Relationship of leptin concentration to gender, body mass index and age in Saudi adults

Rowyda. N. Al-Harithy, *PhD.*

ABSTRACT

Objective: Leptin concentrations are highly correlated with body fat storage and exhibit sexual dimorphism, with women having higher concentrations at every level of relative or absolute adiposity. To test whether or not this relation is consistent across the Saudi population. This study aims to investigate the effect of gender, obesity related parameters, and age on leptin levels from representative samples of Saudi women and men.

Methods: This study was carried out at King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia during the year 2003. Fasting leptin concentrations were determined after an overnight fast in 122 healthy subjects (57 women, 65 men; age 20-75 years; body mass index [BMI] 16-56 kg/m^2). The subjects were separated into female and male groups. To clarify the age and BMI-related changes in leptin levels, each gender was divided into 3 BMI groups (lean 15-24, overweight 25-30 and obese >30 kg/m^2), and 3 age groups (younger 20-34, middle-aged 35-49 and older 50-75 years); and they were treated separately. Anthropometrics measurements (weight, height, waist, and hip circumferences), blood pressure, and fasting glucose levels were taken at the time of the collection.

Results: In the whole group, leptin levels were between 0.16-21.72 ng/ml, and females had higher leptin concentration (6.04 ± 4.71 ng/ml versus 1.72 ± 0.95 ng/ml, $p<0.0001$) than males. Gender differences remained clear when leptin concentrations were divided by BMI or age. In comparing the pattern of changes between the 2 genders, leptin levels were low in lean individuals and rose with increased BMI in both genders. Age-related change in leptin levels showed a tendency toward a non-significant reduction in older women and a significant ($p=0.05$) rise in older men. Correlation analysis between leptin and BMI were highly significant in female (r=0.64; $p=0.0001$) and male (r=0.49; $p=0.0001$) groups independent of age and sex. The findings were further explored using stepwise multiple linear regression analysis with leptin concentrations as the dependent variable and age, BMI, waist hip ratio (WHR), waist, and hip measurements as independent variables. The analysis demonstrated that the determinants of leptin concentrations were BMI and age (r=0.69; $p=0.015$) in women and BMI, age and WHR (r=0.61; $p=0.01$) in men.

Conclusion: The present study demonstrates that there are gender-specific and age-dependent gender-specific differences in leptin concentrations in healthy Saudi individuals. However, this study indicates that there may be unknown variables that may also influence leptin levels in Saudi women and men.

Saudi Med J 2004; Vol. 25 (8): 1086-1090

Since its discovery in 1994,[1] leptin, the protein product of the adipocyte-specific obese gene, has generated much attention in the fields of obesity and metabolic research. The amount of leptin synthesized and secreted by adipose tissue is known to increase in proportion to the accumulation of body fat mass.[2,3] Leptin binds to receptors in the hypothalamus and influences the expression of several neuropeptides that regulate energy intake, energy expenditure, and neuroendocrine function.[4,5] The knowledge of leptin; however, has evolved considerably during the last 5-years and has been

From the Department of Biochemistry, Faculty of Science, King Abdul-Aziz University, Jeddah, *Kingdom of Saudi Arabia*.

Received 20th January 2004. Accepted for publication in final form 27th March 2004.

Address correspondence and reprint request to: Dr. Rowyda. N. Al-Harithy, Assistant Professor, Department of Biochemistry, PO Box 40288, Jeddah 21499, *Kingdom of Saudi Arabia*. Tel. +966 505444238. Fax. +966 (2) 6393640. E-mail: ralharithy@yahoo.com

shown to participate in quite diverse physiologic functions such as reproduction, hematopoiesis, angiogenesis, immunity, blood pressure control and bone formation.[6-9] Although few persons with extreme obesity are leptin deficient, most obese subjects have been found to be resistant to this peptide.[10] At this early junction in the course of leptin research, the physiological role of leptin is not completely understood, but it may be a determinant of obesity and its complications. Although there is considerable variability in serum leptin concentrations among individuals, numerous researchers have studied the gender difference in serum leptin levels and documented the fact that women have higher leptin concentration than men after accounting for adiposity.[11,12] It has been proposed that the higher leptin levels in women involve the different pattern of fat deposition[13,14] or the role of sex hormones;[15] however, the respective roles of these factors in the overall regulation of leptin production have not been fully ascertained. Furthermore, little is known about whether the age-related changes in the hormones known to regulate leptin production might modify circulating leptin levels during aging. Several researchers investigated the effect of age on serum leptin, but results are discordant and sometimes in conflict.[16-21] It has been suggested that the age-related increase in fat mass can be a major confounding factor in studies that examine the relationship between leptin and age, because adiposity is usually the dominant determinant of leptin.[22,23] These studies prompted this researcher to measure leptin levels in a representative, population-based sample from the city of Jeddah and to analyze the data with respect to the relation to gender, body mass index (BMI) and age.

Methods. This study was carried out at King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia during the year 2003. Subjects were from a large age-stratified sample of healthy Saudi women and men. None of the volunteers had been on any medication treatment. Women subjects, who were pregnant, using estrogen replacement therapy, using oral contraceptives, had a history of diabetes or irregular menstruations were excluded. Leptin concentrations were determined from the fasting blood sample using a commercial direct enzyme-linked immunosorbent assay. Human leptin kit according to the procedure provided by the company (Diagnostics Biochem Canada Inc). Blood samples from women were collected on day 3, 10, 17 and 24 from the beginning of menstruation. This was carried out because previous research[24,25] has proven that leptin levels fluctuate during a women's menstrual cycle. From male, blood samples were drawn only once as male leptin levels do not fluctuate. At the time of blood collection, information was recorded for all subjects, including weight, height, waist and hip circumference. Body mass index was calculated as total body-weight in kilograms divided by height in meters squared and waist hip ratio (WHR) as the ratio of the circumference of the narrowest part of the waist divided by the broadest part of the hip.

Statistical analysis was performed using SPSS 10 for Windows. Descriptive statistics were presented as means ± standard deviation. Association between variables was assessed by Bivariate and partial Pearson correlation. Stepwise multiple regression analysis was also performed to establish the independent associations between the variables and leptin levels. Differences between females and males were analyzed using parametric t-test. A difference was considered to be statistically significant when $p<0.05$. The values presented in this paper are 2- tailed.

Results. Two hundred and fifteen volunteers expressed interest in participating in the study. After completion of the screening questionnaire 122 volunteers fit the required criteria for being healthy were recruited. The volunteers covered a wide range of age and BMI; women (n=57) and men (n=65) were considered separately. The leptin mean value for each woman was calculated and used for the analysis. **Table 1** shows the characteristics of the male and female subjects. Females, compared with males, tended to have a significant lower BMI (28.57 ± 5.15 versus 32.58 ± 7.41; $p=0.001$), WHR (0.80 ± 0.58 versus 1.04 ± 0.92; $p=0.0001$), weight (72.40 ± 14.78 versus 85.23 ± 15.54; $p=0.0001$) and waist (85.46 ± 10.49 versus 101.12 ± 16.62; $p=0.0001$). However, hip measurements were significantly ($p=0.0001$) higher in females (106.95 ± 11.0) compared with their male counterparts (97.32 ± 16.99). There were no significant differences in height and age between genders.

In the whole group, leptin levels were between 0.16-21.72 ng/ml and females had higher leptin concentration (6.04 ± 4.71 ng/ml versus 1.72 ± 0.95 ng/ml, $p<0.0001$) than males. Although women had the broadest range of leptin concentrations from 1.15-21.72 ng/ml, 82% of the values were >4 ng/ml. In contrast, 96.9% of the men were <4 ng/ml. Bivariate correlation analysis was performed to assess relationships between leptin, age, BMI, WHR, weight, height, waist and hip circumferences. The correlation coefficients are presented in **Table 1**. Leptin concentration was found to correlate positively and significantly with age (r=0.26; $p=0.03$) independent of BMI in men. In contrast, a non-significant negative (r=-0.14; $p=0.30$) correlation between leptin and age was seen amongst women. Furthermore, BMI, weight, waist, and hip values showed a strong association with leptin levels in both genders. Waist hip ratio

associated strongly with leptin in men (r=0.32, *p*=0.009), but not in women. The findings from the above Bivariate correlation analysis were further explored using stepwise multiple linear regression analysis with leptin concentrations as the dependent variable and age, BMI, WHR, waist and hip measurements as independent variables. The analysis demonstrated that the determinants of leptin concentrations were BMI and age (r=0.69; *p*=0.015) in women; and BMI, age and WHR (r=0.61; *p*=0.01) in men. Body mass index was significant predictor for leptin levels in both genders, explaining the variation in leptin levels in women (64%) and men (49%).

To clarify the age and BMI-related changes in leptin levels, each gender was divided into 3 BMI groups (lean 15-24, overweight 25-30 and obese >30 kg/m^2), 3 age groups (younger 20-34, middle-aged 35-49 and older 50-75 years), and treated separately.

Body mass index and age-related changes. In females, leptin levels were low in lean individuals and rose with increased BMI. Subjects with BMI >30 kg/m^2 had 2 times higher leptin than individuals with BMI <30 kg/m^2. The BMI-dependent rise in leptin levels was found to be highly significant (*p*=0.005) and multiple comparisons showed that the obese group (BMI >30 kg/ml) was significantly (*p*=0.007) higher than the lean group (15-24 kg/ml^2). In considering the age related changes in leptin levels in females, a non-significant negative (r=-0.14; *p*=0.30) decrease in leptin concentrations was observed with age. Females of age 20-34 years had higher levels of leptin than the middle-aged and older groups (35-49 and 50-75 years). The leptin level in subjects aged ≥50 years was reduced (17.7%) compared to that in subjects under age 50 (*p*=0.553). The subsets were not different in terms of WHR and weight, but the older group was different from the other 2 groups (35-49 and 50-75

Table 1 - Characteristics of 65 males and 57 females and their correlation to leptin level.

Gender/ variables	N	Minimum	Maximum	Mean	SD	Correlation to leptin
Female						
Age (years)	57	20.0	75.0	38.02	12.71	-0.14
20-34	26	1.15	21.72	6.50	5.66	-0.23
35-49	20	1.35	17.76	5.91	4.19	-0.26
50-75	11	1.93	11.25	5.21	3.07	0.23
BMI (kg/m^2)	57	16.0	39.0	28.57	5.15	0.64*
15-24	13	1.15	5.45	3.03	1.32	0.61*
25-30	22	1.35	11.25	4.25	2.27	0.33
>30	22	3.22	21.72	9.61	5.53	0.36
WHR (cm)	57	0.66	0.90	0.80	0.58	0.15
Weight (kg)	57	41.0	106.7	72.40	14.78	0.64*
Height (cm)	57	141.0	173.0	159.39	6.34	0.14
Waist (cm)	57	65.0	109.0	85.46	10.49	0.52*
Hip (cm)	57	90.0	130.0	106.95	11.00	0.54*
Leptin (ng/ml)	57	1.15	21.72	6.04	4.71	
Male						
Age (years)	65	23.0	72.0	39.78	11.30	0.26*
20-34	23	0.16	4.18	1.37	0.86	0.01
35-49	26	0.58	3.61	1.77	0.87	-0.05
50-75	16	0.79	4.93	2.12	1.09	-0.06
BMI (kg/m^2)	65	21.0	56.0	32.58	7.41	0.49*
15-24	8	0.28	2.31	1.40	0.68	-0.87*
25-30	18	0.59	2.74	1.33	0.70	0.15
>30	39	0.16	4.93	2.03	0.99	0.37*
WHR (cm)	65	0.87	1.30	1.04	0.92	0.32*
Weight (kg)	65	61.0	127.0	85.23	15.54	0.60*
Height (cm)	65	120.0	190.0	162.69	16.12	-0.01
Waist (cm)	65	67.0	150.0	101.12	16.62	0.46*
Hip (cm)	65	63.0	153.0	97.32	16.99	0.26*
Leptin (ng/ml)	65	0.16	4.93	1.72	0.95	

BMI - body mass index, WHR - ratio of waist-to-hip circumferences.
*Correlation is significant at the 0.05 level (2-tailed)

years) in term of BMI, waist and hip circumferences.

In male subjects, leptin levels changed with BMI. The median leptin level in individuals with BMI >30 kg/ml^2 was 2.03 ng/ml compared with 1.40 ng/ml in subjects <25 kg/ml^2 and 1.33 ng/ml in the subset between 25-30 kg/ml^2. Again, results similar to female's show that leptin levels were low in lean individuals and rose with increasing BMI, but the differences between groups were not significant. The age-related pattern in males was different to that observed in females, a significant rise (p=0.046) in leptin occurred at an older age (50-75 years). The subsets were not different in terms of BMI and WHR, but the older group was different from the younger and middle-aged groups in terms of weight, waist, and hip circumferences.

Gender-dependent and BMI-dependent gender-specific differences in leptin levels. Gender differences did not change when leptin was divided by BMI; a clear gender difference remained. In the lean subjects the mean leptin was 38% higher in lean women than in lean men (3.03 ± 1.32 versus 1.40 ± 0.68; p=0.001), 3 times higher in overweight women than in men (4.25 ± 2.27 versus 1.33 ± 0.70; p=0.001) and 4 times higher in women with BMI >30 than in men (9.61 ± 5.53 versus 2.03 ± 0.99; p=0.001).

Gender-dependent and age-dependent gender-specific differences in leptin levels. There were gender specific and age-dependent gender-specific differences in leptin levels. In group of age between 20-34 years, women had 5 times higher mean leptin than the group of men of similar age (6.50 ± 5.66 versus 1.37 ± 0.86; p=0.0001). Mean leptin concentration of women ages between 35-49 years was 3 times higher than in men of the same age (5.91 ± 4.19 versus 1.77 ± 0.87, p=0.0001). Women >50 years of age had twice higher mean leptin level than their respective parallel BMI categories in men (5.21 ± 3.07 versus 2.12 ± 1.09, p=0.001).

Discussion. The present study examined the influence of gender, BMI, and age on leptin levels in Saudi subjects. Results from a growing number of recent studies demonstrated elevated leptin concentrations in obese humans.[26-28] Also, differences in leptin concentrations have been demonstrated between genders.[29-31] However, no study had been performed examining the BMI and age-related changes in leptin levels in Saudi women and men. To address this issue, a study was performed in a representative group of subjects covering a wide range of BMI and age.

The results of the present study demonstrate that Saudi females have higher leptin concentrations than males. This suggests that gender makes a highly significant independent contribution to the prediction of leptin level. Although this sexual dimorphism in leptin levels had been previously demonstrated, there has been no satisfactory explanation. The data of this study might help in partially explaining this sexual dimorphism. The physical characteristics showed that hip circumference, not waist or WHR, is higher in women and had a significant association with leptin levels in women. Hip circumference reflects glutea-femoral adipose tissue, a major component of peripheral obesity, whereas waist and WHR reflect central/abdominal obesity.[32] This suggested that peripheral obesity was a stronger determinant of leptin concentration than central obesity, giving rise to the higher leptin levels seen in Saudi women.

To clarify the BMI-related changes in leptin levels, subjects were divided into 3 BMI groups. The data showed that leptin concentrations were increased in obese Saudi individuals and reflected the amount of adipose tissue in the body. In women, the rise of leptin levels was found to be highly significant, but in men, the difference between the subgroups was not significant. Gender difference did not change when leptin was divided by BMI; however, women had higher leptin levels than men at every level of adiposity. In comparing the hip measurements between the subgroups, it shows that women have greater hip circumferences than men in each subgroup. This is a striking point that provides further evidence that body fat distribution may be an important determinant of leptin concentrations.

Another interesting point is the relationship between age and leptin levels. Previous studies reported that leptin is reduced,[16-18] unchanged,[19,20] or even increased[21] during aging. Conflicting data exist concerning the relationship between leptin levels and age. The reason for these conflicting results is not understood. Possibly, the age-leptin relationship was not the main focus of most of the studies. In this study, leptin levels showed a tendency toward a reduction in older women and an increase in men subjects. Older women had lower leptin levels regardless of increase in BMI, waist and hip circumferences. Age-related changes over time in leptin levels appear to be more closely related to differences in anthropometric measurements in elderly men than in women. Multiple linear regression analysis demonstrated that BMI was a significant predictor for leptin levels. Body mass index alone explained 64% of the leptin variance in women, and the addition of age to percent BMI increased the explained variance to 68%. In men, BMI alone demonstrated 49% of leptin variance; whereas the addition of age and WHR increased the prediction level to 61%. This result indicates that fat stores are unlikely to be the only factor that regulates leptin concentrations, and other variables must be involved in leptin reduction during the aging process.

The remaining key question is whether the factor that influences the pattern of leptin levels in elderly women and men is the same or if each gender is different in that respect. Thus, more studies are needed to determine what additional factors may play a role in the leptin levels in Saudi women and men.

References

1. Zhang Y, Proenca P, Maffei M, Barone M, Leopold L, Friedom JM. Positional cloning of the mouse obese gene and its human homologue. ***Nature*** 1994; 372: 425-432.
2. Lonnqvist F, Arner P, Nordfors L, Schalling ML. Over-expression of the obese (ob) gene in adipose tissue of human obese subjects. ***Nat Med*** 1995; 1: 950-956.
3. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentration in normal weight and obese humans. ***N Engl J Med*** 1996; 334: 292-295.
4. Fruhbeck G, Jebb SA, Prentice AM. Leptin: physiology and pathophysiology. ***Clin Physiol*** 1998; 18: 399-419.
5. Ahima RS, Flier JS. Leptin. ***Annu Rev Physiol*** 2000; 62: 413-437.
6. Fruhbech G. Pivotal role of nitric oxide in the control of blood pressure following leptin administration. ***Diabetes*** 1999; 48: 903-908.
7. Fruhbeck G. A heliocentrical view of leptin. ***Proc Nutr Soc*** 2001; 60: 301-318.
8. Chehab FF, Qiu J, Mounzih K, Edwart-Toland A, Ogus S. Leptin and reproduction. ***Nutr Rev*** 2002; 60: S39-S46.
9. Lord G. Role of leptin in immunology. ***Nutr Rev*** 2002; 60: S35-S38.
10. Hamilton BS, Paglia D, Kwan AYM, Deitel M. Increased obese mRNA expression in omental fat cells from massively obese humans. ***Nat Med*** 1995; 1: 953-956.
11. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD. Gender difference in plasma leptin concentrations. ***Nat Med*** 1996; 2: 949–955.
12. Rosembaum M, Leibel R. Role of gonadal steroids in the sexual dimorphism in body composition and circulating concentrations of leptin. ***J Clin Endocrinol Metab*** 1999; 84:1784-1789.
13. Mazusaki H. Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M, et al. Human obese gene expression: adipocyte specific expression and regional differences in the adipose tissue. ***Diabetes*** 1995; 44: 855-858.
14. Montague CT, Prins JB, Sanders L, Zhang J, Sewter CP, Digby J, et al. Depot-related gene expression in human subcutaneous and omental adipocytes. ***Diabetes*** 1998; 47: 1384-1391.
15. Rosembaum M, Leibel R. Role of gonadal steroids in the sexual dimorphism in body composition and circulating concentrations of leptin. ***J Clin Endocrinol Metab*** 1999; 84:1784-1789.
16. Rosembaum M, Nicolson M, Hirsch J, Heymsfield SB, Dympna G, Chu F et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. ***J Clin Endocrinol Metab*** 1996; 81: 3424–3427.
17. Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariate. ***J Clin Endocrinol Metab*** 1996; 81: 3909-3913.
18. Ryan AS, Elahi D. The effects of acute hyperglycemia and hyperinsulinemia in plasma leptin levels: its relationship with body fat, visceral adiposity, and age in women. ***J Clin Endocrinol Metab*** 1996; 81: 4433-4438.
19. Perry HM, Morley JE, Horowitz M, Kaiser FE, Miller DK, Wittert G. Body composition and age in African American and Caucasian women: relationship to plasma leptin levels. ***Metabolism*** 1997; 46: 1399-1405.
20. Ahren B, Pacini G. Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. ***J Clin Endocrinol Metab*** 1998; 83: 3350-3356.
21. Baumgartner RN, Walters DL, Morley JE, Patrick P, Montoya GP, Gary P. Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. ***Metabolism*** 1999; 48: 378-384.
22. Saad MF, Riad-Gabriel MG, Khan A, Sharma A, Michael R, Jinagouda SD et al. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. ***J Clin Endocrinol Metab*** 1998; 83: 453-459.
23. Lonnqvist F, Wennlund A, Arner P. Relationship between circulating leptin and peripheral fat distribution in obese subjects. ***Int J Obes Relat Metab Disord*** 1997; 21: 255-260.
24. Riad-Gabriel MG, Jinagouda SD, Sharma A, Boyadjian R, Saad MF. Change in plasma leptin during the menstrual cycle. ***Eur J Endocrinol*** 1998; 139: 528-531.
25. Teirmaa T, Luukkaa V, Rouru J, Koulu M, Huupponen R. Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. ***Eur J Endocrinol*** 1998; 139: 190-194.
26. Lee JH, Reed DR, Price RA. Leptin resistance is associated with extreme obesity and aggregates in families. ***Int J Obes Relat Metab Disord*** 2001; 25: 1471-1473.
27. Mendoza-Nunez VM, Garcia-Sanchez A, Sanchez-Rodriguez M, Galvan-Duarte RE, Fonseca-Yerena ME. Overweight, waist circumference, age, gender, and insulin resistance as risk factors for hyperleptinemia. ***Obes Res*** 2002; 10: 253-259.
28. Chow VT, Phoon MC. Measurement of serum leptin concentrations in university undergraduates by competitive ELISA reveals correlations with body mass index and sex. ***Adv Physiol Educ*** 2003; 27: 70-77.
29. Summer AE, Falkner B, Kushner H, Considine RV. Relationship of leptin concentrations gender, menopause, age, diabetes, and fat mass in African Americans. ***Obes Res*** 1998; 6:128-133.
30. Njelekela M, Kuga S, Hiraoka J, Masesa Z, Ntogwisangu J, Mashalla Y. Determinants of hyperleptinaemia in an African population. ***East Afr Med J*** 2003; 80: 195-199.
31. Smirnoff P, Almiral-Seliger D, Schwartz B. Serum leptin levels in the elderly: relationship with gender and nutritional status. ***J Nutr Health Aging*** 2001; 5: 29-32.
32. Bjorntorp P. Regional obesity. In: Bjorntorp P, Brodoff BN editors. Obesity. Philadelphia (PA): Lippincott; 1992. p. 579-586.