CIRCADIAN VARIATIONS IN ANAEROBIC THRESHOLD

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Abstract:
This study aimed to determine whether certain respiratory and cardiovascular parameters associated with anaerobic threshold (AT), measured during graded exercise testing, occur at lower intensities in the morning than in the evening. Ten healthy Japanese men volunteered to participate in this study, which involved two conditions that were performed at different times of day: morning (M) exercise was performed between 9:00 and 10:00 a.m., and afternoon (A) exercise was performed between 4:00–6:00 p.m. After resting supine for 30 minutes, each subject performed graded cycle ergometer exercise testing comprising 90-second stages. Exercise intensity was initially 10 W and was increased by 10 W for each stage. Heart rate (HR), blood pressure (BP), absolute double product (DP), cardiac autonomic nervous system modulation, and ventilatory volume (VE) were measured during each exercise stage. Ventilatory threshold (VT), the double product breaking point (DPBP), and breaking point of the natural log of high frequency (ln HF) (HFBP) were reached at a lower exercise intensity in the M condition than in the A condition (p<.05). Values for VE at VT intensity, DP, HR, and systolic blood pressure (SBP) at DPBP were significantly lower in the M condition than in the A condition (p<.05). These data suggest that AT is reached at a lower intensity in the morning than in the afternoon, and that relative burden, as indicated by HR and SBP, is greater in the morning than in the afternoon. Exercise prescriptions that incorporate awareness of the circadian rhythms may prevent cardiac or cerebrovascular accidents during exercise.

Key words: circadian rhythm, cardiac autonomic nervous system, double product, ventilatory volume

Introduction
Humans have a daily oscillating circadian rhythm that influences physiological responses. Body temperature is at its lowest immediately before rising from nocturnal sleep (~4:00–6:00 a.m.); it increases thereafter, peaking at ~4:00–6:00 p.m., and declines again at night (Weinert & Waterhouse, 2007). The rhythm of body temperature is the basic circadian rhythm. In the human autonomic nervous system, the parasympathetic nervous system (PNS) is dominant during nocturnal sleep, whereas during rising, the sympathetic nervous system takes over; therefore, during the daytime, the sympathetic nervous system is active, and the PNS is suppressed (Baik, Yamasaki, Nishimura, & Onodera, 2006; Vandewalle, et al. 2007). Risks associated with high-intensity exercise in the early morning have been reported (Atkinson, Jones, & Ainslie, 2010; Muller, Tofler, & Stone, 1989; Shimada, et al., 2001; White, 2000), and cardiac or cerebrovascular events that may induce myocardial infarction and cerebral infarction frequently occur within three hours after rising (Muller, et al. 1989). To date, although the effect of the time of day on aerobic performance appears to be equivocal, the effect of the time of day on anaerobic exercise has been well established, with nadirs in the early morning and peak performances in the late afternoon (Chtourou & Souissi, 2012). The oral temperature and performances during the Wingate, vertical jump, and maximal voluntary contraction tests were higher in the evening than in the morning (Chtourou, et al., 2012). The effects of training and tapering at the same time of the day were observed, and these diurnal variations were blunted in the morning training group and
Persisted in the evening training group. The 2-wk tapering resulted in further time-of-day-specific adaptations and increased in short-term maximal performances (Chtourou, et al., 2012). The time-of-day-specific training increased the children’s anaerobic performances, specifically in the evening (5:00-6:00 p.m.). Moreover, improvement in these performances was greater after the morning than the evening training sessions (Souissi, et al., 2012). The majority of the components in sports performance (e.g. flexibility, muscle strength, and short-term high power output) vary with the time of day in a sinusoidal manner and peak in the early evening close to the daily maximum body temperature (Atkinson & Reilly, 1996).

Heart rate (HR), systolic blood pressure (SBP), and absolute double product (DP) were significantly lower during low-intensity exercise (20% and 40% of maximum oxygen uptake $[\text{VO}_{2\text{max}}]$) performed within three hours after rising than for that performed in the afternoon, whereas no significant difference was observed in these parameters between the morning and afternoon high-intensity exercises (60% of $\text{VO}_{2\text{max}}$) (Nishimura, Takamoto, et al., 2011). This indicates that the influence of the circadian rhythm on physiological responses to exercise varies with exercise intensity, becoming small around the anaerobic threshold (AT). In addition, the ratio of DP at 60% $\text{VO}_{2\text{max}}$ to the resting value was significantly higher in the morning, which supports reported findings on the increased risk associated with high-intensity exercise performed in the morning and on improvement in some exercise parameters in the evening and at night (Atkinson & Reilly, 1996; Hill & Smith, 1991; Ilmarinen, Ilmarinen, Korhonen, & Nurminen, 1980; Winget, DeRoshia, & Holley, 1985; Yanagimoto & Ebisu, 1994). Thus, the physiological parameters of exercise may demonstrate responses that correspond to the circadian rhythm.

During exercise, physiological responses, such as HR, oxygen uptake, DP, blood lactate, etc., are dependent on exercise intensity. At higher workloads, ventilatory volume (VE) and blood lactate levels increase linearly, with an abrupt increase at a point during moderate-intensity exercise (Schneider, McLellan, & Gass, 2000), called the anaerobic threshold (AT), which is defined as the upper limit of aerobic exercise and the point at which anaerobic energy production is mobilized (Wasserman & McIlroy, 1964). An AT-based exercise prescription is part of the exercise guidance given to patients. In such settings, to avoid invasive measurements of blood lactate concentration and VE with expensive equipment, non-invasive techniques such as the determination of the DP breaking point (DPBP) are employed. The DP, which is HR multiplied by SBP, reflects the myocardial oxygen consumption (Tanaka, et al. 1997), and it increases with exercise intensity. DP rises abruptly at a point during moderate-intensity exercise (Tanaka, et al. 1997) called DPBP, which has been shown to be positively correlated with AT with respect to the lactate threshold (LT) and ventilatory threshold (VT). The heart rate variability (HRV) frequency domain spectrum is an index of the cardiac autonomic nervous system (ANS), and the high frequency (HF) components are dependent on exercise intensity. Nishimura et al. defined the workload yielding the highest absolute value of the slope (workload and HF component) as the breaking point of the natural log of HF (In HF) or HFBP (Nishimura, Yoshioka, et al., 2011). Significant relationships between the intensity associated with DPBP and HFBP have been observed, such as the workload at HFBP being significantly lower than that of DPBP. Monitoring for HFBP during graded exercise testing is therefore thought to facilitate a safe exercise prescription in terms of the cardiac PNS-activity endpoint, supporting the use of HF as an index of AT for the purpose of developing exercise prescriptions.

We hypothesized that the VT, DPBP, and HFBP, which are indicators of AT, occur at a lower intensity in the morning than in the evening. This study aimed to verify this hypothesis using HR, the cardiac autonomic nervous system (ANS) activity, BP, and VE as indicators of AT measured during graded exercise testing conducted between 9:00 and 10:00 a.m. and between 4:00 and 6:00 p.m.

**Methods**

**Subjects**

Ten healthy Japanese men (mean±standard deviation [SD]; age, 21.2±0.4 years old; height, 169.0±3.8 cm; body weight, 66.2±12.8 kg; body mass index [BMI], 23.2±4.2 kg/m²; and $\text{VO}_{2\text{peak}}$, 46.1±6.9 mL/kg/min) volunteered to participate in the study. All subjects were normotensive, non-obese nonsmokers with no evidence of cardiovascular disease as based on their medical history and resting electrocardiogram. Seven subjects performed regular exercise and three were sedentary. All subjects demonstrated natural circadian rhythms of oral temperature. All subjects provided their written informed consent prior to study participation. All procedures were reviewed and approved by the Ethics Committee of Kibi International University, and the study protocol conformed to the Helsinki Declaration.

**Measurements**

Each subject rested supine for 30 minutes, and the resting measurements of HR, BP, DP, cardiac ANS modulation, oral temperature, VE, and oxygen uptake were recorded for five minutes. Each subject performed graded exercise, which consisted of 90 seconds of exercise at a pedaling rate of 60 rpm on
an electrically braked cycle ergometer (Aerobike 75XL II, Combi Wellness, Tokyo, Japan). The initial exercise intensity was 10 W, and the intensity was increased by 10 W for each stage. The subjects were asked to indicate their rating of perceived exertion (RPE) for each stage and were instructed to stop the exercise at an RPE of 15–16 or when their HR reached 160 bpm (80% HRmax). The study was conducted under two conditions, but on different days: morning (M) exercise was performed between 9:00 and 10:00 a.m.; and afternoon (A) exercise was performed between 4:00 and 6:00 p.m. All subjects went to bed at 11:00 p.m. and woke at 7:00 a.m. on each experimental day. The subjects did not consume food four hours before the A exercise and did not have breakfast before the M exercise. Caffeine-containing products were not allowed for three hours before the experiments. The room temperature and humidity were 23.3 °C (±1.1 °C) and 60.9 % (±6.1 %), respectively.

The HR, BP, DP, cardiac ANS modulation, VE, oxygen uptake, and oral temperature were measured. Resting HR was calculated from an electrocardiogram rhythm strip and was recorded as the mean value over five minutes of supine rest. During exercise, the HR was calculated between 60 and 90 seconds into each exercise stage. BP was measured using the auscultatory method. Resting BP was recorded as the mean value of measurements taken at 4 and 5 minutes. During exercise, BP was measured between 60 and 90 seconds into each exercise stage. The DP was calculated by multiplying the SBP and HR, and it was used as an index of myocardial oxygen consumption. The cardiac ANS modulation was calculated using the maximum entropy calculation method (Nishimura, Takamoto, et al., 2011; Nishimura, Yoshioka, et al., 2011; Tabusadani, et al., 2001). The HRV frequency domain spectrum was divided into two parts: high frequency (HF; 0.15–0.40 Hz) and low frequency (LF; 0.04–0.15 Hz) (Pomeranz, et al., 1985). The cardiac ANS modulation was transformed into natural logarithm values to obtain a normal distribution. The ln HF was taken as an index of cardiac PNS modulation (Nishimura, Takamoto, et al., 2011). Since the power determined by the spectral analysis of HR variability is influenced by the respiratory frequency, the resting respiratory frequency measurement was reduced to once every four seconds (2-sec inspiration and 2-sec expiration) to exclude the influence of respiratory frequency (Brown, Beightol, Koh, & Eckberg, 1993). The subjects were instructed to breathe naturally when exercising. Their oral temperature was measured using an electronic clinical thermometer (MC-672L; Omron Co., Kyoto, Japan) at rest. Oxygen uptake was calculated using the breath-by-breath method with a gas analyzer (AE300S; Minato Medical Science Co., Osaka, Japan).

The VT was calculated as the point of rapid elevation of VE in relation to workload, and DPBP was defined as the point of rapid elevation of DP in relation to workload. An equation to define the relationship between each exercise workload and ln HF was obtained. The workload that yielded the highest absolute value of the slope was defined as the breaking point of ln HF (HFBP) (Nishimura, Yoshioka, et al., 2011). These parameters were used as indices of AT.

**Data analysis**

All data were expressed as mean ± SD. The Wilcoxon-Mann-Whitney test was used to compare each measured value between the M and A exercise trials. The level of significance was set at p<.05.

**Results**

Table 1 shows the physiological parameters during the M and A exercise conditions. The resting HR, SBP, DP, oral temperature, and VE were significantly lower in the M condition than in the A condition (p<.05). The ln HF and index of cardiac PNS modulation were significantly higher in the M condition than in the A condition (p<.05). The diastolic blood pressure (DBP) and oxygen uptake did not significantly differ between the two measurement conditions. Figure 1 shows a comparison of AT workloads, such as VT (a), DPBP (b), and HFBP (c), between the M and A condition. The VT, DPBP, and HFBP were significantly lower in the M condition than in the A condition (p<.05).

At the intensity associated with the VT, the VE (a) was significantly lower in the M condition than in the A condition (p<.05) (Figure 2). However, at the given intensity of VT, the oxygen uptake (b) did not differ significantly between the two measurement conditions. A comparison of the physiological parameters, such as the DP (a), relative DP (b),

Table 1. The resting physiological parameters under the morning (M) and afternoon (A) conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M condition</th>
<th>A condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>62.2±11.4</td>
<td>68.3±15.3*</td>
</tr>
<tr>
<td>ln HF</td>
<td>6.98±1.06</td>
<td>6.44±1.23*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>111.1±10.4</td>
<td>115.7±8.7*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>58.6±5.1</td>
<td>61.0±7.1</td>
</tr>
<tr>
<td>Double product (mmHg.bpm/100)</td>
<td>69.5±16.0</td>
<td>79.5±21.2*</td>
</tr>
<tr>
<td>Oral temperature (°C)</td>
<td>36.49±0.33</td>
<td>36.88±0.33*</td>
</tr>
<tr>
<td>Oxygen uptake (mL/kg/min)</td>
<td>3.60±0.63</td>
<td>3.81±0.36</td>
</tr>
<tr>
<td>Ventilatory volume (l/min)</td>
<td>9.39±1.90</td>
<td>10.15±1.56*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±standard deviation. * Significance of p<.05 vs. M condition. In HF - natural log of high frequency.
Figure 1. AT workloads at VT (a), DPBP (b), and HFBP (c). *p<.05. A - afternoon; M - morning; AT - anaerobic threshold; DPBP - double product breaking point; HFBP - high frequency breaking point; VT - ventilatory threshold.

Figure 2. At the intensity associated with VT, the VE (a) and oxygen uptake (b) are compared during the exercise conditions. *p<.05. A - afternoon; M - morning; VT - ventilatory threshold; VE - ventilatory volume.

Figure 3. At the intensity associated with DPBP, the DP (a) relative to the resting value of DP (b), HR (c), and SBP (d) are compared during the exercise conditions. *p<.05. A - afternoon; M - morning; DPBP - double product breaking point; DP - double product; HR - heart rate; SBP - systolic blood pressure.
HR (c), and SBP (d), between the M and A conditions at the intensity associated with DPBP is shown in Figure 3. At the intensity associated with DPBP, the DP and relative DP (the ratio to resting DP), HR, and SBP were significantly lower in the M condition than in the A condition (both, p<.05).

**Discussion and conclusions**

The AT, as indicated by the VT, DPBP, and HFBP, was reached at a lower exercise intensity in the morning than in the afternoon. Moreover, values for the VE, HR, SBP, and absolute and resting value ratio of DP at the AT were lower in the morning. These findings indicate that the AT oscillates diurnally, which supports our study hypothesis.

At rest, the HR, SBP, DP, ln HF, oral temperature, and VE in the morning differed significantly from the afternoon values, which is similar to the results of previous studies on the human circadian rhythm (Atkinson & Reilly, 1996; Baik, et al., 2006; Nishimura, Takamoto, et al., 2011; Vandewalle, et al., 2007; Winget, et al., 1985).

During graded exercise testing, the VT, DPBP, and HFBP were significantly lower in the morning than in the afternoon, suggesting that indicators of AT appear at a lower intensity in the morning and that relative exercise intensity is greater in the M condition than in the A condition. The influence of the circadian rhythm observed at rest was also observed during exercise at intensities corresponding to 20% and 40% of the VO$_{2\text{max}}$, not to 60% (Nishimura, Takamoto, et al., 2011). Thus, the influence of the circadian rhythm was present when the exercise intensity was low. The DPBP exercise intensity in this study corresponded to 45.0% of the VO$_{2\text{max}}$ and 44.8% of the HR reserve (HRR). These findings indicate that parameters of AT, such as DPBP and HFBP, showed responses that corresponded to the circadian rhythm. Since the increase in HR during exercise below AT is caused by the suppression of cardiac PNS (Xenakis, Quarry, & Spodick, 1975), we speculate that the high activity level of the cardiac PNS in the morning contributed to the suppression of elevation of HR in exercise below AT. This involvement of the cardiac PNS also likely contributes to the significant difference in the HFBP observed between the two conditions. Furthermore, since no significant difference was observed in ln HF at HFBP between the two conditions, it is likely that the declining slope of the HF component indicates a high value in the M condition. The declining velocity of the HF component and the physical endurance level in the early morning are positively correlated (Tabusadani, et al., 2001). This supports performing high intensity exercise in the morning; however, we consider that elevation of declining velocity of the HF component is a response to the preparation for high-intensity exercise in the early morning, which confirms previous findings (Muller, et al., 1989; Shimada, et al., 2001; White, 2000) on the risks of high-intensity exercise in the early morning.

The VE, absolute value of DP, resting value ratio of DP, HR, and SBP at DPBP intensity were significantly lower in the morning than in the afternoon. These lower morning values are likely due to DPBP being influenced by the circadian rhythm. Therefore, we consider that the anaerobic energy supply mechanism begins to be mobilized during graded exercise in the morning when the VE, HR, SBP, and DP are lower than those in the afternoon.

The present study suggested that AT parameter, such as the VT, DPBP, and HFBP, was reached at lower exercise intensity in the morning than in the afternoon. In addition, the relative value of the HRR at the DPBP workload was 41.1±6.4 % in the morning and 46.7±7.3 % in the afternoon, which was significantly different. A delay in the appearance of parameters associated with AT and relatively higher AT for the same HR to the maximum HR during graded exercise are likely to contribute to improved exercise performance in the afternoon.

The aforementioned findings explain the difference between the morning and afternoon physiological responses. In the morning, anaerobic energy is mobilized at a lower intensity while relative exercise intensity is high; however, in the afternoon, the parameters of exercise performance have higher values. Thus, those accustomed to exercise are likely to achieve high exercise performance and suppress the occurrence of cardiovascular events by exercising in the afternoon. In addition, during morning exercise, an intensity not exceeding AT may be desirable.

The diurnal change in sublingual temperature before the experiment confirmed a circadian rhythm in our subjects; however, to exclude the influence of the exercise on temperature, we did not check this on the day of the experiment. In this study, sleeping hours, physical activities, and diet, which were considered to influence the diurnal change, were controlled. Bedtime and the time of rising were consistent between the conditions. Although the subjects were instructed to rest in the sitting position on the day of the experiment, physical activity was higher in the afternoon. Regarding diet, the subjects were instructed to eat meals no later than 10:00 p.m. the day before the experiment in both conditions. In the A condition, the subjects were instructed to have breakfast at 7:30 a.m. and lunch at noon. Thus, the number of fasting hours differed between the conditions and was a limitation of this study. Further, we could not obtain the chronotype of the participants. However, since all the subjects consistently woke at 7:00 a.m., we think that they preferred an early morning start.
The present study’s findings indicated that performing graded exercise testing in the evening is preferable for calculating the exercise intensity with safety in mind. In addition, when an exercise prescription is provided based on the results of graded exercise testing conducted in the morning, we need to consider the relatively greater exercise intensity. Alternatively, when performing the prescribed exercise in the afternoon after the graded exercise testing in the morning, the relative exercise intensity is lower, indicating a necessity to consider increased load and prolonged exercise duration for obtaining the effect of the exercise. Additionally, in cases where the values of HR and BP are the same in the morning and afternoon, the relative exercise burden on the body is higher in the morning. Exercise prescriptions that correspond to the circadian rhythm after considering the aforementioned facts are expected to prevent accidents during exercise.

In the present study, the relationship between the timing of graded exercise and HR, blood pressure, and respiratory responses during exercise were investigated. Our results demonstrated that VT, DPBP, and HFBP were reached at a lower exercise intensity in the morning than in the afternoon and that VE at VT intensity, absolute value of DP, resting DP ratio, HR, and SBP at DPBP intensity were significantly lower in the morning. This suggests that exercise prescriptions should take into consideration that the morning exercise AT is reached at low intensity and that the relative burden is higher than for the same HR and SBP in the afternoon. Exercise prescriptions that incorporate awareness of the human circadian rhythm may prevent cardiac and cerebrovascular accidents during exercise.

Conflicts of Interest

There are no conflicts of interest to declare.

References


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