Herbal Medicine Applications for Polycystic Ovarian Syndrome

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CRC Press
Taylor & Francis Group

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First edition published 2024
by CRC Press
2385 NW Executive Center Drive, Suite 320, Boca Raton FL 33431

and by CRC Press
4 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN

CRC Press is an imprint of Taylor & Francis Group, LLC

ISBN: 9781032383767 (hbk)
ISBN: 9781032383712 (pbk)
ISBN: 9781003344728 (ebk)

DOI: 10.1201/9781003344728

Typeset in Times
by codeMantra

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13.1 INTRODUCTION

Melatonin (*N-acetyl-5-methoxytryptamine*) is a methoxyindole that is produced endogenously in almost every cell containing mitochondria (Xie et al. 2022). Regardless that the metabolic pathway for synthesizing melatonin is found in most tissues of the body, circulating melatonin originates essentially from the pineal gland. Pineal melatonin synthesis is under the control of the central circadian pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN). After entering the bloodstream, albumin binds about 70% of circulating melatonin (Cardinali et al. 1972). Pharmacodynamically, melatonin has a biexponential half-life in circulation (first distribution half-life: 2 minutes; second distribution half-life: 20 minutes) (Claustrat and Leston 2015)

DOI: 10.1201/9781003344728-13

A high amount of melatonin (92%–97%) is removed from circulation by the liver in a single pass (Moroni et al. 2021). Hepatic cytochrome P450 monooxygenases (isoenzymes CYP1A1, CYP1A2, and to a lesser degree CYP1B1) are responsible for metabolism, completed subsequently to attain the excretory product 6-sulphatoxymelatonin. Liver CYP1A2 and, to a lesser extent, CYP2C19, demethylate melatonin to *N*-acetylserotonin while less specific aryl acylamidases are found in the brain (Moroni et al. 2021). The oxidative pyrrole-ring breakage of melatonin to N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) accounts for one-third of brain's total melatonin metabolism. Arylamine formamidase or heme peroxidase deformylate AFMK to N^1-acetyl-5-methoxykynuramine (AMK) (Hardeland 2010). 2-Hydroxymelatonin and cyclic 3-hydroxymelatonin are other oxidative products. AFMK and AMK are powerful antioxidants scavenging reactive oxygen and nitrogen. Melatonin synthesis in mitochondria occurs at high concentration in the immediate proximity to those reactive oxygen and nitrogen species. Thus mitochondrial electron flow is protected by melatonin and its endogenous metabolites (Tan et al. 2007).

Cloning of a high-affinity membrane melatonin receptor (MT1) was first achieved in a cDNA library from frog melanophores (Reppert 1997). Currently, two Gi-protein-coupled membrane melatonin receptors have been identified in humans (Dubocovich et al. 2010), the amino acid sequence of MT2 receptor being 60% similar to that of the MT1 receptor. GPR50 is a third binding protein with a 45% of the amino acid sequence of MT1 and MT2 but very poor melatonin-binding properties. The ability of GPR50 receptors to form homo- and heteromers with each other and with other GPCRs (Cecon et al. 2018), including the serotonin 5-HT_{2C}, is an intriguing property as functionality may be modified. For instance, the functional features of the heteromers differ from those of the homomers. As one example, melatonin activates Gq signaling by acting on the MT2/5-HT_{2C} heteromer.

Both the phase of the body clock (i.e., internal clock time relative to external clock time) and its amplitude are coded by the melatonin cycle. A remarkable effect of melatonin is to blunt promotion of wakefulness by SCN in the late afternoon, thus "opening the sleep doors" (Lavie 1997). Melatonin also signals the length of the night, i.e. the longer the night, the longer the length of melatonin release. Thus melatonin secretion is the temporal key for seasonal rhythms in most species (Clarke and Caraty 2013)

Nerve terminals from the superior cervical ganglia release norepinephrine into the pineal gland, where it interacts with β (mostly) and α adrenoceptors on the pineal cell membrane, triggering melatonin production. Melatonin is not kept inside the pineal because of its great diffusibility and moves out as soon as it is synthesized. An SCN–melatonin loop referring to the structures that control circadian rhythms has been proposed (Tan et al. 2018). This loop comprises melanopsin-containing retinal ganglion cells, the retino-hypothalamic tract (RHT), the SCN, the paraventricular nucleus, the intermediolateral cell column, the sympathetic cervical ganglia, the pineal gland, and the melatonin rhythm, which feedback to inhibit the SCN. Melatonin is always generated at night, regardless of the nocturnal or diurnal nature of the species studied, a fact that underlies its strong link with the external photoperiod. Light during the night, particularly in the blue range, activates retinal

photoreceptor ganglion cells containing melanopsin as a photopigment and projecting via the RHT. The final event is the inhibition of pineal sympathetic norepinephrine release and, as a result, the decrease or abolition of pineal melatonin production (Lax et al. 2019).

Although as stated above, circulating melatonin in animals and humans is virtually all derived from the pineal, melatonin is generated locally in most cells, tissues, and organs where it may have an autocrine or paracrine role (Acuña-Castroviejo et al. 2014). Indeed, melatonin is synthesized in every animal cell containing mitochondria (Tan and Reiter 2019). Presumably, this is the basis for the anti-inflammatory and cytoprotective actions of melatonin (Hardeland 2018).

Melatonin is an amphiphilic molecule that crosses cell membranes readily. Once in the cytoplasm it interacts with proteins such as tubulin and calmodulin (Jiménez-Rubio et al. 2012). Moreover melatonin can also penetrate to the cell nucleus, where its products can interact with orphan RZR/ROR superfamily receptors. Although RZR/ROR proteins do not bind melatonin directly, the methoxyindole appears to function indirectly through these transcription factors via sirtuin-1 (SRT-1) activation (Hardeland 2019).

Melatonin's cytoprotective efficacy outperforms that mediated by the MT1 and MT2 receptors. Indeed, melatonin levels in virtually all cells are substantially greater than those observed in the blood (Acuña-Castroviejo et al. 2014). Mitochondrial melatonin synthesis has been established, and intramitochondrial melatonin is preserved within that organelle. In mitochondria, the locally produced melatonin can act in part via MT1 receptors to inhibit stress-mediated cytochrome C release. Melatonin dosages required to alter intracellular melatonin concentrations are substantially larger than those of hormonal levels or those used as a chronobiotic (Venegas et al. 2013). Several physiologically relevant melatonin effects in cell cultures are shown at dosages in the 10^{8}–10^{9} M range, which is adequate for virtually entire receptor saturation. However, most animal research on neuroprotective and anti-inflammatory benefits use pharmacological levels of melatonin (Cardinali 2019a). Pro- and anti-inflammatory actions are shown for melatonin (Hajam et al. 2022; Carrillo-Vico et al. 2005; Hardeland 2019) The major medical interest is in anti-inflammatory activities because both high-grade and low-grade inflammation are present in brain damage, ischemia/reperfusion, sepsis, and most chronic disorders.

Anti-inflammatory actions of melatonin reside in the reduction of nuclear factor κB (NF κB) binding to DNA, cyclooxygenase (Cox) (Cardinali et al. 1980) [particularly, Cox 2 (Deng et al. 2006)] and downregulation of the production of inducible nitric oxide synthase receptors (Hardeland et al. 2011) There is also inhibition of NLRP3 inflammasome activation, activation of toll-like receptor-4 and high-mobility group box-1 signaling receptors, and positive regulation of nuclear factor erythroid 2-related factor 2 (Hardeland et al. 2011). The ability to positively regulate SIRT-1 is critical. Melatonin actions include a decrease in proinflammatory cytokines coupled with a greater synthesis of anti-inflammatory cytokines (Carrillo-Vico et al. 2005; Hardeland 2019)

Furthermore, melatonin's known ability to reverse the consequences of increasing insulin resistance (Amaral et al. 2019) is critical for cytoprotection in PCOS.

13.2 MELATONIN IN PCOS

As shown in Table 13.1, a number of studies support the beneficial effect of melatonin in animal models of PCOS. Pinealectomy of rats induces PCOS development, melatonin treatment being effective to counteract it (Prata Lima et al. 2004). Melatonin administration prevented the permanent estrous state found in the PCOS rat (Lombardi et al. 2019).

The metabolic consequences of PCOS were also affected by melatonin. For example, the administrations of melatonin together with metformin were very effective to curtail oxidative stress in livers of PCOS rats (Lemos et al. 2014). Indeed, melatonin exhibits protective effects against most metabolic and reproductive disturbances seen in PCOS (Pai and Majumdar 2014). As seen in PCOS rats, the high circulatory levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α and the downregulation of expression of melatonin receptors (MT1 and MT2), estrogen (ER-α) receptor, and cytokine (IL-2R and IL-6R) receptors in the ovary were counteracted by melatonin treatment (Basheer et al. 2018). Melatonin decreased circulating levels of T3 and T4 and normalized ovarian and thyroid MT1 and ERα receptors in a letrozole-induced PCOS model in rats (Ghosh et al. 2022).

Melatonin administration counteracted the rat endometrial epithelium changes and the increased expression of IGF-1R/IGF-1 and Bcl-2 (Seymen et al. 2021). Likewise, melatonin was effective to inhibit autophagy in the ovarian tissue and to counteract the augmented levels of serum testosterone and inflammatory and apoptosis indexes seen in PCOS rats (Xie et al. 2021)

Supporting its chronobiotic nature, melatonin relieved the hyperinsulinemia and hyperandrogenism of darkness-treated PCOS rats via BMAL1, PER1, and PER2 (Li et al. 2020). In murine models of PCOS, melatonin treatment significantly increased SIRT-1 and decreased the levels of PINK1/Parkin, thus ameliorating mitochondrial dysfunction and PCOS phenotype in granulosa cells (Yi et al. 2020b). In PCOS mice fed a high-fat diet, melatonin administration reduces free radical generation and prevents spindle/chromosome anomalies in oocytes, through aSRT-3-dependent mechanism (Han et al. 2017).

At supraphysiological concentrations (1–10 μM) melatonin improved the quality of murine PCOS oocytes during in vitro maturation by increasing Bcl2 and decreasing Bax gene expression (Nikmard et al. 2017, 2022). Melatonin treatment of PCOS mice decreased mitochondrial permeability transition pore opening, decreased the levels of cytochrome C and Bax and, increased p-PDK 1 and p-Akt. Knocking down SIRT-1 mRNA inhibited the protective effect of melatonin (Zheng et al. 2021).

In a hamster letrozol-induced, PCOS model (Hansda and Haldar 2021), melatonin treatment counteracted endometrial hyperplasia, the increased serum testosterone, leptin and insulin levels, and the decreased uterine insulin receptor/GLUT-4 expression. Increased uterine oxidative load and inflammatory marker expression were also normalized by melatonin PCOS hamsters (Hansda and Haldar 2021)

By applying allometry and normalization of body surface area, the doses of melatonin listed in Table 13.1 can be recalculated to obtain the human equivalent dose (HED) of melatonin for a 75 kg adult (Reagan-Shaw et al. 2008). Noteworthy, theoretical HEDs calculated from most studies in Table 13.1 are significantly greater than those usually employed in humans (Table 13.2). Similar observations have

TABLE 13.1
Melatonin Activity in Animal Models of PCOS

Findings	Melatonin Dose	Daily HED for a 75 kg Adult	Ref.
Rats			
In PCOS rats the combination of metformin hydrochloride plus melatonin is very effective to decrease hepatic oxidative stress and the inflammatory response.	2 mg/kg s.c.	24 mg	Lemos et al. (2014)
In PCOS rats melatonin administration is effective to curtail metabolic and reproductive disturbances.	1–2 mg/kg i.p.	12–24 mg	Pai and Majumdar (2014)
Melatonin was effective to counteract chronic anovulation induced by pinealectomy or continuous light.	2 mg/kg i.m.	24 mg	Prata Lima et al. (2004)
Melatonin treatment was effective in PCOS rats to restore downregulation of melatonin, estrogen, and cytokine receptor expression and to decrease high circulatory level of IL-6 and TNF-α.	2 mg/kg i.p.	24 mg	Basheer et al. (2018)
In PCOS rats melatonin treatment prevents a continuous estrus state.	0.4 mg/kg p.o.	5 mg	Lombardi et al. (2019)
In PCOS rats the administration of melatonin relieves hyperinsulinemia and hyperandrogenism via BMAL1, PER1, and PER2.	10 mg/kg i.p.	120 mg	Li et al. (2020)
In PCOS rats melatonin treatment counteracts the endometrial epithelium alterations and the increased expression of IGF-1R/IGF-1 and Bcl-2.	2 mg/kg s.c.	24 mg	Seymen et al. (2021)
In the ovary of PCOS rats, melatonin treatment inhibits autophagy and inflammatory and apoptosis indexes by increasing PI3K-Akt pathway expression. In addition, serum-free testosterone was decreased.	10 mg/kg p.o.	120 mg	Xie et al. (2021)
In a letrozole-induced PCOS model in rats melatonin decreased circulating level of T3 and T4, normalized ovarian and thyroid MT1 and ERα receptors, without any significant change in the MT2 receptor.	2 mg/kg i.p.	24 mg	Ghosh et al. (2022)
Mice			
In mice fed a high-fat diet melatonin reduces ROS generation and prevents spindle/chromosome anomalies in oocytes, through a SIRT3-SOD_2-dependent mechanism.	30 mg/kg p.o.	180 mg	Han et al., (2017)
Melatonin effect on quality of murine PCOS oocytes when it has been added during in vitro maturation was assessed. Nuclear maturation and cleavage rate of PCOS oocytes were significantly higher at 10^{-5} to 10^{-6} mol/L concentration as compared to untreated oocytes.			Nikmard et al. (2017)

(Continued)

TABLE 13.1 (*Continued*)
Melatonin Activity in Animal Models of PCOS

Findings	Melatonin Dose	Daily HED for a 75 kg Adult	Ref.
In a murine model of PCOS, melatonin treatment increased the protein level of SIRT1 and decreased the levels of PINK1/Parkin, thus ameliorating mitochondrial dysfunction and PCOS phenotype.	10 mg/kg p.o.	60 mg	Yi et al. (2020b)
In a murine model of PCOS, melatonin treatment decreased mitochondrial permeability transition pore opening, decreased the levels of cytochrome C and BAX, and increased p-PDK 1, p-Akt, and SIRT1. Knocking down SIRT1 mRNA inhibited the protective effect of melatonin.	10 mg/kg p.o.	60 mg	Zheng et al. (2021)
In vitro melatonin concentrations (10^{-5} to 10^{-7} M) increased Bcl2 and decreased Bax gene expression in PCOS and control murine oocytes compared to non-treated oocytes.	In vitro.		Nikmard et al. (2022)
Hamster			
In a letrozole-induced PCOS model in hamsters, serum testosterone, leptin, and insulin increased and uterine insulin receptor/GLUT-4 expression decreased, together with endometrial hyperplasia. Augmented uterine oxidative indexes (SOD/catalase/LPO) and inflammatory markers (NFκB/COX-2 expression) were also detected in PCOS animals. Melatonin administration normalized all these parameters.	1 mg/kg p.o.	10 mg	Hansda and Haldar (2021)

Note: The human equivalent dose (HED) of melatonin for a 75 kg adult is calculated by normalization of body surface area (Reagan-Shaw et al. 2008).

been made in animal models of hyperadiposity in which the injection of supraphysiological doses of melatonin normalized most observed alterations and corrected the altered biochemical proinflammatory profile (Cardinali 2019a).

Table 13.2 summarizes the studies that support the relevance of melatonin in human PCOS. A disrupted circadian melatonin rhythm was reported in four consecutive PCOS patients as compared to controls (Tarquini et al. 1996). Urinary excretion of 6-sulfatoxy-melatonin increased (Shreeve et al. 2013), and serum testosterone inversely correlated with this increase in PCOS patients (Luboshitzky et al. 2001, 2004) while the nocturnal plasma levels of melatonin positively correlated with increased circulating testosterone (Jain et al. 2013). Women with PCOS were found to have a significantly smaller mean night–day difference in the concentrations of plasma melatonin and higher melatonin level at 08:00 a.m. in comparison with healthy women (Terzieva et al. 2013). As compared to age-marched controls, patients with PCOS exhibited a longer duration of melatonin secretion, later melatonin offset

relative to sleep timing, and a later clock hour of melatonin offset (Simon et al. 2019). A later melatonin offset after wake time was associated with higher serum-free testosterone levels regardless of group in this study.

PCOS patients can be classified as having hyperandrogenism, amenorrhea, or polycystic ovarian morphology (the phenotypic features of the disease). The number of phenotypic features present showed a significant linear trend with serum melatonin and cortisol as biomarkers of the circadian system (Lim et al. 2019) indicating the occurrence of a significant circadian disruption in PCOS.

Polymorphisms in the genes encoding human melatonin receptors (MTNR1A and MTNR1B) correlated with PCOS (Table 13.2). Impairments in insulin secretion and increased fasting glucose levels have been linked to variants in MTNR1B (MT2) (Li et al. 2011a), while increased risk of developing PCOS is linked to variants in MTNR1A (MT1) (Li et al. 2011b).

In the case of MTNR1B gene, single-nucleotide polymorphisms (SNPs) rs4753426, rs10830963, rs1562444, and rs1279265 were identified (Wang et al. 2010). The frequencies of three genotypes and two allelotypes of the SNP rs10830963 differed between PCOS women and healthy controls (Özcengiz et al. 2011). CC genotype carriers had higher levels of clinical and metabolic features than the TC and TT genotypes. Obese and nonobese women with PCOS showed a significant difference in transmission of allele C of rs2119882 (Song et al. 2015; Yi et al. 2020a). In the case of rs2119882, an increased risk of developing PCOS was found for C allele carriers who were not diagnosed with PCOS while C allele carriers with PCOS had an increased risk of developing obesity (Xu et al. 2019). Collectively, genetic data provide a basis for further studies of melatonin receptor genes in the etiology and diagnosis of PCOS (Yi et al. 2020a).

Melatonin may directly affect human ovarian function: it is synthesized by ovarian cells and is concentrated in human ovarian follicles and it alters granulosa cell steroidogenesis and follicular function in humans (see Olcese 2020; Brzezinski et al. 2021). An insulin resistance cell model was established by treating human ovarian granulosa cell line cells (SVOG) cells with palmitic acid (Guo et al. 2022). Decreased cell viability, promoted apoptosis, and reduced glucose uptake were seen in in SVOG cells exposed to palmitic acid. Downregulation of IRS-1 and GLUT4 mRNA and protein expression and of glucose uptake capacity were seen in PCOS granulosa cells and SVOG cells. Upregulation of IRS-1 and GLUT4 expression, downregulation of p-IRS-1, and improvement in glucose uptake were found in PCOS ovarian cells (Guo et al. 2022).

In 35 women with PCOS undergoing in vitro fertilization (IVF) treatment, the follicular fluid melatonin concentration was lower than in 36 healthy women and was found to be positively correlated with serum basal FSH level (Li et al. 2022). There was no significant difference in the fertilization rate of oocytes between the two groups, but the high-quality embryogenesis rate on the third day of the PCOS group was lower than that of the control group, which showed a positive correlation with the melatonin concentration. Subjectively measured sleep quality indicated that sleep disorders were more likely to exist in the PCOS group (Li et al. 2022).

The supplementation of in vitro culture medium with 10^{-7M} melatonin improved fertilization outcome in PCOS (Kim et al. 2013), while the administration of melatonin (3 mg daily) and myo-inositol augmented oocyte and embryo quality and improved IVF in PCOS patients (Pacchiarotti et al. 2016).

Melatonin treatment (3 mg daily) had a favorable effect on mature follicles, endometrial thickness, as well as chemical and clinical pregnancies in infertile PCOS women undergoing intrauterine insemination (Mokhtari et al. 2019). In women with PCOS undergoing ovulation whose luteinized granulosa cells were treated with melatonin (10^{-7} M), increased conversion of androgen to 17β-estradiol and reduced levels of nitric oxide were found together with improvement of oocyte development potential (Yu et al. 2019). The findings suggest that the addition of melatonin to in vitro fertilization media is an effective way to improve the cytoplasmic maturation of immature oocytes.

Melatonin treatment (2 mg/day for 6 month) of 40 normal-weight women with PCOS resulted in augmented serum FSH and anti-Mullerian hormone levels and decreased serum androgen and 17α-hydroxyprogesterone levels. A significant decrease in low-density lipoprotein (LDL) cholesterol serum levels was also observed (Tagliaferri et al. 2018).The administration of 10 mg melatonin daily to 58 PCOS women in a randomized double-blind, placebo-controlled trial significantly improved insulin levels, quantitative insulin sensitivity check index, HOMA-IR, total- and LDL-cholesterol levels and gene expression of LDL and PPAR-γ receptor (Shabani et al. 2019). A significant reduction in hirsutism, total testosterone, CRP, MDA, and gene expression of IL-1 and TNF-α, and augmentation of TAC and GSH levels, were found in another series of PCOS patients receiving 10 mg of melatonin daily (Jamilian et al. 2019).

In a randomized, double-blind, placebo-controlled trial including 84 women with PCOS aged 18–40 years old, magnesium (250 mg), melatonin (6 mg), and magnesium plus melatonin were administered for 8 weeks. Co-supplementation of magnesium–melatonin decreased serum levels of testosterone, insulin, HOMA-IR, TNF-α, cholesterol, and LDL-cholesterol, and increased HDL-cholesterol levels (Alizadeh et al. 2021). Patients receiving magnesium–melatonin co-supplementation exhibited less hirsutism than the other groups. Magnesium plus melatonin co-administration was associated with a more intense increase in total antioxidant capacity levels (Mousavi et al. 2022).

The administration of melatonin (5 mg/day) to women with PCOS having preinvasive endometrial cancer and receiving a combined hormonal, anti-diabetic, antidopaminergic, and anti-serotoninergic therapy favorably influenced female sexual hormone profile and lipid metabolism and helped to restore normal endometrium (Stanosz et al. 2014).

The data summarized in Table 13.2 agree with many studies supporting the beneficial role of melatonin in patients with metabolic syndrome (Cardinali 2019a). Melatonin treatment ameliorated oxidative stress and inflammatory parameters of obese women (MesriAlamdari et al. 2015) and reduced fat mass and increased lean mass in postmenopausal women (Amstrup et al. 2016). Treatment with melatonin (≤ 5 mg/day) improves the metabolic syndrome seen in bipolar and schizophrenic patients receiving second-generation antipsychotics (Agahi et al. 2018). The administration of melatonin normalizes the metabolic syndrome in elderly hypertensive patients and in patients with alcoholic hepatic steatosis (Cardinali and Hardeland 2017). The combination of melatonin and zinc acetate, when used alone or in combination with metformin, improved the glycemic control in type 2 diabetic patients (Cardinali 2019a).

TABLE 13.2
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
Melatonin levels						
4 Consecutive patients with PCOS. Seven normal women served as controls.	Case–control study.	1 day	Venous blood samples for melatonin assay were taken every 4 hours for 24 hours.	Plasma melatonin levels.	Average concentration of melatonin (mesor) and amplitude of circadian rhythm were higher in patients with PCOS than in controls.	Tarquini et al. (1996)
22 Women with PCOS, 20 women with idiopathic hirsutism, and 15 age-matched women as controls.	Case–control study.	1 day	Fasting blood samples and 24-hour urinary samples were obtained from all participants.	Urinary aMT6s and plasma levels of LH, FSH, testosterone, estradiol, DHEAS, 17 α-hydroxy-progesterone, and insulin.	Higher aMT6s, insulin, LH/FSH ratio, and testosterone were found in PCOS patients as compared to controls or women with idiopathic hirsutism. An inverse correlation was detected between circulating testosterone and aMT6s in PCOS patients.	Luboshitzky et al. (2001)
12 Women with PCOS, 10 women with idiopathic hirsutism, 10 women with late onset adrenal hyperplasia, and 15 age-matched control women were studied.	Case–control study.	Basal and after 4 months of treatment with before and during treatment with cyproterone acetate-ethinyl estradiol.	Fasting blood samples and 24-hour urinary samples were obtained from all participants at baseline and after treatment.	Urinary aMT6s and plasma levels of LH, FSH, testosterone, and DHEAS.	At baseline, women with PCOS and adrenal hyperplasia had significantly higher testosterone and aMT6s values. Treatment significantly decreased testosterone, LH, FSH, and aMT6s values as compared with baseline values.	Luboshitzky et al. (2004)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
50 Women with PCOS and 50 age- and weight-matched healthy women.	Case–control study.	1 day	On day 2 of menstrual cycle, blood samples were collected between midnight and 04:00 a.m.	Plasma levels of melatonin, LH, FSH, TSH, prolactin, insulin, testosterone, and progesterone.	Melatonin levels were increased in all cases of PCOS. Testosterone levels were raised in 72% of patients. Melatonin levels were positively correlated with circulating testosterone.	Jain et al. (2013)
PCOS ($n=26$) and non-PCOS control ($n=26$) women.	Case–control study.	3 days		Sleep quality was recorded by wrist actimetry and specific questionnaires. Urine samples were collected at various time points over a 24-hour period.	Increases of aMT6s and 8-OHdG occurred in the urine of PCOS patients	Shreeve et al. (2013)
30 Women with PCOS and 25 age- and weight-matched healthy women.	Case–control study.	1 day	All hormonal measurements were done at days 3–5 after the last regular menstrual cycle.	Serum levels of melatonin and cortisol were measured at 03:00 a.m and 08:00 a.m. Testosterone, DHEAS, LH, FSH, and insulin levels were measured at 08:00 a.m.	A higher melatonin level at 08:00 a.m. and smaller mean night–day difference were found in PCOS women.	Terzieva et al. (2013)

(*Continued*)

TABLE 13.2 (Continued)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
321 PCOS patients.	Observational study.			All participants underwent a physical examination, ovarian ultrasound scan and follicular-phase blood testing (testosterone, DHEAS, androstenedione, prolactin, estradiol, gonadotropins, sex hormone-binding globulin, glucose, HOMA-IR) and completed a health and lifestyle questionnaire.	All clinical and biochemical characteristics of PCOS, as well as the trend in serum cortisol and melatonin as biomarkers of circadian rhythm disruption, correlated with the number of phenotypic features in the Rotterdam criteria.	Lim et al. (2019)
35 PCOS and 36 non-PCOS women undergoing IVF treatment.	Observational study.			Melatonin concentration in follicular fluid.	Follicular fluid melatonin concentration was lower in PCOS women and correlated with serum basal FSH level. Although there was no significant difference in the fertilization rate of oocytes, the embryogenesis rate in PCOS patients was significantly lower than controls and showed a positive correlation with the follicular fluid melatonin concentration. The PSQI questionnaire indicated that sleep disorders were more likely to exist in the PCOS group.	Li et al. (2022)

(Continued)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
Obese adolescent girls with PCOS ($n=59$) or without PCOS ($n=33$).	Case–control study.			Sleep duration and timing monitored by actigraphy. Dim light salivary melatonin measurement, fasting hormone analysis.	Later melatonin offset relative to sleep timing, later clock-hour of melatonin offset, and longer duration of melatonin secretion in PCOS patients. Higher serum-free testosterone levels were associated with a later melatonin offset after wake time regardless of group.	Simon et al. (2019)
Genomic studies						
364 PCOS cases and 687 healthy nondiabetic women of northern Chinese ancestry.	Observational study.		Plasma testosterone, LH, FSH, and prolactin, and glucose and insulin levels during OGTT were investigated.	The SNPs rs4753426, rs10830963, rs1562444, and rs1279265 in the MTNR1B gene were identified.	Some endocrine and metabolic traits (plasma testosterone, serum glucose and serum insulin concentration at OGTT) were found to be associated with s10830963 only. No single polymorphisms or haplotypes were associated with PCOS risk.	Wang et al. (2010)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
482 Patients with PCOS and 522 healthy Chinese Han women.	Observational study.		The association of plasma glucose and insulin levels during oral glucose tolerance tests (OGTTs) and hormone levels with MTNR1A gene variants was studied. The association of MTNR1A gene variants with plasma glucose and insulin levels during OGTTs and hormone levels was investigated.	The SNP rs2119882 in the MTNR1A gene was detected.	The frequencies of SNP rs2119882 differed significantly between PCOS cases and healthy controls. A higher fasting plasma glucose concentrations and OGTT-induced insulin release and an increased HOMA-IR were associated with SNP rs2119882.	Li et al. (2011b)
526 Patients with PCOS and 547 healthy Chinese Han women.	Observational study.		The association between MTNR1B gene variants and plasma glucose and insulin levels during OGTT was investigated.	MTNR1B gene SNPs rs10830963 and rs10830962 were studied	A higher fasting plasma glucose concentration and increased area under the curve of plasma glucose levels during the OGTT were associated with SNP rs10830963.	Li et al. (2011a)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
263 Family trios (789 participants) were enrolled.	Observational study.		Plasma testosterone, LH, FSH, prolactin, progesterone and estradiol, and glucose and insulin levels during an OGTT were measured.	135 trios of SNPs rs2119882 and 127 trios of rs10830963 were examined.	Only r was found to be associated with a few endocrine and metabolic traits: Serum testosterone, serum glucose concentration at OGTT, and change of glucose and insulin during OGTT were associated with rs10830963. CC genotype carriers had the highest levels of clinical and metabolic features. Obese PCOS patients differed from nonobese PCOS women in transmission of allele C of rs2119882.	Song et al. (2015)
359 PCOS patients classified as obese PCOS or nonobese PCOS group, and 215 oviduct infertile patients who experienced normal ovulation were used.	Observational study.		Plasma testosterone, LH, FSH, and estradiol levels, glycometabolism index and HOMA-IR, and fasting blood lipid levels were measured.	Genotypes of rs2119882 within the MTNR1A gene and of rs10830963 within the MTNR1B were obtained by sequencing.	In the case of rs2119882, C allele carriers who were not diagnosed with PCOS had an increased risk of developing PCOS, and C allele carriers with PCOS had an increased risk of developing obese PCOS. In the case of rs10830963, G allele carriers who were not diagnosed with PCOS had an increased risk of developing PCOS. Protective factors for obese PCOS were the TT genotype in rs2119882 and the CC genotype in rs10830963.	Xu et al. (2019)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
In vitro fertilization						
13 Women with PCOS and 24 age- and weight-matched healthy women.	Case–control study.	Effect of melatonin in culture medium on the clinical outcomes in IVM IVF-embryo transfer.	Melatonin concentrations in the culture media of GC or COC were measured. The outcome after using IVM media with or without 10 μM melatonin was analyzed.	Determination of melatonin in GC and COC by ELISA. PCR analysis of enzymes in melatonin biosynthesis in GC.	Melatonin concentration in follicular fluid of PCOS women was lower than controls. In the culture media of GC or COC, melatonin synthesis and concentration gradually increased. The melatonin-supplemented group exhibited higher implantation and pregnancy rates.	Kim et al. (2013)
198 PCOS patients undergoing intrauterine insemination.	Double-blinded, randomized clinical trial.	14 days	On the 3rd day of menstruation, a 3-mg melatonin tablet or its placebo was given to the patients; this prescription was continued until the day of HCG administration.	The primary outcome was the rate of chemical pregnancies and the secondary outcome was the determination of endometrial thickness on the day of intrauterine insemination.	Melatonin treatment favors maturation of mature follicles, endometrial thickness, and pregnancies in infertile PCOS women.	Mokhtari et al. (2019)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
15 Women with PCOS and 15 with normal menstrual cycles and infertility caused by oviductal dysfunction	Case–control study.	All patients underwent ovulation induction for oocyte maturation and received HCG i.m.	On the day of oocyte retrieval, follicles > 14 mm were collected. Luteinized granulosa cells were treated with melatonin (10^{-7} M) for 24 hours in the presence or absence of luzindole (10^{-7} M, a nonselective MT1/MT2 inhibitor), PD98059 (10^{-6} M, to block ERK activation), ammonium pyrrolidinedithiocarbamate (10^{-5} M, to block the NF-κB pathway), or the Nrf2 inhibitor ML385 (10^{-5} M).	qRT-PCR of mRNA transcripts encoding CYP19A1, Bcl2 and Bax, NADPH oxidase 2, superoxide dismutase, catalase, glutathione peroxidase, and heme oxygenase-1 were studied.	In luteinized granulosa cells melatonin triggered upregulation of CYP19A1, and expression of ERK, NF-E2-related factor-2, and heme oxygenase-1. It augmented the conversion of androgen to 17β-estradiol and reduced the levels of inducible nitric oxide synthase and NO, improving oocyte development potential.	Yu et al. (2019)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
20 Women with PCOS requiring IVF-ET and 20 normal individuals were studied.	Observational in vitro study.		Ovarian granulosa cells isolated from PCOS patients and controls. An insulin resistance cell model was established by treating human ovarian granulosa cell line cells (SVOG) with palmitic acid (PA).	The expression levels of IRS-1 and glucose transporter (GLUT4) were examined using qRT-PCR and western blot. IR was detected in GCs of PCOS patients and SVOG. Cell viability, apoptosis levels, and PI3K/Akt pathway expression were assessed.	In SVOG cells, PA promoted apoptosis, reduced glucose uptake, and decreased cell viability. In PCOS GCs and SVOG cells, mRNA and protein expression of IRS-1 and GLUT4 was downregulated and glucose uptake capacity. Melatonin improved glucose uptake, upregulated IRS-1 and GLUT4 expression, and downregulated p-IRS-1 in PCOS GCs and SVOG cells. Melatonin increased p-PI3K and p-Akt levels while PA decreased PI3K and Akt phosphorylation.	Guo et al. (2022)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
Melatonin treatment						
57 Young PCOS women with concomitant preinvasive endometrial cancer.	Prospective cohort study.	12 months	Patients received (a) Transdermal patches of 17β-estradiol in increasing and decreasing doses to imitate physiological concentrations of estrogens and intravaginal, micronized progesterone in the second phase of the therapeutic cycle. (b) On permanent basis the combined treatment of melatonin 5 mg/day, metformin 850 mg/day, bromocriptine mesylate 2.5 mg/day, and methysergide maleate 3 mg/day	Serum levels of gonadotropins, estrogens, progesterone, total/free testosterone, and prolactin before and after a metoclopramide-stimulating test. DHEA-S, serotonin, and lipid concentration were also assessed.	Improvement of female sexual hormone profile and lipid metabolism and restoration of normal endometrium were seen in preinvasive endometrial cancer of women with PCOS using melatonin, anti-diabetic, anti-dopaminergic, and anti-serotonin therapy, and female sexual hormones.	Stanosz et al. (2014)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
526 PCOS women.	Randomized controlled, double-blind trial.	Treatment from the first day of the cycle until day 14 after embryo transfer.	Patients were divided as controls ($n=195$) receiving only folic acid; group A ($n=165$) receiving folic acid plus a daily dose of 4 g myo-inositol and 3 mg melatonin; group B ($n=166$) receiving 4 g myo-inositol.	The main outcome measures were oocyte and embryo quality, melatonin intrafollicular concentration, clinical pregnancy and implantation rates.	Myo-inositol and melatonin acted synergistically to enhance the number of mature oocytes and of grade I embryos, and to reduce the total administered units of gonadotropin. Melatonin intrafollicular concentrations in the group A were 3–4 times higher than in control or group B.	Pacchiarotti et al. (2016)
40 Normal-weight women with PCOS.	Prospective cohort study.	6 months	Melatonin 2 mg/day	Clinical and hormonal profile, oral glucose tolerance test, and lipids at baseline and after 6 months of melatonin administration.	Melatonin administration decreased androgens, 17α hydroxyprogesterone, anti-Mullerian hormone serum levels, and LDL-C levels, while it augmented FSH levels. Almost 95% of participants experienced an amelioration of menstrual cycles.	Tagliaferri et al. (2018)
56 Patients with PCOS.	Randomized double-blinded, placebo-controlled clinical trial.	12 weeks	10 mg melatonin ($n=28$) or placebo ($n=28$) once a day 1 hour before bedtime. All individuals received metformin (500 mg/day), and 10 mg/day medroxyprogesterone from the 15th until the 25th day of menstrual cycle.	MDA, serum total testosterone, sex hormone–binding globulin, CRP, nitric oxide, TAC, GSH, hirsutism, and expression of inflammatory genes were measured.	Reduction of hirsutism, total testosterone, CRP, MDA, and gene expression of IL-1 and TNF-α, and increase of TAC and GSH levels were found after melatonin administration.	Jamilian et al. (2019)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
58 PCOS women.	Randomized double-blind, placebo-controlled trial.	12 weeks	10 mg melatonin ($n=29$) or placebo ($n=29$) once a day 1 hour before bedtime.	Glycemic control, lipid profiles, and gene expression of PPAR-γ, glucose transporter 1 and low-density lipoprotein receptor were measured. PSQI, Beck´s Depression Inventory index and Beck Anxiety Inventory were assessed.	Mental health parameters, insulin levels, HOMA-IR, quantitative insulin sensitivity check index, total- and LDL-C levels, and gene expression of PPAR-γ and low-density lipoprotein receptor were improved by melatonin.	Shabani et al. (2019)
84 Women with PCOS aged 18–40 years old.	Randomized, double-blind, placebo-controlled trial	8 weeks	Magnesium (250 mg), melatonin (6 mg), magnesium plus melatonin, or placebo.	PSQI questionnaire and serum levels of cholesterol, LDL-C, HDL-C, and insulin, and HOMA-IR.	Treatment Co-ordinator (TCo) supplementation of magnesium–melatonin showed reduced serum testosterone and insulin levels, HOMA-IR, serum cholesterol, LDL-C, and increased HDL-C levels. Mean PSQI score decreased significantly in both co-supplementation and melatonin groups.	Alizadeh et al. (2021)

(*Continued*)

TABLE 13.2 (*Continued*)
Relevance of Melatonin in Human PCOS

Subjects	Design	Study's Duration	Treatment	Measured	Results	Ref.
84 Women with PCOS aged 18–40 years old.	Randomized, double-blind, placebo-controlled trial.	8 weeks	Magnesium (250 mg), melatonin (6 mg), magnesium plus melatonin, or placebo.	Serum levels of TNF-α and total antioxidant capacity.	Significantly more reduction in hirsutism was seen in magnesium–melatonin co-supplementation. Serum levels of TNF-α declined in the melatonin and co-supplementation groups. Co-supplementation of magnesium plus melatonin was associated with a higher total antioxidant capacity level.	Mousavi et al. (2022)

13.3 CONCLUSION

As discussed above, melatonin displays two properties potentially useful in human medicine: chronobiotic and cytoprotection aiming to reduce low-grade inflammation. Melatonin's remarkable evolutionary conservation strongly implies that its cytoprotective actions are important for cell function. However, to change intracellular melatonin levels, dosages substantially greater than those used as a chronobiotic are required as discussed above.

Melatonin as a therapeutic agent in humans is non-toxic, with a high level of safety (Cardinali et al. 2022). Adverse effects in melatonin clinical trials for primary or secondary sleep disorders were typically few, mild to moderate in intensity, and either self-limiting or resolved promptly after treatment discontinuation. Phase 1 studies in humans using up to 100 mg of melatonin showed absence of toxicity (Galley et al. 2014; Andersen et al. 2016). Larger dosages of melatonin have been used in various illnesses without negative consequences; in people, melatonin has a high safety profile and is generally well tolerated (Cardinali 2019a).

Melatonin clinical research has expanded beyond the therapy of sleep problems into various additional possible applications as our understanding of its physiological activities grows. PCOS is an example of this. Generally, it can be postulated that melatonin has potential therapeutic applications in those diseases exhibiting a low-degree inflammation, like cardiovascular or neurodegenerative disorders. To minimize adverse effects and maximize possible advantages in future therapeutic applications, the intricacy of melatonin's interaction with the complete range of human physiological systems must be thoroughly elucidated.

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