European Review for Medical and Pharmacological Sciences 2014; 18: 3010-3015

# In contrast to leptin, serum concentrations of ghrelin are not related to premenstrual syndrome

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**Abstract. – OBJECTIVE: Premenstrual syndrome (PMS) is a disorder related to mood and appetite changes during the premenstrual phase. Unfortunately, the understanding of the pathophysiology of PMS is quite poor. Though, ghrelin and leptin play important roles in the control of food intake. The aim of this study was to evaluate leptin and ghrelin serum concentrations in PMS patients.**

**PATIENTS AND METHODS: Forty-five PMS patients diagnosed according to ICD-10 diagnostic criteria and 45 healthy women as a control group, were included in the study. These groups were matched for age, body mass index and duration of menstrual cycle. Symptoms of the patients were evaluated using "Menstrual Distress Questionnaires". Serum leptin and ghrelin serum concentrations were measured using ELISA in the postmenstrual phase (5-9 days) and 2-3 days before menstruation. Mann-Whitney U test, independent sample *t*-test and Wilcoxon test were used for statistical analyses.**

**RESULTS: In the PMS group, there was no difference in the serum concentrations of ghrelin; however, leptin serum concentrations were 31.05 (± 14.16) and 16.42 (± 15.81) ng/ml during the premenstrual and postmenstrual periods, respectively ($p < 0.05$). Ghrelin serum concentrations in the premenstrual period were 6.9 (± 9.3) ng/ml in the PMS group and 8.8 (± 9.3) ng/ml in the control group, but this difference was not statistically significant ($p = 0.79$).**

**CONCLUSIONS: Ghrelin serum concentrations were not associated with PMS, while leptin serum concentrations were found to be higher in the premenstrual period in PMS patients. Though, these two hormones work antagonistically to control the food intake and body weight, we suggest that this function is not relevant to PMS.**

*Key Words:*
Ghrelin; Leptin; Premenstrual syndrome; Menstrual Distress Questionnaires; Premenstrual period.

## Introduction

Premenstrual syndrome (PMS) is a collective manifestation of behavioural, somatic and physical symptoms that occur repetitively during the luteal phase of the menstrual cycle, but disappear soon after the onset of menstruation, and can interfere with some aspects of woman's life. Although a large range of symptoms have been associated with PMS, the most common symptoms that characterise the syndrome include depression, irritability, mood swings, water retention based symptoms such as breast tenderness and bloating, and change in appetite and food cravings[1]. PMS is a common disorder affecting 20-30% women with regular menstrual cycles. Both genetic and environmental factors play a role in the development of premenstrual symptoms[2,3]. Although the etiology of this disorder remains uncertain, research suggests that altered regulation of neurohormones and neurotransmitters may have some involvement[4]. Though, women with PMS have normal concentrations of serum oestrogen and progesterone, they may have an abnormal response to normal hormonal changes. In the normal menstrual cycle, cyclic fluctuations in luteal phase oestrogen and progesterone concentrations cause marked changes in appetite and/or food cravings, which are considered as characteristics of PMS. In some human studies, an increase in energy intake and appetite during the premenstrual phase, compared to the postmenstrual phase, has been shown[5].

Although leptin was first discovered as an antiobesity hormone, but sooner, it was recognized as a hormonal mediator of adaptation to energy deprivation[6]. Leptin also plays an important role in human reproduction with metabolic regulation of the hypothalamic-pituitary-gonadal axis[7]. A



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randomized, double-blinded, placebo-controlled trial administering human recombinant leptin as replacement resulted in recovery of menstruation and corrected the abnormalities in gonadal functions in hypothalamic amenorrhic patients[8].

Ghrelin is an important regulator of growth hormone secretion, food intake and reproductive function[9,10]. Ghrelin was originally identified in the rat stomach as an endogenous ligand of the growth hormone secretagogue receptor[10,11]. Ghrelin is also known to be related to the hypothalamo-hypophysial-gonadal axis and may play a role in the secretion of gonadotropins, with a predominant inhibitory effect. Especially in animal studies, it has been shown that ghrelin inhibits LH secretions[12].

Interestingly, based on its biological effects and mechanism of action, ghrelin has been proposed to be a functional antagonist of the leptin effects on energy balance. Ghrelin and leptin work antagonistically to control the food intake and body weight[13,14]. Leptin may also play a role in the pathophysiology of PMS[7].

We hypothesized that such a dynamic interaction might also involve the regulation of reproductive function, especially in the premenstrual phase of the menstrual period where appetite and energy intake increase in PMS patients. It is intriguing that does ghrelin play any role in the pathogenesis of this syndrome? Due to the lack of sufficient studies on hormone levels, we aimed to evaluate these two hormones in PMS patients. To our knowledge this is the first study evaluating the relationship of leptin and ghrelin in PMS patients.

## Patients and Methods

### Study Design

From July 2011 to January 2012, in the outpatient clinic of Obstetrics and Gynaecology, 45 PMS patients diagnosed according to the International Classification of Diseases-10 (ICD-10) diagnostic criteria and 45 healthy women as a control group were included in this prospective case-controlled study. These patients, aged between 18-44 years and had regular menstrual cycles of length varying between 23 to 31 days, were admitted to our clinic for dysmenorrhoea or routine control examination.

The two groups were matched for age, body mass index (BMI) and duration of menstrual cycle. An informed consent was obtained from all participants. The presented protocol was approved by the Clinical Research Ethics Committee of Afyon Kocatepe University.

### Exclusion Criteria

Individuals with any systemic or psychiatric disease (hyperthyroidism, hypothyroidism, premature ovarian failure, endometriosis, dysmenorrhoea, substance or alcohol abuse, perimenopausal, major depression, panic attacks, bipolar disorder) or used a drug in the previous three months such as oral contraceptives, antidiabetic, antihypertensive, antiobesity, antihyperlipidemic, glucocorticoids, ovulation induction treatment agents or losing body weight intentionally, taking special diets and taking phytoestrogens as dietary supplements and also women with menstrual irregularities were excluded.

All study participants completed a validated questionnaire consisting of 14 questions that included demographic information. An ICD-10 symptom checklist for PMS was used to identify women with PMS[15]. Only one of these ICD-10 symptoms was required for diagnosis. It was taken into consideration that symptoms must be restricted to the premenstrual phase of the menstrual cycle and should cease with commencement of menstrual flow. Women without these symptoms were selected for the control group while others with complaints including at least one of these symptoms were included in the study group, i.e., women with PMS.

Symptoms of the patients were evaluated using validated "Menstrual Distress Questionnaires (MDQ)" with a face-to-face interview technique. The Menstrual Distress complaint List (MDQ) was developed by Moos[16]. Symptoms of PMS were evaluated separately for 3 groups: the menstrual period, premenstrual period, and intermenstrual period. The prevalence of physical and psychological symptoms used a 47-item Likert-type scale. The maximum score for each period was 188.

### Sample Colletion and Analyses

For the determination of plasma leptin and total ghrelin concentrations, blood samples were collected twice after an overnight fast of 12 hours, in the postmenstrual phase (5-9 days) and premenstrual phase (2-3 days before menstruation). After that the blood samples were centrifuged at 5000 rpm for 10 min and supernatants were stored in Eppendorf tubes at –80 $^{o}$C until measurements were performed. Serum leptin and ghrelin levels

were measured using enzyme-linked immunoassay systems (ELISA kits) for leptin [DRG Leptin (Sandwich) ELISA; DRG Instruments GmbH, Marburg, Germany; catalogue number: EIA-2395; sensitivity: 1.0 ng/ml; Intra and Inter assay CV: 6.95% and 8.66%, respectively] and for ghrelin (Phoenix Pharmaceuticals Inc., Karlsruhe, Germany; catalogue number: EK-031-30; sensitivity: 0.13 ng/ml; Intra and Inter assay CV: 4.85% and 8.18%, respectively). All the women were presumed ovulatory on the basis of regular menstrual cycles. It was further confirmed by mid-luteal plasma progesterone levels.

### Statistical Analysis

Data are presented as mean ± SD for normally distributed variables, median ± IQR for abnormally distributed variables. Comparative analyses between groups were calculated with the Mann-Whitney U-test, independent sample *t*-test and Wilcoxon test. Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Data with $p < 0.05$ were considered statistically significant.

## Results

A total of 90 patients, 45 women in PMS and 45 in control group, were included in this study. Table I shows the demographic characteristics of the participants. The two groups were similar in age, BMI, gravity, parity and length of menstrual cycles (Table I).

In the PMS group there was no difference in the ghrelin concentrations before and after the menstruation, however, leptin concentrations were significantly higher during the premenstrual period (31.05 ± 14.16 vs. 16.42 ± 15.81 ng/ml, respectively) ($p = 0.01$). Ghrelin concentrations in the premenstrual period were 6.9 ± 9.3 ng/ml in the PMS group and 8.8 ± 9.3 ng/ml in the control group, this difference was not statistically significant ($p = 0.79$). Leptin concentrations in the PMS group compared with the control group in the premenstrual period were significantly different (31.05 ± 14.16 ng/ml vs. 11.14 ± 11.89 ng/ml, respectively) ($p = 0.007$), but in the postmenstrual period the differences were not statistically significant (16.42 ± 15.81 vs. 15.76 ± 13.03, respectively) ($p = 0.4$). The postmenstrual ghrelin concentrations were found to be 6.3 ± 5.8 ng/ml and 5.8 ± 7.9 ng/ml in the PMS and control groups, respectively, but this difference was not statistically significant compared to the premenstrual values ($p > 0.05$). Leptin concentrations in the control group were lower during the premenstrual period compared to the postmenstrual period (Table II).

The total average of the scores 68, 46 and 8 were obtained for premenstrual, menstrual and other periods of the menstrual cycle, respectively, when patient MDQ forms were examined (Table III). The MDQ form values in the premenstrual and menstrual phases for PMS patients showed a statistically significant difference compared to the control group ($p = 0.004$ and $p = 0.002$, respectively).

## Discussion

In this work, it was found that serum ghrelin concentrations were not associated with PMS (at least statistically), but that leptin levels were associated with PMS. The ghrelin concentrations during the premenstrual and follicular phases, which have important functions in the reproductive system, were investigated, but no significant difference between the two groups was observed.

**Table I.** Demographic findings of the groups.

| | PMS (n=45) | Control (n=45) | p |
|---|---|---|---|
| Age (year) | 23 (14) | 26 (11) | 0.55[b] |
| Height (cm) | 163 (8) | 163 (7) | 0.88[b] |
| Weight (kg) | 64 (16) | 63 (14) | 0.3[b] |
| BMI | 23 ± 3.8 | 23 ± 3.5 | 0.55[a] |
| Gravidity | 2.8 (2) | 1 (3) | 0.35[b] |
| Parity | 0.89 (2) | 1 (2) | 0.35[b] |
| Menstrual lenght | 26.4 ± 1.9 | 27 ± 2.0 | 0.11[a] |

[a]Independents sample *t*-test was used (value given as mean ± SD); [b]Mann Whitney-U test was used [value given as median (IQR)]: If $p < 0.05$, considered to be statistically significant

**Table II.** Comparison of Ghrelin and Leptin levels between PMS and control groups.

| | | PMS | Control | p |
|---|---|---|---|---|
| Ghrelin (ng/ml) | Premenstrual phase | 6.9 (9.3) | 8.8 (9.3) | 0.79 |
| | Postmenstrual phase | 6.3 (5.8) | 5.8 (7.9) | 0.73 |
| Leptin (ng/ml) | Premenstrual phase | 31.05 (14.16) | 11.14 (11.89) | 0.007* |
| | Postmenstrual phase | 16.42 (15.81) | 15.76 (13.03) | 0.4 |

Mann-Whitney U test was used [value given as median (IQR)]. *Is significant ($p < 0.05$). postmenstrual phase: 5-9. days of menstrual cycle and premenstrual phase: 21-28. days of menstrual cycle.

Investigators have hypothesized an involvement of gonadal steroids in the pathophysiology of PMS because of symptoms that recur during specific phases of the menstrual cycle[17]. Use of ovulation inhibitors, along with the fact that PMS symptoms are not present in non-ovulatory cycles or in ovariectomised patients, supports this hypothesis[18-20]. According to many researchers, progesterone rather than oestrogen can cause PMS symptoms because of a decline in late luteal phase and this has led to consideration of a link to the changes in gamma-aminobutyric acid (GABA) in central nervous system and progesterone metabolites that interact with the GABA-A receptor complex[21,22].

Leptin is produced predominantly in adipose tissue but it is also expressed in a variety of other tissues including placenta, ovaries, mammary epithelium, bone marrow and lymphoid tissues[23,24]. In humans the release of leptin into the circulation is pulsatile and its concentrations follow a circadian rhythm[25]. According to Anim-Nyame et al[7] leptin may play a role in the pathophysiology of PMS[7]. In a PMS group, premenstrual phase levels of leptin were higher than in the premenstrual phase of a control group. Our findings of leptin levels in PMS are consistent with the literature.

Ghrelin is synthesised predominantly in the stomach but it has also been identified in bowel, placenta, hypothalamus, pituitary, kidney, thyroid, lung and lymphatic tissue[26]. The importance of ghrelin in menstrual cycles is suggested by other reports as well. In pig and rat ovary models, ghrelin concentrations were lowest in the proestrous phase and highest during the luteal phase of the cycle, with functional corpus luteum being the major site for ghrelin expression within the ovarian tissue[27,28]. Circulating ghrelin acts through ghrelin receptors on ovarian tissue.

In light of these previous reports, it was expected to find high serum concentrations of ghrelin in PMS patients, since symptoms for this group were present during the luteal phase. But interestingly, the concentrations were found to be equal to those in the postmenstrual phase. The low level of ghrelin and high level of leptin in the luteal phase in the PMS group may indicate a *yin and yang* role in control of the reproductive system (at least for PMS patients) being important in the control of food intake and body weight. Sirotkin et al[29] also found an antagonistic relationship between leptin and ghrelin in rabbit ovarian function.

Another speculation from these findings is concerned with eating habits for the PMS group.

**Table III.** Menstrual Distress Questionaires (MDQ) forms values for premenstrual, menstrual and intermenstrual phases of the menstrual cycle in PMS and control groups.

| | PMS (n=45) median (IQR) | Control (n=45) median (IQR) | z | p |
|---|---|---|---|---|
| Menstrual phase | 68 (40)<br>71.5 ± 28,5 | 15 (10)<br>15.6 ± 8.2 | -7.728 | **0.002*** |
| Premenstrual phase | 46 (44)<br>48.6 ± 22.8 | 11(11.5)<br>12.5 ± 7.3 | -7.123 | **0.004*** |
| Intermenstrual phase | 8 (21.5)<br>15.9 ± 12.3 | 8.5 (9.5)<br>9.4 ± 6.9 | -0.56 | 0.57 |

*Menstrual phase*: during menstrual flow, *premenstrual phase*: the week before the beginning of menstrual flow, *intermenstrual phase*: remainder of cycle. Mann Whitney-U test was used. Values were given as median(IQR) and mean ± SD. *$p < 0.05$.

Dye and Blundell[5] showed, in human, an increase in energy intake and appetite during the luteal phase in comparison to the follicular phase. But there is some evidence that women with anovulatory cycles do not show fluctuations in energy intake. Increases in appetite and/or food cravings are also considered to be characteristics of PMS. Some researchers, in animal and human studies, have found decreasing ghrelin levels after carbohydrate ingestion, whereas significantly increased ghrelin levels after fat, protein, fruit and vegetable ingestion[30,31].

## Conclusions

Our findings in PMS patients suggest that leptin may be related to this disorder whereas ghrelin is not. Although these two hormones play a *yin and yang* role in the control of food intake and body weight, but still this role is not valid in reproductive systems at least for PMS patients. Nevertheless, the central mechanism regulating the interaction of these two hormones is obscure. However, it remains to be determined whether these hormones could have a significant role in PMS or they are mere secondary to other factors not yet described.

### Conflict of Interest

All the authors disclose no financial relationship with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript.

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