

# The Role of Melatonin and Circadian Phase in Age-related Sleep-maintenance Insomnia: Assessment in a Clinical Trial of Melatonin Replacement

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**Summary:** The present investigation used a placebo-controlled, double-blind, crossover design to assess the sleep-promoting effect of three melatonin replacement delivery strategies in a group of patients with age-related sleep-maintenance insomnia. Subjects alternated between treatment and “washout” conditions in 2-week trials. The specific treatment strategies for a high physiological dose (0.5 mg) of melatonin were: (1) EARLY: An immediate-release dose taken 30 minutes before bedtime; (2) CONTINUOUS: A controlled-release dose taken 30 minutes before bedtime; (3) LATE: An immediate-release dose taken 4 hours after bedtime. The EARLY and LATE treatments yielded significant and unambiguous reductions in core body temperature. All three melatonin treatments shortened latencies to persistent sleep, demonstrating that high physiological doses of melatonin can promote sleep in this population. Despite this effect on sleep latency, however, melatonin was not effective in sustaining sleep. No treatment improved total sleep time, sleep efficiency, or wake after sleep onset. Likewise, melatonin did not improve subjective self-reports of nighttime sleep and daytime alertness. Correlational analyses comparing sleep in the placebo condition with melatonin production revealed that melatonin levels were not correlated with sleep. Furthermore, low melatonin producers were not preferentially responsive to melatonin replacement. Total sleep time and sleep efficiency were correlated with the timing of the endogenous melatonin rhythm, and particularly with the phase-relationship between habitual bedtime and the phase of the circadian timing system. **Key words:** Melatonin; sleep; insomnia; aging; circadian rhythms; core body temperature.

IN HUMANS, advancing age is often accompanied by increasing dissatisfaction with the amount and quality of sleep. In the absence of formal classification, the designations *age-related insomnia* or *elderly insomnia* have been used to describe the presence, in otherwise normal older people, of some or all of the following sleep symptoms: (1) advanced sleep onset, (2) sleep-onset insomnia, (3) sleep-maintenance insomnia, and (4) early morning awakening. Sleep-maintenance insomnia is the most prevalent of these

symptoms, affecting approximately 30% of people over the age of 65, followed by sleep-onset insomnia (19.2%), and early morning awakening (18.8%).<sup>1</sup> Whether these sleep symptoms are simply a consequence of aging, or are explained by more specific physiological mechanisms such as circadian rhythm disturbances, subclinical sleep-disordered breathing, periodic limb movement disorder, depression, anxiety, pain, and nocturia, is a matter of some debate and continuing research.<sup>2</sup>

It is well documented that circulating levels of the pineal hormone melatonin decrease with advancing age.<sup>3-7</sup> Melatonin is produced primarily at night in dim light.<sup>8,9</sup> In the mammalian circadian system, melatonin serves as a chemical messenger of the primary circadian pacemaker,

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the suprachiasmatic nuclei (SCN), communicating a hormonal message of “nighttime darkness.”<sup>10,11</sup> Melatonin is thought to function through high-affinity, pharmacologically-specific, G-protein-coupled receptors<sup>12,13</sup> located in both the periphery<sup>14</sup> and in the central nervous system.<sup>15-18</sup> Central melatonin receptors are concentrated primarily in the SCN,<sup>19</sup> where melatonin functions in a feedback loop and has direct phase-shifting properties.<sup>20,21</sup> Melatonin administered to animals<sup>22</sup> and to humans shifts circadian rhythms according to a phase-response curve (PRC).<sup>23,24</sup> Exogenous melatonin is also hypothermic,<sup>25-29</sup> and endogenous melatonin may be responsible for a significant proportion of the nocturnal decline of core body temperature.<sup>30-33</sup>

Melatonin has also been implicated in the sleep-wake process, primarily because of its close temporal association with sleep and sleep propensity in humans and in other diurnal mammals.<sup>34-36</sup> Beyond this temporal association, there are reported correlations between circulating levels of melatonin and sleep. In normal young individuals, for instance, total sleep time (TST) has been correlated with the percentage of nighttime melatonin production<sup>37</sup> (as measured by urinary 6-sulfatoxymelatonin, the major metabolite of melatonin). Inhibition of nighttime melatonin production by beta-adrenergic blockers attenuates the nighttime decline of body temperature<sup>38</sup> and can impair sleep<sup>39</sup>; both of these effects are reversed by low pharmacological doses of melatonin.<sup>38,40</sup> Similarly, nighttime bright light administration, which also suppresses melatonin production,<sup>41</sup> attenuates the nocturnal decline of body temperature and reduces sleep propensity<sup>42-44</sup>; these effects are also reversed by simultaneous melatonin administration.<sup>33,44,45</sup>

Evidence that endogenous melatonin may mediate sleep, coupled with the decline in endogenous melatonin levels with age, have led some to suggest that age-related insomnia may be a result of low melatonin production, and that the sleep of melatonin-deficient elderly individuals may be improved by the administration of melatonin designed to mimic a more “youthful” nighttime profile.<sup>46</sup> This “melatonin replacement hypothesis” has two components: (1) the age-related decline in melatonin in some way contributes to insomnia; and (2) replacement treatment with high physiological doses of melatonin will improve sleep. The present investigation addressed both of these components in a clinical trial of melatonin replacement for age-related sleep-maintenance insomnia.

Both components of the melatonin replacement hypothesis have received some empirical support. Although in younger subjects evidence for a relationship between melatonin production and sleep is mixed,<sup>47-49</sup> in the elderly there is evidence for a relationship between overall 6-sulfatoxymelatonin production and sleep. For

instance, lower 6-sulfatoxymelatonin levels have been reported in elderly groups of self-described poor sleepers, compared to self-described good sleepers,<sup>50</sup> and in elderly women with poor sleep verified by polysomnogram (PSG).<sup>51</sup>

There are also several previous reports demonstrating sleep-promoting effects of melatonin in elderly insomnia subjects. Garfinkel and coworkers<sup>52</sup> administered melatonin (2 mg) in a controlled-release (CR) formulation, 2 hours before bedtime. Twelve elderly subjects, with subjective sleep complaints, were recruited from a lecture at a residential center for seniors. Wrist actigraphy assessed on the last 3 days of each 3-week treatment period showed that melatonin improved mean estimates of sleep efficiency (SE) and wake after sleep onset (WASO), and tended to shorten initial sleep latency (SL). Subjects in this study had various (and sometimes multiple) medical ailments, including cardiovascular disease and Parkinson’s disease. Also, these subjects were concurrently taking one to six other medications, several of which are known to affect melatonin production or sleep quality (eg, beta-adrenergic antagonists, aspirin, and sedative-hypnotics). These methodological shortcomings make the results of this investigation difficult to interpret. At least in the case of medications known to suppress melatonin, it is possible that melatonin treatment simply reversed the untoward effects of these medications.<sup>40</sup>

A study by Haimov and coworkers tested pharmacological doses of melatonin in subjects prescreened for low endogenous melatonin production; inclusion criteria were apparently limited to low production and self-characterization as “poor” sleepers.<sup>53</sup> The placebo-controlled, week-long treatment trials tested 2 mg immediate-release (IR) and 2 mg CR. The IR dose significantly shortened actigraphy estimates of initial SL, and the CR dose increased estimates of SE in the first 6 hours of the night. In a 1-month, open-label, follow-up trial, melatonin (1 mg CR) facilitated estimates of both initial SL and SE. Given the prevalence of early morning awakening and sleep maintenance difficulties in elderly insomnia, it should be emphasized that this study reported only the first 6 hours of sleep opportunity.

Finally, Wurtman and Zhdanova<sup>54</sup> reported results of a melatonin replacement trial in nine subjects complaining of sleep-onset insomnia, sleep-maintenance insomnia, and early morning awakening. The effects of a physiological dose of melatonin (0.3 mg IR), administered for 3 days, were estimated by wrist actigraphy. Melatonin was reported to significantly shorten initial SL, reduce nighttime movements, and reduce the number of awakenings.

When viewed as clinical trials of melatonin replacement, the published investigations had several limitations: (1) Objective assessment of insomnia before treatment was

not done<sup>52,53</sup> or not reported.<sup>54</sup> Besides not screening for primary sleep pathology, these studies did not document the relative contribution of specific insomnia symptoms to their subjects' overall sleep complaints. (2) None of the investigations demonstrated a relationship between endogenous melatonin production and sleep. (3) Treatment outcome assessments did not include PSG recording in any of the investigations. (4) No investigation provided subjective assessments of sleep and daytime alertness. (5) In our opinion, two investigations<sup>52,53</sup> tested doses of melatonin that were too high (>1 mg) to be considered "replacement" doses. The Wurtman and Zhdanova investigation used a physiological dose (0.3 mg IR); however, given the rapid elimination half-life of exogenous melatonin, typically between 45-60 minutes,<sup>55,56</sup> it is not likely that this treatment elevated plasma levels in the second half of the night, and therefore may not have been a true replacement dose.

Based upon the limitations of published investigations, a clinical trial of melatonin replacement therapy for age-related insomnia, including PSG-recorded sleep assessment, was warranted. In the present investigation, a relationship between endogenous melatonin and sleep was evaluated in a group of patients with age-related sleep-maintenance insomnia. In addition, a placebo-controlled, double-blind, crossover design was used to evaluate the sleep-promoting effects of high physiological melatonin replacement (0.5 mg), administered in three distinct delivery strategies.

## METHODS

### Subjects

Insomnia patients between the ages of 55 and 80, with primary complaints of sleep maintenance, were recruited from local retirement communities, senior centers, and from advertisements in area newspapers. Approximately 700 telephone respondents were sent an initial screening questionnaire regarding their sleep history, current sleep patterns, and general medical status. Roughly 64% of the questionnaires were returned (446). Twenty-six candidates were selected and interviewed by the investigators. Medical histories and physical examinations were done on these candidates to verify the absence of major medical disorders. In addition, psychiatric histories and standardized depression and anxiety scales were administered to confirm the absence of psychiatric disorders. To verify the diagnosis of insomnia and to screen for primary sleep pathology, candidates were given a structured insomnia screening interview followed by a diagnostic PSG. Primary diagnostic PSG inclusion criteria were SE less than 80% and WASO greater than 30 minutes. Sleep exclusion criteria included an apnea index greater than 10 and a movement arousal index greater than 15. Candidates found to have

some form of primary sleep pathology were excused from the investigation and referred to the Oregon Health Sciences University (OHSU) Sleep Disorders Clinic for treatment. Candidates were also excluded for taking medications known to affect melatonin or sleep, having a daily caffeine intake greater than 150 mg, smoking, weighing more than 15% over their ideal body weight, and for recent or planned travel outside of the Pacific time zone. In addition, subjects were screened for their ability to comply with treatment protocol restrictions on bedtime, alcohol intake, caffeine intake, and daytime naps. Of the 26 subjects interviewed, 1 candidate had a scheduling conflict, 2 were screened out for symptoms of depression, and 7 were screened out by the diagnostic PSG. Sixteen subjects were admitted into the investigation; however, 2 dropped out after the pre-treatment CRC admission. The final sample, therefore, consisted of 14 subjects, 9 females and 5 males. Each subject completed all four treatment trials. This investigation was approved by the OHSU Institutional Review Board. All subjects gave informed voluntary consent before participating. Subjects were paid for their participation.

### Materials and Technical Procedures

**Melatonin formulation.**—Analytical-grade melatonin was obtained from Regis Chemical Company, Morton Grove, Ill and was formulated under the supervision of Keith Parrott, PharmD at the Oregon State University College of Pharmacy. For the immediate-release doses, melatonin (0.5 mg) was divided equally into two opaque gelatin capsules using a lactose filler (FDA IND 26,318). For the controlled-release dose, melatonin (0.5 mg) was formulated in two opaque capsules using sugar spheres loaded with melatonin and coated with Aquacoat®, an aqueous polymeric ethylcellulose suspension designed to control the release of melatonin (FDA IND 39,681).<sup>57</sup> Placebo capsules, containing only the lactose filler, were identical in appearance. Capsules for each trial were packaged in two bubble-wrap packets, one for the bedtime dose and one for the middle-of-the-night dose. To ensure the double-blind, these packets were labeled with the subject number, the trial number, and either bedtime or middle-of-the-night dose. Subjects took two capsules 30 minutes before bedtime and two more capsules mid-way through their sleep opportunity. To reduce potential confusion, each two-capsule administration (eg, early or late) will be referred to as one dose (0.5 mg melatonin or placebo).

**Melatonin sampling and assays.**—Blood samples (4 ml) were drawn in the CRC from a heparin-lock intravenous catheter inserted into a forearm vein. Light intensity during sampling was 30 lux or less. Plasma melatonin was assayed by radioimmunoassay (RIA) using the antibody developed by Kennaway<sup>58</sup> with reagents supplied by



ALPCO LTD, Windham, NH. The lower limit of sensitivity is 0.5 pg/ml; the coefficient of variability is 10.2% for concentrations of 15 pg/ml. Plasma levels were validated by the gas chromatographic negative chemical ionization mass spectrometric (GCMS) assay developed by Lewy and Markey.<sup>59</sup> The GCMS assay has a lower limit of sensitivity of 0.5 pg/ml; the coefficient of variability is 2.7% for concentrations of 20 pg/ml.

**Core body temperature.**—During the CRC admission on the last night of each treatment, core body temperature was sampled every minute using a rectal temperature probe and ambulatory temperature monitor (Mini Logger, Sunriver, Ore). The maximum core body temperature was determined for each 30-minute block of recording and was averaged by quarters of the sleep episode. The maximum temperature, rather than the mean temperature, was used in order to reduce the masking effects of sleep on core body temperature to test for hypothermic effects of melatonin relatively independent of its effects on sleep.

## Dependent Measures

**Polysomnographic (PSG) recordings.**—Sleep studies were carried out by trained sleep technologists using Sandman® portable PSG systems from Melville Diagnostics (Ottawa, Ontario, Canada). For the initial diagnostic PSG, a standard 16-channel montage was used: four EEG (C<sup>3</sup>A<sup>2</sup>, O<sup>1</sup>A<sup>2</sup>, C<sup>4</sup>A<sup>1</sup>, O<sup>2</sup>A<sup>1</sup>), four EMG (two for chin, one each for right and left legs), one nasal air flow, two ocular movements, two for respiratory muscle movement, one for oximetry, one for body position and one for EKG. This initial recording also served to acclimate subjects to in-home PSG monitoring. Subsequent recordings during treatment omitted the respiratory, oximetry, body position, and airflow channels. All treatment recordings were done in the subjects' homes on nights 11 and 12 of each treatment condition. Occasionally recordings were done on night 13 if repeat recording was necessary. When tested in standardized conditions, we have found in-home PSG monitoring to be a reliable method of assessing elderly sleep. In the year encompassing the present investigation, our failure rate for in-home research PSGs was 3.4%.<sup>60</sup>

All sleep records were hand-scored using standard criteria.<sup>61</sup> Primary dependent measures of sleep included the following: sleep-onset latency (SL), defined conventionally;<sup>61</sup> latency to 10 minutes of persistent sleep; total bed time (TBT), the elapsed time between lights-out and lights-on; sleep period time (SPT), the elapsed time from sleep onset to the last epoch of sleep; total sleep time (TST); sleep efficiency (SE), defined as  $([TST/TBT](100))$ ; total wake time (WT); and wake after sleep onset (WASO).<sup>62</sup> Return-to-sleep onset latencies and return-to-persistent-

sleep onset latencies were also assessed after the obligatory middle-of-the-night awakening.

**Sleep actigraphy.**—Since PSG monitoring was done on only 2 nights of each treatment, wrist actigraphy was used to estimate sleep every night, producing estimates of night-to-night variability as well as assessment of potential acute vs chronic treatment effects. For actigraphy, each subject wore an Actillum® wrist actigraph (Ambulatory Monitoring Inc., Ardsley, NY) around the clock for 13 days of each trial (except when bathing). These four-channel devices monitor wrist movement and ambient light exposure. The internal light channel was particularly useful, as it was more accurate than subject diaries, in determining "lights-out." Actigraph estimates of sleep parameters included SL, TST, WT, WASO, and SE. Sleep parameters were estimated with Action III software (Ambulatory Monitoring Inc., Ardsley, NY). Based upon nights when PSG and Actillum® were used concurrently, the scoring algorithm was calibrated for each subject in order to maximize sensitivity and specificity. As a result of this calibration procedure, there was less than 10% difference between actigraphy estimates and PSG measures of sleep and wake.

**Subjective ratings.**—Daily sleep diaries were used to document self-estimates of sleep parameters, including SL, TST, SE, WASO, and number of awakenings. The sleep diary also contained five-point rating scales for subjective sleep quality. Subjects completed the Visual Analog Scale (VAS)<sup>63</sup> 3 times a day and the Profile of Mood States (POMS)<sup>64</sup> 2 times a day. These self-report scales were used to assess daytime alertness and sleepiness.

### Endogenous melatonin and circadian phase.—

Circadian phase was assessed from endogenous melatonin profiles at the pre-treatment baseline assessment (see below) and from a DLMO at the conclusion of each trial. Melatonin maximum ( $MEL_{Max}$ ) was defined as the highest measured plasma level. The 24-hour area under the curve ( $MEL_{AUC24}$ ) was calculated for each subject by summing the total plasma levels for the entire sampling period. Because of low melatonin production in some elderly subjects, the DLMO was defined as the clock time when the evening onset of plasma melatonin reached 25% of its nighttime  $MEL_{Max}$ . Likewise, melatonin offset (DLMO<sub>off</sub>) was defined as the clock time when the morning offset of melatonin fell below 25% of the  $MEL_{Max}$ . Melatonin duration ( $MEL_{Dur}$ ) was defined as the number of hours that plasma levels remained above this 25% threshold. Finally, phase differences between the DLMO and bedtime ( $_{BT}DLMO$ ) and between bedtime and DLMO<sub>off</sub> ( $_{BT}DLMO_{off}$ ) were calculated.

## PROCEDURES

### Overall study design

Subjects admitted into the investigation underwent a pre-treatment baseline trial that included 1 week of habitual sleep-wake assessment leading into a 26-hour CRC admission for baseline circadian rhythm assessment. The treatment protocol employed a placebo-controlled, double-blind, crossover design. Subjects were tested in four 2-week treatment trials with at least 2 weeks of intervening washout. Melatonin (0.5 mg) was administered in three different treatment strategies, two immediate-release and one controlled-release. The four treatment conditions were: (1) EARLY, 0.5 mg IR taken 30 minutes before bedtime and placebo taken 4 hours after bedtime; (2) CONTINUOUS, 0.5 mg CR taken 30 minutes before bedtime and placebo taken 4 hours after bedtime; (3) LATE, placebo taken 30 minutes before bedtime and 0.5 mg IR taken 4 hours after bedtime; and (4) PLACEBO, placebo taken 30 minutes before and 4 hours after bedtime. Initial treatment order was counterbalanced using Latin square design.

**Pre-treatment assessment.**—At least 2 weeks before the first treatment trial, habitual sleep and wake times were monitored for 1 week by wrist actigraphs and by sleep diaries. Estimates of sleep parameters were used at this time to verify subjective insomnia symptoms. At the end of this pre-treatment week, subjects were admitted to the CRC for a pre-treatment circadian rhythm assessment. Subjects were maintained in dim light (<30 lux) for the entire 26-hour baseline CRC admission. Blood samples (4 ml) were drawn hourly in order to construct a 24-hour melatonin profile. Sampling frequency was increased to every 30 minutes from 1800 until 2400 hours in order to increase resolution around the expected onset of nocturnal melatonin production.

**Treatment protocol assessment.**—As habitual bedtimes were not always constant within an individual, each subject negotiated a bedtime to be fixed throughout the treatment trials. Fixed negotiated bedtimes were strictly enforced and monitored throughout the treatment trials; the mean negotiated bedtime ( $22.04 \pm 0.26$  hour) was not different from the mean pre-treatment habitual bedtime ( $22.05 \pm 0.25$  hour). Although subjects maintained consistent bedtimes throughout the treatment trials, morning lights-on times were not fixed. Each night subjects took two doses, one 30 minutes before bedtime and another 4 hours after bedtime (ie, 4.5 hours after the first dose). While at home, subjects were prompted to take their middle-of-the-night dose by an alarm clock provided by the experimenters. Sleep on nights 1 through 13 was assessed objectively with a wrist actigraph, and subjectively by sleep diary and self-ratings of sleep quality. Sleep was also monitored on nights 11 and 12 with in-home PSG, as described above.

On the last evening of each treatment trial (night 14),

subjects were admitted to the CRC several hours before bedtime. Plasma samples for melatonin pharmacokinetic analyses were obtained 30 minutes before and after the first dose, and then hourly throughout the night. The following day, subjects were free to read and watch television. Subjects were only allowed to leave the CRC for several hours during the middle of this free time. Later that afternoon and evening, subjects underwent a post-treatment circadian phase assessment, using a modification of the DLMO.<sup>65</sup> Blood samples were drawn under dim light (<30 lux) conditions every 30 minutes from 1800 until 0100 (sampling times were sometimes modified to accommodate individual differences in circadian phase).

### Data Analyses

As treatment nights were bisected by an obligatory awakening, PSG and actigraphy sleep data were analyzed by first and second halves of the night, separated by the middle-of-the-night awakening. The two nights of PSG sleep data were averaged for each treatment and analyzed in a series of one-way repeated-measures ANOVAs. Initial analyses of actigraphically-estimated sleep data revealed no differences between the first and second week of any treatment condition. Therefore, actigraphic sleep estimates separated by first and second halves of the night were collapsed across all 13 days and analyzed in a series of one-way repeated-measures ANOVAs. As the results of actigraphy were not appreciably different from PSG, these results are not presented in order to conserve space. Sleep diaries were not split by time of night; therefore, the means of all-night estimates from nights 1 through 13 were analyzed by one-way repeated-measures ANOVA. Of the 196 testing nights, each of the seven nights in which data were lost were replaced with the subject's mean for that condition. Sampling of subjective alertness, sleepiness, and mood (using the VAS) was done in the morning, at midday, and in the evening. These data were analyzed using two-way [treatment condition (4)  $\times$  time of day (3)] repeated-measures ANOVAs. Core body temperature data were averaged every two hours and analyzed in a two-way [treatment condition (4)  $\times$  time of night (4)] repeated-measures ANOVA. Where appropriate, probability values using Greenhouse-Geisser corrections for degrees of freedom are reported. All significant F-values were followed by Tukey post-hoc paired comparisons and were evaluated at the  $p < 0.05$  significance level.

## RESULTS

### Pre-Treatment Assessment

**Sleep.**—Selected demographic information and subjective ratings of insomnia severity are provided in Table 1. The results of the screening insomnia severity question-

naire revealed that subjects rated sleep-maintenance insomnia as the most frequently occurring symptom ( $4.36 \pm 0.24$ ), followed by early morning awakening ( $4.07 \pm 0.30$ ). These two symptoms were rated much higher than symptoms of advanced sleep onset ( $2.86 \pm 0.33$ ) and sleep-onset insomnia ( $2.21 \pm 0.25$ ). Subjective complaints were confirmed by the diagnostic PSG, which yielded the following average sleep parameters:  $SL = 31.18 \pm 7.85$ ,  $TST = 315.11 \pm 15.88$ ,  $SE = 65.85 \pm 3.41\%$ ,  $WASO = 121.82 \pm 23.30$ . The average movement arousal index was  $4.09 \pm 1.66$  per hour of sleep. The average apnea index was  $1.25 \pm 0.54$  per hour of sleep. Pre-treatment sleep diaries also supported subjects' insomnia symptoms. These diaries yielded self-estimates of the following sleep parameters:  $SL = 25.08 \pm 2.90$ ,  $TST = 366.44 \pm 7.92$ ,  $SE = 72.00 \pm 1.90\%$ ,  $WASO = 119.93 \pm 7.80$ . The mean habitual bedtime, determined by sleep diaries, was  $22.14 \pm 0.28$  hour and the average out-of-bed time (ie, lights-on) was  $6.52 \pm 0.27$  hour.

**Melatonin production and circadian phase.**—As illustrated in Fig. 1, plasma levels of endogenous melatonin were quite low in several subjects: seven subjects peaked below 15 pg/ml; of these, four peaked at 10 pg/ml or less, and one peaked just below 5 pg/ml. Even subjects with low melatonin production, however, had distinct rhythms with discernible onsets and offsets and normal durations.<sup>66</sup> Although half of the group could be considered low melatonin producers, several subjects had peak plasma levels comparable to younger subjects. The ranges of maximum melatonin production and total melatonin production revealed individual differences greater than twenty-fold: the range of  $MEL_{Max}$  was 4.7 pg/ml to 100.1 pg/ml; and  $MEL_{AUC24}$  varied from 51.2 pg/ml to 1162.5 pg/ml.

One subject (S05) had a substantially phase-advanced melatonin rhythm (the DLMO occurred sometime before 1700 hours). Since accurate assessments of DLMO and  $MEL_{Dur}$  were not available for this subject, this subject's data were excluded from further analyses of circadian phase and circadian phase differences. For the remaining 13 subjects, the mean DLMO was  $21.04 \pm 0.29$  hour. Although the degree to which habitual bedtimes were in phase with nocturnal melatonin production was variable, the nighttime rise of endogenous melatonin preceded habitual bedtimes in all subjects. Average pre-treatment phase differences were  $_{BT}DLMO = -1.10 \pm 0.25$  hour, and  $_{BT}DLMO_{off} = 9.47 \pm 0.27$  hour. On average, therefore, the DLMO occurred 1 hour and 6 minutes before bedtime and the  $DLMO_{off}$  was 9 hours and 28 minutes after bedtime.

### Treatment Protocol Assessment

**Pharmacokinetics.**—The average plasma melatonin pharmacokinetic data are depicted in Fig. 2. For the EARLY treatment, the mean exogenous melatonin maxi-

mum ( $xMEL_{Max}$ ) was  $838.67 \pm 164.12$  pg/ml; the range varied from 145.70 to 2345.00 pg/ml. The LATE treatment yielded a mean  $xMEL_{Max}$  of  $703.76 \pm 154.14$  pg/ml, with a range of 159.40 to 2117.00 pg/ml. The average  $xMEL_{Max}$  for the CONTINUOUS treatment was within high physiological levels ( $394.50 \pm 81.28$  pg/ml). The range was 135.5 to 1373.00 pg/ml; however,  $xMEL_{Max}$  was below 500 pg/ml for all subjects but one. The CONTINUOUS treatment, therefore, appeared to mimic a youthful profile in most subjects, resulting in roughly physiological levels for 8 to 12 hours (Figure 2). This formulation, however, may have had a long "tail," yielding measurable melatonin levels throughout the next day.

**Phase shifting.**—The average DLMO measured after PLACEBO treatment ( $21.00 \pm 0.24$  hour) revealed that the protocol itself, including the imposed negotiated bedtimes, did not alter circadian phase. For the CONTINUOUS treatment, the unexpected residual melatonin levels masked the next night's DLMO in all but three subjects; therefore, analysis of circadian phase was not available for this treat-

**Table 1.**—Patient demographics and subjective insomnia severity.

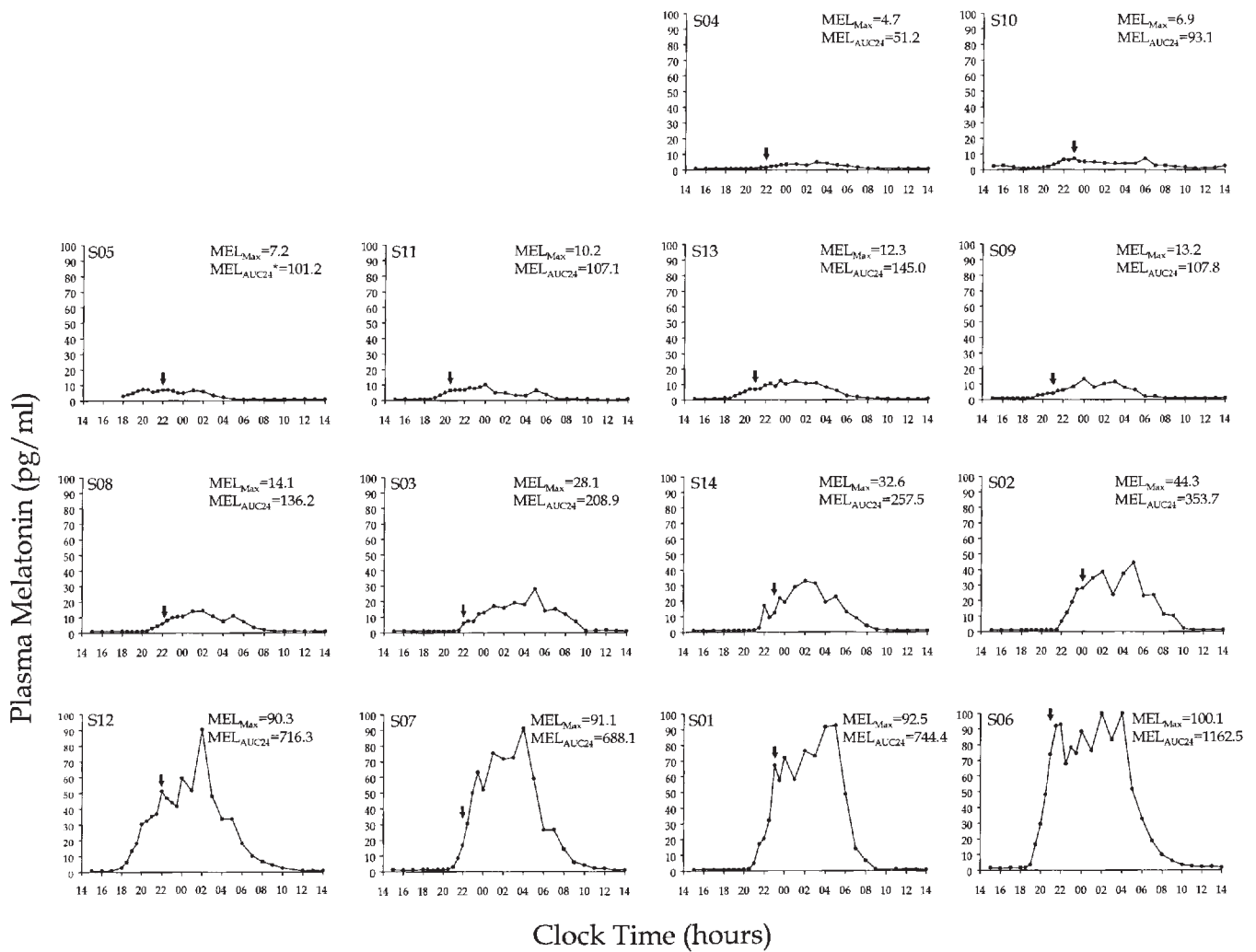
Subject	Demographics			Specific Insomnia Symptom			
	Sex	Age	BMI	SMI	EMA	ASO	SOI
S01	F	71	22.66	5	4	3	3
S02	M	76	23.92	5	4	2	2
S03	F	78	23.00	5	5	1	2
S04	M	57	23.18	5	5	3	1
S05	F	73	25.02	2	1	3	1
S06	F	70	27.43	3	3	2	2
S07	M	65	22.15	5	3	3	1
S08	F	77	27.79	5	5	2	2
S09	F	71	22.31	4	5	5	4
S10	M	79	24.82	4	4	3	2
S11	F	69	24.80	4	4	5	3
S12	F	60	27.46	5	5	3	2
S13	M	76	28.58	4	5	4	2
S14	F	62	27.43	5	4	1	4
mean		70.29	25.04	4.36	4.07	2.86	2.21
sem		1.87	0.58	0.24	0.30	0.33	0.25

BMI = body-mass index; SMI = sleep-maintenance insomnia (ie, WASO > 30 minutes); EMA = early morning awakening; ASO = advanced sleep onset; SOI = sleep onset insomnia (ie, sleep latency > 30 minutes).

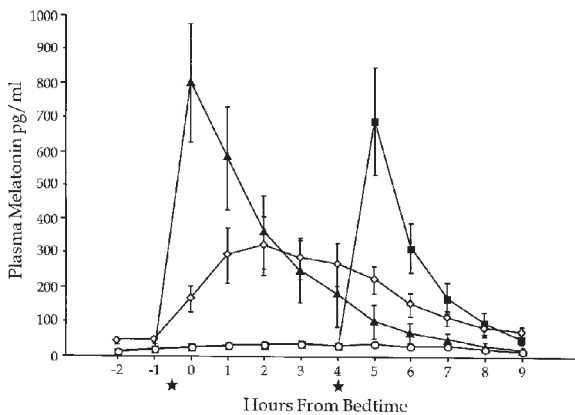
**Insomnia Severity Scale:**

- 1 = **Never:** cannot remember ever having this problem
- 2 = **Seldom:** this happens to me less than once a month
- 3 = **Occasionally:** this happens more than once a month
- 4 = **Frequently:** this happens more than once a week
- 5 = **All the time:** constantly have this problem





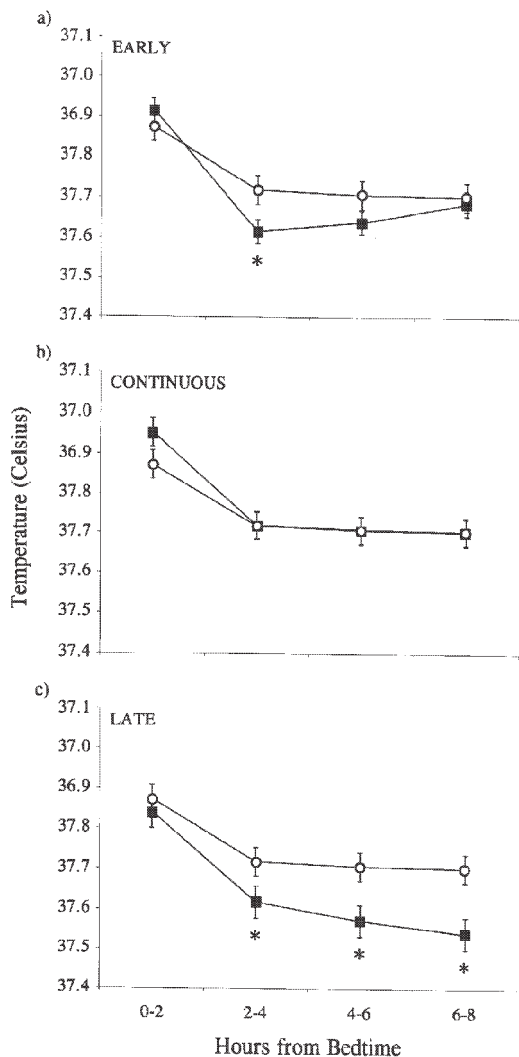
**Figure 1.**—Individual baseline 24-hour plasma melatonin profiles. Arrows represent habitual bedtimes assessed by sleep diary and wrist Actillum monitoring for one week before the pre-treatment admission. Individual maximum plasma melatonin levels (MEL<sub>Max</sub>) and 24-hour area under the curve values (MEL<sub>AUC24</sub>), in pg/ml, are also provided. Asterisk (S05) indicates AUC calculations based on only 20 hours of data collection.



**Figure 2.**—Plasma pharmacokinetic data for PLACEBO (open circles), EARLY treatment (closed triangles), CONTINUOUS treatment (open diamonds) and LATE treatment (filled squares). Sampling occurred on the last night of treatment (Night 14). As bedtimes varied among individuals, data are referenced to bedtime (Time 0). The timing of the first dose (30 minutes before bedtime) and the second dose (four hours after bedtime) are depicted as stars. Data are presented as means  $\pm$  the standard error of the mean (SEM).

ment condition. A one-way repeated-measures ANOVA performed on the PLACEBO, EARLY, and LATE treatment DLMOs yielded no significant differences in phase ( $F(2,24)=1.66$ ). The EARLY treatment ( $21.17 \pm 0.32$  hour) yielded a non-significant 10.2-minute mean phase delay. Although still not statistically significant, the LATE treatment ( $21.46 \pm 0.25$  hour) yielded a larger, 27.6-minute, mean phase delay.

**Core body temperature.**—Core body temperature data are illustrated by quarters of the night in Fig. 3. Hypothermic effects were revealed in a significant treatment by time-of-night interaction [ $F(9,117)=2.02$ ,  $p < 0.05$ ]. Results of Tukey post-hoc comparisons revealed that the EARLY treatment (Figure 3a) significantly reduced core body temperature in the second quarter of the night ( $p < 0.05$ ). The LATE treatment significantly reduced core body temperature in the latter three quarters of the night (Figure 3c) ( $p < 0.05$ ). The CONTINUOUS treatment did not dramatically affect core body temperature (Figure 3b),



**Figure 3.**—The effects of melatonin replacement on nighttime core body temperature. Temperature data were averaged every two hours; bedtime is represented as time 0. Core body temperature data in the PLACEBO condition (open circles) are compared with melatonin (closed squares) in the: a) EARLY treatment, b) CONTINUOUS treatment, and c) LATE treatment. Data are presented as means ( $\pm$  SEM). Asterisks indicate significant Tukey post-hoc comparisons with placebo ( $p < 0.05$ ).

although this may be due to the finding that, at bedtime, core body temperature in this condition was higher than placebo.

**Sleep.**—Polygraphically-assessed sleep data, by the first and second halves of the night, are provided in Table 2. When collapsed across time of night, a significant main effect for treatment condition [ $F(3,39)=3.82$ ,  $p=0.01$ ], followed by Tukey post-hoc paired comparisons ( $p<0.05$ ), revealed that both doses of melatonin given before bedtime significantly shortened the mean latency to 10 minutes of persistent sleep. The EARLY treatment shortened the mean latency to persistent sleep by 11.65 minutes, and the CONTINUOUS treatment shortened it by 10.47 minutes. As a

result of shorter sleep latencies, the EARLY and the CONTINUOUS treatments also increased the average SPT [ $F(3,39)=3.40$ ,  $p<0.05$ ], by 13.46 and 13.82 minutes respectively. Given the unexpected finding that the LATE treatment shortened the initial sleep latency (see Table 2), and since, as dependent measures, initial sleep latency and return-to-sleep latency in the middle of the night are more discrete than other parameters such as TST, additional one-way ANOVAs were performed. Mean initial latencies to persistent sleep as well as return-to-persistent sleep latencies are provided in Fig. 4. Tukey post-hoc paired comparisons performed after a significant ANOVA for the initial latency to persistent sleep [ $F(3,39)=3.80$ ,  $p<0.05$ ] revealed that post-treatment initial sleep latencies were significantly shorter than placebo in both the EARLY and the LATE treatments. A similar analysis, performed on the data from the middle of the night only, revealed that no treatment significantly shortened return-to-persistent-sleep latencies [ $F(3,39)=1.30$ ] at this time. Despite significant effects on sleep induction, no melatonin treatment significantly increased TST or decreased WT. Likewise, there were no significant improvements in measures of sleep consolidation such as SE and WASO. Finally, melatonin treatment did not significantly alter sleep architecture.

**Subjective effects.**—Melatonin treatment did not significantly impact subjects' self-estimates of sleep and sleep quality. Indeed, sleep diary estimates, provided in Table 3, were remarkably similar for all treatment conditions. The VAS data, collapsed across time of day, are provided in Table 4. There were no significant main effects for treatment or for treatment by time-of-day interactions, suggesting that melatonin replacement did not affect daytime mood and alertness. The results of the POMS data were similar to the VAS; therefore, to conserve space, these data are not presented.

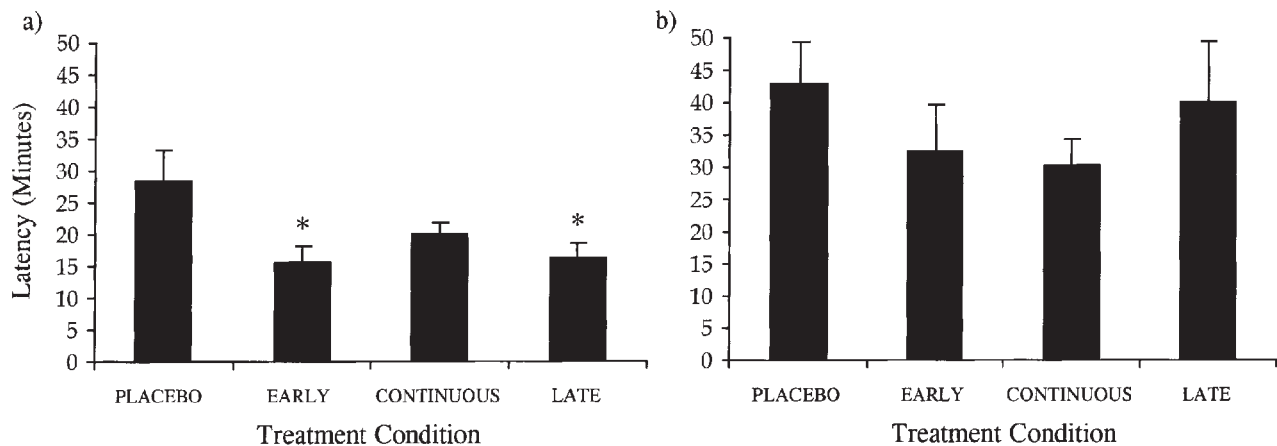
### Endogenous Melatonin and Circadian Phase in Age-Related Insomnia

To test the hypothesis that low endogenous melatonin is associated with insomnia severity, several measures of melatonin production were correlated with parameters of PSG-recorded sleep in the PLACEBO condition. Several sleep parameters were used in these analyses: the clock time of sleep onset, the initial latencies to sleep, TST, and SE. Each of these sleep parameters was correlated with the following measures of melatonin production and melatonin timing: MEL<sub>Max</sub>, MEL<sub>AUC24</sub>, MEL<sub>AUCBT</sub>, MEL<sub>Dir</sub>, DLMO, DLMO<sub>off</sub>, <sub>BT</sub>DLMO, and <sub>BT</sub>DLMO<sub>off</sub>. These correlation coefficients are provided in Table 5. Measures of melatonin amplitude (MEL<sub>Max</sub>) and melatonin production (MEL<sub>AUC24</sub>, MEL<sub>AUCBT</sub>) were not associated with any parameter of sleep. Sleep was significantly correlated with circadian parameters of the melatonin rhythm. The clock time



**Table 2.**—Polysomnography data by half of night (mean ± SEM)

Sleep Parameter	PLACEBO		EARLY		CONTINUOUS		LATE	
	1st-Half	2nd-Half	1st-Half	2nd-Half	1st-Half	2nd-Half	1st-Half	2nd-Half
Latency to Sleep Onset (SL)	15.98 (3.88)	32.46 (4.77)	12.16 (1.94)	25.84 (5.83)	13.79 (2.22)	22.00 (3.51)	10.18 (1.09)	31.55 (8.94)
Latency to 10 min Sleep	28.36 (5.20)	42.93 (5.94)	15.57 (2.43)	32.41 (7.15)	20.11 (2.38)	30.23 (4.10)	16.20 (2.35)	40.00 (9.15)
Lat. to REM Sleep after SO	78.35 (8.01)	49.45 (4.07)	64.95 (3.98)	53.28 (7.36)	64.71 (4.50)	60.89 (6.34)	67.51 (4.38)	51.61 (5.66)
Total Dark Time (TDT)	235.55 (3.80)	257.72 (15.65)	232.38 (6.60)	266.60 (17.51)	243.66 (3.71)	258.38 (12.64)	227.32 (5.54)	259.17 (14.14)
Sleep Period Time (SPT)	210.64 (9.71)	210.46 (17.79)	214.84 (8.66)	233.18 (17.70)	222.48 (4.61)	226.25 (15.09)	208.32 (6.37)	216.61 (17.00)
Total Sleep Time (TST)	185.48 (10.94)	188.21 (16.66)	195.05 (7.86)	202.53 (15.61)	200.66 (5.77)	193.77 (13.95)	188.14 (6.66)	192.39 (16.20)
WASO	25.41 (6.31)	22.89 (3.99)	20.18 (4.31)	31.13 (6.59)	22.91 (3.86)	32.98 (5.57)	19.50 (3.64)	26.01 (5.91)
Total Wake (WT)	50.08 (8.96)	69.50 (6.13)	37.33 (4.85)	64.07 (8.71)	43.00 (4.36)	64.61 (8.07)	39.18 (5.27)	66.78 (9.18)
SE (TST/TDT*100)	78.74 (3.88)	73.03 (2.72)	83.94 (2.09)	75.97 (3.11)	82.35 (1.77)	74.99 (3.30)	82.76 (2.18)	74.23 (3.39)
Total Stage 1 Sleep	26.05 (1.62)	33.70 (4.50)	26.45 (2.10)	33.39 (3.69)	29.66 (2.51)	39.04 (3.18)	27.34 (2.68)	37.92 (5.78)
Total Stage 2 Sleep	123.15 (7.46)	105.56 (9.20)	128.56 (6.98)	120.31 (10.84)	128.48 (5.74)	109.45 (9.01)	124.15 (6.48)	103.84 (8.15)
Total Stage 3-4 Sleep	8.55 (2.00)	0.77 (0.39)	5.73 (2.11)	0.54 (0.28)	8.36 (2.42)	1.48 (0.69)	7.30 (1.91)	0.54 (0.35)
Total REM Sleep	27.71 (3.31)	48.18 (5.90)	34.30 (2.75)	48.30 (4.54)	34.14 (3.25)	43.80 (4.84)	29.35 (3.22)	50.06 (5.14)
Total NREM Sleep	157.75 (9.04)	140.03 (11.85)	160.74 (7.07)	154.24 (13.44)	166.50 (5.07)	149.97 (10.41)	158.79 (5.78)	142.30 (12.43)



**Figure 4.**—Average (+ SEM) latency data for a) the initial latency to persistent sleep and b) the return-to-persistent sleep latency in the middle of the night. Asterisks indicate Tukey post-hoc comparisons significantly different from placebo ( $p < 0.05$ ).

**Table 3.**—Sleep diary data (mean ± SEM).

<i>Sleep Diary Parameter</i>	<i>PLACEBO</i>	<i>EARLY</i>	<i>CONTINUOUS</i>	<i>LATE</i>
Latency to Sleep Onset (SL)	35.73 (9.29)	27.46 (5.79)	29.68 (4.17)	32.66 (5.67)
Total Dark Time (TDT)	507.70 (13.35)	497.67 (14.18)	501.08 (9.78)	502.77 (12.13)
Sleep Period Time (SPT)	437.40 (12.22)	431.37 (13.80)	440.31(6.98)	430.21 (10.72)
Total Sleep Time (TST)	368.42 (15.71)	363.47 (13.63)	379.46 (13.48)	368.52 (15.80)
WASO	75.97 (12.77)	74.00 (10.95)	73.54 (11.00)	65.33 (11.58)
Total Wake (WT)	139.28 (19.32)	134.21 (16.11)	121.62 (16.07)	134.25 (16.94)
SE (TST/TDT*100)	72.57 (3.40)	73.03 (2.90)	75.73 (3.00)	73.30 (3.30)
Number of Awakenings	1.78 (0.11)	1.76 (0.19)	1.87 (0.19)	1.94 (0.19)
Subjective Daytime Fatigue (1-5)	3.59 (0.17)	3.33 (0.12)	3.46 (0.19)	3.53 (0.16)
Subjective Sleep Score (1-5)	3.56 (0.16)	3.31 (0.17)	3.44 (0.19)	3.48 (0.16)
Subjective Rest Score (1-5)	3.55 (0.18)	3.26 (0.16)	3.41 (0.19)	3.45 (0.15)

**Table 4.**—VAS data, collapsed across time of day (mean ± SEM).

<i>Subjective Measure</i>	<i>PLACEBO</i>	<i>EARLY</i>	<i>CONTINUOUS</i>	<i>LATE</i>
Alertness	70.13 (0.47)	65.38 (0.53)	69.05 (0.52)	68.64 (0.47)
Calmness	78.49 (0.45)	75.76 (0.46)	73.64 (0.49)	75.24 (0.47)
Effort	25.11 (0.37)	28.74 (0.40)	26.27 (0.43)	24.47 (0.42)
Happiness	78.89 (0.37)	77.50 (0.40)	75.30 (0.39)	77.55 (0.37)
Sadness	6.47 (0.14)	8.07 (0.16)	7.99 (0.16)	7.80 (0.15)
Sleepiness	27.73 (0.46)	32.37 (0.53)	32.74 (0.49)	29.62 (0.52)
Tension	9.13 (0.17)	10.02 (0.18)	10.08 (0.15)	10.58 (0.17)
Weariness	22.38 (0.39)	27.28 (0.42)	24.76 (0.45)	24.85 (0.46)

of the initial sleep onset was significantly and positively correlated with the DLMO; in all subjects sleep onset occurred after the onset of nocturnal melatonin production. Total sleep time and sleep efficiency were significantly correlated with the timing of the DLMOff and with the phase difference between bedtime and the melatonin rhythm. Total sleep time and sleep efficiency were positively correlated with the amount of time subjects were in bed prior to the offset of their endogenous melatonin rhythm.

To test whether low melatonin producers were preferentially sensitive to melatonin replacement, treatment effects were correlated with measures of endogenous melatonin production. Table 6 provides correlation coefficients for SE treatment response, calculated by subtracting placebo SE from each treatment SE. Sleep efficiency treatment response was not correlated with measures of melatonin production. Treatment response was significantly correlated with measures of melatonin offset. In all conditions, a short duration of endogenous secretion was associated with greater treatment response. Having an early DLMOff was

associated with treatment response in the EARLY and CONTINUOUS treatments. Greater treatment response in the CONTINUOUS and LATE treatments was associated with having shorter time in bed before the DLMOff ( $_{BT}DLMOff$ ).

## DISCUSSION

### Melatonin Treatment of Age-Related Insomnia

**Sleep latency.**—The present investigation is the first to use PSG recordings to document that high physiological doses of melatonin can promote sleep in patients with age-related sleep-maintenance insomnia. The EARLY treatment significantly shortened the initial latency to persistent sleep, consistent with previous reports of facilitation of the initial SL in younger subjects.<sup>67-69</sup> The CONTINUOUS treatment significantly shortened the mean latency to persistent sleep, but only when the data were collapsed across time of night. In contrast to previous reports, actigraphy data did not yield significant treatment effects. This differ-

**Table 5.**—Correlation coefficients for melatonin and sleep in the placebo condition.

Sleep Parameter	MEL <sub>Max</sub>	MEL <sub>AUC24</sub>	MEL <sub>AUCBT</sub>	MEL <sub>Dur</sub>	DLMO	DLMO <sub>Off</sub>	BTDLMO	BTDLMO <sub>Off</sub>
Time of Sleep Onset	-.09	-.26	-.14	-.27	.75*	.40	.45	-.46
Latency to Sleep Onset (SL)	.01	.01	.10	-.21	-.07	-.21	.06	-.19
Latency to 10 min of Sleep	.07	.07	.20	-.21	.02	-.14	.06	-.19
Total Sleep Time (TST)	.11	.27	.14	.37	-.23	.09	-.47	.54*
Sleep Efficiency (TST/TBT)	.05	.08	.00	.48	.35	.63*	-.60*	.70*
Final Awakening	.04	.00	.07	-.19	.40	.18	.05	-.18

\* ( $p < 0.05$ )

ence may be due to the relative prevalence of pathological sleep onset latencies. While other studies have reported mean estimates of initial SL in the PLACEBO condition of 49 minutes,<sup>54</sup> 33 minutes,<sup>52</sup> and 54 minutes,<sup>53</sup> the mean actigraph estimate of the initial SL in present investigation was only 13 minutes.

The initial latency to persistent sleep was also significantly shorter in the LATE treatment. There is no obvious explanation as to why the LATE treatment would shorten the initial sleep latency, as subjects took a placebo dose before bedtime. There was no significant correlation between the treatment response for latency to persistent sleep in the LATE condition and any measure of endogenous melatonin production, circadian phase or phase shift, or core body temperature. The LATE treatment response for this parameter was correlated with latency to persistent sleep in the PLACEBO condition ( $r = -0.90$ ,  $p < 0.05$ ). It may be interesting that the LATE treatment response for this parameter was not significantly correlated ( $r = 0.25$ ) with the subjective treatment response data for sleep latency, calculated from sleep-diary data. A lack of significant correlation between the subjective perceptions obtained from the sleep diary and the objective treatment response may reduce the possibility of a placebo effect. Nevertheless, based upon the present analyses, the finding that the LATE treatment shortened the initial latency to persistent sleep could be explained by a placebo effect or by regression to the mean. The LATE treatment did not facilitate return-to-sleep latencies in the middle of the night, but this dose was given immediately before the re-initiation of sleep, and absorption may not have been fast enough to affect this variable. In fact, both the EARLY and the CONTINUOUS treatments provided higher plasma levels at this time (see Fig. 2, Time 4).

**Sleep and sleep consolidation.**—Despite the significant effect of melatonin on the initial latency to persistent sleep, melatonin did not affect sleep architecture, did not increase total sleep time, and did not improve measures of sleep consolidation such as SE and WASO. Using wrist

actigraphy, two previous investigations have reported SE increases following melatonin treatment.<sup>52,53</sup> Although in older adults, actigraphy has been shown to be sensitive to treatment effects of bed restriction,<sup>70</sup> when bedtimes are held relatively constant, actigraphs significantly overestimate pathological sleep,<sup>71</sup> and particularly sleep in older adults.<sup>72,73</sup> Given the amount of time that elderly insomnia patients may be lying in bed awake but immobile, wrist actigraphy alone (ie, without PSG recording or subjective self-assessment of sleep and daytime functioning) may not be an adequate tool for estimating symptom severity and treatment outcome. In the present investigation, using PSG recording, melatonin did not improve measures of sleep and sleep consolidation. Therefore, there is currently no convincing evidence that melatonin replacement can improve elderly sleep beyond sleep latency. Traditional hypnotics, on the other hand, have been shown to increase measures of nighttime sleep in the elderly.<sup>74,75</sup> Furthermore, in patients with age-related sleep-maintenance insomnia, evening bright-light exposure that delays the circadian timing system has been shown to significantly increase SE.<sup>76</sup> Based upon the current state of the literature, therefore, melatonin replacement for age-related insomnia does not appear to be as efficacious as currently approved hypnotics or evening bright light.

**Subjective sleep and daytime alertness.**—Melatonin did not significantly improve subjective self-estimates of sleep. Including our own, we know of no investigation of elderly insomnia demonstrating improvement in self-rated sleep scores following melatonin treatment. By contrast, traditional hypnotics have been shown to improve subjective reports of the amount and the quality of nighttime sleep.<sup>74,75,77</sup> The lack of subjective improvement with melatonin treatment may be due to the relative degree of anterograde amnesia induced by melatonin<sup>78</sup> and traditional hypnotics.<sup>79,80</sup>

In the present investigation, subjective self-reports of daytime alertness and sleepiness were not improved by nighttime melatonin replacement. In the elderly, however,



improving subjective daytime alertness with nighttime therapeutics is not easily achieved,<sup>74,81</sup> and some hypnotics, particularly those with longer metabolic half-lives, have been shown to impair daytime performance and alertness by carryover effects.<sup>81</sup> This apparent resistance to the daytime alerting effects of nighttime hypnotic treatment may be related to the relative lack of excessive daytime sleepiness in this population. Assessment of daytime mood and alertness in the present investigation demonstrated that, despite their poor nighttime sleep, subjects remained relatively alert until just before bedtime. This lack of excessive daytime sleepiness, in the presence of pathological nighttime sleep, is consistent with previous investigations in this population.<sup>82,83</sup> In summary, when compared with other treatments for elderly insomnia, the effects of melatonin replacement appear to be clinically modest and may be limited to facilitating the initiation of sleep. Although melatonin shortened latencies to persistent sleep, it did not significantly improve measures of sleep and sleep consolidation. Furthermore, melatonin did not improve subjective self-ratings of nighttime sleep and daytime alertness.

**Melatonin pharmacokinetics.**—Plasma pharmacokinetic data documented the formulations used in this investigation. The EARLY and LATE treatments yielded peak plasma levels above physiological levels; at 1 to 2 hours after ingestion they were within high physiological levels. The average elimination half-life for the immediate-release formulations ( $42.42 \pm 2.93$  minutes) was in the same range as that previously reported in younger subjects.<sup>55,56,84</sup> The CONTINUOUS treatment yielded nighttime plasma melatonin levels that approximated a youthful profile. Unfortunately, this formulation was flawed in that—for many subjects—it may have continued to release melatonin throughout the next day so that daytime plasma levels could not return to baseline. Large inter-individual differences in the pharmacokinetic profiles of all formulations revealed that a 0.5 mg dose yields physiological plasma levels in some subjects and low pharmacological levels in others, with a range of peak levels greater than one order of

magnitude.<sup>56</sup> For the EARLY and the LATE immediate-release treatments, differences in  $xMEL_{MAX}$  were significantly correlated with variability in BMI:  $r=0.67$  ( $p<0.05$ ) and  $r=0.64$  ( $p<0.05$ ) respectively. Individual differences in  $xMEL_{MAX}$  were not correlated with treatment response.

**Circadian phase.**—In the present investigation, DLMOs assessed after the PLACEBO condition revealed that the negotiated bedtimes imposed by the treatment protocol did not affect circadian phase. In the pre-treatment and PLACEBO conditions, the mean phase of the DLMO, as assessed by the 25% threshold, was approximately 2100 hours. Compared to the two-standard-deviation method<sup>85</sup> for defining the DLMO, the 25% of nighttime maximum tends to yield a later estimate of phase of up to 1 hour. Therefore, when using the same marker for melatonin onset, our phase data are comparable to previous DLMOs in age-related insomnia<sup>86</sup>; these data are also in agreement with previous reports of an age-related advance of the circadian timing system, as marked by core body temperature rhythm.<sup>87-91</sup> The pharmacokinetic profile of the CONTINUOUS treatment masked DLMO assessment on the night after administration, so the effects of the CONTINUOUS treatment on circadian phase were not determined. The immediate-release treatments did not significantly shift circadian phase, although—as predicted by the melatonin PRC<sup>23</sup>—the LATE treatment did cause a larger phase delay than the EARLY treatment.

**Core body temperature.**—This investigation is the first to demonstrate nighttime hypothermic effects of high physiological doses of melatonin in elderly subjects. The EARLY treatment significantly reduced core body temperature in the second quarter of the night. In the LATE treatment, core body temperature was significantly lower than placebo for most of the night. As with sleep latency, the effect of the LATE treatment on core body temperature occurred before melatonin administration. That core body temperature was also significantly different from placebo in the first half of the night suggests that the LATE treatment may have caused some chronic change in both sleep

**Table 6.**—Correlation coefficients for melatonin, in the placebo condition, and sleep efficiency treatment response.

Treatment Condition	MEL <sub>Max</sub>	MEL <sub>AUC24</sub>	MEL <sub>AUCBT</sub>	MEL <sub>Dur</sub>	DLMO	DLMO <sub>off</sub>	BTDLMO	BTDLMO <sub>off</sub>
EARLY	.07	.06	.07	-.61*	-.27	-.66*	-.02	-.45
CONTINUOUS	-.31	-.35	-.28	-.54*	-.21	-.56*	.52	-.70*
LATE	.00	-.10	-.04	-.75*	.08	-.48	.28	-.72*

\* ( $p<0.05$ )

propensity and core body temperature. The CONTINUOUS treatment did not significantly decrease core body temperature; this may, however, have been a result of higher core body temperature in the beginning of the night.

The LATE treatment significantly reduced core body temperature without improving sleep in the second half of the night. This finding does not support the hypothesis that suppressing the morning rise of core body temperature with high physiological doses of melatonin can significantly improve sleep in elderly insomnia patients. When comparing group averages, dose-response hypothermic effects of daytime melatonin administration have been reported in young subjects.<sup>26,27,29,92</sup> In the present investigation, the magnitude of the mean hypothermic effect was small, less than 0.3°C. Higher doses of melatonin in the second half of the night may have been necessary to reduce core body temperature enough to yield measurable changes in sleep, especially if the elderly are less responsive to the hypothermic effects of melatonin.<sup>93</sup>

### **The Role of Endogenous Melatonin and Circadian Phase in Age-Related Insomnia**

The melatonin replacement hypothesis asserts that the decline in nighttime melatonin production with age mediates age-related insomnia. Two studies have reported a relationship between sleep and 24-hour 6-sulfatoxymelatonin production,<sup>50,51</sup> although urine collection for both of these studies was apparently done in subjects' homes, without strict control of exposure to light. Hajak and colleagues recently reported that 10 patients with chronic primary insomnia had lower nighttime plasma melatonin levels than 5 healthy controls.<sup>94</sup> Given that the control group was significantly younger than the patient group, however, this study cannot be considered compelling evidence for the melatonin replacement hypothesis. In the present investigation, comparing plasma melatonin levels within a group of elderly sleep-maintenance insomnia patients did not reveal a relationship between sleep and any measure of endogenous melatonin production. Furthermore, post-hoc correlations revealed that low endogenous melatonin producers were not preferentially responsive to melatonin replacement.

Although sleep in the present investigation was not associated with absolute levels of melatonin production, sleep was correlated with the timing of the melatonin rhythm. The clock time of sleep onset was significantly correlated with the DLMO, suggesting a temporal association between the onset of the endogenous melatonin rhythm and the time of sleep onset. In young entrained subjects, Zhdanova and coworkers demonstrated a temporal relationship between the onset of endogenous melatonin production and the onset of habitual evening subjective sleepiness.<sup>69</sup> These findings are consistent with previous

reports that the circadian sleep propensity rhythm is temporally linked with the circadian rhythms of plasma melatonin<sup>35,36</sup> and urinary 6-sulfatoxymelatonin.<sup>34,95</sup> Sleep efficiency in the first half of the night was also significantly correlated with circadian phase, as marked by the DLMO and the DLMOff. This finding is consistent with that of Campbell and colleagues, who reported that in patients with age-related insomnia, the amount of sleep in the first hour of the nighttime sleep episode is predicted by the circadian phase of bedtime (as marked by the maximum rate of core body temperature decline).<sup>96</sup>

In the present investigation, other parameters of sleep were strongly correlated with the phase-difference between bedtime and the offset of the endogenous melatonin rhythm ( $_{BT}DLMOff$ ). Total sleep time and sleep efficiency were both significantly correlated with  $_{BT}DLMOff$ . Minimal phase-difference abnormalities (ie, when bedtimes occurred farthest from the DLMOff) were associated with more total sleep time and greater sleep efficiency. The relationship between sleep and the circadian phase of bedtime has been demonstrated in free-running subjects in temporal isolation and in forced desynchrony protocols.<sup>35,97-101</sup> These investigations have consistently demonstrated that sleep is longest and most efficient when bedtimes occur farther away from the temperature minimum, and that TST and SE decrease as bedtimes occur closer to the temperature minimum. In healthy elderly, and in patients with age-related insomnia, several investigators have documented a similar relationship between sleep and circadian phase, as marked by core-body temperature.<sup>82,87,88,90,91</sup> Using 4-hour plasma sampling, MacFarlane and coworkers may have been the first to document abnormal melatonin rhythms in patients with primary insomnia (ages 25-65 years) compared to age- and gender-matched controls.<sup>102</sup> Although difficult to verify with 4-hour sampling, their data appear to be consistent with an advance of the endogenous melatonin rhythm relative to bedtime. The same relationship can be found by recalculating the results of a recent study by Lack and colleagues.<sup>86</sup> These recalculations reveal that, relative to bedtimes, the melatonin rhythms of older patients with early morning awakening are approximately 2 hours phase-advanced, compared with age-matched controls. It appears, therefore, that an abnormally phase-advanced circadian timing system, in relation to bedtimes, may mediate age-related sleep-maintenance insomnia and early morning awakening.

In summary, we report that circadian phase-differences, and not absolute levels of melatonin production, are associated with the severity of age-related sleep-maintenance insomnia. Previous comparisons between sleep and overnight 6-sulfatoxymelatonin levels may have found significant relationships because of a failure to appreciate phase-differences between bedtime and the melatonin

rhythm. Overnight urine collections, for instance, may miss a substantial proportion of nocturnal melatonin production in patients with abnormal phase-relationships such as those reported in the present investigation. Furthermore, investigations demonstrating a relationship between 6-sulfatoxymelatonin production and sleep are typically done in subjects' homes, without strict control of exposure to ambient light. The present comparisons were done in the laboratory using plasma melatonin sampled under strictly controlled lighting conditions, and therefore may better represent the relationship, or lack thereof, between levels of endogenous melatonin production and sleep. On the other hand, given the relatively small sample size, the present investigation may have failed to find a significant relationship between overall melatonin production and sleep because of a Type II error, especially if females are more sensitive than males to a reduction in 6-sulfatoxymelatonin production.<sup>51</sup> Furthermore, the finding that such a high proportion of these patients had very low melatonin production may, in itself, be evidence that melatonin production is associated with age-related sleep-maintenance insomnia. Despite these shortcomings, the results of the present study clearly demonstrate that, within a sample of insomnia patients, circadian phase-differences are more important than levels of melatonin production for predicting severity of age-related sleep-maintenance insomnia.

The apparent consequence of an abnormal phase-relationship between bedtime and the circadian timing system suggests that interventions designed to realign habitual sleep time with circadian time (eg, with appropriately timed bright light) may improve sleep in elderly subjects with sleep-maintenance insomnia and early morning awakening. Although the long-term efficacy of timed exposure to bright light for treating age-related insomnia is unknown, preliminary reports suggest that it is effective in the short-term (for a brief review, see 103). Evening bright light (causing significant phase delays) has been shown to improve sleep in elderly subjects with sleep-maintenance insomnia<sup>76,82</sup> and in slightly younger patients with early morning awakening.<sup>104-106</sup> Although, because of its chronobiotic effects, melatonin may be useful in reestablishing and perhaps maintaining a more appropriate phase-relationship, this treatment would require a morning dose of melatonin capable of shifting circadian phase without causing daytime sleepiness.

There are also age-related changes in the homeostatic properties of the sleep-wake system.<sup>2,107-109</sup> In the present investigation, circadian phase differences were significantly correlated with sleep in the first half and not the second half of the night, suggesting that other processes may have been contributing to poor sleep in the early morning hours. Preliminary analyses of our data are in accordance with previous reports that awakening in the second half of the

night is predicted by a complex interaction between circadian and homeostatic processes.<sup>110</sup> Age-related changes in the homeostatic process of sleep may limit the effectiveness of interventions designed solely to realign the circadian timing system with habitual sleep times.<sup>82</sup> Nevertheless, our results—coupled with the results of others reporting an abnormal phase-relationship between the circadian timing system and sleep, and with the results of bright-light research—suggest that realignment of sleep with the circadian timing system would be an effective treatment of age-related sleep-maintenance insomnia.

## CONCLUSIONS

The present investigation assessed the sleep-promoting and hypothermic effects of melatonin replacement in 14 patients with age-related sleep-maintenance insomnia. Based upon our results, we conclude that high physiological doses of melatonin, given 30 minutes before bedtime, have significant sleep-promoting effects at night, but that these effects may be limited to shortening sleep latency. It is plausible, therefore, that the degree to which melatonin is effective for treating a given patient with elderly insomnia may be determined by the relative contribution of sleep-onset symptoms to that patient's overall sleep complaint. Based upon the results of this investigation, melatonin treatment does not appear to be as efficacious for treating age-related insomnia as currently available hypnotics or bright-light treatment. Nevertheless, our results provide some support for a direct sleep-promoting effect of physiologic melatonin replacement.<sup>28,54,69</sup> That melatonin most consistently facilitates sleep at times when sleep propensity is otherwise low<sup>29</sup> may suggest that the sleep-promoting effects of melatonin are carried out by antagonizing the proposed alerting signal from the SCN.<sup>111</sup> This proposed explanation of the sleep-promoting and hypothermic effects of melatonin is described elsewhere.<sup>112</sup>

In conclusion, the present investigation does not provide strong support for the melatonin replacement treatment strategy for age-related sleep-maintenance insomnia. Absolute endogenous melatonin production was not significantly associated with sleep, and high physiologic melatonin replacement did not yield robust treatment efficacy. Our results suggest that the phase-relationship between habitual bedtime and the phase of the circadian timing system, as marked here by the endogenous melatonin rhythm, may be more important than absolute melatonin production for predicting severity of sleep-maintenance insomnia.

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