systolic BP ≥ 140 and/or diastolic BP ≥ 90 mmHg or current antihypertensive drug treatment [18].

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. Body mass index (BMI) was measured according to the formula weight (kg)/height² (m). Excess body weight was defined as a BMI ≥ 25 kg/m². Waist circumference (WC), expression of abdominal adiposity, was measured at the level of the umbilicus with the subject standing erect, the abdomen relaxed, arms at the sides, and feet together; measurements were performed to the nearest 0.1 cm with a flexible inextensible plastic tape.

Physical activity level was expressed according to whether the participant habitually engaged at least 4 h per week of aerobic exercise.

Statistical analysis

All statistical analyses were performed using the SPSS software, version 20 (SPSS inc, Chicago, Ill). Because the distribution of LPT was skewed, the log-transformed values were used in the analyses. Changes in the participants' main characteristics were calculated as final minus basal measurements. The Chi-squared test was used to evaluate differences between categorical variables. Bivariate relationships between the variables under investigation were evaluated by Pearson correlation analysis. Paired t-tests were used to assess differences between baseline and follow-up visit in any of the variables under investigation. The multivariable linear regression analysis was used to determine the independent effect of LPT on changes in HOMA index over time, adjusting for the main potential confounders. The analysis of variance (ANOVA) was used to assess differences in main baseline characteristics and their changes between the group who developed IR (IR[+]) (HOMA index > 2.77 UI) and the one who did not (IR[-]), and to compare baseline characteristics between participants who developed diabetes (fasting blood glucose level ≥ 126 mg/dL or current anti-diabetic therapy) or impaired fasting glucose (IFG) (fasting blood glucose level between ≥ 100 and ≤ 126 mg/dL without current anti-diabetic therapy) and those who did not. The binary logistic regression analysis was used to estimate the role of LPT on the development of IR, adjusting for the main potential confounders. Given the strong relationship between BMI and WC ($r = 0.78$,

$p < 0.01$), multivariate analyses were separately adjusted for BMI or WC. In addition, baseline HOMA index was transformed into rank to include them as covariate in linear regression analysis. The results are reported as mean or geometric mean (with standard deviation –SD) as percentages or as odds ratio (OR) and 95% confidence interval (95% CI), unless otherwise indicated.

It was estimated that 41 subjects were required in each group to detect a true difference of 2 ng/mL (SD = 2.8) in LPT between group that developed IR and those not, and to provide 90% power at 5% probability level (two-sided). Two-sided P values below 0.05 were considered statistically significant.

Results

The baseline characteristics of the study participants are reported in Table 1. The mean age was 51.2 (7.6) years and mean BMI was 26.5 (2.8) kg/m², with 70% having excess body weight.

The analysis of the correlation between LPT levels and the most relevant characteristics of participants at baseline showed a significant and positive association with HOMA index ($r = 0.23$, $p < 0.01$), glucose ($r = 0.17$, $p < 0.01$), insulin ($r = 0.19$, $p < 0.01$), BMI ($r = 0.51$, $p < 0.01$), WC ($r = 0.51$, $p < 0.01$) and BP (systolic: $r = 0.12$, $p < 0.01$; diastolic: $r = 0.12$, $p < 0.01$), but not with age ($p > 0.05$).

At the eight-year follow-up, a significant increase in BMI (0.5 kg/m²), WC (3.6 cm) and HOMA index (0.3 UI) was observed, and an overall incidence of IR of 20% and of diabetes of 4.4% was detected, and 16 new cases of IFG were found. The analysis of the correlation underlined a significant positive association of basal LPT levels with changes in HOMA index ($r = 0.25$, $p < 0.01$) and insulin ($r = 0.29$, $p < 0.01$), but not with changes in BMI and WC. The multivariate analysis confirmed the predictive role of LPT on changes in HOMA index, after accounting for baseline age, HOMA index, and BMI (for 1-SD increase in log-LPT [2.3 ng/mL]: $\beta = 0.22$, 95%CI = 0.08–0.35; $p = 0.001$) or WC ($\beta = 0.25$, 95%CI = 0.12–0.39; $p = 0.001$). Furthermore, in the model including changes in BMI or WC over time as covariate, LPT levels also

remained significantly associated with changes in HOMA index ($p < 0.01$).

We also stratified the whole sample by IR after the eight year follow-up; Table 2 shows the differences in baseline features between IR[+] and IR[-] participants. The IR[+] group had higher LPT, BMI and WC, with higher prevalence of excess body weight than the IR[-] group. Also, baseline BP, glucose, insulin and HOMA index were significantly higher in IR[+] participants than in IR[-], while no difference was detected for age. The logistic regression analysis confirmed the significant positive association between baseline LPT and development of IR over time (OR: 2.6; 95% CI: 1.9–3.4) (Table 3). The association remained statistically significant also accounting for baseline age, HOMA index, physical activity, and BMI or WC (Table 3). Although during follow-up BMI and WC changes were significantly more marked in the IR[+] group than in the IR[-] (changes in BMI: +1.3 vs + 0.4 kg/m², $p < 0.01$; changes in WC: +5.2 vs + 3.2 cm, $p < 0.01$), the models adjusted for these

Table 2 Baseline characteristics of the participants stratified by development of insulin resistance at follow-up.

	IR[-]	IR[+]
N. of participants	422	105
Age (yrs)	51.3 (7.8)	50.5 (6.6)
BMI (kg/m ²)	26.1 (2.7)	28.1 (2.6)*
Excess body weight (%)	65	89*
Waist Circumference (cm)	92.2 (7.9)	97.4 (6.6)*
Systolic BP (mmHg)	127.4 (15.9)	128.9 (17.5)
Diastolic BP (mmHg)	82.9 (9.6)	83.9 (9.1)
Hypertension (%)	33	39
Glucose (mg/dL)	94.2 (8.6)	98.4 (9.6)*
Insulin (μ U/mL)	7.0 (2.3)	8.4 (2.1)*
HOMA index (U)	1.6 (0.5)	2.0 (0.5)*
LPT (ng/mL) [‡]	2.2 (2.3)	4.1 (2.0)* [†]

Data are expressed as means (SD) or as percentages; BMI: body mass index; BP: Blood Pressure; HOMA: homeostasis model assessment; IR[-]: not insulin resistance, IR[+]: insulin resistance; LPT: leptin. * IR[+] vs IR[-]; $p < 0.001$; [‡] Data expressed as geometric mean. [†] analysis performed on log-transformed variable.

Table 3 Eight year risk of incident insulin resistance for 1-SD increase in log-Leptin (logistic regression analysis).

	Risk of Insulin Resistance OR (95% CI)	P-value
1-SD \uparrow in log-Leptin*		
Unadjusted	2.6 (1.9–3.4)	<0.001
Multivariable Model ^a	1.9 (1.4–2.7)	<0.001
Multivariable Model ^b	2.0 (1.4–2.8)	<0.001
Multivariable Model ^c	2.6 (1.9–3.6)	<0.001
Multivariable Model ^d	2.5 (1.8–3.4)	<0.001

OR: Odds Ratio; SD: standard deviation; BMI: body mass index; HOMA: homeostasis model assessment; *1-SD log-Leptin = 2.3 ng/mL.

^a Adjusted for baseline age, BMI, Homa, physical activity.

^b Adjusted for baseline age, waist circumference, Homa, physical activity.

^c Adjusted for baseline age, Homa, physical activity, and BMI changes.

^d Adjusted for baseline age, Homa, physical activity, and waist circumference changes.

Table 1 Baseline characteristics of the study participants.

N. of participants	527
Age (yrs)	51.2 (7.6)
BMI (kg/m ²)	26.5 (2.8)
Excess body weight (%)	70
Waist Circumference (cm)	93.3 (8.0)
Systolic BP (mmHg)	127.7 (16.2)
Diastolic BP (mmHg)	83.1 (9.5)
Hypertension (%)	34
Glucose (mg/dL)	95.0 (9.0)
Insulin (μ U/mL)	7.3 (2.3)
HOMA index (U)	1.7 (0.6)
LPT (ng/mL) [‡]	2.5 (2.3)

Data are expressed as means (SD) or as percentages; BMI: body mass index; BP: Blood Pressure; HOMA: homeostasis model assessment; LPT: leptin [‡] Data expressed as geometric mean.

changes detected similar results (Table 3). In addition, also when we considered only participants who did not have weight increase over the 8 years (52.2%, $n = 275$), baseline LPT remained predictive of a reduction in insulin sensitivity ($\beta = 0.38$, 95%CI = 0.22–0.53; $p < 0.01$) and IR development (OR: 3.1; 95%CI: 1.8–5.3; $p < 0.01$).

We also carried out separate analyses after stratification by baseline body weight. At the end of follow-up, the groups with excess body weight had a significantly greater incidence of IR than normal weight participants (25% vs 7%; $p < 0.01$). Baseline LPT was significantly higher in IR[+] than IR[-], both in normal and excess body weight subjects (Fig. 1). The predictive role of LPT on IR development was confirmed in the analysis when adjusting for age and baseline HOMA index (for 1-SD increase in log-LPT, normal weight- OR: 5.7, 95%CI: 2.0–16.5; excess body weight- OR: 1.9, 95%CI: 1.4–2.7). Although during follow-up there was a significantly greater increase in anthropometric indices in IR[+] compared to IR[-] in both the normal and excess body weight group, when the analysis was adjusted for changes in BMI (normal weight- OR: 5.8, 95%CI: 1.8–18.7; excess body weight- OR: 2.0, 95%CI: 1.4–2.9) or WC (normal weight- OR: 6.7, 95%CI: 2.0–21.9; excess body weight- OR: 2.0, 95%CI: 1.4–2.8) the results were similar.

Finally, we analysed LPT stratified by IFG and diabetes development. LPT was significantly higher in participants with IFG than in those without (3.80 (2.08) vs 2.29 (2.34) ng/ml, $p = 0.02$). On the other hand, LPT was slightly higher in those who developed diabetes than in the other participants (2.63 (2.51) vs 2.51 (2.34) ng/ml, $p = 0.8$). Conversely, when those who developed diabetes ($n = 23$) were matched one-by-one for age, BMI and central adiposity, LPT was significantly greater in the new diabetic participants (Appendix Table 1).

Discussion

To our knowledge, this is the first study directly relating baseline LPT levels to changes in insulin sensitivity over time, in a relatively large middle-aged sample of

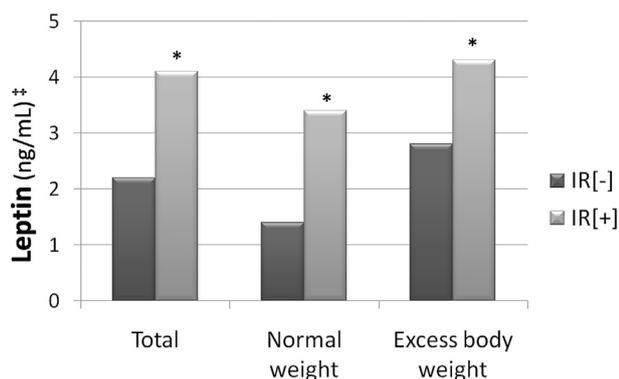


Figure 1 Baseline leptin levels and development of insulin resistance, in the total sample, stratified by normal weight and excess body weight subjects. IR[-]: not insulin resistance, IR[+]: insulin resistance. †Data expressed as geometric mean. * $p < 0.001$.

individuals without diabetes or IR at baseline, observed for a reasonably long period. In particular, our results indicate that LPT levels are positively associated with changes in insulin sensitivity over an eight year period and are predictive of IR development, also after accounting for potential confounders at baseline and at follow-up, such as age, insulin sensitivity, physical activity and anthropometric measures. The predictive role of baseline LPT levels was also confirmed in subjects who did not have any weight increase over the years, and in both normal and excess body weight participants, separately. In addition, as far as we know, this is also the first time that a study analyzes the development of IR in a group of normal weight subjects and without IR at baseline. LPT levels were also higher in those who developed IFG or diabetes, in particular when matched for age and adiposity indices. Despite the low incidence of IFG and diabetes and the small sample size, these results support the predictive role of LPT on altered glucose homeostasis.

One prospective study detected an unfavourable association between baseline LPT and changes in insulin levels after 10 years of follow-up, in both male and female participants [15]. However, limitations of this study are that IR individuals at baseline were not excluded from the analysis and insulin levels are a weak expression of IR.

The key role of LPT on glucose homeostasis is already supported by a wealth of evidence. Indeed, LPT administration improves insulin functions in normal and diabetic rats [19,20]. Moreover, LPT reduces gene expression and the activity of negative regulators involved in the glucose transporter GLUT4 in skeletal muscle [21]. Other experimental studies have shown that LPT increases both basal and insulin stimulated glucose uptake and oxidation in isolated muscles [22]. However, studies carried out in pathological conditions – such as obesity – have pointed out the negative metabolic effect of LPT [3], indicating that LPT resistance may be due to defects in LPT signalling [4]. The signalling attenuation could be mediated by different mechanisms including the up-regulation of the suppressor of cytokine signalling (SOCS3) in the cytoplasm and of the protein tyrosine phosphatase-1B (PTP1B) in the endoplasmic reticulum, both involved in the regulation of the JAK2/STAT3 pathway [7–10]. Furthermore, other experimental studies support the hypothesis that LPT regulates peripheral insulin sensitivity via the effects on the liver, and that the mechanism underlying this effect is dependent on the hepatic sympathetic and parasympathetic branches of the autonomic nervous system [23,24]. Such pathway alterations might contribute to IR [7–10] by affecting the inverse relationship with insulin secretion [3,5,6,25].

Also some classes of drugs could have a beneficial action on LPT levels, which in part would explain its favourable effect on cardiovascular risk [26–29]. Experimental and clinical studies have shown a reduction of LPT with the administration of different antidiabetic classes. Metformin (a biguanide) expresses beneficial effects thought different ways: it decreases LPT, upregulates the expression of LPT receptors in the liver, improves the LPT

hypothalamic sensitivity, and reduces LPT-related reactive oxygen species production [26]. Sitagliptin (a dipeptidyl peptidase-4 inhibitor) reduces LPT in animals and humans [30,31], as do pioglitazone (a thiazolidinedione) [32] and empagliflozin (a sodium-glucose co-transporter 2) [33]. Conversely, liraglutide (a glucagon-like peptide-1 receptor agonist) improves LPT resistance, in addition to reducing LPT during treatment [34,35]. Also statin therapy seems to be associated with a reduction in LPT [27,36,37], as recently confirmed by an experimental study showing a favourable effect of simvastatin and atorvastatin on LPT expression in primary human adipocytes [38]. Moreover, the results of a meta-analysis on nine randomized controlled trials show that orlistat (an anti-obesity drug) seems to have a beneficial effect on LPT (28). On the other hand, the results of a meta-analysis (including only two studies) indicated a non-significant reduction in LPT during ezetimibe added to statin therapy (29). Of note, based on such evidence, LPT levels in our sample were not affected by therapy, as none of the participants were on anti-diabetic or on statins therapy at baseline.

In previous epidemiological studies, LPT levels were higher than in our sample [2,39,40]. Indeed, this difference may be due to the exclusion of subjects with diabetes and IR, and to the low prevalence of obesity in our population sample (10%). Although, LPT resistance is essentially present in obese or overweight individuals, our results show that LPT levels are predictive of IR development in excess body weight participants, as expected, but also underline that such predictive role is present in normal weight humans, particularly in subjects who did not experience weight increase over the years. Noteworthy is that LPT levels in IR[+] normal weight men were higher than in excess body weight IR[-] participants.

In the absence of a threshold for the LPT resistance, our results suggest that for a 2.3 ng/mL increase in LPT levels there is two-fold increased risk to develop IR, independently of body weight.

The results of this paper are in line with our previous studies on the relationship between LPT and cardio-metabolic and cardiovascular risk [11–13].

The strengths of our study are: a) the prospective design; b) the relatively long follow-up period; c) the inclusion of untreated non-diabetic participants without IR; d) the careful standardization of data collection at both baseline and follow-up examination; e) the fact that none of the participants had taken any treatment affecting baseline LPT levels.

Nonetheless, the study has some limitations: the first one is the participation of only adult white male individuals, which makes our results only generalizable to male Caucasian people. Another limitation is the lack of intermediate parameters measured during the 8-year follow-up period, with the consequent inability to perform a time-to-event analysis relative to changes in insulin sensitivity. Finally, serum LPT interacting proteins and circulating leptin-receptor were not assessed.

In conclusion, the present analysis of a selected sample of adult male population drawn from the Olivetti Heart

Study database, indicated that high LPT levels predict the development of IR, independently of the main potential confounders, in particular body weight at baseline and its changes over the years. In addition, LPT levels were also higher in participants who developed diabetes, independently of body weight; however, definitive conclusions on the predictive role of LPT on diabetes cannot be drawn due to the small sample size.

Our study adds sound evidence to the hypothesis accumulated through previous studies in favour of a causal role of LPT on cardio-metabolic risk over time. Thus, given also the strong role of IR on cardiovascular risk, further investigations are needed to better clarify the mechanisms involved, in particular in individuals with normal insulin sensitivity at baseline.

Disclosures

The authors have no conflict of interest to disclose.

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Author contributions

Conception and design: FG. Analysis and interpretation of the data: LD, FG. Drafting of the article: LD, FG. Critical revision of the article for important intellectual content: LD, PS, RI,OR,FG. Final approval of the article: all Authors. Statistical expertise: LD,FG. Administrative, technical, or logistic support: PS,FG. Collection and assembly of data: LD,RI,OR.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2018.10.003>.

References

- [1] Haynes WG. Role of leptin in obesity-related hypertension. *Exp Physiol* 2005;90:683–8.
- [2] Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;334:292–5.
- [3] Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med* 2008;14:741–51.
- [4] Lahlou N, Clement K, Jean-Claude C, Christian V, Lotton C, Bihan YL, et al. Soluble leptin receptor in serum of subjects with complete resistance to leptin: relation to fat mass. *Diabetes* 2000; 49:1347–52.
- [5] Holz GG, Habener JF. Signal transduction crosstalk in the endocrine system: pancreatic beta-cells and the glucose competence concept. *Trends Biochem Sci* 1992;17:388–93.
- [6] Kline AD, Becker GW, Churgay LM, Landen BE, Martin DK, Muth WL, et al. Leptin is a four-helix bundle: secondary structure by NMR. *FEBS Lett* 1997;407:239–42.
- [7] Reed AS, Unger EK, Olofsson LE, Piper ML, Myers Jr MG, Xu AW. Functional role of suppressor of cytokine signaling 3 upregulation

- in hypothalamic leptin resistance and long-term energy homeostasis. *Diabetes* 2010;59(4):894–906.
- [8] Münzberg H, Myers Jr MG. Molecular and anatomical determinants of central leptin resistance. *Nat Neurosci* 2005 May; 8(5):566–70.
 - [9] Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, et al. SOCS3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet induced obesity. *Nat Med* 2004;10(7): 739–43.
 - [10] Kievit P, Howard JK, Badman MK, Balthasar N, Coppari R, Mori H, et al. Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. *Cell Metab* 2006;4(2):123–32.
 - [11] Galletti F, D'Elia L, Barba G, Siani A, Cappuccio FP, Farinaro E, et al. High-circulating leptin levels are associated with greater risk of hypertension in men independently of body mass and insulin resistance: results of an eight-year follow-up study. *J Clin Endocrinol Metab* 2008;93(10):3922–6.
 - [12] Galletti F, D'Elia L, De Palma D, Russo O, Barba G, Siani A, et al. Hyperleptinemia is associated with hypertension, systemic inflammation and insulin resistance in overweight but not in normal weight men. *Nutr Metab Cardiovasc Dis* 2012;22(3):300–6.
 - [13] Galletti F, Barbato A, Versiero M, Iacone R, Russo O, Barba G, et al. Circulating leptin levels predict the development of metabolic syndrome in middle-aged men: an 8-year follow-up study. *J Hypertens* 2007;25(8):1671–7.
 - [14] D'Elia L, Manfredi M, Perna L, Iacone R, Russo O, Strazzullo P, et al. Circulating leptin levels predict the decline in renal function with age in a sample of adult men (The Olivetti Heart Study). *Intern Emerg Med* 2018 Aug 16. <https://doi.org/10.1007/s11739-018-1924-9>.
 - [15] Franks PW, Brage S, Luan J, Ekelund U, Rahman M, Farooqi IS, et al. Leptin predicts a worsening of the features of the metabolic syndrome independently of obesity. *Obes Res* 2005;13(8):1476–84.
 - [16] Iacone R, Russo O, Russo P, Venezia A, Varriale V, Gerardi MC, et al. Plasma leptin measurements in epidemiological investigation: comparison of two commonly used assays and estimate of regression dilution bias. *Nutr Metab Cardiovasc Dis* 2002;12(2): 71–9.
 - [17] Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, et al. Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes* 1998;47:1643–9.
 - [18] Mancía G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension [ESH] and of the European Society of Cardiology [ESC]. *J Hypertens* 2013;31: 1281–357.
 - [19] Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* 1997; 138(8):3395–401.
 - [20] Chinookoswong N, Wang JL, Shi ZQ. Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. *Diabetes* 1999;48(7):1487–92.
 - [21] Sáinz N, Rodríguez A, Catalán V, Becerril S, Ramírez B, Lancha A, et al. Leptin reduces the expression and increases the phosphorylation of the negative regulators of GLUT4 traffic TBC1D1 and TBC1D4 in muscle of ob/ob mice. *PLoS One* 2012;7(1):1–12.
 - [22] Ceddia RB, William Jr WN, Curi R. Comparing effects of leptin and insulin on glucose metabolism in skeletal muscle: evidence for an effect of leptin on glucose uptake and decarboxylation. *Int J Obes Relat Metab Disord* 1999;23(1):75–82.
 - [23] German J, Kim F, Schwartz GJ, Havel PJ, Rhodes CJ, Schwartz MW, et al. A neurocircuit involving the vagus nerve. *Endocrinology* 2009;150(10):4502–11.
 - [24] Tanida M, Yamamoto N, Morgan DA, Kurata Y, Shibamoto T, Rahmouni K. Leptin receptor signaling in the hypothalamus regulates hepatic autonomic nerve activity via phosphatidylinositol 3-kinase and AMP-activated protein kinase. *J Neurosci* 2015 Jan 14;35(2):474–84.
 - [25] Aijälä M, Malo E, Ukkola O, Bloigu R, Lehenkari P, Autio-Harmainen H, et al. Long-term fructose feeding changes the expression of leptin receptors and autophagy genes in the adipose tissue and liver of male rats: a possible link to elevated triglycerides. *Genes Nutr* 2013;8(6):623–35.
 - [26] Katsiki N, Mikhailidis DP, Banach M. Leptin, cardiovascular diseases and type 2 diabetes mellitus. *Acta Pharmacol Sin* 2018 Jul; 39(7):1176–88.
 - [27] Bellia A, Rizza S, Lombardo MF, Donadel G, Fabiano R, Andreadi K, et al. Deterioration of glucose homeostasis in type 2 diabetic patients one year after beginning of statins therapy. *Atherosclerosis* 2012;223:197–203.
 - [28] Derosa G, Maffioli P, Sahebkar A. Improvement of plasma adiponectin, leptin and C-reactive protein concentrations by orlistat: a systematic review and meta-analysis. *Br J Clin Pharmacol* 2016 May;81(5):819–34.
 - [29] Dolezelova E, Stein E, Derosa G, Maffioli P, Nachtigal P, Sahebkar A. Effect of ezetimibe on plasma adipokines: a systematic review and meta-analysis. *Br J Clin Pharmacol* 2017 Jul;83(7):1380–96.
 - [30] Saad MI, Kamel MA, Hanafi MY. Modulation of adipocytokines production and serum nefa level by metformin, glimepiride, and sitagliptin in HFD/STZ diabetic rats. *Biochem Res Int* 2015;2015: 138134.
 - [31] Li S, Li H, Wang R, Zhang JP. The effect of sitagliptin on obese patients with insulin treatment-induced diabetes mellitus. *Eur Rev Med Pharmacol Sci* 2017;21:3490–5.
 - [32] Negrotto L, Farez MF, Correale J. Immunologic effects of metformin and pioglitazone treatment on metabolic syndrome and multiple sclerosis. *JAMA Neurol* 2016;73:520–8.
 - [33] Vickers SP, Cheetham SC, Headland KR, Dickinson K, Grempler R, Mayoux E, et al. Combination of the sodium-glucose cotransporter-2 inhibitor empagliflozin with orlistat or sibutramine further improves the body-weight reduction and glucose homeostasis of obese rats fed a cafeteria diet. *Diabetes Metab Syndr* 2014;7:265–75.
 - [34] Li N, Zhao Y, Yue Y, Chen L, Yao Z, Niu W. Liraglutide ameliorates palmitate-induced endothelial dysfunction through activating AMPK and reversing leptin resistance. *Biochem Biophys Res Commun* 2016;478:46–52.
 - [35] Frössing S, Nylander M, Chabanova E, Frystyk J, Holst JJ, Kistorp C, et al. Effect of liraglutide on ectopic fat in polycystic ovary syndrome: a randomized clinical trial. *Diabetes Obes Metab* 2018;20: 215–8.
 - [36] Sun YM, Li J, Luan Y, Wang LF. Effect of statin therapy on leptin levels in patients with coronary heart disease. *Peptides* 2010;31: 1205–7.
 - [37] Buldak L, Dulawa-Buldak A, Labuzek K, Okopien B. Effects of 90-day hypolipidemic treatment on insulin resistance, adipokines and proinflammatory cytokines in patients with mixed hyperlipidemia and impaired fasting glucose. *Int J Clin Pharmacol Ther* 2012;50:805–13.
 - [38] Singh P, Zhang Y, Sharma P, Covassin N, Soucek F, Friedman PA, et al. Statins decrease leptin expression in human white adipocytes. *Physiol Rep* 2018 Jan;6(2). <https://doi.org/10.14814/phy2.13566>.
 - [39] Owecki M, Nikisch E, Miczke A, Pupek-Musialik D, Sowiński J. Leptin, soluble leptin receptors, free leptin index, and their relationship with insulin resistance and BMI: high normal BMI is the threshold for serum leptin increase in humans. *Horm Metab Res* 2010 Jul;42(8):585–9.
 - [40] Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, et al. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996;348:159–61.