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Correlation of leptin and sex hormones with endocrine changes in healthy Saudi women of different body weights

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BACKGROUND: A relationship between estrogen and leptin has been described during the follicular phase of both spontaneous menstrual cycles and cycles stimulated with exogenous follicle-stimulating hormone (FSH), which suggest that leptin has either a direct effect on or is regulated by gonadal steroids in the human ovary [AUTHOR: Please accept this sentence or write an introductory sentence explaining the background for the study]. To examine the changes in plasma leptin levels during the menstrual cycle, we studied the association between plasma leptin and reproductive hormones in young, healthy Saudi women.

SUBJECTS AND METHODS: Sixty-five young women between 19 to 39 years of age, with a normal menstrual cycle, were grouped into 33 overweight and obese females of BMI >25 kg/m^2, and 32 lean females of BMI <25 kg/m^2. Anthropometrics measurements were made at the time of the collection. Samples were analyzed for leptin, progesterone, estradiol, FSH, luteinizing hormone (LH), cortisol, and testosterone concentrations.

RESULTS: Overweight and obese women, compared with lean, tended to have a significantly higher plasma leptin levels (11.38± 4.06 vs. 6.22± 2.87 ng/mL; P=0.05). In overweight and obese subjects, circulating leptin concentrations showed a direct correlation with BMI (r=0.53; P=0.002), hip (r=0.32; P=0.005), waist-hip ratio (r=0.37; P=0.042), weight (r=0.41; P=0.021), and E_2 on day 3 (r=0.35; P=0.048). In all correlation analyses, leptin levels did not correlate with cortisol or testosterone. In lean subjects, a bivariate correlation analysis showed that plasma leptin concentrations were directly correlated to hip circumference (r=0.43; P=0.012). Moreover, a direct correlation was found with progesterone on day 10 (r=0.43; P=0.014) and E_2 on day 24 (r=0.47; P=0.007).

CONCLUSION: There is a link between plasma leptin and progesterone concentrations during the menstrual cycle, and the variation in circulating estradiol concentrations may have an influence in circulating leptin in female subjects.

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Leptin is a multifunctional adipostatic hormone that is produced by the obese (ob) gene.[1] Although white fat cells are its main producers, it is now known that it is made by several other kinds of cells as well.[2] Leptin plays a key role in regulation of food intake and energy expenditure.[3] Studies in humans and animals have shown that leptin concentrations in the blood correlate with the amount of adipose tissue in the body, acting as a sensing hormone in a negative feedback control from adipose tissue to the hypothalamus.[4] There is considerable interindividual variation in plasma leptin concentrations among individuals with a comparable degree of obesity, suggesting that

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other factors are involved in the regulation of leptin production. Furthermore, human subjects with leptin gene mutations are markedly obese[5] and lose weight in response to exogenous administration of leptin.[6] However, human obesity is not usually associated with leptin deficiency, but largely with leptin resistance.[7] Recent investigations have shown that leptin receptors are widely distributed in a variety of tissues, and that any defects in the genes encoding leptin receptors lead to a spectrum of manifestations, including diabetes.[8,9] In humans, there is also evidence that leptin is involved in reproduction function, including puberty, and with the maintenance of normal menstrual cycles.[10] A number of studies have proposed that if leptin is the signal that there are adequate fat stores to start and maintain ovulation and menstruation, it may account for these changes through its effects on the ovary[11-13] or on the brain.[14] Another study suggested that, at the level of the central nervous system, leptin may stimulate gonadotropin-releasing hormone (GnRH) release from the hypothalamus, and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from the pituitary, probably by acting on its own receptor and promoting nitric oxide release.[15]

Studies have found that high leptin levels during the luteal phase of spontaneous menstrual cycles are correlated with LH levels.[16-19] Also, a relationship between estrogen and leptin has been described during the follicular phase of both spontaneous cycles and cycles stimulated with exogenous FSH.[20] This suggests that leptin either has a direct effect or is regulated by gonadal steroids in the human ovary. Given this background, we sought to examine the changes in plasma leptin levels during the menstrual cycle and study the association between plasma leptin, body fat mass (using body mass index, BMI), fat distribution (waist, hip, waist-hip ratio, WHR), and reproductive hormones in young, healthy Saudi women.

Subjects and Methods

Experiments were carried out in 65 healthy female volunteers, aged 19-39 years, who had regular menstrual cycles between 27 and 30 days in length (Table 1). Their ovulatory cycles were assessed according to the information recorded about their last eight menstrual cycles. None had received drugs known to interfere with hormonal concentrations for at least 3 months before the study, and women with polycystic ovary syndrome (PCOS) were excluded. All subjects in the study provided informed consent for all procedures. Blood samples were collected after an overnight fast, on day 3, 10, 17, and 24 from the beginning of the menstruation from all the participants. All blood samples were taken between 08:00 and 10:00 AM after an overnight fast. At each time of collection, information was recorded for all subjects, including weight, height, waist, and hip circumference.

BMI was calculated by dividing the body weight (in kilograms) by the square of the height (in meters). The waist circumference was taken as the largest standing horizontal circumference between the ribs and the iliac crest; the hip circumference was taken as the largest horizontal circumference of the buttocks. WHR was calculated by dividing the waist by the hip circumference.

The concentration of leptin in plasma was determined by a solid-phase sandwich ELISA with an affinity-purified polyvalent antibody immobilized in microtiter wells using a commercial Direct ELISA human leptin kit according to the procedure provided by the company (Diagnostics Biochem, Canada). Assays were performed in duplicate. The concentration of cortisol was determined in duplicate using a commercial DRG ELISA kit (DRG Diagnostics, Germany). Serum progesterone, estradiol, FSH, LH, and testosterone were measured by electrochemiluminescence ECLIA methods (Roche Diagnostics, Mannheim, Germany) on the Roche Elecsys 2010 Immunoassay Analyzer (Tokyo, Japan).

Statistical analysis was performed using SPSS 10 for Windows. One-way ANOVA, paired-sample and independent-sample t tests were applied where appropriate. Correlations were studied by Pearson's method. Multivariate analysis was performed using a multiple linear regression model. All comparisons or regression parameters were considered statistically significant at a P level less than 0.05.

Results

Compared with lean women, overweight and obese women tended to have a significantly higher mean BMI, WHR, body weight, waist, serum cortisol, and plasma leptin levels (Table 1). There were no significant differences in hip circumference, height, and testosterone between the two groups. Overweight and obese individuals had a serum leptin level between 7.05 and 21.73 ng/mL and lean women had a serum leptin between 2.04 and 15.61 ng/mL.

There was a physiological fluctuation in leptin concentrations during the menstrual cycle, with the

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Table 1. Characteristics of the study subjects. [AUTHOR: Data are mean±SD?]

Variables	Lean (n=33)	Obese (n=32)	P (t test)
Body weight (kg)	53.70±6.64	79.87±11.72	0.003
Height (m)	1.60± 0.06	1.61±0.06	NS
BMI (kg/m²)	20.94±2.14	30.57±3.90	0.004
Hip circumference (cm)	96.07±5.37	112.80±7.49	NS
Waist (cm)	67.24±4.64	85.19±9.69	0.002
WHR	0.70± 0.03	0.76± 0.07	0.0001
Serum cortisol (ηmol/L)	357.86±210.8	331.68±145.0	0.023
Serum testosterone (ηmol/L)	0.61±0.35	0.56±0.41	NS
Plasma leptin (ng/mL)	6.22±2.87	11.38±4.06	0.05

Plasma and serum concentrations were measured during the fasting state. Body mass index (BMI). Waist-to-hip ratio (WHR). Non-significant (NS).

Table 2: Values (mean ± S.D.) of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone during the menstrual cycle of obese and lean women.

Hormones	Days of the menstrual cycle			
	Day 3	Day 10	Day 17	Day 24
FSH (mIU/mL)				
Obese	5.99±1.47	5.02±1.85	4.34±2.18*	2.74±1.45**
Lean	7.19±2.93	5.79±3.07**	4.99±2.16**	4.35±2.62**
LH (mIU/mL)				
Obese	4.19±1.91	8.56±3.94**	15.86±14/53**	8.74±7.74*
Lean	5.88±2.68	9.79±11.73*	15.21±13.42**	9.34±9.05*
Progesterone (ηmol/L)				
Obese	4.52±7.61	1.21±1.33**	4.64±9.38	7.89±6.73**
Lean	3.15±5.25	2.37±1.01	5.56±6.13	9.83 ±7.72**
Estradiol (ηmol/L)				
Obese	222.63±174.56	487.75±421.74**	829.05±606.75**	739.06±448.06**
Lean	138.08±79.02	425.32±287.04**	857.11±571.33**	632.53±314.26**
Leptin (ng/mL)				
Obese	10.58±5.47	7.65±2.99*	9.60±4.09**	12.67±13.57**
Lean	6.70±4.27	8.07±5.83**	7.77±3.80**	10.01±5.18**

All comparisons with day 3 values, two-tailed test; *P<0.05; **P<0.01.

lowest values during the early follicular phase and the highest during the luteal phase in the two groups (Table 2). In the overweight and obese groups, the mean plasma leptin level on day 3 of the menstrual cycle was 10.58± 5.47 ng/mL, which varied significantly with day 10 (P= 0.02), 17 (P= 0.009) and 24 (P=0.001). In the same group, estradiol, FSH, and LH fluctuated with a significant increase (P= 0.0001) during the ovulating phase (day 17) with respect to day 3. Leptin concentration peaks during the luteal phase (day 24) were coincident with maximal progesterone levels on day 24. Progesterone showed significant variations on day 17 (P=0.052) and day 24 (P=0.0001) with respect to day 3.

To provide an overview of the relationships between leptin, body composition and hormones, a bivariate correlation was performed. Table 3 shows bivariate and partial correlations between leptin levels and specific variables in both groups. In the partial analyses, successive adjustments were made for BMI and hip circumference in separate analyses. In overweight and obese subjects, circulating

Table 3: Bivariate and partial correlations of mean leptin concentration with selected variables.

Variables	Lean (n=33)			Obese (n=32)		
	Bivariate correlation (r)	Partial correlations adjusted for BMI	Partial correlations adjusted for hip	Bivariate correlation (r)	Partial correlations adjusted for BMI	Partial correlations adjusted for hip
Body mass index (BMI)	0.26	-	-0.15	0.53*	-	0.60
Weight	0.22	-0.01	-0.32	0.41*	-0.13	0.31
Hip circumference	0.43*	0.39	-	0.32*	-0.21	-
Height	-0.01	-0.02	-0.22	-0.06	-0.1	-0.23
Waist circumference	0.25	0.09	-0.11	0.50	0.11	0.41
Waist to hip ratio (WHR)	-0.14	-0.19	-0.11	0.37*	0.18	0.39
Fasting serum cortisol	0.21	0.14	0.11	0.01	0.05	0.06
Fasting serum testosterone	0.16	0.64	-0.01	-0.03	-0.11	-0.04
Progesterone on day 3	0.01	0.02	0.04	0.06	-0.05	0.18
Progesterone on day 10	0.43*	0.42*	0.35*	-0.07	0.21	-0.21
Progesterone on day17	-0.31	-0.31	-0.31	0.18	-0.11	-0.13
Progesterone on day 24	0.26	0.29	0.36*	-0.39	-0.35	-0.5
Estradiol on day 3	-0.09	-0.07	0.01	0.35*	-0.09	0.16
Estradiol on day 10	-0.31	-0.35	-0.35	0.12	-0.09	0.06
Estradiol on day17	0.27	0.22	0.24	-0.27	-0.08	-0.33
Estradiol on day 24	0.47**	0.49**	0.47**	-0.17	-0.18	-0.36
FSH on day 3	-0.09	-0.15	-0.21	-0.35	-0.18	-0.21
FSH on day 10	-0.09	-0.11	-0.08	-0.01	0.2	-0.05
FSH on day 17	0.04	0.03	0.03	0.25	0.42	0.19
FSH on day 24	-0.12	-0.22	-0.23	0.43	0.49*	0.34
LH on day 3	-0.12	-0.07	-0.04	-0.4	0.043	-0.46
LH on day 10	-0.1	-0.09	-0.05	0.17	0.16	0.06
LH on day 17	0.23	0.21	0.19	-0.12	0.05	-0.25
LH on day 24	0.24	0.18	0.11	0.41	0.46*	0.32

Two-tailed test: *$P<0.05$; **$P<0.01$.

plex. On the other hand, a stimulatory action of E_2 appears to be most likely, as the increase in E_2 concentrations precedes that of leptin. A previous study had suggested that the relation between leptin and E_2 may depend on other factors.[34]

There are some limitations to this study. The design of this study did not permit us to confirm the stimulatory action of E_2 and progesterone on leptin secretion. Further studies in vitro and in vivo are needed to explore these relationships. Second, there may be a selection bias of enrolled subjects due to the relatively small sample size in this study.

In conclusion, the results suggest that there is a link between plasma leptin and progesterone concentrations during the menstrual cycle, and the variation in circulating estradiol concentrations may have an influence in circulating leptin in female subjects. The challenging question is: Does leptin need additional molecules and hormones to regulate energy metabolism and reproduction? Undoubtedly, the years to follow will increase our understanding of the relationship between leptin and sex hormones.

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