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RESEARCH ARTICLE

Leptin as a possible link between obesity, hypertension and insulin resistance in Egyptian females

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Abstract

Leptin is a peptide expressed and secreted from adipocytes. It serves an adipostat function that regulates fat mass by decreasing food intake and increasing energy expenditure or thermogenesis. Leptin is found to be related to obesity, hypertension and insulin resistance in some studies. Our aim was to investigate leptin as a possible link between obesity, hypertension and insulin resistance in Egyptian females. Eighty adult females were recruited, blood pressure and body measures were recorded and a fasting blood sample was obtained for the quantitation of glucose, lipid profile, insulin and leptin. Running several statistical analyses, we found that leptin was significantly elevated (all at $p<0.001$) in case of insulin resistance and obesity when classified upon body mass index, waist circumference or waist/height ratio but not upon waist/hip ratio nor conicity index. On the other hand, hypertension and metabolic syndrome did not show leptin variation in cases than in controls.

Introduction

Leptin is a 16 kDa peptide consisting of 167 amino acids expressed and secreted from white adipose tissue as part of a feedback loop where changes in adipose tissue mass are signaled to CNS centers to regulate fat mass by decreasing food intake and increasing energy expenditure or thermogenesis (Haynes et al., 1997; Chakraborty and Chakraborty, 2012), so it serves an "adipostat" function (Eikelis et al., 2003). It is named leptin after the Greek leptos for "thin" (Haynes et al., 1997). It is generally thought that human obesity is a state of relative leptin resistance and plasma leptin levels were proportionally greater in the obese than in their leaner counterparts. This may be attributed to both greater leptin production per unit of body fat "due to resistance" and to increased production of leptin from the increased size of body fat mass (Ahrén et al., 1997). Variations in total body fat content and fat distribution explain approximately 50% of circulating leptin level variance (Nawata et al., 2008).

Leptin increases sympathetic activity causing a slow but steady rise in arterial pressure (usually minimal effect) and increased heart rate. However, these effects are thought to be attenuated through stimulation of endothelium-derived nitric oxide (Kuo et al., 2001) and through the action of leptin on the renal tubules to promote natriuresis and diuresis (Haynes et al., 1997). This issue is controversial, a study states that a graded statistically significant association between plasma leptin concentration and systolic and diastolic blood pressure exists independently of body mass and of abdominal adiposity (Barba et al., 2003), while another study shows no correlation at all between blood pressure and leptin (Franks et al., 2005). The level of circulating leptin also correlates with insulin secretion, explaining 2% of its variance (Saad et al., 1997) although no abnormalities in circulating leptin have been associated with glucose intolerance or diabetes (Ahrén et al., 1997). Long-term therapy with subcutaneous injections of

recombinant leptin provided sustained beneficial effects on lowering fat mass, hyperinsulinemia, and hyperlipidemia in human obese subjects (Cheung and Mao, 2012).

Over the few recent years, several studies addressed leptin and its association with diseases like obesity (Schinzari et al., 2013), hypertension (Galletti et al., 2012; Moraes et al., 2013), insulin resistance (Askari et al., 2010) and metabolic syndrome (Esteghamati et al., 2011; Martins et al., 2012). Nevertheless, none was conducted on Egyptians yet. So, the main aim of the present study was to investigate the variations in leptin levels within several disorders such as obesity, hypertension and insulin resistance in a sample of Egyptian females.

Subjects and Methods

Eighty adult non-pregnant females were chosen from an outpatient clinic, Kasr El-Aini Hospital, affiliated to Cairo University. Subjects suffering hematologic, renal, hepatic or thyroid disorders were excluded. For each participant, full medical and family history were checked, anthropometric measurements and blood pressure were recorded, in addition to taking a fasting blood sample. A written informed consent was obtained from all participants before enrollment in the study. The research protocol was approved by the Research Ethical Committee of the Faculty of Medicine, Cairo University. The research also conformed to the ethical guidelines of the Declaration of Helsinki.

Anthropometric measurements

Body weight was measured using an electronic scale while wearing light clothes and no shoes. Non-stretchable tape was used to measure height in an upright standing position without shoes, waist circumference (WC) was measured at the midpoint between the bottom of the ribs and the top of the iliac crest, and finally, hip circumference was measured at the largest posterior extension of the buttocks.

Blood pressure

For systolic and diastolic blood pressure (SBP and DBP), auscultation method using a mercury sphygmomanometer and a stethoscope was performed in sitting position after resting for at least 10 min, following the recommendations of the American Heart Association (Pickering et al., 2005).

Blood sampling

A 12 hours-fasting blood sample was taken and divided into two portions for further analysis: 1 ml was fluorinated for determination of fasting blood glucose level, and 2 ml was prepared into serum for assay of insulin, leptin and lipid profile.

Biochemical laboratory investigations

Enzymatic colorimetric methods were used to determine plasma glucose level (Trinder, 1969), serum triglycerides (TG) level (Megraw et al., 1979) and serum total cholesterol (TC) level (Allain et al., 1974). Serum high density lipoproteins (HDL) level was determined using a precipitating kit (Grove, 1979). Low density lipoproteins (LDL) level was calculated using the equation: LDL=TC – HDL – (TG/5) (Friedewald et al., 1972). All kits were supplied by PIONTE SCIENTIFIC Inc. (Canton, MI, USA).

Enzyme-linked immunosorbent assay (ELISA)

The kits used for determination of leptin and insulin levels were supplied by DiaSource Europe S.A (Nivelles, Belgium). Serum leptin level was determined using solid phase Enzyme Amplified Sensitivity Immunoassays (EASIA). Its detection limit was 0.04 ng/ml, the inter-assay coefficient of variance (CV) was 10.2–12.7% and the intra-assay CV was 3.5–13.3%. Insulin was assayed using an ELISA kit that employed a quantitative sandwich enzyme immunoassay technique. Its detection limit was 0.881 pmol/L, the inter-assay CV was 6.9–7.5% and the intra-assay CV was 3.7–4.0%.

Calculated indices

Several indices were calculated for each subject. The two indices for blood pressure were mean arterial pressure (MAP) and pulse pressure (PP**) (Chae et al., 1999)**. Insulin resistance indices were homeostasis model assessment (HOMA), quantitative insulin sensitivity check index (QUICKI) and fasting glucose insulin ratio (FGIR) (Keskin et al., 2005). Adiposity indices were body mass index (BMI), waist/hip ratio (WHR) (Abolfotouh et al., 2011), waist/height ratio (WHtR) (Abdullah et al., 2009), and conicity index (C-index**)** (Chakraborty and Chakraborty, 2012). Finally, lipid ratios were TC/HDL and LDL/HDL (Avenue, 1992).

Subjects grouping

The studied individuals were classified into three groups based on both BMI and leptin level [obese patients with high leptin level (n=26), obese patients with low leptin level (n=25) and lean control subjects (n=29)]. The cut-off used for leptin categorization was 11 ng/ml (Esteghamati et al., 2011). Subjects were also divided into 2 categories based on each of the following cut-offs for hypertension (SBP ≥ 140 or DBP ≥ 90 (Pickering et al., 2005)), BMI (≥ 26 (Masuo et al., 2000)), WC (≥ 92 cm (Ibrahim et al., 2011)), WHR (≥ 0.85 (Deurenberg and Yap, 1999)), WHtR (≥ 0.52 (Abdullah et al., 2009)**)**, HOMA (≥ 3.5 (Ramadan, 2013)) and metabolic syndrome (patients having at least 3 of 5 criteria (WC ≥ 92 cm, SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg, fasting glucose ≥ 100 mg/dl, TG ≥ 150 mg/dl and HDL < 50 mg/dl) (Sliem et al., 2012)).

Statistical analyses

All the statistical analyses were carried out using SPSS (Chicago, IL, USA) software, version 17.0 for Microsoft Windows. Data were presented as mean ± standard deviation for quantitative variables, and as numbers and percentages for categorical variables. Comparisons of the continuous parameters were made with independent sample t-test between two groups and ANOVA between three groups. Continuous variables were converted into categorical values to assess the association between leptin and some metabolic parameters using the chi-square test. A value of $p<0.05$ (two-tailed) was considered significant.

Results

We first classified our sample of Egyptian females into 2 groups based on different parameters as shown in Table 1 in order to investigate leptin levels among the different disorders under study. Leptin was significantly elevated in obese subjects when classified upon BMI, WC or WHtR but not WHR nor C-index. Also, insulin resistance was accompanied by significantly higher leptin level. On the other hand, hypertension and metabolic syndrome did not show leptin variation in cases than in controls.

Using ANOVA, we then analyzed our sample when stratified based on BMI and leptin level into three groups; obese subjects with higher leptin levels than 11 ng/ml (leptin: 17.99 ± 5.27), obese subjects with leptin levels below 11 ng/ml (leptin: 7.06 ± 2.52) and normal control subjects (leptin: 4.10 ± 1.83). As shown in ***Table*** 2, all anthropometric parameters showed a highly significant association with leptin levels at $p < 0.001$, except WHR and C-index that were not significantly different between the three groups. The differences in blood pressure parameters were non-significant although a pattern of lower blood pressure was noticed in lean subjects. Also, obese subjects having higher leptin levels had slightly lower blood pressure than obese subjects having lower leptin levels. By comparing the lipid profile between the three groups, it was found that triglycerides were significantly higher in the obese high leptin group than in the lean group. Also, the obese high leptin group showed a significant difference in HDL and cholesterol ratios compared to subjects with lower leptin levels, whether they were obese or lean. Although levels of TC and LDL showed the same pattern, they did not reach statistical significance. As for the indices of insulin resistance: glucose, insulin and HOMA showed significant elevation in obese subjects with high leptin compared to lean subjects, while QUICKI was significantly higher in both obese groups than the lean group, while FGIR was not different between the three groups.

To further investigate the association of leptin with hypertension, insulin resistance and metabolic syndrome, we used chi-square to check the frequencies of subjects for any significant difference between groups, but none was found, as shown in ***Table 3***.

We also checked the possible association between obesity and hypertension and whether leptin affected that relationship. No significant association was found between hypertension and obesity based on all anthropometric parameters used, except WHR. As shown in ***Table 4***, high WHR was associated with a significantly higher prevalence of hypertension than normal WHR subjects both having high or low leptin levels, but the odds were higher in case of leptin < 11 ng/ml (OR= 11.00 vs 3.94).

Discussion

In this study on a sample of Egyptian females, our aim was to investigate the variations in leptin levels within several disorders such as obesity, hypertension and insulin resistance. Our study was sex-specific and included only females since gender is a significant determinant of leptin level explaining 28% of its variance, where plasma leptin levels are higher in females than males, independent of BMI and age (Takizawa et al., 2001; Martins et al., 2012),

probably because of the fact that leptin levels have a higher correlation with subcutaneous fat -which is higher in females- than with visceral fat levels and the fact that leptin levels are inversely correlated with testosterone levels (Shi and Clegg, 2009).

Stratification of obese subjects having high BMI according to their leptin level showed that leptin level highly paralleled the anthropometric indices: weight, WC, hip, BMI and WHtR, but not WHR nor C-index. The higher the leptin level, the higher these indices were, being significantly higher in severely obese high leptin group than in the obese low leptin group which was also significantly higher than the lean control group. This reflects resistance to leptin effects on appetite suppression that increases with higher leptin levels making obesity more severe. Previous studies also stated similar findings that leptin level correlates significantly with body weight, BMI and percent body fat but not with WHR (Saad et al., 1997; Martins et al., 2012).

As for hypertension, the pattern was different, although all were non-significant. Blood pressure was noticed to be lower in control lean subjects. On the other hand, obese subjects having higher leptin levels had slightly lower blood pressure than obese subjects having lower leptin levels. Also, considering the possible association between obesity and hypertension and whether leptin affected that relationship, no significant association was found between hypertension and obesity based on all anthropometric parameters measured except WHR that showed possible association. The prevalence of hypertension was significantly higher in subjects having high WHR than in subjects having normal WHR, at both levels of leptin, but the odds were higher in case of leptin levels <11 ng/ml. This higher association between hypertension and obesity (based on WHR) at lower leptin elevation and the slightly higher blood pressure at lower leptin elevations, may both be attributed to slight increase of blood pressure with increasing leptin followed by resistance to leptin effects on blood pressure at higher leptin values. It was previously found that elevated leptin level causes only minimal stimulation of sympathetic activity in obese subjects which may be explained by obesity-associated leptin resistance on the hypothalamus, thus, resistance to the effects of leptin both on satiety and sympathetic activity (Kuo et al., 2001; Schinzari et al., 2013). Also, the lack of an acute pressor effect of leptin may be due to opposing depressor effects, such as stimulation of endothelial-derived nitric oxide, which offsets the effects of increased sympathetic activity (Kuo et al., 2001). Recent studies tied elevated plasma leptin with high blood pressure only to special conditions such as in overweight and obese subjects (Galletti et al., 2012) or in uncontrolled resistant hypertension not in well-controlled patients (Moraes et al., 2013).

In our study, lipid profile parameters were found to be related to leptin levels not to BMI classification. Subjects having high BMI and high leptin level showed a significant difference in lipid parameters (mainly TG and cholesterol ratios) than subjects with lower leptin, regardless of their BMI. On the other hand, levels of TC and LDL showed the same pattern, but they did not reach statistical significance. This probably suggests that leptin level should be taken in consideration when evaluating the relationship between obesity and lipid profile. Unlike our results, previous studies showed no significant correlations between plasma leptin levels and lipids (Leyva et al., 1998; Franks et al., 2005).

As for the indices of insulin resistance: glucose, insulin and HOMA showed significant elevation in the obese high leptin than the lean subjects, while QUICKI was significantly different in both obese groups than the lean group. In addition, leptin differed significantly between the insulin resistant and the insulin sensitive subjects, suggesting a relationship between leptin and insulin resistance. It seemed that Quicki was the insulin resistance parameter to differ significantly in response to obesity at the slightest increase of leptin than normal, while glucose, insulin and HOMA were significantly elevated only in the obese high leptin than the controls. On the other hand, FGIR was not a sensitive indicator of insulin resistance in our sample of Egyptian females. It was previously mentioned that serum insulin levels are directly related to leptin levels regardless of gender and age (Thomas et al., 2000; Askari et al., 2010; Martins et al., 2012) or in females only (Takizawa et al., 2001; Cnop et al., 2002), while another study stated that leptin may not be directly related to glucose intolerance (Saad et al., 1997).

Conclusions

In the currently studied sample of Egyptian females, anthropometric indices showed highly significant variations matching leptin levels except WHR and C-index. Blood pressure did not vary significantly between subjects with high or low leptin levels. Insulin, HOMA and QUICKI were the best insulin resistance parameters that significantly mirrored leptin levels, unlike FGIR. TG, HDL and cholesterol ratios were the best to match leptin-based differences, unlike TC or LDL.

Tables

Table 1: Comparison of leptin level (ng/ml) based on different groupings

Group (n)	**Leptin**	Group (n)	**Leptin**	**t**	**Sig.**
Obese BMI (51)	12.05 ± 7.07	Normal BMI (29)	4.60 ± 4.17	5.54	< 0.001
Large WC (53)	11.26 ± 7.30	Normal WC (27)	3.97 ± 2.07	6.48	< 0.001
Large WHtR (69)	10.29 ± 7.20	Normal WHtR (11)	3.23 ± 0.83	7.34	< 0.001
Large WHR (55)	9.21 ± 6.35	Normal WHR (25)	9.77 ± 8.74	-0.27	0.79
Large C-index (44)	8.73 ± 6.46	Normal C-index (36)	10.22 ± 7.92	-0.87	0.39
Hypertensive (40)	9.13 ± 7.44	Normotensive (40)	9.64 ± 6.90	-0.30	0.77
Insulin Resistant (62)	10.31 ± 7.53	Insulin Sensitive (18)	5.70 ± 3.42	3.39	0.001
MetS (45)	9.89 ± 7.58	No MetS (35)	8.83 ± 6.65	0.62	0.54

Data are presented as mean ± standard deviation.
BMI: body mass index, C-index: conicity index, MetS: metabolic syndrome, Sig.: significance, t: t-test statistic, WC: waist circumference, WHR: waist/hip ratio, WHtR: waist/height ratio.

Table 2: Comparison of different indices between studied groups via ANOVA

	Obese subjects with leptin > 11 (26)	**Obese subjects with leptin < 11 (25)**	**Lean subjects (29)**	**F**	**Sig.**
Leptin *	17.99 ± 5.27 [1]	7.06 ± 2.52 [2]	4.10 ± 1.83 [3]	117.33	< 0.001
Age	48.81 ± 6.74	49.24 ± 5.42	47.52 ± 11.42	0.31	0.74
Weight *	98.37 ± 16.25 [1]	80.50 ± 15.42 [2]	53.91 ± 9.03 [3]	72.84	< 0.001
Height	156.27 ± 5.10	155.92 ± 6.75	153.66 ± 7.40	1.31	0.28
Waist *	113.31 ± 11.27 [1]	104.24 ± 10.32 [2]	85.48 ± 10.63 [3]	48.28	< 0.001
Hip *	125.12 ± 13.14 [1]	113.48 ± 9.61[2]	96.21 ± 8.23 [3]	53.35	< 0.001
BMI *	40.30 ± 6.48 [1]	33.00 ± 5.44 [2]	22.70 ± 2.58 [3]	85.33	< 0.001
WHR	0.91 ± 0.09	0.92 ± 0.08	0.89 ± 0.09	0.99	0.38
WHtR *	0.73 ± 0.08 [1]	0.67 ± 0.07 [2]	0.56 ± 0.07 [3]	41.12	< 0.001
C-index	1.315 ± 0.087	1.34 ± 0.099	1.328 ± 0.124	0.35	0.71
SBP	130.77 ± 15.67	134.60 ± 17.26	128.97 ± 22.26	0.62	0.54

DBP	84.23 ± 9.87	86.00 ± 10.99	81.21 ± 11.78	1.34	0.27
MAP	99.74 ± 11.26	102.20 ± 12.53	97.13 ± 14.90	1.02	0.37
PP	46.54 ± 9.46	48.60 ± 10.16	47.67 ± 12.65	0.23	0.80
TC	168.62 ± 35.09	166.20 ± 34.66	156.31± 20.80	1.27	0.29
TG *	96.69 ± 37.83 [1]	86.40 ± 35.17	69.83 ± 13.92 [2]	5.51	0.006
LDL	115.31 ± 30.64	110.44 ± 30.39	103.24 ± 24.94	1.24	0.29
HDL *	33.58 ± 8.02 [1]	38.88 ± 8.25 [2]	39.59 ± 9.03 [2]	3.99	0.022
TC/HDL *	5.18 ± 1.17 [1]	4.39 ± 1.04 [2]	4.17 ± 1.20 [2]	5.77	0.005
LDL/HDL *	3.56 ± 1.08 [1]	2.93 ± 0.93 [2]	2.81 ± 1.12 [2]	3.97	0.023
Glu *	110.69 ± 17.26 [1]	105.64 ± 17.89	96.79 ± 21.91 [2]	3.69	0.03
Insulin *	20.15 ± 6.36 [1]	18.56 ± 4.05	16.58 ± 2.96 [2]	4.11	0.02
HOMA *	5.61 ± 2.38 [1]	4.80 ± 1.19	3.95 ± 1.09 [2]	7.00	0.002
QUICKI *	0.302 ± 0.015 [1]	0.305 ± 0.01[1]	0.314 ± 0.012 [2]	7.64	0.001
FGIR	5.93 ± 1.75	5.99 ± 1.86	6.06 ± 1.89	0.03	0.97

Data are presented as mean ± standard deviation.
BMI: body mass index, C-index: conicity index, DBP: diastolic blood pressure, F: ANOVA statistic, FGIR: fasting glucose insulin ratio, Glu: glucose level, HDL: high density lipoproteins, HOMA: homeostasis model of assessment of insulin resistance, LDL: low density lipoproteins, MAP: mean arterial pressure, PP: pulse pressure, QUICKI: quantitative insulin sensitivity check index, Sig: significance, SBP: systolic blood pressure, TC: total cholesterol, TG: triglycerides, WC: waist circumference, WHR: waist/hip ratio, WHtR: waist/height ratio, *: significant difference, superscripted letters: summary of Duncan post-hoc tests.

Table 3: Association of leptin with different disorders using chi-square test

	Hypertension		**Insulin Resistance**		**Metabolic Syndrome**	
	Hypertensive (40)	**Normotensive (40)**	**Resistant (62)**	**Sensitive (18)**	**Yes (45)**	**No (35)**
Leptin > 11 (26)	12 (46.2%)	14 (53.8%)	23 (88.5%)	3 (11.5%)	16 (61.5%)	10 (38.5%)
Leptin < 11 (54)	28 (51.9%)	26 (48.1%)	39 (72.2%)	15 (27.8%)	29 (53.7%)	25 (46.3%)
	χ^2=0.23, p=0.81		χ^2=2.65, p=0.15		χ^2=0.44, p=0.63	

The frequency of subjects in each group was presented as n (%).
χ^2: chi-square statistic, p: significance level.

Table 4: Effect of leptin on the association of hypertension and obesity as classified by waist/hip ratio (WHR)

	Leptin > 11		**Leptin < 11**	
	Large WHR (18)	**Normal WHR (8)**	**Large WHR (37)**	**Normal WHR (17)**
Hypertensive (40)	11 (91.7%)	1 (8.3%)	23 (82.1%)	5 (17.9%)
Normotensive (40)	7 (50.0%)	7 (50.0%)	14 (53.8%)	12 (46.2%)
	χ^2=5.00, p=0.04* OR=3.94, CI=1.145-13.58		χ2=5.27, p=0.036* OR=11.00, CI=1.103-109.67	
The frequency of subjects in each group was presented as n (%). CI: 95% confidence interval, OR: odds ratio, p: significance level, χ^2: chi-square statistic, *: significant difference.				

References

1. Abdullah, A., Abd El-baky, A., Bakry, S., & Abd-el Hay, E. (2009): Adiponectin and Leptin Levels and Their Risk in Coronary Artery Diseases in Obese Egyptian Men. Research Journal of Medicine and Medical Sciences, 4(2): 526–532.
2. Abolfotouh, M. A., Sallam, S. A., Mohammed, M. S., Loutfy, A. A., & Hasab, A. A. (2011): Prevalence of elevated blood pressure and association with obesity in egyptian school adolescents. International Journal of Hypertension, 2011(i): 1–8.
3. Ahrén, B., Larsson, H., Wilhelmsson, C., Birgitta, N., & Olsson, T. (1997): Regulation of Circulating Leptin in Humans. Endocrine, 7(1): 1–8.
4. Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., & Fu, P. C. (1974): Enzymatic Determination of Total Serum Cholesterol. Clinical Chemistry, 20(4): 470–475.
5. Askari, H., Tykodi, G., Liu, J., & Dagogo-Jack, S. (2010): Fasting plasma leptin level is a surrogate measure of insulin sensitivity. Journal of Clinical Endocrinology and Metabolism, 95(8): 3836–3843.
6. Avenue, G. (1992): Joint Effects of Serum Triglyceride and LDL Cholesterol and HDL Cholesterol Concentrations on Coronary Heart Disease Risk in the Helsinki Heart Study. Implications for treatment. Circulation, 85: 37–45.
7. Barba, G., Russo, O., Siani, A., Iacone, R., Farinaro, E., Gerardi, M. C., … Strazzullo, P. (2003): Plasma leptin and blood pressure in men: graded association independent of body mass and fat pattern. Obesity Research, 11(1): 160–166.
8. Chae, C. U., Pfeffer, M. A., Glynn, R. J., Mitchell, G. F., Taylor, J. O., & Hennekens, C. H. (1999): Increased Pulse Pressure and Risk of Heart Failure in the Elderly. The Journal of American Medical Association, 281(7): 634–639.
9. Chakraborty, B. M., & Chakraborty, R. (2012): Bioinformatics of Obesity. In Handbook of Statistics (Vol. 28, pp. 433–477): Elsevier Inc.
10. Cheung, W. W., & Mao, P. (2012): Recent advances in obesity: genetics and beyond. ISRN Endocrinology, 2012: 1–11.
11. Cnop, M., Landchild, M. J., Vidal, J., Havel, P. J., Knowles, N. G., Carr, D. R., … Kahn, S. E. (2002): The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations : distinct metabolic effects of two fat compartments. Diabetes, 51(4): 1005–1015.
12. Deurenberg, P., & Yap, M. (1999): The assessment of obesity : methods for measuring body fat and global prevalence of obesity. Baillière's Clinical Endocrinology and Metabolism, 13(1): 1–11.
13. Eikelis, N., Schlaich, M., Aggarwal, A., Kaye, D., & Esler, M. (2003): Interactions Between Leptin and the Human Sympathetic Nervous System. Hypertension, 41: 1072–1079.
14. Esteghamati, A., Zandieh, A., Zandieh, B., Khalilzadeh, O., Meysamie, A., Nakhjavani, M., & Gouya, M. M. (2011): Leptin cut-off values for determination of metabolic syndrome: third national surveillance of risk factors of non-communicable diseases in Iran. Endocrine, 40(1): 117–123.
15. Franks, P. W., Brage, S., Luan, J., Ekelund, U., Rahman, M., Farooqi, I. S., … Wareham, N. J. (2005): Leptin predicts a worsening of the features of the metabolic syndrome independently of obesity. Obesity Research, 13(8): 1476–1484.
16. Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, 18(6): 499–502.
17. Galletti, F., D'Elia, L., De Palma, D., Russo, O., Barba, G., Siani, a., … Strazzullo, P. (2012): Hyperleptinemia is associated with hypertension, systemic inflammation and insulin resistance in overweight but not in normal weight men. Nutrition, Metabolism and Cardiovascular Diseases, 22(3): 300–306.
18. Grove, T. H. (1979): Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clinical Chemistry, 25(4): 560–564.
19. Haynes, W. G., Sivitz, W. I., Morgan, D. A., Walsh, S. A., & Mark, A. L. (1997): Sympathetic and Cardiorenal Actions of Leptin. Hypertension, 30(3): 619–623.
20. Ibrahim, M. M., Elamragy, A. a, Girgis, H., & Nour, M. a. (2011): Cut off values of waist circumference and associated cardiovascular risk in Egyptians. BMC Cardiovascular Disorders, 11(53): 1–8.
21. Keskin, M., Kurtoglu, S., Kendirci, M., Atabek, M. E., & Yazici, C. (2005): Homeostasis Model Assessment Is More Reliable Than the Fasting Glucose/Insulin Ratio and Quantitative Insulin Sensitivity Check Index for Assessing Insulin Resistance Among Obese Children and Adolescents. Pediatrics, 115(4): 500–503.
22. Kuo, J. J., Jones, O. B., & Hall, J. E. (2001): Inhibition of NO Synthesis Enhances Chronic Cardiovascular and

Renal Actions of Leptin. Hypertension, 37(2): 670–676.
23. Leyva, F., Godsland, I. F., Ghatei, M., Proudler, A. J., Aldis, S., Walton, C., … Stevenson, J. C. (1998): Hyperleptinemia as a Component of a Metabolic Syndrome of Cardiovascular Risk. Arteriosclerosis, Thrombosis, and Vascular Biology, 18(6): 928–933.
24. Martins, M. do C., Lima Faleiro, L., & Fonseca, A. (2012): Relationship between leptin and body mass and metabolic syndrome in an adult population. Portuguese Journal of Cardiology, 31(11): 711–719.
25. Masuo, K., Mikami, H., Itoh, M., Ogihara, T., & Tuck, M. L. (2000): Sympathetic activity and body mass index contribute to blood pressure levels. Hypertension Research : Official Journal of the Japanese Society of Hypertension, 23(4): 303–310.
26. Megraw, R. E., Dunn, D. E., & Biggs, H. G. (1979): Manual and continuous-flow colorimetry of triacylglycerols by a fully enzymic method. Clinical Chemistry, 25(2): 273–278.
27. Moraes, C. de H., Figueiredo, V. N., de Faria, A. P. C., Barbaro, N. R., Sabbatini, A. R., Quinaglia, T., … Júnior, H. M. (2013): High-circulating leptin levels are associated with increased blood pressure in uncontrolled resistant hypertension. Journal of Human Hypertension, 27(4): 225–230.
28. Nawata, K., Ishida, H., Uenishi, K., & Kudo, H. (2008): The relationship between serum leptin concentration and the percentage of body fat in Japanese high school students. Asia-Pacific Journal of Public Health, 20: 180–188.
29. Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., … Roccella, E. J. (2005): Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans. Hypertension, 45(1): 142–161.
30. Ramadan, R. A. (2013): Establishment of Reference Interval for Homeostasis Model Assessment of Insulin Resistance in Healthy Adult Males: a Pilot Study in Alexandria Governorate, Egypt. Iranian Journal of Diabetes and Obesity, 5(3): 91–97.
31. Saad, M. F., Damani, S., Gingerich, R. L., Riad-gabriel, M. G., Khan, A., Boyadjian, R., … Kamdar, V. (1997): Sexual Dimorphism in Plasma Leptin Concentration. Journal of Clinical Endocrinology and Metabolism, 82(2): 579–584.
32. Schinzari, F., Tesauro, M., Rovella, V., Di Daniele, N., Mores, N., Veneziani, A., & Cardillo, C. (2013): Leptin stimulates both endothelin-1 and nitric oxide activity in lean subjects but not in patients with obesity-related metabolic syndrome. J Clin Endocrinol Metab, 98(3): 1235–1241.
33. Shi, H., & Clegg, D. J. (2009): Sex differences in the regulation of body weight. Physiology & Behavior, 97(2): 199–204.
34. Sliem, H. A., Ahmed, S., Nemr, N., & El-Sherif, I. (2012): Metabolic syndrome in the Middle East. Indian Journal of Endocrinology and Metabolism, 16(1): 67–71.
35. Takizawa, H., Ura, N., Saitoh, S., Wang, L., Higashiura, K., Takagi, S., … Shimamoto, K. (2001): Gender Difference in The Relatioships Among Hyperleptinemia, Hyperinsulinemia and Hypertension. Clinical and Experimental Hypertension, 23(4): 357–368.
36. Thomas, T., Burguera, B., Melton, L. J., Atkinson, E. J., O'Fallon, W. M., Riggs, B. L., & Khosla, S. (2000): Relationship of serum leptin levels with body composition and sex steroid and insulin levels in men and women. Metabolism, 49(10): 1278–1284.
37. Trinder, P. (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry, 6(1): 24–27.