

Melatonin Phase-Shifts Human Circadian Rhythms with No Evidence of Changes in the Duration of Endogenous Melatonin Secretion or the 24-Hour Production of Reproductive Hormones

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The pineal hormone melatonin is a popular treatment for sleep and circadian rhythm disruption. Melatonin administered at optimal times of the day for treatment often results in a prolonged melatonin profile. In photoperiodic (day length-dependent) species, changes in melatonin profile duration influence the timing of seasonal rhythms. We investigated the effects of an artificially prolonged melatonin profile on endogenous melatonin and cortisol rhythms, wrist actigraphy, and reproductive hormones in humans. Eight healthy men took part in this double-blind, crossover study. Surge/sustained release melatonin (1.5 mg) or placebo was administered for 8 d at the beginning of a 16-h sleep opportunity (1600 h to 0800 h) in dim light. Compared with placebo, melatonin ad-

ministration advanced the timing of endogenous melatonin and cortisol rhythms. Activity was reduced in the first half and increased in the second half of the sleep opportunity with melatonin; however, total activity during the sleep opportunities and wake episodes was not affected. Melatonin treatment did not affect the endogenous melatonin profile duration, pituitary/gonadal hormone levels (24-h), or sleepiness and mood levels on the subsequent day. In the short term, suitably timed sustained-release melatonin phase-shifts circadian rhythms and redistributes activity during a 16-h sleep opportunity, with no evidence of changes in the duration of endogenous melatonin secretion or pituitary/gonadal hormones. (*J Clin Endocrinol Metab* 88: 4303–4309, 2003)

MELATONIN, AN INDOLEAMINE hormone, is widely and indiscriminately used in countries in which it is freely available for a variety of purposes including the treatment of insomnia and combating jet lag. In 1996 it was estimated that sales of melatonin in the United States exceeded those of vitamin C, and apparently there was a global shortage of the synthetic hormone (1).

Melatonin is synthesized and secreted by the pineal gland mainly during the biological night, in both diurnal and nocturnal organisms (2). It is well established that in several mammalian species the timing of melatonin synthesis, in particular its duration, is used to organize seasonal functions that respond to changes in day length (photoperiod) such as reproduction (2). Although the rhythm of melatonin in humans has been shown to respond to changes in photoperiod (3), the precise physiological role of melatonin in humans has yet to be determined. This remains an important question because administration of the hormone in doses frequently used (2–5 mg) will normally result in a (artificially) prolonged melatonin profile.

Acute administration of melatonin during the daytime, when endogenous concentrations are low, leads to an increase in sleep propensity (4–12) and shifts in the timing of the endogenous rhythm of melatonin (5, 13–16). Timed

chronic administration of melatonin to blind individuals, in whom circadian rhythms are not synchronized to the external light-dark cycle, can resynchronize these rhythms to the 24-h day (17, 18). These properties are potentially useful for the treatment of circadian rhythm and sleep disorders. Beneficial effects have indeed been reported in patients with non-24-h sleep wake disorder and delayed sleep-phase syndrome, shift workers, and travelers suffering from jet-lag (12, 19–22). However, not all studies report phase-shifting effects (23, 24), probably because of melatonin not being administered at the correct circadian time.

The lack of consistency in currently available data and the possible undesirable effects of chronic melatonin administration on human endocrine function are major impediments to the therapeutic applications of this hormone. We therefore devised a protocol to establish unequivocally whether an artificially prolonged melatonin profile does or does not influence its own secretion, and that of other endocrine parameters in controlled conditions, with simultaneous wrist activity recording and subjective assessments of mood and sleepiness.

Subjects and Methods

Experimental subjects

Eight male subjects (body mass index 23.8 ± 3.0 kg/m²; age 24.4 ± 4.4 yr), who were paid volunteers, were studied. Females were excluded because this study required assessment of circadian phase on two oc-

Abbreviations: CR, Constant routine; CV, coefficient of variation; D, study day; LD, limit of detection; PRL, prolactin.

casions approximately 10 d apart. Because body temperature rhythms are substantially different between the follicular and luteal phase (25, 26), female volunteers were not included in this study in case this would compromise the reliability of phase assessments.

Subjects were nonsmokers, unmedicated, and in good general health, as determined by self-report, medical and sleep questionnaires, history taking, physical examination, electrocardiography, and biochemical and hematological screening tests. They had no history of psychiatric illness, drug or alcohol dependence, sleep disorder, or recent shift work and transmeridian travel. Maximum habitual caffeine and alcohol consumption did not exceed 300 mg/d and 90 g/wk, respectively. Sleep was assessed by polysomnography during a laboratory adaptation night. The study was approved by the Defense Evaluation Research Agency Centre for Human Sciences Ethics Committee (Farnborough, UK).

Design

The study had a double-blind, crossover design, balanced according to the order of treatment. Subjects attended two 13-d trials (Fig. 1), separated by at least 14 d. During each trial either melatonin (1.5 mg, surge-sustained release, Penn Pharmaceuticals Ltd., Tredegar, Gwent, UK) or placebo was administered orally in gelatin capsules for 8 consecutive days. The surge-sustained release preparation was specifically designed for the purposes of the study to raise melatonin levels immediately and then to maintain high levels until endogenous melatonin secretion occurred. This ensured that circulating melatonin levels were elevated throughout the 16-h sleep opportunity. The period of treatment administration was chosen because although effects of melatonin on LH secretion in men may be manifested rapidly (27), a significant reduction was seen after 7 d of treatment with melatonin when using prolactin (PRL) as an index of a photoperiodic response (28). Furthermore, 7 d of treatment has been used in successful alleviation of jet lag symptoms (29).

Protocol

The light-proof, sound-attenuated, temperature- and humidity-controlled environmental scheduling facility (QinetiQ, Farnborough, Hampshire, UK) consisted of individual bedrooms, communal living and kitchen areas, showers, and lavatories. Ambient light was provided by broad-spectrum fluorescent tubes (Osram Biolux, Langley, Berkshire, UK). Throughout the study dim light was on average 2.4 ± 0.1 lux (mean \pm SEM) in the angle of gaze and 5.0 ± 0.2 lux looking directly at the light. Before each session, subjects were instructed to abstain from

caffeine for 7 d and alcohol for 24 h and to maintain a regular sleep-wake cycle (sleep 2300 h–0700 h) for 10 d. Adherence to the sleep-wake schedule was verified by wrist actigraphy (Actiwatch-L, Cambridge Neurotechnology, Cambridge, UK).

After an initial baseline sleep opportunity [day (D)1, 2300 h to 0715 h] in dim light, subjects underwent a constant routine (CR) for 29 h (CR1) (D2–3, Fig. 1). During the CR, subjects were instructed to remain semi-recumbent and awake in dim light and received identical nutrients every 2 h (30). Following the CR, subjects remained in bed in dim light during daily scheduled 16-h sleep opportunities (D3–D11, 1600 h to 0800 h) with knowledge of clock time but no access to recreational material such as books and television. Light levels during wake episodes were 147.6 ± 4.3 lux in the angle of gaze. A standardized meal was provided each day at 1300 h (~ 1500 kJ), and light snacks were available at other times. Melatonin or placebo was administered at 1600 h (D3–D10). On D11, all subjects took placebo at 1600 h (single-blind). A second 29-h constant routine (CR2) was imposed on D12–D13, followed by a 16-h recovery sleep opportunity.

Outcome measures

Blood samples (2–4 ml) were collected into lithium-heparin containers via iv cannulae at 1-h intervals during the CRs to assess circadian rhythm profiles and for 24 h on D3 to assess melatonin pharmacokinetics. Plasma was immediately separated by centrifugation and stored at -20 C. These samples were later assayed for melatonin, cortisol, PRL, FSH, LH, testosterone, TSH, and GH.

Wrist activity levels were continuously measured throughout the study using Actiwatch-L actigraphy monitors (Cambridge Neurotechnology). Computerized versions of the Karolinska Sleepiness Scale (31) and a series of 12 subjective mood (visual analog) scales were administered at 2-h intervals during the wake episodes (0800 h–1400 h). The mood scales reported here are as follows: agitation, concentration, aggression, depression, and anxiety.

Other parameters recorded during the study were sleep via polysomnography during all scheduled sleep opportunities and CRs, tests of cognitive performance during wake episodes and CRs, and core body temperature during the entire study. These parameters will be reported elsewhere.

Data analyses and statistics

Data are expressed as means \pm SEM. Plasma melatonin and cortisol concentrations were measured by RIA (Stockgrand Ltd., Guildford, Surrey, UK). For the melatonin assay, the limit of detection (LD) was 6.5 pg/ml and the interassay coefficients of variation (CVs) were 14% at 27 pg/ml, 10% at 55 pg/ml, 13% at 145 pg/ml, and 13% at 244 pg/ml. For the cortisol assay, the LD was 6.3 nmol/liter and the interassay CVs were 15% at 60 nmol/liter, 10% at 511 nmol/liter, and 8% at 918 nmol/liter. Plasma samples obtained during CR2 were also assayed for pituitary/gonadal hormones. PRL, FSH, LH, testosterone, and TSH concentrations were measured by automated chemiluminescence (Bayer ACS 180+, Bayer Centaur, Newbury, Berkshire, UK), and GH concentrations were measured by dissociation enhanced lanthanide fluoroimmunoassay (Chemical Pathology Laboratory, Southampton University Hospitals NHS Trust, Southampton, Hampshire, UK). The assay parameters were as follows: CVs, PRL, 12.7%, 4.3%, and 6.0% at 93, 243, and 577 mU/liter; LH, 5.5%, 6.8%, and 6.8% at 5.2, 19, and 40 IU/liter; GH, 8.8% at 1.12 mU/liter; TSH, 4.5%, 4.5%, and 4.5% at 0.65, 4.4, and 19 mU/liter; testosterone, 10.4%, 4.2%, 5.1%, and 4.1% at 2.0, 3.3, 10.1, and 26.6 nmol/liter; and FSH, 5.4%, 7.7%, and 5.0% at 8.0, 24, and 57 IU/liter. LDs were: PRL 6 mU/liter, LH 0.1 IU/liter, GH 0.1 mU/liter, TSH 0.01 mU/liter, testosterone 0.3 nmol/liter, and FSH 0.3 IU/liter. Normal reference ranges were PRL 150–500 mU/liter, LH 0.8–12.0 IU/liter, GH 0–5 mU/liter (fasting), TSH 0.35–5.5 mU/liter, testosterone 8.0–30.0 nmol/liter, and FSH 0.8–11.5 IU/liter. Reference ranges were derived in-house except for TSH and testosterone in which values were derived from the kit manufacturer.

Plasma samples collected during the first 4 h of the CR were excluded to eliminate possible masking effects from the preceding sleep and changes in posture. The onset and offset of the plasma melatonin rhythm and the fitted maximum of the cortisol rhythm were used as markers of the phase of the endogenous circadian pacemaker (32, 33). Peak levels

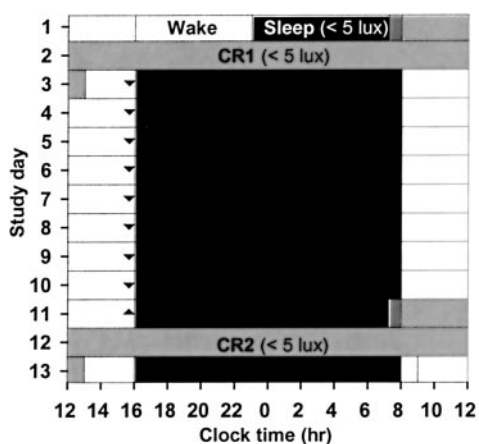


FIG. 1. Diagrammatic representation of study protocol for one treatment trial. Successive days are shown beneath each other, and clock time (24 h plotted from 1200 h) is shown on the horizontal axis. The trial commenced at 1600 h on study D1 and ended at 0900 h on D14. Scheduled sleep opportunities are represented in black, wake in white, and CRs in gray. Treatment administration time (indicated by downward-pointing arrows) was 1600 h from D3 to D11. On D11, all subjects received a placebo capsule (indicated by upward-pointing arrow) single blind.

of plasma melatonin were calculated as the mean of the five highest values for each subject. Melatonin onset and offset were defined as the times corresponding to when plasma levels rose or declined to 25% of the observed peak values, respectively. This method of determining melatonin rhythm phase was selected to overcome problems with individual differences in melatonin secretion levels and was based on previous studies (34, 35). Because of the episodic nature of the cortisol rhythm profile, onset and offset measures would be obscured. Therefore, the cortisol rhythm was quantified by cosinor analysis, a method used in previous studies (36). Phase shifts were determined by calculating the difference in timing of the melatonin onset and cortisol peak in CR1 and CR2. The duration of high plasma melatonin was defined as the number of hours between melatonin onset and melatonin offset.

Data were statistically analyzed by repeated measures ANOVA or paired samples *t* test, as appropriate. Where ANOVA was applied, a full model was used and main effects and interaction effects were included. For plasma melatonin and cortisol phase markers, two-way ANOVA was carried out with factors treatment trial and CR. For wrist actigraphy and subject mood and sleepiness assessments, two-way ANOVA was applied with factors treatment trial and time of day. $P = 0.05$ was used to determine significance. Significant effects revealed by ANOVA were subjected to *post hoc* analysis using paired samples *t* test. Statistical analyses were performed using either the SAS (version 8.0, SAS Institute Inc., Cary, NC) or SPSS (version 10.05, SPSS Inc., Chicago, IL) statistical software packages.

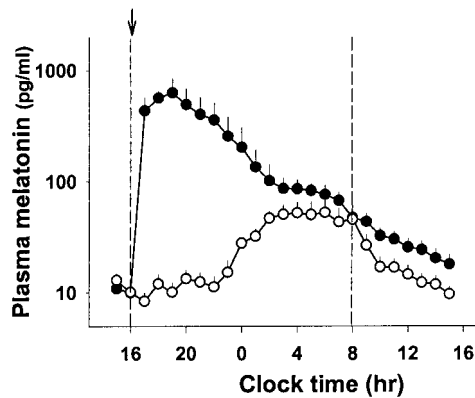
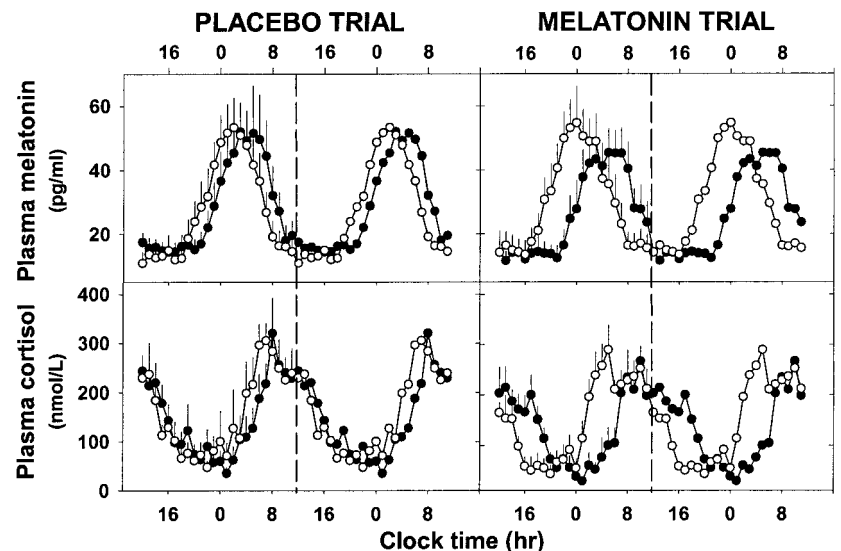


FIG. 2. Mean (\pm SEM) plasma melatonin concentrations (pg/ml) 1 h before and 23 h after the first administration of the melatonin (closed circles) and placebo (open circles) treatments. Clock time is shown on the horizontal axis, and the vertical arrow indicates the time of treatment administration (1600 h). The two dashed lines represent the beginning and end of the scheduled sleep opportunity.

FIG. 3. Mean (\pm SEM) profiles of plasma melatonin and plasma cortisol for the placebo trial (left) and melatonin trial (right), in CR1 (closed circles) and CR2 (open circles). Clock time is shown on the horizontal axis. Data are double-plotted (i.e. 24-h data are plotted twice) for clarity, and the double-plot segment is indicated by a vertical dashed line and the absence of error bars.



Results

Melatonin pharmacokinetics

On the first day of melatonin administration (D3), plasma melatonin levels rose rapidly in the first hour to, on average, 431 ± 135 pg/ml (maximum 626 ± 212 pg/ml, 3 h post administration) and remained elevated above the placebo nocturnal values for the duration of the sleep opportunity (Fig. 2).

Melatonin and cortisol rhythms

During the initial circadian phase assessments (CR1), all subjects exhibited normal 24-h profiles of plasma melatonin and cortisol during both treatment trials (Figs. 3 and 4).

The timing of melatonin onset and the cortisol peak were significantly advanced after both melatonin (5.3 h and 4.4 h, respectively, $P < 0.01$) and placebo (2.4 h and 1.8 h, respectively, $P < 0.05$) treatment. However, the advances following melatonin were significantly greater than after placebo ($P < 0.05$; Table 1).

In CR1, the duration of high plasma melatonin was 9.0 ± 0.6 h and 9.5 ± 0.4 h in the melatonin and placebo trials, respectively. The duration of high melatonin in CR2 was identical in the melatonin and placebo trials (8.9 ± 0.5 h). No significant change was observed from CR1 to CR2 in either trial.

Wrist actigraphy

Mean activity levels at hourly intervals, as determined by wrist actigraphy, were calculated for sleep opportunities of all treatment administration days except D3. Data from D3 were excluded because of the possible influence of the period of extended wakefulness associated with CR1. Mean activity levels were higher in the placebo trial than in the melatonin trial during the first half of the sleep opportunity and lower in the placebo trial than in the melatonin trial during the second half of the sleep opportunity (Fig. 5). A significant treatment trial by time interaction was observed ($P < 0.001$). *Post hoc* analysis revealed that between 1900 h and 2300 h,

activity levels during the placebo trial were significantly greater than during the melatonin trial ($P < 0.05$), and between 0200 h and 0300 h activity levels were significantly higher in the melatonin trial ($P < 0.05$).

Mean total activity levels calculated across the treatment period (D4 to D10) for the sleep opportunities and the sched-

uled wake episodes separately did not differ between the melatonin and placebo trials ($P > 0.05$; Fig. 6).

Pituitary/gonadal hormones

Mean 24-h levels of pituitary/gonadal hormones, assessed during CR2, were found to be within normal range (37) in both treatment trials (Table 2), except for one subject, who showed abnormally high TSH levels in both trials (>5 sds from mean values). This subject's TSH data were excluded from the analysis. Average levels of all hormones measured did not vary significantly between the treatment trials (Table 2). Although there was no significant difference in PRL levels between the two trials, the level after melatonin treatment was slightly lower than after placebo (difference = 18.3 mU/liter; $P = 0.07$, 95% confidence interval -38.9 to 2.3).

Self-assessment of daytime sleepiness and mood

As for the actigraphy analysis, data from D3 were excluded for self-assessed sleepiness and mood. There was not a significant effect of melatonin administration on daytime sleepiness or mood scores (D4-D11, Table 3). A main effect of time of day was found for sleepiness scores ($P < 0.001$), with scores at 0800 h being significantly higher than other times.

Discussion

The present study provides clear and convincing evidence that melatonin (1.5 mg at 1600 h) is able to substantially advance the timing of endogenous melatonin and cortisol rhythms with no evidence of changes in the duration of endogenous melatonin secretion or changes in the pituitary/gonadal axis. Actigraphic data indicate that the distribution of activity was profoundly altered during melatonin administration such that lower activity occurred during the first half of the sleep opportunity after melatonin and during the second half after placebo. However, total activity levels during the scheduled sleep opportunities and during the wake episodes were not altered by melatonin administration. Similarly, daytime sleepiness and mood were not affected.

The magnitude of the phase advances induced by melatonin are consistent with previous reports (5, 13–15). Small phase advances were also observed in the placebo trial, and these are likely to be caused by the alteration in light/dark exposure, in particular the lack of high light levels during the evening (38) and possibly the daily regimen of enforced bed

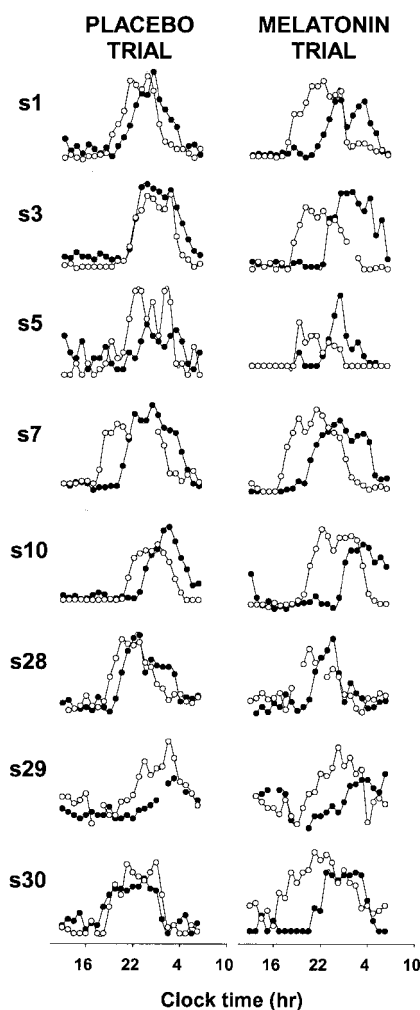


FIG. 4. Plasma melatonin profiles for individual subjects (s1, s3, s5, s7, s10, s28, s29, and s30) in the placebo trial (left) and melatonin trial (right), in CR1 (closed circles) and CR2 (open circles). The vertical axis represents plasma melatonin levels (exact values not given), and clock time (h) is shown on the horizontal axis.

TABLE 1. Circadian phase shifts

| | Melatonin trial | Placebo trial | 95% CI CR1 vs. CR2 | 95% CI Mel vs. Plac |
|-------------------------------------|---------------------------|---------------------------|-------------------------------------|---------------------|
| Plasma melatonin onset ^a | +5.3 h (0.4) ^d | +2.4 h (0.7) ^c | Mel: 4.3 to 6.2 Plac: 0.7 to 4.0 | 0.6 to 5.2 |
| Plasma cortisol peak ^b | +4.4 h (0.5) ^d | +1.8 h (0.5) ^c | Mel: 3.2 to 5.5 Plac: 0.7 to 2.9 | 1.1 to 4.1 |

Mean (\pm SEM) phase shifts (h) observed for plasma melatonin onset (25% crossing) and cortisol peak. Positive values indicate advances in the timing of the phase marker from constant routine (CR)1 to CR2. These data were analyzed by 2-way repeated measures ANOVA (factors, treatment trial and CR). ANOVA revealed significant main effects for CR (melatonin onset $P < 0.001$; cortisol peak $P < 0.001$) and significant treatment trial \times CR interaction effects (melatonin onset $P < 0.05$; cortisol peak $P < 0.01$). These interactions were further analyzed by paired samples *t* tests. Significant differences in the magnitude of shifts between the melatonin (Mel) and the placebo (Plac) trial are indicated as ^a if $P < 0.05$ and ^b if $P < 0.01$. Significant advances from CR1 to CR2 are shown as ^c if $P < 0.05$ and ^d if $P < 0.01$. For each comparison (CR1 vs. CR2, or Mel vs. Plac), the 95% confidence interval (CI) for the difference is shown.

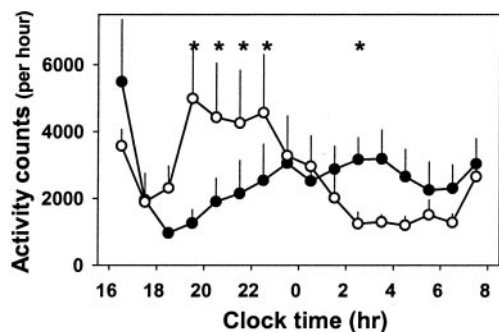


FIG. 5. Mean (\pm SEM) wrist activity levels (1-h intervals) recorded during sleep opportunities calculated across all treatment days except study D3 in the melatonin trial (closed circles) and the corresponding day of the placebo trial (open circles). Clock time is shown on the horizontal axis. Significant differences between the melatonin and placebo trials, revealed by repeated-measures ANOVA and subsequent paired samples *t* tests, are indicated by asterisks ($P < 0.05$).

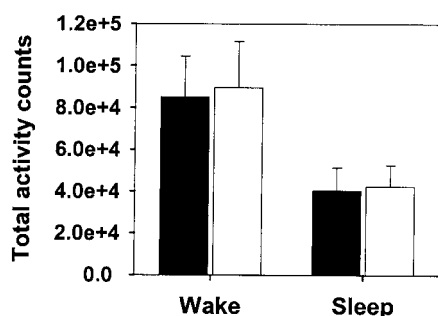


FIG. 6. Mean (\pm SEM) total wrist activity levels during scheduled sleep opportunities and wake episodes, calculated across all treatment days except study D3 in the melatonin trial (black columns) and the corresponding day of the placebo trial (white columns). Paired samples *t* tests did not reveal significant differences between the treatment trials ($P > 0.05$).

rest. Melatonin evidently reinforced the effects observed in the placebo condition.

The redistribution of wrist activity levels by melatonin administration occurred without altering the total activity levels during the sleep opportunities or the wake episodes. These findings are in contrast to those of Zhdanova *et al.* (39), who showed that melatonin (40–320 μ g/kg) administered to three species of nonhuman primates resulted in increased nocturnal (actigraphically assessed) activity levels and reduced daytime activity levels. A number of methodological differences between the previous study and present study may account for the divergent findings, including species; light-dark and activity-rest cycles; whether there was enforced recumbency during melatonin treatment; and the timing, dose, and formulation of melatonin.

Artificially prolonged melatonin profiles usually occur in protocols that use melatonin as a phase-shifting agent because to achieve maximal shifts, the optimal time of administration will be several hours before or after the time when endogenous melatonin levels are high (40). It is therefore important to ascertain whether a prolonged melatonin profile results in altered circadian and pituitary/gonadal functioning in humans, as is the case in many seasonally breeding

TABLE 2. Pituitary/gonadal hormone concentrations

| | Melatonin trial | Placebo trial |
|---------------------------|-----------------|----------------|
| PRL (mU/liter) | 230.64 (9.33) | 248.93 (15.09) |
| LH (IU/liter) | 4.28 (0.41) | 4.58 (0.70) |
| GH (mU/liter) | 2.14 (0.39) | 2.57 (0.81) |
| TSH (mU/liter) | 2.94 (0.36) | 3.03 (0.39) |
| Testosterone (nmol/liter) | 18.83 (1.75) | 18.54 (1.25) |
| FSH (IU/liter) | 3.69 (1.32) | 3.83 (1.41) |

Mean (\pm SEM) 24-h pituitary/gonadal hormone levels assessed during CR2 in the melatonin and placebo trials. Hormone levels were measured hourly for 24 h, and mean levels were determined. Administration of melatonin (1.5 mg) for 8 consecutive days did not significantly alter any of the hormone levels.

mammals (2). Prolonged exposure of human subjects to a long-night, short-day cycle results in changes in the profile of some circadian rhythms including the melatonin rhythm and slightly increased duration and redistribution of sleep (3, 41). These findings are reminiscent of the effects of photoperiod in other mammalian species (42, 43). In our study, 9 d of exposure to 16-h sleep opportunities did not produce photoperiodic changes in melatonin duration. Comparing these data with those of Wehr and colleagues (3), it may be suggested that the length of time required to observe human photoperiodic responses is between 9 d and 4 wk. Alternatively, it is possible that the sample size used in the present study was not sufficient to detect statistically significant human photoperiodic responses.

In this short-term study of the effects of an artificially prolonged melatonin profile, we did not observe changes in the duration of endogenous melatonin and found no significant changes in 24-h levels of pituitary/gonadal hormones. A previous study showed that long-term melatonin administration (1 month, 6 mg) to six healthy male subjects did not alter mean nocturnal LH, FSH, testosterone, and inhibin- β secretion (44). The trend to lower PRL after melatonin treatment in the present study may, however, indicate a comparable photoperiodic response in humans should treatment be prolonged. PRL declines rapidly under short days (or long duration melatonin) in photoperiodic species (45). The non-significant decline in PRL observed in the present study is if anything reassuring because increased PRL is associated with a number of problems including infertility in women (46), with some evidence for such an association in men (47).

A surge-sustained release capsule containing melatonin (1.5 mg) was used in the present study to achieve high circulating levels for at least the duration of the sleep opportunity (16 h) at close to physiological levels. Whether the concentrations achieved using this formulation of melatonin (maximum 626 ± 212 pg/ml) could be considered a pharmacological or a physiological treatment is a matter for discussion, and further data are required on the concentration of melatonin at its target sites (48).

Our findings that melatonin administration does not have deleterious effects on subjective assessments of daytime sleepiness and mood on the day following treatment support the short-term use of melatonin for the treatment of sleep complaints. Particularly in situations in which the circadian clock is misaligned from external time cues, *e.g.* after rapid time zone change and when employed on changing shift

TABLE 3. Self assessments of daytime sleepiness and mood

| | Melatonin trial | | | | Placebo trial | | | |
|---------------|-----------------|-------------|-------------|-------------|---------------|-------------|-------------|-------------|
| | 0800 h | 1000 h | 1200 h | 1400 h | 0800 h | 1000 h | 1200 h | 1400 h |
| Sleepiness | 4.2 (0.3) | 3.0 (0.3) | 3.1 (0.3) | 3.0 (0.2) | 4.0 (0.3) | 2.9 (0.3) | 2.8 (0.2) | 3.2 (0.2) |
| Agitation | 79.5 (5.8) | 71.7 (10.9) | 73.3 (10.9) | 75.7 (10.3) | 74.8 (7.2) | 72.9 (9.7) | 70.9 (9.8) | 70.7 (9.7) |
| Concentration | 65.7 (6.3) | 67.3 (9.6) | 65.5 (9.0) | 68.1 (7.7) | 65.7 (5.0) | 64.9 (8.4) | 65.3 (8.7) | 64.8 (8.6) |
| Aggression | 75.7 (6.6) | 70.0 (10.1) | 72.3 (10.6) | 72.7 (10.0) | 70.8 (7.1) | 70.4 (8.8) | 65.6 (9.3) | 67.7 (9.3) |
| Depression | 59.5 (6.1) | 56.9 (9.3) | 56.7 (9.4) | 59.2 (9.2) | 59.4 (7.0) | 58.9 (9.0) | 59.5 (8.8) | 60.1 (9.0) |
| Anxiety | 71.4 (9.2) | 69.7 (11.3) | 69.5 (11.3) | 71.0 (11.3) | 68.2 (7.9) | 71.3 (10.5) | 69.1 (10.3) | 67.7 (11.0) |

Mean (\pm SEM) subjective assessments of sleepiness (Karolinska Sleepiness Scale, 0–9) and mood (visual analogue scales, 0–100) during the treatment phase of melatonin and placebo trials. Assessments were recorded at 2-h intervals, from 0800 h to 1400 h each day, and the mean for each time was determined (study days 4 to 11). No significant differences were observed between the melatonin and placebo trials. For all scales except concentration, higher is worse.

schedules (49), melatonin administration, under appropriate conditions, may have substantial benefits. Non-24-h sleep-wake disorder of the blind is a lifetime problem requiring long-term treatment. Melatonin is probably the treatment of choice in these patients (17, 18). In this case long-term safety studies are desirable (50), and the present findings should be confirmed in clinical populations. We also note that if desired sleep time is not for several hours after the time of melatonin administration (*e.g.* 1600 h), alertness levels may be compromised during the intervening period.

In summary, these findings provide unequivocal evidence that, in the short term, timed melatonin treatment in suitable conditions is a highly effective means of phase-shifting circadian rhythms, without indication of deleterious effects on major aspects of human endocrine function, or daytime sleepiness and mood on the day following administration.

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