Circadian phase-shifting effects of nocturnal exercise in older compared with young adults

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SLEEP COMPLAINTS ARE REPORTED in ~20–60% of elderly individuals (1, 18, 19) and are associated with a variety of mental, physical, and behavioral health problems (19). With increasing age, circadian rhythm changes (including circadian phase advance, change in the amplitude of circadian rhythms, change in the phase angle between sleep and other circadian rhythms, etc.) also can occur (11, 12, 14, 15, 29, 31, 41) and may contribute to the sleep complaints reported by this population.

In older age, the phase of circadian rhythms tends to become more advanced relative to the environment (11, 12, 14, 29, 41). This circadian phase advance may be a normal part of aging, but for some people it can be so severe that their sleep/wake schedule is not in synchrony with the rest of the world (i.e., they fall asleep in the early evening and wake very early in the morning). Bright light in the evening can phase delay the circadian rhythms of older adults (9, 10, 26). Although bright light is a strong synchronizing agent (zeitgeber) for the circadian clock and has been shown to improve sleep complaints in older adults, it may not always be a feasible treatment. Ophthalmological conditions (such as retinal disease, narrow-angle glaucoma, cataracts, macular degeneration, retinal blindness, etc.) may contraindicate the use of bright light or result in the light being less effective by interfering with the path it takes to affect the circadian pacemaker. Thus other potential zeitgebers, such as exercise, may be useful for phase shifting the circadian rhythms of older adults.

Several studies have examined the effects of exercise on the circadian rhythms of young, healthy humans. Some studies used “re-entrainment” designs, in which subjects underwent a shift of the sleep/dark period (e.g., simulated night shift work) and exercise was used as a stimulus to accelerate entrainment to the new sleep/wake cycle. Results of these studies indicated that exercise accelerated entrainment to the new sleep/dark schedule; exercise at night accelerated phase delays (17, 34), and daytime exercise accelerated phase advances (28). Of the studies that did not involve a shift in the sleep/dark schedule (6, 7, 28, 32, 38) nocturnal exercise was associated with circadian phase delays.

Two studies used exercise in combination with bright light to phase delay circadian rhythms of young, healthy humans (2, 43). Both studies found that exercise neither facilitated nor inhibited the phase shifts produced by bright light.

This study examined whether a single bout of nocturnal, low-intensity exercise could phase delay the circadian rhythms of older adults. To date, all other investigations of the phase-shifting effects of exercise have been conducted in young humans or in animals.
Subjects completed the Horne-Ostberg Morningness-Eveningness Questionnaire (Ref. 23 and Table 1) before beginning the study. Subjects signed informed consent forms and were paid for their participation.

Methods

Subjects

We tested 8 healthy young adults (20–32 yr old) and 10 healthy older adults (55–73 yr old; Table 1). Subjects were recruited from the Aging Research Registry of the Buehler Center on Aging at Northwestern University, by word of mouth and by flyers.

Initial screening of potential subjects involved a phone interview that excluded subjects with dementia, major psychiatric disorder, and major physical or medical disabilities that would contraindicate exercise. Potential subjects who passed the initial screening underwent psychological testing and physical evaluation to determine eligibility for enrollment in the study. Exclusion criteria for the study were as follows: 1) any acute or unstable medical problems, especially those that contraindicate exercise; 2) body mass index >30; 3) abnormal mood as assessed by the Geriatric Depression Scale (for older adults; see Ref. 42) or by the Beck Depression Inventory (for young adults; see Ref. 5), young and older subjects being given different questionnaires for assessment of depression because these are the validated screening instruments for these particular age groups; 4) abnormal personality (any clinical elevations with T >65) as measured by the Minnesota Multiphasic Personality Inventory—2 (22); 5) possible dementia (score <26 out of 30) as determined by the Mini-Mental Status Examination (20); 6) having a daily caffeine intake >200 mg; 7) smoking; 8) sleep disorder as assessed by a comprehensive sleep questionnaire; 9) shift work or travel across more than one time zone within 30 days of beginning the study; and 10) use of psychoactive medications or any medications or herbs known to affect melatonin or sleep.

During participation in the study, two older women were on estrogen replacement, three older men were taking aspirin, and one older man was on a diuretic. Three young women took oral contraceptives, and they were studied during the placebo pill week of their cycles. The young woman not taking oral contraceptives was studied in the early follicular phase of her menstrual cycle.

Subjects followed while they were admitted to the General Clinical Research Center (GCRC) of Northwestern Memorial Hospital. The GCRC is a National Institute of Health-funded inpatient facility with nurses, dietitians, laboratory facilities, and support staff who were specifically trained to assist in this study. Subjects were admitted to the GCRC 8 h before their baseline sleep-onset time on day 1 of the study. Upon admission, they commenced constant conditions (consistent semirecumbent posture, dim lighting, and food intake) with the head of the bed at a 45° angle during waking hours and the bed flat during sleep. During waking hours, subjects were allowed to read, watch television or videos, play cards, and do other quiet activities while remaining in the semirecumbent position. Subjects received 200- to 250-kcal snacks at 2-h intervals, and water was available ad libitum.

At admission, subjects received a catheter for intravenous blood sampling, an Actiwatch-L, and a Mini-Logger (Mini-Mitter, Sunriver, OR) connected to a disposable rectal probe to monitor core body temperature at 1-min intervals. Blood sampling began 5 h before baseline sleep onset on day 1. Samples were collected at 20-, 30-, or 60-min intervals and

Fig. 1. Sample protocol, based on a 2200–0600 baseline sleep schedule. Timing of sleep and exercise were based on each individual’s habitual schedule. Black bars represent scheduled sleep times. Hatched bars represent the exercise bout or the time during the control session that subjects sat upright in a chair. Vertical lines represent blood sampling. The timing of the sample collection depended on when the subject normally slept and increased in frequency during the times that melatonin was expected to rise.

Table 1. Descriptive statistics for young and older subjects

<table>
<thead>
<tr>
<th></th>
<th>Young Subjects</th>
<th>Older Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>8 (4 men, 4 women)</td>
<td>10 (4 men, 6 women)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27.1 ± 4.3</td>
<td>64.5 ± 6.8</td>
</tr>
<tr>
<td>Morningness-eveningness score†</td>
<td>55.5 ± 14.7</td>
<td>62.6 ± 8.8</td>
</tr>
<tr>
<td>Mini mental status exam score*</td>
<td>29.6 ± 0.5</td>
<td>28.0 ± 1.2</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23.3 ± 2.9</td>
<td>23.9 ± 3.6</td>
</tr>
<tr>
<td>VO₂ max score, ls/min</td>
<td>1.77 ± 0.46</td>
<td>1.45 ± 0.64</td>
</tr>
<tr>
<td>40% Peak VO₂ heart rate*, beats/min</td>
<td>109.8 ± 10.78</td>
<td>89.9 ± 8.32</td>
</tr>
<tr>
<td>60% Peak VO₂ heart rate, beats/min</td>
<td>126.3 ± 10.33</td>
<td>102.2 ± 7.28</td>
</tr>
<tr>
<td>Average min s/session spent in target heart rate zones</td>
<td>12:55 ± 0:46</td>
<td>12:13 ± 0:49</td>
</tr>
</tbody>
</table>

Values are means ± SD; N, no. of subjects. †P < 0.05 for young vs. older by t-test. *Higher scores = more morningness.
were more frequent when melatonin was expected to rise (Fig. 1). Blood samples were centrifuged, and plasma samples were stored in the freezer at −70°C before analysis.

Light levels in the GCRC were maintained at a dim level (<50 lux) during waking hours and reduced to dark levels (<5 lux) during sleep episodes. Light levels in the GCRC were periodically sampled using a light meter and were continuously monitored using the Actiwatch-L. Subjects were required to remain in their rooms and use the restroom attached to their rooms. A night light was installed in the restroom, and the overhead light was not illuminated to keep the lighting conditions in the restroom the same (or darker) as in the bedrooms.

On day 2 of the exercise session, subjects were kept awake for 4 h after their baseline bedtime. A 3-h exercise bout (described below) began 0.5 h after the baseline bedtime (see Fig. 1). One-half hour after the exercise, the lights were extinguished, and subjects were allowed to sleep for 6 h. Within the 1st h after waking on day 3, subjects were allowed a brief (<5 min) cool shower. Constant conditions continued throughout day 3, and subjects were allowed to sleep during their habitual time. During the control session, subjects followed the same protocol as in the exercise session but sat upright in a chair during the same period as the exercise occurred in the exercise session. This procedure was used to control for the possibility that phase shifts in the exercise condition could be caused by the sleep deprivation, extended waking, and/or postural changes.

Exercise

Before being admitted for the exercise portion of the study, subjects completed baseline exercise testing to determine exercise capacity. A symptom-limited maximal ergometer test with a 10- to 40-W/min step protocol was used to measure maximum oxygen uptake (\(\dot{V}O_2\)max) for each subject. The amount of work was tailored to individual capacity, so that the subject reached anaerobic threshold after 5–15 min of exercise. The heart rate that corresponded to 40 and 60% of peak VO2 was measured (see Table 1), which determined the exercise level subjects would be asked to maintain during the exercise bout (see below). The baseline exercise testing also served as a screening process to exclude potential subjects with cardiac abnormalities or exercise intolerance.

In the exercise condition, subjects pedaled on a stationary cycle ergometer. A Polar Accurex II Heart Rate Monitor was used to monitor heart rate, and a pulse oximeter monitored oxygen saturation during the exercise. The exercise took place in the same room and under the same lighting conditions as the rest of the study. Subjects completed five consecutive 36-min pulses consisting of 15 min of cycle ergometer exercise at 40% of \(\dot{V}O_2\)max, followed by 15 min at 60% of \(\dot{V}O_2\)max, followed by a 1-min cool down and a 5-min rest. Because the exercise was tailored to each individual based on his or her own capacity, the relative difficulty of the exercise was the same for both young and older subjects and for those who were fit or sedentary. During the exercise stimulus, subjects were asked to keep their heart rate within 5% of the target heart rate during the exercise bout (see Table 1).

Data Analyses

Melatonin. Melatonin levels in plasma samples were analyzed using a double-antibody RIA procedure (Stockgrand, Surrey, UK) with reagents that are commercially available. The antimelatonin antiserum was also supplied by Stockgrand and has been validated for the direct assay of melatonin in human plasma using an iodinated melatonin tracer (NEN Life Science Products). Each sample was analyzed two times. The lower limit of detection for the assay was 2.60 pg/ml, and the upper limit of detection for the assay was 293.00 pg/ml. The intra-assay coefficient of variation averaged 15.2% for values <10 pg/ml, 15.7% for values 10–50 pg/ml, and 8.5% for values >50 pg/ml. The interassay coefficient of variation averaged 18.3% for values <10 pg/ml, 13.5% for values 10–50 pg/ml, and 12.8% for values >50 pg/ml.

The dim-light melatonin onset (DLMO) was used as the marker of circadian phase and was the time of the first plasma melatonin level to cross the DLMO threshold. The threshold was defined as 20% of the maximum raw value averaged for days 1 and 3. For each subject, there was a threshold for the exercise session and a threshold for the control session. During the exercise session (day 2), melatonin values were greater than on any other day (this finding will be discussed in RESULTS). We did not want these high values to influence the DLMO threshold. Thus the peak value on day 2 was not included in the determination of the DLMO thresholds.

The DLMO on day 1 was a baseline (or before the stimulus) circadian phase marker. The DLMO on day 2 was not originally planned to be used as a baseline phase marker because it was possible that the exercise could have begun before the DLMO on day 2. However, the exercise began after the DLMO on day 2 for all subjects. Thus there were two DLMOs before the intervention for each session, and we averaged them to obtain the baseline DLMO. The phase shift of the melatonin rhythm was calculated by comparing the difference in the timing of the baseline DLMO (the average of days 1 and 2) and the DLMO on day 3 (after the manipulation) for each condition. Phase shifts of the DLMO were analyzed using ANOVA with factors condition (control vs. exercise) and age (young vs. older). The baseline DLMOs were compared by Student’s t-test for young vs. older subjects.

There was one older male (subject 28) who was a low melatonin secretor and had very large phase advances of the DLMO in both the control and exercise conditions. Because of the fact that he was a low secretor and was an outlier compared with other subjects for DLMO shift, his data were excluded from the melatonin phase summary statistics and from Figs. 2–4. There was one older female (subject 22) with 4 h of missing data on day 3 of the control session, corresponding to when the melatonin levels rose, and her melatonin phase-shifting data were also excluded.

The data were analyzed to quantify whether the exercise acutely raised the melatonin levels in the 3-h exercise condition compared with the control condition. The nine time points when blood was drawn over the course of the exercise (and the corresponding time during the control condition) were analyzed using ANOVA with factors condition (control vs. exercise) and age (young vs. older).

The amplitude of the melatonin rhythm was determined for each individual on each day by calculating the difference between the peak value and the minimum value. The melatonin rhythm amplitude was analyzed using a four-factor ANOVA, with between-subjects factors age (young vs. older) and sex and within-subjects factors condition (exercise vs. control) and day of experiment (days 1, 2, and 3).

Temperature. The core body temperature minimum (\(T_{min}\)) was also used as a circadian phase marker. Measurements of daily circadian temperature phase were made for each subject for day 1 (baseline) and day 3 (after the manipulation) using a curve-fitting procedure. The body temperature data on the second night of GCRC admission were not used for phase-estimation purposes because of the increase in tem-
temperature associated with exercise. For each 24-h section, temperature data were averaged into 15-min sections and missing data interpolated. The 24-h sections began 3 h before baseline bedtime on day 1 and 1 h after wake time on day 3. It was necessary to start the 24-h sections at different times on day 1 and day 3 so that each contained one sleep episode and neither contained time in which temperature data were directly affected by exercise. A 24-h cosine curve was fit to the data for each day. The minimum of the fitted temperature curve was used as the phase marker for each day. Mathematical demasking of the temperature curve was not necessary to estimate the endogenous T\textsubscript{min} because subjects slept in-phase with their temperature rhythms, i.e., the T\textsubscript{min} occurred during the sleep period.

Phase shifts of the fitted temperature data were calculated by comparing the change in the time of the T\textsubscript{min} from night 1 to night 3 for each condition. Phase shifts of the T\textsubscript{min} were analyzed using ANOVA with factors condition (control vs. exercise) and age (young vs. older).

There were two older females (subject 22 and subject 34) whose temperature data could not be analyzed for amount of phase shift because too many data points were missing (because of probe slips) to accurately fit the cosine curves to their data.

Correlations between different circadian phase markers. To determine how well the between-subject melatonin and temperature phase estimations corresponded to each other, correlations were performed on circadian phase for baseline and after the manipulation for both the control and exercise conditions and on the overall phase shifts in both the control and exercise conditions.

Sleep. Total sleep time was estimated using the data collected from the Actiwatch using the Actiware Sleep 3.2 program. These data were analyzed for each night of sleep in the GCRC using ANOVA with factors condition (control vs. exercise) and age (young vs. older). Statistics are reported as means ± SD.

RESULTS

Phase-Shifting Effects of Exercise

Melatonin. Complete DLMO phase-shifting data were available for eight young and eight older subjects (the data from subject 22 and subject 28 were excluded; see METHODS). Figure 2 shows that, for the young subjects in the control condition, there were both small phase advances and small phase delays. In contrast, during the exercise condition, there were only delays. On average, young subjects phase delayed more in the exercise than in the control condition (−0.75 vs. −0.25 h; Table 2). For the older subjects, there were both advances and delays in both conditions, but on average the exercise condition produced a phase delay, whereas there was virtually no phase shift in the control condition (−0.50 vs. +0.08 h).

The ANOVA showed a main effect of condition [F(1,14) = 6.53, P < 0.05] but no effect of age and no interaction. Thus, on average, there were larger phase delays of the circadian rhythm of melatonin in the exercise condition compared with the control condition, and older subjects did not shift more or less than young subjects. Within the older adults, there was no significant relationship between age and the magnitude of the circadian rhythm phase shift in the exercise condition (r = −0.25).

Temperature. Complete T\textsubscript{min} phase-shifting data were available for eight young and eight older subjects (the data from subject 22 and subject 34 were excluded; see METHODS). Although exercise produced significant delays of the melatonin rhythm compared with the control condition, there was no difference between the
Table 2. **DLMO phase shifts in hours**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control Phase Shift</th>
<th>Exercise Phase Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>−1.00</td>
<td>−0.50</td>
</tr>
<tr>
<td>4</td>
<td>+0.49</td>
<td>−0.66</td>
</tr>
<tr>
<td>5</td>
<td>−0.83</td>
<td>−0.50</td>
</tr>
<tr>
<td>7</td>
<td>+0.49</td>
<td>−1.67</td>
</tr>
<tr>
<td>10</td>
<td>−0.16</td>
<td>−0.66</td>
</tr>
<tr>
<td>11</td>
<td>−0.33</td>
<td>−0.49</td>
</tr>
<tr>
<td>14</td>
<td>−0.49</td>
<td>−1.17</td>
</tr>
<tr>
<td>15</td>
<td>−0.16</td>
<td>−0.33</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>−0.25 ± 0.55</td>
<td>−0.75 ± 0.45</td>
</tr>
</tbody>
</table>

Older subjects

| 21       | −0.66               | +0.50               |
| 24       | +0.51               | 0.00                |
| 29       | −0.67               | −1.83               |
| 31       | +0.84               | +0.17               |
| 32       | +0.35               | −1.00               |
| 34       | +0.16               | −1.17               |
| 36       | +0.66               | −0.16               |
| 37       | −0.50               | −0.50               |
| Mean ± SD | +0.08 ± 0.61        | −0.50 ± 0.78        |

| All subjects |                     |                     |
| Mean ± SD    | −0.08 ± 0.59        | −0.62 ± 0.63        |

DLMO, dim-light melatonin onset. Positive numbers represent circadian phase advances, and negative numbers represent circadian phase delays.

two conditions when temperature was used as the circadian phase marker; the average for all subjects showed a delay of −0.5 h in both the control and the exercise conditions (Table 3). ANOVA indicated no effect of condition [F(1,14) = 0.01, P ≤ 0.94] or age [F(1,14) = 0.77, P ≤ 0.40] and no interaction [F(1,14) = 1.07, P ≤ 0.32].

**Correlations Between Different Circadian Phase Markers**

There were strong correlations between the timing of the DLMO and the timing of the Tmin during baseline and after the manipulation for both the control (baseline: r = 0.72, P < 0.05, N = 15; postmanipulation: r = 0.84, P < 0.05, N = 15) and exercise (baseline: r = 0.83, P < 0.05, N = 15; postmanipulation: r = 0.86, P < 0.05, N = 15) sessions. Although circadian phase as assessed by the DLMO and Tmin were highly correlated, circadian rhythm phase shifts as assessed with these methods were not correlated significantly (control: r = 0.03, n = 15; exercise: r = 0.18, n = 15).

**Timing of Exercise Relative to Circadian Phase**

The magnitude of the DLMO phase shift was not significantly related to the circadian time of the exercise bout for young or older subjects, or for all subjects combined (r = −0.54, P = 0.17 for young, r = −0.32, P = 0.45 for older subjects, and r = −0.34, P = 0.19 for all subjects).

**Acute Effects of Exercise on Melatonin**

Melatonin levels were higher during the exercise pulse than they were during same period of the control condition (Fig. 3). However, the overall difference failed to reach significance [F(1,16) = 2.96, P = 0.11]. Because the difference appeared to be greatest during the last hour of the exercise in the older subjects, a post hoc t-test on the last hour of data was conducted, which revealed a significant difference between the control and exercise condition for the older subjects [t(9) = 2.51, P < 0.05].

**Baseline DLMO Circadian Phase**

The baseline DLMO occurred later for young subjects in the control session [2150 ± 01:14 (mean ± SD) in local clock hours] and the exercise session (2117 ± 01:28) than for older subjects in the control session (2038 ± 00:58) and the exercise session (2034 ± 00:52). These differences were significant in the control condition only [t(16) = 2.31, P < 0.05].

The phase angle between baseline DLMO and baseline bedtime was not statistically significantly different in young compared with older subjects (1.5 ± 0.7 h in control session and 1.9 ± 0.9 h in exercise session for young subjects, and 1.8 ± 1.4 h in control session and 1.8 ± 1.2 h in exercise session for older subjects).

**Melatonin Amplitude**

The melatonin amplitude was larger for young subjects than for older subjects [Fig. 4; F(1,14) = 6.00, P < 0.05]. There was also an interaction between age and day [F(2,28) = 3.54, P < 0.05], with the average amplitude of the melatonin rhythm becoming significantly larger over successive days for young subjects (193.6, 213.1, and 224.1 pg/ml on days 1, 2, and 3, respectively)

Table 3. **Tmin phase shifts in hours**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control Phase Shift</th>
<th>Exercise Phase Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>−1.73</td>
<td>−0.68</td>
</tr>
<tr>
<td>4</td>
<td>−2.25</td>
<td>+0.01</td>
</tr>
<tr>
<td>5</td>
<td>−0.78</td>
<td>−0.56</td>
</tr>
<tr>
<td>7</td>
<td>−0.36</td>
<td>−0.45</td>
</tr>
<tr>
<td>10</td>
<td>−1.23</td>
<td>+0.08</td>
</tr>
<tr>
<td>11</td>
<td>+0.69</td>
<td>+0.75</td>
</tr>
<tr>
<td>14</td>
<td>−0.31</td>
<td>−1.37</td>
</tr>
<tr>
<td>15</td>
<td>−0.69</td>
<td>−2.11</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>−0.83 ± 0.91</td>
<td>−0.54 ± 0.89</td>
</tr>
</tbody>
</table>

Older subjects

| 21       | −0.25               | +1.10               |
| 24       | −0.40               | +0.69               |
| 28       | −2.17               | −1.66               |
| 29       | +0.82               | −0.40               |
| 31       | +0.20               | −0.50               |
| 32       | +1.77               | +0.18               |
| 36       | −1.10               | −1.48               |
| 37       | −0.10               | −1.85               |
| Mean ± SD | −0.15 ± 1.18        | −0.49 ± 1.11        |

Tmin, core body temperature minimum. Positive numbers represent circadian phase advances, and negative numbers represent circadian phase delays.
but not for older subjects (128.6, 147.7, and 127.0 pg/ml on days 1, 2, and 3, respectively). There was no effect of sex or condition and no other interactions.

Sleep (Assessed by Activwatch)

Subjects slept on average 11 min less on night 2 during the exercise session than during the control session \([F(1,14) = 11.51, P < 0.05]\). Total sleep time for night 1 and night 3 did not differ between sessions, there were no age differences on any night, and there were no interactions between condition and age on any night.

DISCUSSION

The circadian rhythm of melatonin phase delayed more with the nocturnal exercise in both the young and older subjects compared with the control condition. The fact that both young and older subjects had exercise-induced DLMO phase shifts suggests that the exercise phase-shifting pathway is preserved in older age. Research in rodents has indicated that this activity-induced phase-shifting pathway is different from the pathway taken by light to the circadian clock (the retinohypothalamic tract) and involves the intergeniculate leaflet and the geniculohypothalamic tract (24, 25). The mechanism by which activity might work to phase shift or entrain the circadian clock is not yet known. However, some theories have suggested that the activity itself may produce effects on the circadian clock, whereas others have suggested that the increase in body temperature, motivation to run, arousal, or hormonal changes are the critical variables responsible for the effects (30). Research on the effects of aging on activity-induced phase shifts in hamsters produced mixed results; one study found a reduction in the phase-shifting effects of activity (39), but the other did not (16). These conflicting results raise the question of...
whether exercise has the same phase-shifting properties in older vs. young humans. In the present study, there was no significant difference between young and older subjects in the magnitude of the DLMO phase shift in response to exercise. Further research with larger sample sizes is needed to determine whether older subjects are less responsive to the circadian phase-shifting effects of exercise.

Notable was that, in the exercise condition, all of the young subjects experienced delays in the circadian rhythm of melatonin, whereas in the older subjects there was slightly more (although not significantly more) variability in the response (see Fig. 2). Two older subjects had small advances, and three had relatively large phase delays. The magnitude of the exercise-induced phase shift within the older adult group was not related to the age of the subject or the timing of the stimulus relative to the circadian phase. The reason for large shifts in some older subjects and small shifts in others is unknown. However, it is possible that age-related reductions in the amplitude of circadian rhythms (4, 12, 29, 40, 41) affected the ability to phase shift the rhythm. More specifically, a lower amplitude may indicate a weaker oscillator, with greater ability to phase shift.

Despite the fact that exercise produced phase delays of the melatonin rhythm, there was no difference between the two conditions when temperature was used as the circadian phase marker. Average phase delays of ~0.5 h were observed in both the control and exercise conditions. The reason why exercise produced phase delays that were greater than the control using the DLMO as the phase marker but not using the Tmin as the phase marker may be because of the lower sensitivity of temperature as a phase marker. The phase shifts observed in the present study, by design, were very small, and the difference in the magnitude of phase shift between the control and exercise conditions using the DLMO was also very small (<1 h). Thus it was important to utilize a very accurate marker of the circadian rhythm to detect subtle differences in phase shifts.

Previous research has suggested that temperature may be a less sensitive phase marker than melatonin (21, 27). Temperature had a larger within-subject variability than melatonin, and the SD of a phase assessment within a subject was 0.60 h for the Tmin but only 0.17 h for the DLMO in one study (21) and was 0.78 h for the Tmin but only 0.23–0.35 h for the DLMO in another study (27). In the present study, the mean phase shifts as assessed by the two markers (DLMO and Tmin) did not differ by >1 h. Specifically, the disagreement in phase shift between the two markers was 0.58 h in the control session and 0.21 h in the exercise session for young subjects and was 0.23 h in the control session and 0.01 h in the exercise session for older subjects (see Tables 2 and 3). Thus the difference between the two measures of the phase shift was within what would be considered by the previous studies to be an expected amount of error in measurement for the Tmin.

The timing of the DLMO and Tmin correlated strongly at baseline and after the manipulation (correlations ranged from 0.72 to 0.86). These robust correlations are consistent with other investigations that found strong relationships between phase, as assessed by the DLMO and the Tmin (8, 35, 37). Unfortunately, the phase shifts using the two methods were not significantly correlated. As stated above, it is possible that error in measurement accounted for this discrepancy.

Given that temperature has a larger variability than melatonin in estimating circadian phase, it is a less reliable method to measure small circadian rhythm changes. Overall, it appears that temperature may be a good gross estimate of circadian phase but may be less effective than melatonin for detecting small changes in circadian phase. Recently, investigators have been dropping temperature as the marker for circadian phase in favor of using the more precise, although more expensive, measure of melatonin as the phase marker (26). Given that the DLMO has been found to be a more reliable measure of circadian phase, in combination with the general consensus it is more accurate, the results from the melatonin data have been emphasized in this report.

Melatonin levels were higher during the exercise pulse than they were during the corresponding time in the control condition, and the difference was greatest in the older subjects during the last hour of the exercise pulse. It is possible that body temperature mediated the relationship between exercise and the acute rise in melatonin. In other studies of young subjects, a 3-h pulse of low-intensity nocturnal exercise did not significantly raise plasma melatonin (6, 38), but a 1-h pulse of high-intensity nocturnal exercise did produce a significant increase (6). Body temperature was monitored in the present study, but many subjects had missing temperature data during the exercise stimulus because of probe slippage. Thus an accurate measure of body temperature during the exercise stimulus compared with the corresponding time in the control session was not available. Although the intensity of exercise in the present study was similar to the low-intensity exercise in previous studies (6, 38), the equipment was different. In previous studies, subjects cycled on equipment with a built-in fan that cooled them as they exercised, but there was no fan in the present study. In addition, unlike the previous study, the present study included older adults, and it is possible that it was more difficult for the older subjects to lose heat during the exercise (accounting for the fact that older subjects vs. young subjects had significantly higher temperature during the last hour of exercise). Thus body temperature could be mediating the relationship between the acute effects of exercise on melatonin. To date, no studies could be found that investigated the direct effect of an increase in body temperature on levels of melatonin (in the absence of exercise). Future research should address whether an acute rise in body temperature itself can affect levels of melatonin.

The baseline DLMO occurred about 1 h earlier for older subjects than for young subjects. The difference is
consistent with well-established previous findings that, with older age, there is a circadian phase advance (11, 12, 14, 29, 41). Previous reports from one laboratory found that older subjects slept on an earlier part of their circadian cycle. In other words, there was a smaller phase angle difference between circadian phase markers (temperature minimum or melatonin peak) and wake in older compared with young subjects (14, 15). In contrast, another study did not find a difference between young and older subjects in the phase angle between the temperature minimum and wake (11), and another did not find a difference in the phase angle between the DLMO and sleep onset (3). We did not find a difference between young and older subjects in the baseline phase angle between the DLMO and bedtime. Thus, although all studies agree that the sleep schedules and circadian rhythms of older subjects occur at an earlier clock time compared with younger subjects, there is less agreement on whether there is a change in the phase relationship between sleep and the other circadian rhythms in aging. It is interesting to note that previous research revealed a smaller phase angle difference between the temperature minimum and wake in young evening types vs. morning types; evening types slept on an earlier part of their circadian cycle (4, 13). It has been hypothesized that evening types are driven by social pressures (e.g., pressure to get up in the morning and go to work) to adopt an earlier sleep-wake schedule than their circadian clock would produce naturally (4). It is not clear why older adults (who tend to be more advanced) might also go to bed earlier relative to their circadian cycle (the phase angle of older subjects is similar to that of young evening types). It does not seem that social pressures would be influencing this phase relationship in older adults, as it may be in young evening types. More research is necessary to determine under what circumstances older people might go to bed earlier relative to their internal clocks. One possible explanation is that, in some cases, there may be a greater homeostatic drive by the end of the day that is causing older adults to go to bed earlier than the circadian clock may dictate.

The melatonin amplitude was smaller for older subjects than for young subjects. This difference is consistent with previous reports that have shown a reduction in melatonin amplitude with increasing age (33, 36), but not with another study (44). Of note is that the studies that found the age difference between young and older in the amplitude of the melatonin rhythm included men and women in both age groups, whereas the study that did not find this difference compared the melatonin amplitude of older men and women with those of young men only. The present study did not find any sex differences in the amplitude of the melatonin rhythm (the relatively small number of subjects may make detection of sex differences difficult), but future studies should address whether sex differences could account for these differential results. There was an interaction between day of the study and age, with the melatonin amplitude becoming larger over the 3 days of the study for young subjects but not for older subjects. It is possible that constant dim light exposure over successive days increased the melatonin amplitude for the young subjects. However, it is unclear why this relationship would exist for young subjects, but not for older subjects.

In conclusion, this study found that nocturnal exercise is capable of delaying the circadian melatonin rhythms of older adults, suggesting that the phase-shifting effects of exercise are preserved in healthy older adults. It is possible that exercise may eventually prove useful for delaying the circadian rhythms of older adults who have advanced sleep-wake cycles, resulting in a sleep-wake schedule that is more in synchrony with the external physical and social environment.

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REFERENCES

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