ALTERATIONS IN SERUM MELATONIN AND SLEEP IN INDIVIDUALS IN A SUB-ARCTIC REGION FROM WINTER TO SPRING

Trond Bratlid, M.D., Ph.D.¹, Björn Wahlund, M.D., Ph.D.²

ABSTRACT

Methods. In a sub-arctic region at 69º N, seven individuals with self-reported insomnia during the 'dark period' and seven without, were followed with repeated measures of melatonin and questioned on ten different sleep variables, from the beginning of January to the vernal equinox in March.

Results. The distribution of melatonin over a 24-hour period (five time points) indicated an increase in melatonin levels in both groups in the middle of January and a decrease at the time of year when the sun first rises over the horizon (23rd-24th of January). Moreover, an indication of a delayed phase shift of melatonin secretion was found during the dark period, which returned to "normal" secretion during the night at the equinox in March. Individuals with sleep problems had a slower return to "normal" melatonin secretion than those without sleep problems. A positive correlation between morning tiredness and morning levels of melatonin was found among individuals with sleep disturbances, but not in controls.

Conclusion. This study indicates changes in the internal circadian rhythm in humans at the end of the annual dark period of winter when there is a rapid increase in the number of hours of sunlight. For vulnerable individuals, the disturbance in sleep, and in particular morning tiredness, lasts at least until the vernal equinox in March.

Keywords: dark, light, melatonin, rhythm sleep, sub-arctic

¹Psychiatric Department, University of Tromsø, Norway
²Huddinge University Hospital, Institution of Clinical Neuroscience, Sweden
INTRODUCTION

Seasonal changes in human behaviour and physiology have been recognized since ancient time. With its extreme northern geographical position at 69º N, Tromsø, Northern Norway, provides a unique natural laboratory for studying the effects of season on humans, keeping in mind the influence of artificial light. People living north of the Arctic Circle are exposed to marked seasonal changes in the light-darkness ratio, ranging from the darkness of the "polar night", to constant daylight during the midnight sun period. The sub-arctic climate in Tromsø has special characteristics. In Tromsø, the "darkness period" in wintertime lasts from November 25 until January 17. Due to the surrounding mountains, the sun does not rise above the horizon in the centre of Tromsø from November 22 until January 20. In the middle of this period, there is almost total darkness all day, except for a few hours of dim daylight on clear days around noon. These marginal light conditions have been related to various sleep problems, especially insomnia during the dark period, but also seasonal affective disorder (SAD), or "winter blues", extending over several ‘long’ winter months, as previously reported (1,2) for the same geographic region as that figuring in the present study.

The pineal hormone melatonin is thought to play an important role in the circadian and seasonal regulation of sleep and many other biological rhythms. Melatonin is mainly secreted during the night and is alleged to act as a "darkness signal". Some studies have revealed seasonal variations in melatonin secretion patterns (3-7). Light at night suppresses melatonin secretion. Photoperiodic alterations in the secretion during the year are significantly influenced by latitude (4). Seasonal complaints increase with distance from the Equator and it is believed that some people are vulnerable to these fluctuations in day length (8,9). Phase delay of melatonin rhythms in SAD (10) and insomniac patients have been reported (11).

In previous studies, patients with insomnia during the "dark period" in northern Norway were investigated in explorative, controlled trials with drugs (12) and light treatment (1) and their serum melatonin and cortisol levels were determined before and after artificial light treatment (2).

To study changes in the natural light-dark conditions in the region, we followed a group of individuals who complained of insomnia du-
ring the dark period and a control group of healthy individuals, from
the beginning of January until the vernal equinox in March. Blood
samples were studied for morning and evening melatonin levels and
sleep questionnaires were completed during the dark period and after
the sunlight had reappeared.

The main purpose of the study was to investigate changes in mela-
tonin levels and reported sleep problems during the rapid dark-light
shift from January to the vernal equinox in March.

MATERIAL AND METHODS

Material
The insomnia patient group consisted of seven individuals (five men
and two women) who complained of sleep difficulties during the dark
winter period. The mean age was 38, with a range of 20-64 years. The
control group consisted of seven individuals (three men and four wo-
men) who reported that they slept normally during the dark period.
The mean age was 29, with a range of 21-44 years.

Sunlight conditions
The rapid increase in environmental light during the study period is il-
lustrated by the number of hours of sunlight for each month as recor-
ded by The Norwegian Meteorological Institute, Forecasting Division
for Northern Norway at the Tromsø latitude of 69° N: in January 5.6
h, in February 20.8 h, and in March 86.5 h.

Clinical assessments
Individuals in the insomnia patient group were recruited after reading
information in the media about ongoing studies at our hospital concern-
ing sleep problems in the dark period. Individuals in the control
group consisted of staff at the hospital and some of their family mem-
bers and friends.

All individuals were screened using a questionnaire with the follow-
ing 10 items:
1. Time for going to bed,
2. Sleep latency,
3. Sleep interruptions,
4. Total sleep time,
5. Wake onset, 
6. Sleep need, 
7. Sleep depth, 
8. Sleep quality. 

We also used two visual analogue scales (VAS) relating to: 
9. Tiredness at bedtime (VAS_{p.m.}) and 
10. Tiredness in the morning (VAS_{a.m.}). 

The VAS was a self-evaluated, 100-millimetre scale with endpoints "no tiredness" and "extreme tiredness". 

During the study, all subjects filled in the self-rated questionnaire relating to the upcoming night, in the evening while blood samples were taken. The next morning, again during the collection of blood samples, they completed the 10-item questionnaire relating to the previous night. 

All individuals were interviewed before the study and none of the participants had any apparent, or known psychiatric, or somatic disease. None of the individuals used medication, or drank alcohol during the days, or nights, before sampling. 

**Melatonin assay**

Blood samples for melatonin analysis were collected in the evenings and the following mornings on the dates Jan. 9^{th}-10^{th}, Jan. 16^{th}-17^{th}, Jan. 23^{rd}-24^{th}, Jan. 30^{th}-31^{st} and March 19^{th}-20^{th}, at the hours 22:00, 22:45 and 23:30 and 07:30 and 08:15. The serum samples were analysed by the method of Wetterberg et al. (13). The lower limit of detection was 0.01 nmol/l. The intra-assay coefficient of variance (CV) was 7.4 % and the inter-assay CV for samples (N = 60) above 0.15 nmol/l was 4.8 %. 

**Statistical analysis**

To capture potential changes in evening and morning melatonin over the different dates, from the 9^{th}-10^{th} of January until the 19^{th} - 20^{th} of March, we used three different statistical measures of melatonin. One estimate of melatonin was to calculate the Mean ± Standard Error of the Mean (SEM) of the three time points in the evening and of the two time points in the morning. Another estimate of melatonin was to calculate the Mean ± Standard Deviation (SD) of the area under the curve (AUC). The third was to determine the Mean ± SD of the ma-
ximal melatonin value (MTmax). The MTmax of the five time-points 22:00, 22:45, 23:30, 07:30 and 08:15 hours were selected for each individual. The areas under the curves of melatonin levels for different time points were calculated for the hours 22:00, 22:45 and 23:30 (MT_{AUC \text{ evening}}), as well as for 07:30 and 08:15. (MT_{AUC \text{ morning}}).

The means were compared between groups, using Wilcoxon rank scores for two groups. Correlation analysis was performed according to Spearman. A defined probability with less than, or equal to 0.05 is taken as statistically significant. All statistical calculations were performed using the JMP™ software (14), version 3.2.2, developed by the Statistical Analysis Systems (SAS) institute.

RESULTS

Correlations
The melatonin levels at the times 22:00, 22:45 and 23:30 were correlated, as were those at 07:30 and 08:15 (p < 0.01). A positive correlation was found between M_{tmax}, as well as morning values of MT_{AUC \text{ morning}}, and morning tiredness in group 1 (rho = 0.39, p < 0.05 and rho = 0.48, p < 0.01, respectively). No other items of sleep correlated with single melatonin levels (at the different time points), MT_{max}, MT_{AUC \text{ evening}}, or MT_{AUC \text{ morning}}.

Comparisons between patients and controls of melatonin levels at different time points and dates
Individuals in the insomnia patient group had lower melatonin levels, 0.11 ± 0.02 (Mean ± SEM) at 23:30 h compared to controls 0.18 ± 0.09, (p < 0.01) on January 9th-10th (Figure 1a).

On the 30th-31st of January, individuals in the insomnia patient group also had lower levels of melatonin than individuals in the control group at 23:30 h (0.16 ± 0.07; 0.32 ± 0.13, respectively, p < 0.05) (Figure 1d).

Comparisons within patients of melatonin levels at different time points and dates
The melatonin level (mean ± SEM) was significantly lower at 23:30 h when comparing the dates Jan 9th-10th (0.11 ± 0.08) with Jan 30th-31st (0.32 ± 0.13; p < 0.01) (Figure 1a and 1d).
Figure 1a-e Melatonin levels (mean ± SEM) in insomnia patients (●) and controls (○) at 22:00, 22:45, 23:30, 07:30 and 08:15 h, and on at the 9th-10th of January (Figure 1a), the 16th-17th of January (Figure 1b), the 23rd-24th of January (Figure 1c), the 30th-31st of January (Figure 1d) and the 19th-20th of March. Melatonin is expressed as nmol/l. Bars indicates SEM. Significant differences between patients and controls are indicated by (*).
The melatonin level was significantly higher at 8:15 h on Jan 9th–10th (0.27 ± 0.04) than on March 19th–20th (0.16 ± 0.05; p < 0.05) (Figure 1a and 1e).

In the insomnia patient group, the evening mean value of melatonin levels (mean ± SEM) on the 16th–17th of January was significantly higher than on the 9th–10th of January (Figure 2).

A higher evening level of melatonin was also found on 23rd–24th of January as compared with the 30th–31st of January (Figure 2).

Morning mean values of melatonin levels were significantly higher on the 16th–17th of January as compared to mean value of melatonin levels on the 19th–20th of March (Figure 2).

The control group showed a significant rise in the average melatonin level in the evening of the 23rd–24th of January as compared to the 9th–19th of January. No other significant differences were found (Figure 3).

MTmax was significantly higher in the insomnia patient group on the 16th–17th and 23rd–24th of January as compared with Mtmax in March (Table I).
Comparisons within controls of melatonin levels at different time points and dates

The control group differed from the insomnia patient group by their melatonin levels at 23:00 h between Jan 9th-10th and March 19th-20th (0.18 ± 0.07 and 0.41 ± 0.08, respectively; p < 0.05), (Figure 1a and 1e).

The control group also showed a tendency of phase advance when light returned during the later half of January (Figure 1c and 1d) as compared with the insomnia patient group.

The control group had a significantly higher Mtmax on the 23rd-24th of January as compared with Mtmax in March (Table I).

Comparison between means of sleep variables

The VAS scores for ‘morning tiredness decreased significantly in the control group, but not in the insomnia patient group, when comparing the VASa.m. on the 9th-10th of January with the VASa.m. in March. When comparing the mean VASa.m. of the four dates in January with the VASa.m. in March, a significantly lower level was found in

---

Figure 3. Evening (E) and morning (M) melatonin levels in controls during the dark period. For explanation of the bars and their significance, see Figure 2. A significant rise in evening melatonin level was found in on the 23rd-24th of January as compared to the 9th-19th 10th of January. No other significant differences were found. Melatonin is expressed as nmol/l. The line on each bar indicates standard error of mean.
Table I. Mean of maximum melatonin (MTmax) ± standard deviation (SD) and mean of morning tiredness, expressed as Visual Analogue Scale (VASa.m.) ± SD, for each group and both groups together. The left column indicates at which dates melatonin was sampled and sleep variables were recorded. Bold figures indicate significant differences between the means of MTmax, or VASa.m., in January as compared to values in March. Significant differences between groups are indicated with italics and with the symbol ‡. Serum melatonin was expressed in nmol/l and VASa.m. in millimetres. Wilcoxon signed rank test.

| Dates   | Insomnia patient group (n = 7) | Control group (n = 7) |
|---------|--------------------------------|----------------------|
|         | MTmax                           | VASa.m.              | MTmax | VASa.m. |
| Jan. 9-10 | 0.49 ± 0.35                | 67 ± 20               | 0.39 ± 0.18 | 56 ± 31 * |
| Jan. 16-17 | 0.85 ± 0.35*               | 66 ± 22               | 0.62 ± 0.28 | 41 ± 32  |
| Jan. 23-24 | 0.75 ± 0.31*               | 71 ± 17 ‡            | 0.96 ± 0.38* | 35 ± 32 ‡ |
| Jan. 30-31 | 0.40 ± 0.13                | 54 ± 26               | 0.40 ± 0.09 | 40 ± 26  |
| March 19-20 | 0.43 ± 0.27                | 48 ± 29 ‡            | 0.47 ± 0.21 | 19 ± 17 ‡ |

Level of significance of differences in MTmax, or VAS, between January and March *p < 0.05.
Level of significance between the insomnia patient group and the control group ‡p < 0.05.

March in the control group, (VASa.m.January: 48 ± 29 and VASa.m.March: 19 ± 17, p < 0.05).

The insomnia patient group had significantly higher rated scores of morning tiredness on the 23rd-24th of January, as well as in March, when compared with individuals of the control group (see Table I).

We thus found that the rapid change in environmental lighting conditions, at the time period when daylight returned on the 21st of January, was related to an increase in melatonin in both the insomnia patient group and the control group. All three statistical measures, i.e. the mean of evening and morning melatonin levels separately, the AUC and the MTmax, gave very similar results with a correlation coefficient > 0.98.

In this paper, we have presented the MTmax and selected sleep variables in Table I and the mean melatonin levels in Figure 1, Figure 2 and Figure 3.
DISCUSSION

The present study was unique in the sense that natural light-dark conditions were investigated from complete lack of daylight (0 h) in the middle of January, to 12 h of sunlight per 24 h (at the end of March). Using three statistical measures of melatonin (mean melatonin evening and morning levels, AUC and MTmax) gave very similar results in this pilot study. However, with a more complex research design and more time points, it may be of crucial importance to examine the data with all three measures, i.e. variables emphasizing different aspects of the endocrine changes. This becomes even more important when several variables are included in the study (15,16).

The time-points of the melatonin assays in this study are discontinuous and do not include the night-time measures (1-3 hrs) during which, under "normal" conditions, the nocturnal peak secretion of melatonin occurs. However, by repeated measures of melatonin on different dates and during the period of time when there is a considerable change in dark-light conditions, we believe that potential changes in phase and amplitude will be detected with late evening and early morning measures.

The investigation was performed during a limited, three-month period of the year and we were, thus, able to limit the variation in subjective reports, which may occur in individuals within the subarctic region. Lund and Hansen (9) studied the average ratings for seasonal affective symptoms of 200 individuals in northern Norway using a Seasonal Pattern Assessment Questionnaire and found that reported symptoms differed according to the season in which the questionnaire was completed. The average score for seasonal affective disorder changed over the year, with the highest score in March and the lowest in September.

The significant rise in melatonin during the middle of January is rapid and of short duration. This was obviously not detected in another study of melatonin and season, when only monthly sampling of urine was analysed for melatonin concentration (4), but it agrees with the experimental results of Midwinter et al. (17) and Owen et al. (18). They found that the effect of light on melatonin was more efficient in the latter part of the night in winter and this was particularly documented for the effect of dim light.
The significant decrease in melatonin when hours of sunlight return to Tromsø in January may be explained by a shift of timing for melatonin onset.

Given that the endogenous circadian period of the human circadian pacemaker is slightly longer than 24 h, the findings suggest that a naturalistic dawn signal is sufficient to prevent this natural delay drift. Zeitgeber transduction and circadian system response may be tuned to the time-rate-of-change of naturalistic twilight signals, as recently reported in an experimental study by Danilenko et al. (19). Actually, the same research group (20) showed that, under controlled conditions of a modified constant routine protocol, a single dawn signal was sufficient to phase advance the timing of the onset of secretion of melatonin. The significant phase advance of melatonin was enhanced from 20 to 34 minutes after three consecutive dawn signals. This is small but appears to be of sufficient magnitude to entrain the human circadian pacemaker, which has an endogenous period of about 24.2 h.

Individuals in both the insomnia patient group and the control group reported morning tiredness in January, while individuals of the control group reported a decrease in morning tiredness with increasing day length and hours of sunshine. However, the insomnia patients still had sleep problems in March and, in particular, reported that they still experienced morning tiredness. This may be due to an endogenous vulnerability of this group of persons in their ability to adapt their internal biological rhythm to the rapid light change during the last part of January. This was demonstrated by a slower return of the higher melatonin secretion in the morning to the higher and "normalised" secretion of melatonin during night-times in March, as compared with individuals without sleep problems. The finding of a significant correlation of morning tiredness and morning melatonin is in line with the finding of a slower adaptation to the rapid external light changes in insomnia patients.

Therefore, it is conceivable that individuals with sleep problems during the dark period may benefit from treatment with light, or from oral melatonin administration.

Both individuals with sleep problems and controls reported morning tiredness in January. One possible reason for the group difference may be differences in exercise patterns, as Weydahl (21) has shown that melatonin levels in January are influenced by hours of
habitual exercise during the autumn among individuals living in a sub-arctic region in Norway. Another possible explanation may be different sensitivities of retinal light receptors between the two groups – a hypothesis that may now be tested. This explanation is interesting in light of the recent finding by Lucas et al. (22), who suggested the existence of a melanopsin-associated system, complementary in function to the classical rod/cone system.

Other possible underlying mechanisms may also participate in the regulation of the sleep-wake cycle, as recently reviewed by La-vie (23).

Acknowledgements
The authors would like to thank Professor Lennart Wetterberg (at the time of the writing of this paper, he was chief of the PFLP, Tromsö) for his generous help and support.

REFERENCES

1. Lingjaerde O, Bratlid T, Hansen T. Insomnia during the "dark period" in northern Norway. An explorative, controlled trial with light treatment. Acta Psychiatr Scand 1985; 71: 506-512.
2. Hansen T, Bratlid T, Lingjaerde O, Brenn T. Midwinter insomnia in the sub-arctic region: evening levels of serum melatonin and cortisol before and after treatment with bright artificial light. Acta Psychiatr Scand 1987; 75: 428-434.
3. Arendt J, Wirz-Justice A, Bradtke J, Kornemark M. Long-term studies on immunoreactive human melatonin. Ann Clin Biochem 1979; 16: 307-312.
4. Wetterberg L, Bratlid T, von Knorring L, Eberhard G, Yuwiler A. A multinational study of the relationships between night time urinary melatonin production, age, gender, body size, and latitude. Eur Arch Psychiatry Clin Neurosci 1999; 249: 256-262.
5. Beck-Friis J, von Rosen D, Kjellman BF, Ljunggren J-G, Wetterberg L. Serum melatonin in relation to body measures, sex, age, seasonal variation and the use of drugs in patients with major depressive disorders and healthy humans. Psychoendocrinology 1984; 9: 261-277.
6. Kauppila A, Kivelä A, Pakarinen A, Vakkuri O. Inverse seasonal relationship between melatonin and ovarian activity in humans in a region with strong seasonal contrasts in luminosity. J Clin Endocrinol Metab 1987; 65: 823-828.
7. Wehr TA, Giesen HA, Moul DE, Turner EH, Schwartz PJ. Suppression of men's responses to seasonal changes in day length by modern artificial lighting. Am J Physiol 1995; 269: 173-178.
8. Rosen LN, Rosenthal NE. Seasonal variations in mood and behaviour in the general population: a factor-analytic approach. Psychiatry Res 1991; 38: 271-283.
9. Lund E, Hansen V. Responses to the Seasonal Pattern Assessment Questionnaire in Different Seasons. Am J Psychiatry 2001; 158: 316-318.
10. Thalén BE, Kjellman BF, Morkrid L, Wetterberg L. Melatonin in light treatment of patients with seasonal and nonseasonal depression. Acta Psychiatr Scand 1995; 92: 274-284.
11. Partonen T, Vakkuri O, Lamberg-Allardt C, Lonnqvist J. Effects of bright light on sleepiness, melatonin, and 25-hydroxyvitamin D(3) in winter seasonal affective disorder. Biol Psychiatry 1996 May 15; 39(10): 865-872.

12. Lingjaerde O, Bratlid T. Triazolam (Halcion) versus flunitrazepam (Rohypnol) against midwinter insomnia in Northern Norway. Acta Psychiatr Scand 1981; 64: 260-269.

13. Wetterberg, L., Eriksson, O., Friberg, Y., Vangbo, B. A simplified radio immunoassay for melatonin and its application to biological fluids. Preliminary observations on the half-life of plasma melatonin in man. Clin Chim Acta 1978; 86: 169-177.

14. Lehman A, Sall JP, Cole M J MPTM User’s Guide. Introductory Edition, SAS Institute, Inc., Cary, North Carolina, USA (1995).

15. Wahlund, B., Säaf, J., Wetterberg, L. Classification of patients with affective disorders using platelet monoamine oxidase, serum melatonin and post dexamethasone cortisol. Acta Psychiatr Scand 1995; 91: 313-321.

16. Wahlund B., Säaf J., Grahn H., Wetterberg, L. Diagnostic subgrouping of depressed patients by principal component analysis and visualized pattern recognition. Psychiatry Res Dec 1998; 14; 81(3): 393-401.

17. Midwinter MJ, Arendt J. Adaptation of the melatonin rhythm in human subjects following night-shift work in Antarctica. Neurosci Lett 1991; 122: 195-198.

18. Owen J, Arendt J. Melatonin suppression in human subjects by bright and dim light in Antarctica: time and season-dependent effects. Neurosci Lett 1992; 137: 181-184.

19. Danilenko KV, Wirz-Justice A, Krauchi K, Cajochen C, Weber JM, Fairhurst S, Terman M. Phase advance after one or three simulated dwawns in humans. Chronobiol Int 2000a; 17: 659-668.

20. Danilenko KV, Wirz-Justice A, Krauchi K, Weber JM, Terman M. The human circadian pacemaker can see by the dawn’s early light. J Biol Rhythms 2000b; 15; 437-446.

21. Weydahl, A. Evening melatonin in January after changes in hours of habitual exercise during fall among youths living in the sub-arctic. Arctic Med Res 1994; 53: 146-151.

22. Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, Yau KW. Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science 2003 Jan 10; 299(5604): 245-247.

23. Lavie, P. Sleep-wake as a biological rhythm. Annu Rev Psychol 2001; 52: 277-303.