

Fluctuation of Serum Leptin Through Menstrual Cycle in Young Fertile Women in Mosul City

Rajaa A. yonis* , Fadhila S. Al-Doski*

*Department of Physiology, College of Medicine, University of Mosul, Mosul.

Correspondence: rajaa2009@yahoo.com

(Ann Coll Med Mosul 2019; 41 (2):125-131).

Received: 11th April. 2019; Accepted: 21st July. 2019.

ABSTRACT

Background: Leptin is a hormone secreted by adipocytes , it is a protein encoded by the ob gene. Mutations in the ob gene in animals cause obesity , leptin affects nutritional homeostasis and reproductive function.

Objectives : a followup study was done to investigate serum leptin levels in 30 fertile healthy normal weight women with regular menstrual cycle aged (8-45) years in follicular and luteal phase of menstrual cycle.

Methods: Fasting blood samples were obtained from normal weight fertile women (BMI 19.5-24.9 Kg/m²) to measurement serum concentrations of leptin, which measured by using (ELISA) in follicular phase (2nd day of menstrual cycle) then ask the women to come back and give another blood sample in 21st- 23rd day of the cycle to measure serum progesterone and leptin in luteal phase of the menstrual cycle.

Results: The mean age \pm SD of the normal fertile women was (29.1 \pm 8.4) years, the mean weight value \pm SD was (53 \pm 5)kg and the mean height value \pm SD was (158 \pm 6.2) cm and the mean BMI \pm SD was (21.2 \pm 1.3).

Leptin was higher in luteal phase than follicular phase in normal weight fertile women ,this was statistically significant ($p \leq 0.000$), the mean \pm SD of progesterone hormone was (7.8 \pm 3.2) in day 21-23 of the cycle this was the indicator for ovulation and starting of luteal phase.

Conclusion: A great variation in serum leptin levels throughout the menstrual cycles with lower levels in the follicular rather than luteal phase in normal weight fertile women is found.

Keywords: Leptin , fertile women, menstrual cycle.

تقلب مستوى هرمون اللبتين خلال الدورة الشهرية لدى النساء الخصبات في مدينة الموصل

رجاء احمد يونس* ، فضيلة شهوان الدوسكي*
* فرع الفلسفة الطبية ، كلية الطب ، جامعة الموصل، الموصل

الخلاصة

الخلفية: هرمون اللبتين هو ناتج أل(وب) جين يفرز من الخلايا الدهنية . لوحظ ان الحيوانات التي لديها تغير في هذا الجين تعاني من السمنة , هرمون اللبتين لا يؤثر على التوازن الغذائي فقط بل على الأحداث التكاثرية في دراسة تتابعية تم فحص هرمون اللبتين في ثلاثون امرأة في سن الإخصاب أعمارهن 18-45 سنة ولديهن دورة شهرية منتظمة بعد ان تم تقييم الوزن الطبيعي بواسطة مؤشر كتلة الجسم .

الاهداف : في دراسة تتابعية تم فحص هرمون اللبتين في ثلاثون امرأة في سن الإخصاب أعمارهن 18-45 سنة ولديهن دورة شهرية منتظمة بعد ان تم تقييم الوزن الطبيعي بواسطة مؤشر كتلة الجسم .

طريقة العمل: تم سحب عينات الدم من النساء قبل تناول الفطور وذلك من اجل قياس هورمون اللبتين في الطور الجريبي من الدورة الشهرية بعد اخذ الوزن والطول للمريضة وحساب مؤشر كتلة الجسم على ان يكون وزن المرأة ضمن المؤشر الطبيعي (19.9-24.9 كغم)² وتم سحب عينة اخرى من نفس النساء في الطور الاصفر من الدورة (21-23) من الدورة من اجل اخذ عينة اخرى وقياس هورمون اللبتين والبروجستيرون لتأكيد عملية الاباضة .

النتائج: كان متوسط \pm الانحراف المعياري للعمر (31.6 \pm 7.9) سنة و متوسط \pm الانحراف المعياري للوزن في المجموعة (5 \pm 53) كغم و متوسط \pm الانحراف المعياري للطول (158 \pm 6.8) cm و متوسط \pm الانحراف المعياري لمؤشر كتلة الجسم (21.2 \pm 1.3) هورمون اللبتين كان اعلى في الطور الاصفر منه في الطور الجريبي بفرق إحصائي واضح (p \leq 0.000) وكان متوسط \pm الانحراف المعياري لهورمون البروجستيرون (7.8 \pm 3.2) في يوم (21-23) من الدورة الشهرية مما يدل على وجود اباضة.

الاستنتاجات: تبين وجود اختلاف كبير في مستوى هورمون اللبتين في مصل الدم خلال الدورة الشهرية , حيث كان المستوى الأدنى في الطور الجريبي عنه في الطور الاصفر لدى النساء الخصبات ذوات الوزن الطبيعي .

الكلمات المفتاحية: اللبتين ، النساء المنجبات ، الدورة الشهرية.

INTRODUCTION

At Jackson Laboratory in 1950 the effects of leptin were observed by studying mutant obese mice that arose at random within a mouse colony¹. These mice were very obese and extremely voracious. , many strains of laboratory mice have been found to be homozygous for single-gene mutations that cause them to become grossly obese, and they fall into two classes: "ob\ob", those having mutations in the gene for the protein hormone leptin, and "db\ob", those having mutations in the gene that encodes the receptor for leptin. When ob\ob mice are treated with injections of leptin, they lose their extra fat and return to normal body weight. Leptin itself was discovered in 1994 by Jeffrey M. Friedman and colleagues at the Rockefeller University through the study of such mice^{2,3}.

Leptin is a 16 KDa protein produced by adipose tissue and secreted into the peripheral blood^{2,3,4}.

It is apparently paradox that leptin which was named after the Greek word (Leptos) for lean is encoded by the obesity gene (ob). This unfortunate misnaming occurred because it is leptin deficiency that was discovered first in obese mice^{2,5,6}.

Leptin interface with the reproductive axis at multiple sites, it has stimulatory effects at the hypothalamus and pituitary and inhibitory actions at the gonads^{7,8}.

In women the reproductive system, not like that of men, has regular cyclic changes which

may be regarded as periodic preparations for fertilization and pregnancy. In humans and other primates, the cycle is a menstrual cycle, and its most distinct feature is the periodic vaginal bleeding that occurs with the take of the uterine mucosa (menstruation)⁹.

In advanced researches, leptin has been found to play a role in other target reproductive organs such as the endometrium, placenta and mammary gland; with corresponding influences on important physiologic processes such as menstruation, pregnancy and lactation^{7,9}.

It may act as the important connection between adipose tissue and the reproductive system, suggesting there are adequate energy reserves are present for good and normal reproductive function, suboptimal nutritional status as functional hypothalamic amenorrhea, exercise-induced amenorrhea and eating disorders are associated with low serum leptin levels and conditions with extra energy stores or metabolic disturbances such as obesity and polycystic ovarian syndrome which usually have high serum leptin levels, making the possibility that moderate leptin deficiency or resistance may be at least responsible for the reproductive abnormalities that occur with these conditions^{7,10}.

AIMS OF THE STUDY

- 1.Measurement of serum leptin in follicular phase in young fertile women with regular menstrual cycle.
- 2.Measurement of serum leptin and serum progesterone in luteal phase of the same group.
- 3.Comparing serum leptin level in two phases of menstrual cycle.

MATERIALS AND METHODS

Subjects & Study Design

The study was conducted at the women health center of al-khansa hospital in 2013 a follow up prospective study included thirty fertile apparently healthy women in reproductive age (18 - 45) years old.

All women included in the study were interviewed and the general information was taken to fill the questionnaire .

A verbal informed consents were taken from all participants

They had regular menstrual cycle for the last 6 months ago , and did not used any hormonal medication for at least 2 months before the study. None of them had a history of coronary heart disease, diabetes mellitus, Hypertension, they were neither pregnant nor lactating.

For every women height was measured to the nearest millimeter using a standard stadiometer, with the participant bare foot and wearing light weight clothing, weight was measured to the nearest (0.1)kg using a standard digital scale and BMI (kg/m^2) was computed by dividing the weight in kilogram (kg) over the height in square meters (m^2).

SPECIMENS

The subjects were instructed to have an overnight fasting, through antecubital venepuncture blood samples were obtained from all women participated in the study. Two milliliter (2ml) of venous blood had been collected in plain tube in 2nd -3rd day of menstrual cycle then incubated for 37°C for 15 minutes in water bath, after centrifugation for 10 minutes at 3000 rotation per minute (rpm), aspiration of supernatant sera were put in plane tube , then frozen at -20°C till time of assay to measure leptin hormone .

Then the women were instructed to come back and give another blood sample in 21st-23nd day of the cycle to measure serum progesterone and leptin in luteal phase of the menstrual cycle. .

METHODS

1. Leptin Measurement

Leptin was measured by using kit provided by DRG company, (Germany) Based on a solid phase ELISA of a sandwich type. The analytical sensitivity of zero standard was equal to (1.0ng/mL) and the assay dynamic range was(1-100ng/mL).

2. Progesterone Measurement

VIDAS progesterone kit provided by Biomerieux (France) is automated quantitative test for measurement of progesterone in human serum or plasma using the ELFA technique (Enzyme linked fluorescent Assay) .

Statistical Analysis

SPSS version 11.5 was used for the statistical analysis.

1. Descriptive results were represented as mean \pm standard deviation (SD).
2. The comparisons within the group were done by student t- test .

RESULTS

1. The anthropometric parameters of 30 normal weight fertile women is shown on Table1 .

Table 1 anthropometric measures of the (30) normal weight fertile women.

Parameters	Normal weight	
	No.(30)	
	Mean±SD	
Age	29.1±8.4	
No. of children	2.8±1.3	
Weight(kg)	53±5	
Height(cm)	158±6.2	
BMI (kg/m ²)	21.2± 1.3	

2. Leptin in follicular phase and luteal phase of menstrual cycle in normal fertile women **Table 2**

Serum leptin was higher ($p \leq 0.000$) in luteal phase than follicular phase in normal weight women, the mean \pm SD of serum progesterone hormone was (7.8 \pm 3.2) ng/ml in day 21-23 of the cycle this was the indication for ovulation and luteal phase starting table 2 .

Table 2 Leptin in normal weight with regular menstrual cycle women in the follicular phase and luteal phase.

Normal weight with regular cycle women			
(n = 30)			
Parameters	Mean± SD at 2nd day of cycle (follicular phase)	Mean± SD in (luteal phase)	*p –value
Leptin ng/ml	12.47 ± 6.7	14.98±7.31	0.0001

* p-value \leq 0.05 is significant , ** p-value = 0.01 is highly significant.

DISCUSSION

Leptin adipocyte-derived hormone is one of these cues which have been studied carefully in the regulating the reproductive physiology. Humans and mice lacking leptin or leptin receptor are infertile. Administration of Leptin to leptin-deficient subjects and ob/ob mice induces puberty and restores fertility ¹¹ .

The role of leptin in the preservation of body weight, related with various other regulatory functions in endocrine systems including the hypothalamo-pituitary gonadal axis which is well recorded. New works reveal that leptin is also involved in reproduction and responsible for fertility of humans and animals ¹² .

Leptin level enlightin the brain that metabolic reserve are adequate for reproduction¹³ .

It appears obvious that a minimum threshold of serum leptin is essential for a regular ovulatory function¹⁴ . The mechanism of high serum leptin in luteal phase is not known exactly . It could be by increased a adipocyte production of leptin hormon due to high caloric intake or hypothalamic neuropeptide Y production. Alternatively, this increase may indicate the release of leptin from mature ovarian follicles¹⁵ .

Low serum leptin is well known in amenorrhea which found in conditions such as in highly trained women or in women with anorexia nervosa. Thus, leptin clearly appears to be linked to the reproductive system, and menstrual cycle¹⁴ .

In the present study, a thirty fertile women with normal bodyweight and regular cycles, in whome luteal phase confirmed by serum progesterone estimation, luteinization and ovulation were considered to have taken place when serum progesterone concentration exceeded 5 ng/ml^{16,17} .

The mean \pm SD of progesterone level was (7.8 \pm 3.2) ng\ ml in the (21 -23) day of the cycle .

The serum leptin level in the 2nd day of the cycle (follicular phase) was lower than it in the 21-23days (luteal phase)of the menstrual cycle , this difference was statistically significant ($p < 0.0001$) , so this study demonstrated for the first time in our locality that healthy women in mosul city with normal weight having significantly higher serum leptin level during luteal in contrast to the follicular phase.

This result is in agreement with a number of other studies^{15,17,18,19,20,21,22,23,24} had shown considerable variation in serum leptin levels throughout the menstrual cycles with higher levels in the luteal rather than follicular phase .

Einollahi *et al.*, in 2010 studied 42 Iranian women with regular ovulatory cycle their age were between (18 -35) years, BMI (18-25) kg\ m² the mean serum leptin concentration \pm SD during the follicular phase (13.1 \pm 1.6)ng\ml were lower compared to the luteal phase (16.5 \pm 1.6) ng\ml ($p < 0.015$) which is stately significant¹⁷ .

Leptin homone act as mediator of cell proliferation and with respect to the presence of leptin receptors in the endometrium, its rational that the luteal phase of menstrual cycle has a high leptin levels, also preovulatory surge of estrogen might increase the circulating leptin which in turn could provoke the gonads to produce more leptin^{25,26,27} .

Data of the present study seem to contradict the finding of other studies, which found no statiatical significant difference in serum leptin level in follicular and luteal phase, Shafi and Afzal in 2009 study 83 women in reproductive age (13-45)years, 37 were in follicular phase ,47 in luteal phase no significant difference found in leptin level (54.6 \pm 33.4 ; 53.5 \pm 38.8) respectively ($p \leq 0.25$) but when they classified them according to BMI they found no significant difference in overweight women and only normal weight women show higher serum leptin in follicular phase²⁸ .

Some other studies also have found either small but insignificant swing toward higher leptin levels at the end of the cycle²⁹ . Serum leptin concentrations were the same at the beginning of the cycle, around the time of the anticipated ovulation and at the end of the cycle (no fluctuation at all)^{30,31} .

Recommendations and Further Work

- 1.To study leptin hormone in fertile and non fertile women.
- 2.To study leptin hormone in pregnant women and after delivary.

REFERENCES

1. Ingalls AM ,Dickie MM ,Snell GD. (1950).Obese, anew mutation in the house mouse. J Hered. 41(12):317-318 .(abstract)
2. Zhang Y, Proenca R, Brone M, Leopld L,Friedman JM, et al.(1994). Positional cloning of the mouse obese gene and its human homologue. Nature .372(6505):425-432.
3. Murakami T and Shima k (1995).Cloning of rat obese cDNA and its expression in obese rats. Biochemical and biophysical research communications. 209(3):944-952.(abstract)

4. Saad M F, Khan A, Sharma A, Micheal R, Jinagonda SD, et al. (1998). Diurnal and Ultradian rhythmicity of plasma leptin: effects of gender and adiposity . *J Clin Endocrinol Metab.* 83: 453–459.
5. Larsen P, Kronenberg H ,Bresser C, Melmed S, Polonsky K. (2003).Williams textbook of Endocrinology.10th ed. Elsevier Saunders Company, Philadelphia, USA: pp 138-140 ; 602-634.
6. Saleh HA, El-Nwaem MA , El-Bordiny MM, El-Mohandes AA, Eldaqaqet EM, et al. (2004).Serum leptin elevation in obese women with PCOS a continuing controversy. *Journal of assisted reproduction and genetics .* 21(10):361-366.IVSL
7. Moschos S,Chan JL, Manzoros CS.(2002).Leptin and reproduction :a review. *Fertility sterility.*77(3):433-444.
8. Popovic V and Casanueva FF.(2002). Leptin, nutrition and reproduction: new insights. *Hormones .* 1(4):204-217.
9. Barrett KE , Boitano S, Barman SM, Brooks HL. (2010). Ganong's review of Medical Physiology, 23rd Edition. McGraw-Hill Companies, Inc, USA: pp 411-421.
10. Messinis IE and Milingos SD.(1999).Leptin in human reproduction.Human reproduction update . 5(1) :52-63.
11. Donato JJ , Roberta MR , Frazão C, Elias CF .(2011). Hypothalamic Sites of Leptin Action Linking Metabolism and Reproduction. *Neuroendocrinology .* 93 : 9–18.
12. Farooq R, Lutf Ullah S and Ishaq H.(2013).Relation of serum leptin with sex hormone of obese infertile men and women. *Journal applied pharmaceutical science .* 3(10) :060-065.
13. Yamada M, Matsuzaki T, Iwasa T, Shimizu F, Tanaka N, et al. (2003).Serum leptin level in women throughout life; relation to body mass index and serum estradiol levels. *J reproductive endocrinology.*8:55-60.IVSL
14. Cella F, Giordano G, Cordera R.(2000). Serum leptin concentrations during the menstrual cycle in normal-weight women: effects of an oral triphasic estrogen–progestin medication. *European Journal of Endocrinology .* 142 :174–178.
15. Riad-Gabriel MG, Jinagouda SD, Sharma A, Saad MF and Boyadjian R.(1998). Changes in plasma leptin during the menstrual cycle. *European Journal of Endocrinology .*139 :528–531.
16. Pirwany IR , Fleming R,Sattar N,Greer IA and Wallace AM.(2001).Circulating leptin concentration and ovarian function in polycystic ovary syndrome. *European Journal of Endocrinology.*145:289-294.
17. Einollahi N , Dashti N and Nabatchian F.(2010). Serum Leptin Concentrations during the Menstrual Cycle in Iranian Healthy Women. *Acta Medica Iranica .* 48(5) : 300-303.
18. Hardie L, Trayhurn P, Abramovich D and Flower P.(1997). Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clinical Endocrinology .*47, (1) : 101–106. IVSL
19. Messinis IE, .Milingo S , Zikopoulos K, .Kollio G , .Seferiadi K and Lolis D. (1998). Leptin concentrations in the follicular phase of spontaneous cycles and cycles superovulated with follicle stimulating hormone . *Human Reproduction .* 13(5): 1152–1156.
20. Quinton ND, Laird SM, Okon MA, Li TC, Smith RF, et al.(1999). Serum leptin levels during the menstrual cycle of healthy fertile women. *Br.J.Biomed Sci.* 56(1):16 -19. (abstract)IVSL
21. 2l. Anim-Nyame N, Domoney C, Panay N, Jones J , Studd JWW and Zadeh J.(2000). Plasma leptin concentration are increased in women with premenstrual syndrome. *Human Reproduction.*15(11):2329 -2332.
22. Al-Wunder DM, Yared M, Bersinger NA, Widmer D, Kretschmer R, et al.(2006). Serum leptin and C-reactive protein levels in the physiological spontaneous menstrual cycle in reproductive age women. *European journal of endocrinology.*155:137-142.
23. Al-Harithy RN, Al- Doghaither H and Abualnaja K. (2006). Correlation of leptin and sex hormones with endocrine changes in healthy Saudi women of different body weights. *Annals of Saudi medicine .* 26(2): 110-115.

24. Baig M.(2007).Role of serum leptin in primary infertility in females. In partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry. College of Medicine, University of Karachi .pp150-153.
25. Mannucci E, Ognibene A, Becorpi A, Cremasco F, Massi G, et al.(1998).Relationship between leptin and oestrogens in healthy women. European Journal of Endocrinology.139.198-201.
26. Wolf G, Hamann A, Han DC, Helmchen U, Thaiss F,Ziyadeh FN, et al. (1999). Leptin stimulates proliferation and TGF-beta expression in renal glomerular endothelial cells: potential role in glomerulosclerosis . Kidney International .56(3):860-872.
27. Sirotkin AV and Grossmann R. (2007). Leptin directly controls proliferation, apoptosis and secretory activity of cultured chicken ovarian cells. Comp Biochem Physiol A Mol Integr Physiol .148(2):422-429. (abstract)IVSL.
28. Shafi R and Afzal MN.(2009). Leptin Levels during Follicular and Luteal Phases of Menstrual Cycle. Rawal Med J. 34(2):180-182.
29. Teirmaa T, Luukkaa V, Rouru J, Koulu M and Huupponen R.(1998). Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. European Journal of Endocrinology . 139: 190–194.
30. Stock SM, Sande EM and Bremme KA. (1999). Leptin levels vary significantly during the menstrual cycle, pregnancy, and in vitro fertilization treatment: possible relation to estradiol. Fertility Sterility .72:657– 62.
31. Lin KC. (1999). Changes of circulating leptin levels during normal menstrual cycle: relationship to estradiol and progesterone. Kaohsiung J Med Science .15:597– 602.