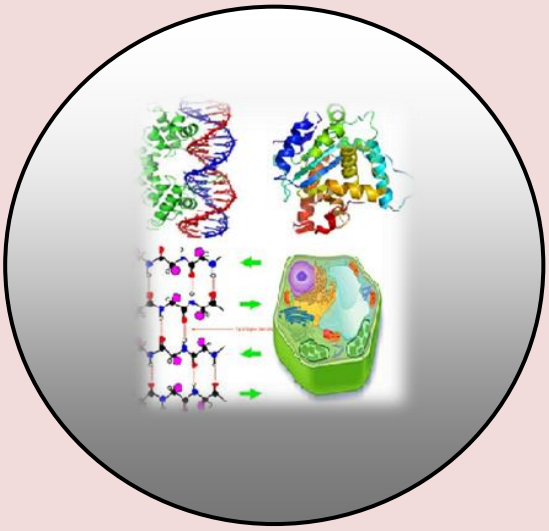
Serum Pattern of Circulating Leptin during Menstrual Cycle: Relationship to Estradiol and Progesterone

By
Jana Chakrabarti

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1), Part C, 2019 Pages No. 48-55



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1, Part C: 48-55, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/2523/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

Dr. J. Chakrabarti
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER
Received: 05/04/2019 Revised: 15/04/2019 Accepted: 16/04/2019

Serum Pattern of Circulating Leptin during Menstrual Cycle: Relationship to Estradiol and Progesterone

Jana Chakrabarti

Department of Zoology, A.P.C Roy Government College, Himachal Vihar, Matigara, Siliguri – 734010, West Bengal, India

ABSTRACT

Ovary is an ever changing tissue and dynamic multi-compartmental organ which is under the chief regulatory control of hypothalamic pituitary principles. The hypothalamic-pituitary control over ovarian functions however is precisely governed by a plethora of external factors and internal peripheral principles including many of ovarian origin. A critical body mass of adipose tissue is essential for the normal development of female reproductive functions. The mechanistic link between body mass and reproductive functions; however, is not clearly elucidated. Leptin, an adipocyte derived peptide has emerged as a potential regulator of many reproductive functions including gametogenic and steroidogenic potential of ovary. Available literature propose leptin as a signal or playing permissive role in this regard, but perplexing controversies over every aspect of leptin effects left us nowhere to form a conclusive concept on the dynamic interrelationship between leptin and ovarian functions. Present investigation thus attempts to explore the possible interrelationship between gonadotropins, ovarian steroids and leptin. To address the issue, fasting serum leptin levels were evaluated in a cohort of regularly cycling eugonadal women with or without luteal phase deficiency. Leptin exhibits a distinct pattern of change during different phases of menstrual cycle. It gradually increases from baseline day through the ovulatory phase and reaches the maximum level in the luteal phase. The possible stimulatory influence of estrogen cannot be ruled out but no deviation of leptin pattern in luteal phase deficient women from that with normal luteal phase discount any possible leptinotropic effect of progesterone.

***Key words:** Gonadotropin, Hypothalamo-Pituitary-Gonadal Axis, Leptin, Menstrual Cycle and Ovarian Steroids.*

INTRODUCTION

A critical body mass of adipose tissue is essential for the normal development of female reproductive functions (Tataranni et al. 1997). The mechanistic link between body mass and reproductive functions, however, is not clearly elucidated (Chu et al. 2002). Leptin, an adipocyte derived multifactorial 16KDa polypeptide, consists of 167 amino acids and encoded by the '*Ob*' gene has been proposed as the peripheral signal indicating the adequacy of nutritional status for reproductive functions (Almog et al. 2001).

Female reproductive function is exquisitely sensitive to the alteration in body's metabolic states. Leptin is important in regulating energy homeostasis, and by this virtue impacts the reproductive systems in diverse ways (Moschos et al. 2002). Leptin perhaps exerts direct regulatory action on ovarian folliculogenesis (Spicer and Francisco 1997). It is also reported to regulate ovarian function through its modulating effects on the hypothalamo-pituitary-ovarian (HPO) axis and contributing to the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and gonadotropins from pituitary and thus modulate ovarian steroidogenesis (Licino et al. 1998). Leptin concentrations showed fluctuations during menstrual cycle (Hardie et al. 1997). Moreover, leptin mRNA and its protein production have been documented in granulosa cells, oocytes, and early cleavage stage embryos (Cioffi et al. 1996; Luoh et al. 1997; Karlsson et al. 1997; Cioffi et al. 1997; Considine et al. 1996). However, though leptin is widely present in reproductive tissues, its relationship to reproductive hormones is still poorly understood. Estrogens are reported to stimulate the release of leptin in the humans (Bray and York 1997). Estrogens are considered among the factors accounting for higher concentration of leptin in women than in men, and also for cyclic elevations in leptin concentrations during the menstrual cycle (Saad et al. 1997; Sumner et al. 1998; Hardie et al. 1997; Hadji et al. 2000). It has also been demonstrated that preovulatory human follicles synthesizes leptin. However, the overall data on the relationship between gonadotropins, sex steroids and leptin are not uniform. There are reports that leptin levels in menopausal women do not undergo alteration (Gower et al. 2000), increase (Rosenbaum et al. 1996), or decrease (Shimizu et al. 1997). Progesterone is also known to stimulate leptin release, regardless of estrogen status (Messinis et al. 2000). Natural progesterone can stimulate leptin release and perhaps accounts for the higher concentration of leptin in the luteal phase of the menstrual cycle (Licinio et al. 1997). Steroid hormone levels undergo cyclic variation during different phases of menstrual cycle. This provides a unique opportunity to evaluate the effects of reproductive hormones on leptin, provided the variables are corrected for confounding factors. Present study is therefore, centered on the objective of evaluating the cyclic variation of leptin in regularly cycling women in concert with cyclic changes in the hormonal levels with reference to factors like obesity and luteal phase defect.

MATERIAL AND METHODS

Study subjects

A total of 21 regularly menstruating women between the ages of 27-34 years old, attending the Institute of Reproductive Medicine (IRM) infertility clinic for male factor infertility volunteered for the study. The subjects were included in the study only after receiving consent from them. The investigations were performed with the approval from the Institutional Research Ethics Board. All subjects had documented ovulation during the past cycles preceding the study cycle. None of the women were taking any medication and none had any systemic disease or symptoms related to polycystic ovarian syndrome (PCOS). There was no history of weight gain or loss more than 6% during the past three months. Moreover, 15 patients diagnosed with luteal phase defect (LPD) were also recruited for this study, since the objective of our study was to monitor the cyclic variation of leptin in concert with the cyclic changes of hormonal levels. They were selected on the basis of luteal phase length (<11days), the mid-luteal serum progesterone level (<10ng/ml) and the follicular growth pattern. Body Mass Index (BMI) of all subjects was recorded at the beginning.

Outcome measures

Fasting blood samples were taken by venipuncture between 08:00 ± 30 A.M for every woman, every alternate day until the next menstruation started. Between day 9 and 16 of the cycle, transvaginal USG assessed folliculometry was performed to document the time of ovulation. The day of the mid-cycle gonadotropin surge was identified on the basis of day of LH/FSH peak, day of estradiol peak, and the day of ovulation as documented by fluid in pouch of Douglas (POD).

Biochemical assays

Patients were reviewed for glucose, leptin, insulin, luteinising hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), prolactin (PRL), Estrogen and Progesterone.

FSH (assay sensitivity: 0.3μIU/ml), LH (assay sensitivity: 0.07μIU/ml), TSH (assay sensitivity: 0.01μIU/ml) and prolactin (assay sensitivity: 0.3ng/ml) were measured by a two-site Chemiluminescence sandwich immunoassay System. Estradiol (assay sensitivity: 9pg/ml) and Progesterone (assay sensitivity: 0.25ng/ml) were assayed through Enzyme Linked Fluorescent Assay (ELFA). Insulin level (assay sensitivity: 1.3μIU/ml) was analyzed by Radioimmuno assay (RIA). All serum samples were assayed for leptin with immunoradiometric assay (IRMA) with assay sensitivity of 0.10ng/ml. Estimation of blood glucose was done by glucose oxidase method.

Statistical analysis

Results were expressed as mean ± standard error of mean (SE). All statistical analyses were done using PRISM Statistical Software Package (PRISM Version 4.03@1992- 2005; GraphPad Software Inc). $P < 0.05$ was considered significant.

RESULTS

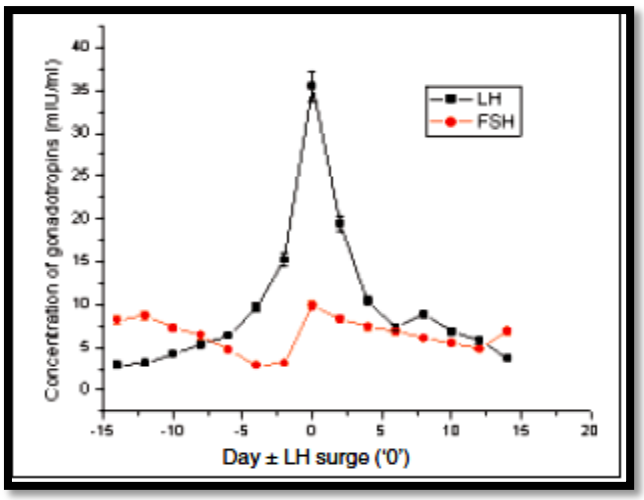
Table 1 represents baseline characteristics and hormone levels, which were comparable between the two categories of women, except luteal phase progesterone that was significantly lower in the LPD group. In all subjects, the study cycles were ovulatory. The gonadotropins, estradiol and progesterone showed the expected changes during different phases of menstrual cycle. In regularly menstruating women, serum leptin levels increased gradually from the onset of menses through the late follicular phase to reach a plateau by the time of mid-cycle gonadotropin surge. There was a subsequent rise in the leptin level during the luteal phase followed by decline to reach the baseline value by the start of the next menstrual cycle. The changes in serum leptin levels apparently correlated with those of changes in, estradiol (Fig. 1). In women with luteal phase defect (LPD), progesterone levels dropped 4 days earlier (Day +10), but increase in leptin levels followed the same pattern as it was in the regularly menstruating women (Fig 2). However, leptin levels in the LPD women during the postovulatory mid-luteal phase were of significantly lower magnitude as compared to those of the regularly cycling group.

Table 1. Baseline characteristics and hormone levels of the regularly menstruating women population with normal luteal phase and luteal phase deficiency

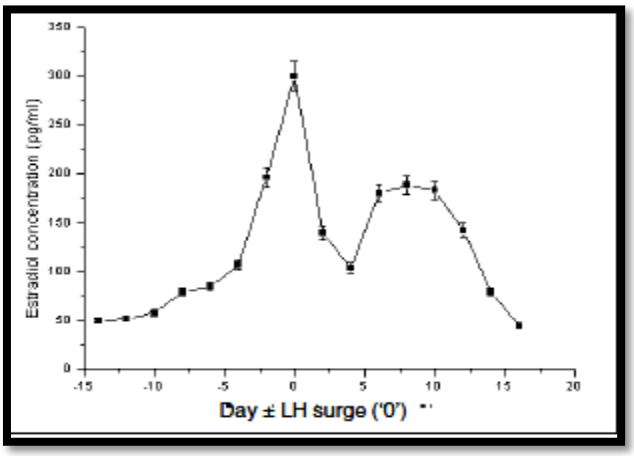
Regularly Menstruating Women		
Parameters	**Normal Luteal Phase (n= 21)**	**Luteal Phase Deficient (n=15)**
Age (years)	33.00±1.03	32.0±0.90
Body Mass Index (kg/m^2)	22.86±0.60	23.2±0.80
Serum Glucose (mg/dL)	81.50±1.70	77.2±2.10
Basal FSH (mIU/mL)	5.71±0.31	5.21±0.30
Basal LH mIU/mL)	4.82±0.34	4.32±0.50
Basal E_2 (pg/mL)	43.80±3.52	28.7±5.32
Basal TSH (μIU/ml)	2.84±0.38	2.70±0.40
Basal PRL (ng/ml)	13.24±1.37	11.38±1.40
Insulin (μg/ml)	14.24±1.44	14.87±1.52
Luteal phase Progesterone (ng/ml)	18.3±1.80	8.2±2.10*

* $P<0.001$ *vs*. nomal luteal phase

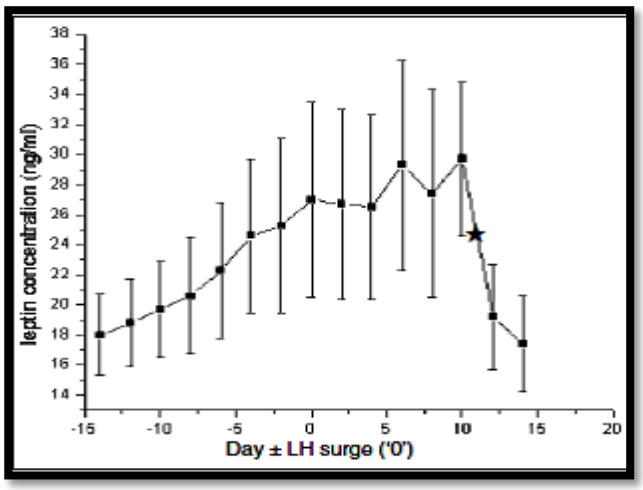
[FSH: Follicle stimulating hormone; LH: Luteinising hormone; E_2: Estradiol; TSH: Thyroid Stimulating hormone; PRL: Prolactin]



A

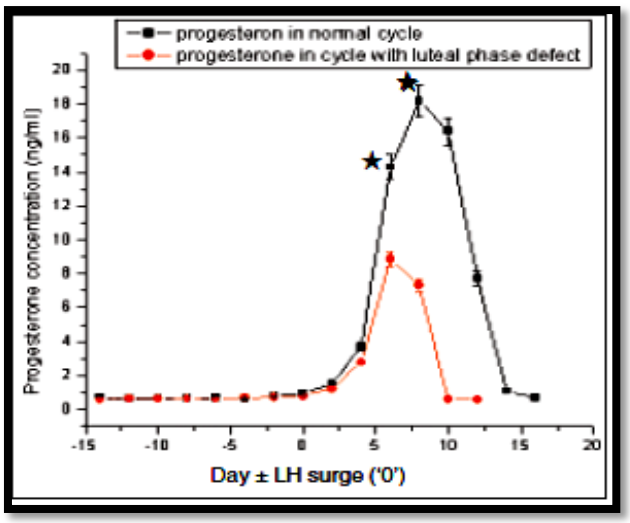


B

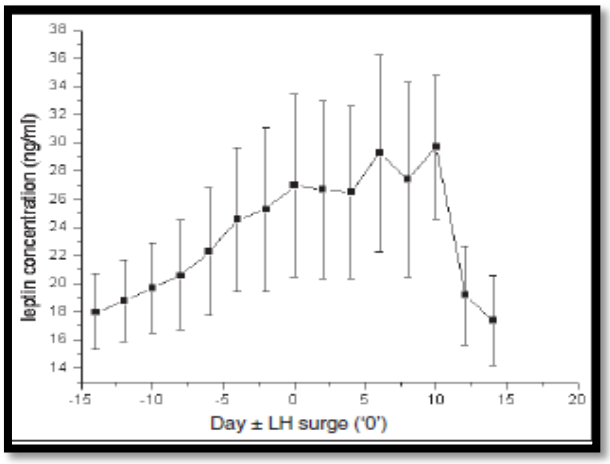


C

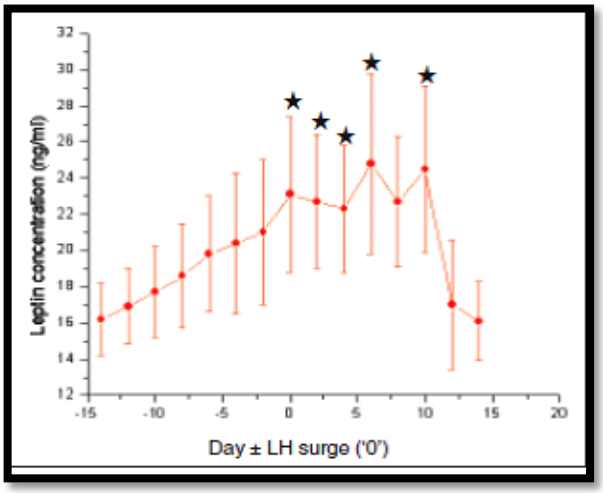
Figure 1: *Changing pattern of serum concentrations of (A) LH (■) and FSH (■), (B) estradiol and (C) leptin during different phases of menstrual cycle in women with normal luteal phase. The day of mid-cycle gonadotropin surge is considered as day '0'. Note: increase in leptin levels is parallel with increase in estradiol concentrations. The peak (day −1) estradiol level is associated with a sharp rise in leptin levels which are followed by a decrease and subsequent increase of both estrogen and leptin. A second rise of leptin concentration is noted on +day10 in association with an increase in post ovulatory E_2. However, this E_2 increase is also accompanied by a parallel increase in progesterone level (Figure 'C').*



A



B



C

Figure 2: *Changing pattern of serum concentrations of (A) Progesterone and (B) leptin in women with normal luteal phase (■) and luteal phase deficiency (■). For easy comparison, the leptin profile in normal women has been reproduced from the figure (1C) of previous Figure. Luteal phase defect is clearly marked by early drop in progesterone level however leptin pattern is comparable to that of women with normal luteal phase. Nevertheless, the leptin levels were significantly lower in the LPD women at all post LH points.*

DISCUSSION

Ovary is an ever changing tissue and dynamic multi-compartmental organ which is under the chief regulatory control of hypothalamic pituitary principles. The hypothalamic-pituitary control over ovarian functions, however, is precisely governed by a plethora of external factors and internal peripheral principles including many of ovarian origin. Leptin, an adipocyte derived hormone has emerged as a potential regulator of many reproductive functions including gametogenic and steroidogenic potential of ovary. The execution of leptin's effect involves almost all compartments of HPO axis with direct effects on the hypothalamus, pituitary and ovary. Ob-R has been found at all points along the HPO axis (Considine et al. 1996). Leptin is considered a possible link between nutrition and reproduction (Clark et al 1995). Serum leptin level is positively correlated with BMI (Paul et.al 2011). Much evidence has accumulated which suggest that reproductive potential in women undergoes adverse alteration following severe changes in nutritional status and energy availability in both directions. These adaptive changes are reversible when nutritional status is normalized (Lonnqvist et al. 1995; Rogers and Mitchell 1952; Mitchell and Rogers 1953). Moreover, reduction of circulating leptin concentrations following food restriction is associated with reduced secretion of LH and FSH (Cunningham et al. 1996). Treatment with exogenous leptin can reverse the effects of under-nutrition on the reproductive axis and advance sexual maturation in feed restricted animals (Cheung et al. 1997; Ahima et al. 1996). Leptin and leptin receptors are found in reproductive tissues. Ovarian functional state is reported to have impacts on leptin concentrations (Butzow et al. 1999). Earlier studies have demonstrated conflicting results pertaining to cyclic variation in leptin levels in women during reproductive years. The major discrepancy in the results is perhaps attributed to sample collection at varied time of the day, which might have affected the results. Leptin secretion exhibits a circadian pattern. Serum concentrations steadily increase from a nadir in the morning to peak concentrations after midnight (Laughlin and Yen 1997; Cella et al. 2000). Taking this into consideration, in the present study, blood samples were collected at a fixed point of time. The present results clearly demonstrate a distinctive pattern of change in leptin levels during menstrual cycle. Data from animal experimentation suggests that gonadal steroids might modulate leptin expression in isolated mature rat adipocyte (Murakami et al. 1995; Messinis et al. 1999). Estradiol increases leptin release, while ovariectomy (Messinis et al. 2001) attenuates leptin expression in adipose tissue. So, increased leptin level during the ovulatory phase may be a consequence of preovulatory rise in estrogen levels (Riad-Gabriel et al. 1998). Most of the earlier authors agreed that in regularly menstruating women circulating leptin is higher in the luteal phase than in the follicular phase. This effect was believed to be under the stimulatory influence of luteal phase increase in progesterone (Lukaszuk et al. 1998; Hardie et al. 1997; Messinis et al. 2000). Moreover, with respect to the presence of leptin receptors in the endometrium, it is possible that the leuteal phase of the menstrual cycle is accompanied by increased leptin levels (Einollahi et al. 2010).

Present investigation showed distinct changes in leptin concentration in concert with changes in gonadal steroid and gonadotropin concentrations. But it is of particular importance to note that in the present investigation, women with luteal phase insufficiency, despite premature drop in progesterone level had the pattern of leptin secretion that was comparable with that of normal luteal phase. Moreover, Cella et al. (2000) demonstrated that exogenous administration of progestins did not influence serum leptin.

Taken together, the observations made in the study may be interpreted to mean that progesterone exerts no stimulatory effect in leptin secretion. Apparently, in this mechanism, peripheral signals by the adipocytes do not play a major role because no significant changes in fat mass likely during one menstrual cycle. It seems plausible that with regard to the reproductive system, leptin mainly acts through or is under the central control of the hypothalamic-pituitary-axis. Increase in serum leptin level in the ovulatory phase and further in luteal phase may be connected to the fact that leptin may have a role in preparing the body for the metabolic demands of pregnancy (Ajala OM et al. 2013). But small sample size in this study limited the ability to draw inferences regarding the precise role of leptin in the reproductive physiology of female. However the observations are preliminary and therefore should be viewed as a prelude to what future holds.

ACKNOWLEDGEMENTS

This study was supported financially partly by University Grants Commission, New Delhi, India.

REFERENCES

Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B. and Maratos-Flier, E. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature*; 382:250–252.

Ajala, O.M., Ogunro, P.S., Elusanmi, G.F., Ogunyemi, O.E. and Bolarinde, A.A. (2013). Changes in serum leptin during phases of menstrual cycle of fertile women: relationship to age groups and fertility. *Int J Endocrinol Metab*; 11(1): 27-33.

Almog, B., Gold, R., Tajima, K., Dantes, A., Salim, K., Rubinstein, M., Barkan, D., Homburg, R., Lessing, J.B., Nevo, N., Gertler, A. and Amsterdam, A. (2001). Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. *Mol Cell Endocrinol*; 183(1-2): 179 - 191.

Bray, G.A. and York, D.A. (1997). Leptin and clinical medicine: a new piece in the puzzle of obesity. *J. Clin. Endocrinol Mteab.*, 82 (9): 2771-2776.

Butzow, T.L., Moilanen, J.M., Lehtovirta, M., Tuomi, T., Hovatta, O., Siegberg, R., Nilsson, C.G. and Apter D. (1999). Serum and follicular fluid leptin during in vitro fertilization: Relationship among leptin increase, body fat mass, and reduced ovarian response. *J. Clin. Endocrinol Metab*, 84; (9): 3135-3139.

Cella, F., Giordano, G. and Cordera, R. (2000). Serum leptin concentrations during the menstrual cycle in normal-weight women: effects of an oral triphasic estrogen-orogestin medication. *European Journal of Endocrinology*; 142: 174-178.

Cheung, C.C., Thornton, J.E., Kuijper, J.L., Weigle, D.S., Clifton, D.K. and Steiner, R.A. (1997). Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology*; 138:855–858.

Chu, N.F., Shen, M.H., Wu, D.M. and Shieh, S.M. (2002). Decreased leptin levels in normal weight women with hypothalamic amenorrhea: The effects of body composition and nutritional intake. *Clin. Biochem*; 35:287-292.

Cioffi, J.A., Shafer, A.W., Zupancic, T.J., Smith-Gbur, J., Mikhail, A., Platika, D. and Snodgrass, H.R. (1996). Novel B219 /OB receptor isoforms: Possible role of leptin in haematopoiesis and reproduction. *Nat Med*; 2(5): 585-589.

Cioffi, J.A., Van Blerkom, J., Antczak, M., Shafer, A., Wittmer, S. and Snodgrass, H.R. (1997). The expression of leptin and its receptors in pre-ovulatory human follicles. *Mol Hum Reprod*; 3 (6): 467-472.

Clark, A.M., Ledger, W., Galletly, C., Tomlinson, L., Blaney, F., Wang, X. and Norman, R.J. (1995). Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Hum Reprod*; 10 (10): 2705-2712.

Considine, R.V., Considine, E.L., Williams, C.J., Hyde, T.M. and Caro, J.F. (1996). The hypothalamic leptin receptor in humans: Identification of incidental sequence polymorphisms and absence of the db/db mouse and fa/fa rat mutations. *Diabetes*; 45: 992-994.

Cunningham, M.J., Clifton, D.K. and Steiner, R.A. (1999). Leptin's actions on the reproductive axis: perspectives and mechanisms. *Biol Reprod*; 60 (2): 216–222.

Einollahi, N., Dashti, N. and Nabatchian, F. (2010). Serum leptin concentrations during the menstrual cycle in Iranian healthy women. *Acta Med Iran*.; 48(5):300-303.

Gower, B.A., Nagy, T.R., Goran, M.I., Smith, A. and Kent, E. (2000). Leptin in Postmenopausal Women: Influence of Hormone Therapy, Insulin and Fat Distribution. *J. Clin. Endocrinol. Metab*; 85(5): 1770-1775.

Hadji, P., Hars, O., Bock, K., Sturm, G., Bauer, T., Emons, G. and Schulz, K.D. (2000). The influence of menopause and body mass index on serum leptin concentrations. *European Journal of Endocrinology*; 143 (1): 55-60.

Hardie, L., Trayhurn, P., Abramovich, D. and Fowler, P. (1997). Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clinical Endocrinology*; 47 (1): 101-106.

Karlsson, C., Lindell, K., Svensson, E., Bergh, C., Lind, P., Billig, H., Carlsson, L.M. and Carlsson, B. (1997). Expression of functional leptin receptors in the human ovary. *J Clin Endocrinol Metab*; 82 (12): 4144 - 4148.

Laughlin, G.A. and Yen, S.S. (1997). Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. *Journal of Clinical Endocrinology and Metabolism*; 82 (1): 318-321.

Licinio, J., Mantzoros, C., Negrao, A.B., Cizza, G., Wong, M.L., Bongiorno, P.B., Chrousos, G.P., Karp, B., Allen, C., Flier, J.S. and Gold, P.W. (1997). Human leptin levels are pulsatile and inversely related to pituitary-adrenalfunction. *Nature Medicine*; 3 (5): 575-579.

Licino, J., Negaro, A.B., Mantzoros, C., Kaklamani, V., Wong, M.L., Bongiorno, P.B., Mulla, A., Cearnal, L., Veldhuis, J.D., Flier, J.S., McCann, S.M. and Gold, P.W. (1998). Synchronicity of frequently sampled 24 h concentrations of circulating leptin, luteinizing hormone and estradiol in healthy women. *Proc Natl Acad Sci USA*; 95 (5):2541-2546.

Lonnqvist, F., Arner, P., Nordfors, I. and Schalling, M. (1995). Over expression of the obese gene in the adipose tissue of human obese subjects. *Nature Medicine*; 1(9): 950-953.

Lukaszuk, K., Liss, J., Kusiak, E. and Wjcikowski, C.Z. (1998). Serum leptin concentration increase during luteal phase in healthy premenopausal women. *Hormone and Metabolic Research*; 30: 172 - 174.

Luoh, S.M., Marco, F.D., Levin, N., Armanini, M., Xie, M.H. and Nelson, C. (1997). Cloning and characterization of human leptin receptor using a biologically active leptin immunoadhesin. *J Clin Endocrinol Metab*; 18:77-85.

Messinis, I.E., Milingos, S.D., Alexandris, E., Kariotis, I., Kollios, G. and Seferiadis, K. (1999). Leptin concentrations in normal women following bilateral ovareictomy. *Hum Reprod*; 14: 913-918.

Messinis, I.E., Kariotis, I., Milingos, S., Kollios, G. and Seferiadis, K. (2000). Treatment of normal women with oestradiol plus progesterone prevents the decrease of leptin concentrations induced by ovariectomy. *Hum. Reprod.*; 15 (11): 2383- 2387.

Messinis, I.E., Papageorgiou, I., Milingos, S., Asprodini, E., Kollios, G. and Seferiadis, K. (2001). Oestradiol plus progesterone treatment increases serum leptin concentrations in normal women. *Hum Reprod.*; 16 (9): 1827-1832.

Mitchell, (Jr.) G.W. and Rogers, J. (1953). The influence of the weight reduction on amenorrhea in obese women. *N Eng J Med.*; 249(21): 835-837.

Moschos, S., Chan, J.L. and Mantzoros, C.S. (2002). Leptin and reproduction: A review. *Fertil Steril*; 77 (3): 433-444.

Murakami, T., Iida, M. and Shima, K. (1995). Dexamethasone regulates obese expression in isolated rat adipocytes. *Biochem Biophys Res Commun*; 214: 1260-1267.

Paul, RF., Hassan, M., Nazar, HS., Gillani, S., Afzal, N., Qayyum, I. (2011). Effect of body mass index on serum leptin levels. *J Ayub. Med. Col. Abbottabad*; 23(3): 40-43

Riad-Gabriel, M.G., Jinagouda, S.D., Sharma, A., Boyadjian, R. and Saad, M.E. (1998). Changes in plasma leptin during the menstrual cycle. *European Journal of Endocrinology*; 139 (5): 528-531.

Rogers, J. and Mitchell, (Jr.) G.W. (1952). The relation of obesity to menstrual disturbances. *N Eng J Med.*; 247(2): 53-55.

Rosenbaum, M., Nicolson, M. and Hirsch, J. (1996). Effects of gender, body composition and menopause on plasma concentrations of leptin. *J. Clin. Endocrinol. Metab.*; 81 (9): 3424-3427.

Saad, M., Damani, S. and Gingerich, R. (1997). Sexual dimorphism in plasma leptin concentration. *J.Clin.Endocrinol Metab.*; 82 (2): 579-584.

Shimizu, H., Shimomura, Y., Nakanishi, Y., Futawatari, T., Ohtani, K., Sato, N. and Mori, M. (1997). Estrogen increases *in vivo* leptin production in rats and in human subjects. *J. Endocrinol.*; 154 (2): 285-292.

Spicer, L.J. and Francisco, C.C. (1997). The adipose gene product, leptin: Evidence of a direct inhibitory role in ovarian function. *Endocrinology*; 138 (8): 3347-3379.

Sumner, A.E., Falkner, B., Kushner, H. and Considine, R.V. (1998). Relationship of leptin concentration to gender, menopause, age, diabetes and fat mass. *Obese. Res.*; 6(2): 128-133.

Tataranni, P.A., Monroe, M.B., Dueck, C.A., Traub, S.A., Nicolson, M. and Manore, M.M. (1997). Adiposity, plasma leptin concentration and reproductive function in active and sedentary females. *Int J Obes Relat Metab Disord*; 21:818-821.

Corresponding author: Dr. Jana Chakrabarti, Associate Professor, Department of Zoology, A.P.C Roy Government College, Himachal Vihar, Matigara, Siliguri – 734010, West Bengal, India
Email: janachakrabarti@gmail.com