INTERRELATIONS BETWEEN LEPTIN & OBESITY

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ABSTRACT

Objective: Excess body weight is the sixth most important risk factor contributing to the overall burden of disease worldwide. The circulating leptins have been found to play a vital role in the regulation of appetite, glucose homeostasis and body fat. Therefore, this study was designed to measure serum leptin and insulin resistance in non obese and obese young adults and to correlate them with obesity parameters: body mass index, waist circumference, waist-to-hip ratio and metabolic indices.

Methods: A total of 43 non- obese subjects, 20 male and 23 female aged 20- 25 years and 46 obese subjects, 25 male and 21 female with age 28- 37 years were studied. All subjects selected for study were normotensive and non-diabetic. Variables measured were Body Mass Index (BMI), waist to hip ratio(WHR), blood pressure, serum leptin, insulin, fasting blood glucose, oral glucose tolerance test (OGTT), and lipid profile.

Results: Serum leptin was significantly higher in females than males 8.8 ± 2.10 SEM and 2.2 ± 0.26 ng/ml SEM respectively in non-obese. As well, Serum leptin was significantly higher in females than males 23.0 ± 3.98 SEM and 12.5 ± 2.24 ng/ml SEM respectively in obese group. Serum leptin was significantly higher in obese males, and obese females compared to non- obese subjects. Serum leptin significantly, and positively correlated with BMI ($r=0.440$), hip circumference ($r=0.425$), insulin ($r=0.334$), and homeostasis Model of Assessment - Insulin Resistance (HOMA-IR) $r=0.334$ whereas HOMA-IR positively correlated with BMI, waist Circumference, fasting insulin, fasting blood glucose, triglycerides (TG), TG/HDL-Cholesterol ratio and negatively correlated with HDL-Cholesterol.

Conclusions: Serum leptin levels increase with obesity, and are higher in females in both non-obese and obese individuals. Serum leptin significantly correlated with hip circumference. Increased serum leptin, especially in obese subjects, should be taken as a warning sign of energy imbalance, poor diet, hyperinsulinemia, insulin resistance, or changes in other metabolic risk factors that are strongly associated with cardiovascular diseases and type 2 diabetes.

Key words: Anthropometry, body mass index, insulin resistance, leptin, obesity,

INTRODUCTION

Obesity is the sixth most significant risk factor contributing to the overall burden of disease worldwide. 1.1 billion adults and 42 million children under the age of five are now classified as overweight or obese¹. The major undesirable consequences of obesity are cardiovascular disease, type 2 diabetes, and several cancers².

BMI, waist-to-hip ratio (WHR) and waist circumference (WC) are the typical obesity parameters. Based on BMI values, obesity is classified into three grades: first BMI 30-34.9 kg/m², second BMI 35-39.9 kg/m² and third BMI = 40 kg/m²³. Abdominal obesity, which is described

by WHR and WC, has been found to be more strongly correlated to cardiovascular events than BMI⁴.

The discovery of leptin in 1994 revolutionized the pathophysiology of obesity⁵. Leptin, a 167 amino acid 16-kDa peptide product of obese(OB) gene, is secreted by the adipose tissue in direct proportion to amount of body fat⁶. Leptin plays an important role in the regulation of appetite, glucose homeostasis and body fat⁷. Leptin levels are enhanced in obesity implicating leptin resistance but there is a scarcity of data regarding mutations in leptin and leptin receptor genes⁸ and leptin has been under investigation as a risk marker for future

complications. This study was conducted to measure the levels of serum leptin and insulin resistance in normal weight and obese young adults and correlate them with obesity parameters: BMI, WC, and WHR and metabolic indices.

MATERIALS AND METHODS

The study protocol was approved by the Local Bioethical Committee and informed consent was obtained from all subjects. Subjects between 20-25 years of age with normal BMI and obese with BMI above 30 were recruited. Subjects with hypertension, diabetes, psychiatric disorders, pregnancy, cancer, stroke, severe hepatic or renal disease and acute cardiovascular events, or with a history of abdominal surgery, which could have an impact on abdominal fat distribution, were excluded from the study. Blood samples were obtained in the morning after an overnight fast. Serum specimens were stored at -20°C until analysis. Fasting blood glucose and 2 hour Oral glucose tolerance test after giving 75 gm. Glucose were performed immediately after collection.

ANTHROPOMETRIC AND BLOOD PRESSURE MEASUREMENTS

Height and weight was measured using Detecto scale to the nearest 0.5 cm and 0.1 kg respectively. BMI was defined as the weight in kilograms divided by the square of the height in meters³. Waist circumference was measured at the high point of the iliac crest and hip circumference at the maximum circumference of the buttocks. Blood pressure (BP) was measured using Dinamap compact T by Johnson & Johnson. BP measurement was carried out in the lying, sitting, and standing positions. The mean systolic and diastolic BP was calculated.

BIOCHEMICAL AND HORMONAL MEASUREMENTS

Serum leptin was measured by enzyme linked immunosorbant assay sandwich method, (DRG Instruments GMBH, Germany). The lowest limit of detection was 1 ng/ml. The intra- and interassay coefficients of variation were 3.3- 5.4 % and 6.7- 8.4% respectively. Serum glucose, total cholesterol, triglycerides, and HDL-cholesterol were measured by Dimension RXL analyzer by Dade Behring. LDL-

cholesterol was calculated by the formula:

Total cholesterol mg/dl - (HDL-c mg/dl + triglycerides mg/dl).

Serum insulin was measured by microenzyme immunoassay (IMX analyzer by Abbott diagnostics). The scores HOMA- IR were calculated with the formula, fasting serum insulin ($\mu\text{U/ml}$) $\times$ fasting serum glucose (mmol/l)/22.5⁹.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS statistical software. Student t-test, and Mann-Whitney test were carried out according to the results of Levene test of homogeneity of variances as appropriate. Pearson correlation coefficients were measured.

RESULTS

Table 1 shows the characteristics of the subjects.

	Non obese			Obese		
	Males	Females	V ^P value	Males	Females	P Value
Number	n = 20	n = 23		n = 25	n = 21	
Age (years)	25.8 $\pm$ 0.53	23.9 $\pm$ 1.9	n.s	29.4 $\pm$ 7.6	28.8 $\pm$ 6.2	n.s
Body mass index	23.1 $\pm$ 1.4	23.0 $\pm$ 1.8	n.s	35.5 $\pm$ 5.7	35.6 $\pm$ 4.4	n.s
Waist (cm)	81.2 $\pm$ 7.3	72.8 $\pm$ 8.9	0.002	109.8 $\pm$ 13.1	100.0 $\pm$ 13.4	0.016
Hip (cm)	100.9 $\pm$ 15.6	95.9 $\pm$ 7.2	n.s	118.7 $\pm$ 13.1	119.6 $\pm$ 11.7	n.s
WHR	0.818 $\pm$ 0.116	0.758 $\pm$ 0.066	0.04	0.929 $\pm$ 0.063	0.836 $\pm$ 0.080	0.0001
Mean systolic BP (mm Hg)	124.2 $\pm$ 12.1	103.5 $\pm$ 9.9	0.0001	125.6 $\pm$ 12.8	113.4 $\pm$ 14.4	0.004
Mean diastolic BP (mm Hg)	76.1 $\pm$ 7.5	68.7 $\pm$ 7.8	0.003	78.9 $\pm$ 10.1	75.5 $\pm$ 9.0	n.s

Mean $\pm$ SEM

n.s = means not significant

n = number of subjects

SUBJECTS WITH NORMAL BMI

20 males and 23 females were studied. There was no gender difference with regard to the mean age or BMI. Females had lower waist circumference and waist to hip ratio (WHR) than males with p value 0.002 and 0.04 respectively. There was no difference in mean hip circumference. The mean systolic and diastolic BP were normal but significantly higher in males.

OBESE OBJECTS

25 males and 21 females were studied. There was

no gender difference with regard to the mean age or body mass index. Females had lower waist circumference and WHR than males with p value 0.016 and 0.0001 respectively. The mean systolic and diastolic blood pressures were normal, but systolic blood pressure was significantly higher in males.

Table 2 shows the concentrations of serum leptin and other metabolic variables.

	Non-obese		P value	Obese		P value
	Males n = 20	Females n = 23		Males n=25	Females 21	
Number (n)						
Leptin (ng/ml)	2.2 ± 0.26	8.8 ± 2.10	0.049	12.5 ± 2.24	23.0 ± 3.98	0.021
Insulin (uU/ml)	8.3 ± 0.72	8.8 ± 0.70	n.s	18.7 ± 1.69	13.6 ± 1.08	0.019
HOMA - IR	1.90 ± 0.18	1.94 ± 0.17	n.s	4.40 ± 0.47	3.14 ± 0.27	0.032
Fasting glucose (mg/dl)	91.6 ± 1.4	87.6 ± 1.3	0.047	93.7 ± 1.7	93.0 ± 2.3	n.s
2h glucose post 75g OGTT (mg/dl)	92.4 ± 4.4	95.1 ± 4.1	n.s	99.7 ± 5.1	108.6 ± 3.8	n.s
Total cholesterol (mg/dl)	173.8 ± 5.9	168.1 ± 5.7	n.s	80.6 ± 7.5 120.7 ± 11.7	188.6 ± 6.0	n.s
Triglyceride (mg/dl)	98.6 ± 9.6	57.1 ± 3.5	0.0001	120.7 ± 11.7 20.7 ± 11.7	80.7 ± 8.3	0.020
HDL - Cholesterol (mg/dl)	47.4 ± 2.0	65.6 ± 2.9	0.0001	42.4 ± 1.2	59.6 ± 2.6	0.0001
TG / HDL - C ratio	2.18 ± 0.25	0.93 ± 0.09	0.0001	2.94 ± 0.33	1.48 ± 0.22	0.0001
LDL - Cholesterol (mg/dl)	106.7 ± 5.4	91.0 ± 4.2	0.024	114.1 ± 6.6	112.8 ± 6.0	n.s

Mean ± SEM

n.s = means not significant.

n = number of subjects.

SUBJECTS WITH NORMAL BMI

Serum leptin was significantly higher in females

than males with p value 0.049. Females had significantly lower mean fasting blood glucose (FBG), triglyceride, triglyceride to HDL-cholesterol ratio and LDL-cholesterol. Males had significantly lower HDL-cholesterol. There was no gender difference with regard to mean fasting insulin, HOMA- IR, or total cholesterol.

OBESSE SUBJECTS

Serum leptin was significantly higher in females than males. There was no gender difference in mean fasting blood glucose, 2 hour glucose post 75g OGTT, total cholesterol, and LDL-cholesterol. Females had significantly lower fasting insulin, HOMA- IR, triglyceride, and triglyceride to HDL-cholesterol ratio but higher HDL-cholesterol.

Gender and obesity had an effect on serum leptin values overall. Serum leptin of all 44 females (23 non-obese and 21 obese) was significantly higher when compared with that of all 45 males (20 non-obese and 25 obese) $15.61 \pm \text{SEM } 2.42$ and $7.92 \pm \text{SEM } 1.46$ ng/ml respectively with p value 0.006. Serum leptin was significantly higher in 21 obese females when compared to that of 23 non-obese females, with p value 0.002. Serum leptin was as well significantly higher in 25 obese males when compared with 20 non-obese males, with p value 0.0001. There were individual variability in serum leptin within the same sex with similar BMI; 6 male and 6 female with normal weight and 2 obese males subjects had serum leptin < 1 ng/ml.

Table 3: Compares the Pearson correlation coefficients for serum leptin and HOMA- IR with the anthropometric and blood pressure measures.

	Leptin		HOMA -IR	
	r	P value	r	P value
Age (years)	0.061	n.s.	0.068	n.s.
Body mass index	0.440	0.0001	0.589	0.0001
Waist (cm)	0.284	0.007	0.594	0.0001
Hip (cm)	0.425	0.0001	0.496	0.0001
WHR	-0.042	n.s.	0.367	0.0001
Mean systolic BP (mmHg)	-0.100	n.s	0.230	0.030
Mean diastolic BP (mmHg)	-0.124	n.s.	0.187	n.s.

r = is the Pearson correlation coefficient,
n.s = means not significant.

SERUM LEPTIN

There was significant positive correlation with BMI ($r = 0.440$) and hip circumference ($r = 0.425$), both with p values 0.0001. There was no correlation with age or mean systolic and diastolic BP.

HOMA- IR

There was significant positive correlation with all measures of obesity including BMI ($r = 0.589$), waist circumference ($r = 0.594$), hip circumference ($r = 0.496$), and WHR ($r = 0.367$), with p value 0.0001. HOMA- IR correlated best with waist circumference and BMI, while serum leptin correlated best with BMI and hip circumference.

Table 4: Compares the Pearson correlation coefficients for serum leptin and HOMA- IR with metabolic variables.

	Leptin		HOMA- IR	
	r	P value	r	P value
Leptin (ng/ml)			0.344	0.001
Insulin (uU/ml)	0.334	0.001	0.982	0.0001
HOMA -IR	0.344	0.001		
Fasting glucose (mg/dl)	0.208	n.s.	0.520	0.0001
2h glucose post 75g OGTT (mg/dl)	0.027	n.s.	0.107	n.s.
Total cholesterol (mg/dl)	0.002	S n.s	0.204 0.204 S^S	n.s.
Triglyceride (mg/dl)	-0.006	n.s	0.55 0.204 S^S	n.s *
HDL -Cholesterol (mg/dl)	-0.032	n.s.	-0.365	0.0001
TG / HDL -C ratio	0.036	n.s.	0.606	0.0001
LDL -Cholesterol (mg/dl)	0.019	n.s.	0.217	0.041

SERUM LEPTIN

Significant positive correlation was only present with fasting insulin ($r = 0.334$) and HOMA- IR ($r = 0.344$) with p value 0.001.

HOMA- IR

The strongest positive correlation was with fasting insulin ($r = 0.982$). There was significant positive correlation with FBG ($r = 0.520$), TG ($r = 0.555$),

and TG / HDL-cholesterol ratio ($r = 0.606$) with p value 0.0001. There was a significant negative correlation HDL-cholesterol ($r = -0.365$) with p value 0.0001. These variables are indirectly measures of insulin resistance.

DISCUSSION

It is well recognized that obesity enhances the risk for metabolic syndrome¹⁰, type 2 diabetes mellitus and atherosclerotic coronary heart diseases¹¹. Obesity is related to insulin receptor resistance, that in turn results from the decreased insulin binding to tissue receptors, especially in adipose tissue and muscles. Measurement of insulin resistance predicts future type 2 diabetes and cardiovascular diseases in children and adolescents even in the absence of hyperglycemia and diabetes^{12,13}. We found significantly increased BMI, WHR in obese subjects than non obese subjects and this describes the association between adiposity and insulin resistance. These findings are in agreement with those of Mariusz et al¹⁴. Nilsson et al¹⁵ also showed a positive correlation between WC and insulin resistance. These findings were also validated by some other authors, who found WC as an independent predictor of insulin resistance^{16,17}.

Leptin is an adipokine with complex and multidirectional action¹⁸. Our findings are in accordance with the results of other studies showing elevated serum leptin levels in obese, normotensive patients⁴. Serum leptin was found to be higher in females than males in obese as well in lean individuals. There was about 2-4 fold elevations in female subjects as reported by others¹⁹. BMI as an index of overall adiposity was strongly associated with serum leptin with high statistical significance ($p < 0.001$) as was also found in other studies⁴.

Our finding that leptin levels correlated strongly with fasting insulin levels are in agreement with those of Askari H et al²⁰ and with insulin resistance as measured by HOMA-IR are in agreement with those of Josef et al²¹. Therefore we might anticipate that hyperleptinaemia may play a role in insulin resistance. Insulin has been found to stimulate both leptin biosynthesis and secretion

from adipose tissue as an endocrine adipo-insular feedback loop "adipo-insular axis". Also leptin has been found to increase peripheral insulin sensitivity and modulates pancreatic β -cell function²². Thus increased leptin secretion may be the response to hyperinsulinemia

HOMA-IR correlated with all variables associated with obesity. These included BMI, waist circumference, WHR, BP, serum TG, and TG/HDL-cholesterol ratio. Increased cardiovascular risk and hyperinsulinemia are known to be associated with excess central visceral fat, which is clinically assessed by increased waist circumference and high WHR. Serum leptin correlated significantly with hip circumference which reflects peripheral (subcutaneous) fat. Central (visceral) fat is associated with hyperinsulinemia and insulin resistance²³, while peripheral (subcutaneous) fat is associated with hyperleptinemia²⁴.

CONCLUSION

We conclude that serum leptin levels increase with obesity, higher in females in both lean and obese individuals. Enhanced serum leptin, especially in obese individuals, should be considered as a warning sign of energy imbalance, poor diet, hyperinsulinemia, insulin resistance, or alteration in other metabolic risk factors that are strongly associated with cardiovascular disease and type 2 diabetes.

REFERENCES

1. Cali AMG, Caprio S. Obesity in children and adolescents. *J Clin Endocrinol and Metab.* 2008; 93: s31-s36
2. Haslam DW, James WP. Obesity. *Lancet* 2005; 366:1197-209.
3. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. WHO Technical Report Series 894. Geneva: World Health Organization, 2000.
4. Stepień M, Wlazeł RN, Paradowski M, Banach M, Rysz M, Misztal M and Rysz J. Serum concentrations of adiponectin, leptin, resistin, ghrelin and insulin and their association with obesity indices in obese normo- and hypertensive patients – pilot study. *Arch Med Sci* 2012; 8: 431–36.
5. Brito N, Fonseca M, Dinis I and Mirante A. Metabolic factors in obesity. *J Pediatr Endocrinol Metab.* 2010; 23:97-100.
6. Kelesidis T, Kelesidis L, Chou S and Mantzoros CS. Narrative Review: The Role of Leptin in Human Physiology: Emerging Clinical Applications. *Ann of Intern Med* 2010; 152: 93-100
7. Austin J, Marks D. Hormonal Regulators of Appetite. *International J Pediatr Endocrinol* 2009; Article ID 141753
8. Dubey S, Kabra M, Bajpai A, Pandey, Hassan M and Gautam RK. Serum Leptin Levels in Obese Indian Children: Relation to Clinical and Biochemical Parameters. *Indian pediatr* 2007; 44:257-62
9. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia*; 1985; 28: 412-19.
10. Beigh SH, Jain S. Prevalence of metabolic syndrome and gender differences. *Bioinformation.* 2012; 8: 613–16.
11. Nikolopoulou A, Kadoglou N. Obesity and metabolic syndrome as relate to cardiovascular disease. *Expert Review of Cardiovascular Therapy.* 2012; 10(7):933-39
12. Asato Y, Katsuren K, Ohshiro T, Kikawa K, Shimabukuro T, Ohta T. Relationship between lipid abnormalities and insulin resistance in Japanese school children. *Arterioscler Thromb Vasc Biol.* 2006; 26: 278-6.
13. Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ et al. Retinol-binding protein 4 and insulin resistance in lean, obese and diabetic subjects. *N Engl J Med* 2006; 354(24): 2552- 63.
14. Stepień M, Rosniak-Bak K, Paradowski M, Misztal M, Kujawski K, Banach M and Rysz J. Waist circumference, ghrelin and selected adipose tissue-derived adipokines as predictors of insulin resistance in obese patients: Preliminary results. *Med Sci Monit* 2011; 17:13-8.
15. Nilsson G, Hedberg P, Jonason T, Lonnberg I, Tenerz A, Forberg R and Ohrvik J. Waist circumference alone predicts insulin resistance as good as the metabolic syndrome in elderly women. *Eur J Intern Med*, 2008; 19: 520-6
16. Tabata S, Yoshimitsu S, Hamachi T, Abe H, Ohnaka K, and Kono S. Waist circumference and

- insulin resistance: a cross-sectional study of Japanese men. *BMC Endocrine Disorders*, 2009; 9: 1-7
17. Lee S, Bacha F, Gungor N, Arsianian SA: Waist circumference is an independent predictor of insulin resistance in black and white youths. *J Pediatr*, 2006; 148: 188-94
 18. Oswal A, Yeo G. Leptin and the Control of Body Weight: A review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. *Obesity* 2010; 18: 221-9.
 19. Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnkiv OP, Koniaris A. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* 2011; 30:E567-84.
 20. Askari H, Tykodi G, Liu J, Dagogo-Jack S. Fasting plasma leptin level is a surrogate measure of insulin sensitivity. *J Clin Endocrinol Metab* 2010; 95: 3836-43.
 21. Silha JV, Kersk M, Skrha JV, Sucharda P, Nyomba BL and Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* 2003; 149:331-5.
 22. Martins MD, Lima FL, Fonseca A. Relationship between leptin and body mass and metabolic syndrome in an adult population. *Rev Port Cardiol* 2012; pii: S0870-2551(12)00194-1.
 23. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Iribeck T, et al. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. *Obesity (Silver Spring)* 2010; 18: 2191-8
 24. Dardeno TA, Chou SH, Moon HS, Chamberland JP, Fiorenza CG, Mantzoros CS. Leptin in human physiology and therapeutics. *Front Neuroendocrinol*. 2010; 31: 377-93.

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