

Poor sleep in PCOS; is melatonin the culprit?

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STUDY QUESTION: Are daily cycles in urinary melatonin and oxidative stress marker levels (8-hydroxydeoxyguanosine) altered in PCOS, and is this associated with changes in sleep quality?

SUMMARY ANSWER: There is an association between elevated nighttime melatonin and 8-hydroxy-2-deoxyguanosine (8-OHdG) levels, and poor sleep quality in our PCOS study group.

WHAT IS KNOWN ALREADY: Women with PCOS are known to have poorer sleep. However, there have been few studies examining the possible association between melatonin levels and sleep quality in women with polycystic ovarian syndrome (PCOS).

STUDY DESIGN, SIZE, DURATION: This is a case–control study of PCOS ($n = 26$) and non-PCOS control ($n = 26$) subjects recruited from a tertiary gynaecological centre.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The participants were requested to complete sleep questionnaires for a month. In a subgroup from these cohorts (PCOS, $n = 15$; controls, $n = 18$), urine samples were also collected at various time points over a 24-h period. In addition, their sleep patterns and lighting environment were monitored for 3 consecutive days and nights using a wrist-mounted Actiwatch device.

MAIN RESULTS AND THE ROLE OF CHANCE: PCOS women had significantly elevated night-time urinary levels of the melatonin metabolite 6-sulfatoxymelatonin (aMT6s) and of 8-OHdG (both at $P < 0.05$), as well as significantly reduced sleep quality ($P < 0.05$), compared with the controls.

LIMITATIONS, REASONS FOR CAUTION: Due to the small sample size of the study, further studies will be required to confirm our findings.

WIDER IMPLICATIONS OF THE FINDINGS: Our preliminary work provides a possible new insight into the interactions between melatonin, increased oxidative stress and sleep in women with PCOS.

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Key words: PCOS / melatonin / ovary / sleep / oxidative stress

Introduction

Polycystic ovarian syndrome (PCOS) is the most prevalent form of an-ovulatory infertility and one of the most common endocrine disorders, affecting between 5 and 10% of women of reproductive age (Moreira *et al.* 2010). Despite its prevalence, the aetiology of the condition is still poorly understood. It has been suggested that the hormone

melatonin might play an important role in the pathophysiology of PCOS (Tamura *et al.*, 2009).

Melatonin (*N*-acetyl-5-methoxytryptamine) is an indoleamine and is known as a hormone that regulates the sleep–wake cycle. It is mainly secreted by the pineal gland in the brain, and its release is activated by darkness and suppressed by light (Macchi and Bruce 2004). Elevated circulating night-time levels of melatonin have been reported to be

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associated with increased propensity for sleep (Shochat et al., 1998). Women with PCOS, on the other hand, have increased sleep disturbances (Vgontzas et al., 2001) and abnormal sleep architecture (de Sousa et al., 2010). PCOS women have also been found to have a higher prevalence of obstructive sleep apnea, which is independent of their body mass index (BMI) (Gopal et al., 2002). Daytime concentrations of the urinary melatonin metabolite 6-sulfatoxymelatonin (aMT6s) in women with PCOS have been shown to be elevated compared with that in women with normal fertility (Luboshitzky et al., 2001) and women with idiopathic hirsutism (Luboshitzky et al., 2003). Moreover, melatonin administration in women can cause features mimicking those of PCOS, such as enhanced LH secretion, pulse amplitude and response to exogenous gonadotrophin-releasing hormone (GnRH) (Cagnacci et al., 1995). Melatonin has also been reported to reduce peripheral tissue insulin sensitivity and glucose tolerance, both of which are metabolic features of PCOS (Cagnacci et al., 2001).

Melatonin is known to be an effective anti-oxidant and free-radical scavenger (Tamura et al., 2009), and its interaction with reactive oxidative species (ROS) in the reproductive system is widely acknowledged (Adriaens et al., 2006). PCOS women are known to have increased oxidative stress (Kuşçu and Var 2009). 8-hydroxy-2-deoxyguanosine (8-OHdG), which is detectable in the urine, is a known biomarker of oxidative stress, which commonly referred to as an imbalance between oxidants and antioxidants (Karihtala and Soini 2007). 8-OHdG is a major product of oxidation of guanine, the purine base most prone to oxidative damage (Cooke et al., 2005). Thus, it is possible that 8-OHdG may play a role in the pathogenesis of PCOS.

To our knowledge, the association between circulating levels of melatonin, biomarkers of oxidative stress and sleep quality in women with PCOS has not been elucidated. Our hypothesis is that if an altered melatonin profile is associated with sleep disturbances and oxidative stress in women with PCOS, exogenous synthetic melatonin could potentially be beneficial (Rios et al., 2012). Thus, the aims of the study were (i) to investigate whether there are differences in urinary melatonin levels between women with and without PCOS; (ii) to determine whether the sleep–wake pattern of women with PCOS is different from that of non-PCOS subjects and (iii) to determine the associations between melatonin and 8-OHdG levels and sleep quality in women with PCOS.

Materials and Methods

Subjects

Women attending gynaecological outpatient clinics at a tertiary university hospital were invited to participate in this case–control study. Female patients aged between 18–40 years and diagnosed with PCOS (based on the 2003 Rotterdam Criteria) were eligible for the study group. Eligibility for the study group required two out of the following three clinical findings, i.e. biochemical hyperandrogenism (manifest clinically as acne, hirsutism, etc.), polycystic ovaries detected through ultrasound scanning and/or oligomenorrhoea (≥ 35 days between cycles). The designated control group consisted of females aged between 18 and 40 years with regular menstrual cycles and no history of subfertility. Night shift workers and pregnant women were excluded from the study, as were women who have additional related pathologies, such as malignancy, thyroid disease, hyperprolactinemia or adrenal gland disease. Any

participants with psychiatric disorders were also excluded. On total 52 women were recruited to the study: 26 with PCOS and 26 controls. All subjects provided informed consent, and were appropriately recruited in accordance with local policy. Ethical approval was granted by the Isle of Wight, Portsmouth and South East Hampshire (UK) research ethics committee (reference 08/H0501/84).

Assessing sleep quality

All participants were asked to complete a validated sleep questionnaire and a daytime sleepiness scale. Basic demographic data and information on sleep patterns were also collected on the sleep questionnaire. Sleep quality was determined using the Pittsburgh Sleep Quality Index (PSQI) questionnaire and daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS) questionnaire.

The Pittsburgh Sleep Quality Index

This self-rated, validated questionnaire (Buysse et al., 1989) assesses sleep quality and disturbances over a 1-month period. It consists of 19 questions with seven component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. Each component score is rated from 0, indicating no difficulty, to 3, indicating severe difficulty, and is aggregate to a global score with a maximum difficulty score of 21. A score above 5 indicates poor sleep quality.

Epworth sleepiness scale (ESS)

The ESS measures a person's usual level of daytime sleepiness (Johns 1991). It asks the examinee to rate their propensity to fall asleep in eight different situations. The score reflects average sleep propensity (ASP). ASP is a measurement of a person's general level of daytime sleepiness and can be used to identify people with sleep apnoea. A score above 16 indicates a high level of daytime sleepiness and a score of between 9 and 16 suggests inadequate sleep.

Monitoring sleep–wake patterns

The sleep–wake patterns in a subgroup of women from the two cohorts ($n = 15$ PCOS and $n = 18$ control subjects) were monitored using a wrist-mounted Actiwatch device (Phillips Healthcare, Respironics, The Netherlands) for 3 consecutive days and nights. Evidence suggests that the Actiwatch 3-day aggregate is comparable to the values obtained over 7- or 14-day aggregates (McCrae et al., 2008). Actiwatchers are small, actigraphy-based data loggers that record a digitally integrated measure of gross motor activity. The device also records white light exposure and activity levels and provides a reliable indicator of sleep–wake state in healthy populations (Littner et al., 2003). Exposure of the participants to white light was taken into account as white and blue light exposure has been reported to be a key determinant of the sleep–wake cycles and melatonin release (Kayumov et al., 2005), and all white light contains some blue light. Unlike polysomnography, which is the gold standard neurophysiological method for detection of sleep, actigraphy is non-invasive and can be used more cost-effectively for long-term monitoring. A previous study compared these two methods and found that actigraphy correctly predicted sleep and wake periods 86.6% of the time in adults, compared with polysomnography (Pollak et al., 2001). For the purposes of our study, we focused on two key sleep variables: (i) total sleep time in minutes (defined as time from sleep onset to morning waking that is recorded as sleep) and (ii) sleep efficiency (defined as a percentage of the total sleep period, i.e. time from sleep onset to sleep offset that is recorded as sleep). Both variables provide complementary information about sleep quality, and were calculated using sleep analysis software (Phillips Healthcare, Respironics, The Netherlands).

Collection of urinary samples

A one-off daytime sample (between the hours of 09.00 h and 17.00 h) of the subject's urine was collected at the time of recruitment to the study. The same women who were used for monitoring sleep-wake patterns ($n = 15$ PCOS and $n = 18$ control subjects) were also asked to collect all their urine passed over five pre-set periods for 24 h. After an initial 24 h acclimatization period, aliquots (about 5 ml) were taken from volumes collected between bedtime and waking (~ 08.00 h) (sample 1), between 08.00 and 12.00 h (sample 2), from 12.00 to 16.00 h (sample 3), from 16.00 to 20.00 h (sample 4) and from 20.00 h to bedtime (~ 23.00 – 00.00 h) (Sample 5). The acclimatization period allows the participant to become accustomed to the collection protocol and reduces the chances of the study protocol inducing stressors which may impact on the participants' hormonal profile. The participants were asked to record the total volume of urine collected from each sampling period. Collected samples were initially kept and then transported to the research centre at 4°C , where they are eventually stored at -20°C until being analysed for melatonin.

Hormone assays

The melatonin metabolite 6-sulfatoxymelatonin (aMT6s) concentrations in urine samples were measured in duplicate by radioimmunoassay (analysed by Stockgrand Ltd, University of Surrey, UK). The aMT6s value for each sample was corrected for total volume of urine collected over the pre-set sampling periods. Limit of detection for the urinary aMT6s assays was 0.48 ± 0.19 ng/ml. Intra-assay variation for urinary aMT6s melatonin was 3.3%.

8-OHdG levels were measured as per the manufacturer's protocol with a competitive enzyme-linked immunoassay (ELISA) kit (Stressmarq Biosciences, Inc., Victoria, Canada, 2008). This particular assay utilized an anti-mouse IgG-coated plate, a tracer consisting of an 8-OHdG-acetylcholinesterase conjugate and a specific 8-OHdG monoclonal antibody. Overnight and daytime urine samples of 32 participants (PCOS = 16, control = 16) were assayed in duplicate to ensure accuracy and reproducibility of results. Intra-assay variance was 4.5%.

Statistical analysis

All data were analysed for normality by Shapiro–Wilk testing. Group differences were evaluated using independent samples *t*-test for normally distributed data, with Mann–Whitney *U* tests were used for abnormally distributed data. Bivariate correlations were drawn by Spearman's rho tests. Statistical significance was set at $P < 0.05$. All data were analysed using the SPSS statistical package version 17.0 (SPSS, Chicago, IL, USA). Data values are presented as mean \pm SD, or as median (and interquartile range) for variables that are not normally distributed.

Results

The 52 participants recruited to the study had a mean age of 28.0 ± 5.1 years. In the PCOS group ($n = 26$), 71% were diagnosed with polycystic ovaries on ultrasound scanning and 57% reported having oligo- or amenorrhoea, including two patients who reported complete amenorrhoea. Their hormonal profiles are listed in Table I. There was no significant difference in BMI values between the PCOS and control groups (29.3 ± 8.2 versus 24.6 ± 3.3 , respectively). However, there was a significant difference in age when comparing the study group to the control group (PCOS: 29.8 ± 3.7 versus controls: 26.3 ± 5.6 years, $P = 0.04$).

Sleep parameters

The PCOS group had a higher percentage of self-reported 'bad sleep' compared with the controls (19.6% versus 10.7%, respectively, $P = 0.054$) (Table II). PCOS women were also found to be far more lethargic during the day as a consequence of poor sleep compared with the controls ($P < 0.05$). The total PSQI score for the PCOS group was higher than the controls, with 56% of the PCOS women having overall poor sleep with a PSQI score > 5 ($P < 0.05$) compared with 31% of women in the control group. There was also a trend towards a higher mean ESS score in PCOS women compared with controls ($P = 0.052$). There was no correlation between sleep efficiency and BMI in the PCOS group ($P = 0.97$, $r = -0.014$).

Actiwatch data

The Actiwatch data were generated by averaging three consecutive nights of sleep. The data generated revealed lower sleep quality in the PCOS group compared with controls. Sleep efficiency was significantly lower in the PCOS group compared with the control group ($P < 0.05$, Table II). However, we did not observe any differences in day or night white light exposure between the two groups (data not shown).

Table I Hormonal profile of women with PCOS.

Hormones	Mean \pm SD ($n = 26$)	Normal range
Testosterone (nmol/l)	2.3 ± 0.2	0.7–2.8
Free androgen index	10.1 ± 3.5	7–10
Progesterone (Day 21, nmol/l)	34.6 ± 9.6	17–92
Luteinizing hormone (IU/l)	8.1 ± 1.2	20–75
Follicle-stimulating hormone (IU/l)	6.9 ± 0.6	6–26
Sex hormone-binding globulin (nmol/l)	46.8 ± 13.2	40–120
Oestradiol (pmol/l)	78.3 ± 50.5	70–500

Table II PSQI score, ESS score and actiwatch data in PCOS and non-PCOS (control) individuals.

	PCOS	Controls	P-value
Sleep questionnaires (PCOS $n = 26$, controls $n = 26$)			
Quality of sleep			
Fairly bad (%)	19.6	10.7	$P = 0.054$
Adverse effect on daily enthusiasm (%)	39.6	27.8	$P < 0.05$
Total PSQI > 5	15 (56%)	8 (31%)	$P < 0.05$
Mean ESS score (\pm SD)	7.96 ± 4.57	5.56 ± 3.61	$P = 0.052$
Actiwatch data (PCOS $n = 15$, controls $n = 18$)			
Sleep efficiency (\pm SD)	82.8 ± 4.7	85.6 ± 4.3	$P < 0.05$

PSQI, Pittsburgh Sleep Quality Index (PSQI); ESS, Epworth Sleepiness Scale.

Hormone assays

The daytime salivary melatonin and urinary aMT6s levels were similar in both groups. In the 24 h serial sampling study, we did not observe any significant differences in aMT6s levels taken in all subjects during their waking hours (samples 2, 3, 4 and 5; see Materials and Methods for details) and so we combined them and presented the data as the daytime aMT6s values (Fig. 1a). However, the night-time aMT6s level (sample 1) was 1.6-fold higher in the PCOS group when compared with the controls (60.3 ± 30.6 pg/ml versus 37.7 ± 21.5 pg/ml, respectively; $P < 0.05$).

Mean night-time 8-OHdG levels (Fig. 1b) were found to be 1.4-fold higher in the PCOS group compared with the control group (120.5 ± 42.1 ng/ml versus 84.0 ± 40.8 ng/ml, respectively; $P < 0.05$). On the other hand, the mean daytime 8-OHdG levels were not significantly different.

In the PCOS group, the mean night-time 8-OHdG levels were almost 2-fold greater than corresponding daytime levels (116.5 ± 40.8 ng/ml versus 63.1 ± 45.1 ng/ml; $P < 0.05$). In the control group, however, there was no significant difference between night-time and daytime 8-OHdG levels.

Correlation analysis

Night-time melatonin levels in both the control and PCOS groups were not found to be correlated with sleep efficiency ($r = -0.237$,

$P = 0.436$). We also examined for possible correlations between the hormone profiles (see Table 1) or urinary aMT6s levels and sleep quality in the PCOS group, but did not find any.

Curve estimation was carried out to determine if a non-linear relationship existed between night-time 8-OHdG and aMT6s levels. In the PCOS group, there was a very strong and significant quadratic association between the two variables ($r = 0.86$, $P < 0.05$), while no association was found in the controls.

Discussion

Melatonin is a well-known marker of the individual's circadian rhythm (Klerman et al., 2012), and our results show that the day–night changes in urinary melatonin (i.e. low during the day and high at night) can be observed in both control and PCOS groups. This indicates that the circadian rhythm is not disrupted in either group. However, serial urine collections over a 24 h period, has revealed novel observations that night-time melatonin and 8-OHdG levels are significantly elevated in PCOS women compared with the non-PCOS controls. The elevated night-time levels of melatonin in the PCOS group could potentially be acting as a free radical scavenger for the increased oxidative stress, as indicated by the elevated 8-OHdG levels during this period. Crucially, sleep efficiency, which is an overall measure of sleep quality, was found to be significantly lower in the PCOS group. Nevertheless, the causal role of melatonin with respect to the pathogenesis of PCOS, in the presence of an elevated oxidative stress levels, is still unclear. Some researchers have advocated systematic evaluation of sleep disturbances in all women with PCOS and our data offers further support to this recommendation (Tasali et al., 2008a,b).

There was a difference between the mean age of our control and study groups; however, we are unaware of any studies currently indicating age directly as a significant factor in melatonin's effect on circadian rhythms or female reproductive physiology.

The significantly raised night-time urinary aMT6s levels that we observed in the PCOS patients lend further insight into the relationship between melatonin and sleep behaviour in these patients. The possible relationship between poor sleep quality in women with PCOS in our study is likely to be complex. Polysomnographic analysis of women with PCOS has shown an increased sleep onset latency period and disturbed sleep architecture (de Sousa et al., 2010). Melatonin in the evening may be more important in inducing sleepiness than in maintaining sleep. Whereas an earlier study has demonstrated a rise in total 24 h urinary aMT6s in women with PCOS (Luboshitzky et al., 2001), it did not delineate the pattern of melatonin secretion or the associated effect on sleep quality. Also many studies do not take into account the effect of shift work or the light environment, which are crucially important variables in melatonin secretion.

Our results showing the occurrence of both poor sleep quality and high melatonin levels among the PCOS patients at first appear counterintuitive, as melatonin is a hormone that promotes sleep. Although studies have reported that exogenous melatonin increases rapid eye movement (REM) sleep or deep sleep (Kunz et al., 2004), increased REM sleep itself can be followed by increased wakefulness in the latter half of the sleep episode (Cajochen et al., 1997). The observed elevated night-time melatonin levels in our PCOS group may be in response to an increased duration of their sleep latency.

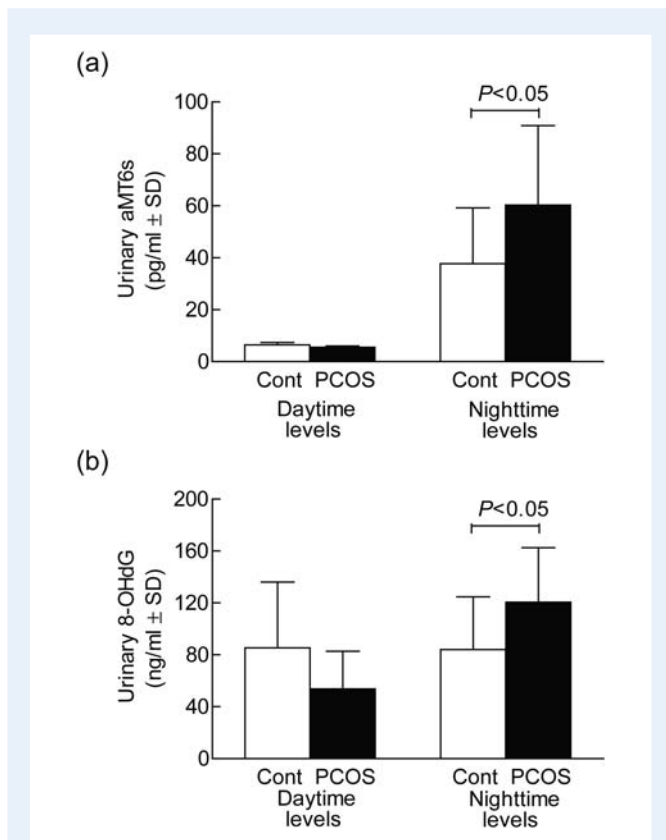


Figure 1 Daytime and night-time urinary aMT6s (a) and 8-OHdG (b) levels in PCOS and non-PCOS (Cont) groups. Values are expressed as means \pm SD.

Polysomnography together with more detailed investigation of night-time serum melatonin levels will provide better understanding of the association of sleep architecture and serum melatonin alternations.

It is well established that melatonin directly acts on the ovary (Rönnberg *et al.*, 1990) and has a role in follicular development, oocyte maturation and ovulation (Reiter *et al.*, 2005). Furthermore, follicular fluid melatonin concentrations have been shown to be higher than, and are inversely correlated with, peripheral serum levels (Rönnberg *et al.*, 1990). Intra-follicular melatonin concentrations have been found to be significantly lower in PCOS, regardless of high circulating melatonin levels (Tamura *et al.*, 2009). It would have been useful to delineate the relationship between melatonin secretion and ovulatory status in our PCOS group. However, as only two of our participants reported complete amenorrhoea, a statistical subgroup analysis would have been impossible to perform. We believe that an analysis of this relationship would be of great importance to future studies of this nature.

There is emerging evidence that oxidative stress is raised in PCOS (Sathyapalan *et al.*, 2012). 8-OHdG is a known biomarker of oxidative stress, mainly because of its relatively easy detectability in urine (Karihtala and Soini, 2007). It is a marker of oxidative damage to DNA, as well as generalized cellular oxidative stress (Cooke *et al.*, 2000). Fairly conclusive evidence has demonstrated that the principal source of urinary 8-OHdG *in vivo* is oxidative stress-derived DNA damage and is not confounded by diet, cell turnover or artefacts (Cooke *et al.*, 2005). Melatonin is known to be a scavenger of ROS. When we compared overnight aMT6s levels with 8-OHdG levels in the PCOS participants, we saw a distinct and significant curved relationship. This indicates that PCOS women with high 8-OHdG levels, and thus high oxidative stress levels, are producing more melatonin (and significantly more than controls), possibly in an attempt to neutralize excess ROS. Although these associations by no mean prove causality, it is certainly a direction for future studies.

In the future, perhaps translational work could start to explore the use of systemic versus local synthetic melatonin administration as a potential therapeutic option in women with PCOS reporting sleep disturbances.

Conclusion

This study is the first to simultaneously measure urinary melatonin and 8-OHdG levels, as well as assess the sleep patterns in women with PCOS. The significance of increased oxidative stress in PCOS is unknown, but its role in the melatonin-sleep paradigm could be of importance in our understanding of the disease process. Our results show two novel findings, that the night-time urinary melatonin levels are raised in women with PCOS and that this is associated with lower sleep quality and an increase in oxidative stress. Whilst these findings do not imply any causal link between sleep disturbance, oxidative stress and raised melatonin secretion in women with PCOS and the pathogenesis of PCOS, they do suggest that an association exists. We believe that the clinical and physiological importance of such an association requires further elucidation.

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Authors' roles

Y.C. and F.C. conceived the study and contributed in the study design, execution, analysis, critical discussion, and in drafting and revising the manuscript. N.S., K.S., M.T. and A.H. contributed in recruiting patients, collecting and analysing data and samples, critical discussion and in drafting and revising the manuscript. C.M.H., N.B. and N.M. contributed in critical discussion, and in drafting and revising the manuscript. There were no known conflicts of interest.

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Conflict of interest

None declared.

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