Serum Leptin Levels in Menopause and Impact of Hormone Replacement Therapy

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ABSTRACT: *Leptin, an adipocyte derived peptide hormone has emerged as a potential regulator of many female reproductive functions including gametogenic and steroidogenic potential of ovary. To address the issue of interrelationship between leptin, adiposity and ovarian steroid hormones during menopause, leptin and fat distributions were evaluated in post-menopausal women with or without hormone replacement therapy in the forms of estrogen only or estrogen-progestin combination. Leptin levels were significantly lower in the postmenopausal women as compared with the premenopausal counterpart. During the post-menopausal course, all estimates of body fat increased, but without any significant increase in circulating leptin. Leptin levels correlated positively with fat mass and negatively with lean mass. Differential correlation between leptin and ovarian steroids in premenopausal and post menopausal women raises the possibility of ovary as an important site of leptin production.*

Key Words: *Adiposity, Hormone replacement therapy, Leptin, Menopause*

Introduction

A critical body mass of adipose tissue is essential for the normal development of female reproductive functions (Tataranni et al. 1997). But the mechanistic link between body mass and reproductive functions is not clearly elucidated (Chu et al. 2002). Leptin, an adipocyte derived multifactorial 16 KDa polypeptide consists of 167 amino acids and encoded by *'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 modulate the ovarian functions through its modulating effects on hypothalamo-pituitary-ovarian (HPO) axis and contributing to the release of gonadotropin releasing hormone (GnRH) from the hypothalamus and gonadotropins from pituitary (Licino et.al. 1998). Leptin concentrations show fluctuations during menstrual cycle (Hardie et.al. 1997) and it exerts direct regulatory action on ovarian folliculogenesis (Spicer and Francisco 1997). However, though leptin is widely present in reproductive tissues, its relationship to reproductive hormones is still poorly understood. Moreover, controversial results have been reported during hormone replacement therapy (HRT), oral contraceptive intake and ovulatory disorders (Carmina et.al 1999, Brannian et.al 2001). Menopause is a period during which women tend to gain body fat. Ovarian hormones influence body composition through several potential mechanisms. Estrogens inhibit the action of lipoprotein lipase that hydrolyzes circulatory triglycerides and permits the uptake of fatty acids into adipocytes (Poehlman et.al 1995). It also stimulates the adipocyte release of leptin that affect energy intake and expenditure (Stern and Murphy 1972). Despite all these data, no systemic study is available that uncovered a significant relationship between gonadal steroids and circulating leptin in menopausal women. There are reports that leptin levels do not undergo alteration (Gower et al. 2000), increase (Rosenbaum et.al. 1996), or decrease (Shimizu et.al. 1997) in menopausal women. Moreover, adipose tissue seems heterogeneous, with respect to leptin production (Murakami et.al 1995, Montague et.al 1997). Present study is therefore, centered on the objectives to explore serum leptin levels during menopause and the effects of HRT on body fat distribution and corresponding leptin status.

Materials and Methods

Study subjects and selection criteria

Fifty (50) postmenopausal women aged between 48-52 years, were recruited for this study. Women were included in the study only after receiving consent from them. They were selected on the basis of attending menopause minimum of three months along with persistent high level of follicle stimulating hormone (FSH) (>40mIU/ml) and low level of estradiol (E_2) (<20pg/ml) on two occasions. They were grouped as HRT users

and non-users according to their plan to undergo HRT or not. All participants were healthy and none were being treated with drugs which influence adipose tissue metabolism. Moreover, these women had not been exposed to ovarian hormonal therapy because menopause. This study was approved by the Ethics Committee of Menopausal Society of India, kolkata chapter.

Along with this postmenopausal group, with the approval of the Research Ethics Board of the Institute of Reproductive Medicine (IRM), Kolkata, fifteen (15) eugonadotropic regularly menstruating women aged between 27-34 years, attending the infertility clinic for male factor infertility were recruited for this study. Women were included for the study after receiving consent from them. All subjects had documented ovulation during the past cycles preceding the study cycle. None had any systemic disease or symptoms related to polycystic ovarian syndrome (PCOS).

Intervention

Of the total 50 postmenopausal women recruited for this study, 23 of them were grouped as HRT users in future and rest 27 were as non-user of HRT. Furthermore, women wished to have HRT were again sub-divided in to two categories. Women with intact uterus received conjugated equine estrogens (CEE) 0.625mg/day + medroxyprogesterone acetate (MPA) 2.5mg/day while hysterectomized women were treated with estrogens only. All were followed up to three years. Postmenopausal women not using HRT were regarded as 'control' for this study. Body fat composition and hormonal status were measured at the time of their reporting and after three years of study.

Outcome measures

Evaluation of body composition

Baseline characteristics were measured in both premenopausal and postmenopausal women. All were reviewed for their body mass index (BMI). Body composition was evaluated by dual energy X-ray absorptiometry (DEXA) using Prodigy DXA system; Software version 8.80; manufactured by GE Medical Systems, LUNAR (GE Healthcare, South Asia). The following measurements were performed: total fat mass (FM; this is the sum of fat elements of soft tissue); relative fat mass (FM%) and lean body mass (LBM: the sum of the chemical fat-free elements of soft tissue). Moreover, fat localized on both legs (LF) and the relative amount of fat on the legs (LF%) were also measured.

Biochemical assays

Fasting blood samples were collected from all the volunteers after 30 minutes rest in supine position. Serum samples were stored at - 40^{o}C until assayed for endocrine milieu including estradiol, insulin and for leptin. Estradiol was assayed through Enzyme Linked Fluorescent Assay (assay sensitivity: 9pg/ml). Insulin were analysed through radioimmunoassay (assay sensitivity: 1.3μIU/ml) and serum samples were measured for leptin with immunoradiometric assay (assay sensitivity: 0.10ng/ml).

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 1A: Baseline Characteristics of Participants

	Premenopausal women (n=15)		Postmenopausal women (n=50)	
Parameters	BMI≤25 (n=9)	BMI›25 (n=6)	BMI≤25 (n= 28)	BMI›25 (n= 22)
Age	32.6±0.7	31.9±1.2	50.1±2.2	49.3±2.1
Year Since menopause	NA	NA	1.8±0.4	1.8±0.5
BMI (Kg/m^2)	23.2±0.8	27.2±2.2	23.4±1.1	28.2±1.6
TFM (Kg)	16.2±1.3	24.2±1.4	17.8±1.4	27.6±2.1
Relative FM (%)	33.3±1.3	38.1±1.6	34.6±1.8	37.3±2.2
Leg fat (Kg)	8.2±0.8	8.9±0.8	9.2±0.6	9.8±0.8
LBM(Kg)	38.4±2.8	38.8±2.6	39.2±2.8	38.7±2.6

Visceral Fat (cm^2)	95.3±25.3	105.7±25.2	108.7±37.2	118.5±37.5
Fasting Insulin(μIU/ml)	13.5±5.2	15.4±8.2	14.6±6.2	16.3±8.4
Serum Estradiol (pg/ml)	43.8±3.5	51.9±8.1	14.8±3.4	16.2±2.7
Serum Leptin (ng/ml)	15.8±1.5	24.6±1.0	11.2±0.8	18.8±1.1

Table 1B: Descriptive Data of Participants

PARAMETER	Postmenopausal Women (n=50)											
	Non-HRT (n=27)				HRT (n=23)							
					Estrogen (n=11)				Estrogen-Progestin (n=12)			
	BMI≤25 (n= 15)		BMI›25 (n= 12)		BMI ≤25 (n = 6)		BMI›25 (n= 5)		BMI≤ 25 (n= 7)		BMI›25 (n= 5)	
	'0'	Term	'0'	Term	'0'	Term	'0'	Term	'0'	Term	'0'	Term
Age	50.1 ±2.2	53.1 ±2.2	49.3 ±2.1	52.2 ±2.1	49.7 ±2.2	52.6 ±2.1	49.8 ±1.9	52.6 ±1.8	50.2 ±2.4	53.2 ±2.4	49.9 ±2.4	52.8 ±2.5
Year Since menopause	1.8 ±0.4		1.8 ±0.5		1.4 ±0.4		1.6 ±0.5		1.7 ±0.4		1.5 ±0.6	
BMI (Kg/m^2)	23.4 ±1.1	24.3 ±1.4	28.2 ±1.6	29.0 ±1.8	23.5 ±1.2	24.0 ±1.4	28.1 ±2.1	28.7 ±2.3	23.8 ±1.3	24.4 ±1.1	27.8 ±1.2	28.3 ±2.1
TFM (Kg)	17.8 ±1.4	20.2 ±1.6[a]	27.6 ±2.1	30.8 ±2.3	18.1 ±1.3	19.7 ±1.4	28.1 ±1.9	29.6 ±1.7	18.6 ±1.2	19.8 ±1.3	28.3 ±1.8	29.7 ±2.1
Relative FM (%)	34.6 ±1.8	38.6 ±2.1[a]	37.3 ±2.2	41.2 ±2.3[a]	34.2 ±1.9	36.8 ±1.9[b]	38.3 ±2.1	40.4 ±2.2[b]	33.9 ±1.6	36.8 ±1.6[b]	38.5 ±2.2	41.4 ±2.3[b]
Leg fat (Kg)	9.2 ±0.6	10.0 ±0.7	9.8 ±0.8	11.2 ±0.8[a]	10.1 ±0.7	10.8 ±0.7[b]	11.3 ±1.0	12.0 ±1.0[b]	10.6 ±0.7	11.1 ±0.7[b]	11.4 ±0.9	12.2 ±0.9[b]
LBM (Kg)	39.2 ±2.8	39.6 ±2.8	38.7 ±2.6	39.1 ±2.6	38.8 ±2.9	39.4 ±2.9	38.2 ±2.4	38.8 ±2.6	37.8 ±2.6	38.2 ±2.6	38.1 ±2.8	38.6 ±2.9
Visceral Fat (cm^2)	108.7 ±37.2	117.3 ±32.6	118.5 ±37.5	138.2 ±43.4	105.4 ±33.4	111.3 ±36.5	121.6 ±41.3	127.7 ±37.4	105.3 ±36.2	109.4 ±35.5	123.4 ±39.2	126.4 ±41.1
Fasting Leptin (ng/ml)	11.2 ±0.8	10.6 ±0.7	18.8 ±1.1	18.3 ±1.1	11.8 ±0.7	12.1 ±0.8	19.2 ±1.3	18.6 ±1.3	12.2 ±0.8	11.4 ±0.7	17.3 ±1.1	17.0 ±0.9

a: *P‹ 0.01 vs.* respective '0' year; b: *P‹ 0.05 vs.* respective '0'year.

Descriptive data of subjects are presented in Table 1A and 1B. Postmenopausal women of all subcategories had significantly lower level of estradiol at '0' year. Irrespective of BMI, both HRT users and non-users, had significantly lower level of leptin at '0' year as compared with respective subcategories (based on BMI) of premenopausal regularly menstruating women. At 'term', estradiol level in HRT users increased significantly as expected, but the levels were still significantly lower than the respective premenopausal group. Moreover, despite increase in circulating estradiol over its baseline levels in the HRT group, no significant increase in leptin level was noted in relation to '0' year. Also, there were no difference in the leptin levels between the estrogen only and estrogen-progestin combined HRT treatments. At term of the study, there were significant increases in all estimates of body fat both in non-HRT as well as HRT user groups. The magnitude of increase was however, more significant in the non-users, particularly with respect of absolute or relative (%) fat mass. Table 2 and Table 3 represent the correlations between leptin, ovarian steroid and all individual body fat regions examined.

Table 2: Outcome measures at inclusion time and at term in the HRT- user and non-user groups.

Parameter	Non-User		User	
	'0'year	Term	'0' year	Term
BMI (Kg/m^2)	24.2±0.5	25.6±0.5[a]	24.3±0.4	24.9±0.3[b]
Total body mass (Kg)	21.2±0.4	22.4±0.3[a]	21.2±0.3[a]	21.8±0.3[b]
Lean body mass(Kg)	39.1±1.0	39.7±1.1[a]	38.8±0.9	39.2±0.9[a]
Fasting leptin (ng/ml)	14.4±1.1	14.1±1.2	14.7±0.9	14.2±1.0

a: *P ‹ 0.0001; b: ‹ 0.001 vs*. respective '0' year population

While leptin levels correlated positively with fat mass, it had negative correlation with lean body mass.

Table 3: Pearson coefficient of correlation (r) between leptin and different estimates of body fat and estradiol

Corrected for age		
	r	***p***
BMI	0.78	‹ 0.001
TFM	0.76	‹ 0.001
LBM	0.36	‹ 0.001
Estradiol	0.16	‹ 0.05

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 is considered as a peripheral signal and a possible link between nutrition and reproduction (Clark et.al 1995). Serum leptin level is positively correlated with BMI (Paul et.al 2011). The execution of leptin's effect involves almost all compartments of HPO axis with direct effects on hypothalamus, pituitary and ovary. Ob-R has been found at all points along the HPO axis (Considine et.al. 1996). The present investigation clearly demonstrates that menopause led to a decrease in leptin concentration, the condition however, is not reversed by HRT. Estrogens are reported to stimulate the release of leptin in humans (Russell et.al 1998). Estrogen has thus been considered one of the factors accounting for higher concentration of leptin in women than in men and also for cyclic variations (Casebielli et.al 1998, Bray et.al 1997, Sumner et.al 1998, Hardie et.al 1997). But these effects of estrogens have been contradicted by other investigations. In the present investigation, healthy postmenopausal women with or without HRT, however, show that leptin correlated well with most estimates of adipose tissue. When adjusted for body fats, leptin level did not vary between the control or HRT group. Hormone use status did not influence serum leptin. Even within the HRT group, the changes in total fat mass as well as leptin were equal in the 'estrogen only' and 'estrogen-progestin' group. This observation goes well with the fact that adipose tissue possesses no progesterone receptor (Saad et.al 1997). But contradictory report suggests a close association between leptin and serum progesterone levels (Pederson et.al 1996). During gestations, serum leptin concentrations show a positive correlation with progesterone (Hardie et.al 1997). This significant dichotomy between the findings of different investigations may be explained by extra adipocytic production of leptin. *In vitro* studies have documented that leptin mRNAs are expressed in the granulosa cells (Cioffi et.al 1997). A possibility therefore, exits that additional site of leptin production in women during reproductive years may be the ovary itself. So, exogenous administration of E_2 and/or progestin could increase the serum leptin when the ovaries are in the functional state. This explains that why postmenopausal women suffer from decreased leptin concentrations and HRT has no significant impact on leptin status. But it is also important to recall that reports are available that leptin levels undergo significant reduction following menopause while HRT causes increase in leptin over its menopausal level (Dedeoğlu et.al 2009). Discrepancy between different reports is perhaps attributed to some other factors having modulatory effects on leptin secretion. Another possible explanation towards paradoxical effects of steroid hormones on leptin may be the differential distribution of fats in pre and postmenopausal women. The relationship between gonadal steroids and serum leptin could be mediated through the effects of gonadal hormones on body fat content and concentrations (Hickey et.al 1998). It has been demonstrated in this investigation that postmenopausal increase in adiposity is associated with proportionately greater increase in visceral rather than subcutaneous adipose tissue. But serum leptin level is associated with subcutaneous not visceral adipose tissue volume (Paolisso et.al 1998). Further investigations on expression of ovarian leptin mRNA in parallel with increased production of granulosa cell estrogen or luteal progesterone could probably explain the situation.

Conclusion

Taken together, observations made in this study may be interpreted to mean that menopause led to a decrease in leptin concentration. The condition however, is not reversed by hormone replacement therapy (HRT) which further indicates that regulation of leptin's synthesis and actions are governed by a plethora of controlling factors and ovary appears as an additional site of leptin synthesis. Small size of the study population limits the statistical power to judge precise role of leptin during menopause. The observations presented herein should be viewed as a prelude to what future holds.

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