Annual changes in 6-sulphatoxymelatonin excretion in man

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Abstract: A recently developed RIA for 6-sulphatoxymelatonin, the major urinary metabolite of melatonin, has been used to investigate the annual change in melatonin secretion in humans. Twenty plasma samples were taken from 18 volunteers throughout a 24-h period and simultaneous 6-hourly urine samples were also collected. Plasma melatonin and urinary 6-sulphatoxymelatonin were measured by RIA. 6-Sulphatoxymelatonin assayed in the urine samples was shown to be a good index of the rhythmic characteristics of the plasma melatonin secretion. To study annual changes in excretion four sequential 6-hourly urine samples were collected at monthly intervals from 16 normal volunteers for 13 months. Cosinor curves were fitted to the 6-sulphatoxymelatonin excretion data and the 24-h rhythm was described by the cosinor parameters: amplitude, mesor and acrophase. Significant differences in the acrophase were found during the year. The summer acrophase was phase advanced relative to the winter acrophase by about 1.5 h while intermediate phase positions were observed in spring/autumn. The 24-h excretion of urinary 6-sulphatoxymelatonin was remarkably consistent and there was no annual rhythm. In contrast, the daytime 6-sulphatoxymelatonin excretion between 12.00–18.00 h showed a statistically significant seasonal rhythm, with peaks in December/January and in July.

The secretion of the pineal neurohormone melatonin is directly under the control of the light-dark cycle in many animal species. The nature of the nocturnal increase in melatonin varies with the seasons and it has been suggested that the pattern of melatonin secretion conveys information about the environment to the brain; with subsequent effects on the reproductive axis and other physiological systems (Goldman 1983; Arendt 1986).

For a long time human melatonin production was considered to be insensitive to light because normal domestic intensities failed to suppress the nocturnal increase in melatonin. When light of a sufficiently high intensity was used, however, man was found to be similar to other animals, in that light was demonstrated to have an acute suppressive effect on melatonin (Lewy et al. 1980). Recent work has shown that man is even more sensitive to light than previously thought. When light is administered under the right conditions, light of an intensity as low as 300 lux can suppress nocturnal plasma melatonin in normal volunteers (Bojkowski et al. 1987a).

Experiments on subjects in environmental isolation have demonstrated another important effect of light, that of entraining human circadian rhythms (Wever et al. 1983). Recent evidence has shown that bright light can also phase shift the human melatonin rhythm (Broadway et al. 1987; Lewy et al. 1987) and the human temperature and cortisol rhythms (Czeisler et al. 1986).

This recent evidence of the effects of light on human physiology prompted us to re-examine the annual variation in melatonin secretion. Seasonal variations in melatonin have been reported, however, the data are largely incomplete. Single time point sampling or few yearly time points have been used. Plasma melatonin levels have been determined at different times of the year (Arendt et al. 1979; Touitou et al. 1984; Martikainen et al. 1985; Illnerova et al. 1985) and seasonal differ-
ences have been found; with peaks in summer and winter, and troughs in spring and autumn (Arendt et al. 1979) and a phase shift in the melatonin profiles, with the summer profile phase advanced by 1 h relative to the winter profile (Illnerova et al. 1985).

A recently developed RIA for 6-sulphatoxy-melatonin (aMT6s) (Arendt et al. 1985), the major urinary metabolite of melatonin has been employed in this study. Urinary aMT6s is a good index of plasma melatonin levels, provides an integrated picture of melatonin secretion and samples can be collected at frequent intervals. The assay has recently been used by Kennaway & Royles (1986) to investigate the circadian rhythm in aMT6s at two time points (winter and summer) during the year.

In this report urinary aMT6s is shown to be a reliable indicator of the rhythmic characteristics of the plasma melatonin profile and we describe the detailed annual changes in aMT6s excretion at 52°N.

Subjects and Methods

Sixteen apparently healthy volunteers (9 men and 7 women aged 19–29 years at the beginning of the experiment, mean ± sd, 25.5 ± 3.3 years) recruited from the laboratory staff collected 6-hourly urine samples for 24 h, starting at midday. During the collection period subjects remained in their normal social environments. All the volunteers had similar daytime routines and were all subjected to approximately the same light intensity. Natural daylight varies from 16.4 h in June to 7.5 h in December at 52°N. Throughout the year in the late evenings normal domestic intensity (300–500 lux) artificial lights were used. The samples were collected at local time between the 10th and 16th day of each month over a period of 13 months, commencing in November 1985.

Subjects were asked to abstain from alcohol on sampling days and to record any medication taken on or up to a week before sampling days. Three of the female subjects were taking oral contraceptives. One of the subjects was prescribed β-blockers for 2 months; no samples were collected during this period and the mean of the 2 adjoining months was used for the purposes of calculation. Three subjects took flights across several time zones; in these cases, samples were collected 3 weeks after the return to England.

Urine was collected into plastic containers. The volume of each sample was recorded and 5-ml aliquots were stored frozen at −20°C until analysis. aMT6s has been found to be extremely stable without preservative for at least 5 days at room temperature and for at least 2 years at −20°C and no special precautions were necessary during sample collection (Bojkowski et al. 1987b).

aMT6s was measured by RIA (Bojkowski et al. 1987b). At a dilution of 1:50, 200 µl of urine sample was assayed directly; where necessary, samples were re-assayed at a dilution of 1:100. All the samples from any one subject were measured in the same assay. The order in which the samples for each month were measured in successive assays was formally randomised for each assay, to eliminate any within assay trends. Sensitivity was 7.9 fmol per tube (1.98 nmol/l urine). The inter-assay coefficients of variation were 11.0%, 8.4% and 9.7% (N = 32 each) at concentrations of 10.7, 23.8 and 78.3 nmol/l, respectively. Intra-assay coefficients of variation were less than 5%.

In a previously described experiment 6-hourly urine samples were collected from 18 apparently healthy volunteers for 24 h and simultaneous blood samples were taken at 2-hourly intervals during the day and at hourly intervals at night. Full experimental details are given elsewhere (Bojkowski et al. 1987b). Melatonin was measured in the plasma samples by RIA (Fraser et al. 1983) and aMT6s was assayed in the urine samples. The data from this experiment was analysed using a cosine-curve fitting programme (Monk & Fort 1983). The results obtained from the cosinor analysis of the plasma melatonin data were compared with the results obtained from the cosinor analysis of the urinary metabolite data.

Statistical analysis

The 24-h aMT6s excretion data were analysed by a two-way analysis of variance with replication. Close examination of the raw data demonstrated large differences in the variances within one particular time period throughout the year. A Bartlett's test for homogeneity of variances (Steel & Torrie 1981) indicated the presence of significant differences and therefore the 6-hourly aMT6s excretion data and the total 24-h aMT6s excretion data were analysed non-parametrically using the Friedman two-way analysis of variance (Siegel 1956). In order to eliminate the effect of the considerable interindividual differences in aMT6s excretion, the monthly aMT6s excretion values were converted to percentages of the individual annual means. These transformed data were analysed as described above. The variabilty in the excretion of aMT6s over 24 h for each individual was calculated (coefficient of variation = sd/annual mean). The group means were calculated and the results for the male subjects and the female subjects were compared.

In order to estimate the parameters of the aMT6s rhythm of each subject in each month, cosine curves were fitted by a least-squares method to each set of four 6-hourly samples (Monk & Fort 1983). For each 24-h
time span the rhythmic characteristics of the excretion are described by the following parameters: the acrophase (the estimated peak of the rhythm data); the amplitude (the measurement of the peak of the rhythm above the mean level); and the mesor (the mean value of the fitted cosine curve).

The 24-h plasma melatonin profiles and simultaneous urinary aMT6s levels obtained in a previous experiment were also subjected to cosinor analysis. The linear correlation coefficients between the acrophases, amplitudes and mesors obtained for plasma melatonin and the acrophases, amplitudes and mesors for the urinary metabolite, obtained from simultaneous 6-hourly urine collections were

The cosinor parameters for the urinary aMT6s data throughout the year were analysed by a two-way analysis of variance. Further statistical significance was determined with the Student-Newman-Keuls test (Steel & Torrie 1981). In mid-March, British Summer Time was introduced and the clocks were advanced by 1 h until the end of October when Greenwich Mean Time resumed. All cosinor analysis for the acrophases was calculated on the basis of Greenwich Mean Time. All other calculations for aMT6s excretion refer to local time.

Results

There were good correlations between the parameters obtained from the cosinor analysis of the plasma melatonin data and the cosinor parameters obtained from the simultaneous 6-hourly urinary aMT6s measurements. One subject had no melatonin levels above the detection limit of the assay throughout the 24-h blood sampling period and was excluded from the analysis. In the remaining 17 subjects the mean (± SD) acrophase for the urinary aMT6s data was 03.67 ± 01.34 h and the mean (± SD) acrophase for the plasma melatonin data was 03.15 ± 01.00 h. The correlations between the acrophases, amplitudes and mesors obtained from the four 6-hourly urine samples and the acrophases, amplitudes and mesors from the 24-h plasma melatonin data were $r = 0.73$ ($P = 0.0012$), $r = 0.76$ ($P = 0.0007$), $r = 0.77$ ($P = 0.0005$), respectively (N = 17 each).

In all the urinary aMT6s excretion data a similar pattern was observed, with low levels during the day and a peak in excretion during the night. Two-way analysis of variance showed no significant differences in the amplitude and mesor throughout the year. In contrast a very significant difference was found in the acrophase of the aMT6s rhythm $F = 3.66, P = 0.00015$ (df 12, 180). Student-Newman-Keuls test showed significant differences in the acrophases between December and: April, June, July, October ($P < 0.05$), August, September ($P < 0.01$) and between January and: August, September ($P < 0.05$). The mean monthly values for the acrophase, amplitude and mesor are shown in Fig. 1. In December/January the mean acrophase was 04.69 h, whereas in August/September it was 03.22 h; a phase advance of about 1 h and 30 min in summer. The 3-month moving averages of the aMT6s acrophases together with the annual variation in night-length are shown in Fig. 2. The linear correlation coefficient between the 3-month moving averages of the aMT6s acrophases and the night-time duration for each month was $r =$

![Fig. 1.](image-url)

Group means ± SEM for the acrophases, amplitudes and mesors, respectively, of the aMT6s rhythm throughout the period of one year. The capital and the small form of the letter indicate a statistically significant difference between these points ($P < 0.05$, **$P < 0.01$).
0.83, \( P = 0.002 \) (\( N = 11 \)). The linear correlation coefficient between the mean monthly aMT6s acrophases and the night-time duration for each month was \( r = 0.76 \), \( P = 0.003 \) (\( n = 13 \)).

Two-way analysis of variance with replication, of the raw aMT6s excretion data showed a very significant time of day effect \( F = 210 \), \( P < 0.0001 \) (df 3, 780) but no variation with months \( F = 0.13 \), \( P = 0.99 \) (df 12, 780). Barlett's test showed that the variances in the 12.00–18.00 h time group were not homogeneous (chi-squared = 46.5, 12 df, \( P < 0.005 \)). The results were therefore analysed non-parametrically. The Friedman two-way analysis of variance showed a significant seasonal variation in aMT6s excretion for the 12.00–18.00 h time period (\( P < 0.015 \)). Peaks were observed in December/January and in July and troughs were seen in spring and autumn. No other significant differences were observed in any of the other time periods, including the total 24-h excretion. The mean aMT6s excretion in each of the four 6-hourly collection periods throughout the year is shown in Fig. 3.

When the data were transformed so that each monthly aMT6s level represented the percentage of the individual annual means, identical results were obtained on statistical analysis. Expressed this way, the mean total aMT6s excretion over 24 h was shown to be very consistent throughout the year and ranged from 94.5 ± 3.4% to 107.3 ± 3.1% (mean ± SEM, \( N = 16 \) each).

The means ± SD of the individual subjects' coefficients of variation for consistency of aMT6s excretion over 24 h for 13 months were 18.5 ± 5.4% (\( N = 9 \)) for male subjects, 16.0 ± 2.3% (\( N = 7 \)) for female subjects and 17.4 ± 4.4% (\( N = 17 \)) for the whole group.

Discussion

This study reports the detailed parameters of the annual aMT6s rhythm, that is acrophase, amplitude and mesor together with data on total urinary aMT6s excretion. Previous studies have shown that urinary aMT6s excretion is a good index of plasma melatonin levels (Arendt et al. 1985; Markey et al. 1985; Bojkowski et al. 1987b) and it has now been shown that aMT6s measured in 6-hourly urine collections also provides a good measure of rhythmic characteristics of the nocturnal increase in plasma melatonin.

The collection of urinary samples has several major advantages. Extensive field studies can be carried out. Subjects can act as their own controls and collections are possible in the volunteers' normal social environment with the minimum of inconvenience. Previous studies using plasma measurements have been restricted to single time point sampling or only few yearly time points have been used. In this study collections were made throughout the year so that any effects due to...
The mean (± SEM) urinary aMT6s excretion for each of the four 6-hourly collections in each month throughout the year. The only statistically significant difference in excretion during the year was observed for the 12.00–18.00 h collection period (P < 0.015).

Fig. 3.

extremes of daylength in winter and summer could be observed, together with effects due to rapid changes in daylength in spring and autumn. An accurate measure of seasonal trends in urbanized man was achieved and in a sense the subjects could be considered as a 'wild-captured' species.

The annual change in the acrophase for aMT6s excretion is in agreement with the findings of other workers (Illnerova et al. 1985; Kennaway & Royles 1986; Arendt & Broadway 1986), who all reported phase advances in summer. The greatest phase advance, that of 2 h, occurred under the extreme lighting conditions found in the Antarctic (Arendt & Broadway 1986). The relatively small change in the phase position found in the Antarctic is surprising and suggests that there may be entraining cues other than light, such as social factors, which influence the phase position of the melatonin secretion profile. Whether the change in acrophase observed in our study is a masking effect due to the phase advance in the rest-activity cycle caused by the introduction of British Summer Time, has to be considered. This remains unlikely since the phase advance observed (1.5 h) was greater than the change in the rest-activity cycle due to the change in clock-time and secondly because similar results have been observed in the Antarctic, where there is no manipulation of clock-time (Arendt & Broadway 1986). It is likely therefore that the phase-advance observed is the result of environmental factors.

The shift in acrophase correlates closely with the duration of darkness, suggesting that the phase-shift is a photoperiod-related phenomenon. Studies in the Antarctic have shown that the application of a 12.5 h skeleton spring photoperiod of 2500 lux in winter, produces a phase advance of the melatonin rhythm comparable to the phase advance seen in the Antarctic summer (Broadway et al. 1987). These results demonstrate that a residual photoperiodic response at least as far as melatonin is concerned, is retained in man. The possible physiological relevance of the annual change in melatonin secretion has yet to be determined. Whether other human seasonal changes are day length and melatonin-dependent remains to be seen.

Recent work on the suppression of nocturnal plasma melatonin by light has shown that light of an intensity as low as 300 lux can suppress melatonin secretion in humans (Bojkowski et al. 1987a). Work in animals has shown that previous lighting history is likely to be an important factor in determining an individuals' sensitivity to light (Reiter et al. 1983). If this is the case, then a variation in the sensitivity to light which will suppress melatonin levels may occur throughout the year and the total aMT6s excretion would be expected to change with the seasons. However, the ubiquitous use of indoor lighting may prevent the observation of any of the above changes.

In agreement with other workers we found no changes in total melatonin production throughout the year (Beck-Friis et al. 1984; Griffiths et al. 1986; Sack et al. 1986). A seasonal variation in
aMT6s excretion was observed for the 12.00–18.00 h collection period, with peaks in summer and winter. This is similar to the reports made by other workers who used daytime sampling (Arendt et al. 1979; Martikainen et al. 1985). Why this variation exists only in the daytime and not at other time points remains puzzling. It may be that during the daytime when aMT6s excretion is low, other factors such as dietary constituents may contribute to the aMT6s output. Peaks in daytime plasma tryptophan levels have been reported in January and July (Wirz-Justice et al. 1977). The amplitude of the observed daytime rhythm was low and it was not reflected in the 24-h aMT6s excretion rhythm.

Throughout the year a very stable excretion of aMT6s over 24 h was observed. The mean coefficients of variation for consistency of aMT6s excretion over 24 h were 18.5% for male subjects and 16.0% for female subjects. The coefficient of variation for the whole group was 17.4% (N = 16), which is very similar to the coefficient of variation of 13.7% previously reported when 18 volunteers collected urine samples over 4 consecutive days (Bojkowski et al. 1987b). No major differences were observed between the male and female subjects suggesting that there were no major menstrual cycle effects on aMT6s excretion. Variations in melatonin secretion with the menstrual cycle have been reported (Wetterberg et al. 1976) and it is possible that subtle changes in aMT6s may occur during the cycle with or without contraceptive steroid treatment. In order to assess any such changes, however, it would be necessary to take frequent samples from the same individuals in the course of the cycle.

Large interindividual differences in the aMT6s excretion were again observed, emphasizing the need to use subjects as their own controls. Nighttime peaks in aMT6s were always observed and the acrophase for aMT6s was relatively stable between volunteers. The large interindividual differences in aMT6s excretion, together with the marked seasonal variation in the acrophase of aMT6s excretion, suggest that perhaps it is the precise phase of melatonin secretion rather than the amplitude of the rhythm which conveys messages about the environment to the human brain. In future studies it may be of greater value to study abnormalities of phase position rather than differences in the total amount of melatonin secreted.

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References

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