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Melatonin Treatment May Be Able to Restore Menstrual Cyclicity in Women With PCOS: A Pilot Study

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Abstract

The objective of the study was to investigate the effects of 6 months of melatonin administration on clinical, endocrine, and metabolic features of women affected by polycystic ovary syndrome (PCOS). This is a prospective cohort study including 40 normal-weight women with PCOS between January and September 2016, enrolled in an academic research environment. Ultrasonographic pelvic examinations, hirsutism score evaluation, hormonal profile assays, oral glucose tolerance test, and lipid profile at baseline and after 6 months of melatonin administration were performed. Melatonin treatment significantly decreased androgens levels (free androgen index: $P < .05$; testosterone: $P < .01$; 17 hydroxyprogesterone: $P < .01$). Follicle-stimulating hormone levels significantly raised ($P < .01$), and anti-Mullerian hormone serum levels significantly dropped after 6 months of melatonin treatment ($P < .01$). No significant changes occurred in glucoinsulinemic and lipid parameters after treatment except a significant decrease of low-density lipoprotein cholesterol. Almost 95% of participants experienced an amelioration of menstrual cycles. Until now, only few data have been published about the role of melatonin in women with PCOS. This is the first study focused on the effects of exogenous oral melatonin administration on the clinical, endocrine, and metabolic characteristics of patients with PCOS. After 6 months of treatment, melatonin seems to improve menstrual irregularities and biochemical hyperandrogenism in women with PCOS through a direct, insulin-independent effect on the ovary. Based on our results, melatonin could be considered a potential future therapeutic agent for women affected by PCOS.

Keywords

melatonin, PCOS, menstrual irregularities, insulin

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting about 7% to 10% of women in reproductive age. The clinical expression of the syndrome is characterized by the heterogeneous combination of irregular menstrual cycles, infertility, hirsutism/acne, central obesity, and by the appearance of "polycystic" ovaries at the ultrasound.[1,2] Although the precise etiology still remains unknown, an intrinsic prooxidant state, resulting from an imbalance between the excessive development of oxidants in the presence of limited antioxidants defenses, was recently proposed to play an additional role in determining, maintaining, and/or worsening the reproductive and metabolic manifestations observed in women with PCOS.[3-6]

Melatonin is an old and ubiquitous molecule in nature, controlling a variety of important central and peripheral mechanisms related to circadian rhythms and reproduction.[7] In mammals, melatonin is mainly synthesized by the pineal gland and the retina,[8] but it is measurable also in human preovulatory follicular fluid where its concentration has been reported to be 3 times higher than in peripheral blood.[9-11] In addition, the 2 enzymes, arylalkylamine *N*-acetyl-transferase and hydroxyindole-O-methyltransferase, that play a key role in the synthesis of melatonin have been detected in ovarian tissue.[12] For these reasons, a local production of this hormone by the ovary has been taken into account by several authors, and it is

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conceivable that melatonin may directly affect ovarian function. In vitro models, indeed, show that melatonin regulates steroidogenesis, folliculogenesis, and oocyte maturation within the ovary.[13] Actually, melatonin seems to protect follicles against oxidative stress, thanks to its documented properties of antioxidant and free radical scavenger, and to rescue them from atresia, thus promoting the correct follicular maturation and, finally, ovulation.[14] Several studies in rats also demonstrated a role of melatonin in the regulation of gonadotropin release hormone (GnRH) secretion in the hypothalamus and gonadotropin release in the pituitary gland.[4] Interestingly, the reduction in melatonin levels due to pinealectomy seems to induce the development of some characteristics of PCOS in rats. Conversely, studies in rat models of the syndrome have shown increased progesterone (P) and androgen production in preantral follicles after incubation with melatonin for 12 days.[15] However, the role of melatonin in influencing the hypothalamic–pituitary–gonadal axis in humans is less clear.

Until now, only few data have been published about the role of melatonin in women with PCOS. Serum melatonin concentrations were found to be higher in PCOS in respect to healthy controls. Nonetheless, lower levels of this hormone have been detected in the ovarian follicles of women affected by the syndrome, and this finding has been related to the anovulation and the putative poor oocyte quality that characterize PCOS.[16-17]

Based on this rationale, the aim of the present study was to investigate the effects of 6 months of melatonin administration on clinical, endocrine, and metabolic features of women affected by PCOS. In particular, the primary end point of the study was to explore the possible role of melatonin treatment in restoring the menstrual cyclicity of normal-weight participants with PCOS.

Materials and Methods

This is a prospective study conducted from January to September 2016 at the Department of Obstetrics and Gynecology of the Università Cattolica Sacro Cuore, Rome.

A total of 40 normal-weight women with PCOS were enrolled and completed the study therapy without protocol violations. All participants were volunteers of fertile age (mean: 23.25 [4.07] years) and gave informed consent to participate in this study, which was approved by our local ethics committees (institutional review board of Department of Obstetrics and Gynaecology, Catholic University of Sacred Heart).

In accordance with the Rotterdam Consensus Conference (Rotterdam ESHRE/ASRM 2004), PCOS was diagnosed in the presence of at least 2 of the following criteria—irregular menstrual cycles (or amenorrhea), clinical and/or biochemical evidence of hyperandrogenism, and ultrasound assessment of polycystic ovaries (bilateral normal or enlarged ovaries with at least 10 micro cysts [2-8 mm diameter] from the inner margin to the outer margin in longitudinal cross section, associated with an augmented stromal area/total area ratio on ultrasonography).[18] We considered the following clinical findings as exclusion criteria: pregnancy, history of cardiovascular disease, diabetes mellitus (or impaired glucose tolerance as determined by a standard 75 g oral glucose tolerance test [OGTT]), hypertension, significant liver or renal impairment, other hormonal dysfunctions, neoplasms, unstable mental illness, shift-workers women, and obesity. We decided to exclude obese women in order to avoid possible confounding factors. All women were euthyroid, and none had taken medications known to affect plasma sex steroids for at least 3 months before entering the study. In all patients showing high levels of 17 hydroxyprogesterone (17-OHP), an adrenocorticotropic hormone test was performed (250 μg intravenous of Synacthen; Ciba-Geigy, Basel, Switzerland) to exclude the presence of late-onset adrenal enzyme defect, according to the criteria defined by New et al.[19]

Study Protocol

The clinical and laboratory workup was conducted during the early follicular phase (days 3-7) of the menstrual cycle. In patients with amenorrhea, menstrual bleeding was induced by medroxyprogesterone acetate administration (10 mg/d for 5 days). In the absence of menstrual bleeding, patients were studied at least 15 days after the last progestin administration. The frequency of menstrual bleedings in the previous 6 months was recorded.

The following anthropometric characteristics were measured—weight, height, and waist and hip circumference for the determination of waist-to-hip ratio (WHR). The body mass index (BMI) was calculated as the ratio of weight (kg) to height 2 (m^2). A BMI ranging from 19 to 25 kg/m^2 was considered suggestive of normal weight. Waist and hip circumference were measured as previously referred,[20] and cutoff point for high WHR was set at 0.80.[21] The grade of hirsutism was evaluated using the Ferriman-Gallwey (FG) score.[22] The evaluation of the FG score was performed at each visit by the same 2 components of our medical staff, and the mean between the 2 determinations was considered for the analysis of the data. Patients were asked not to epilate for at least 1 month before each visit. Women with an FG score >8 were considered hirsute. After fasting overnight for 10 to 12 hours, blood samples were collected for the following hormonal assays—anti-Mullerian hormone (AMH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), P, prolactin (PRL), testosterone (T), androstenedione (A), 17-OHP, sex hormone–binding globulin (SHBG), and dehydroepiandrosterone sulfate (DHEAS). Lipid assay was performed to measure total cholesterol levels, high-density lipoprotein (HDL) cholesterol levels, low-density lipoprotein (LDL) cholesterol levels, and triglyceride levels. The ratio of T × 100/SHBG was used to calculate the free androgen index (FAI).

On the same day, patients underwent a 75-g OGTT. The OGTT was performed as follows—at 9 AM after overnight fasting, an indwelling catheter was inserted into the antecubital vein of 1 arm. Blood samples were collected basally and 30,

60, 90, and 120 minutes after ingestion of 75 g glucose in 150 mL water. Insulin and glucose were assayed in all samples. Insulin and glucose responses to the stimuli are expressed as the area under the curve (AUC). The AUC was calculated by the trapezoidal rule method and reported as μIU/mL times 120 minutes for insulin and as mg/dL times 120 minutes for glucose. A normal glycemic response to OGTT was defined according to the criteria of the National Diabetes Data Group.[23] A normal insulinemic response to OGTT was defined by a threshold AUC value of 7000 μIU/mL/120 min, as previously described.[24,25]

Evaluation of insulin sensitivity was made by the homeostatic index (HOMA), which was calculated as follows—HOMA = (fasting insulin [μU/mL] × fasting glucose [mmol/L]/22.5).[24] Insulin resistance was defined as an HOMA-IR value >3.8 based on a cohort of white women with PCOS.[26]

A transvaginal pelvic ultrasound was performed on each patient using a 6.5 MHz endovaginal probe (Esaote, AUC5). Ovarian volume was calculated for each ovary using the formula for a prolate ellipsoid: $\pi/6$ (D1 × D2 × D3), where D represents the maximum diameter in transverse, anteroposterior, and longitudinal axes.[27]

After the basal evaluation, patients with PCOS were prescribed treatment with melatonin (Armonia Fast 1 mg; Nathura, Montecchio [TR], Italy) 2 tablets a day in the evening before going to bed for 6 months. Patients were asked not to change their diet and physical exercise habits. After a 6-month treatment, on the third day of a spontaneous or progestin-induced menstrual bleeding, patients returned to hospital and repeated the basal clinical and laboratory workup. During the second hospitalization, patients' compliance to treatment was checked with a questionnaire about side effects and a subjective evaluation about tolerability of the administered drug; we asked patients whether they had correctly followed the scheduled treatment and to report any missed administration.

Measurements

Plasma samples for hormone determination were maintained at −20°C until assayed. The serum AMH concentration was assessed using a second generation ultrasensitive immunoenzymometric assay (Beckman-Coulter, Marseilles, France). The limit of detection (defined as the lowest detectable amount of AMH) was calculated at 0.08 ng/mL. All remnant hormonal assays were performed with electrochemiluminescence immunoassay kits (Roche Diagnostics, Mannheim, Germany). Glucose was measured within 24 hours of blood collection using a glucose oxidase method (Beckman Glucose Analyzer; Beckman Instruments, Fullerton, California). Samples contained in test tubes lacking heparin were immediately centrifuged in a refrigerated centrifuge, and the sera obtained were stored at –20°C until assayed. Insulin assay was performed by RIA. The intra-assay and interassay coefficients of variation were <6% for all hormones. The intra-assay and interassay coefficients of variation were <3% for TSH.

Total cholesterol and triglyceride concentrations were determined by an enzymatic assay (Bristol, Paris, France). High-density lipoprotein concentrations were determined after precipitation of chylomicrons, VLDL, and LDL (Roche, Mannheim, Germany), and VLDL was separated (as the supernatant) from LDL and HDL by lipoprotein ultracentrifugations. A magnesium chloride/phosphotungstic acid technique was used to precipitate LDL from the bottom fraction after ultracentrifugation. All lipid assays were performed according to standardized laboratory procedures, as previously reported.[28]

Statistical Methods

All results are presented as mean (standard deviation). Distribution of the data was tested by the Kolmorov-Smirnov test to verify whether the samples follow a specified distribution. We found that the variables were not normally distributed. The significance of differences between the same tests performed before and after treatment was assessed using the nonparametric Wilcoxon rank sum test. $P < .05$ was considered statistically significant.

Results

Forty women were enrolled in this study, and all completed the study therapy without protocol violations. The treatment was generally well tolerated, with no relevant side effects reported by the patients; in particular, none of the patients experienced any sleep disturbances.

Table 1 shows the clinical, anthropometrical, and hormonal features of the study group at baseline and after 6 months of therapy. All patients were of normal weight, according with inclusion criteria, and with a normal body fat distribution. All participants were oligo-amenorrhoeic and showed a condition of mild hirsutism. After 6 months of treatment, a slight, yet significant, decrease in body weight ($P < .01$) and, consequently, in BMI ($P < .01$) was observed. Waist-to-hip ratio values did not significantly change. Patients reported an improvement in menstrual cycle frequency with a significant increase in the number of cycles/6 months ($P < .01$). Melatonin treatment significantly decreased androgens levels (FAI: $P < .05$; T: $P < .01$; 17-OHP: $P < .01$). We observed an increase in SHBG levels, though these data did not reach a statistical significance. Follicle-stimulating hormone levels significantly raised ($P < .01$). By contrast, no significant changes occurred in LH, E2, PRL, thyroid hormones, DHEAS, and A.

Anti-Mullerian hormone serum levels, which were above the normal range at baseline, significantly decreased after 6 months of melatonin treatment ($P < .01$). The ovarian volume, as estimated at the ultrasound scan, was not affected by the treatment.

Table 2 shows the metabolic features of participants before and after 6 months of therapy. None of the studied participants exhibited impaired glucose tolerance at baseline, and mean insulinemic response to the OGTT resulted in the normal range.

Table 1. Clinical, Anthropometrical, Hormonal, and Ovarian Features of the Studied Patients at Baseline and After 6 Months of Treatment.[a,b]

Parameters	Baseline	After 6 months
Age, years	23.25 (4.07)	
BMI, kg/m^2	21.74 (2.09)	21.05 (1.79)[c]
WHR	0.74 (0.04)	0.74 (0.04)
FG score	9.74 (3.79)	8.79 (3.93)
Cycles in 6 months	2.56 (1.84)	4.04 (1.68)[d]
FSH, mUI/mL	5.17 (1.27)	6.10 (1.59)[d]
LH, mUI/mL	6.30 (3.74)	6.83 (6.50)
E2, pg/mL	40.42 (16.06)	43.71 (18.76)
PRL, ng/mL	11.23 (6.45)	9.60 (4.42)
SHBG, nmol/L	55.02 (18.21)	61.38 (24.73)
A, ng/mL	2.17 (1.15)	2.04 (1.07)
T, ng/mL	1.79 (0.88)	1.33 (0.54)[d]
FAI	4.01 (3.89)	2.67 (1.75)[c]
17-OHP, ng/mL	0.94 (0.42)	0.75 (0.37)[d]
DHEAS, ng/mL	2957.46 (1015.35)	2837.54 (1581.79)
AMH, ng/mL	11.68 (5.40)	8.26 (5.10)[d]
TSH, mUI/mL	1.43 (0.67)	1.34 (0.66)
fT4	10.49 (0.8)	10.75 (1.27)
Ovarian volume, cm^3	7.69 (5.06)	7.40 (3.09)

Abbreviations: A, androstenedione; AMH, anti-Mullerian hormone; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E2, estradiol; FAI, free androgen index; FG, Ferriman-Gallwey; FSH, follicle-stimulating hormone; fT4, free tiroxine 4; LH, luteinizing hormone; 17-OHP, 17 hydroxyprogesterone; PRL, prolactin; SHBG, sex hormone–binding globulin; T, testosterone; TSH, thyroid-stimulating hormone; WHR, waist-to-hip ratio.
[a]N = 40.
[b]Data are presented as mean (SD).
[c]$P < .05$.
[d]$P < .01$.

Table 2. Metabolic Features of the Studied Patients at Baseline and After 6 Months of Treatment.[a,b]

	Baseline	After 6 months
AUC glucose, mg/dL/120 min	13 376.88 (2570)	12 545.88 (2358.04)
AUC insulin, μUI/mL/120 min	5531.31 (2812.51)	5394.44 (2303.75)
HOMA-IR	1.12 (0.45)	1.25 (0.59)
Total cholesterol, mg/dL	168.0 (35.24)	158.92 (25.33)
HDL cholesterol, mg/dL	64.83 (16.39)	61.71 (11.51)
LDL cholesterol, mg/dL	97.92 (30.55)	87.96 (24.30)[c]
Triglycerides, mg/dL	51.08 (20.04)	44.13 (15.34)

Abbreviations: AUC, area under the curve; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.
[a]N = 40.
[b]Data are presented as mean (SD).
[c]$P < 0.05$.

The evaluation of HOMA-IR documented a normal peripheral insulin sensitivity. After therapy, we did not observe a significant modification in the insulin secretion during the OGTT, and the treatment did not affect peripheral insulin sensitivity in our group of patients. All patients had a normal lipid profile at baseline; total cholesterol, HDL, and triglycerides plasma levels remained unvaried after 6 months of treatment, whereas LDL-cholesterol plasma concentrations showed a significant reduction.

Discussion

The first report on the possible role of melatonin in PCOS dates back to 2001: Luboshitzky and colleagues[29] observed a significantly higher excretion of 6-sulfatoxymelatonin, the major enzymatic metabolite of melatonin, in women affected by the syndrome compared with controls. Further studies reported higher serum melatonin concentrations associated with ovarian intrafollicular deficiency in patients with PCOS.[16] Therefore, PCOS disclose high blood levels but also high urinary excretion of melatonin. This imbalance could explain the smaller mean night–day difference in the concentrations of this hormone compared to that of healthy women, thus suggesting an altered secretory circadian rhythm.[30] This was the reason for which we decided, in an ostensible paradox, to use melatonin in women with already apparent high blood levels of this hormone.

Indeed, it could be borne in mind that these serum melatonin fluctuations in blood may play a role in the ovulatory dysfunction and altered steroidogenesis of PCOS.

Our study is the first evaluating the effects of the treatment with exogenous melatonin on the clinical, endocrine, and metabolic characteristics of patients with PCOS (English-based search from PubMed through October 2016. The search terms "melatonin," "polycystic ovary syndrome," "hirsutism," and "insulin" were used).

The main finding of our study was the significant amelioration of the hormonal picture and menstrual pattern observed in our treated women. The reduction in FAI, T, and 17-OHP levels may be due to the effect of melatonin on steroidogenesis,[14] which is believed to be exerted through 2 different, though synergistic, mechanisms at the intraovarian level. First, a direct modulation of the steroid biosynthesic pathway by melatonin is supported by previous evidence of its inhibitory effect on the expression of steroidogenic acute regulatory protein and of the second messenger cAMP in granulosa cells (GCs).[31,32] Second, melatonin may act as a power-free radical scavenger with a broad antioxidant spectrum of action.[33,34] In PCOS, ROS generation from mononuclear cells and serum lipid peroxidation products are significantly elevated[5,6]; oxidative stress may cause GCs and oocyte damage and may have a role in influencing steroidogenesis.[35] Interestingly, melatonin receptors have been found on GCs, indicating that this may be an additional site of melatonin activity.[31]

It is well known that both the intraovarian hyperandrogenism and the oxidative stress are considered possible determinants of the disturbances of follicular development and ovulation which are typical of PCOS. The consequent chronic anovulation is the physiopathologic background of the menstrual irregularities, which represent one of the major complaints of affected women. The premature arrest of folliculogenesis leads to the accumulation of small antral follicles,

which are responsible for a hyperproduction of AMH.[36-38] Accordingly, women with PCOS enrolled in our study showed, at baseline, menstrual cycle disturbances and high AMH levels. After 6 months of melatonin treatment, all participants experienced an improvement in menstrual cyclicity, which was concomitant with the reduction in androgens and AMH circulating levels.

Although preliminary, our in vivo data confirm the previous in vitro observations of a direct effect of the pineal hormone on the ovary: melatonin seems able to mitigate the vicious cycle of hyperandrogenism–follicular atresia–hyperAMH–anovulation that is a hallmark of PCOS.

As is common knowledge, follicular development is a complex process involving not only paracrine and autocrine factors but also endocrine mechanisms. The growth beyond the late preantral/early antral stage, which is impaired in patients with PCOS, is critically dependent on circulating FSH levels,[39] the role of which is one of the most debated aspects of PCOS physiopathology. Even if FSH response to GnRH stimulus as well as FSH pulsatility is comparable to that of normoovulatory controls,[40] FSH levels were reported to be slightly decreased in women with PCOS[41] and are estimated to be below the threshold level required during the early follicular phase to stimulate normal follicle maturation.[42] In our study, we observed a statistically significant increase of FSH and a mild reduction in LH levels. These findings are in line with possible central effects of melatonin on the hypothalamic pituitary axis, being capable of modifying the release of gonadotropins and GnRH.[43,44]

For what concerns metabolic parameters, melatonin treatment induced with a slight but statistically significant decrease in mean BMI value. This result was obtained in the absence of significant lifestyle modifications as reported by the participants. Previous experimental studies performed in young animals documented that melatonin supplementation is able to reduce both long-term body weight gain and the size of visceral fat deposits independently of food intake.[45] Furthermore, unpublished data by Castro et al showed that pinealectomy leads to overweight in rats, whereas the daily rhythmic melatonin replacement is able to completely reverse this effect. In our pilot study, we decided to recruit only normal-weight patients in order to avoid interference by metabolic factors; nevertheless, the observation of such modification could be of potential interest in the future for overweight/obese women with PCOS.

Beyond the presumed direct effect of melatonin on the reproductive axis, it could not be ignored that the reduction in adiposity may have played a marginal role in the improvement of menstrual irregularities and biochemical hyperandrogenism in our patients with PCOS. However, such effect in PCOS is generally mediated by an improvement in peripheral insulin sensitivity and/or insulin secretion, which did not occur in our participants after melatonin treatment. Previous reports on the influence of melatonin on glucose and insulin metabolism are extremely controversial. Some authors reported that melatonin may potentiate central and peripheral insulin action either due to regulation of GLUT-4 expression or triggering the insulin-signaling pathway.[46] It was also documented that pinealectomy of rodents causes hyperinsulinemia[47] and that the condition of diabetes is associated with lower melatonin levels.[48] On the other hand, Cagnacci and colleagues in 2001concluded that in postmenopausal women, melatonin may reduce peripheral tissues sensitivity to insulin.[49] Our study is the first evaluating the possible effect of melatonin on the metabolic characteristics of PCOS in human and the lack of a beneficial effect of melatonin on the insulin response to glucose load and insulin sensitivity observed in our population could be partially attributed to the high percentage of normoinsulinemic women in the studied group.

In conclusion, our study has analyzed for the first time the effect of a 6-month supplementation with melatonin on the clinical, hormonal, and metabolic features of women affected by PCOS. Our results suggest that long-term treatment with melatonin may improve menstrual irregularities and biochemical hyperandrogenism in these women. The absence of changes in insulin secretion and insulin sensitivity suggests that melatonin may directly interfere with follicular development and steroidogenic activity, with a mechanism that could be insulin independent. In our study, patients with PCOS are considered their own controls ("before" and "after" comparison); in the absence of an adequate control or placebo group, the beneficial effects observed may be explained by the phenomenon of regression toward the mean. Nonetheless, the current study has suggested a potential role of melatonin as a new therapeutic agent for women affected by PCOS, though further double-blind randomized placebo-controlled trials on a larger sample size with different metabolic features are needed to confirm our results.

Authors' Note

Valeria Tagliaferri and Daniela Romualdi substantially contributed to the design of the study and the draft of the article. Elisa Scarinci, Simona De Cicco, Christian Di Florio, Valentina Immediata, Anna Tropea, and Carla Mariaflavia Santarsiero substantially contributed to acquisition of data and analysis of data. Antonio Lanzone and Rosanna Apa revised the article for important intellectual content and gave final approval of the version to be published. Rosanna Apa and Antonio Lanzone contributed equally to this work.

Declaration of Conflicting Interests

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