Decrease in melatonin precedes follicle-stimulating hormone increase during perimenopause

Olli Vakkuri1, Aarre Kivelä2, Juhani Leppäluoto1, Maija Valtonen3 and Antti Kauppila2

Departments of Physiology1 and Obstetrics and Gynecology2, University of Oulu, Kajaanintie, Oulu, Finland; Veterinary Research Station2, University of Kuopio, Kuopio, Finland


Melatonin, the hormone of the pineal gland, which in animal studies has been found to inhibit aging processes, is secreted in smaller amounts towards senescence. Menopause, an aging process in women, is known to be associated with typical changes in gonadotropin and sex steroid secretion. Our main objective was to study the possible role of melatonin in the hormonal regulation of menopause. This study focused on detailed changes in melatonin and follicle-stimulating hormone (FSH) secretion cross-sectionally in pre- to postmenopausal females. Special attention was paid to females aged around 50 years, which is the mean menopausal age. Seventy-seven healthy female volunteers aged 30–75 years were the subjects of this study. Melatonin was measured radioimmunologically from nocturnal urine collected between 20.00 and 08.00 h, and FSH and melatonin from blood samples taken at 09.00 h. Nocturnal urinary excretion of melatonin was found to decline significantly from premenopause to postmenopause. The youngest premenopausal women (age group 30–39 years) excreted the highest amounts of melatonin (21.1 ± 2.2 pmol/h, mean ± SEM, N = 17). In the age group 40–44 years the excretion declined by 41% (p < 0.05). The second significant decline (35%, p < 0.05) took place between the age groups 50–54 years and 55–59 years. A declining trend as a function of age was also seen in morning serum melatonin. Serum FSH rose sharply to high levels before the age of 50 (p < 0.01) and remained at a high level thereafter. Urinary melatonin correlated negatively with serum FSH (r = −0.32, p < 0.05). In conclusion, the inverse changes in melatonin and FSH secretion during the perimenopausal years, with the sharpest decline in nocturnal excretion of melatonin far before menopause, suggest that melatonin may be permissively linked to the initiation of menopause. O Vakkuri, Department of Physiology, University of Oulu, Kajaanintie 52 A, 90220 Oulu, Finland

Menopause is a unique phenomenon in that female reproductive competence goes away as a result of cessation of ovarian function far before senescence. It involves several hormonal changes, of which a decrease in the synthesis of ovarian steroids and inhibit and an increase in the synthesis and release of pituitary FSH and LH are the most typical (1, 2). Although it has been proposed that the sensitive ovarian follicles are exhausted by menopause (3), it is not known what factors determine the timing of menopause and what mechanisms trigger the menopausal development.

Synthesis and secretion of melatonin, the pineal hormone, takes place during the night, the dark time of the day. Thus, it mediates rhythmic information of night length to the organism (4). In addition to its diurnal and seasonal significance, melatonin may be involved essentially in the timing of puberty. Circulating melatonin levels have been observed to decrease during childhood, from under 5 years up to puberty, which may be critical for the initiation of puberty (5–7). Melatonin secretion has been reported to decrease also after puberty as a function of age (6, 8–10). It is not known whether this decrease in adults is gradual and associated with normal aging or whether it is linked to the cessation of female reproduction during menopause. In previous studies mentioned above, data on menopausal melatonin changes are fragmentary. Fernandez et al. (11) reported a decrease in morning serum melatonin but, controversially, no changes in urinary excretion of melatonin in some cohorts of menopausal women as compared with fertile women. Therefore, we were interested to study the pattern of melatonin secretion throughout the perimenopausal years more thoroughly.

Subjects and material
The subjects of this study were female volunteers aged 30–75 years who gave their informed consent. Most of the women were healthy but some were treated for a benign disease (due to uterine fibroma, genital prolapse or urinary incontinence) not related to ovarian function. The subjects were asked to give their menstrual records in order to assess the reproductive state

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(premenopausal and postmenopausal women). The subjects received no hormonal treatment for at least 1 year before the study. The total number of subjects was 77. The data were pooled according to six age groups: 30–39 (N = 17), 40–44 (N = 15), 45–49 (N = 13), 50–54 (N = 14), 55–59 (N = 8) and over 60 (N = 10) years.

Melatonin secretion was followed by measuring the nocturnal excretion of melatonin into urine. This was collected between 20.00 and 08.00 h. Additionally, blood samples were taken for serum FSH and melatonin measurements at 09.00 h. In fertile women, sampling was carried out without any menstrual timing because according to our (12) and other studies (13, 14) melatonin does not significantly vary during the menstrual cycle. The whole collection procedure was carried out over a 2-month period (October–November).

Urinary and serum melatonin were determined radio-immunologically as described previously (15) using [2,125I]melatonin (16) as tracer. The purity of the tracer at the time of use was 96%. Serum FSH was analyzed with a commercial time-resolved FIA kit developed for human FSH (Delfia, Wallac, Turku, Finland).

Statistical analyses were carried out using one-way analysis of variance. Correlation analyses and curve fittings of the raw data were based on the FigP graphics program.

Results

A scattergram with curve fittings of the results of the urine melatonin and serum FSH analyses is presented in Fig. 1A. Urinary excretion of nocturnal melatonin was approximately 20 pmol/h in premenopausal women of < 40 years but about 8 pmol/h in postmenopausal women of > 60 years. The 60% decrease was significant (p < 0.01). The best curve fitting for this decrease is cubic spline (Fig. 1A).

The melatonin data of Fig. 1A divided into six age groups (Fig. 1B) shows that melatonin excretion decreased by 41% (p < 0.05) from 21.1 ± 2.2 pmol/h (mean ± SEM. N = 17) in premenopausal women of < 40 years to 12.5 ± 2.2 pmol/h (N = 15) in women of 40–44 years. Thereafter, melatonin excretion decreased to 11.2 ± 1.3 (N = 13) in women of 50–54 years (p > 0.05 compared with age groups 40–44 and 45–49 years) and then by 35% (p < 0.05) to 7.3 ± 0.9 (N = 8) pmol/h in women of 55–59 years.

Serum FSH level was generally < 10 IU/l before menopause and > 40 IU/l after the age of 50 years. These FSH observations could be curve-fitted according to the exponential sigmoid model (an exponential increase between two plateaus). More exactly, serum FSH rose from 4.0 ± 0.6 IU/l (40–44 years) to 15.3 ± 4.0 IU/l (45–49 years) and then furthermore to 41.8 ± 7.8 IU/l (50–54 years); p < 0.01 in both cases (Fig. 1B). Thus, the FSH rise started about 5 years later than the decline in urinary melatonin. The changes in serum FSH during perimenopause correlated negatively to the corresponding changes in urinary

Fig. 1. Nocturnal melatonin (solid triangle) excretion into urine and serum FSH (open circle, sampled at 09.00 h) as a function of female age, presented as a scatter-plot with curve fittings (A) and in six age groups (B, solid column for melatonin and open column for FSH; means ± SEM). *p < 0.05 for melatonin; #p < 0.01 for FSH.

Fig. 2. Serum melatonin as a function of female age in a scattergram presentation with linear regression analysis (y = -0.13x + 18.2). All serum samples were collected at 09.00 h.
excretion of melatonin \(y = -5.38x + 37.9; r = -0.32, p < 0.05\).

A scattergram of serum melatonin levels at 09.00 h (Fig. 2) shows that the morning level in the youngest age group was 53.3 ± 7.3 pmol/l, whereas the level in the oldest age group was 33.5 ± 6.5 pmol/l. The difference was not significant. The curve fitting used in Fig. 2 for serum melatonin data is based on linear regression analysis \(y = -0.13x + 18.2\). The negative correlation \((r = -0.12)\) between serum melatonin and age was not statistically significant \((p > 0.05)\).

**Discussion**

In the present study we observed a clearcut decrease (60%) in nocturnal melatonin excretion from fertile premenopausal ages of < 40 years to postmenopause of > 60 years. This finding is in accordance with and confirms the previous results on changes in circulating melatonin as a function of human age. After the first controversial results in the early 1980s (17–23), an age-related decline in melatonin secretion and a negative correlation between melatonin secretion and age from childhood to senescence has been reported unambiguously in several studies (6, 8, 9, 24, 25). The different findings reported in the early 1980s may be partly due to methodological reasons, to sampling only during daytime or to a limited age range monitored for melatonin secretion.

Our study focused especially on the rate and pattern of the decline in melatonin excretion from premenopause to postmenopause, i.e. throughout the mean menopausal age, which has been observed to be 51 years in Finnish women (26). In our curve-fitting calculations the cubic spline was the best descriptor of the age-related changes in urinary excretion of nocturnal melatonin. This indicates that the decline was not steady. Furthermore, when presented as age groups of 5 years we observed two steps in the decline: the first one (41%) when moving from premenopausal women (< 40 years) to women of 40–44 years, and the second one (35%) when moving from women of 50–54 years to women of 55–59 years. In contrast to our results, Fernandez et al. (11) did not find any changes in urinary excretion of melatonin during menopause, possible because they had only three age groups which did not cover the whole age range between fertile and postmenopausal ages.

The rate, gradual or stepwise, of the melatonin decrease with age is interesting and may have physiological significance. According to Waldhauser et al. (5, 6), the decline in melatonin levels is biphasic: the first decline taking place from early childhood to adolescence is steep (75–80%), whereas the second one seen in adults is much smaller. In both cases the decline was found to be steady without any clear steps. Physiologically, the first decline has been connected to intense growth and therefore to increasing body size in children, rather than to decreased secretion of melatonin per day (6, 27), when taking into account also lack of pineal growth during childhood (28). In adults the decrease is likely to be associated with a decreasing activity of the pineal gland, which is a process associated with aging. The corresponding decline has also been observed in GH and several other hormones (29, 30).

Our results on an age-related decrease in urinary melatonin are in general agreement with those on serum melatonin by Waldhauser et al. (6). However, a direct comparison is not possible because Waldhauser et al. (6) had only four adult age groups with an age range of 15–20 years/group. They found decreased levels in nocturnal serum melatonin after menopause in older women, but not premenopausally. Due to the wide age limits of the cohorts, closer inspection about the association of melatonin to menopause was not possible. It must be pointed out also that Waldhauser et al. (6) measured nocturnal serum melatonin from a single blood sample, which might therefore not be as informative as whole nocturnal urine is.

We measured also morning serum melatonin in conjunction with FSH measurements. Morning melatonin gives only limited information because, in contrast to high nocturnal levels, morning levels (at 09.00 h, 1–2 h after awakening) are fairly low and very close to low daytime levels (31). Additionally, we performed the study in autumn when day length is getting shorter (in November, < 8 h in Oulu) and therefore there might have been differences in the diurnal rhythmic phase between the subjects. However, a trend to lower serum melatonin levels was observed as a function of age. It should be pointed out that Fernandez et al. (11) found a significant decrease in morning serum melatonin, but only when the comparison was carried out to the melatonin level in the follicular phase, not to that of the luteal phase. This was surprising because, according to recent observations, circulating melatonin has no menstrual variation (12–14).

The inverse change in melatonin as compared to FSH from premenopause to postmenopause is the most interesting result of the present study. The first decrease seen in nocturnal melatonin levels (5, 6) in the course of childhood and the corresponding increase in nocturnal FSH levels may be closely associated with changes occurring during growth and finally with the triggering of pubertal development. The slower and more gradual decrease in adult people has been connected to aging. The age-related decrease in melatonin secretion may have some clinical implications. Decreased circulating melatonin may increase the susceptibility of the organism to oxidative damage, because recent studies refer to a natural anti-aging hormone character of melatonin, protecting the organism against the aging processes of free radicals and thus also against cancer (10, 32). Normal melatonin secretion also appears to be
necessary for keeping up the circadian pacemaker system (33). According to recent in vitro studies, melatonin is involved in programmed cell death (apoptosis) via interleukins (34). Additionally, in rats the injection of synthetic melatonin has been shown to increase their physical condition and life time (35). However, due to pharmacological doses of melatonin used, especially in the older studies, all results cannot be regarded as physiologically relevant.

The relationships of melatonin, FSH (and LH) and sex steroids take a new steady state menopauserally. In particular FSH increases sharply during menopause, as seen also in the present study. This increase is a consequence of a decreased secretion of estradiol and other sex steroids, as well as in inhibit (1,2). A rise in FSH is one of the best menopausal indicators. It has been observed that administration of melatonin to postmenopausal women suppresses LH levels (36), which is also in accordance with the inverse relationship between melatonin and gonadotropins. Melatonin has been found to be anti-oestrogenic but stimulatory to progestosterone production (37). According to our results, the clearest changes in melatonin secretion take place before menopause, i.e. in women just over the age of 40 years. Thus, the changes in melatonin and FSH are analogous to those in puberty, but not simultaneous. Because the changes in melatonin precede those in FSH, melatonin may have a permissive role in the development of gonadal atrophy. Thus, melatonin may be one factor in the circadian timing mechanisms of the menopause, although the exact mechanism (i.e. the relationship between melatonin, hypophysis and ovary with exhausting follicles) cannot be evaluated in this study.

In conclusion, our results on premenopausal decline in urinary excretion of melatonin provide some evidence for a connection between melatonin and menopause. Based on inverse changes between melatonin and FSH, and also taking into account temporal relationships, i.e. the early premenopausal decrease in melatonin, we suggest that melatonin may trigger menopausal development, as concluded analogously with respect to puberty.

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