# Phase-Dependent Treatment of Delayed Sleep Phase Syndrome with Melatonin

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**Study Objective:** Delayed sleep phase syndrome (DSPS) is a circadian-rhythm sleep disorder characterized by abnormally late sleep and wake times. Melatonin, taken in the evening, advances sleep and circadian phase in patients with DSPS. However, little is known about the most effective dose or time of administration. In the present study, we tested the effectiveness of melatonin to advance the timing of sleep and circadian phase in individuals with DSPS.

**Design:** Following baseline assessment of sleep and circadian phase, subjects were randomly assigned to 1 of 3 treatment groups. The administration of melatonin (0.3 or 3.0 mg) or placebo was double-blinded.

**Setting:** All procedures were conducted on an outpatient basis.

**Participants:** Thirteen subjects with DSPS, recruited via flyers, advertisements, and referrals from the Sleep Clinic, completed this study.

**Interventions:** Melatonin (0.3 or 3.0 mg) or placebo was administered between 1.5 and 6.5 hours prior to dim light melatonin onset for a 4-week period.

**Measurements and Results:** Both doses of melatonin advanced the circadian phase of endogenous melatonin. The magnitude of phase advance in dim-light melatonin onset correlated strongly with the time of melatonin administration, with earlier times being more effective ($r^2$ = 0.94, P < .0001). Similar, though weaker, relationships were obtained between the timing of melatonin administration and changes in sleep time.

**Conclusions:** These results indicate that melatonin advances the circadian clock and sleep in patients with DSPS in a phase-dependent manner. This is the first study that reports a relationship between timing of melatonin administration and phase changes in patients with DSPS.

**Keywords:** Circadian rhythms, melatonin, delayed sleep phase syndrome

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## INTRODUCTION

DELAYED SLEEP PHASE SYNDROME (DSPS) IS A CIRCADIAN-RHYTHM SLEEP DISORDER CHARACTERIZED BY UNUSUALLY LATE SLEEP AND WAKE TIMES WITH an inability to fall asleep or wake up at conventional times.[1] The inability to maintain a conventional sleep phase results in symptoms of chronic insomnia and excessive daytime sleepiness, which affect education, work, mood, and social life.[2-4] Although the exact prevalence is unknown, it is believed that approximately 10% of patients with chronic insomnia may have DSPS.[4,5] In addition to sleep, melatonin and core body temperature (CBT) rhythms are also delayed.[6-8] Thus, evidence indicates that the delay in timing of sleep is at least in part due to a delay in circadian timing relative to the light-dark cycle.

Treatment of DSPS focuses on aligning the endogenous sleep rhythm with conventional sleep times, as dictated by a socially acceptable schedule. Currently accepted modes of treatment include chronotherapy, early-morning bright light, and evening oral melatonin. Chronotherapy involves delaying sleep onset for approximately 3 hours every day until sleep occurs at the desired sleep time.[2] While effective, the need for a controlled environment and the length of treatment decrease the practicality of chronotherapy. Furthermore, in some patients with DSPS, chronotherapy can induce free-running circadian rhythms.[9] Bright light, usually administered for 1 to 2 hours in the early morning, has also been shown to be effective.[6] However, due to the need for somewhat lengthy daily treatment, compliance is generally poor.

Because of the practical limitations of both light and chronotherapy, melatonin taken orally in the evening has been increasingly investigated as a potential treatment for DSPS. As with other treatments that target the circadian system, melatonin affects circadian timing in a phase dependent manner. The phase response curve (PRC) to melatonin in humans reveals advances in phase when melatonin is administered in the evening and delays in phase when melatonin is given in the morning.[10] In healthy volunteers, maximal phase advances were obtained when melatonin (0.5 mg for 4 days) was administered 3 hours before the dim-light melatonin onset (DLMO).[10] It should be noted, however, that the published PRC to melatonin is based on internal circadian time, rather than external clock time. Given that the circadian time of patients with DSPS is later than that of unaffected controls, the clock time of melatonin administration may be substantially later and should be adjusted to each patient's own circadian phase.

In patients with DSPS, melatonin given in the evening is reported to advance sleep, temperature, and melatonin rhythms; decrease sleep latency; and improve quality of life.[7,8,11-15] Typically, a dose of 5 mg of melatonin has been administered for at

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least 4 weeks in patients with DSPS, with the time of administration ranging from 5:00 PM to 10:00 PM. These times of administration were determined by either using a fixed time for all subjects or individualizing treatment based on baseline sleep time, estimated DLMO, or measured DLMO.[7,8,11-15] It remains unclear if individualized timing is more effective than administration at standard times or whether efficacy differs with low and high doses of melatonin. Thus, there is a lack of a uniform approach to the use of melatonin in the treatment of DSPS. In the present pilot study, we assessed the effectiveness of 2 doses of melatonin (0.3 or 3.0 mg) to advance circadian and sleep phase, with times of administration ranging between 1.5 and 6.5 hours prior to measured DLMO.

## METHODS

### Study Protocol

Subjects with DSPS were recruited via flyers, advertisements, and referrals from the Northwestern Medical Faculty Foundation Sleep Clinic, Chicago, Ill. All subjects met the criteria for DSPS according to the International Classification of Sleep Disorders criteria.[16] All subjects reported a delay in sleep and wake times relative to the demands of society (work and school schedules) and an inability to fall asleep at conventional or desired times, as well as difficulty awakening in the morning. Although it is well recognized that behavior can influence the expression and perpetuate the condition, subjects were not further classified as having intrinsic or extrinsic DSPS in this study. The delayed sleep and wake times were assumed to be predominantly due to a misalignment between circadian timing and the desired or conventional sleep and wake times. A detailed medical and psychiatric history was obtained from all subjects, and medical records were reviewed when available. Patients on hypnotic, antidepressant, or other psychotropic medications were excluded from the study. Patients with a history of shift work within 1 month of enrollment or travel across 1 or more time zones during the study period were excluded. This study was approved by the Northwestern University Institutional Review Board, and informed consent was obtained prior to participation.

A total of 22 subjects with DSPS were enrolled and randomly assigned to treatment with 0.3 mg of melatonin, 3.0 mg of melatonin, or placebo. One subject, assigned to the melatonin treatment, withdrew due to nausea. Eight subjects withdrew or were discontinued from the study due to noncompliance with the study procedures. Reasons for withdrawal included discomfort from the rectal temperature monitoring and the time required for the study. The group sizes of the 13 subjects (8 men, 5 women) who completed the protocol were 5 for 0.3 mg of melatonin, 4 for 3.0 mg of melatonin, and 4 for placebo. The average age was 28.15 ± 5.74 years. Demographic information is presented in Table 1.

Subjects maintained their usual daily routine throughout the study, attending classes or work. They were free to go to bed and wake up at the time of their own choosing. All procedures and data collection were conducted on an outpatient basis. The baseline assessment included a clinical evaluation and determination of circadian phase by 48-hour rectal temperature monitoring (Actilume, Ambulatory Monitoring, Inc., Ardsley, NY), as well as collection of salivary DLMO on 2 consecutive evenings and determination of sleep and wake times by sleep diary along with a wrist-worn activity-monitoring device (Actiwatch, Mini Mitter, Inc., Bend, Ore) for 1 week. Subjects were instructed to collect saliva samples under dim light (see specific instructions below). There were no restrictions associated with the portable CBT monitoring. The baseline and posttreatment assessments for CBT and salivary melatonin were conducted on 2 consecutive weekdays (Monday to Thursday) or a combination of 1 weekday and 1 weekend day (eg Sunday and Monday or Thursday and Friday). Differences in sleep times between weekdays (Monday to Thursday) and weekends (Friday to Sunday) at baseline, as well as after treatment, were compared to assess any significant differences within individuals.

Following the baseline assessment, subjects were instructed to take the provided capsules for 2 weeks. The administration of melatonin or placebo capsules was double blinded. Initially, subjects were instructed to take melatonin at 8:00 PM. However, preliminary analysis of data indicated large variability in the timing of DLMO and a phase-dependent response to the effect of melatonin treatment.[17] DLMO was subsequently measured prior to group assignment, and subjects were instructed to take the

**Table 1**—Subject Demographics

| | Age | Gender | Ethnicity | Onset (age) | Occupation | Medical History |
|---|---|---|---|---|---|---|
| AS | 27 | M | Asian | Childhood | Student | GERD, Mitral Valve Prolapse |
| EC | 25 | M | Caucasian | Childhood | Technician | None |
| MH | 25 | M | Caucasian | Childhood | Attorney | Scoliosis |
| MW | 24 | F | Caucasian | Young Adult (22) | Consultant | None |
| SP | 36 | F | Caucasian | Childhood | Consultant | None |
| RK | 23 | F | Asian | Childhood | Student | None |
| DM | 33 | M | Caucasian | Childhood | Student | None |
| JP | 29 | F | Caucasian | Adolesence | Student | None |
| GH | 42 | M | Caucasian | Young Adult (27) | Computer Systems | None |
| RC | 24 | M | Caucasian | Childhood | Library Assistant | None |
| JA | 22 | M | Caucasian | Childhood | Student | History of Seizures, Other hypersomnia |
| EP | 29 | M | Caucasian | Adolescence | Educator | Eczema, Hiatal Hernia |
| SC | 26 | F | Caucasian | Adolescence | Student | None |

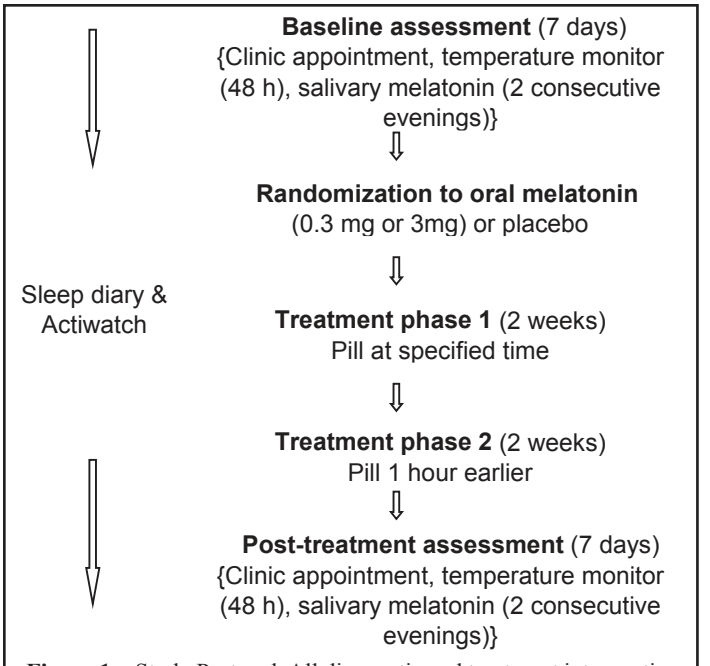



**Figure 1**—Study Protocol. All diagnostic and treatment interventions were clinic based. Following baseline assessment, subjects were instructed to take the provided pill at a specified time for 2 weeks (Treatment Phase 1). The time of administration was advanced by 1 hour for the 2 subsequent weeks (Treatment Phase 2). After completion of the treatment period, subjects underwent a posttreatment evaluation, which was identical to the baseline assessment. Details of the baseline and posttreatment phase assessments are described in methods.

capsules at earlier times relative to their own DLMO but remaining within the phase-advance region of the melatonin PRC.[10,18] The actual range in the time of treatment was 1.5 to 6.5 hours before DLMO, which corresponded to clock times of 3:00 PM to 9:30 PM. During the second treatment phase, patients were instructed to take the capsule 1 hour earlier for the next 2 weeks. A 2-stage treatment was utilized to maximize the phase-advancing effects of melatonin (as the circadian clock advances, the timing of melatonin advances), based on our previous findings from a mouse model.[19] For clarity, treatment times are described as the time melatonin was taken during the first 2-week period. After completing the second treatment period, subjects underwent posttreatment evaluation. The procedures for the posttreatment evaluation were identical to the baseline assessment (Figure 1).

### Assessment of Circadian Phase

Saliva was collected in dim light every half hour beginning about 5 hours before usual sleep time, on 2 consecutive evenings, for determination of the DLMO. Subjects were instructed to collect the saliva samples with cotton swabs and specially designed collection tubes (Salivettes, Sarstedt, Germany). Subjects were instructed to collect the melatonin under dim light, by using only a side lamp while watching TV, and to refrain from eating or drinking in the 20 minutes prior to each sample collection. Subjects were provided with a small cooler in which to keep the samples on ice during the collection period. Samples were returned to the laboratory the next day on ice, centrifuged, and frozen for later analysis by radioimmunoassay. The level of melatonin in saliva was assayed with the Buhlman Saliva-direct kit (ALPCO, Windham, NH). The intraassay precision at 3.56 pg/mL expressed as the coefficient of variance was 4.1%, while the interassay precision at 3.39 pg/mL expressed as the CV was 7.5%. The assay limit of analytical sensitivity was 0.2 pg/mL and the functional sensitivity was 0.9 pg/mL. Average melatonin levels over the 2 nights (pg/mL) were used to determine melatonin phase. DLMO was defined as the time point at which melatonin levels rose greater than 2 standard deviations above the mean of the baseline.[20] Assessment of DLMO was conducted in a blinded manner.

Core body temperature (CBT) was sampled every minute for 48 hours with a flexible rectal thermometer connected to a lightweight data-recording unit. The 24-hour profiles of temperature were edited to remove obvious artifacts and quantitatively described using simple Cosine analysis (software provided by C. Eastman).[21] Demasking procedures were used to counterbalance the decrease in CBT associated with sleep, according to the method of Martin and Eastman.[21-23] The phase of the rhythm was characterized by the timing of the fitted nocturnal temperature minimum (Tmin).

### Assessment of Sleep Phase

Subjects maintained sleep diaries and wore activity monitors (Actiwatch) throughout the study. No specific instructions regarding sleep were provided, and light exposure was not measured. Sleep parameters were assessed from actigraphy data (Actiware-Sleep, Mini Mitter Co., Inc, Bend, Ore). The bedtimes/lights-out times from the sleep-diary data were used to calculate sleep latency from the actigraphy data. The averages for sleep onset, sleep offset, sleep efficiency, and sleep latency were calculated for the baseline week and the fourth week of treatment.

### Melatonin Capsules

The study was conducted under an "Investigational New Drug" protocol for melatonin (Food and Drug Administration Protocol # 52606). Melatonin for use in humans was purchased from Regis Technologies, Inc. (Morton Grove, Ill). The Northwestern Memorial Hospital pharmacist filled gelatin capsules with lactose (placebo pills) or lactose and melatonin (0.3 or 3.0 mg). To ensure accurate dosage and stability of the melatonin after packing, the amount of melatonin was determined by high-pressure liquid chromatography with ultraviolet detection. Melatonin levels in the capsules (0.3 and 3.0 mg/capsule) were measured every 3 months and remained stable during the course of the study (mg/capsule [n=3]: 0.35 ± 0.01 and 3.32 ± 0.12 at the beginning; 0.3 ± 0.01 and 3.05 ± 0.17 at the end, respectively). Melatonin identity was assessed by mass spectrometric analysis (Biomedical and Bioorganic Mass Spectrometry Resource, Washington University, St. Louis, MO) in capsules selected at random for the duration of the study. A prominent ion of mass 233.1 ± 0.1 corresponding to protonated melatonin was always present. Mass ions corresponding to oxidized or deacylated products were absent.

### Statistical Analysis

Changes in circadian phase were assessed by the difference in the time of the phase marker (DLMO and Tmin) from baseline to posttreatment for each subject. Changes in sleep phase were assessed by the difference in the time of sleep onset and sleep offset from baseline to the last week of treatment for each subject. To evaluate possible differences in sleep parameters on weekend versus weekdays, weekend and weekdays were analyzed both

**Table 2**—Weekday and Weekend Sleep Parameters at Baseline

**A. Placebo**

| | Weekday Sleep Times | | | Weekend Sleep Times | | |
|---|---|---|---|---|---|---|
| **Subjects** | **Sleep onset** | **Sleep offset** | **Sleep latency** | **Sleep onset** | **Sleep offset** | **Sleep latency** |
| 7 | 23.78 | 6.90 | 0.08 | 0.32 | 7.83 | 0.18 |
| 12 | 4.92 | 9.08 | 0.12 | 2.25 | 7.47 | 0.25 |
| 14 | 1.28 | 7.42 | 0.73 | 1.38 | 7.27 | 1.07 |
| Mean | 1.99 | 7.80 | 0.31 | 1.32 | 7.52 | 0.50 |
| SD | 2.64 | 1.14 | 0.37 | 0.97 | 0.29 | 0.49 |

**B. Melatonin**

| | Weekday Sleep Times | | | Weekend Sleep Times | | |
|---|---|---|---|---|---|---|
| **Subjects** | **Sleep onset** | **Sleep offset** | **Sleep latency** | **Sleep onset** | **Sleep offset** | **Sleep latency** |
| 1 | 5.21 | 12.54 | 0.40 | 2.45 | 11.4 | 0.22 |
| 2 | 1.84 | 10.36 | 0.17 | 3.59 | 12.35 | 0.07 |
| 4 | 0.22 | 7.82 | 0.53 | 0.66 | 8.04 | 0.38 |
| 13 | 0.01 | 9.41 | 0.32 | 2.06 | 9.41 | 0.17 |
| 15 | 0.88 | 7.99 | 1.33 | 1.44 | 9.73 | 1.95 |
| 16 | 23.79 | 5.49 | 0.40 | 1.15 | 8.91 | 0.68 |
| 19 | 3.2 | 10.72 | 0.17 | 1.79 | 9.56 | 0.02 |
| 21 | 2.38 | 8.86 | 0.00 | 2.95 | 9.83 | 0.07 |
| Mean | 1.69 | 9.15 | 0.41 | 2.01 | 9.90 | 0.44 |
| SD | 1.87 | 2.14 | 0.41 | 0.96 | 1.37 | 0.65 |

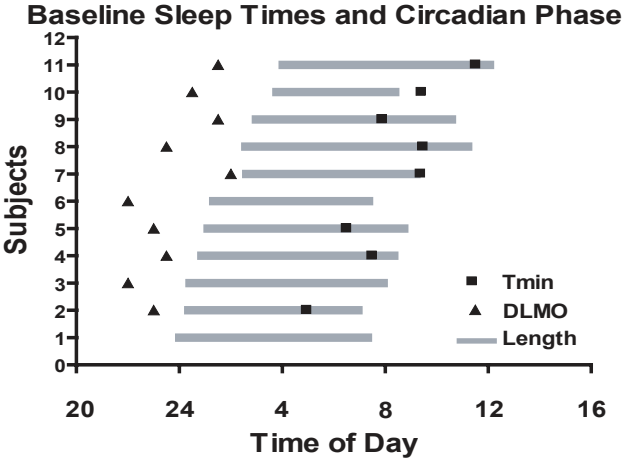
separately and together (Table 2). As there was no significant difference in the average sleep parameters between weekend (Friday to Sunday) and weekday (Monday to Thursday) in this population (paired t tests), only the full-week analysis is presented here. Phase angles between circadian markers and the sleep schedule were calculated as the interval between the time of the phase marker (DLMO or Tmin) and the time of sleep onset or sleep offset. For missing values, remaining data were included in the average circadian phase (Table 3, baseline and posttreatment) but not in the calculation of phase change or phase relationships. Changes in the timing of sleep and circadian phase markers between baseline and following treatment were compared using paired 2-tailed t tests. Correlation coefficients between the variables were calculated using Pearson rank correlation coefficient.

## RESULTS

Average baseline sleep onset for the entire group was 1.83 ± 1.42 hours and sleep offset was at 8.90 ± 1.66 hours as measured by actigraphy (Table 3). The baseline DLMO was 23.46 ± 1.62 hours, and the average time of the Tmin occurred at 7.96 ± 2.21 hours.

Phase relationships between the timing of sleep and circadian phase markers at baseline are illustrated in Figure 2. On average, melatonin levels began to rise 2.16 ± 0.93 hours prior to sleep onset or 9.21 ± 1.35 hours before sleep offset (or 14.79 hours after sleep offset). Tmin occurred 1.09 ± 1.29 hours prior to sleep offset.

Administration of placebo had no effect on the timing of DLMO, whereas melatonin (0.3 and 3.0 mg) advanced DLMO and Tmin (Table 3). There was no difference in the average magnitude of phase shift induced by the 2 doses of melatonin; therefore, we combined the 2 groups for further analyses. DLMO and Tmin advanced by an average of more than 1.5 hours for the




| Phase Relationships | Average (h ± sd) |
|---|---|
| DLMO - Sleep Onset | 2.16 ± 0.93 |
| DLMO - Sleep Offset | 9.21 ± 1.35 |
| DLMO - Tmin | 8.02 ± 1.43 |
| Sleep Onset - Tmin | 6.03 ± 1.05 |
| Tmin - Sleep Offset | 1.09 ± 1.29 |

**Figure 2**—Baseline Phase Relations. The baseline phase relations between dim-light melatonin onset (DLMO) (▲), sleep times (bar), and temperature nadir (■) in individual subjects are depicted in the left panel. Temperature nadir for 3 of the subjects could not be obtained. Average group values (± SD, n) for phase relations are described in the panel on the right.

**Table 3**—Effect of Treatment on Sleep Measures and Circadian Phase

| | Baseline | Post-treatment | Change |
|---|---|---|---|
| **DLMO** | | | |
| Melatonin | 23.67 ± 1.70 (9) | 22.25 ± 0.80 (8) | 1.75 ± 0.89*** |
| Placebo | 22.83 ± 1.44 (3) | 23.17 ± 2.02 (3) | -0.33 ± 0.58 |
| **Tmin** | | | |
| Melatonin | 7.78 ± 2.29 (8) | 6.15 ± 1.86 (8) | 1.63 ± 1.79* |
| Placebo | N.A. | N.A. | N.A. |
| **Sleep Onset** | | | |
| Melatonin | 1.88 ± 1.36 (8) | 1.43 ± 1.56 (8) | 0.45 ± 0.68 |
| Placebo | 1.71 ± 1.90 (3) | 1.38 ± 1.72 (3) | 0.33 ± 0.27 |
| **Sleep Offset** | | | |
| Melatonin | 9.36 ± 1.75 (8) | 8.57 ± 1.15 (8) | 0.79 ± 1.03# |
| Placebo | 7.67 ± 0.60 (3) | 8.14 ± 1.75 (3) | -0.47 ± 1.17 |
| **Total Sleep Time** | | | |
| Melatonin | 7.49 ± .68 (8) | 7.13 ± .69 (8) | -0.36 ± 1.11 |
| Placebo | 5.97 ± 1.34 (3) | 6.88 ± .09 (3) | 0.91 ± 1.35 |
| **Sleep Efficiency** | | | |
| Melatonin | 81.75 ± 11.32 (8) | 80.25 ± 10.07 (8) | -1.50 ± 7.56 |
| Placebo | 79.51 ± 2.33 (3) | 81.32 ± 3.53 (3) | 1.80 ± 2.14 |
| **Sleep Latency** | | | |
| Melatonin | 0.42 ± 0.51 (8) | 0.30 ± 0.30 (8) | -0.11 ± 0.28 |
| Placebo | 0.39 ± 0.42 (3) | 0.34 ± 0.14 (3) | 0.05 ± 0.29 |

Average time in hours ± sd, (n) By convention, advances in phase are depicted as positive, and delays in phase are depicted as negative. (* $p < 0.05$, *** $p < 0.001$, # $p = 0.067$). N.A. Temperature data was not available for three of the four subjects in the placebo group.

melatonin group (1.75 ± 0.89 hours, $P < .001$ and 1.63 ± 1.79 hours, $P < .05$, respectively). DLMO on the second evening of assessment after melatonin treatment was 0.42 ± 0.67 hours earlier than on the first evening of assessment, suggesting no significant aftereffects of the medication. The days of the week of the baseline and posttreatment circadian-phase assessments and the respective DLMO are shown in Table 4. There was no difference in the average DLMO between weekdays (23.46 ± 1.53 hours) and weekends (23.29 ± 1.39 hours). Although there was a tendency for subjects to wake up earlier (0.79 hours, $P = .067$) following treatment with melatonin, this change did not reach statistical significance. Sleep onset was comparable between the 2 groups. Melatonin administration did not affect total sleep time, sleep efficiency, or sleep latency, as determined by actigraphy (Table 3).

Advances in the timing of DLMO were dependent on the time of melatonin administration at initiation of treatment, such that the earlier the time of melatonin administration, the greater the magnitude of the phase advance ($r^2 = 0.94$, $P < .0001$) (Figure 3A). When melatonin was taken approximately 2 hours prior to DLMO, phase advances were of relatively small magnitude (1 hour), whereas, when melatonin was taken between 5 and 7 hours before baseline DLMO, phase advances of more than 2.5 hours were obtained. As shown in Figure 3B, the linear correlations (between the time of administration and phase shifts in DLMO) were similar for the 2 doses of melatonin (0.3 mg vs 3.0 mg). The timing of melatonin administration and phase shifts in Tmin were not significantly correlated.

The timing of melatonin administration and advances in the timing of sleep offset were also correlated. When changes in sleep offset were plotted according to the timing of melatonin administration, the data revealed a modest correlation (Figure 4B, time of melatonin administration relative to sleep offset: $r^2 = 0.5$, $P < .05$), with the effectiveness of melatonin increasing with earlier times of administration.

## DISCUSSION

This pilot study assessed the effectiveness of individually timed melatonin (0.3 or 3.0 mg) to advance sleep and circadian phase in subjects with DSPS. We found that both doses of melatonin advanced the circadian rhythms of melatonin and CBT. For the melatonin treatment group as a whole, 4 weeks of melatonin administration induced robust advances in DLMO and Tmin. A common alternative explanation for the advance in circadian phase following melatonin administration in the evening is that the phase change is secondary to falling asleep and waking earlier (and subsequently advancing the light-dark cycle). In the present study, there was a trend for earlier wake times, but sleep-onset time did not change following treatment with melatonin. Although we cannot exclude the possibility that the phase advance was secondary to alterations in light exposure, it is unlikely that the 47-minute advance in sleep offset-light onset can entirely account for the 1-hour 45-minute advance in the timing of DLMO. Furthermore, recent studies indicate that variations of this magnitude in the timing of sleep and wake have relatively minor effects on the timing of clock-controlled phase markers.[24,25]

This is the first study to investigate the relationship between the circadian time of melatonin administration and the magnitude of phase shifts in patients with DSPS. When oral melatonin was administered 1.5 hours to 6.5 hours prior to measured DLMO, the magnitude of phase advances correlated strongly with the time of administration, such that the earlier the administration, the greater the magnitude of the phase advances. Larger phase advances with early administration times is supported by other studies.[7,8,11,12] Based on the published melatonin PRC in normal subjects, we predicted that melatonin taken 2 to 4 hours before DLMO would result in the greatest phase advances.[10,18] However, in our study, maximal phase shifts occurred when melatonin administration began 6.5 hours before DLMO.

There are several differences in the protocols between the present study and the melatonin PRC in normal subjects published by Lewy et al.[10,18] The present study was conducted in patients with DSPS, and the duration of treatment was longer (4 weeks vs 4 days). The most likely explanation is that, since internal circadian phase moves progressively through the broad phase-advance region of the melatonin PRC, the earlier in the phase-advance region of the PRC that melatonin is taken, the more potential there is for phase advances. Theoretically, for a subject who receives melatonin at 5:30 pm (initially 6 hours before DLMO), his or her DLMO would gradually phase advance over the 4 weeks, thereby effectively receiving treatment at -6, then -5, then -4, and eventually -3 hours before DLMO. In contrast, a subject who receives melatonin initially 1 hour prior to DLMO would advance by 1 hour and then would rapidly be out of the phase-advance region, thus limiting any further advances. Data shown in Figure 3 support this hypothesis, as there was a strong linear correlation between the time of melatonin administration

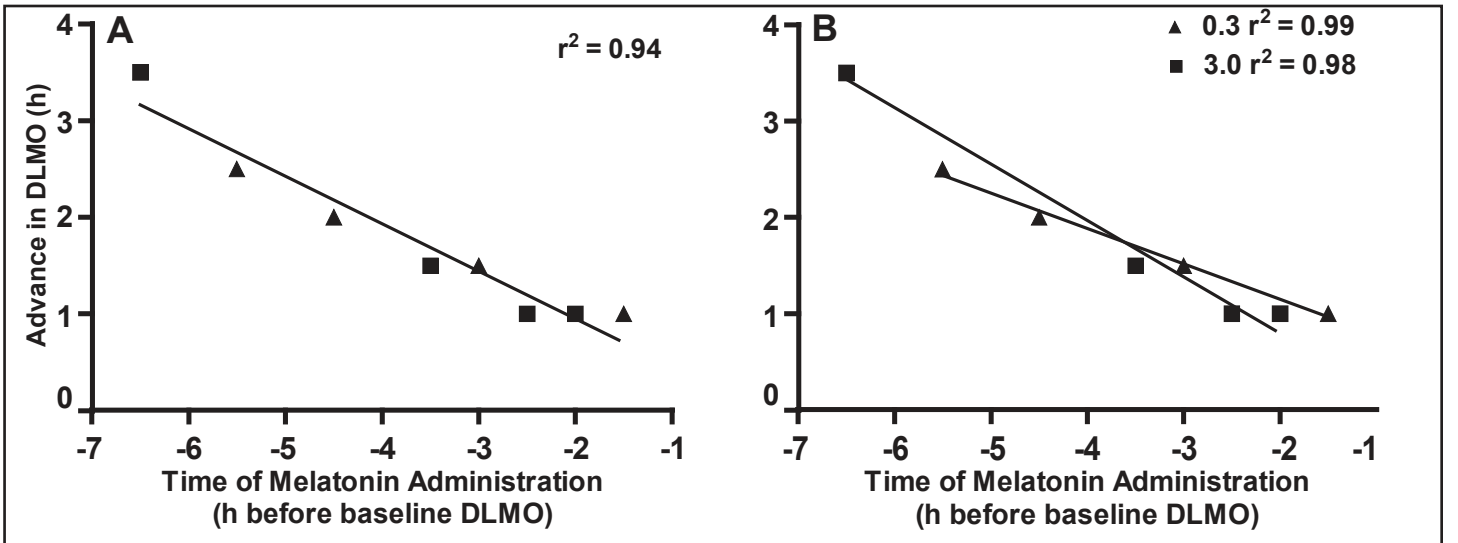



**Figure 3**—Correlation between advance in dim-light melatonin onset (DLMO) and time of administration of melatonin. Melatonin, (0.3 ▲ or 3.0 mg ■ in a double-blind fashion) was administered at various circadian times. (A) There was a strong correlation between time of circadian administration and the magnitude of phase advance. Slope = -0.49 ± 0.05, $r^2$ = 0.94, $P$ < .001. (B) There was no difference in the effectiveness of the 2 doses of melatonin to advance DLMO. 0.3 mg: Slope = -0.36 ± 0.02, $r^2$ = 0.9918; 3 mg: Slope = -0.58 ± 0.05, $r^2$ = 0.98

**Table 4**—Days of the week of circadian phase assessments

**A. Placebo**

| Subjects | Baseline DLMO | Days Assessed | Post-tx DLMO | Days Assessed |
|---|---|---|---|---|
| 9 | 22.0 | Mon/Tues | 22.0 | Tues/Wed |
| 12 | 24.5 | Sun/Mon | 1.5 | Thu/Fri |
| 14 | 22.0 | Sun/Mon | 22.0 | Thu/Fri |
| Mean | 22.83 | | 23.17 | |
| SD | 1.44 | | 2.02 | |

**B. Melatonin**

| Subjects | Baseline DLMO | Days Assessed | Post-tx DLMO | Days Assessed |
|---|---|---|---|---|
| 1 | 1.5 | Thurs/Sun | 23.5 | Wed/Thu |
| 2 | 23.5 | Sun/Mon | 22.0 | Wed/Thu |
| 4 | 22.0 | Tues/Wed | 21.0 | Sun/Mon |
| 13 | 23.5 | Sun/Mon | 22.5 | Thu/Fri |
| 15 | 23.0 | Mon/Tues | 21.5 | Mon/Tues |
| 16 | 23.0 | Sun/Mon | 22.0 | Thu/Fri |
| 19 | 1.5 | Mon/Tues | 23.0 | Tues/Wed |
| 20 | 21.0 | Sun/Mon | | Thu/Fri |
| 21 | 2.0 | Sun/Mon | 22.5 | Wed/Thu |
| Mean | 23.67 | | 22.25 | |
| SD | 1.70 | | 0.80 | |

Time of DLMO (hour) and the days of the week on which it was assessed.

and the magnitude of phase advances. The subjects in our study who received melatonin earlier relative to DLMO had the largest phase advances.

In this study, we did not assess whether an advancing schedule was more effective than a fixed time of administration. In addition, it remains possible that even greater advances may be obtained if subjects take melatonin earlier than 6.5 hours before DLMO. In support of this hypothesis, robust average advances of nearly 2 hours in sleep and wake times have been reported when melatonin was taken between 4 and 8.5 hours prior to estimated DLMO.[11] These authors did not examine the relation between the time of melatonin administration and the magnitude of phase advances in individual subjects. Thus, additional studies are required to define the most effective time and type of schedule of melatonin administration in individuals with DSPS.

With this small sample size, we saw no evidence of a difference in the ability of the 2 doses, 0.3 and 3.0 mg of melatonin, to phase advance DLMO when administered over the course of 4 weeks. This is consistent with the finding that low doses of melatonin can effectively entrain circadian rhythms in the blind.[26,27] Since the maximal phase-advancing effects were seen when melatonin was administered in the late afternoon or early evening, low doses that are less somnogenic[28-30] may be preferable at those times. However, the sleep-promoting effects of melatonin given closer to bedtime could potentially contribute to the treatment of sleep-onset difficulties in patients with DSPS.

In our study, melatonin induced robust advances in circadian rhythms, without statistically significant advances in the timing of sleep. On average, subjects fell asleep 27 minutes earlier and woke up 47 minutes earlier than before treatment. The 47-minute advance in sleep offset, though not reaching statistical significance, could be clinically relevant for patients with difficulty in meeting work and school schedules. The lack of an effect on sleep onset may be due to the range in the timing of melatonin administration and/or variability in the unrestricted sleep schedule. Also, given the early time of administration together with the short half-life of melatonin, a hypnotic effect would not have been expected near bedtime. Our finding of a greater effect of melatonin on sleep offset is consistent with recent evidence of a stronger relationship between circadian timing and sleep offset than between circadian timing and sleep onset.[25,31,32]

Alterations of circadian timing are commonly believed to underlie DSPS. Although the circadian clock directly affects circadian rhythms of melatonin and temperature, it shares its influence on the timing of sleep with homeostatic drive and social constraints.[33] Thus, it is not surprising that the timing of sleep would be less affected by melatonin administration than would

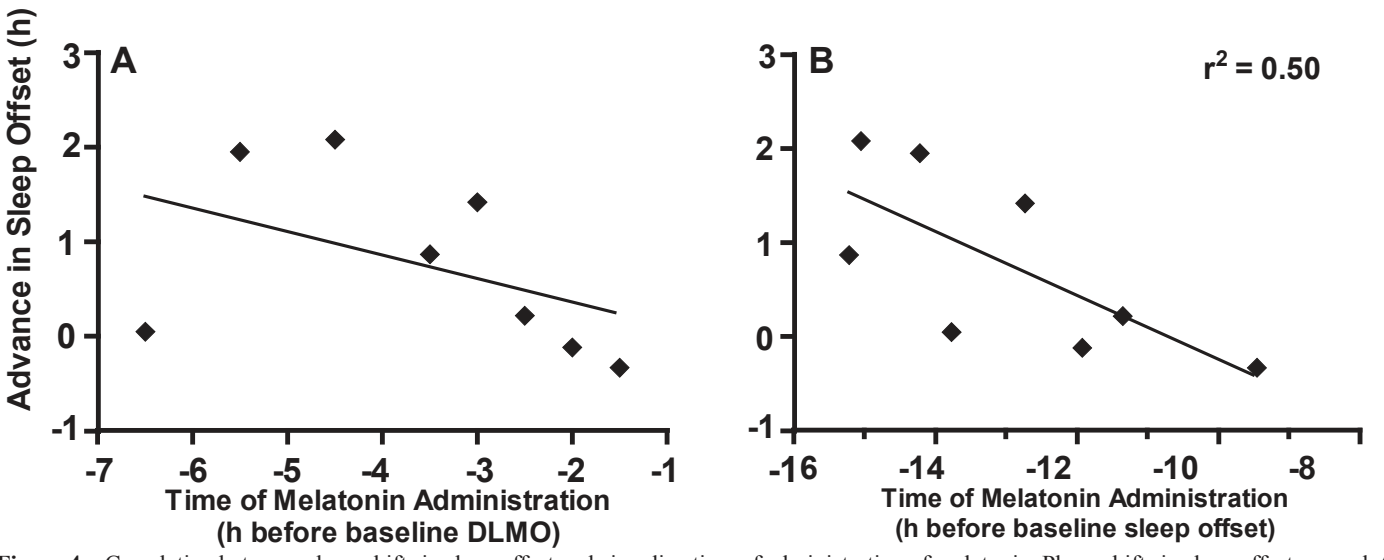



**Figure 4**—Correlation between phase shifts in sleep offset and circadian time of administration of melatonin. Phase shifts in sleep offset were plotted relative to baseline dim-light melatonin onset (DLMO) (A) or baseline sleep offset (B). Exclusion of 1 data point in Figure 4A from a subject given melatonin 6.5 hours prior to DLMO changed the correlation coefficient from $r^2 = 0.20$ ($P > .5$) to $r^2 = 0.85$ ($P < .01$).

be the DLMO and Tmin. In addition, recent evidence suggests that patients with DSPS recover poorly from sleep deprivation.[34] These results suggest that alterations in the interaction between circadian and homeostatic processes may play an important role in the pathophysiology of DSPS. Additional studies are needed to assess the influence of these factors on the timing of sleep in patients with circadian rhythm sleep disorders.

We, like others, found no evidence of changes in sleep parameters following melatonin treatment, other than the tendency to advance the time of sleep.[11,12,14,16] Overall, these results suggest that the primary effect of melatonin under this protocol is to advance the timing of the circadian clock. The group of subjects in the present report had an average sleep onset at approximately 2 am, with DLMO around 11:30 pm and Tmin at nearly 8 am. While these times were several hours delayed relative to healthy control subjects, the relationship between DLMO and sleep onset in this population was similar to that reported for young control subjects (ie, average DLMO of 2 to 3 hours prior to sleep onset).[25,35-37] In contrast, the interval between DLMO or Tmin and sleep offset at baseline (Figure 2) was shorter than that typically reported for controls of similar age.[25,38,39] This finding is consistent with reports that evening types wake at an earlier circadian phase than do morning types.[40,41] It is notable that, following melatonin treatment, the DSPS patients in the present study were waking at a later, more normal, circadian phase. Thus, in the treatment of DSPS, in addition to aligning circadian timing with the external clock time, it may be equally important to normalize the internal phase relationship between circadian rhythms and sleep.

Alterations in phase relations between melatonin and temperature rhythms relative to sleep have previously been reported in subjects with DSPS.[38,39,42] However, while we found a shortened interval between circadian phase markers (DLMO and Tmin) and sleep offset, other studies have found a longer interval.[38,39,42] The most parsimonious explanation is differences in the total sleep duration. Uchiyama and colleagues consistently report longer sleep durations in patients with DSPS (eg, 8.6 hours vs 7.1 hours for controls).[38,39,42] In the present study, the sleep duration of subjects with DSPS was 7 hours, which is similar to both nonaffected controls and to other DSPS subject populations, as measured by actigraphy at home.[8,25,38,39,42] The difference in sleep duration between the studies may be due to differences in subject selection and the setting in which the studies were conducted. Uchiyama and colleagues reported that their subjects' sleep schedules were not markedly constrained by their work schedules.[38,39,42] In contrast, many of our subjects reported an inability to obtain adequate sleep and difficulty meeting the constraints of their work and school schedules.

In summary, both low (0.3-mg) and high (3-mg) doses of melatonin, taken daily for 1 month, advanced circadian rhythms of melatonin and CBT in subjects with DSPS. The advance in circadian phase was dependent on the circadian time of administration. These results support the view that timed administration of oral melatonin, taken daily, can be an effective treatment to advance circadian rhythms in individuals with DSPS. The advance in circadian phase allowed individuals with DSPS to awaken at a more appropriate circadian time. Treatment based on determination of circadian phase (DLMO or Tmin) is, however, generally impractical in clinical practice. Thus, more studies are needed to determine the phase-dependent response to melatonin in clinical populations and to establish more practical methods to assess circadian timing in clinical settings.

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## REFERENCES

1. Baker S and Zee PC. Sleep Wake Cycle Disorders. In:Principles and Practice of Sleep Medicine, ed Kryger M, Roth T, Dement W. Philadelphia: WB Saunders Co; 2000:606-14.

2. Czeisler CA, Richardson GS, Coleman RM, et al. Chronotherapy: resetting the circadian clocks of patients with delayed sleep phase insomnia. Sleep 1981;4:1-21.
3. Regestein QR. Relationship between patterns of chronic insomnia and insomnia diagnoses in sleep clinic patients. Sleep Res 1983;12:276.
4. Regestein QR and Monk TH. Delayed sleep phase syndrome:a review of its clinical aspects. Am J Psychiatry 1995;152:602-8.
5. Lewy AJ, Sack RL, Singer CM. Treating phase typed chronobiologic sleep and mood disorders using appropriately timed bright artificial light. Psychopharmacol Bull 1985;21:368-72.
6. Rosenthal NE, Joseph-Vanderpool JR, Levendosky AA, et al. Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. Sleep 1990;13:354-61.
7. Dahlitz M, Alvarez B, Vignau J, et al. Delayed sleep phase syndrome response to melatonin. Lancet 1991;337:1121-4.
8. Tzischinsky O, Dagan Y, Lavie P. The effects of melatonin on the timing of sleep in patients with delayed sleep phase syndrome. In: Touitou Y, Arendt J, Pevet P, eds. Melatonin and the Pineal Gland: From Basic Science to Clinical Application. Amsterdam: Elsevier; 1993:351-4.
9. Oren DA, Wehr TA. Hypernyctohemeral syndrome after chronotherapy for delayed sleep phase syndrome. N Engl J Med 1992;327:1762.
10. Lewy AJ, Bauer VK, Ahmed S, et al. The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. Chronobiol Int 1998; 15:71-83.
11. Oldani A, Ferini-Strambi L, Zucconi M, et al. Melatonin and delayed sleep phase syndrome:ambulatory polygraphic evaluation. Neuroreport 1994;6:132-4.
12. Nagtegaal JE, Kerkhof GA, Smits MG, Swart AC, Van Der Meer YG. Delayed sleep phase syndrome:A placebo-controlled cross-over study on the effects of melatonin administered five hours before the individual dim light melatonin onset. J Sleep Res 1998;7:135-43.
13. Dagan Y, Yovel I, Hallis D, Eisenstein M, Raichik I. Evaluating the role of melatonin in the long-term treatment of delayed sleep phase syndrome (DSPS). Chronobiol Int 1998;15:181-90.
14. Kayumov L, Brown G, Jindal R, Buttoo K, Shapiro CM. A randomized, double-blind, placebo-controlled crossover study of the effect of exogenous melatonin on delayed sleep phase syndrome. Psychosom Med 2001;63:40-8.
15. Nagtegaal JE, Laurant MW, Kerkhof GA, et al. Effects of melatonin on the quality of life in patients with delayed sleep phase syndrome. J Psychosom Res 2000;48:45-50.
16. The International Classification of Sleep Disorders: Diagnostic And Coding Manual. Rochester, MN: American Sleep Disorders Association; 2000.
17. Benloucif S, He J, Dubocovich ML, Zee PC. Melatonin induces phase dependent advances in circadian timing in subjects with delayed sleep phase syndrome. The Physiologist 1999;42:A-10.
18. Lewy AJ, Ahmed S, Latham-Jackson JM, Sack RL. Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiol Int 1992;9:380-92.
19. Benloucif S, Dubocovich ML. A novel dosing regimen enhances the facilitation of reentrainment by melatonin in mice. The Physiologist 1995;38:A-23.
20. Voultsios A, Kennaway DJ, Dawson D. Salivary melatonin as a circadian phase marker:validation and comparison to plasma melatonin. J Biol Rhythms 1997;12:457-66.
21. Martin SK, Eastman CI. Medium-intensity light produces circadian rhythm adaptation to simulated night-shift work. Sleep 1998;21:154-65.
22. Carrier J, Monk TH. Estimating the endogenous circadian temperature rhythm without keeping people awake. J Biol Rhythms 1997;12:266-77.
23. Minors DS, Waterhouse JM. Investigating the endogenous component of human circadian rhythms:A review of some simple alternatives to constant routines. Chronobiol Int 1992;9:55-78.
24. Burgess HJ, Eastman CI. Early versus late bedtimes phase shift the human dim light melatonin rhythm despite a fixed morning lights on time. Neurosci Lett 2004;356:115-8.
25. Benloucif S, Guico MJ, Reid KJ, et al. Stability of melatonin and temperature as circadian phase markers and their relation to sleep times in humans. J Biol Rhythms 2005; 20:178-88.
26. Sack RL, Brandes RW, Kendall AR, Lewy AJ. Entrainment of free-running circadian rhythms by melatonin in blind people. N Engl J Med 2000;343:1070-7.
27. Lewy AJ, Bauer VK, Hasler BP, et al. Capturing the circadian rhythms of free-running blind people with 0.5 mg melatonin. Brain Res 2001;918:96-100.
28. Deacon S, Arendt J. Melatonin-induced temperature suppression and its acute phase-shifting effects correlate in a dose-dependent manner in humans. Brain Res 1995;688:77-85.
29. Mishima K, Satoh K, Shimizu T, Hishikawa Y. Hypnotic and hypothermic action of daytime-administered melatonin. Psychopharmacology 1997;133:168-71.
30. Satomura T, Sakamoto T, Shirakawa S, et al. Hypnotic action of melatonin during daytime administration and its comparison with triazolam. Psychiatry Clin Neurosci 2001;55:303-4.
31. Burgess HJ, Savic N, Sletten T, et al. The relationship between the dim light melatonin onset and sleep on a regular schedule in young healthy adults. Behav Sleep Med 2003;1:102-14.
32. Martin SK, Eastman CI. Sleep logs of young adults with self-selected sleep times predict the dim light melatonin onset. Chronobiol Int 2002;19:695-707.
33. Dijk DJ, Lockley SW. Integration of human sleep-wake regulation and circadian rhythmicity. J Appl Physiol 2002;92:852-62.
34. Uchiyama M, Okawa M, Shibui K, et al. Poor recovery sleep after sleep deprivation in delayed sleep phase syndrome. Psychiatry Clin Neurosci 1999;53:195-7.
35. Sack RL, Lewy AJ, Blood ML, Keith LD, Nakagawa H. Circadian rhythm abnormalities in totally blind people: incidence and clinical significance. J Clin Endocrinol Metab 1992;75:127-34.
36. Yang CM, Spielman AJ, D'Ambrosio P, et al. A single dose of melatonin prevents the phase delay associated with a delayed weekend sleep pattern. Sleep 2001;24:272-81.
37. Duffy JF, Zeitzer JM, Rimmer DW, et al. Peak of circadian melatonin rhythm occurs later within the sleep of older subjects. Am J Physiol Endocrinol Metab 2002;282:E297-303.
38. Ozaki S, Uchiyama M, Shirakawa S, Okawa M. Prolonged interval from body temperature nadir to sleep offset in patients with delayed sleep phase syndrome. Sleep 1996;19:36-40.
39. Uchiyama M, Okawa M, Shibui K, et al. Altered phase relation between sleep timing and core body temperature rhythm in delayed sleep phase syndrome and non-24-hour sleep-wake syndrome in humans. Neurosci Lett 2000;294:101-4.
40. Duffy JF, Dijk DJ, Hall EF, Czeisler CA. Relationship of endogenous circadian melatonin and temperature rhythms to self-reported preference for morning or evening activity in young and older people. J Investig Med 1999;47:141-50.
41. Baehr EK, Revelle W, Eastman CI. Individual differences in the phase and amplitude of the human circadian temperature rhythm: with an emphasis on morningness-eveningness. J Sleep Res 2000;9:117-27.
42. Shibui K, Uchiyama M, Okawa M. Melatonin rhythms in delayed sleep phase syndrome. J Biol Rhythms 1999;14:72-6.