Morning and evening physical exercise differentially regulate the autonomic nervous system during nocturnal sleep in humans

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Yamanaka Y, Hashimoto S, Takasu NN, Tanahashi Y, Nishide S, Honma S, Honma K. Morning and evening physical exercise differentially regulate the autonomic nervous system during nocturnal sleep in humans. Am J Physiol Regul Integr Comp Physiol 309: R1112–R1121, 2015. First published September 2, 2015; doi:10.1152/ajpregu.00127.2015.—Effects of daily physical exercise in the morning or in the evening were examined on circadian rhythms in plasma melatonin and core body temperature of healthy young males who stayed in an experimental facility for 7 days under dim light conditions (<10 lux). Sleep polysomnogram (PSG) and heart rate variability (HRV) were also measured. Subjects performed 2-h intermittent physical exercise with a bicycle ergometer at ZT3 or at ZT10 for four consecutive days, where zeitgeber time 0 (ZT0) was the time of wake-up. The rising phase of plasma melatonin rhythm was delayed by 1.1 h without exercise. Phase-delay shifts of a similar extent were detected by morning and evening exercise. But the falling phase shifted only after evening exercise by 1.0 h. The sleep PSG did not change after morning exercise, while Stage 1+2 sleep significantly decreased by 13.0% without exercise, and REM sleep decreased by 10.5% after evening exercise. The nocturnal decline of rectal temperature was attenuated by evening exercise, but not by morning exercise. HRV during sleep changed differentially. Very low frequency (VLF) waves increased without exercise. VLF, low frequency (LF), and high frequency (HF) waves increased after morning exercise, whereas HR increased after evening exercise. Morning exercise eventually enhanced the parasympathetic activity, as indicated by HRV, while evening exercise activated the sympathetic activity, as indicated by increase in heart rate in the following nocturnal sleep. These findings indicated differential effects of morning and evening exercise on the circadian melatonin rhythm, HRV, and HRV.

Physical exercise; melatonin; body temperature; autonomic nervous system; sleep EEG; heart rate variability

Timed physical exercise was reported to facilitate phase shifts of the human circadian rhythms to a new time schedule, suggesting a circadian clock resetting capability of physical exercise (5, 22). However, the mechanism of resetting by physical exercise is not well understood. Previously, a single trial of physical exercise phase shifted the human melatonin rhythm in a phase-dependent manner (7), suggesting that physical exercise acts as a time cue for the human circadian pacemaker similar to a bright light pulse. However, an exercise pulse under dim light conditions failed to shift the circadian melatonin rhythm (22). On the other hand, daily physical exercise accelerated the reentrainment of the sleep-wake cycle but not of the circadian melatonin rhythm to 8-h phase-advanced sleep-schedule under dim light conditions (39). These findings indicated differential effects of physical exercise on the circadian pacemaker and sleep-wake cycle in humans. Interestingly, reentrainment of both sleep-wake cycle and circadian melatonin rhythm was accelerated by physical exercise under bright light conditions (38). Physical exercise was interpreted to reset the circadian melatonin rhythm indirectly through stimulation of the sympathetic activity, which enhanced the light inputs to the circadian pacemaker.

Effects of physical exercise on the following nocturnal sleep were controversial in the literature (10, 40). Physical exercise in the evening was reported to deteriorate sleep structure by increasing arousal and inadequate sleep hygiene (4, 15). A more recent study indicated that heart rate increased and relaxation time decreased in subsequent sleep after evening exercise, while there was no change in the sleep polysomnogram (PSG) (23). On the other hand, physical exercise in the daytime was reported to have no effect on the following nocturnal sleep (15) or to increase non-rapid eye movement (NREM) sleep (32). The discrepancy in the effect of physical exercise on sleep was ascribed to a number of factors, including the strength and the time of physical exercise (40). The effects of physical exercise could also depend on whether the subjects are physically trained or not (19). Physical exercise is well known to change the activity of autonomic nervous system, which is involved in a variety of physiology, such as circulation, endocrinology, metabolism, and thermoregulation and are under the control of circadian pacemaker (36). Thus, the effect of physical exercise on the nocturnal sleep could well depend on the time of exercise.

Physical exercise stimulates the autonomic nervous system to various extents (27). Heart rate variability (HRV) has been used to evaluate the effect of physical exercise on the autonomic nervous activity. However, the wide variety of results was reported on the effect of physical exercise on the autonomic nervous system as measured by HRV (29).

In the present study, effects of daily physical exercise in the morning or in the evening were examined, focusing on the circadian rhythms in plasma melatonin and rectal temperature of young male subjects, who stayed in a living facility for 6 nights. The effects were also examined on sleep PSG and HRV. We found that physical exercise had differential effects on these parameters depending on the time of day.
MATERIALS AND METHODS

Subjects and Facility

A total of 22 healthy male subjects (age, 22.0 ± 1.8 years; means ± SD; body mass index, 20.9 ± 2.2 kg/m²) participated in the present study as paid volunteers. They had neither a job in the early morning or late at night nor did they go abroad 4 wk before the experiment. The subjects were not athletes specifically trained for competitive sports, but had a habit of light exercise, such as cycling, swimming, and jogging, once or twice a week. The subjects gave written informed consent, which allowed them to withdraw from the experiment at any time they wanted. This study was approved by the Ethical Committee of Hokkaido University Graduate School of Medicine (no. 04-001).

The experiment was carried out in a living facility. Details of the facility have been reported elsewhere (13). Briefly, the facility consists of four rooms that are isolated from natural day-night alternations. Each room is equipped with a bed, desk, comfortable chair, bicycle ergometer, compact disk player, kitchen, and bathroom. The main illumination is supplied from the ceiling by fluorescent tubes and controlled by the experimenter. Communication with the subject was performed by letter through the anteroom or by intercom. There was no indication of time in the room. In addition, the subjects were not allowed to use timing devices such as a watch, television, radio, and cellular phone in the facility.

The light intensity in the waking period was less than 10 lux measured on the forehead of standing subject throughout the experiment. There were accessory dim lights of less than 10 lux at bedside and bathroom. The main illumination and accessory lights were turned...
off automatically during the sleeping period. The room temperature and humidity were kept in a narrow range (24 ± 2°C and 60 ± 10%).

Experimental Protocols

The subjects were instructed to keep a regular sleep-wake cycle schedule with 8 h sleep (0000 to 0800) for 2 wk before the experiment. Sleeping time was recorded by the subjects themselves and movements by an ambulatory device, Actiwatch (Actiwatch-L, Minimitter, USA). One week before the experiment, the subjects stayed in the facility from 1700 to 1200 on the next day. They were asked to go to bed at 0000 and wake up at 0800. To examine the circadian peak phase of plasma melatonin, a serial blood sampling was performed every 2 h from 1800 to 1000 under dim light conditions (≤10 lux) (Pre-Exp1). To adjust the sleep-wake cycle among the subjects in reference to the circadian cycle, the times of retiring to bed and wake-up were fixed in such a way that the retirement time should be 4 h before the melatonin peak, which was determined in Pre-Exp1 and sleep length should be 8 h. And they were expressed as zeitgeber time (ZT), where the times of wake-up, retirement to bed, and the melatonin peak were defined as ZT0, ZT16, and ZT20, respectively.

The experimental protocols were illustrated in Fig. 1. One day before the experiment (Pre-Exp2), the subjects came to the facility at 1700 for the measurement of sleep PSG. In the next morning, after conducting the symptom-limited exercise test (11), the subjects returned to their home and came back to the facility at 1700 on the same day (day 1). The subjects participated in one of three experiments (groups) as follows.

Subjects were instructed to keep the sleep-wake schedule consisting of an 8-h sleep and a 16-h wake (Fig. 1). A phone call was given to the subjects at 30 min before the scheduled bed time (ZT16) and wake-up call at the scheduled wake-up time (ZT0). Three meals were supplied at fixed times at ZT1, ZT7, and ZT13. The time of the meal was announced by phone. They were allowed to drink water and caffeine-free tea anytime. They took a shower regularly at ZT12. During the waking period, the subjects were requested to take a computer-based performance test and fill out a questionnaire (35) every 2 h from the time of waking up to 2 h before bedtime. Each test was finished in about 10 min. In their free time, they were allowed to read books, listen to music, and watch videos. Smoking, napping, and physical exercise was prohibited.

From day 3 to day 6 of the experiment, the subjects in the exercise group performed physical exercise for 2 h with a bicycle ergometer from ZT3 to ZT5 (morning exercise, n = 7) or from ZT10 to ZT12 (evening exercise, n = 8), respectively. Subjects without physical exercise (control, n = 7) were instructed to rest during the waking period.

Measurements and Data Analysis

Plasma melatonin. Serial blood sampling was performed at 1-h intervals for 24 h through an indwelling catheter with a heparin lock placed in the forearm. The blood sampling was done from 1800 in local time on day 1 to 1800 on day 2 and 1800 on day 6 to 1800 on day 7 (Fig. 1). Thus, the actual sampling time was slightly different from subject to subject since subjects' daily schedule was based on ZT. Immediately after blood sampling, whole blood was centrifuged to separate plasma, which was frozen at −40°C until melatonin assay. Plasma melatonin was determined by a double-antibody radioimmunoassay (14). The sensitivity of the assay was 1.56 pg/ml, and the intra-assay and inter-assay variances were 6.6 and 7.0%, respectively.

The phase of plasma melatonin rhythm was determined by a geometric method, as described previously (35). Briefly, the original melatonin rhythm was smoothed by a three-point moving average method. The difference between the maximum and minimum levels was defined as the amplitude of rhythm. The onset and offset phases of nocturnal melatonin rise were defined as the time at which the horizontal line of 20% amplitude crossed the ascending and descending portions, respectively. The peak melatonin phase was defined as the midpoint between the onset and offset phases. The phase shift of melatonin rhythm was calculated by comparing the differences in the phases (onset, peak, offset) on day 1 and on day 6.

Core body temperature. Rectal temperature was continuously measured by a thermistor probe inserted into the rectum by 15 cm from the anus, except when the subjects were taking a shower or defecating. The temperature data were fed into a computer every 30 s and were averaged at every 10-min interval. The trough phase of temperature rhythm was determined by a geometric method, as described previously (9). The amplitude of temperature rhythm was defined as the difference between the maximum and minimum levels. The trough phase and amplitude of temperature rhythms were calculated on day 1 and day 6. Time-to-time difference in body temperature was calculated between nocturnal sleep on day 2 without physical exercise and on day 5 with physical exercise.

Sleep-wake cycle. The sleep-wake cycle was recorded by three different methods: bed sensors (weight sensor), bed lamp, and wrist actograph. The subjects were instructed to use the bed only for sleep, to turn off the bed lamp when they went to bed, and to turn on the bed lamp when awakened. Signals from these monitoring tools were continuously recorded and fed into a computer system.

Sleep polysomnogram. PSG consisting of single-channel (C3-A2) electroencephalogram, electrooculogram, and electromyogram was recorded on the Pre-Ex2 day and at night on day 5. The data were stored in an electronic computer by the data-acquisition program, SleepSign (KisseiComtec, Nagano, Japan), with a resolution of 128 Hz. The sleep stages were visually scored in every 20-s epoch, according to the standard criteria (26). The sleep latency was defined as the time until the appearance of stage 1 sleep, which was followed by stage 2. NREM sleep contains four stages (stage 1, 2, 3, and 4). The total sleep time (TST) was defined as the sum of NREM sleep [S1 + S2, slow-wave sleep (SWS)] and rapid eye movement (REM) sleep times. The sleep period time (SPT) was defined as the time from

<table>
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<th>Groups</th>
<th>Onset, ZT, h</th>
<th>Peak, ZT, h</th>
<th>Offset, ZT, h</th>
<th>Amplitude, pg/ml</th>
<th>Duration, h</th>
<th>AUC, pg/ml</th>
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<tr>
<td>Control (n = 7)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 1</td>
<td>14.0 ± 1.1</td>
<td>19.7 ± 1.2</td>
<td>1.2 ± 1.4</td>
<td>93.5 ± 34.1</td>
<td>11.2 ± 0.8</td>
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<td>Day 6</td>
<td>15.1 ± 1.1*</td>
<td>20.5 ± 1.2*</td>
<td>1.8 ± 1.4</td>
<td>89.7 ± 42.5</td>
<td>10.7 ± 1.0</td>
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<td>Morning exercise (n = 7)</td>
<td>14.2 ± 1.0</td>
<td>19.6 ± 1.3</td>
<td>1.2 ± 1.9</td>
<td>54.6 ± 20.6</td>
<td>11.1 ± 1.1</td>
<td>414 ± 162</td>
</tr>
<tr>
<td>Day 1</td>
<td>15.4 ± 1.4*</td>
<td>20.6 ± 1.1*</td>
<td>1.3 ± 1.0</td>
<td>57.8 ± 19.9</td>
<td>9.9 ± 1.2</td>
<td>388 ± 132</td>
</tr>
<tr>
<td>Day 6</td>
<td>14.7 ± 1.5</td>
<td>19.7 ± 1.2</td>
<td>0.6 ± 1.1</td>
<td>67.6 ± 34.0</td>
<td>9.9 ± 0.9</td>
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<tr>
<td>Evening exercise (n = 8)</td>
<td>16.0 ± 2.0*</td>
<td>20.8 ± 1.4*</td>
<td>1.6 ± 0.9*</td>
<td>59.3 ± 30.7</td>
<td>9.6 ± 1.4</td>
<td>397 ± 220</td>
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</tbody>
</table>

Values are expressed as the means ± SD. AUC, area under the curve; ZT, zeitgeber time. *P < 0.05 vs. day 1 by one-way repeated-measures ANOVA test with post hoc t-test.
Fig. 3. Twenty-four-hour profiles of rectal temperature rhythms. Circadian rhythms of rectal temperature are plotted in the control (top), morning exercise (middle), and evening exercise (bottom) groups, respectively (A). Solid horizontal bars at the bottom of each column indicate the sleep period. Values are expressed as the mean and SD. Arrows indicate the time of taking a shower at ZT12. Open column in the middle and bottom panel indicate the time of 2-h physical exercise in the morning (ZT 3–5) and the evening (ZT 10–12). Values are expressed as the means and SD; control, n = 7; morning exercise, n = 7; evening exercise, n = 8. Mean rectal temperatures were plotted in the control, morning, and evening exercise groups on day 1 (gray) and day 6 (black), respectively (B). Asterisks with horizontal bars indicate the time when significant differences were detected between day 1 and day 6 (*P < 0.05, one-way repeated-measures ANOVA test with post hoc t-test).
the sleep onset to sleep end, including the time of wake after sleep onset. The sleep efficiency was defined as the percentage of TST to SPT (37). EEG record was missed in two subjects (a subject in the morning and a subject in the evening exercise group) because of technical error.

**HRV during sleep**. HRV (variability of R-R intervals) during the sleep period was determined on the Pre-Ex2 and day 5. Heart rate was recorded by a portable heart rate monitor with a resolution of 1 ms (active tracer 300, GMS, Tokyo, Japan). HRV was analyzed with commercially available software (CHIRAM, Suwa-Trust, Tokyo, Japan), which is based on the maximum entropy, and the nonlinear least squares method (31). Artifacts caused by premature contractions or by body movements were automatically detected and replaced by estimated values through a linear interpolation method (30). Heart rate (HR), the power density of the high-frequency wave (HF: 0.15 –0.40 Hz), the very-low-frequency wave (VLF; below 0.03 Hz), the low-frequency wave (LF: 0.04 –0.15 Hz), and the ratio of LF and HF (LF/HF) were calculated every 5 min. Sleep-stage dependency of HRV was examined according to Ako et al. (1). Sleep stages were determined in 5-min bins. Since SleepSign evaluates sleep stages every 20 s, a sleep stage was defined as the stage that appeared more than 70% in a bin (70% rule). A pair of 5-min HRVs and the 5-min sleep stage was pooled from all subjects.

**Questionnaires for exhaustion and sleepiness**. Subjective sleepiness and tiredness were evaluated with the Questionnaire for Subjective Symptoms in 2002 (QSS2002) (35), which was conducted every day at 2-h intervals during the waking period.

**Physical exercise**. To evaluate the exercise capacity in individual subjects, the symptom-limited exercise test (11) with bicycle ergometer was done from 1000 to 1200 on the next day of the Pre-Ex2. The mean maximum heart rate (HRmax) was 190 ± 8 beats/min (n = 22; mean ± SD). Subjects were randomly assigned to the control and exercise experiments. Subjects in the two exercise groups performed an interval exercise for 2 h with a bicycle ergometer from day 3 to day 6 (Fig. 1, B and C). The 2-h exercise session consisted of 10 min of warming-up, 45 min of exercise, 10 min of rest on a chair, 45 min of exercise, and 10 min of cooling-down. During the exercise session, HR was monitored by a heart rate monitor (Polar S610i; POLAR, Kempele, Finland) every 1 min, and the subjects were instructed to keep their HR at 65–75% HRmax during exercise.

**Statistical Analysis**

Temporal changes in the circadian phases, amplitudes, and sleep parameters along with the experiment were evaluated by a one-way repeated-measures ANOVA test with post hoc t-test for dependent measures or two-way factorial ANOVA test with post hoc t-test for independent measures. The normality and iso-variance of measures were evaluated before testing. The significance level was set at P < 0.05.

### Results

**Plasma Melatonin Rhythms**

Figure 2 illustrates the circadian rhythms in plasma melatonin on day 1 (open circle) and day 6 (closed circle). The melatonin levels in individuals were standardized by transformation of the value to percentage of the maximum level on day 1. The mean phase, amplitude, and duration of nocturnal rise and area under the curve (AUC) of melatonin rhythm were summarized in Table 1. In all groups, the onset and peak phases of melatonin rise on day 6 were significantly delayed relative to those on day 1 (control, onset, −1.1 ± 0.7 h, peak, −0.8 ± 0.4 h, means ± SD, P < 0.05; morning exercise, onset, −1.2 ± 1.0 h, peak, −1.0 ± 0.3 h, P < 0.05; and evening exercise, onset, −1.3 ± 0.9 h, peak, −1.0 ± 0.5 h, P < 0.05, one-way repeated-measures ANOVA with post hoc t-test). In contrast, the offset phase was significantly delayed in the evening groups (−1.0 ± 0.8 h, P < 0.05) but not in the control and morning group (Table 1). There was no significant difference in the amplitude, duration, or AUC of melatonin rhythm between day 1 and day 6.

**Rectal Temperature Rhythms**

Figure 3 illustrates the mean rectal temperature of each group throughout the experiment (Fig. 3A) and the comparison of the rectal temperature during nocturnal sleep between the 1st (day without exercise) and 6th night (day with exercise). The trough phase was not significantly changed in any group. The nocturnal temperature decline was significantly attenuated in the evening exercise group (P < 0.05, one-way repeated-measures ANOVA with post hoc t-test), but not in the control or in the morning exercise group (Fig. 3B). The change in rectal temperature during 8-h sleep was also evaluated from the difference in the AUC where rectal temperatures in 10-min bins were individually summed and averaged for each group. The AUC in the evening exercise group was significantly increased by 9.2 ± 10.7°C/8 h when compared with that on the 1st night (P < 0.05, one-way repeated-measures ANOVA), while those in the control and the morning exercise group were not changed.

**Sleep Parameters**

Table 2 demonstrates the mean sleep parameters of each group on the Pre-Ex2 and on day 5. The amount of Stage 1 + 2 sleep in the control group significantly decreased by 13.0 ±

### Table 2. Sleep parameters

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Morning Exercise (n = 6)</th>
<th>Evening Exercise (n = 8)</th>
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<tbody>
<tr>
<td></td>
<td>PreEx2</td>
<td>Day 5</td>
<td>PreEx2</td>
</tr>
<tr>
<td>Sleep latency, min</td>
<td>16 ± 19</td>
<td>24 ± 29</td>
<td>16 ± 17</td>
</tr>
<tr>
<td>Stage 1 + 2, min</td>
<td>264 ± 45</td>
<td>229 ± 44*</td>
<td>270 ± 26</td>
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<tr>
<td>SWS, min</td>
<td>105 ± 39</td>
<td>107 ± 26</td>
<td>62 ± 27</td>
</tr>
<tr>
<td>REM sleep, min</td>
<td>82 ± 29</td>
<td>100 ± 30</td>
<td>125 ± 24</td>
</tr>
<tr>
<td>WASO, min</td>
<td>11 ± 10</td>
<td>14 ± 12</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>TST, min</td>
<td>453 ± 23</td>
<td>442 ± 27</td>
<td>458 ± 18</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>97.7 ± 2.2</td>
<td>97.0 ± 2.6</td>
<td>98.8 ± 0.9</td>
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</tbody>
</table>

Data are expressed as the means ± SD. SWS, slow-wave sleep; REM, rapid eye movement; WASO, wake after sleep onset; TST, total sleep time. *P < 0.05 vs. PreEx2 by one-way repeated-measures ANOVA.
Fig. 4. Heart rate variability during the sleep period. Mean powers of HRVs [very low frequency (VLF), low frequency (LF), high frequency (HF), and LF/HF] during the sleep period are plotted in the control (n = 7), the morning exercise (n = 7), and the evening exercise (n = 8) groups on the Pre-Ex2 (gray) and day 5 (black) (A). Differences of HRVs between Pre-Ex2 and day 5 were plotted in the control (CTL; white, n = 7), the morning exercise (ME; gray, n = 7) and the evening exercise (EE; black, n = 8) groups (B). Asterisks indicate a significant difference (*P < 0.05, **P < 0.01, one-sample t-test).
10.2% (P < 0.05, one-way repeated-measures ANOVA) and of REM sleep in the evening group by 10.5 ± 9.7% (P < 0.05) on day 5 compared with those on the Pre-Ex2, respectively. In the morning group, there was no significant change in the sleep parameters examined.

**HRV During Sleep**

The HR during 45-min physical exercise was 145 ± 4 beats/min (74 ± 3% of HRmax, means ± SD) on average in the morning and 143 ± 6 beats/min (76 ± 5% of HRmax) in the evening of the 4-day exercise session. There was no significant difference between them. The intensity of physical exercise in the present study was regarded as a hard level, according to the classification of physical activity intensity by American College of Sport Medicine (ACSM) (25).

Figure 4 illustrates the time course of mean HRV in each group plotted in 5-min bins on the Pre-Ex2 (blue) and day 5 (red) (Fig. 4A) and the differences of HRV between the two nights (Fig. 4B). HR increased significantly in the evening group (P < 0.05, one sample t-test), and VLF increased significantly in the control and morning group (P < 0.05). On the other hand, LF and HF increased significantly in the morning group (P < 0.01).

Figure 5 illustrates a representative HRV (HR, VLF, LF, and HF) during nocturnal sleep on the Pre-Ex2 (Fig. 5A) and the differences of HRV at each sleep stage. HR was significantly low in S1, S2, SWS, and REMS compared with that in wake (P < 0.001, one-way repeated-measures ANOVA test with post hoc t-test; Fig. 5B). VLF and LF were significantly lower in SWS (P < 0.05, P < 0.001). HF was significantly high during sleep (P < 0.01). LF/HF was significantly high in wake compared with those in sleep (P < 0.001). During sleep, LF/HF was significantly lower in SWS (P < 0.001). In this analysis, some fractions (16–34%) of sleep stages were not used for estimating correlations with HRV, because of the 70% rule. The validity of the estimation was guaranteed by using in the analysis only the bins occupied with a particular sleep stage by 100% and found essentially the same results.

Figure 6 illustrates sleep-stage-dependent changes in HRV. In the control, VLF significantly increased in S1+S2 (P < 0.05, one sample t-test), and LF/HF in REM sleep (P < 0.01). In the morning group, VLF, LF, and HF...
exercise, which showed a dark-pulse type phase-response curve with a phase-advance portion during the evening and a phase-delay portion during the midnight to the morning (6, 7). However, we failed to confirm these results (22), and in the present study, the circadian melatonin rhythm phase delayed in a similar amount regardless of physical exercise. The discrepancy between the present and previous studies could be due to the design of experiments (four trials on successive days vs. single trial), and/or to the light intensity (<10 lux vs. 42 lux) during exercise. With respect to the intensity and duration of exercise, there were slight differences between the two studies: 40 min at 75% \( \dot{V}O_2 \text{max} \) is equivalent to 85% HRmax (34) in the previous study, whereas 90 min at 75% HRmax is equivalent to 60% \( \dot{V}O_2 \text{max} \) in the present study. According to the classification of physical activity intensity by ACSM (25), however, the intensities of exercise in both studies are classified similarly as a hard level of exercise (70–89% HRmax). Therefore, the discrepancy between the two studies is unlikely due to the intensity of physical exercise.

Physical exercise in the evening produced slight but significant phase-delay shifts in the offset of nocturnal melatonin rise (Table 1), indicating some difference due to the time of physical exercise. It was reported that the onset and offset of melatonin rise responded differentially to phase shifting stimuli such as bright light (12, 35). The time of day effect of physical exercise could affect the offset phase. The discrepancy between the previous studies and present one could be due to the existence of a strict sleep-wake schedule in the present study. Strict sleep-wake schedule is reported to reset the circadian rhythms in totally blind subjects (17) and in sighted subjects who stayed in constant dark conditions (2). The circadian system entrained by a strict sleep-wake schedule could be resistant to perturbing stimuli. A lack of significant phase-shifts in the circadian temperature rhythm may support the above interpretation.

Rectal temperature during nocturnal sleep was increased by evening exercise. A similar result was reported previously (33). Nocturnal decline of core body temperature is mainly due to an increase of heat loss from the skin of extremities (3). Peripheral vasodilatation contributes to nocturnal heat loss, in which the autonomic nervous system is involved (18). Physical exercise in the late evening prevents the nocturnal decline of core body temperature, probably through the sympathetic nerve activation. On the other hand, the nocturnal decline of rectal temperature was not changed or even accelerated by the morning exercise, although statistical evidence is lacking. The above interpretation is supported by the changes of nocturnal HRVs by physical exercise (Fig. 4). HR during nocturnal sleep was significantly increased by the evening exercise, while it was not changed by the morning exercise. Since HR is stimulated by the sympathetic nerve activation, the evening exercise may continuously stimulate the sympathetic nervous system several hours after physical exercise. On the other hand, morning exercise significantly increased VLF, LF, and HF during the nocturnal sleep. HF is widely accepted as a marker of cardiac parasympathetic activity (8), while the significance of VLF, LF, and LF/HF is a matter of discussion in relation with the autonomic nervous functions (8). HRV is dependent to some extent on sleep stage (Fig. 5). However, the increases of VLF, LF, and HF by the morning exercise were independent of the sleep stages (Fig. 6). Since physical exercise in the morning did

**DISCUSSION**

Previous studies demonstrated phase-dependent phase shift of plasma melatonin rhythm by a single trial of physical exercise, which showed a dark-pulse type phase-response curve with a phase-advance portion during the evening and a phase-delay portion during the midnight to the morning (6, 7). However, we failed to confirm these results (22), and in the present study, the circadian melatonin rhythm phase delayed in a similar amount regardless of physical exercise. The discrepancy between the present and previous studies could be due to the design of experiments (four trials on successive days vs. single trial), and/or to the light intensity (<10 lux vs. 42 lux) during exercise. With respect to the intensity and duration of exercise, there were slight differences between the two studies: 40 min at 75% \( \dot{V}O_2 \text{max} \) is equivalent to 85% HRmax (34) in the previous study, whereas 90 min at 75% HRmax is equivalent to 60% \( \dot{V}O_2 \text{max} \) in the present study. According to the classification of physical activity intensity by ACSM (25), however, the intensities of exercise in both studies are classified similarly as a hard level of exercise (70–89% HRmax). Therefore, the discrepancy between the two studies is unlikely due to the intensity of physical exercise.

Physical exercise in the evening produced slight but significant phase-delay shifts in the offset of nocturnal melatonin rise (Table 1), indicating some difference due to the time of physical exercise. It was reported that the onset and offset of melatonin rise responded differentially to phase shifting stimuli such as bright light (12, 35). The time of day effect of physical exercise could affect the offset phase. The discrepancy between the previous studies and present one could be due to the existence of a strict sleep-wake schedule in the present study. Strict sleep-wake schedule is reported to reset the circadian rhythms in totally blind subjects (17) and in sighted subjects who stayed in constant dark conditions (2). The circadian system entrained by a strict sleep-wake schedule could be resistant to perturbing stimuli. A lack of significant phase-shifts in the circadian temperature rhythm may support the above interpretation.

Rectal temperature during nocturnal sleep was increased by evening exercise. A similar result was reported previously (33). Nocturnal decline of core body temperature is mainly due to an increase of heat loss from the skin of extremities (3). Peripheral vasodilatation contributes to nocturnal heat loss, in which the autonomic nervous system is involved (18). Physical exercise in the late evening prevents the nocturnal decline of core body temperature, probably through the sympathetic nerve activation. On the other hand, the nocturnal decline of rectal temperature was not changed or even accelerated by the morning exercise, although statistical evidence is lacking. The above interpretation is supported by the changes of nocturnal HRVs by physical exercise (Fig. 4). HR during nocturnal sleep was significantly increased by the evening exercise, while it was not changed by the morning exercise. Since HR is stimulated by the sympathetic nerve activation, the evening exercise may continuously stimulate the sympathetic nervous system several hours after physical exercise. On the other hand, morning exercise significantly increased VLF, LF, and HF during the nocturnal sleep. HF is widely accepted as a marker of cardiac parasympathetic activity (8), while the significance of VLF, LF, and LF/HF is a matter of discussion in relation with the autonomic nervous functions (8). HRV is dependent to some extent on sleep stage (Fig. 5). However, the increases of VLF, LF, and HF by the morning exercise were independent of the sleep stages (Fig. 6). Since physical exercise in the morning did
not alter the sleep quality, the changes in HRV during sleep was not secondary to the changes in sleep stages. It is unlikely that the morning exercise activated the parasympathetic nerve activity per se. The activity of autonomic nervous system was possibly altered in a long run after physical exercise in the morning. It is well known that bright lights in mammals, including humans, stimulate the sympathetic activity for several hours, even after the cessation of stimulation (24, 28). Differential effects of bright light exposure on melatonin secretion were reported in humans. Nocturnal bright lights suppressed the circadian rise of melatonin level (20), while daytime bright lights enhanced the nocturnal melatonin level (21, 35). Similar differential effects were reported for thermal stimulation on nocturnal decline of rectal temperature. Thermal stimulation in the morning accelerated the nocturnal decline of core body temperature, while the stimulation in the late evening suppressed the nocturnal decline (16). These findings indicate that sympathetic stimulation could produce an opposite effect in a long-run depending on the time of stimulation. The present results lead us to the hypothesis that although physical exercise stimulates, in general, the sympathetic nervous system, the activity of autonomic nervous system changed afterward depending on the time lapse. The sympathetic activity is kept at the high level for a few hours after stimulation (24, 28), while the parasympathetic activity becomes predominant by the following nocturnal sleep. Changes in the number of receptors after physical exercise could be involved in this alternation. The hypothesis waits to be tested.

In conclusion, daily physical exercise of moderate strength produced differential effects on circadian melatonin rhythm, rectal temperature during nocturnal sleep, sleep stages, and HRV, depending on the time of exercise. The morning exercise increased LF and HF during nocturnal sleep, while the evening exercise shifted the offset phase of nocturnal melatonin rise, suppressed nocturnal decline of rectal temperature, and increased HR during sleep. The morning exercise may eventually enhance the parasympathetic activity at the following nocturnal sleep, while the evening exercise keeps the increased sympathetic activity during the following sleep.

**Perspectives and Significance**

The present findings suggest that morning and evening exercises differentially affect nocturnal body temperature and cardiac activity during sleep. These findings could answer the question at which time of day physical exercise should be done for better sleep. The morning exercise would improve the quality of nocturnal sleep by increasing the parasympathetic nerve activity, whereas high-intensity exercise in the evening should be avoided. We do not know yet the mechanism of the time-dependent effect of physical exercise. The specific characteristics of the autonomic nervous system could be responsible.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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