Melatonin in perimenopausal and postmenopausal women: associations with mood, sleep, climacteric symptoms and quality of life

Running Title: Melatonin in perimenopause and postmenopause

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Abstract

Objective: Melatonin synthesis and secretion are partly modulated by estrogen and progesterone. Changes in melatonin concentrations, possibly related to the menopausal transition, may be associated with climacteric mood, sleep and vasomotor symptoms. The aims of this study were to compare the serum concentrations of melatonin in perimenopausal and postmenopausal women, and to evaluate its influence on mood, sleep, vasomotor symptoms and quality of life. **Methods:** We analyzed data of 17 perimenopausal (43-51 years) and 18 postmenopausal (58-71 years) healthy women who participated in a prospective study. During the study night (21:00-09:00 hr) serum melatonin was sampled at 20-minute (21:00-24:00 hr; 06:00-09:00 hr) and one-hour (24:00-06:00 hr) intervals. Questionnaires were used to assess depression (Beck Depression Inventory, BDI), anxiety (State-Trait Anxiety Inventory, STAI), insomnia and sleepiness (Basic Nordic Sleep Questionnaire, BNSQ), subjective sleep quality, vasomotor symptoms, and the quality of life (EuroQoL). **Results:** Postmenopausal women had lower nighttime serum melatonin concentrations than perimenopausal women. The duration of melatonin secretion tended to be shorter in postmenopause, while the melatonin peak time did not differ. Mean melatonin concentrations and exposure levels did not correlate with FSH or E2, BMI, BDI, STAI, BNSQ insomnia, BNSQ sleepiness, subjective sleep, climacteric vasomotor score or the quality of life. In perimenopause, the later the melatonin peak, the higher the level of anxiety (p=0.022), and the longer the melatonin secretion, the better the quality of life (p<0.001). **Conclusions:** Longitudinal research is needed to better understand the possible contributive role of menopause on lower melatonin levels.

Keywords:

melatonin, perimenopause, postmenopause, mood, sleep, quality of life.

Introduction

Melatonin is a hormone produced primarily by the pineal gland and by the retina, skin and gastrointestinal tract.[1] The synthesis and secretion of melatonin follow a circadian rhythm and are indirectly regulated by the light/dark cycle, with light having an inhibitory and darkness a stimulatory effect.[2] According to animal and human studies, estrogen and, at a less extent, progesterone[3-6] also contribute to direct and indirect modulation of melatonin synthesis and secretion. In addition, melatonin concentration is influenced by administration of gonadotropin-releasing hormone agonists,[7,8] and in general, the circulating melatonin concentrations seem to be high when endogenous estrogen levels are low, like in women using oral contraception.[9] Reciprocally, it has been suggested that melatonin has a regulatory,[10] mostly inhibitory, effect on reproduction, probably by down-regulating the hypothalamic-pituitary-ovarian axis and modulating estrogen synthesis in peripheral tissues,[11] such as in the breast, muscles and adipose tissue. For instance, Voordouw et al.[12] found a reduced ovarian function, decreased luteinizing hormone and estrogen and progesterone levels after 4-month melatonin (or melatonin-progestin combinations) administration in adult women. Elevated levels of plasma/serum nocturnal melatonin (concentration, area under curve AUC, peak amplitude) have also been associated with reproductive malfunctions[13] such as amenorrhea in women [7,14]and hypogonadism in men.[15]

Although the peak serum melatonin concentrations, as well as the total amount of urinary melatonin, decrease physiologically with age both in men and women,[16,17] probably due to pineal aging, it is possible that in women the mean nocturnal melatonin concentrations, as well as the melatonin peak time, the amplitude and duration of melatonin secretion vary during the menopausal transition in relation to changes in gonadal hormone levels. However, the literature on this issue is sparse and inconsistent.[18-20]

Because of this possible reciprocal relationship between melatonin and gonadal hormones, and since long-term administration of melatonin was found to partly improve the quality of life (with regard to the physical domain) in perimenopausal women,[21] it is possible to hypothesize that melatonin may also contribute to mitigate other symptoms associated with the perimenopausal hormonal fluctuations. Among others, depressive and anxiety symptoms and disorders are common during the perimenopause.[22-24] Impaired sleep quality is also common during the menopausal transition and in postmenopause.[25-29] Melatonin is known to influence mood and sleep. In fact, even with some inconsistencies, melatonin secretion seems to be lower in individuals having depression,[30-35] and melatonin or melatonin receptor agonists have antidepressants, anxiolytic and sleep-promoting effects.[36-39] It is therefore plausible that changes in melatonin concentrations related to both aging and transition to menopause may at least partly explain depressive, anxiety and sleep symptoms as well as climacteric vasomotor symptoms, typically experienced by women during perimenopause and postmenopause.

Given the general lack of data on the association between melatonin concentration and secretion pattern and the transition to menopause, the aim of this work was to describe the melatonin levels in perimenopausal and postmenopausal women. We hypothesized that postmenopausal women have lower mean nighttime serum melatonin concentrations, as well as lower melatonin exposure levels and shorter duration of nighttime secretion than perimenopausal women. Our further aim was to evaluate the relationship between melatonin levels and depressive, anxiety, sleep and vasomotor symptoms, as well as quality of life in perimenopausal and postmenopausal women. We hypothesized that low melatonin concentrations and exposure levels, and shorter duration of secretion associate with more symptoms, especially in postmenopausal women.

Methods

The current study was part of a larger survey evaluating the effects of menopause on sleep and cognition. The women were recruited through advertisements in the local newspapers in the area of Turku, Finland; 17 of them were perimenopausal (aged 43-51 years), and 18 postmenopausal (aged 58-71 years). Perimenopausal status was defined by the serum follicle stimulating hormone (FSH) level (< 23 IU/mL) and an ongoing regular or irregular menstrual cycle. Postmenopause was determined by age (≥ 58 years) and at least 12 months of amenorrhea.

The exclusion criteria included presence of a mental, cardiovascular (with the exception of drug-treated balanced hypertension), endocrine (with the exception of drug-treated balanced hyperlipidemia), pulmonary, neurological or specific sleep disorder; malignancies; alcohol abuse, smoking, excessive caffeine intake (>5 cups per day) and use of other substances that are known to affect the central nervous system. In addition, women suffering from other conditions possibly affecting sleep (e.g. fibromyalgia and anemia) were excluded. All women had normal levels of blood hemoglobin, leukocytes, thrombocytes and serum thyrotropin. One perimenopausal woman and 13 postmenopausal women had previously used hormone therapy (HT), and a washout period of at least 12 months was required. More details about the data collection and study design have already been described elsewhere.[40] After receiving oral and written information, all participants gave written informed consent. The study was approved by the Ethics Committees of Turku University Hospital and of University of Turku, Finland.

The participants kept a sleep diary during the three weeks before and one week after the study to verify their sleep-wake rhythms. All women had regular sleep-wake schedules (from 22:00-23:00 hr to 06:00-07:00 hr). Travelling abroad, as well as use of alcohol and caffeine was prohibited one week before and during the study. Coffee-drinkers were provided with decaffeinated beverages.

The blood samples were collected all throughout the year; in detail, 13 of the 17 perimenopausal women, and 10 of the 18 postmenopausal women were studied during winter time (October to March). The participants spent one adaptation night (from 19:30 to 08:00 hr; lights-off at 23:00, lights-on at 07:00 hr) in the sleep laboratory. In the following morning, a blood sample was taken for baseline serum FSH and estradiol (E2) measurements. On the following evening, the women returned to the laboratory at 19:30 hr for the baseline sleep recording (lights-off at 23:00, lights-on at 07:00 hr), which was repeated also through the third night. During the night only red light was allowed for illumination if needed. Therefore, during the study period the participants spent their time inside a building, in a dark room without windows, with strictly controlled nighttime illumination levels; this has limited the possible influence of different photoperiods in different subjects. The study was performed by similar timetable in all subjects and food was provided by the sleep laboratory.

On the evening before the third night an indwelling catheter was inserted into a forearm vein to permit a 24-hour blood sampling at 20-minute intervals, starting from 21:00 hr. At night (from 21:00 to 07:00 hr) the catheter was connected to a plastic tube extending into an adjacent room: this allowed repeated blood sampling without disturbing the woman's sleep. The catheter was kept patent with a slow heparinized saline infusion. Thus, melatonin measurements were available for 20-minute interval samples between 21:00 and midnight, and from 06:00 to 09:00 hr; measurements on one-hour interval samples were available between midnight and 06:00 hr. All perimenopausal women were examined in the beginning of their menstrual cycle (i.e., in the follicular phase).

The blood samples were drawn into EDTA tubes and placed in the refrigerator for 20 min. Thereafter, they were centrifuged to separate serum, which was frozen at 70° C until assayed. The inter-assay coefficients of variation were 2.3% for FSH at a concentration of 44.8 IU/l and 8.5% for E2 at a concentration of 0.18 nmol/l, and the analytical sensitivities were 0.05

IU/l and 0.05 nmol/l respectively. For melatonin analyses the serum samples were first extracted with chloroform and then assayed by radioimmunoassay with an iodinated melatonin tracer and a melatonin-specific antiserum.[41] The lowest detectable concentration by the method was 1.3 pg/ml (5.7 pmol/l), and the intra-assay and inter-assay coefficients of variation were from 6.7 to 9.5% and from 9.8 to 12.5%, respectively.

The following melatonin indicators were derived: 1. the mean nighttime serum melatonin concentration from lights-off (at 23:00 hr) to lights-on (at 07:00 hr); 2. the range and mean of maximum and minimum levels of nighttime serum melatonin concentration (from lights-off to lights-on); 3. the nighttime melatonin exposure level: after the interpolation of melatonin exposure level curve, the area under melatonin exposure curve (AUC, from lights-off to lights-on) was calculated for each individual, and the mean, quartile and median values of melatonin exposure levels were calculated; 4. the duration of nighttime melatonin secretion: the total amount of time (in hours) when serum melatonin levels (circulating melatonin) were ≥10 pg/ml, where 10 pg/ml is the usual threshold for melatonin onset;[42,43] 5. the melatonin peak time: the clock time of the peak of melatonin secretion; and 6. the time from lights off to melatonin peak time (in hours).

Questionnaires

Depressive symptoms during the past four weeks were evaluated with the Beck Depression Inventory (BDI, a sum score, with the range of 0-63),[44] and current anxiety level with the State-Trait Anxiety Inventory (STAI, a sum score, with the range of 20-80).[45] Insomnia (a sum score, with the range of 5-25) and sleepiness (a sum score, with the range of 5-25) during the past three months were evaluated using the Basic Nordic Sleep Questionnaire (BNSQ),[46] with lower score referring to better sleep (i.e., low levels of sleeping problems and sleepiness; see Appendix 1, Supplemental Digital Content 1, which reports the questions concerning

insomnia and sleepiness of the BNSQ). In addition, the subjective sleep score (a sum score with the range of 6-20) of the preceding blood-sampling night in the laboratory was assessed in the morning by questions on sleep quality, sleep efficiency, sleep latency, number of awakenings, too early morning awakening and morning tiredness, with lower number referring to better sleep or to a low level of sleeping problems (see Appendix 2, Supplemental Digital Content 2, which reports the questions concerning the sleep of preceding night used to calculate the subjective sleep score). Climacteric vasomotor symptoms were scored with two questions on the past six months (night sweats and hot flashes). The frequency of the symptoms (a sum score, with the range of 2-8) was determined on the following four-point scale: one ("seldom or never"), two ("approximately once a month"), three ("approximately once a week"), four ("almost every day"). The current quality of life (an index score, with the range of from -0.011 to +1) was assessed using the EuroQoL quality of life questionnaire (EQ-5D) and the EQ-5D visual analogy scale (VAS, a scale score, with the range of 1-100).[47] The EQ-5D index was calculated through a specific algorithm which considers a weight for each dimension.[48] The questionnaires were administered during the day after the blood sampling.

Statistical analysis

After testing for normality of the distribution (the Kolmogorov–Smirnov test), bivariate analyses were calculated to study the differences between perimenopausal and postmenopausal women using Student's *t*-test for comparison of the mean values. A *p*-value of <0.05 was considered significant. The mean, maximum and minimum levels of nighttime serum melatonin concentrations, the mean nighttime melatonin exposure level, the duration of nighttime melatonin secretion and the melatonin peak time in perimenopausal *vs.* postmenopausal women were compared by means of t-test and Wilcoxon rank-sum test.

Bivariate Pearson correlation analyses were performed separately in perimenopausal women and in postmenopausal women between nighttime serum melatonin concentrations, melatonin exposure level, duration of nighttime melatonin secretion and the time from lights off to melatonin peak, *vs*. independent variables including FSH, E2, body-mass index (BMI), BDI, STAI, BNSQ insomnia, BNSQ sleepiness, subjective sleep score, climacteric vasomotor symptom score and quality of life (EQ-5D index and EQ-5D VAS). Interaction analyses were performed to test the associations between nighttime melatonin exposure (AUC) and, alternatively, each of the independent variables that differed (or tended to differ) between perimenopausal and postmenopausal women (i.e., FSH levels, E2 levels, BMI, BDI, BNSQ insomnia and climacteric vasomotor scores). Menopausal status was entered in each model as a controlling variable. The statistical analyses were performed using the SPSS/PASW software version 18.0 (SPSS Inc., Chicago, IL, USA) and the R program.[49]

Results

The basic characteristics of the participants are described in Table 1. As determined, perimenopausal women were younger and had lower FSH levels and higher E2 levels than postmenopausal women. In addition, perimenopausal women had lower BMI and less climacteric vasomotor symptoms. A tendency towards lower BDI and BNSQ insomnia scores was found in perimenopausal women. No differences were found in respect to anxiety scores on the STAI, sleepiness scores on the BNSQ or subjective sleep questionnaire, or the quality of life on the EQ-5D index or EQ-5D VAS.

Data on melatonin levels was available for 17 perimenopausal and 17 postmenopausal women (data missing from one postmenopausal woman). Values of melatonin indicators in perimenopausal and postmenopausal women are reported in Table 2. Mean nighttime serum melatonin concentrations, maximum and minimum levels, as well as mean nighttime

melatonin exposure level (AUC) were lower in postmenopausal compared with perimenopausal women (Table 2 and Figure 1). Although the melatonin peak time did not differ between the two groups, the duration of the nighttime melatonin secretion (serum level ≥10 pg/ml; 43.1 pmol/l) approached the significant level for a longer duration in perimenopausal women than in postmenopausal women (6 h 47 min vs. 6 h 22 min, respectively; p=0.058).

The mean nighttime melatonin concentration and age did not correlate. Further, mean nighttime serum melatonin concentration did not correlate with FSH levels or E2 levels, BMI, BDI, STAI, BNSQ insomnia, BNSQ sleepiness, subjective sleep of the preceding night, climacteric vasomotor score or the quality of life (EQ-5D score and EQ-VAS) either in perimenopausal or postmenopausal women. No correlations were found between nighttime melatonin exposure level (AUC) and any of the independent variables either in perimenopausal or postmenopausal women. In the perimenopausal group, the time from lights off to melatonin peak correlated with scores on the STAI (r=0.55, p=0.022), i.e., the later the melatonin peak, the higher the anxiety level. In addition, the duration of nighttime melatonin secretion (serum levels ≥10 pg/ml; 43.1 pmol/l) correlated with the EQ5D-VAS scores (r=0.74; p<0.001), i.e. the longer the duration of nighttime melatonin secretion, the better the quality of life. No correlations were found in the postmenopausal group.

In interaction analyses, no association was found between nighttime melatonin exposure level (AUC) and FSH or E2 levels, BMI, BDI, BNSQ insomnia or climacteric vasomotor scores after controlling for the menopausal status.

Discussion

From these results using serial blood draws, we are among the first to observe that as compared to perimenopausal women, postmenopausal women had reduced nighttime

melatonin concentrations at each time point. However, the nighttime pattern of rise and fall in melatonin levels, including the melatonin peak time was similar for both groups. Although not reaching statistical significance, the nighttime duration of melatonin secretion (the circulating melatonin concentrations equal to or more than 10 pg/ml) was longer in the perimenopausal women. Postmenopausal women had more depressive, insomnia and climacteric vasomotor symptoms than perimenopausal women, but there was no evidence that these symptoms were related to the melatonin levels for either group.

Our findings of lower nighttime melatonin concentrations in postmenopausal women may be a reflection of the well-known age-related decrease of melatonin levels,[16,17] where the transition into menopause may be itself considered as a dimension of aging, in specific of the hypothalamus-pituitary-ovarian system. In this respect, it is possible that the menopause-related hormonal alterations, or the accompanying mood, sleep and vasomotor symptoms, may modulate melatonin activity, e.g. by accelerating its reduction. On the other hand, it is also possible that the age-related changes in melatonin, whether or not attributable to the hypothalamus-pituitary-ovarian-axis-related hormone changes, contribute themselves to the modulation of ovarian hormone fluctuations, or to the menopause-associated mood, sleep and vasomotor symptoms.

To date, only a few works have focused on this issue. In the study of Okatani et al.[20] the nighttime serum melatonin concentration (measured via 2-hour interval samples between 20.00 and 08:00 hr) was higher in the oldest premenopausal women (aged 46 to 50 years, i.e. likely perimenopausal) compared to younger premenopausal women with or without oophorectomy. Additionally, they found that nocturnal melatonin concentration and secretion decreased steeply in the first 15 years since the beginning of menopause, and continued to decrease more gradually thereafter. In that study reproductive state was determined on the basis of menstrual records. Vakkuri et al.[19] studied 77 women aged 30 to 75 years, dividing

them into premenopausal and postmenopausal groups on the basis of their menstrual records, and thereafter into six further age groups. Nocturnal (20.00-08.00 hr) urinary excretion of melatonin and morning (09.00 hr) serum melatonin were measured. Urinary melatonin levels were found to decline during the menopausal transition, the most significantly in women aged 40-44 years, followed by those over 50 years; also, the serum morning melatonin levels tended to be lower in women aged 60 years and over compared to women younger than 40 years. Keeping in mind the different age ranges of the participants, as well as the different melatonin sampling (nocturnal urinary and morning serum samples in the Vakkuri et al. study,[19] vs. serial nocturnal serum samples in our current work), these results are in line with our findings of decreased melatonin concentration in postmenopause. Since the drop in melatonin levels was most notable far before menopausal age, the authors concluded that it could be permissively linked to the initiation of menopause. Frequently, changes in the hypothalamus-pituitary-ovarian function start several years before the actual cessation of menstrual periods, i.e. at the age where the drop in melatonin level was more evident. Further, Vakkuri et al.[19] reported a negative correlation between urinary melatonin and serum FSH. Fernandez et al.[18] found the lowest values of morning serum (but not urinary) melatonin levels in postmenopausal women. Moreover, they found no correlation between melatonin and FSH, E2 or progesterone levels during the perimenopausal period, but a negative correlation between FSH and melatonin levels in the postmenopausal women. We did not find any association between melatonin and FSH or E2 levels in either perimenopausal or postmenopausal women. A plausible reason for the lack of such correlation was a considerable inter-individual variation in melatonin secretion in our study. However, it may not be ignored that we used the mean value of repeated nocturnal serum samples, instead of an overnight urinary sample as in the study of Vakkuri et al.[19] or a single morning serum sample as in the study of Fernandez et al.[18] Even though urinary melatonin (or its metabolite)

sampling technique is among the most practical ones for the assessment of melatonin secretion, given its limited possibility of repeated samples, it may lack in precision. Saliva sampling is also a practical and reliable technique, which allows repeated samples; however, it can hardly be used for overnight assessment. On the contrary, repeated blood samples can be more easily taken at frequent intervals during night without or with limited sleep disruption. In addition, the levels of melatonin are higher in the plasma than in the saliva, thus implying a better resolution and sensitivity. In detail, overnight blood sampling at frequent intervals seems to be the most informative technique for the assessment of melatonin profile.[50]

Our results also suggest that the nighttime pattern of rise and fall in melatonin levels, including the melatonin peak time, does not significantly differ between perimenopausal and postmenopausal women. This finding is in contrast with the earlier report of Walters et al.[51] on the advanced phase of melatonin secretion in postmenopausal compared with premenopausal women during one-night sleep deprivation. However, even though in the same study the melatonin onset was found to precede the onset of subjective sleepiness equally in the premenopausal and postmenopausal women, the time between the melatonin onset and that of sleepiness was longer in postmenopausal than premenopausal women.[51] Possible explanations for these different outcomes may be the different study designs, since Walters et al.[51] assessed salivary melatonin from samples collected hourly for 22 hours during sleep deprivation under constant routine conditions, and studied younger groups (premenopausal aged 38-46 years, postmenopausal aged 53-57 years), whereas we used repeated serum samples and older study groups (perimenopausal aged 43-51 years, postmenopausal aged 58-71 years) in normal sleeping condition.

Melatonin concentration and secretion pattern seemed not to be related to climacteric vasomotor symptoms or to BMI either in perimenopausal or in postmenopausal women. As

expected, postmenopausal women had more climacteric vasomotor symptoms than perimenopausal women. They also had a higher BMI, which is known to increase with the transition to menopause, even with small differences according to the type of menopause.[52] It is possible that, besides other known factors such as changes in estrogen and FSH levels, the menopause- and age-related changes in melatonin levels also contribute to these symptoms and body changes in postmenopausal women. A recent animal study showed that administration of melatonin was more effective than estrogen therapy in reversing the glycemic and lipid dysregulation as well as in restoring the increased BMI after the ovariectomy.[53] According to another study administration of melatonin to perimenopausal and postmenopausal women led to a tendency of reduction in climacteric symptoms.[54] However, other studies did not support these results.[55,56] Our correlation and interaction analyses did not find any association between melatonin and climacteric symptoms or BMI, even after controlling for the effect of menopausal status.

Also, melatonin concentration and secretion pattern seemed not to be related to depressive symptoms or sleep disturbances either in perimenopausal or in postmenopausal women. In our sample, postmenopausal women tended to have more depressive and insomnia symptoms. Instead, anxiety and the quality of life scores did not differ between perimenopausal and postmenopausal women. This is in line with the well-known increased risk of mood symptoms and disorders[22] and decrease in sleep quality[57-60] during the menopausal transition. In early (<5 years) versus late (>5 years) postmenopausal women, Hachul et al.[61] found more depression, anxiety and sleepiness among the latter group. In general, it has been reported that perimenopausal and especially postmenopausal women suffer from subjective sleep problems more than premenopausal women.[57-60] In this respect, a prospective study[62] showed that even after controlling for age and other confounding factors, women had higher odds for both moderate and severe self-reported sleep problems when transiting from premenopause to

perimenopause, and even higher odds when transiting to postmenopause. However, perhaps because of the small sample size, correlation and interaction analyses did not detect any significant associations between melatonin and BDI or BNSQ insomnia scores. Hence, these findings do not support our original hypothesis that low melatonin levels associate with depressive, anxiety or sleep symptoms in postmenopausal women. It is of note that melatonin levels was associated with other parameters in the perimenopausal group: a delayed peak time was associated with a higher level of anxiety, while a longer duration of melatonin secretion was associated with better quality of life. It is possible that higher levels of anxiety postponed the onset of sleep and subsequently the peak of melatonin secretion, whereas longer durations of melatonin secretion could improve sleep quality and subsequently the quality of life.

This study was the first one to use repeated serum sampling technique to evaluate the interrelationship of melatonin secretion and menopausal status. This seems to be the best technique as assessment of melatonin phase, duration and amplitude are concerned, in particular when frequent (20-30 minutes) samples are provided.[50] Even if the study was not carried out under constant routine conditions, which would have been the most appropriate technique, nevertheless the high-frequency collection of serum samples under strictly controlled sleep laboratory conditions ensured the good quality of the samples in order to monitor the pattern of melatonin concentrations. In addition, several confounding factors were effectively ruled out by the accurate exclusion criteria, such as irregular sleep-wake schedules, the use of HT and other medications, smoking as well as the use of alcohol or drugs.

The main limitation of our study is a rather small sample size in the context of a convenience sampling design. This, along with the elevated inter-individual variability in melatonin levels, may partly explain the absence of any significant correlation between melatonin and FSH or E2 levels, or most of the mood and sleep symptoms. However, the sample size was

comparable with that of other studies in the field, and even with this limited sample size, significant differences in melatonin levels were detected. The study was carried out on a healthy population, preventing the generalization of the results to populations with common diseases. Further, some of the women in the perimenopausal group had regular menstruation, categorizing them as premenopausal. In addition, it must be noticed that mood, sleep and climacteric symptoms and quality of life were retrospectively assessed with self-reported questionnaires that covered different timeframes of recall (ranging from current quality of life to climacteric symptoms in the past six months), being a potential weakness in the measurement of self-reported data. However, the reliability and validity of most of these instruments have been tested and found to be reliable.[46,63,64] The melatonin sampling took place throughout the year, likely influencing the results. However, during the visit in the sleep laboratory the participants spent their time inside the building and the nighttime illumination levels were strictly controlled. Finally, the cross-sectional design of the study did not allow any causal conclusions.

Conclusions

Our results confirm lower nighttime serum melatonin concentrations and exposure levels in postmenopausal women compared with perimenopausal women, with no difference in melatonin peak time. There was also a tendency towards longer duration of melatonin secretion in perimenopausal women. Further research with prospective follow-up studies during menopausal transition is needed to better understand the nature of these differences. Changes in melatonin levels were not related to mood, sleep quality, vasomotor symptoms or quality of life either in perimenopause or postmenopause. This finding needs to be confirmed in larger studies. Whether the beneficial effect of HT on alleviation of these symptoms in menopause is partly regulated via melatonin needs to be verified.

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Figure 1. Mean nighttime serum melatonin concentrations (pg/ml) of perimenopausal and postmenopausal women.

List of Supplemental Digital Content

- Supplemental Digital Content 1. Appendix that reports the BNSQ questions concerning insomnia and sleepiness
- Supplemental Digital Content 2. Appendix that reports the questions concerning the sleep of preceding night used to calculate the subjective sleep score