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Seasonal pattern of melatonin excretion in humans: relationship to daylength variation rate and geomagnetic field fluctuations

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Abstract. In order to investigate the influence of various environmental parameters on melatonin excretion, the night-time urinary melatonin excretion of 16 healthy volunteers was measured in samples collected monthly over a period of one year. No significant interindividual differences were detected in the monthly rate of change of melatonin excretion. A seasonal bimodal pattern did, however, emerge. Peak values were observed in June and November. In these months a combination of high daylength stability and low values of the vertical component of the geomagnetic field was recorded. Trough values were found in April and August–October when low daylength stability was combined with high values of the vertical component of the geomagnetic field. We propose that the daylength variation rate, and the fluctuations of the vertical component of the geomagnetic field, interact to induce the changes in melatonin secretion which signalize the different seasons in humans.
Key words. Melatonin; excretion; seasonal pattern; daylength variation rate; geomagnetic field.

Melatonin is a hormone synthesized and secreted by the pineal gland[1]. In mammals, including humans, and other vertebrates, melatonin synthesis and secretion exhibits a circadian rhythm. It shows maximal values during darkness and is almost undetectable during daylight[2]. Furthermore, melatonin displays annual fluctuations. These are well documented in animals[3] and have been reported in humans[4–7], suggesting the existence of an additional substantial seasonal rhythm[3–7]. This rhythm has been attributed to the adjustment of the circadian pattern of melatonin secretion – either its duration or its phase shift[8] – to the changing daylength throughout the year. It thus provides the organism with time-of-year information[9]. These seasonal fluctuations are claimed to entrain essential biological phenomena such as reproduction[10–12], sexual maturation[13–15], hibernation[16], thermoregulation[17], immunomodulation[18,19] and possibly other important functions and behaviors[20]. On the other hand, perturbations of melatonin secretion have been implicated in pathological conditions like cancer[21–23], depressive and other psychiatric disorders[24,25] and neuroendocrine dysfunctions[26], and in the aging process[27–29].

Over the last two decades several studies have shown an apparent sensitivity of the pineal gland to static and extremely low frequency electromagnetic fields, of the magnitude of the earth's field, which implies that exposure to artificial magnetic fields, as well as to variations in natural and artificial light[30–32], can alter the secretion of melatonin and perturb its rhythmicity[33–41]. These findings suggest that beyond the proven photosensitivity of the pineal gland, there may be other external factors which also entrain its activity throughout the year[42–43]. Disclosing such factors and decoding their mode of action could provide important information on melatonin's multiple interactions with the central nervous system and numerous endocrine and immune processes. Furthermore, the investigation of the nature of mammalian magnetosensitivity could contribute to new insight into the way environmental changes influence physiological and behavioral parameters[44].

The aim of the present study was to measure the seasonal variation in overnight urinary melatonin excretion in healthy individuals, and to investigate its correlation with environmental factors such as the daylength variation rate and geomagnetic field fluctuations.

Materials and methods

Urine samples for the estimation of night-time urinary melatonin were collected on the first Wednesday of each month (± one day) over a period of 12 months (July 1988–June 1989) from 16 healthy volunteers (mean age 34.38 ± 1.60 years, 10 males and 6 females). Subjects were medically and psychiatrically screened to exclude those with a major physical or mental disease, as well as those taking medication continuously (except for contraceptives). For each subject, demographic data and additional information on several environmental and social parameters were also obtained for each month and for the week preceding the urine collection. The total urine volume produced during the night, after discarding the last micturition before bedtime, and including the first morning urination, was collected. The total urine volume was recorded and a portion was stored frozen at −20 °C. At the end of the study all urine samples were transported to Stockholm (Sweden) on dry ice, and analyzed using a specific radioimmunoassay developed for use with urine and blood samples[45]. Previous studies have shown that urinary melatonin is stable under these conditions of storage and transfer[46]. Urinary melatonin concentration in samples collected in this way correlates highly with the 2 a.m. peak value of serum melatonin[47]. The assay had a sensitivity of 0.01 nmoles/l. Interassay variability was 4.8% for melatonin levels above 0.15 nmol/l. Melatonin values were expressed as the concentration in nmol/l.

The monthly rate of change in melatonin excretion was defined as the percentage increment or decrement from the value of the preceding month. Daylength was considered to be the time (in minutes) from dawn to dusk (Athens local time, latitude 37° 52′ North). The daylength variation rate (in minutes) was calculated by subtracting the daylength of the first day from that of the last day of each month, and dividing by the total number of days in the particular month. Variation rate values below two minutes per day were codified as low, indicating a high daylength stability for the specific month, while higher values were codified as high, indicating a low daylength stability. Monthly mean values of the horizontal and vertical components of the geomagnetic field (in nanoTesla) were calculated from data from 'Istituto Nazionale di Geofisica, Osservatorio Geomagnetico L'Aquila, latitude 42° 23′ N, Italy' (published in "Risalti delle osservazioni magnetiche" 1988 and 1989). Values of the vertical component of the geomagnetic field above 38579 nT (one nT above the mean value for the specific year and specific place) were codified as high values.

In the statistical analysis, Student's t-test was used for comparisons between means (±SE), the Chi square test was applied for comparisons of non-parametric variables, and correlation coefficients were calculated for the detection of inter-group differences in fluctuating values. Manova for repeated measurements was applied to detect differences between months, and one-way Anova was used for the detection of interindividual differences. Multiple linear regression analysed the potential relation between the monthly rate of change in melatonin and independent parameters. Bivariate spectral (Fourier) analysis, which uncovers the correlation between two time series at different frequencies, was used in order to investigate the possible time synchronization (periodicity) of the dependent variable with the independent one. In all cases the spectral estimates were smoothed with a Parzen window of width 3. Finally, analysis of variance was used to define the possible interactions between daylength variation rate, the vertical component of the geomagnetic field and the monthly rate of change in melatonin excretion.

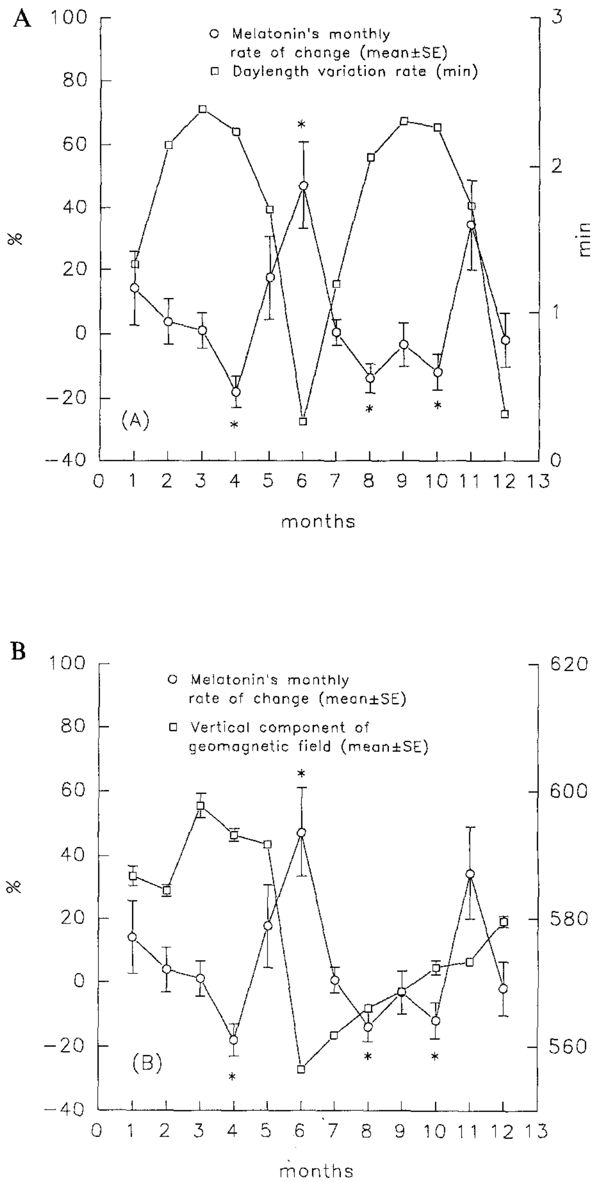

Figure. *A)* Monthly rate of change of melatonin excretion ± SE (○) and daylength variation rate (□) across the year. *B)* Monthly rate of change of melatonin excretion ± SE (○) and monthly means of the vertical component of the geomagnetic field ± SE (□) across the year, p* < 0.05.

Results

No significant difference in the monthly rate of change of melatonin excretion among individuals was observed (Anova, F Ratio = 0.8242, $p < 0.62$). The regression analysis showed no correlation between the monthly rate of change and various parameters (time of falling asleep, waking-up time, total sleep time, sleep quality, overnight total urine volume, time of urine collection in the morning, travelling, presence of minor sickness, medication use and events that changed the daily rhythm; regression coefficient, $R^2 = 0.27$, NS). Manova for repeated measurements showed that the monthly rate of change in melatonin excretion exhibited signifi-

Table 1. Analysis of variance of the monthly rate of change of melatonin excretion by daylength stability rate (high-low), and by the vertical component of the geomagnetic field (high-low) covariate by person.

Source of variation	SS	DF	F	Sig of F
Day variation rate (low/high)	14,107.67	1	9.68	0.002
Vertical component of geomagnetic field (low/high)	72.34	1	0.05	0.824
Vertical component × Day variation	6208.25	1	4.26	0.040
Person × Day variation	1076.42	1	0.74	0.391
Vertical component × Person	1502.26	1	1.03	0.311
Person	1.57	1	0.00	0.974
Explained	41,075.23	6	4.70	0.000
(Total)	310,783.97	191		

SS = sums of squares, DF = degree of freedom, Sig = significance of F, Explained = total sum of squares for the main effect and interaction terms in the model.

cantly higher values in June, a tendency towards high values in November, and significantly lower values in April, August and October (figure A, B). These values correspond to the values for melatonin excretion for the beginning of July, December, May, September and November. There was no seasonal pattern in overnight urine production (Manova) and no correlation was found between the amount of monthly overnight urine production and the monthly rate of change in melatonin excretion (correlation coefficient, $r = -0.12$, NS).

The monthly rate of change of melatonin excretion showed a significant negative correlation with the daylength variation rate (correlation coefficient, $r = -0.54$, $p < 0.03$) but no correlation with the photoperiod expressed as the mean monthly daylength (correlation coefficient, $r = 0.07$, $p < 0.4$). Moreover, the daylength variation rate had a high negative but not significant relation to the horizontal component of the geomagnetic field (correlation coefficient, $r = -0.39$, $p < 0.1$) and a similarly high but not significant positive one to the vertical component of the geomagnetic field (correlation coefficient, $r = 0.40$, $p < 0.1$).

In the bivariate cross spectrum analysis the dependence of the cross-amplitude versus the frequency distribution was checked. The frequency components of these two time series, i.e daylength variation rate considered as the independent variable, and the monthly rate of change of melatonin excretion considered as the dependent variable, covary, exhibiting a high cross-amplitude component at the period of 6 months and a lower one at 3 months. Furthermore, a high cross-amplitude component at the period of 6 months and a lower one at 2.5 months was observed between the vertical component of the geomagnetic field considered as the independent variable and the monthly rate of change in melatonin considered as the dependent variable. Likewise, the covariation of either the mean monthly daylength or the horizontal component of the geomagnetic field and the monthly rate of change of melatonin showed a high cross-amplitude at the period of 12 months.

The analysis of variance of the monthly rate of change of melatonin with two factors covariate by person showed a robust statistically significant relationship to the daylength variation rate when codified as high or low, and a significant interaction between the daylength variation rate and the vertical component of the geomagnetic field codified as high or low (table). The lowest values over the year for the monthly rate of change in melatonin excretion synchronize with high values of the daylength variation rate and high values of the vertical component of the geomagnetic field; this situation occurs close to the spring and fall equinoxes. In contrast, the highest values occur close to the summer and winter solstices, coinciding with low values of the daylength variation rate and low values of the vertical component of the geomagnetic field (figure A, B).

Annual means of the monthly rate of change in melatonin excretion did not differ between males and females (5.54 ± 1.34 vs 6.22 ± 1.23, NS), while their annual rhythm was highly correlated (correlation coefficient, $r = 0.81$, $p < 0.001$). It should be noted that the men and women in the study did not differ in demographic and personal characteristics, except that the males were significantly heavier and taller, as expected (77.00 ± 3.07 vs 63.67 ± 2.95 kg, $p < 0.01$; 177.00 ± 1.92 vs 168.33 ± 2.72 cm, $p < 0.03$).

Discussion

This study has revealed the existence of a biannual rhythm in the monthly rate of change of melatonin excretion at latitude 37° North. The monthly rate of change exhibits a bimodal curve with one clear peak in June when the photoperiod is the longest. There is a second, though not statistically significant, peak in November, when the photoperiod is short. Two significant troughs are present in April and August–October. Fairly similar results, showing a bimodal seasonal variation in the morning level of plasma melatonin[4–6], in urinary 6-sulphatoxymelatonin concentrations[7], and midnight plasma melatonin concentrations[5] have been previously reported in healthy subjects. Moreover, a post mortem study on human pineal glands pointed to the same pattern of melatonin synthesis[48]. The excretion rhythm shows a strong negative correlation with the daylength variation rate. Thus, the high values of the rate of change in excretion are observed during periods which have different daylength, but all have a very low daylength variation rate, i.e. periods with a high daylength stability. Inversely, the troughs are observed during periods with a high daylength variation rate, i.e. a low daylength stability. The daylength variation rate is a constant with a periodicity of 6 months, and expresses the changing acceleration of the earth rotating round the sun. That the changing acceleration by itself may be the direct trigger of the observed seasonal fluctuations of melatonin remains in the field of speculation, since neither this particular study nor any other study, to our knowledge, provides the necessary experimental data for this calculation to be made. It seems more likely that the relative stability of the photoperiod in the summer and winter solstices, and its rapid change in the spring and fall equinoxes, modify the excretion of melatonin. It should be noted that no correlation was found between changes in melatonin excretion rates and the mean monthly daylength. A prominent circadian rhythm characterizes urinary melatonin concentrations, which peak in the middle of the night, and the nyctohemeral acrophases are remarkably stable (around 3 a.m.) whatever the time of the year[27]. The seasonal rhythmicity of melatonin seems to be dependent on the daylength stability, which influences the magnitude of its secretion independently of its duration.

Furthermore, a negative correlation and a significant cross-amplitude on a semiannual basis were found between the monthly rate of change in melatonin excretion and the vertical component of the geomagnetic field. A correlation between the horizontal component of the geomagnetic field and melatonin excretion in animals has been recently reported. However, no strict correspondence was found to the actual values of the particular year, but only to monthly averages calculated over a few years[42]. Yet in our study, neither a correlation, nor a cross-amplitude between the monthly rate of change in melatonin levels and the horizontal component could be demonstrated. As far as we know from experimental animal studies, manipulations of either the horizontal or the vertical component of the natural magnetic field may alter the activity of the pineal, causing a marked decrease in melatonin synthesis[35,36,40,41]. The intensity of the vertical component of the earth's magnetic field has been found to act as a weak Zeitgeber in house sparrows[49], while elimination of the vertical component of the Earth's field influences the orientation ability of the European robin[50]. In support of our findings are reports suggesting that the pineal gland cells responded with a depression of their electrical activity when a magnetic stimulus was added to the vertical component of the earth's magnetic field[33,51]. Generally, it seems that many biological parameters and behaviors are somewhat influenced by magnetic and electromagnetic fields in humans and animals[52–60]. The sensitivity of the pineal gland to static and extremely low frequency electromagnetic fields of the magnitude of that of the earth has been demonstrated[40], although the magnetic parameters involved have not been elucidated. The above findings, along with the observed seasonal secretion of melatonin in the absence of a natural strong light/dark cycle in polar latitudes[6], implies that it is also entrained by exogenous factors other than light. We propose that the vertical component of the geomagnetic field could act as such an external synchronizer, influencing the pineal gland's seasonal synthesis of melatonin irrespective of the daylength.

The large interindividual differences in the monthly excretion of overnight urinary melatonin is well known[61–63]. In spite of this, there was no difference in the monthly rate of change among the subjects, and various situational factors did not alter this rate significantly. This finding supports the notion that melatonin's seasonal fluctuations should be determined by its monthly rate of change than by its absolute value. Age will hardly have influenced melatonin excretion in our study, because the age range of the subjects was narrow. The mean body weights and heights of the males involved in this study were greater than those of the females, but this difference does not seem to have influenced the monthly rate of change of melatonin excretion, because it remained stable over the year. The negative correlation between height and urinary melatonin which was found in a previous study refers to the actual melatonin values, and refer to the whole population rather than male or female separately[64]. Yearly and monthly means of the monthly rate of change of melatonin excretion exhibited no difference between males and females, a fact indicating that the seasonal production rhythm is controlled by factors influencing the activity of the pineal equally in both genders, and independently of the constitutional capacity for synthesis and secretion. The total monthly urine volume and the monthly rate of change of melatonin excretion showed no significant correlation with each other. Although only a small percentage of the melatonin secreted by the pineal is excreted in the urine[65,66], melatonin values in the morning collection of overnight urine analyzed by our method have been found to correlate highly with the 02.00–03.00 hr secretory peak of the hormone in serum[47]. Occasional use of analgesics, antibiotics, antiinflammatory drugs and minor anxiolytics for short periods (mostly 1–2 days), did not have a significant influence on the monthly rate of change of melatonin excretion in the regression analysis.

In conclusion, we suggest that independent of the duration of the underlying secretion, the level of the overnight urinary melatonin exhibits a clear seasonal bimodal pattern at latitude 37° North, where there are four well-defined seasons. The peaks are present in June/November and are triggered by the high daylength stability in combination with low values of the vertical component of the geomagnetic field. The troughs are present in April/August–October when a low daylength stability is combined with high values of the vertical component of the geomagnetic field. Therefore, we propose that the daylength variation rate and the fluctuations of the vertical component of the geomagnetic field, interacting with each other, induce the changes in melatonin secretion which signalize the different seasons to the organism.

The influence of the above parameters on melatonin secretion seems to be so strong that it practically eliminates all the other environmental, social or constitutional noise to which individuals are exposed. However, the relative importance of each of these two factors in the process triggering melatonin's seasonal fluctuations remains to be elucidated by studies of subjects living at different latitudes, with various light and geomagnetic conditions.

To our knowledge, this is the first report pointing to a relationship between the daylength variation rate, geomagnetic field fluctuations and melatonin excretion in humans. Considering the involvement of melatonin in multiple physiological and pathological processes, a field of rich implications is to be opened for future research.

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