BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2011. Vol. **8**(2), p. 885-891

Plasma Leptin and its Correlation with Anthropometric Variables in the Saudi Population

Arjumand S. Warsy*, Nashwa Othman[1], Maha Saleh Alsulaimani*, Zeinab Habib[2], Mona Halawani[3], Mohammad Addar[2], Sooad Al-Daihan*, Abeer Al-Dbass and Mohammad Al Anazi

*Department of Biochemistry, College of Science; [1]Central Laboratory, Center for Science and Medical Studies for Girls, [2]Department of Ob/Gyn., [3]Department of Dermatology, College of Medicine, King Saud University King Saud University, Riyadh, (Saudi Arabia).

(Received: 12 November 2011; accepted: 27 December 2011)

Leptin, a 16 kDa non-glycosylated polypeptide also referred to as obesity hormone, is a product of the LEP gene and is mainly secreted by adipose tissue having a role in regulating food intake and energy expenditure. The levels of leptin in the body are an indication of the body fat stores. Studies have shown ethnic variations in leptin levels in different populations. The aim of this study was to determine the levels of leptin in Saudi males and females and to analyze the effect of weight changes on leptin levels.

We investigated 31 healthy Saudi males and 168 females. The ages, weight, height and BMI were recorded into specially designed forms and leptin levels were estimated using radioimmunoassay methods (Linco Research USA).

The mean ± SD for leptin in Saudi males was found to be 11.95 + 7.22 ng/ml and for females was 16.4 ± 7. ng/ml which are significantly higher than those reported from other populations. Statistically significant positive correlations of leptin levels were found with age, weight and BMI in both males and females.

The results indicate that leptin levels increase with an increase in age and BMI suggesting that most obese persons are insensitive to endogenous leptin production, in hence controlling their weight.

Key words : Leptin, Saudi Arabia, age, gender, Body Mass Index.

Leptin, a 16 kDa non-glycosylated polypeptide also referred to as the obesity hormone, is a adipose tissue peptide hormone, which plays an important role in the regulation of body fat and is the product of the LEP gene[1,2]. First it was thought that this hormone is produced only by adipocytes and is involved only in modulating satiety and energy homeostasis[2,3,4] but, now it is known to be produced in many tissues and is also associated with the advent of reproductive maturity and fertility[5,6], neuroendocrine function[7] angiogenesis[8, 9], bone formation[10]. It acts centrally to regulates food intake and energy expenditure[11], and mediates it's action through its functional long form receptor (OB-Rb; LRb) in the hypothalamus[12,13,14]. Leptin regulates body weight through inhibiting food intake and

* To whom all correspondence should be addressed.
E-mail: aswarsy@ksu.edu.sa

stimulating energy consumption[15]. The secretion of leptin is affected by food intake, total body fat and serum levels of several hormones[11], including insulin, and to a lesser extent other peptide hormones. Leptin brings about its regulatory role by binding to its receptor which is expressed mainly in the hypothalamus as well as in peripheral tissues like the lungs, placenta and gastric mucosa[16, 17].

Initially it was believed that reduced leptin levels may be the cause of obesity in humans[18] and indeed several obese individuals were identified with low leptin levels[11]. However, further investigations showed that in most obese individuals, leptin levels are either normal or higher than in normal individuals[19]. These results indicate that in obese individuals there may be leptin resistance[20]. It is now generally accepted that leptin levels are an indication of body fat stores. These levels are also affected by gender, where women have 3 to 4 times higher leptin levels than men[21]. This may be due to the fact that women have a higher amount of body fat and a different fat distribution compared to their male counterparts. It is known that subcutaneous fat produces more leptin mRNA than intra-abdominal fat[11]. Other factors affecting leptin production include hormones like insulin, which increases leptin secretion and cathecholamines, which have a negative effect on leptin secretion[22]. Sex hormones also play a role in leptin production[23,24]. Smoking[25], physical activity[26], body fat distribution[27], age[23], pre- and postmenopausal changes[23], alcohol[28] and cholesterol/fat components[29], may also effect serum leptin levels.

Leptin is synthesized mainly in white adipose tissue and is the gene product of the ob gene[30]. White adipose tissue stores energy in the form of triglycerides and releases energy in the form of free fatty acids[31]. On the other hand, brown adipose tissue is responsible for the expenditure of fatty acid derived energy for maintenance of the organism's thermal stability, dissipating energy as heat[32]. Obesity is a term used to indicate excessive deposition of fat in the body. It is mainly a multifactorial disorder caused by both genetic and environmental factors, where genetic susceptibility is necessary for the environmental factors to precipitate the disease. The genetic factors are mainly multiple genes as seen in the common form of obesity (polygenic), but in a few rare types, monogenic obese phenotypes are known[33].

Variation in leptin levels have been found in many populations[34, 35].This study was carried out in the Saudi population to determine the effect of gender, age and body weight on leptin levels in body.

MATERIAL AND METHODS

We investigated 194 subjects out of which 31 were males and 168 were females. The study was approved by the ethical committee of the institution and informed consent was obtained from all participants. All individuals were normal, but were attendeding the outpatient clinics of King Khalid University Hospital for minor illness. Height and weight were measured in light clothing without shoes. BMI was calculated as follows: [Weight(kg)/ height^2(m2)]. The history and the essential information (age, height and weight) were recorded on specially designed forms. Blood was extracted by venipuncture in EDTA tubes, from each individual following an 8-12 hours fast.

The plasma was separated by centrifugation at 4000 rpm for 5 minutes at room temperature. The plasma samples were stored frozen at -20 °C until required for analysis. The cells were discarded. Leptin levels were estimated using Radioimmunoassay technique by using kits from Linco Research, St Charles, MO, USA.

Statistical Analyses

All data were analysed by the SPSS statistical package Version 17. Mean, median and mode, standard deviation, the standard error of mean, skewness, kurtosis and variance were calculated. The range was calculated using parametric (mean ± 2SD) and non parametric (2.5th 97.5th percentile) methods. Frequency distribution histograms were obtained separately for males and females (not shown). Any two groups were compared using student (t) test. $P < 0.05$ was considered statistically significant. Regression analysis was carried out and correlation coefficient (r) was calculated.

RESULTS

The demographic data (age, weight, height and BMI) for both genders are presented in Table 1. For both groups age, weight and BMI did not show any statistically significant difference.

Table 1. The demographic data (age, weight, height and BMI) for males and so aalhinesal gender

Parameter	Mean ± SD		
	Males (31)	Females (168)	Significance(P)
Age (Years)	36.55 + 16.31	33.68 + 11.43	N.S.
Weight (kg)	66.7 + 10.38	67.31 ± 13.08	N.S.
Height (m)	1.64 ± 0.079	1.59 + 0.08	P<0.05
BMI (kg/m2)	25.07 ± 4.51	26.87 ± 5.42	N.S

NS = Non- significant

Table 2. The distribution of serum leptin level in the total study population, and separately in males and in females

		Total Population (199)	Males (31)	Females (168)
Mean(ng/ml)		15.7	11.95*	16.4*
Std. error of mean (SEM)		0.55	1.3	0.59
Median (ng/ml)		14.77	11.4	15.5
Mode (ng/ml		12.29	0.65	12.29
Std. Deviation (SD)		7.71	7.22	7.61
Variance		59.44	52.2	58
Skewness		0.63	0.33	0.72
Std. error of Skewness		0.17	0.42	0.19
Kurtosis		0.71	-0.44	0.81
Std. error of Kurtosis		0.34	0.81	0.37
Minimum		0.65	0.65	2.99
Maximum		46.98	26.75	46.98
Percentile	2.5#	2.99	0.65	4.1
	97.5#	31.71	26.75	32.79

*p= 0.003

Table 3. Levels of leptin in the total study population and males and females, grouped into normal, overweight and Obese individuals, according to BMI

Weight gp	Leptin: Mean ± SD			
	Total study group	Males	Females	P
Normal weight	*10.92 ± 5.12**	*7.89 ± 5.32**	*11.63 ± 4.84**	0.008
Overweight	*16.87 ± 5.75***	*17.29 ± 6.63***	*16.81 ± 5.69***	0.84
Obese	**21.56 ± 8.18***	**15.37 ± 6.77***	**22.57 ± 8***	0.02

·Significance of the difference in the results of the males and females in different weight groups.

* The leptin levels in normal and overweight individuals in the total study population are significantly different (p = 0.0001), as well as in males and females (p = 0.002)
(p = 0.0001), respectively.

** The leptin levels in normal and obese individuals in the total study population are significantly different (p = 0.0001) . This is seen in the females (p = 0.0001) and to a lesser extent in males (p = 0.07).

*** The leptin levels in overweight and obese individuals in the total study population are significantly different (p = 0. 001); the same was observed in females(p = 0.0001), while there was no significant difference between overweight and obese males.

Leptin levels were analysed separately for the males and females and the results are presented in Table 2. The mean, median and mode values for leptin were higher in females (16.4, 15.5 and 12.29 ng/ml, respectively) compared to males (11.95, 11.4 and 0.65 ng/ml, respectively). The minimum and maximum values for leptin in males were 0.65 ng/ml and 26.75ng/ml, whereas in females the minimum value was 2.99 ng/ml and the maximum was 46.98 ng/ml. In males the leptin parametric range was 0-26.39 ng/ml and the non-parametric range was 0.65-26.75 ng/ml. In females, on the other hand, the parametric range was 1.18-31.62 ng/ml and the non-parametric range was 4.1-32.79 ng/ml. The results in the males and females showed a statistically significant difference in leptin levels, with significantly higher levels in the females compared to males (p=0.003).

Both the male and female were grouped according to their BMI values into normal weight (BMI≤24.9 Kg/m2), overweight (BMI 25-29.9Kg/m2) and obese (BMI≥30) individuals and leptin values were obtained. The results are shown in Table 3. In the total study population the mean of leptin in the normal weight, over weight and obese groups was 10.92, 16.87 and 21.56 ng/ml respectively and the difference in the levels in the different weight groups was statistically significant. This effect of weight on leptin level was obvious both in the males and the females. In the former group, the mean of leptin in the normal weight, overweight and obese groups was 7.89, 17.29 and 15.37 ng/ml, respectively. Whereas for females the mean of leptin in the different weight groups was 11.63, 16.81 and 22.57 ng/ml, respectively. A statistically significant difference in leptin levels in the normal and obese weight groups was noticed between males and females ($p < 0.05$).

Leptin levels were correlated with the anthropometric data in the males and females and Pearson correlation coefficient (r) was obtained. Table 4 summarizes the correlation of leptin with age, height, weight and BMI. A significant correlation was obtained between leptin and age, leptin and weight and leptin and BM1 in both the males and females.

Table 4. Correlation between leptin and age, height, weight and BMI

Correlations between	Total Population		Males		Females	
leptin &	r	p	R	P	R	P
Age	0.203	0.004	0.221	0.233	0.23	0.002
Weight	0.567	0.0001	0.340	0.061	0.609	0.0001
Height	-0.195	0.06	-.0506	0.004	-0.96	0.215
BMI	0.629	0.0001	0.56	0.001	0.631	0.0001

DISCUSSION

The results of our investigations showed that leptin levels were significantly different between the two sexes with significantly elevated level in females. These results are in line with all studies reported in literature[21,36,37,38] and have shown that leptin levels are higher in females compared to males. This gender difference may be explained by the fact that females tend to have more body fat than males and have a different fat distribution compared to males[39]. In addition, it is well documented that hormonal factors play a role and have an effect on adipose tissue distribution[11,23,24]. Since adipose tissue is more active in synthesizing leptin compared to visceral tissue, and adipose tissue is represented more in females than in males, this may be one of the causes of the difference seen in the male and female results.

When the levels of leptin were analyzed separately in the individuals with different body weights (normal, overweight and obese), it was observed that there was a significant difference in leptin levels between the three weight groups and that leptin levels increased significantly from the normal weight group to the obese weight group showing a positive and statistically significant correlation. The correlation results were the same

when the two genders were analyzed separately, and positive correlations remained significant between the leptin levels and BM1 in both males and females. In all groups leptin levels were significantly different between normal weight and obese Saudi males and females where the females had a significantly higher leptin levels compared to the male counterpart in the same BMI group

Among the female group, none of the normal weight females had very low levels of leptin and the maximum leptin level was 24.07 ng/ml. Among the overweight and obese females the levels were significantly higher and the maximum levels were 26.05 and 46.98 ng/ml, respectively. Two obese females had leptin levels less than 4.00 ng/ml and both were suffering from diabetes. One very obese female had a leptin level of 39.27 ng/ml, and was suffering from hypertension. Among the males, two normal weight males had leptin levels < 2.0 ng/ml and the highest level was 16.35 ng/ml. One 17 year old male had a very low leptin level of 0.65 ng/ml, and was having a very low weight and a BMI of 19.46 Kg/m‘. While in the overweight and obese males, none had very low levels of leptin, while the highest levels were 26.75 and 26.66 ng/ml respectively. This indicates that overall, leptin levels increase with an increase in BMI and that most obese persons had elevated leptin levels being insensitive to endogenous leptin production, they possibly have leptin resistance.

These results are in line with results reported in a study by Lucantoni *et al* (2000) [40]. The results of this study show definitely that in Saudis, obesity is not a leptin deficient state and none of the obese or overweight individuals had leptin deficiency. A leptin resistance state seems to exist in many, but not all, of the obese and overweight individuals investigated during this study. As mentioned earlier, it was believed by several investigations that obesity was a leptin deficient state and some of the earlier reports stressed the need for leptin therapy for the treatment of obesity[11,18,20]. Recombinant leptin preparations were marketed and several clinicians discussed its use in the management of obese patients. However, recent studies have confirmed that very few obese individuals have leptin deficiency, the majority of the obese individuals have either normal or even elevated leptin levels indicating that the leptin production in these individuals was normal if not higher than in normal weight individuals.

CONCLUSION

The exact mechanism of leptin resistance in the overweight and obese individuals and the mechanism leading to significant differences between the males and females need further studies.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-068"

Disclosure of competing interests

Authors declare that they do not have any competing interests with any group.

Authors contributions

MSS, NO and ASW designed the experiment, analyzed the data and wrote the manuscript. [2] ZB, MH and MA collected samples from their patients and contributed to discussion of results and preparation of the manuscript. SD and MA helped in data analysis, discussion of results and preparation of the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; **392**(6674): 398-401.
2. Myers MG Jr. Leptin receptor signaling and the regulation of mammalian physiology. *Recent Prog Horm Res* 2004; **59**:287–304.
3. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; **89**: 2548-2556
4. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. *Nature.* 2006; **443**: 289-295
5. Henson MC, Castracane VD, Edwards DE. Leptin: from satiety signal to reproductive regulator. In: Henson MC, Castracane VD, (eds.), Leptin and Reproduction. New York: Kluwer Academic Press/Plenum Press Publishers, 2003: 3-11.

6. Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: a review. *Fertil Steril* 2002; **77**:433–444.
7. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest*. 1997; **100**:270-278.
8. Sierra-Honigmann MR, Nath AK, Murakami C, et al. Biological action of leptin as an angiogenic factor. *Science*. 1998; **281**: 1683-1686.
9. Bouloumie A, Drexler HC, Lafontan M, Busse R. Leptin, the product of *ob* gene, promotes angiogenesis. *Circ Res*. 1998; **83**: 1059-1066.
10. Ducy P, Amling M, Takeda S, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell*. 2000; **100**: 197-207.
11. Mantzoros C.S, Moschos S.J. Leptin: in search of role(s) in human physiology and pathophysiology. *Clin. Endocrinol*. 1998; **49**: (5): 551
12. Burguera B, Couce M.E, Curran, G.F, Jensen M.D, Lloyd R.V, Cleary M.P, Poduslo J.F. Obesity is associated with a decreased leptin transport across the blood-brain barrier in rats. *Diabetes* 2000; **49**(7): 1219-1223.
13. Burguera B, Couce M.E, Long j, Lamsam j, Laakso K, Jemsem M.D, Paresi J.E, Lloyd R..V. The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. *Neuroendocrinology* 2000; **71**(3):187-95
14. Myers, M.G., Cowley, M.A. & Munzberg, H. Mechanisms of leptin action and leptin resistance. *Annu. Rev. Physiol*. 2008; **70**: 537–556.
15. Muoio D. and Dohm G. Lynis. Peripheral metabolic actions of leptin. *Best Pract Res Clin Endocrinol Metab*. 2002; **16** : 653-666.
16. ORourke L, Yeamen S.J, Shepherd P.R. Insulin and leptin acutely regulate cholesterol ester metabolism in macrophages by novel signaling pathways. *Diabetes* 2001; **50**: 955-961.
17. Lu H, Li C. Leptin: a multifunctional hormone. Cell Res. 2000; **10**: 81-92.
18. Davies L, Marks J.L. Role of hypothalamic neuropeptide Y gene expression in body weight regulation. *Am. J. Phsiol*. 1994; **226**: 1687-1691.
19. Oksanen J, Kaprio P, Mustajoki and Kontula K. A common pentanucleotide polymorphism of the 3'-untranslated part of the leptin receptor gene generates a putative stem-loop motif in the mRNA and is associated with serum insulin levels in obese individuals. Inter. *J. of Obes. Relat. Metab. Disord*. 1998; **22**: 634-640.
20. Igel M, Becker W, Herberg L, Joost H.J. Hyperleptinemia, leptin resistance and polymorphic leptin receptor in the new Zealand obese mouse. *Endocrinology.* 1997; **138** (10): 4234-4.
21. Rosenbaum M, Pietrobelli A, Vasselli JR, Heymsfield SB, Leibel R. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. *Int J Obes Relat Metab Disord* 2001; **25**: 1365-71.
22. Kim S, Moustaid- Moussa N. Secretory, endocrine and autocrine/paracrine function of the adipocyte. *J. nutrition* 2000; **130**: 3110S-3115S.
23. Baumgartner RN, Waters DL, Morley JE, Patrick P, Montoya GD, Garry PJ. Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. *Metabolism* 1999; **48**: 378-84
24. Roemmich JN, Clark PA, Berr SS, *et al.* Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. *Am J Physiol* 1998; **275**: E543-551.
25. Li MD, Parker SL, Kane JK. Regulation of feeding-associated peptides and receptors by nicotine. *Mol Neurobiol* 2000; **22**: 143- 65.
26. Kraemer RR, Chu H, Castracane VD. Leptin and exercise. Exp Biol Med 2002; 227: 701-8.
27. Ruhl CE, Everhart JE, Ding J, *et al.* Aging, and Body Composition Study. *Am J Clin Nutr* 2004; **80**: 576-83.
28. Perkins KA, Fonte C. Effects of smoking status and smoking cessation on leptin levels. Effects of smoking status and smoking cessation on leptin levels. *Nicotine Tob Res* 2002; **4**: 459-66
29. Njelekela M, Kuga S, Hiraoka J, *et al.* Determinants of hyperleptinaemia in an African population. *East Afr Med J* 2003; **80**: 195-9.
30. MacDougald 0.A, Hwang C.S., Fan H, Lane M.D. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc. Natl. Acad.* 1995: **92** (20): 9034-9037.
31. Pankov I.A. Leptin-a peptide hormone from adipocytes. Sensation of the 23rd FEBS Meeting. Bioorg. *KHim.* 1996; **22**: 228-233
32. Fielding P.E., Fielding C.J. Dynamics of lipoprotein transport in the human circulatory system. In: Biochemistry of lipids, lipoproteins and membranes. Edited by Vance D.E., Vance J.E. Elsevier Science. New York. 1997.
33. Bougneres P. Genetics of obesity and type 2 diabetes: tracking pathogenic traits during the predisease period. *Diabetes* 2002; **51**(3): 295-202.
34. Bribiescas, RG. and Hickey, M S. Population variation and differences in serum leptin

independent of adiposity: a comparison of Ache Amerindian men of Paraguay and lean American male distance runners. *Nutrition & Metabolism* 2006, **3**: 34.

35. Lindeberg S. Soederberg S, Ahren B and Olsson T. Large differences in serum leptin levels between nonwesternized and westernized populations: the Kitava study. Journal of Internal Medicine 2001; **249**: 553-558.
36. Considine R.V, Sinha M.H, Heiman M.L, Kriauciunas A, Stephens T.W, Nyce M.R, Ohannesian J.P, Marco C.C, McKee L.J, Bauer T.L, Caro J.F. Serum immunoreactive leptin concentrations in normal weight and obese humans. *New Engl. J. Med.*1996; **334**(5): 292-295.
37. Flier J.S. What's in a name? In search of Leptins physiologic role. *J. Clin. Endocrinol. Met* 1998; **83**(5): 1407-1413.
38. Havel P.J. Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance. *Proc. Nutr. Soc.* 2000; **59**(3): 359-71
39. Allison D.B, Faith M.S. Genetic and environmental influences on human body weight: implication for the behaviour therapist. *Nutr. Today* 2000; **35**: 18-21.
40. Lucantoni R, Ponti E, Berselli M.E, Savia G, Minocci a, Calo' G, De Medici C, Liuzzi A, Di Blasio. The AI9G polymorphism in the 5' untranslated region of the human obese gene does not affect leptin levels in severely obese patients. *J. Endocrinol. Met.* 2000; **85**(10): 3589-3591.