Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase

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Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase. Am J Physiol Regul Integr Comp Physiol 284: R714–R724, 2003; 10.1152/ajpregu.00355.2002.—To examine the immediate phase-shifting effects of high-intensity exercise of a practical duration (1 h) on human circadian phase, five groups of healthy men 20–30 yr of age participated in studies involving no exercise or exposure to morning, afternoon, evening, or nocturnal exercise. Except during scheduled sleep/dark and exercise periods, subjects remained under modified constant routine conditions allowing a sleep period and including constant posture, knowledge of clock time, and exposure to dim light intensities averaging (±SD) 42 ± 19 lx. The nocturnal onset of plasma melatonin secretion was used as a marker of circadian phase. A phase response curve was used to summarize the phase-shifting effects of exercise as a function of the timing of exercise. A significant effect of time of day on circadian phase shifts was observed (P < 0.004). Over the interval from the melatonin onset before exercise to the first onset after exercise, circadian phase was significantly advanced in the evening exercise group by 30 ± 15 min (SE) compared with the phase delays observed in the no-exercise group (−25 ± 14 min, P < 0.05). Phase shifts in response to evening exercise exposure were attenuated on the second day after exercise exposure and no longer significantly different from phase shifts observed in the absence of exercise. Unanticipated transient elevations of melatonin levels were observed in response to nocturnal exercise and in some evening exercise subjects. Taken together with the results from previous studies in humans and diurnal rodents, the current results suggest that 1) a longer duration of exercise exposure and/or repeated daily exposure to exercise may be necessary for reliable phase-shifting of the human circadian system and that 2) early evening exercise of high intensity may induce phase advances relevant for nonphotic entrainment of the human circadian system.

DURING THE PAST decade, studies in several rodent species have shown that nonphotic stimuli are capable of altering mammalian circadian rhythms. Perturbation analyses of the circadian locomotor rhythms of nocturnal rodents revealed that exposure to single nonphotic stimuli, such as pulses of induced activity during constant darkness (25, 26, 28, 31) or pulses of darkness during constant light, which also induce increased activity (7, 16), results in phase shifts that are dependent on the timing of stimulus presentation. Detailed studies of the nonphotic component of the rodent circadian system have led to an understanding of the types of stimuli that lead to phase shifts and/or alterations of photic entrainment, as well as model systems for examining the neural pathways and genes involved in these processes.

In contrast, the nonphotic component of the human circadian system has received comparatively little attention. Early attempts to determine the effects of various nonphotic stimuli used repeated exposure to social cues, such as regular gong sounds (4) or regularly scheduled performance tests, meals, and bedtimes (3). The results of these early studies were confounded by the limitations of the circadian phase measures used and the conditions under which circadian phase was estimated. More recent studies involving exposure to a single stimulus over a background of constant conditions demonstrated that exposure of humans to nocturnal exercise results in significant phase delays of circadian hormonal rhythms (8, 24, 30). Consistent with the phase-delaying effects of exposure to single bouts of nocturnal exercise, a field study examining the entrainment of night workers to a daytime sleep schedule found that subjects exposed to nocturnal exercise experienced phase delays in the rhythm of body temperature greater than those observed in a control group not exposed to exercise, although statistical significance was only achieved if the morningness–eveningness of the control group was taken into account (15). In a study of 15 blind individuals without ocular photoreception who did not suppress melatonin in response to bright light (i.e., have no photic input to the circadian system), nine subjects exhibited a 24.0-h period of their circadian rhythms of melatonin and jet lag; shift work

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body temperature in their normal environment. These findings are consistent with the interpretation that their entrainment to a 24-h schedule occurred via nonphotic means as the human free-running circadian period is generally not exactly 24.0 h. One of these blind subjects with a 24.1-h endogenous circadian was further studied in a 23.8-h nonphotic schedule that included a 10-min daily ride on a stationary bicycle that occurred ~6 h after awakening (18). The melatonin and body temperature rhythms entrained to this nonphotic schedule. Taken together, these findings support the hypothesis that appropriately timed nonphotic stimuli such as exercise or other forms of arousal could facilitate adaptation to acute changes in the light-dark cycle.

Evidence suggesting that appropriately timed exposure to exercise could also result in circadian phase advances has recently been obtained in a study that demonstrated partial entrainment to a 23.5-h light-dark and sleep-wake schedule in healthy volunteers exercising at a moderate intensity twice daily (at midday and late afternoon) over 2 wk (24). Although these exercising subjects did not exhibit the average 30-min/day phase advance that would be necessary for complete entrainment to the 23.5-h day, they did advance on average 10 min/day more than control subjects who did not exercise. These observations raise the possibility that phase advances leading to complete nonphotic entrainment of the human circadian system, or reentrainment to a shifted schedule by daily phase advances, could be observed if the timing and intensity of the exercise stimulus were optimized.

In the first attempt to rigorously examine the effects of a single exposure to exercise on human circadian rhythmicity (30), the selection of exercise duration and intensity (i.e., 3 h at moderate intensity) was based on extrapolations from rodent studies. Although this protocol represented a compromise between duration and intensity consistent with observations in animal studies, a 3-h exercise session is obviously impractical in most circumstances where phase-shifting human rhythms under real-life conditions would be desirable. In a subsequent study (8), high-intensity nocturnal exercise of only 1-h duration appeared to be as effective as low-intensity exercise of 3-h duration in causing delays of two independent markers of circadian phase.

The present study was designed to systematically examine the immediate phase-shifting effects of exercise of a practical duration (1 h) presented across the range of circadian times not explored in the previous studies—morning, afternoon, and evening—using the modified constant routine conditions as in our previous study and using the timing of the onset of nocturnal melatonin (22, 27, 29) as the marker of human circadian phase.

METHODS

Subjects. A total of 38 healthy men aged 20–30 yr (mean age ± SD = 24.0 ± 3.3 yr) with normal body weight (mean body mass index ± SD = 24.2 ± 2.5 kg/m²) and regular sleep/wake habits was studied in the Clinical Research Center (CRC) of the University of Chicago. By self-report, subjects had a regular sleep/wake schedule of 0000–0800 (±30 min). They were instructed to maintain that schedule for the week before the study. Compliance was verified by analysis of continuous wrist activity recordings (Activitv activity monitors with data analyzed using Rhythmwatch software; Minimitter, Sunriver, OR; or Gahwiler Electronics, Hombrektikon, Switzerland). Subjects with a personal history of psychiatric illness, endocrine illness, sleep disorders, smoking, drug use, night work, or transmeridian travel in the previous 6 wk were excluded. All subjects engaged in cardiovascular conditioning exercise of at least moderate intensity during the daytime (between 8 AM and 8 PM) on a regular basis (3–5 times/wk). Some subjects also engaged in moderate strength training. None were competitive athletes, and there was no association of fitness level or habitual timing of exercise with the phase shifts elicited in response to experimental exercise exposure. All subjects provided informed written consent and were compensated $600 for their participation. The University of Chicago Institutional Review Board approved all procedures. The work fully conforms to the “Guiding Principles for Research Involving Animals and Human Beings” (1).

Before the beginning of the study, exercise capacity was determined during a physician-supervised outpatient visit to the University of Chicago Cardiac Exercise Physiology Laboratory using the same exercise equipment (Climbmax stair-climber, Tectrix, Irvine, CA) as in the study. All tests were performed in the morning. To evaluate peak oxygen uptake (VO₂ max), each subject performed a symptom-limited maximal upright ergometer exercise test after 3 min of rest in a sitting position using a 25-W/min ramp protocol. All subjects had normal cardiac function and a mean VO₂ max ± SD of 2.997 ± 0.367 ml/min.

Experimental protocol. Thirty subjects participated in 3-day studies involving the presentation of a 1-h, high-intensity exercise session during the daytime or a no-exercise study involving continuous bed rest under modified constant routine conditions allowing a sleep period but including constant posture, knowledge of clock time, and exposure to dim light (19). These data were combined with results previously obtained from eight subjects in 2-day studies involving nocturnal exposure to the same high-intensity, 1-h exercise session on the same exercise equipment (8). The clock time of exercise for the daytime exercise studies was randomized, but the timing of exercise presentation relative to the timing of the circadian onset of melatonin secretion was calculated a posteriori from the plasma melatonin profiles (as described in results) for assignment to a treatment group based on the circadian time of exercise.

Subjects were admitted to the CRC by 1800 on day 0 for habituation purposes. Bedtime hours were 0000–0800, consistent with the habitual schedule of the subjects for the week before admission. Prestudy light-dark conditions and entrained phase were thus stabilized for the week before the study while the subjects were at home, and for the laboratory night preceding the initiation of serial blood sampling. Exposure to ordinary (indirect) fluorescent light averaging 40 ± 19 lx (SD) in intensity was maintained throughout all waking periods for the remainder of the study after admission, including during exercise. The room lighting was indirect using shielded, downward-directed fixtures located above head level on the wall behind the volunteers. Thus, the light was not in the line of gaze even when subjects faced straight upward. These fixtures employed standard indoor fluorescent bulbs (Sylvania F40/WW, 2 × 40 W, General Electric). Lighting levels were measured hourly with a digital light meter
(model YF-170, Yu Fong Electronics) at the subject’s angle of gaze that was typically horizontal, although slight upward or downward angles of gaze occurred depending on the subject’s activity. A television (≥5 ft away) did not provide additional illumination at levels detectable with our instrumentation.

On day 1, subjects had breakfast between 0830 and 0900. An intravenous glucose infusion at a constant rate of 5 g·kg\(^{-1}·24·h\)^{-1} was begun at 1000 via a catheter inserted into the antecubital vein of the dominant arm. This glucose infusion constituted the only source of caloric intake until the end of the study. The subjects had access to water and sugar-free, caffeine-free sodas. A second catheter was then inserted in the nondominant arm for 2-ml blood samples to be drawn at 20- to 30-min intervals. For the 30 subjects who participated in the present study, the duration of sampling was 64 h beginning at 1600 on day 1. For the eight subjects who participated in our previous nocturnal exercise study (8), sampling started at 1600 on day 1 and continued for 36 h.

From 1000 on day 1 until the end of the study, the subjects remained recumbent in bed with the head of the bed at a 45° angle and continuously awake in constant dim light except during the scheduled dark sleep and exercise periods. They were allowed to read, work on a computer, watch television, use the telephone, have visitors, and play board games. Estimations of baseline (i.e., prestimulus) circadian phase were derived from measurements of plasma melatonin during the evening and first part of the night of day 1 obtained under these modified constant routine conditions (i.e., constant dim light exposure and recumbence, constant caloric infusion, and constant wakefulness). In the 3-day studies, bedtime hours in total darkness on day 2 were 0000–0800 for the morning, afternoon, and evening exercise groups and 0200–0800 for the no-exercise group. In the 2-day studies with nocturnal exercise exposure, bedtime hours were 0400–0800. At the end of the study, the subjects were allowed to sleep as long as they wished and were released later in the morning after they had eaten a meal, their glucose infusion had been discontinued, and their glucose levels had stabilized.

Procedures for exposure to exercise. Subjects exercised for 1 h on a stairclimber on day 2 of the study. All exercise sessions took place in the same room and under the same lighting conditions as the constant routine part of the studies. Lighting conditions during exercise were controlled by maintaining gaze away from the indirect light source. There were no differences in light exposure measured at the angle of gaze while the subjects were on the exerciser compared with sitting in bed. Stairclimber exercise involved a 10-min warm-up at 25% of \(\dot{V}O_2\) max, 40 min of constant exercise at 75% of \(\dot{V}O_2\) max, and then a 10-min cool-down at 25% of \(\dot{V}O_2\) max. Exercise sessions occurred under close supervision and with continued venous sampling.

Assays. Plasma melatonin levels were measured with a double-antibody RIA using commercially available reagents (Stockgrand, Guilford, Surrey, UK) as previously described (29). The lower limit of sensitivity of the assay was 2.5 pg/ml. The intra-assay coefficient of variation averaged 17.5% for values <10 pg/ml, 8.6% in the range of 10–30 pg/ml, and 5.2% for values >30 pg/ml. The interassay coefficient of variation averaged 20% for values <10 pg/ml and 13.5% for values >10 pg/ml. Samples from the same subject were measured in the same assay.

Estimations of circadian phase. The timings of the onset of the nocturnal elevation of plasma melatonin (Fig. 1) were used to estimate circadian phase before and after exposure to the nonphotic stimulus. Phase shifts were defined as the difference between the prestimulus phase estimations on day 1 and the poststimulus phase estimations on days 2 and 3. By convention, phase advances are expressed as positive numbers and phase delays are expressed as negative numbers.

In the majority of individual studies, daytime melatonin levels were below the limit of sensitivity of the assay (i.e., 2.5 pg/ml). Therefore, the onset of nocturnal melatonin secretion was estimated as the time (defined by interpolation between blood sampling times) when the plasma level of melatonin reached 10.0 pg/ml that was not followed by a return to concentrations <10.0 pg/ml. One subject in the evening exercise group had low melatonin levels in the range of 5–20 pg/ml without consistent circadian variation. Because meaningful secretion onsets and phase shifts could not be calculated in this individual, data from this subject were excluded from the analysis. One subject in the evening exercise group exhibited large acute effects of exercise on melatonin levels (see Results). A higher threshold for the timing of the melatonin onset, i.e., 20 pg/ml, was used to estimate phase shifts in this subject. In one no-exercise subject, daytime melatonin
levels were consistently in the range of 5–12 pg/ml rather than near the limit of detection of the assay. A “basal” level was therefore calculated from the steadily low concentrations measured during the 1800–2200 time interval, and the onset of nocturnal melatonin secretion was then estimated as the timing of the first plasma level exceeding that mean basal level ±1 SD not followed by a return to concentrations below this value. One other no-exercise subject discontinued participation on the final evening of the study for nonmedical reasons and thus circadian phase assessments could be ascertained only for days 1 and 2.

In summary, nine no-exercise subjects were available for phase shift comparisons from day 1 to day 2, and for eight of these no-exercise subjects, phase shifts from day 1 to day 3 could be estimated. Comparisons of the results from the no-exercise group were made vs. those from morning (n = 7), afternoon (n = 7), evening (n = 7), or nocturnal (n = 8) exercise groups.

**Estimations of acute effects of exercise on melatonin levels.** The acute effects of exercise on melatonin levels (delta) were calculated as the difference between the mean level in the hour before exercise and the value at the end of exercise. To dissociate the effects of “treatment” (i.e., exercise or no-exercise) and time of day, changes in the levels of melatonin in the no-exercise group were similarly estimated using the melatonin values from no-exercise subjects at the same circadian time.

**Statistical tests.** Comparisons of phase shifts from day 1 to day 2 between the no-exercise group and the groups with morning, afternoon, evening, and nocturnal exercise timing were performed by factorial ANOVA. When the overall P level was significant at a level of 0.05, phase shifts observed in each of the exercise groups were compared with phase shifts observed in all other groups using the Tukey-Kramer procedure. These calculations were repeated for phase shifts from day 2 to day 3 and for phase shifts from day 1 to day 3.

The impact of time of day on the acute effects of exercise on melatonin was compared between nocturnal, morning, afternoon, and evening exercise groups using a two-way ANOVA with treatment (exercise or no-exercise) and time of day (nocturnal, morning, afternoon, and evening) as factors. If the interaction of treatment and time of day was significant, groups were stratified by time of day and comparisons of exercise and no-exercise groups were then performed for each time of day using a separate variances-independent t-test (2-tail). Unless otherwise indicated, all group data are expressed as means ± SE. Statistical analyses employed Prism (version 3.0, Graphpad Software, San Diego, CA) and SPSS (version 10.0, SPSS, Chicago, IL).

**RESULTS**

**Estimation of timing of exercise relative to melatonin onset.** Subjects exposed to exercise were grouped a posteriori according to the start of the circadian time of exercise, i.e., the time of exercise relative to the melatonin onset on day 1, resulting in four 6-h bins of circadian timing of exercise starting at 0, 6, 12, and 18 h after the melatonin onset. The mean clock time of the melatonin onsets before stimulus exposure and the timing of the exercise sessions relative to these onsets are listed in Table 1. Group differences in the timing of the prestimulus melatonin onsets were not significant.

**Acute effects of exercise on melatonin levels.** The acute effects of exercise on melatonin levels (delta) varied depending on time of day. The interaction of treatment × time of day was significant (P < 0.001; Fig. 2).

Nocturnal exercise was associated with a robust stimulation of melatonin secretion (Figs. 1 and 2). Melatonin levels were elevated starting 30 min after the beginning of exercise and remained elevated for at least 80 min in all cases. The mean increases in melatonin levels (delta ± SD) were 20.3 ± 12.1 vs. 5.7 ± 10.6 pg/ml (P < 0.02) at the same circadian time in the no-exercise group. Peak levels were attained 70 min after initiation of exercise in four subjects, after 50 min in three subjects, and after 30 min in one subject. The magnitude of these elevations is much larger than those expected from postural changes alone (12). Similar findings are obtained when the melatonin levels are referenced to clock time rather than circadian time.

In response to morning exercise, the mean decrease in melatonin levels (delta ± SD) was −21.8 ± 15.3 vs. −5.2 ± 7.4 pg/ml (P < 0.02) at the same circadian time in the no-exercise group. This difference likely reflects the fact that three subjects in the morning exercise group had high melatonin levels that began to decline before exercise and continued to fall during the course of morning exercise (see Fig. 1; note differences in ordinate scale).

### Table 1. Timing of exercise and melatonin secretion onsets

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Melatonin Onset</th>
<th>Exercise Start</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Clock time, h)</td>
<td>(Clock time, h)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>After Onset</td>
</tr>
<tr>
<td></td>
<td>(Day 1)</td>
<td>(h ± min)</td>
</tr>
<tr>
<td></td>
<td>(h ± min)</td>
<td></td>
</tr>
<tr>
<td>Nocturnal (0033 ± 01)</td>
<td>8</td>
<td>2236 ± 21</td>
</tr>
<tr>
<td>Morning (0936 ± 33)</td>
<td>7</td>
<td>2318 ± 41</td>
</tr>
<tr>
<td>Afternoon (1256 ± 53)</td>
<td>7</td>
<td>2231 ± 28</td>
</tr>
<tr>
<td>Evening (1824 ± 24)</td>
<td>7</td>
<td>2243 ± 15</td>
</tr>
<tr>
<td>No exercise</td>
<td>9</td>
<td>2339 ± 35</td>
</tr>
</tbody>
</table>

Values are means ± SE. n.a., Not applicable.
In response to afternoon exercise, the mean change in melatonin levels (delta ± SD) measured at the end of the exercise period relative to the level immediately preceding exercise was 1.0 ± 2.9 vs. 0.6 ± 1.5 pg/ml [not significant (ns)] at the same circadian time in the no-exercise group.

The acute responses to evening exercise were more variable. In one subject (Fig. 3A), melatonin levels during exercise exceeded the melatonin onset threshold level for several hours. In two subjects (e.g., Fig. 3B), melatonin levels increased briefly at the end of exercise. Melatonin concentrations in another subject (Fig. 3C) increased briefly and slightly during exercise before returning to undetectable levels. The remaining four subjects in the evening exercise group had negligible or no acute increases of melatonin levels during exercise. On average, evening exercise resulted in only a small increase in melatonin levels. Relative to the level immediately preceding exercise, the mean change in melatonin levels (delta ± SD) measured at the end of evening exercise was 8.7 ± 10.2 vs. 0.0 ± 1.2 pg/ml (ns; \( P < 0.07 \)) at the same circadian time in the no-exercise group.

Phase shifts of melatonin onsets. Mean phase shifts of the melatonin onset for all study groups are depicted in Fig. 4. Over the interval day 1-day 2, the differences in phase shifts across subject groups were significant (\( P < 0.004 \); Table 2). The phase advance of melatonin in the evening group (30 ± 15 min) was significantly different from the phase delays of the no-exercise group. Subjects exercising in the morning (A; ●) and afternoon (B; ●) exhibited phase delays of melatonin not different from no-exercise subjects and the SEs were thus omitted for clarity. Subjects exercising in the evening (C; ●) exhibited significant phase advances of melatonin onsets over the interval days 1-2. Nocturnal exercise subjects (D; *) exhibited relatively larger phase delays than no-exercise subjects, but these differences did not reach statistical significance.
the overall differences in phase shifts across subject groups were significant (\(P < 0.05\); Table 2). Phase shifts of the evening exercise group (−66 ± 9 min) in the delaying direction were significantly larger than the phase delays in the no-exercise group (\(P < 0.05\); Table 2). No other pair-wise contrast was significant.

Over the interval day 1–day 3, differences in phase shifts between the exercise and no-exercise groups were not significant (\(P < 0.05\)).

**Regression analysis of the exercise-induced phase shifts** indicated that the phase shifts (\(\gamma\)) varied significantly according to the circadian timing of the exercise session (\(x\)), from phase delays at the beginning of the night to phase advances at the end of the subjective day. Curve-fitting procedures exploring linear and non-linear models determined that a line (\(\gamma = 3.12x − 57.3\) min) was the simplest model that best fit the data and that the slope of the line is significantly different from 0 (\(R^2 = 0.21, P < 0.02\)).

**DISCUSSION**

Two previous studies from our laboratory have demonstrated that exposure to nocturnal exercise in the evening (8) and daytime exercise (9) altered the phase of melatonin secretion. The magnitude of the phase shift of the melatonin onset over the interval day 1–day 2 vs. the timing of the start of the exercise relative to the preexercise melatonin onset (Fig. 5).

**Table 2. Phase shifts of melatonin secretion onsets**

<table>
<thead>
<tr>
<th>Treatment Group (Clock time exercise began, h ± min)</th>
<th>n</th>
<th>Phase Shift Days 1–2, min</th>
<th>Phase Shift Days 2–3, min</th>
<th>Phase Shift Days 1–3, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nocturnal (0033 ± 01)</td>
<td>8</td>
<td>−49 ± 13</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Morning (0936 ± 33)</td>
<td>7</td>
<td>−23 ± 14</td>
<td>−37 ± 17</td>
<td>−61 ± 12</td>
</tr>
<tr>
<td>Afternoon (1256 ± 53)</td>
<td>7</td>
<td>−43 ± 12</td>
<td>−22 ± 15</td>
<td>−65 ± 22</td>
</tr>
<tr>
<td>Evening (1824 ± 24)</td>
<td>7</td>
<td>30 ± 15</td>
<td>−66 ± 9</td>
<td>−38 ± 8</td>
</tr>
<tr>
<td>No exercise</td>
<td>8</td>
<td>−25 ± 14</td>
<td>−19 ± 6</td>
<td>−43 ± 21</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Regression analysis of the exercise-induced phase shifts indicated that the phase shifts (\(\gamma\)) varied significantly according to the circadian timing of the exercise session (\(x\)), from phase delays at the beginning of the night to phase advances at the end of the subjective day. Curve-fitting procedures exploring linear and non-linear models determined that a line (\(\gamma = 3.12x − 57.3\) min) was the simplest model that best fit the data and that the slope of the line is significantly different from 0 (\(R^2 = 0.21, P < 0.02\)).
healthy young men maintained under modified constant routine conditions results in a phase delay of the onset of nocturnal melatonin secretion on the following day (8, 30). These phase delays appeared to be more consistent when the subjects were submitted to a 3-h session of moderate-intensity exercise than when they performed a 1-h session of high-intensity exercise (8). Similar phase delays of the melatonin onset following exposure to a single 2-h exercise session initiated at midnight were recently reported by other investigators (24). The present study provides the first evidence for phase-advancing effects of acute exposure to evening exercise on the human circadian system. Early evening 1-h, high-intensity exercise sessions (at ~1830) resulted on the following day in phase advances significantly different from the phase delays observed in response to morning, afternoon, and nocturnal exercise and in no-exercise subjects who did not exercise.

Figure 6 combines the results of the present study and the results from our two previous studies of 3-h, low-intensity exercise stimulus (8) in a human PRC to exercise. Data from a total of 86 constant routine studies (of at least 36 h in duration) obtained in subjects who exercised in the laboratory at various times of day or who remained continuously at bed rest are summarized. Bed rest under these conditions resulted in a significant delay of circadian phase (mean phase shift of $-18 \pm 6$ min, $n = 33$, $P < 0.005$, $t$-test against phase shift $= 0$), despite the fact that these no-exercise subjects were not in temporal isolation and were thus fully aware of time of day. Despite the wide variability of responses to continuous bed rest or exercise at all

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**Fig. 6.** PRC observed in response to exercise at various times of day. Phase shifts in response to high-intensity, 1-h nocturnal exercise (8) and daytime exercise are depicted by $\bullet$. Phase shifts in response to low-intensity, 3-h exercise sessions from 2 previous studies are depicted by $\triangle$ (8) and $\bigtriangleup$ (30) at top and by $\bullet$ at bottom. Line depicts significant relationship between phase shifts and circadian time of exercise ($y = 3.87x - 70.7; R^2 = 0.28$, $P = 0.0003$; slope significantly different from 0). Dashed curves depict 95% confidence intervals of the slope of the line.
circadian phases, a clear trend for phase-dependent effects of exercise emerged. Exercise in the morning or afternoon was not associated with significant phase shifts compared with no-exercise conditions. The timing of the dim light melatonin onset (which occurred under baseline conditions at 2231 ± 8 min in the 86 studies shown in Fig. 6) appears to represent a sharp break point between the phase advance and phase delay regions of the human PRC to exercise.

Remarkably, this break point in the exercise PRC coincides with the timing of the opening of the “sleep gate” (20) that has been hypothesized to correspond to an abrupt shift of circadian function from generating a waking signal opposing the build up of homeostatic sleep pressure to generating a sleep signal facilitating the consolidation of nocturnal sleep (14). Vigorous exercise may thus advance circadian sleep propensity when performed in the early evening, i.e., during the few hours before the melatonin onset, but instead delay circadian sleep propensity when performed late at night. In contrast, exercise in the morning and afternoon, i.e., at a time when higher levels of physical activity often occur in real life, appears to have no consistent effect on circadian phase. We can only speculate as to the adaptive significance of a cross-over point at this particular phase. Phase advances in response to high levels of activity in the early evening could tend to encourage subsequent sleep and the associated physiological recovery processes at an earlier time. In contrast, a high intensity of activity in the hours after the onset of melatonin at a time opposing a high sleep pressure suggests that the activity is forced by external circumstances rather than spontaneous. The resulting delay of circadian phase may be associated with a delay of the timing for sleep so as to accommodate this challenge and prepare for the occurrence of this obligatory activity at the same time on subsequent nights.

An interaction of exercise with the homeostatic regulation of sleep has been suggested by a recent study in hamsters that indicated that the phase-shifting effects of a nonphotic stimulus may be due solely to the maintenance of wakefulness during the normal sleep period (2). Maintenance of wakefulness by gentle handling in the absence of substantial activity elicited phase shifts comparable to sustained wheel-running. Because a smaller number of required rousing interventions predicted greater phase shifting, the authors speculated that the “arousing” nature of the stimulus may be the most salient component of phase shifts elicited by increased physical activity during the habitual rest period (2). Careful measures of the wake and sleep EEG of humans exposed to putative nonphotic zeitgebers will be needed to determine the relevance of these findings to human circadian regulation.

The melatonin onsets in subjects exposed to 1-h, high-intensity nocturnal exercise were, on average, phase delayed by 24 min more than in no-exercise subjects. This delay failed to reach significance, however, in contrast to the significant phase-delaying effects of 3-h, moderate-intensity exercise at the same time of day in previous studies (8, 30). This discrepancy reflected the fact that the phase delays elicited by 3-h, moderate-intensity exercise pulses were more consistent across individuals, because, on average, the phase delay was of similar magnitude for both types of exercise sessions. When the phase shifts from all studies with nocturnal exercise exposure (mean phase shift of −62 ± 7 min, n = 22) are compared with the data from all no-exercise studies with continuous bed rest (mean phase shift of −18 ± 6 min, n = 33), the exercise-induced phase delay is significantly larger than no exercise (P < 0.0001, unpaired 2-tailed t-test).

We previously characterized the small daily phase delays (20 min on average) that we observe in circadian phase-shifting studies in the absence of treatment as a “drift” in circadian phase. This small delay is attributable to a free run at a normal circadian period slightly greater than 24 h that can occur under these dim light conditions, or a possible small phase-delaying effect of the dim light itself. The small differences in the lighting conditions between groups in the present study (dim light from 0200 to 0400 on day 1 for the nocturnal exercise but dark/sleep for the no-exercise group at that time) are unlikely to represent a significant confound. If dim light had been maintained until 0400 in the no-exercise group, we may have observed slightly greater phase delays in this group. The original study did, however, demonstrate these phase delays against a true “control” for the nocturnal exercise group (8). There were also small differences in lighting conditions between the no-exercise group and the other daytime exercise conditions (dim light from 0200 to 0400 on day 1 for the no-exercise group but dark/sleep for the daytime exercise groups at that time). However, because the additional light for the no-exercise group would result in a putatively greater phase delay, this would only increase the differences between the no-exercise and the evening exercise groups’ phase advances and yield a similar qualitative interpretation.

The advance shifts of melatonin onsets observed in the present study in response to evening exercise were attenuated by another day in constant conditions of dim light and bed rest and were not significantly different from the phase shifts observed in no-exercise subjects over the interval day 1–day 3. The delaying effects of the continued dim light conditions may have attenuated exercise-induced phase advances to a greater extent than the small phase delay drift in the no-exercise condition over the same interval. Alternatively, the relatively brief, single exercise stimulus may not have been sufficient to induce detectable steady-state phase shifts of the human circadian system. An exercise-induced masking of “true” circadian phase estimated from melatonin profiles has never been described but cannot be excluded.

Repeated, appropriately timed exposure to exercise may therefore be needed to obtain reliable stable shifts of circadian phase. Two studies involving repeated exposure to exercise have provided support for this
hypothesis, and in both studies, the direction of the phase shifts was consistent with the PRC illustrated in Fig. 6. In 1995, a field study showed that adaptation to night work may be facilitated by exposure to nocturnal exercise, which resulted in larger phase delays in the rhythm of body temperature than those observed in a control sedentary group (15). In a more recent study, entrainment to a 23.5-h light-dark and sleep-wake cycle was examined in healthy volunteers who either exercised at a moderate intensity twice daily (at midday and late afternoon) over 2 wk or maintained sedentary habits. Entrainment or synchronization to a schedule with period <24 h is highly unlikely to occur in the absence of a stimulus that provides a daily phase advance to the circadian pacemaker. Although exercising subjects did not exhibit the approximate 30-min/day phase advance necessary for complete entrainment to the 23.5-h day, they did advance on average 10 min/day more than the nonexercising control group by the end of the study (24). These findings are consistent with phase-advancing effects of late afternoon or early evening exercise on the human circadian clock. Taken together, the findings from these studies of circadian entrainment raise the possibility that repeated exposure to appropriately timed exercise sessions may facilitate the adaptation of human circadian rhythmicity to both phase delay and phase advance shifts, as may occur in jet lag and shift work. This hypothesis is further supported by data from a recent study of nonphotic entrainment in the diurnal European ground squirrel (Citellus spermophilus). Repeated exposure to a 3-h confinement in a running wheel every 23.5 h, to which the squirrels respond by substantially increasing locomotor activity, resulted in unambiguous entrainment in a majority of squirrels (17). The net daily phase advance required to elicit entrainment in this paradigm was consistently observed when the induced activity occurred in the late subjective day, i.e., the same circadian time at which evening exercise in the present study resulted in immediate phase advances. Given the >24-h endogenous period of humans (11) and the net daily phase advance required for stable entrainment, evening exercise, particularly repeated daily exposure, may be a promising avenue by which to facilitate entrainment in humans.

Acute increases in melatonin levels in some subjects in response to early evening exercise, i.e., when concentrations usually remain at low daytime levels, were an additional novel finding of the present study. This observation contradicts the findings of a 1990 study by Monteleone et al. (21) in which seven young men exercised on a stationary bicycle for 20 min starting at 2200 or remained sedentary over the same time period (baseline). In that study, melatonin levels 90 min after the initiation of exercise were found to be ~50% lower than under baseline conditions. In our previous studies (8, 30), nocturnal exercise of moderate intensity and 3-h duration resulted in phase delays in the absence of acute changes in melatonin levels, suggesting that increased melatonin levels are not required for nocturnal exercise to induce phase shifts. Nevertheless, in the present study, the slight increases in melatonin levels near the end of exercise and the phase advance of melatonin 2–5 h later could be related since repeated, low-dose exogenous melatonin administration in the evening for 4 days in subjects maintained on a consistent light-dark cycle is associated with phase advances of the melatonin onset (21). It cannot be excluded that the phase advances of melatonin onset in response to evening exercise detected in the present study reflect the phase-shifting effects of small melatonin elevations.

The mechanism by which a robust stimulation of melatonin levels occurs in response to high-intensity exercise at night but not in response to high-intensity exercise at other times of day is not known and cannot be inferred from data we collected in this experiment. However, because low-intensity, 3-h nocturnal exercise sessions elicit reliable phase shifts without any effect on melatonin levels, it seems likely that the acute effect of high-intensity nocturnal exercise occurs downstream of the suprachiasmatic nucleus. We previously reported body temperature and cortisol increases in response to nocturnal exercise of high and moderate intensities and speculated that thermoregulatory mechanisms may play a role in exercise-induced increases in melatonin levels since melatonin has been shown to have hypothermic effects at this time of day (9, 10). That there are minimal effects of exercise on melatonin levels at other times of day suggests that the system may be deactivated and thus unresponsive to hyperthermic challenge during the daytime. Although high-intensity nocturnal exercise elicited sharp increases in body temperature and cortisol, these changes were not correlated with increases in melatonin levels (9), suggesting that increased sympathetic activation of the pineal gland is not an obvious candidate mechanism.

Several studies have examined the role of increased physical activity across the waking period on circadian rhythms. Studies performed under “forced desynchrony” conditions of light-dark and sleep-wake cycles of 20-h period designed to dissociate the periodicities in the environment and the internal circadian pacemaker provided no evidence for a modulation of circadian period by short (<1 h) but substantial increases in physical activity repeated at regular intervals throughout the waking periods compared with a sedentary condition (6). However, changes in circadian period as a result of increased activity were not likely to be identified over a study duration of only 5 days and the role of the circadian timing of exercise could not be examined. In a recent, simulated night work study, intermittent exercise sessions at an intensity of 50–60% of maximum heart rate and for 40 min in duration were presented over the waking periods during the course of adaptation to night work and daytime sleep schedule. Exercise for 3 consecutive days did not induce phase shifts different from the control condition (5). Thus, brief repeated bouts of exercise occurring at
many circadian times across the waking period do not appear sufficient to alter the period or phase shift human circadian rhythms. Further studies are needed to demonstrate alterations of circadian period or entrainment pattern by exercise exposure at specific circadian times.

The present study demonstrates that appropriately timed exercise can phase advance or phase delay the human circadian system, adding a non-pharmacological stimulus to manipulate circadian time in situations where this may be beneficial, such as shift work and jet lag. Importantly, the immediate phase shifts induced by single sessions of exercise are of the same order of magnitude as those observed following a single exposure to a pulse of bright light and are considerably larger than those achieved after 4 days of melatonin administration. One of the goals of this study was to develop a human PRC to acute exercise providing the basis for future studies of nonphotic entrainment and modification of endogenous circadian period by nonphotic stimuli. Our findings are limited to healthy young men and research efforts should be expanded to include groups of men and women of different ages. The relevance of nonphotic stimuli for circadian function should be explored when stimulus exposure occurs on a daily basis and in the presence of a light-dark cycle. The present findings suggest that such studies are warranted.

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REFERENCES

