Serum Pattern of Circulating Adipokines throughout the Physiological Menstrual Cycle


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Abstract. This study investigated the serum levels of resistin, adiponectin and leptin during the physiological menstrual cycle. Sixteen women (age: 19-30 years; body mass index: 19.46-24.9) with regular menstrual cycles participated. Fasting blood samples were collected on alternate days throughout a full menstrual cycle. Mean resistin concentrations were slightly higher during the luteal phase (5.30±0.23 ng/ml) compared to the follicular (4.68±0.07 ng/ml) and midcycle (4.86±0.09 ng/ml) phases (p=0.032). Mean leptin concentrations during the follicular phase (18.14±0.28 ng/ml) were significantly lower compared to the midcycle (21.79±0.29 ng/ml, p=0.006) and luteal phases (23.75±0.64 ng/ml, p<0.001).

The variation of adiponectin concentrations throughout the menstrual cycle was not significant. According to the results, circulating resistin, likewise leptin concentrations vary significantly during the physiological menstrual cycle presenting with higher values during the luteal phase. This pattern, although its physiological importance is not clear, suggests that resistin, likewise to leptin, may have a role in the regulation of cyclic female reproductive functions. The stable adiponectin concentrations throughout the menstrual cycle indicate that this adipokine probably does not play a considerable role in female reproductive functions.

Key words: resistin, adiponectin, leptin, menstrual cycle, reproduction

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Adipose tissue has been recognized not only as a reservoir of energy-rich molecules but also as an important and highly specialized, endocrine and paracrine organ producing an array of cytokines called adipokines. Adipokines have local and systemic biological effects influencing a plethora of functions of the human body and they are also implicated in the development of a variety of diseases [1-4].

Leptin, resistin, and adiponectin are among the cytokines produced by adipose tissue. They participate in the control of food intake, glucose and lipid homeostasis [1-4].

During the last decade, studies provided evidence on the indirect or direct participation of leptin in human reproductive functions. Leptin plays a role in puberty, gonadal function, early embryogenesis and fat metabolism during pregnancy [5-7]. Mean circulating leptin levels are higher in women compared to men [5-7]. At the same time, circulating leptin levels vary during the physiological spontaneous menstrual cycle presenting with lower values during the follicular and higher values during the luteal phase [8-13]. This fact supports the notion that leptin plays an important role in female reproductive functions and it fuels questions whether leptin levels are associated with gonadotropin or ovarian steroid levels. These questions still remain open.

The participation of leptin in human female reproductive functions raises the question whether other adipokines are related to these functions, too. The present study was designed to investigate the serum levels of...
resistin and adiponectin throughout the menstrual cycle of endocrinologically normal young women. In addition, the levels of total leptin were measured to investigate its relationships with the other two adipokines.

Namely, the present research aimed to explore the following questions:

- Are the circulating levels of resistin and adiponectin associated with the cyclic female reproductive function?
- Is there any association between the ovarian steroids, or gonadotropins and adipokines?
- Are there correlations between leptin, resistin and adiponectin serum levels throughout the menstrual cycle?

**Materials and methods**

**Subjects and collection of sera**

Sixteen healthy female students with a mean age of 22.73±0.95 years (range: 19-30 years) participated in the study. Written consent was provided by all. None of the volunteers had taken any medication for at least two months prior to participation in the study. All had regular menstrual cycles, normal thyroid function, normal basal hormone levels for estradiol (E2), testosterone, dehydroepiandrosterone-sulfate (DHEA-S), PRL, progesterone (P), FSH and LH on cycle day 3 (table 1). In addition, haematological and chemical laboratory analyses, physical examination and the results from gynecological ultrasound were normal. All had normal blood pressure. Body mass index (BMI) was between 19.46 and 24.9 with a mean value of 22.78±0.55. During the study, they did not receive any medication and they did not consume alcohol. The study was conducted following approval by the School of Medicine, Democritus University of Thrace.

Blood samples were obtained by venipuncture, after overnight fasting, between 8:00 and 9:00 h every second day throughout a full menstrual cycle. Each blood sample was allowed to clot, it was centrifuged within 60 minutes following venipuncture and the extracted serum was divided into 1ml aliquots and immediately stored at -25°C until the measurements were performed.

**Measurements**

In every serum sample, resistin, adiponectin and total leptin were quantified with enzyme immunoanalytical methods (ELISA) as follows: Leptin: RD191001100 (BioVendor Laboratory Medicine Inc., Brno, Czech Republic), intra-assay precision (intra-AP) <7.5%, inter-assay precision (inter-AP) <9.2%, limit of detection (LD) = 0.5ng/ml; Resistin RD191016100R (BioVendor Laboratory Medicine Inc., Brno, Czech Republic), intra-AP <3.4%, inter-AP <6.9%, LD=0.1ng/ml; Adiponectin RD195023100 (BioVendor Laboratory Medicine Inc., Brno, Czech Republic), intra-AP <7%, inter-AP <8.2%, LD=210ng/ml.

Homeostasis model assessment of insulin resistance (HoMA-iR) was also calculated as glucose (mg/dl) X insulin (pmol/l)/2813.

**Statistical analysis**

Menstrual cycle profiles for all subjects were aligned with reference point the day of the LH surge (day 0). The day of LH surge was considered as the day where LH>10 mIU/ml followed by a marked in-
Results in all participating women, the fluctuations of FSH, LH, E2 and P were as expected (Table 2). Glucose and insulin concentrations were normal; their fluctuations, along with those of HOMA-iR, are presented in Figure 1. Resistin, adiponectin and leptin concentrations varied considerably among women at each sampling day. The mean values of serum resistin, adiponectin and leptin throughout the menstrual cycle are presented in Table 3.

Here, the results for each adipokine are presented in detail.

Resistin

There was a statistically significant variation in resistin concentration throughout the menstrual cycle (repeated measures ANOVA, p=0.032); the mean resistin concentration was 4.48±0.33 ng/ml at the beginning and fluctuated around that level (ranging from 4.60 to 5.16 ng/ml) during the follicular (days -14 to -6) and midcycle phase (days -4 to +4); the mean resistin concentration was 4.94±0.31 ng/ml at the day of LH surge (day 0) (Figure 2). The mean resistin concentrations started to increase in the luteal phase, reaching a peak value of 5.97±0.98 ng/ml on day +10.
AsiMAKOPOULOS et al. (r=-0.23, p=0.001) and leptin (r=0.22, p=0.002) (Table 4). In multivariate analysis, the changes in p and in -sulin remained significantly associated with those in resistin, explaining only the 3.0% and 5.0% of its variance, respectively.

Adiponectin

The mean adiponectin serum concentration was 12.57±1.44 ng/ml at the beginning of the menstrual cycle. The fluctuations in adiponectin concentrations over time were not significant (repeated measures ANOVA, p=0.23). The mean adiponectin concentration was 13.72±0.78 ng/ml at the follicular, (p<0.05 compared to all other measurements). With respect to the three phases of the menstrual cycle, there was a trend towards slightly higher concentrations during the luteal phase (5.30±0.23 ng/ml) compared to follicular (4.68±0.07 ng/ml) and midcycle (4.86±0.09 ng/ml) phases.

The statistical analysis between subjects showed a marginal correlation of resistin concentrations with those of leptin (r=-0.49, p=0.06) (Table 4), which was stronger in the second half of the menstrual cycle (r=-0.60, p=0.02).

Within subjects, the changes in resistin concentrations correlated with those in FSH (r=-0.16, p=0.03), P (r=0.30, p<0.001), insulin (r=-0.23, p=0.001), glucose (r=-0.23, p=0.001) and leptin (r=0.22, p=0.002) (Table 4). In multivariate analysis, the changes in P and insulin remained significantly associated with those in resistin, explaining only the 3.0% and 5.0% of its variance, respectively.

Table 3. Concentrations of resistin, adiponectin and leptin of sixteen healthy women during a full menstrual cycle. Mean values±SEM are presented. Day 0 indicates the day of LH surge.

<table>
<thead>
<tr>
<th>Day of the cycle</th>
<th>Resistin (ng/ml)</th>
<th>Adiponectin (ng/ml)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-14</td>
<td>4.48±0.33</td>
<td>12.57±1.44</td>
<td>17.68±2.48</td>
</tr>
<tr>
<td>-12</td>
<td>4.77±0.34</td>
<td>14.46±2.18</td>
<td>19.17±2.70</td>
</tr>
<tr>
<td>-10</td>
<td>4.60±0.33</td>
<td>12.18±1.82</td>
<td>18.24±2.68</td>
</tr>
<tr>
<td>-8</td>
<td>4.90±0.37</td>
<td>16.45±2.98</td>
<td>17.62±2.00</td>
</tr>
<tr>
<td>-6</td>
<td>4.68±0.33</td>
<td>12.94±2.20</td>
<td>17.96±1.85</td>
</tr>
<tr>
<td>-4</td>
<td>4.63±0.30</td>
<td>13.39±2.15</td>
<td>21.27±2.31</td>
</tr>
<tr>
<td>-2</td>
<td>4.67±0.36</td>
<td>13.01±1.56</td>
<td>22.34±2.72</td>
</tr>
<tr>
<td>0</td>
<td>4.94±0.31</td>
<td>12.36±1.66</td>
<td>21.57±2.67</td>
</tr>
<tr>
<td>+2</td>
<td>5.16±0.44</td>
<td>12.05±1.57</td>
<td>21.17±2.32</td>
</tr>
<tr>
<td>+4</td>
<td>4.89±0.34</td>
<td>12.05±1.74</td>
<td>22.59±2.36</td>
</tr>
<tr>
<td>+6</td>
<td>4.98±0.36</td>
<td>16.07±3.10</td>
<td>23.12±2.92</td>
</tr>
<tr>
<td>+8</td>
<td>5.51±0.44</td>
<td>14.58±2.32</td>
<td>25.17±2.53</td>
</tr>
<tr>
<td>+10</td>
<td>5.97±0.98</td>
<td>14.01±2.55</td>
<td>24.32±3.47</td>
</tr>
<tr>
<td>+12</td>
<td>4.63±0.57</td>
<td>12.38±2.81</td>
<td>24.58±3.20</td>
</tr>
<tr>
<td>+14</td>
<td>5.41±1.01</td>
<td>14.96±4.31</td>
<td>21.57±4.87</td>
</tr>
</tbody>
</table>

Table 4. The (a) between- and (b) within- subjects correlation of adipokines and hormones during the menstrual cycle

<table>
<thead>
<tr>
<th>(a)</th>
<th>Resistin</th>
<th>Adiponectin</th>
<th>Leptin</th>
<th>FSH</th>
<th>E2</th>
<th>P</th>
<th>Glucose</th>
<th>Insulin</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistin</td>
<td>-</td>
<td>-0.39</td>
<td>-0.49</td>
<td>0.33</td>
<td>-0.08</td>
<td>0.11</td>
<td>-0.23</td>
<td>-0.08</td>
<td>-0.07</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-</td>
<td>-0.23</td>
<td>-0.06</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td>-0.11</td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>-</td>
<td>-0.66**</td>
<td>-0.27</td>
<td>-0.31</td>
<td>0.14</td>
<td>0.07</td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b)</th>
<th>Resistin</th>
<th>Adiponectin</th>
<th>Leptin</th>
<th>FSH</th>
<th>E2</th>
<th>P</th>
<th>Glucose</th>
<th>Insulin</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistin</td>
<td>-</td>
<td>0.06</td>
<td>0.22**</td>
<td>-0.16*</td>
<td>0.11</td>
<td>0.30***</td>
<td>-0.23***</td>
<td>-0.23***</td>
<td>-0.04</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-</td>
<td>0.02</td>
<td>-0.04</td>
<td>-0.01</td>
<td>0.13</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.05</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>-</td>
<td>-0.30***</td>
<td>0.19**</td>
<td>0.20**</td>
<td>0.03</td>
<td>0.03</td>
<td>0.24***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01; *** p<0.001.
significant over time (p<0.001). In particular, the mean leptin serum concentration was 17.68±2.48 ng/ml at the beginning of the menstrual cycle and fluctuated around that level (ranging from 17.62 to 19.17 ng/ml) during the follicular phase. At the beginning of the midcycle phase, the mean leptin concentration increased to 21.27±2.31 ng/ml and remained approximately at the same level throughout this phase (ranging from 21.27 to 22.59 ng/ml); the mean leptin concentration was 21.57±2.67 ng/ml at the day 0. Slightly higher mean leptin concentrations (ranging from 23.12 to 25.17 ng/ml) were observed during the luteal phase, with the exception of the last day of this phase, when mean leptin concentration declined to 21.57±4.87 ng/ml (Figure 4).

Overall, the statistical analysis between subjects did not reveal significant correlations between adiponectin concentrations and the concentrations of the other adipokines and hormones (Table 4).

**Leptin**

As a whole, ANOVA for repeated measures indicated that the changes in mean leptin serum concentrations, after adjustment for BMI, were statistically significant over time (p<0.001). In particular, the mean leptin serum concentration was 17.68±2.48 ng/ml at the beginning of the menstrual cycle and fluctuated around that level (ranging from 17.62 to 19.17 ng/ml) during the follicular phase. At the beginning of the midcycle phase, the mean leptin concentration increased to 21.27±2.31 ng/ml and remained approximately at the same level throughout this phase (ranging from 21.27 to 22.59 ng/ml); the mean leptin concentration was 21.57±2.67 ng/ml at the day 0. Slightly higher mean leptin concentrations (ranging from 23.12 to 25.17 ng/ml) were observed during the luteal phase, with the exception of the last day of this phase, when mean leptin concentration declined to 21.57±4.87 ng/ml (Figure 4).
Regarding the three phases of the menstrual cycle, the mean leptin concentrations during the follicular phase (18.14±0.28 ng/ml) were significantly lower compared to the midcycle (21.79±0.29 ng/ml, p=0.006) and luteal phase (23.75±0.64 ng/ml, p=0.001). No significant difference was observed between the midcycle and the luteal phase (p=0.32).

The statistical analysis between subjects showed that in general leptin concentrations correlated with FSH (r=-0.66, p=0.03) and marginally with resistin (r=-0.49, p=0.06) concentrations. Particularly, in the second half of the cycle, leptin concentrations were also significantly correlated with resistin concentrations (r=-0.60, p=0.02).

Within subjects, the changes in leptin concentrations correlated with those in FSH (r=-0.30, p<0.001), E2 (r=0.19, p=0.006), P (r=0.20, p=0.006), resistin (r=0.22, p=0.002) and HOMA-IR (r=0.24, p=0.001) (Table 4). In a multiple regression model that included all variables, the changes in FSH (p<0.001), E2 (p=0.01), HOMA-IR (p=0.007) and resistin (p=0.04) were significantly associated with those of leptin concentrations, explaining 3.9%, 1%, 1.2% and 0.5% of its variance, respectively.

Discussion

In this study we investigated for the first time, the resistin circulating concentrations throughout the physiological menstrual cycle. To our knowledge, it was also the first time where the three adipokines were investigated altogether during the menstrual cycle. We demonstrated that resistin concentrations moderately increase in the luteal phase, while mean adiponectin concentrations remained practically stable during the menstrual cycle. Leptin concentrations increased during the midcycle and luteal phases.

Significant variations of leptin during the menstrual cycle, with higher concentrations at the luteal phase have also been reported by several groups [8-13]. However, the associations of leptin levels to those of gonadotropins or ovarian steroids, during the menstrual cycle, are controversial. In the present study, leptin levels in each woman participated in the study, correlated with those of FSH, E2 and P, however this correlation was weak. Multiple regression model analysis showed that the changes in FSH and E2 accounted only for 4.9%, in total, of the variations in leptin concentrations. Additionally, the statistical analysis between subjects showed a significant correlation between leptin and FSH.

Many different opinions have been reported regarding this issue. Riad-Gabriel et al reported that the variation in P accounted for only 5% of the variation in leptin [8]; Ludwig et al did not find any correlation between leptin and E2 or P concentrations [10]; Fernández-Real et al found LH to correlate with leptin concentrations during the midcycle and luteal phases [11]; Cella et al reported positive correlation of leptin with both E2 and P concentrations [9]; whereas, Wunder et al did not find any correlation of leptin with ovarian steroid hormones but they reported a significant correlation with free testosterone [13]. Furthermore, taking into consideration in-vitro studies showing that leptin influences ovarian steroidogenesis [16-19] we could assume that an association of leptin levels to those of gonadotropins and ovarian steroids does exist though weak, as the present and previous studies have shown.

The statistical analysis revealed that adiponectin concentrations were not associated either with the concentrations of ovarian steroids and gonadotropins or the concentrations of the other adipokines. This finding, along with the stable concentrations of adiponectin throughout the menstrual cycle, suggests that this adipokine probably is not related to female reproductive functions such as follicular maturation, ovulation or corpus luteum function. Recently, Kleiblova et al, in a small study including six healthy women, found insignificant changes of serum adiponectin concentrations throughout the menstrual cycle [20]. They also reported that changes in sex hormones do not seem to affect circulating adiponectin concentrations [20].

On the other hand, the pattern of resistin concentrations during the menstrual cycle was quite similar to that of leptin, showing an elevation at the luteal phase, though the concentrations of resistin varied less than those of leptin.

The role of resistin in humans is not certain. Though it is considered as an adipokine, it seems that, in humans, the main resource of resistin is blood mononuclear cells. Resistin expression by macrophages and monocytes indicates a potential role in inflammation. Moreover, it has been shown that its expression is enhanced by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 [4]. Whereas in rodents it is clear
that resistin is linked with insulin resistance, clinical studies, in humans, have failed to show a consistent link between resistin levels and insulin resistance. It is worth noting that human resistin is only 64% homologous with the murine counterpart thus it is possible that human resistin does not play a similar role as murine resistin. However, recent studies indicated that a resistin gene promoter single nucleotide polymorphism (SNP) at -420 is related with higher resistin plasma levels and insulin resistance [21]. Namely, resistin levels in subjects with the G/G genotype of resistin SNP-420 ranged between 1.9 to 52.7 ng/ml; in subjects with the G/C genotype, resistin levels ranged between 2.2 to 46.2 ng/ml whereas in subjects with the C/C genotype the levels ranged between 2.2 to 35.2 ng/ml [21]. In the present study, the genotype of resistin SNP-420 of the volunteers participating was not investigated mainly for two reasons: first, there is a large inter-covering of the range of resistin levels between the different genotypes thus limiting their value to distinguish individuals of high and low resistin levels. Secondly, the present study was focused rather on the fluctuation of resistin levels during the menstrual cycle and not on the precise values.

The importance and the role of the fluctuations in resistin or in leptin concentrations during the menstrual cycle are not known. It has been suggested that the increase in leptin concentrations during the luteal phase is due to increased caloric intake [8]. It is known that in women, appetite and food intake is usually greater during the luteal phase [22], hence it could be suggested that adipocytes secrete larger amounts of leptin during this phase. Consequently, the increased concentrations of leptin acting at hypothalamus, control food intake but also contribute to the regulation of hypothalamic-pituitary-gonadal tropin release and ovarian steroidogenesis. In this notion, leptin participates in the regulation of menstrual cycle. The role of leptin in the regulation of menstrual cycle is further supported by studies demonstrating that menstrual irregularities and amenorrhea are common in cases with very high or very low leptin concentrations [6-7]. Moreover, it has been shown that leptin influences the secretion of growth factors and hormones from ovarian theca and granulosa cells [5-7].

Circulating resistin also increases in cases of increased caloric intake [23]. Additionally, it has been reported that resistin influences the production of various hypothalamic transmitters involved in central energy metabolism [24]. Therefore, we could suggest that resistin concentrations increase during the luteal phase as a response to increased feeding and then they act in the hypothalamus to regulate the energy metabolism further. The weak point of this hypothesis and at the same time the main limitation of our study is that the caloric intake of the subjects participating was not monitored.

Another possible explanation for the peri- and post-ovulatory elevation of leptin and the post-ovulatory elevation of resistin concentrations may lie on their inflammatory actions. Ovulation is considered as an inflammatory-like process [25]. LH surge induces the expression of various inflammatory mediators within the follicle which lead to breakdown of extracellular matrix, increased blood flow and vascular permeability. Macrophages seem to play key roles in these processes [26]. Many studies conducted with women following controlled ovarian hyperstimulation have documented the presence of proinflammatory cytokines in follicles at the time of ovulation [27-33]. On the other hand, it is known that leptin exerts pro-inflammatory actions: it is produced by inflammatory cells, its secretion is increased by inflammatory stimuli such as IL-1, IL-6 and lipopolysaccharide (LPS), whereas it modulates white blood cells [4]. As for resistin, its expression by blood mononuclear cells per se indicates a possible role in inflammation. Resistin levels have been associated with markers of inflammation [4]. It has been reported that in human peripheral blood mononuclear cells, resistin production is induced and induces IL-6 and TNFα production, whereas LPS induces resistin production in human macrophages [4]. Therefore, it could be postulated that the elevation of resistin concentrations during the luteal phase and the elevation of leptin concentrations during the midcycle and luteal phases was a response to inflammatory conditions related to ovulation. From this point of view, it would be interested to investigate the levels of pro-inflammatory cytokines (such as IL-1, IL-6 and TNFα) during the menstrual cycle as well as their possible correlation with the levels of leptin and resistin.

In any case, the cyclic variation of resistin concentrations throughout the menstrual cycle constitutes a noteworthy indication that resistin is involved in cyclic female reproductive functions.

The statistical analysis revealed weak associations between the changes in resistin concentrations and
those of other hormones. In each woman participated in the study, the fluctuations of P and insulin explained only a small part of the variance of resistin concentrations. HOMA-IR did not significantly correlate with resistin.

The association between leptin and resistin seems to be of particular interest. The statistical analysis showed that during the second half of the cycle, women with high levels of leptin tended to have lower levels of resistin ($r=-0.60, p=0.02$). However, in each woman, the changes in resistin concentrations followed a similar pattern to those of leptin, especially during the luteal phase where both elevated (within subjects $r=0.22, p=0.002$). This is another interesting finding, since it is known that both hormones act through the cellular nutrient-sensing AMP-activated protein kinase (AMPK) signaling pathway; leptin activates whereas resistin decreases AMPK activity [3].

Further investigation is needed on the relation of the two cytokines.

As a conclusion, the present study provided evidence and stimulated questions on the mode and nature of the involvement of resistin in female reproductive functions showing that its concentrations slightly increase during the second half of the menstrual cycle. This finding marks resistin as an interesting target for further investigation aiming to clarify the possible role of resistin on ovarian functions. This study also confirmed previous findings on the cyclic pattern of leptin concentrations throughout the physiologically menstrual cycle which support the notion that leptin does play a role in reproductive functions. Finally, the stable adiponectin concentrations throughout the menstrual cycle indicated that this adipokine does not seem to play a considerable role in female cyclic reproductive functions.

References


