ABSTRACT
There has been a surge of interest in recent years in studying the changes of serum melatonin concentrations in disorders that are associated with insulin resistance such as diabetes mellitus type 2 and polycystic ovary syndrome (PCOS).

AIM: The present study was designed to investigate the day-time and night-time levels of serum melatonin and the cortisol rhythm in women with PCOS and compare them with those of healthy women.

PATIENTS AND METHODS: This is a case-control study which included 30 women with PCOS and 25 healthy women. All hormonal measurements in both the study group and controls were carried out between days 3 and 5 counted from the beginning of the last regular menstrual cycle; they included serum levels of melatonin and cortisol at 03:00 a.m and 08:00 a.m, total testosterone (T), dehydroepiandrosterone sulfate (DHEA-S), luteinizing hormone (LH), follicle stimulating hormone (FSH), and immunoreactive insulin at 08:00 a.m.

RESULTS: Women with PCOS were found to have a significantly higher melatonin level at 08:00 a.m. and smaller mean night-day difference in the concentrations of melatonin in comparison with those of healthy women (natural log (Ln) night-day difference 0.60 ± 0.10 pg/ml versus 1.15 ± 0.14, p < 0.002). Melatonin to cortisol ratios at 03:00 a.m. and 08:00 a.m. showed no statistically significant differences between the two groups (Ln melatonin/cortisol at 03:00 a.m., 1.01 ± 0.06 versus 1.05 ± 0.05; Ln melatonin/cortisol at 08:00 a.m., 0.62 ± 0.01 versus 0.56 ± 0.03, p > 0.05).

CONCLUSION: The results we obtained about the changes of melatonin in women with PCOS could help in elucidating the complex pathophysiological pattern of this disease.

Key words: serum melatonin, polycystic ovary syndrome, melatonin night-day difference

INTRODUCTION
The polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders – it has complex pathophysiological characteristics not yet fully understood.1 The disorder affects about 5-10% of women of reproductive age and is a leading cause of infertility.2 PCOS is characterized by a wide phenotypic diversity of hormonal and metabolic alterations. The most common laboratory findings include high levels of androgens (total and/or free testosterone, androstenedione, DHEA-S), increased LH with low/normal FSH, and increased LH/FSH ratio, insulin resistance with compensatory hyperinsulinemia. Abnormal cortisol metabolism has been suggested as one of the possible reasons for enhanced adrenal androgen production.2

Melatonin is the main hormone produced by the pineal gland. It is secreted in a pronounced circadian rhythm – melatonin levels are high during the night and low during daytime. Melatonin secretion can be affected by exogenous (e.g. light, medication) and endogenous (e.g. age) factors which makes its serum levels fluctuate. The scope of knowledge about this hormone’s significance for healthy people is constantly changing and therefore is growing up. There is also increasing interest in
the role melatonin is believed to play in various pathological conditions. Current knowledge suggests that melatonin is considered as circadian rhythms synchronizer and can regulate sleep and body temperature. Currently melatonin is widely researched as to its implication in the processes of sexual maturation and reproduction, immune response, ageing, and tumour growth. Melatonin serum levels have been found to change in sleep disorders, depressive syndromes, and dementia. Recent studies suggest that the changes in the melatonin secretion rhythm can play a role in some cardio-vascular diseases and malignant disorders. Particular interest has been recently drummed up by the importance of melatonin for diseases associated with insulin resistance such as type 2 diabetes and PCOS. It is believed that women with PCOS present with disruption of melatonin secretion, although data in the literature concerning alterations in melatonin secretion in this disease are scanty.

AIM

The aim of the present case-control study was to investigate the day-time and night-time levels of serum melatonin alongside with the cortisol rhythm of secretion in women with PCOS and compare them with those in clinically healthy women.

MATERIAL AND METHODS

The study included 30 women in reproductive age diagnosed with PCOS and hospitalized in the Clinic of Endocrinology and Metabolic Diseases at St. George University Hospital in Plovdiv. The diagnosis of PCOS was made according to the joint criteria of the European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine, Rotterdam, 2003. The women in the study sample had received no medication in the previous 3 months that may have interfered with the hormonal and metabolic parameters (hormonal contraceptives, β-blockers, steroids, psychotropic agents, insulin sensitizers, lipid-lowering agents, etc.). The exclusion criteria were hypo- and hyperthyroidism, Cushing’s syndrome, congenital adrenal hyperplasia (including the forms with late onset), androgen-secreting tumors, prolactinomas, severe acute and chronic concomitant diseases.

A control group of 25 age-matched clinically healthy women was used in the study for comparison. All hormonal samples in the study group and controls including serum levels of melatonin and cortisol, total testosterone (T), dehydroepiandrosterone sulfate (DHEA-S), luteinizing hormone (LH), follicle stimulating hormone (FSH) and immunoreactive insulin were carried out between days 3 and 5 counted from the beginning of the last regular menstrual cycle.

Blood samples for determination of melatonin level were taken in a separate room by the same medical staff in order to standardize the conditions of taking venous blood samples. During the day the room was illuminated by normal day light. During night time to ensure complete darkness the windows were covered with black paper. All light sources were turned off at 10:00 p.m. The women were tested twice at 03:00 a.m. and 08:00 a.m. They were instructed to keep their eyes closed during the blood sampling at 03:00 a.m. The latter was performed by a small torch with a red light source for 3-5 minutes. Serum melatonin concentrations were calculated in SirioS Microplate Reader (SEAC, Italy) using ELISA Kit (IBL-Hamburg, Germany). The limit of detection of the assay was 1.6 pg/ml. The intra- and inter assay coefficient of variation (CV) were < 11.4% and < 19%, respectively.

Concentrations of LH, FSH, immunoreactive insulin, cortisol and total T were measured on Axsym™ system (Abbott, USA). DHEA-S was measured using ELISA Kit (DRG, Germany).

STATISTICS

The variables of the cases and controls were assessed by methods of descriptive statistics and tests of normality (Kolmogorov-Smirnov). The variables with skewed distribution were normalized by log-transformation. The statistical analysis was performed using SPSS v.17. The quantitative parameters were presented as mean ± SEM. The comparison between groups was performed using the independent samples t-test. The significance level for the null hypothesis was set at p < 0.05.

RESULTS

The women with PCOS were between 18 and 40 years of age. The mean age of PCOS women was 25.07 ± 1.10 years, and that of the women from the control group 26.24 ± 1.097 years (the difference did not reach statistical significance, t = 0.748, p = 0.458). The hormonal and anthropometric parameters of women from both groups are presented in Table 1.

As expected, women with PCOS had a significantly higher weight and BMI compared with controls (p < 0.05). Total T and DHEA-S concentrations also differed significantly between the two groups - in women with PCOS these parameters...
were about 2 times as high as that of the controls, which suggests hyperandrogenism. In this comparative study LH and the FSH/LH ratio were statistically significantly higher in women with PCOS in comparison with controls. We found no statistically significant differences between the groups in the levels of FSH and basal insulin.

Table 2 presents the mean values of melatonin and cortisol at 03:00 and 08:00 hr, the ratios between them and the night-day difference of melatonin in women with PCOS and healthy women.

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Table 2. Mean values of melatonin and cortisol at 03:00 and 08:00 hr, the ratios between them and the night-day difference of melatonin in women with PCOS and healthy women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCOS (n = 30)</th>
<th>Controls (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin, 03:00 a.m., (pg/ml)</td>
<td>88.63 ± 7.22</td>
<td>103.32 ± 8.51</td>
</tr>
<tr>
<td>Melatonin, 08:00 a.m., (pg/ml)</td>
<td>40.97 ± 3.79</td>
<td>42.91 ± 9.38</td>
</tr>
<tr>
<td>Night-day difference, (pg/ml)</td>
<td>39.42 ± 7.12</td>
<td>60.42 ± 10.12</td>
</tr>
<tr>
<td>Cortisol, 03:00 a.m., (nmol/l)</td>
<td>164.92 ± 35.7</td>
<td>113.15 ± 16.0</td>
</tr>
<tr>
<td>Cortisol, 08:00 a.m., (nmol/l)</td>
<td>479.22 ± 27.8</td>
<td>480.72 ± 36.7</td>
</tr>
<tr>
<td>Melatonin/cortisol, 03:00 a.m.</td>
<td>1.75 ± 0.57</td>
<td>1.83 ± 0.58</td>
</tr>
<tr>
<td>Melatonin/cortisol, 08:00 a.m.</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.03</td>
</tr>
</tbody>
</table>

DISCUSSION

Although melatonin was discovered more than 40 years ago, there is yet no clear explanation of its involvement in various physiological and pathological processes. There is a growing body of evidence that melatonin is implicated in the pathogenesis of a number of reproductive disorders in women such as endometriosis, PCOS and premature ovarian decline.8

Melatonin has been found experimentally to exert neuroendocrine control through directly affecting the GnRH neurons of the hypothalamus
either by downregulating their gene expression or by regulating the G protein-coupled melatonin receptors. Thus, the higher levels of melatonin inhibit the pulsatility of hypothalamic GnRH, and cause a series of changes in the reproductive function. There is also a direct effect on the pituitary gland to suppress the secretion of LH and FSH.

In 2001, Woo et al. advanced the hypothesis that melatonin directly affects the function of the ovaries and may take part in the intraovarian regulation of steroidogenesis. The hypothesis is supported by the higher concentrations of melatonin in the follicular fluid than those in the venous blood and the presence of melatonin receptors on the membrane of the granulosa-luteal cells. The authors demonstrate in their study that the melatonin receptor expressed in the granulosa cells changes the stimulated secretion of progesterone, the values of mRNA of LH and of GnRH receptor. All things considered, melatonin has an inhibitory effect on hypothalamic-pituitary-gonadal axis, and the hypothalamus is regarded as the likely site of melatonin-mediated regulation of gonadal activity.

Because of the pronounced night-day fluctuations of the secretion, the hormone concentration is about 10 times higher at night. This rhythm is under the immediate control of the suprachiasmatic nucleus and is affected by signals of light from the surrounding environment. Night melatonin level can drop under the influence of strong light. Experimental data show that removal of the pineal body reduces the body’s ability to adapt to new photoperiods and disrupts a number of physiological functions. A specific feature of melatonin rhythm is its extraordinary permanence for individuals, with significant differences in its nocturnal plasma levels between individuals.

Searching for alterations in the melatonin secretion in PCOS patients we have found that the main difference between healthy controls and women with PCOS in this study was the significantly lower night-day difference of melatonin as a result of the higher levels in the morning and the trend towards lower night-time levels. Our results are in full accordance with the results of Arkhypkina et al., which found elevated morning levels of melatonin in women with PCOS in comparison with healthy controls (75.9 ± 5.2 versus 49.9 ± 5.4 pmol/l, p < 0.05), as well as reduced night peak of melatonin (52.0 ± 3.1 versus 78.8 ± 5.9 pmol/l, p < 0.05).

Given the fact that nocturnal melatonin levels in patients with coronary pathology have been found to be lower than those in healthy individuals, a correlation between impaired melatonin secretion and the proven increased cardiovascular risk in PCOS patients can be suggested. In young women with PCOS, for instance, we found in a previous study that the non-dipper pattern of night fall of arterial

### Table 3. Mean values of melatonin and cortisol at 03:00 a.m. and 08:00 a.m., the ratios between them and the night-day difference of melatonin in women with PCOS and healthy women after logarithmic transformation of data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>n</th>
<th>mean ± SEM</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln Melatonin, 03:00 a.m. (pg/ml)</td>
<td>PCOS</td>
<td>30</td>
<td>4.36 ± 0.10</td>
<td>1.28</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>4.54 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln Melatonin, 08:00 a.m. (pg/ml)</td>
<td>PCOS</td>
<td>30</td>
<td>3.77 ± 0.10</td>
<td>1.99</td>
<td>0.05*</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>3.39 ± 0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln night-day difference (pg/ml)</td>
<td>PCOS</td>
<td>30</td>
<td>0.60 ± 0.10</td>
<td>3.19</td>
<td>0.002**</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>1.15 ± 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln cortisol, 03:00 a.m. (nmol/l)</td>
<td>PCOS</td>
<td>30</td>
<td>4.60 ± 0.20</td>
<td>0.56</td>
<td>0.574</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>4.45 ± 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln cortisol, 08:00 a.m. (nmol/l)</td>
<td>PCOS</td>
<td>30</td>
<td>6.12 ± 0.05</td>
<td>0.21</td>
<td>0.829</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>6.10 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln melatonin/cortisol, 03:00 a.m.</td>
<td>PCOS</td>
<td>30</td>
<td>1.01 ± 0.06</td>
<td>0.59</td>
<td>0.555</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>1.05 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ln melatonin/cortisol, 08:00 a.m.</td>
<td>PCOS</td>
<td>30</td>
<td>0.62 ± 0.01</td>
<td>1.66</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>25</td>
<td>0.56 ± 0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Differences are statistically significant at * p < 0.05, ** p < 0.01.
pressure occurs more commonly, and there is evidence that the less pronounced night melatonin increase is associated with smaller nocturnal blood pressure fall.

The correlation between cortisol and melatonin is also worth studying. In patients with hypercortisolemia and depression the highest nocturnal melatonin concentrations in peripheral blood are lower than those in healthy persons. It should be emphasized that women with PCOS can present with elevated levels of cortisol compared with healthy women, as well as disruption of its rhythm, which may expectedly lead to a change in the levels of melatonin. In this study we found no significant differences in cortisol levels and the melatonin/cortisol ratio at 03:00 and 08:00 hr between healthy women and those with PCOS, which does not suggest a direct link between the secretion of melatonin and secretion of cortisol. However, to make definitive conclusions further studies are needed on greater contingent of women with the syndrome with different phenotypic characteristics.

Similarly, in the present study we found no direct correlation between the alterations in melatonin secretion and insulin (insulin levels are similar in controls and women with PCOS), which is consistent with the results of Luboshitzky et al.

There are some studies in the current literature that assess the 24-hour excretion of melatonin in women with PCOS. The research in this respect carried out by Luboshitzky et al., showed that women with PCOS excrete more intensely the main melatonin metabolite, 6-sulfatoxy-melatonin (6-SMT), in 24-hour urine, which correlates with high serum testosterone. This type of changes cannot be found in women with idiopathic hirsutism or in healthy women. In this study no distinction was made between the day-time and night-time melatonin excretion, and based on most of the data we can speculate that the increased 24-hour excretion is due to the elevated daily hormone levels.

It has been found that melatonin is involved in the regulation of energy metabolism and weight control in experimental animals (rats). It is quite possible that melatonin promotes the recruitment of brown adipose tissue (BAT) as well as enhances its activity. As a result this raises the basal metabolic rate by stimulating thermogenesis, and generation of heat through uncoupling oxidative phosphorylation in mitochondria. Clinical studies have found a correlation between a high BMI and low urine excretion of 6-SMT. Travis et al. found a significant inverse correlation (p = 0.04) between 6-SMT and BMI in women under 50 years of age. In another study, women with a BMI under 21 kg/m² were shown to have a higher average level of 6-SMT than women with a BMI ≥ 29 kg/m² (20.8 ng/mg versus 11.8 ng/mg creatinine, p < 0.0001). Obviously, melatonin level is correlated with the body mass index. The lower night increase of the hypnotic hormone melatonin and the resulting sleep disorders may affect body weight. The women in the present study had a significantly higher BMI than that of healthy controls, in whom we observed statistically significantly lower morning melatonin concentration and a pronounced night-day difference.

CONCLUSIONS

Our study showed that night-day difference of melatonin levels in women with PCOS was smaller than that in healthy controls and that there was a tendency towards lower melatonin at 03:00 hr, which could contribute to weight gain and increased cardiovascular risk. Based solely on these results here, we are not able to explain what causes these changes of melatonin in PCOS – whether they are the result of primary neuroendocrine disorder inherent to the syndrome, or can be associated with a hyperandrogenic condition and metabolic disorders. Further studies are needed in this respect that would elucidate the etiopathogenesis of PCOS.

REFERENCES


СЫВОРОЧНЫЙ МЕЛАТОНИН У ЖЕНЩИН СО СИНДРОМОМ ПОЛИКИСТОЗНЫХ ЯЧНИКОВ

Д. Терзиева, М. Орбецова, М. Митков, Н. Матева

РЕЗЮМЕ

Введение. В последние годы увеличился интерес к изучению изменений сывороточного мелатонина при заболеваниях, протекающих с инсулиновой резистентностью как сахарный диабет типа 2 и синдром поликистозных яичников (PCOS).

Цель: Настоящее исследование ставит себе целью изучить дневные и ночные уровни сывороточного мелатонина и кортизола у женщин с PCOS и сравнить их с эталонными данными у здоровых женщин.

Материал и методы: Обследовано 30 женщин с PCOS и 25 здоровых женщин. Гормональные исследования и патологическая, и контрольной групп женщины проведены в рамках 3-его – 5-ого дня от начала последнего регулярного менструального цикла и включают измерения сывороточных уровней мелатонина и кортизола в 3:00 и в 8:00 ч., общего тестостерона (T), DHEA-S, LH, FSH и иммунореактивного инсулина в 8:00 ч.

Результаты: Установлено, что женщины с PCOS имеют сенситивно более высокий мелатонин в 8:00 ч. и соответственно меньшую ночное-дневную разницу мелатонина по сравнению со здоровыми женщинами (Ln ночное-дневная разница 0.60 ± 0.10 pg/ml по отношению к 1.15 ± 0.14 pg/ml, P < 0.002). Отношение мелатонин/кортизол в 3:00 и в 8:00 ч. не различается статистически значимо между обеими группами (Ln мелатонин/кортизол в 3:00 ч. 1.01 ± 0.06 по отношению к 1.05 ± 0.05; Ln мелатонин/кортизол в 8:00 ч. 0.62 ± 0.01 по отношению к 0.56 ± 0.03, P > 0.05).

Заключение: Полученные авторами данные об изменениях мелатонина у женщин с PCOS могли бы способствовать выявлению сложной патофизиологии этого заболевания.