ORIGINAL PAPER

Serum Leptin Levels in Women throughout Life; Relationship to Body Mass Index and Serum Estradiol Levels

Masayo YAMADA, Toshiya MATSUZAKI, Takeshi IWASA, Fumi SHIMIZU, Naoko TANAKA, Rie OGATA, Machiko KIYOKAWA, Toshiyuki YASUI, Minoru IRAHARA and Toshihiro AONO

Department of Obstetrics and Gynecology, The University of Tokushima, School of Medicine, Tokushima 770-8503, Japan

Abstract. In order to investigate serum leptin levels and the influence of estradiol on these levels throughout the female lifespan, leptin and estradiol levels in sera of females that ranged from adolescent to old age (pre-menarche, post-menarche, menstrual cycle, pregnancy, post-partus, pre-menopause, post-menopause) were examined using radioimmunoassays. The serum leptin level was positively correlated with the BMI and the Rohrer index ($p < 0.01$) in the various age groups from schoolgirls to menopausal women. Serum leptin levels during the normal menstrual cycle negligibly changed, and no differences were detected between pre-menopause and post-menopause women. Moderate changes of estradiol levels in sera during menarche, normal menstrual cycle and menopause had no influence on the serum leptin levels. As expected, serum leptin levels in pregnant women were higher than that in non-pregnant women.

The present results are summarized as follows: 1) Serum leptin levels correlated with BMI at all ages. 2) Moderate increases in serum estradiol levels had no effect on serum leptin levels. 3) Serum leptin levels are significantly increased during pregnancy, possibly due to an excessive increase of leptin production from maternal adipose tissue that is stimulated by high concentrations of serum estradiol and leptin production from the placenta.

Key words: Leptin, Estradiol, Body mass index

Introduction

Leptin is produced in adipose tissues and secreted into the peripheral blood [1, 2]. Leptin acts on the hypothalamus, which contains leptin receptors [3], and it plays an important role in the maintenance of energy homeostasis and the regulation of metabolic activities.

Previous studies have shown that the serum concentration of leptin in human sera is well correlated with body mass index (BMI) and the percentage of body fat. It is also known that the concentrations of leptin are significantly higher in women than in men of the same BMI [4, 5, 6, 7]. In addition, it has been demonstrated that serum leptin levels are higher in pregnant women than in puerperal women and that factors other than fat mass could be responsible for the modulation of serum leptin levels in women of reproductive age [8]. However, the cause of higher leptinemia in women remains unclear.

Administration of leptin or factors, which regulate ob mRNA expressions such as glucocorticoids and insulin, correct reproductive functions of female ob/ob mice, inducing ovulation, pregnancy and parturition [9, 10, 11]. In earlier studies, we reported that women who received ovarian hyperstimulation through IVF/ICSI showed higher serum leptin levels when serum estradiol (E2) levels were greatly elevated [12], and that E2 increased the expression of ob mRNA in adipose tissue of ovariectomized rats [13] and in cultured rat adipose cells [11]. These findings indicate that E2 levels could influence the serum leptin levels. Since serum E2 concentrations change dramatically throughout the female lifespan, serum leptin levels might change in parallel to E2 levels.

In this study, we measured the serum leptin levels in women of various ages and conditions, and analyzed the relationship between the leptin and E2 levels.

Accepted: August 19, 2003
Correspondence: Toshiya MATSUZAKI, Department of Obstetrics and Gynecology, The University of Tokushima, School of Medicine, 3-18-15, Kuramoto-cho, Tokushima 770-8503, Japan
TEL: +81-886-33-7177
FAX: +81-886-31-2630
E-mail: mat@clin.med.tokushima-u.ac.jp

Materials and methods

1) Subjects and blood sampling

The subjects of this study were 216 females, aged 6 to 67 years. None of the subjects were taking any medication nor had any evidence of metabolic diseases aside from simple obesity. All subjects provided written informed consent for the procedures of this study. The subject categories were as follows: (1) Peri-menarche girls whose age ranged from 6 to 15 years old (n = 22, 10.8 ± 0.5 years old, mean ± SEM), and who were further divided into pre-menarche (n = 11), and post-menarche (n = 11) groups. (2) Reproductive-age females who had a normal menstrual cycle as evidenced by basal body temperature (n = 22, 30.0 ± 1.0 years old). Blood samples were collected three times from each subject at the early follicular, pre-ovulatory and mid-luteal phases in this group. (3) Pregnant females (n = 61) and age-matched puerperal females (n = 14), who were divided into four groups (1st trimester: 8.9 ± 0.4 weeks, 2nd trimester: 22.7 ± 1.5 weeks, 3rd trimester: 35.4 ± 0.5 weeks, and one month after delivery, mean ± S.D., respectively). (4) Peri-menopausal women, whose age ranged from 40 to 67 years old (n = 95, 49.8 ± 0.5 years old), and who were further divided into pre-menopause (n = 29) and post-menopause (n = 66) groups.

The weight and height of the subjects were measured in light clothing without shoes. Body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters, kg/m^2) and the Rohrer index in the case of schoolgirls (calculated as weight in grams divided by the cube of height in centimeters multiplied by 100, g/cm^3 X 100) were used as indexes of overall adiposity. Serum samples were frozen at -40℃ until assayed.

2) Measurement of leptin and estradiol in serum

A specific radioimmunoassay for serum leptin using human recombinant leptin as a tracer was performed as previously described by Iida et al [14]. The minimum value of detection was 0.5 ng/mL. The intra-assay standard coefficient of variation (CV) was 7.8 %, and the inter-assay CV was 5.6 %. Samples were assayed in duplicate.

Serum concentrations of E2 were measured using a

Table 1. Body mass index and serum leptin and estradiol concentrations

Subjects	N	Age	BMI(kg/M2) (Rohrer index g/cm2x100)	Estradiol(pg/ml)	Leptin (ng/ml)
Peri-manarche girls					
Pre-menarche	11	9.1±0.7	17.3±0.8 (132.2±5.5)	5.2±1.0	19.4±3.5
Post-menarche	11	12.5±0.5*	21.5±2.3 (143.0±15.1)	39.7±8.9*	21.3±4.3
Reproductive age women					
Follicular phase				79.4±17.4"	24.4±2.7^
Pre-ovulatory phase	22	30.0±1.0	20.9±0.5	243.1±10.3	24.1±2.5
Luteal phase				154.2±31.7	24.6±2.6
Pregnant and puerperal women					
1st Trimester	22	30.0±1.0	21.8±0.6#	1065.8±152.5"	35.9±4.2
2nd Trimester	14	28.3±1.3	25.1±1.3	8778.7±1433.6#	49.7±5.6
3rd Trimester	25	29.0±1.0	26.3±0.6	22051.4±2477.9	46.6±4.8
One month after birth	16	31.4±4.6	22.6±0.7	72.7±9.5"	23.9±2.9^
Peri-memopausal women					
Pre-menopause	29	45.8±0.8	24.1±0.8	103.4±10.1	30.1±2.8
Post-menopause	66	52.1±0.7**	23.5±0.4	10.4±1.2**	30.0±2.0

*P<0.01 vs Pre-menarche
^p<0.05 vs 1st, 2nd &3rd trimester
#p<0.01 vs 3rd trimester
"p<0.01 vs 2nd &3rd trimester
**p<0.01 vs Pre-menopause

coated-tube immunoassay kit (Diagnostic Products Corp., Los Angeles, CA) according to the manufacturer's protocols. The minimum value of detection was 8 pg/mL. The intra-assay CV was 8.1%, and the inter-assay CV was 5.0%. Serum samples from pregnant women were diluted two- and fourfold with the buffer included in the kit. Samples were assayed in duplicate.

3) Statistical analysis

All data were expressed as the mean ± SE. Differences among the groups were tested by one-way ANOVA. When $P < 0.05$ was detected by a one-way ANOVA, a Scheffe's test was also performed. Pearson product-moment correlations were calculated to test the association among the variables. Partial correlation tested for the associations between the two variables and was performed independently of the covariate. Repeated measurements were analyzed by a Student's paired t-test.

Results

1) The levels of leptin in sera of peri-menarche girls

The BMI of peri-menarche females ranged from 13.7 to 36.2 (19.3 ± 2.2: mean ± SE) kg/m^2, and their Rohrer index ranged from 89.6 to 244.5 (137.3 ± 7.6) g/cm^3 X 100. Leptin levels were not significantly different between the pre-menarche and post-menarche groups, but the levels of E2 in the post-menarche group were significantly higher than those in the pre-menarche group (Table 1). Serum leptin levels were significantly correlated with BMI (Fig. 1 and Table 2), and were not significantly correlated with age.

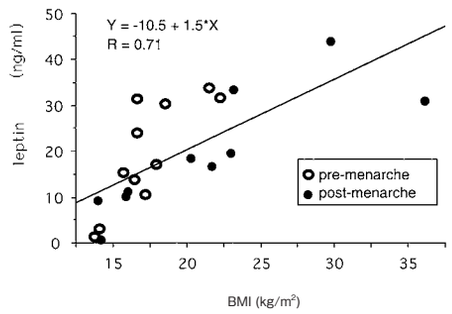

Fig. 1. Relationship between serum leptin and BMI ($r = 0.71$, $p < 0.01$, $y = -10.5 + 1.5 * X$) of 22 peri-menarche girls
Serum leptin levels were significantly correlated with BMI.

2) The changes of serum leptin levels during normal menstrual cycle

The BMI of reproductive-age females ranged from 16.6 to 26.5 (20.9 ± 0.5) kg/m^2. Although the levels of E2 were significantly changed during the menstrual cycle, no significant change in leptin levels was observed (Table 1). Serum leptin levels were significantly correlated with BMI, but were not significantly correlated with age or estradiol (Table 2).

Table 2. Correlations between serum leptin concentrations and various parameters

Subjects	Number	Age		BMI		Estradiol	
		r	p	r	p	r	p
Peri-manarche girls							
Pre-menarche	11	0.16	n.s.	0.81	<0.01	0.21	n.s.
Post-menarche	11	0.07	n.s.	0.84	<0.01	0.04	n.s.
Reproductive age women							
Follicular phase	22	0.12	n.s.	0.60	0.01	0.41	n.s.
Pre-ovulatory phase	22	0.11	n.s.	0.64	<0.01	0.12	n.s.
Luteal phase	22	0.46	<0.05	0.54	0.01	0.09	n.s.
Pregnant and puerperal women							
1st Trimester	22	0.03	n.s.	0.60	<0.01	0.12	n.s.
2nd Trimester	14	0.19	n.s.	0.78	<0.01	0.65	<0.05
3rd Trimester	25	0.30	n.s.	0.72	<0.01	0.09	n.s.
One month after birth	16	0.25	n.s.	0.70	<0.01	0.02	n.s.
Peri-memopausal women							
Pre-menopause	29	0.10	n.s.	0.77	<0.01	0.12	n.s.
Post-menopause	66	0.25	<0.05	0.64	<0.01	0.04	n.s.

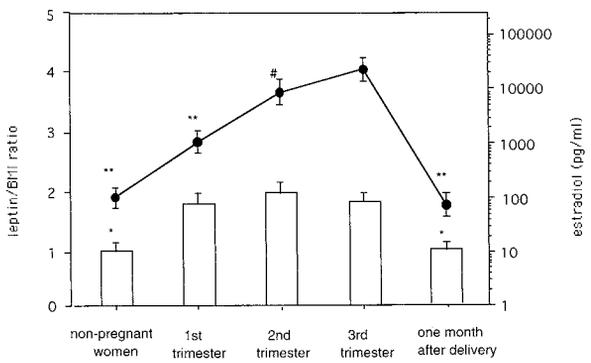

Fig. 2. The change of serum leptin and E2 levels in pregnant, puerperal and non-pregnant women (follicular phase)
□, leptin concentration; ●, estradiol concentration. Error bars depict SD. *p < 0.05 vs. 1st, 2nd & 3rd trimester, #p < 0.01 vs. 3rd trimester, **p < 0.01 vs. 2nd & 3rd trimester. The estradiol levels were higher in the pregnant women than in the non-pregnant and puerperal women. Ratio of leptin to BMI was higher in pregnant women than in the non-pregnant and puerperal women.

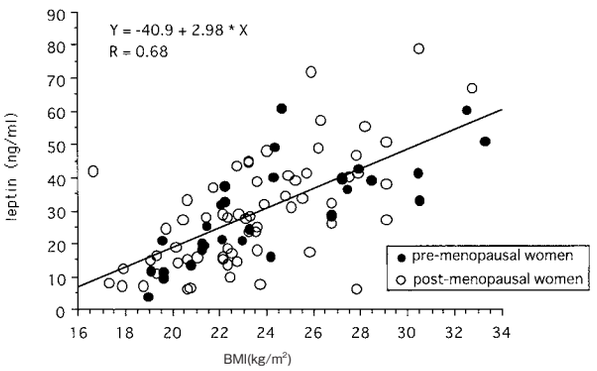

Fig. 3. The relationship between serum leptin levels and BMI in peri-menopausal women (r = 0.68, p < 0.01, y = -40.9 + 3.0 * X)
Serum leptin levels were significantly correlated with BMI.

3) The levels of leptin in pregnant women

BMIs, levels of leptin and estradiol in pregnant and age-matched non-pregnant women (follicular phase) are shown in Table 1. The mean BMI prior to pregnancy in the pregnant group was 21.2 ± 3.5 and this was not significantly different from that of the normal menstrual group. As shown in Table 1, the mean BMI in the 3rd trimester group was higher than that seen in the 1st trimester and in non-pregnant women. Serum estradiol levels were significantly higher in the pregnant women than in the follicular phase women and puerperal women, and gradually increased during pregnancy. Serum leptin levels were also significantly higher in the pregnant women than in the follicular phase and puerperal women. Leptin levels in pregnant women were correlated with BMI, but not correlated with age or estradiol (Table 2).

To eliminate the influence of increased BMI in pregnancy, the relationship between the estradiol and leptin/BMI ratio was examined. As shown in Fig. 2, the leptin/BMI ratios of the 3 pregnancy groups were significantly higher than those for the non-pregnant or puerperal women, respectively.

4) Leptin levels in peri-menopausal women

The relationship between BMI and serum leptin level in the peri-menopausal women is shown in Fig. 3. Their BMI ranged from 16.6 to 33.3 (23.7 ± 0.4) kg/m^2. Serum leptin levels were significantly correlated with BMI and were not correlated with age or estradiol levels (Fig. 3, Table 2). Serum leptin levels were not significantly different between the pre-menopausal and post-menopausal women, while E2 levels were significantly higher in the pre-menopausal women ($p < 0.01$) than in the post-menopausal women (Table 1).

Discussion

Serum leptin primarily originates from the fat tissue and its level is an important signal for the brain with regard to regulating appetite and energy homeostasis. Present findings demonstrated that serum leptin levels are correlated positively with BMI and the Rohrer index at all ages, ranging from peri-menarche to peri-menopausal females. Leptin levels were not different between pre-menarche and post-menarche, pre-menopause and post-menopause, or during the normal menstrual cycle (follicular, pre-ovulatory, and luteal phases). Our data indicates that adipose cells always produce regular amounts of leptin irrespective of age or menstrual status. It is reasonable to assume that the serum leptin level accurately reflects the amount of fat storage in the body.

Elevation of the serum leptin level, which precedes puberty, is thought to play an important role in the onset of puberty. Our data clearly shows that elevation of the serum leptin level during puberty does not reflect the capacity of leptin production by each adipose cell, but rather indicates fat mass stored in the body. Constant synthesis of

leptin from each adipose cell enables the brain to know the exact amount of energy that is stored in the body.

Our study found that serum leptin levels were highly correlated with body composition (BMI or percent body fat). Other studies have reported that serum leptin levels are higher in women than in men, in the fetus [15, 16], in children [17, 18], and in adults [5, 6, 7]. Interestingly, it is reported that E2 stimulates the production of ob mRNA in both cultured rat adipose cells [11] and adipose tissues of ovariectomized rats [13].

It might be conceivable that E2 has a stimulatory effect on the production of leptin in women. However, the E2 effect on leptin is controversial. Our data suggests that serum leptin levels are not correlated with the E2 levels seen throughout women's lifetimes. Havel et al. has also reported that absolute and adiposity-corrected plasma leptin levels were independent of both age and reproductive status in women [7]. In addition, Havel et al. [7] and Kohrt et al. [19] reported that leptin levels were not affected by hormone replacement therapy (HRT). However, Rosenbaum et al. reported that leptin levels were significantly higher in pre-menopausal women than in post-menopausal women [20]. We previously reported that serum leptin levels became elevated when serum E2 levels rose in ovarian hyperstimulated women receiving IVF-ET [12]. These data suggest that E2 might induce leptin production under conditions of high concentrations of E2.

The serum leptin levels and leptin/BMI ratios during pregnancy were significantly higher than those in non-pregnant women, and increased from the 1st to the 3rd trimester. The leptin levels were lower in puerperal women than in pregnant women, and were the same as those seen in non-pregnant women. BMI does not necessarily reflect body fat mass in pregnant women, and the remarkable increase of the serum leptin level during pregnancy is not explicable by an increase of fat mass alone. It has been suggested that the incremental increase of leptin during pregnancy is caused by production in the placenta [21].

Ob mRNA is expressed in the placenta, and the placental leptin may contribute to an increase in serum leptin levels in the mother [15]. However, placental leptin cannot fully explain the elevation of leptin levels during pregnancy because placental weight does not correlate with maternal leptin levels [22]. As the placenta produces a large amount of E2, the E2 level in the mother's serum increases during pregnancy, but decreases rapidly after delivery. This change parallels the changes of serum leptin during and after pregnancy. It seems likely that a large quantity of E2 has an effect on the leptin production in the adipose tissues of the mother. Therefore, the source of high leptin levels in pregnant women may not only be from production in the placenta but also from the maternal adipose cells that are stimulated by a high concentration of E2 originating from the placenta.

In summary, the measurement of serum leptin levels obtained throughout women's lifetimes disclosed the following findings: (1) Serum leptin levels are correlated with BMI at all ages. (2) A moderate increase in the serum estradiol level has no effect on serum leptin levels. (3) Serum leptin levels significantly increase during pregnancy, possibly due to an excessive increase of leptin production from the maternal adipose tissue that is stimulated by a high concentration of serum estradiol and leptin production from the placenta.

References

1. Zhang Y, Proenca Y, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425-32.
2. Murakami T, Shima K (1995) Cloning of rat obese cDNA and its expression in obese rats. Biochem Biophys Res Commun 209, 944-952.
3. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263-1271.
4. Ma Z, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M (1996) Radioimmunoassay of leptin in human plasma. Clinic Chem 42, 942-946.
5. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, Mckee L J, Bauer TL, Caro JF (1996) Serum immnoreactive concentrations in normal-weight and obese humans. New Engl J Med 334, 292-295.
6. Ostlund, Jr. RE, Yang JW, Klein S, Gingerich R (1996) Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 81, 3909-3913.
7. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD (1996) Gender differences in plasma leptin concentrations. Nature Med 2, 949-950.
8. Butte NF, Hopkinson JM, Nicolson MA (1997) Leptin in human reprodution: Serum levels in pregnant and lactating women. J Clin Endocrinol Metab 82, 585-589.
9. Slieker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F,

Manatta J, Bue-Valleskey, Stephens TW (1996) Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J Biological Chemist 271, 5301-5304.
10. Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF (1996) Acute and chronic effects of insulin on leptin production in humans. Diabetes 45, 699-701.
11. Murakami T, Iida M, Shima K (1995) Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem Biophys Res Commun 219, 1260-1267.
12. Yamada M, Irahara M, Tezuka M, Murakami T, Shima K, Aono T (2000) Serum leptin profiles in the normal menstrual cycles, gonadotropin treatment cycles. Gynecol Obstet Invest 49, 119-123.
13. Yoneda N, Saito S, Kimura M, Yamada M, Iida M, Murakami T, Irahara M, Shima K, Aono T (1998) The influence of ovariectomy on ob gene expression in rats. Horm Metabl Res 30, 263 - 265.
14. Iida M, Murakami T, Yamada M, Sei M, Kuwajima M, Mizuno M, Noma Y, Aono T, Shima K (1996) Hyperleptinemia in chronic renal failure. Horm Metabol Res 28, 724 - 727.
15. Hassink SG, Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, Opentanova I, Dostal K, Spear ML, Leef K, Ash M, Spitzer AR, Funanage VL (1997) Placental leptin: An important new growth factor in intrauterine and neonatal development? Pediatrics 100, E1.
16. Matsuda J, Yokota I, Iida M, Murakami T, Naito E, Ito M, Shima K, Kuroda Y (1997) Serum leptin concentration cord blood: Relationship to birth weight and gender. J Clin Endocrinol Metab 82, 1642-1644.
17. Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI (1997) Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. J Clin Endocrinol Metab 82, 2148-2152.
18. Hassink SG, Sheslow DV, Lancey E, Opentanova I, Considine RV, Caro JF (1996) Serum leptin in children with obesity: Relationship to gender and development. Pediatrics 98, 201-203.
19. Kohrt WM, Landt M, Birge Jr. SJ (1996) Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. J Clin Endocrinol Metab 81, 3980-3985.
20. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, Leibel R (1996) Effects of gender, body composition and menopause on plasma concentrations of leptin. J Clin Endocrinol Metab 81, 3424-3427.
21. Senaris R, Garcia-Caballero T, Casabiell X, Gallego R, Castro R, Considine RV, Dieguez C, Casanueva F (1997) Synthesis of leptin In human placenta. Endocrinol 138, 4501-4504.
22. Schubring C, Kiess W, Englaro P, Rascher W, Dotsch J, Hanitsch S, Attanasio A, Blum WF (1997) Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal, placental weight. J Clin Endocrinol Metab 82, 1480-1483.