Diurnal variation in the control of ventilation in response to rising body temperature during exercise in the heat

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Tsuji B, Honda Y, Kondo N, Nishiyasu T. Diurnal variation in the control of ventilation in response to rising body temperature during exercise in the heat. Am J Physiol Regul Integr Comp Physiol 311: R401–R409, 2016. First published June 22, 2016; doi:10.1152/ajpregu.00484.2015.—We investigated whether heat-induced hyperventilation during exercise is affected by time of day, as diurnal variation leads to higher core temperatures in the evening. Nineteen male subjects were divided into two experiments (protocol 1, n = 10 and protocol 2, n = 9). In protocol 1, subjects performed cycle exercise at 50% peak oxygen uptake in the heat (37°C and 50% RH) in the morning (0600) and evening (1800). Results showed that baseline resting and exercising esophageal temperature (RH) in the morning (0600) and evening (1800). Results showed that cycle exercise at 50% peak oxygen uptake in the heat (37°C and 50% RH) in the morning (0600) and evening (1800). Results showed that baseline resting and exercising esophageal temperature (RH) in the morning (0600) and evening (1800). Results showed that baseline resting and exercising esophageal temperature (Tesar) were significantly (0.5°C) higher in the evening than morning. Minute ventilation (VE) increased by 10.2 ± 0.3 l/min from 54.3 ± 7.9 l/min at 10 min to 71.4 ± 8.1 l/min at 48.5 min in the morning and evening, respectively (both P < 0.01). Time of day had no effect on VE (P = 0.44). When TE as the output response was plotted against Tes, as threshold input, the Tes threshold for increases in VE was higher in the evening than morning (37.2 ± 0.7°C vs. 36.6 ± 0.6°C, P = 0.009), indicating the ventilatory response to the same core temperature is smaller in the evening. In protocol 2, the circadian rhythm-related higher resting Tes seen in the evening was adjusted down to the same temperature seen in the morning by immersing the subject in cold water. Importantly, the time course of changes in VE during exercise were smaller in the evening, but the threshold for VE remained higher in the evening than morning (P < 0.001). Collectively, those results suggest that time of day has no effect on time course hyperventilation during exercise in the heat, despite the higher core temperatures in the evening. This is likely due to diurnal variation in the control of ventilation in response to rising core temperature.

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reflecting a higher temperature threshold for hyperventilation in the evening (protocol 1). We also hypothesized that when the higher resting core temperature seen in the evening is adjusted down to the level in the morning prior to exercise, the time course of the increases in Ve during subsequent exercise would be smaller, but the threshold for hyperventilation would remain higher in the evening than morning (protocol 2).

**METHODS**

**Ethical Approval**

Written informed consent was obtained from all participants. The present procedure was approved by the Human Subjects Committee of the University of Tsukuba, and conformed to the provisions of the Declaration of Helsinki.

**Subjects**

Nineteen healthy males volunteered to participate in this study. The participants were nonsmokers, and none were taking any medication. Subjects were divided into two experiments: protocol 1 (n = 10) and 2 (n = 9). Subjects were asked to abstain from strenuous exercise, alcohol, and caffeine for 24 h before the experimental testing.

**VO2peak Test**

To determine VO2peak, subjects were asked to perform an incremental exercise to volitional fatigue on a bicycle ergometer (818E, Monark, Sweden; customized for semirecumbent cycling) in an environmental chamber (Fujikka, Chiba, Japan) maintained at 25°C and 50% relative humidity. Subjects first performed a light warm-up (30 W, 60 rpm) for 3 min, which was followed, 1 min later, by the incremental cycling exercise. The exercise was started at 60 W, and the load was increased by 15 W every 1 min thereafter. The pedaling rate was set at 60 rpm, and volitional fatigue was defined as an inability to pedal at more than 50 rpm. Expired gas was analyzed using a metabolic cart (RM300i; Minato Medical Science, Osaka, Japan). The flow sensor was calibrated using an air syringe able to blow a fixed volume (2 liters) of air. The O2 and CO2 sensors were calibrated using room air and reference gas of known concentration (15% O2-5% CO2-N2 balance). Ve, oxygen uptake (VO2), and carbon dioxide output (VCO2) were calculated at 60-s intervals.

**Experimental Design**

After performing the VO2peak test on a separate day, subjects performed two bouts of cycle exercise at 50% of VO2peak in the heat, one in the early morning and the other in the evening (Exercise-Heat test protocol 1 and 2; see below for details). The two trials were conducted in random order and were separated by at least 6 days. Exercise at 50% of VO2peak intensity in a hot condition was previously shown to elicit gradual increases in Tes and hyperventilation, whereas VO2 and blood lactate concentrations (<2 mmol/l) were maintained virtually constant (20). Exercise at that intensity can, thus, induce heat-related hyperventilation that is minimally affected by metabolic factors. Before each exercise, resting Tes was reduced by immersing the subject in cold water. This enabled detection of the Tes threshold for hyperventilation during exercise (46, 47). We previously reported that this threshold, which was around 37°C, could be detected only after preexercise Tes was lowered by precooling the subjects. This precooling is needed because the threshold is at normothermia (~37°C), and data for core temperatures below the threshold (i.e., <37°C) are needed to detect the inflection point that indicates the threshold. Consequently, when exercise is performed without precooling, the threshold cannot be observed due to insufficient data below the threshold. As a result, Ve appears to increase linearly with rising Tes without a threshold (47). To standardize the hydration status, for the early morning trial, the subjects were asked to drink 500 ml of water on the night before the experiment and the morning of the experiment. For the evening trial, they consumed a light meal and 500 ml of water 3 h before the experiment and an additional 500 ml of water before arriving at the laboratory.

**Exercise-Heat Test in the Early Morning and Evening**

**Protocol 1. Effect of time of day.** Ten male subjects [age 24.5 ± 2.1 years, height 172.6 ± 4.7 cm, weight 64.0 ± 5.6 kg, peak O2 uptake (VO2peak) 52.2 ± 5.5 ml·kg⁻¹·min⁻¹] participated in this protocol. The subjects came to the laboratory at 0500 or 1700, and rested in an environmental chamber (ambient temperature = 25°C, relative humidity = 50%). During this time, the subjects themselves inserted a thermocouple for measuring esophageal temperature (Tes) and then voided urine, after which body weight was recorded. After putting on shorts and shoes, the subjects moved to another environmental chamber (ambient temperature = 37°C, relative humidity = 50%, and wind speed <0.2 m/s), where they sat in a semirecumbent position on the seat of the cycle ergometer and rested for 30–40 min. During this time, the remaining instrumentation was attached. At 0600 or 1800, baseline resting measurements were started while the subjects sat for another 10 min. They then moved into a water-filled tank situated next to the ergometer in the environmental chamber and remained there for 25 min while immersed to the level of the axillae in water at 18°C. We previously found that this immersion reduced Tend by ~0.5°C, and that Tend was reduced by another ~0.5°C during subsequent exercise. These reductions in Tend were adequate to obtain the data for Tend below the Tes threshold for hyperventilation during exercise (i.e., to detect the threshold), as described previously (47). In previous pilot experiments, in which the immersion duration and water temperature were varied, we confirmed that a 25-min water immersion at 18°C was suitable. During the immersion, the subjects did not visibly shiver. After the immersion, the subjects towed themselves dry, voided urine, and sat in the chair of the ergometer. Resting variables were again recorded for 1–2 min, after which the subjects performed the cycle exercise at 50% of VO2peak. The transition period from the time the subjects finished the immersion to the start of the exercise was 10.3 ± 1.5 min. The exercise was terminated when Tend reached 39.0°C or the subject could no longer pedal at 60 rpm.

**Protocol 2. Effect of preexercise core temperature.** Nine male subjects (24.7 ± 2.1 years, 172.3 ± 4.9 cm, 64.1 ± 5.9 kg, 52.3 ± 5.8 ml·kg⁻¹·min⁻¹) participated in protocol 2. This protocol was designed to examine how the time course of Ve during exercise and core temperature threshold for hyperventilation are affected when the higher preexercise resting core temperature seen in the evening is adjusted down to the same level seen in the morning. To accomplish this, the subjects were immersed in water (18°C) for 25 min before the early morning exercise and for 50 min before the evening exercise. The 25-min immersion in the morning was needed to detect the Tend threshold for hyperventilation, as shown in protocol 1, while the 50-min immersion, twice as long as that required in protocol 1, was needed to offset the circadian rhythm-related ~0.5°C difference in Tend between the morning and evening. In preliminary experiments, we confirmed that cold-water immersion for 25 min in the morning and 50 min in the evening reduced Tend by ~0.5 and ~1.0°C, respectively. Other than the immersion duration, the experimental procedures and exercise were the same as in protocol 1.

**Measurements**

**Body temperatures.** Tes and skin temperatures were measured using copper-constantan thermocouples and recorded on a com-
Cardiorespiratory variables. Heart rate was recorded every 5 s using a heart rate monitor (Vantage NV, Polar, Finland). Blood pressure was measured from the upper left arm every 1 min using an automated sphygmomanometer (STBP-780, Nippon Colin, Japan). Mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure. Expired gas was measured using the same analyzers used in the VO₂ peak test, and VT, tidal volume (VT), which were adjusted to BTPS, respiratory frequency (f), VO₂, VCO₂, end-tidal CO₂ pressure (PETCO₂), and the respiratory exchange ratio were recorded breath-to-breath.

Other variables. Ratings of perceived exertion were measured every 5 min using Borg’s scale. Body weight was measured using a platform scale with an accuracy of ±10 g (Yamato scale, Hyogo, Japan) before and after the experiment.
Data Analysis

Core temperature thresholds for increases in $V_e$ and $f$ were selected as important outcome data for the comparison of means. The minimum sample size was calculated on the basis of 80% power and a significance level of 0.05 using time-of-day-related differences and standard deviations from our pilot experiments. The minimum sample sizes were estimated to be 8 and 6 for $V_e$ and $f$, respectively. The smallest sample size used in this study ($n = 9$) was, thus, adequate for our analysis.

To compare the time course of changes in each variable between the morning and evening, 5-min averaged data were used. In some instances, the length of time a subject exercised differed between the morning and evening. In those cases, only the data obtained up to the earlier termination time, in the morning or evening, were compared. For each subject, therefore, all variables were compared between the morning and evening at the same time points during the exercise. However, the exercise times varied among subjects, and the mean exercise times differed somewhat between the two protocols (48.5 ± 8.5 min in protocol 1 and 55.0 ± 11.5 min in protocol 2).

To estimate the relationship between core temperature and ventilatory variables ([$V_e$, $V_{\text{CO}_2}$, $V_r$, $f$, and $P_{\text{ETCO}_2}$]), we plotted the ventilatory variables as a function of $T_e$ and conducted linear regression analyses (14, 20). For these analyses, 30-s averaged data were used. In addition, to exclude the fast component of $V_e$ kinetics, only data obtained after the first 5 min of the exercise were analyzed. Using these data with a computer algorithm (14), we determined the thresholds for the increase or decrease in the respective ventilatory variables as the inflection point where two calculated regression lines crossed. To calculate the two best-fit regression lines, we chose the two lines with the smallest residual sums of squares. We also divided the $T_e$ range into subranges spanning the temperatures below and above the thresholds, and the sensitivities of $V_e$, $V_{\text{CO}_2}$, $V_r$, $f$, and $P_{\text{ETCO}_2}$ to increasing $T_e$ above their thresholds were determined by calculating the slope of the regression lines.

Statistical Analysis

Two-factor repeated-measures ANOVA was used to analyze the time-dependent data using time-of-day (levels: morning and evening) and exercise duration (protocol 1: 0, 5, 10, 15, 20, 25, 30, 35, 40 and 48.5 min; protocol 2: 0, 5, 10, 15, 20, 25, 30, 35, 55 and 60 min) as factors. When an interaction or main effect was detected, post hoc paired $t$-tests with the Bonferroni correction for multiple comparisons were performed to identify pairwise differences. Between-trial comparisons of baseline data, thresholds, and sensitivities were performed using paired $t$-tests. All data are reported as means ± SD. Values of $P < 0.05$ were considered significant, except that $P < \alpha$/number of comparisons between exercise durations (0.05/10 = 0.005 and 0.059 = 0.006 in protocol 1 and 2, respectively) was considered significant in the post hoc comparison. All statistical analyses were performed using the SPSS statistics package (version 19.0, SPSS, Chicago, IL).

RESULTS

Protocol 1

Exercise duration and body weight. Exercise duration did not significantly differ between the morning and evening trials (54.0 ± 8.1 vs. 50.0 ± 11.5 min, $P = 0.17$). Baseline body weight and the weight lost over the course of the morning trial was nearly identical to those in the evening trial ($P = 0.35$ and 0.51, respectively).

Baseline resting data. Baseline resting $T_s$ (36.8 ± 0.2 vs. 36.3 ± 0.1°C), $T_{sk}$ (35.8 ± 0.3 vs. 35.5 ± 0.3°C), $V_e$ (9.0 ± 1.1 vs. 8.0 ± 1.5 l/min), $V_{O2}$ (261 ± 19 vs. 230 ± 40 ml/min), and $V_{CO2}$ (222 ± 26 vs. 190 ± 37 ml/min) were all higher in the evening than in the morning (all $P < 0.04$).

Time Course of Changes in Physiological Responses During Exercise

Body temperatures, cardiovascular responses and ratings of perceived exertion. Cold water immersion reduced $T_s$ by 0.6°C in both the morning and evening ($P < 0.01$). The time-of-day related difference in $T_s$ was similar before and after immersion ($P = 0.42$). There were significant main effects of time of day ($P < 0.01$) and exercise duration ($P < 0.01$) on $T_s$, which was significantly higher in the evening than in the morning (all $P < 0.01$) (Fig. 1A). There was also a significant main effect of exercise duration on $T_{sk}$ ($P < 0.01$), but there was no effect of time of day ($P = 0.61$). During cold-water immersion, $T_{sk}$ declined to 30.1 ± 0.9 and 30.4 ± 0.7°C in the morning and evening, respectively. Then during exercise, $T_{sk}$ increased to 34.8 ± 0.7 and 34.7 ± 0.6°C in the morning and evening, respectively (both $P < 0.001$).

Heart rate significantly increased to 166 ± 15 and 174 ± 15 beats/min at 48.5 min in the morning and evening, respectively, without a main effect of time of day ($P = 0.11$). Also without an effect of time of day ($P = 0.53$), mean arterial pressure increased for the first 10 min of exercise (morning vs.

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<th>Table 1. Core temperature thresholds and sensitivities calculated after plotting the indicated variables against esophageal temperature in protocol 1</th>
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Values are expressed as means ± SD (95% confidence interval). $n = 10$. *Significant difference from morning, $P < 0.05$. †Calculation using data from nine subjects who showed a core temperature threshold for increases in minute ventilation.
evening: 105 ± 7 vs. 104 ± 7 mmHg), after which it was maintained constant at the 10-min level until 48.5 min (morning vs. evening: 99 ± 7 vs. 100 ± 10 mmHg; \( P > 0.87 \)). Ratings of perceived exertion increased from 12.3 ± 1.6 at 5 min to 17.0 ± 0.9 at 48.5 min in the morning, and from 12.7 ± 1.4 to 16.6 ± 2.5 in the evening (both \( P < 0.001 \)), without a significant main effect of time of day (\( P = 0.52 \)).

**Ventilatory responses.** The time course of changes in respiratory variables are shown in Fig. 1B (\( \dot{V}E \)) and C (\( f \)). There was a significant main effect of exercise duration on \( \dot{V}E, V_T, f, PETCO_2, VO_2, VCO_2, \dot{V}E/VO_2, \dot{V}E/VCO_2, \) and the respiratory exchange ratio (all \( P < 0.01 \)), while time-of-day had no main effect on any of these variables (\( \dot{V}E, P = 0.44; V_T, P = 0.61; f, P = 0.51; PETCO_2, P = 0.44; VO_2, P = 0.32; VCO_2, P = 0.22; \dot{V}E/VO_2, P = 0.81; \dot{V}E/VCO_2, P = 0.62; \) and respiratory exchange ratio, \( P = 0.31 \)). This indicates that ventilatory responses during prolonged exercise in the heat are unaffected by time of day.

**\( T_{es} \)-Dependent Changes in Ventilatory Responses**

Figure 2 shows the relationships between \( T_{es} \) and ventilatory variables in protocol 1 (30-s averaged data for 10 subjects). The \( T_{es} \) threshold and the sensitivity of the variables are shown in Table 1. Nine of the ten subjects showed a \( T_{es} \) threshold for increases in \( \dot{V}E \) in both the morning and evening, and \( \dot{V}E \) increased linearly with rising \( T_{es} \) above that threshold. The threshold for increases in \( \dot{V}E \) was 0.6°C higher in the evening than the morning, which is a significant change. This indicates that ventilatory responses to the same core temperature are lower in the evening than in the morning. In addition, there was no time-of-day difference between the relative increase in \( T_{es} \) from preexercise resting level to the level at which increases in \( \dot{V}E \) occurred (morning vs. evening, 1.0 ± 0.6 vs. 1.2 ± 0.4°C, \( P = 0.29 \)). The \( T_{es} \) thresholds for increases in \( f, \dot{V}E/VO_2 \) and \( \dot{V}E/VCO_2 \), and for decreases in \( PETCO_2 (n = 10) \) were all higher in the evening than in the morning. The sensitivities of \( \dot{V}E, \dot{V}E/VO_2, \) and \( PETCO_2 \) were all greater in the evening.

**Protocol 2**

**Baseline resting data.** Baseline resting \( T_{es} \) (36.9 ± 0.2 vs. 36.3 ± 0.2°C), \( T_{sk} \) (35.6 ± 0.5 vs. 35.1 ± 0.3°C), \( \dot{V}E \) (9.4 ± 0.8 vs. 8.7 ± 1.1 l/min), and \( f \) (17.6 ± 2.9 vs. 16.2 ± 2.1 breaths/min) were higher in the evening than in the morning (all \( P < 0.02 \)).

**Time Course of Changes in Physiological Responses During Exercise**

**Body temperatures and cardiovascular responses.** Because the preexercise cold-water immersion was longer in the evening (50 min) than the morning (25 min), preexercise resting \( T_{es} \) after the immersion was similar in morning and evening (both 35.6 ± 0.7°C, \( P = 0.90 \)). \( T_{es} \) during subsequent exercise also did not significantly differ between morning and evening (all \( P > 0.06 \)) (Fig. 3A). However, there was a significant interaction between time-of-day and exercise duration that affected \( T_{sk} (P < 0.01) \), which was slightly but significantly higher in the evening than the morning at 30 to 35 min.

There was a significant main effect of exercise duration (\( P < 0.01 \)), but not time of day (\( P = 0.09 \)), on heart rate. There was also a significant interaction between time of day and exercise duration that affected mean arterial pressure (\( P < 0.01 \)), which was significantly higher in the evening at 0 to 5 min.

**Ventilatory responses.** The time course of changes in respiratory variables are shown in Table 2 and Fig. 3B (\( \dot{V}E \)) and C (\( f \)). There were significant interactions between time-of-day and exercise duration that affected \( \dot{V}E, f, PETCO_2, VO_2, VCO_2, \dot{V}E/VO_2, \dot{V}E/VCO_2 \), and the respiratory exchange ratio (all \( P < 0.02 \)). \( \dot{V}E \) was significantly lower in the evening than the morning at 5 min and 35 to 55 min (Fig. 3B). \( f \) was significantly lower in the evening than the morning at 15 min and 55 min (Fig. 3C). \( \dot{V}E/VO_2 \) was significantly lower and \( \dot{V}E/VCO_2 \) tended to be lower in the evening than the morning at 55 min (\( P = \)
Quantitatively compare the control of ventilation in response to rising core temperature (4, 13, 14, 20, 46, 47, 50). Our main finding of a higher $T_{es}$ threshold for increases in $V\dot{E}$ in the evening is adjusted down to the level seen in the morning. Resting $T_{es}$ is smaller in the evening than the morning. Resting level to the level at which increases in $V\dot{E}$ occurred was normally higher in the evening than morning due to natural diurnal variation. $T_{es}$-dependent changes in ventilatory responses. 

| $T_{es}$-dependent changes in ventilatory responses. Figure 4 shows the relationships between $T_{es}$ and the ventilatory variables during protocol 2 (30-s averaged data from nine subjects). The $T_{es}$ threshold and the sensitivity of the variables are shown in Table 3. The results showed that although the preexercise $T_{es}$ was similar in the morning and evening, the threshold for $V_{E}$ was higher in the evening than in the morning. In addition, the relative increase in $T_{es}$ from the preexercise resting level to the level at which increases in $V_{E}$ occurred was higher in the evening than in the morning (morning vs. evening, 1.0 ± 0.7 vs. 1.6 ± 0.8°C, $P < 0.01$). The thresholds for $f$, $V_{E}/V_{O_{2}}$, $V_{E}/V_{CO_{2}}$, and $PET_{CO_{2}}$ were higher in the evening. The sensitivity of $V_{E}$ was greater in the evening than the morning, which was also the case in protocol 1.

**DISCUSSION**

The present study investigated the effect of time of day on heat-induced hyperventilation during prolonged exercise. We found that 1) time of day has no effect on the time course of the increase in $V_{E}$ during prolonged exercise in the heat, and 2) the $T_{es}$ threshold for hyperventilation during exercise is higher in the evening than the morning, which means the ventilatory response to the same core temperature is smaller in the evening than in the morning. Resting $T_{es}$ is normally higher in the evening than morning due to natural diurnal variation. 3) We found that when resting $T_{es}$ in the evening is adjusted down to the level seen in the morning prior to exercise, the time course of the increase in $V_{E}$ during exercise is smaller in the evening than morning. 4) Moreover, the threshold for hyperventilation remains higher in the evening. These findings demonstrate that the absence of a time-of-day effect on the time course of the hyperventilatory response observed during prolonged exercise in the heat can be attributed to diurnal variation in the ventilatory response to the same core temperature.

**Time-of-Day Effect on the Time Courses of the Changes in Core Temperature and the Ventilatory Response**

Consistent with earlier studies, which showed diurnal rhythms for core temperature at rest (27, 40–42) and during prolonged exercise (2, 24, 31, 44) in both thermoneutral and hot environments, baseline resting and exercising $T_{es}$ were ~0.5°C higher in the evening than the early morning. Reilly and Brooks (37, 38) detected a diurnal rhythm in resting $V_{E}$, while others found that $V_{E}$ exhibits no diurnal variation during exercise at a constant workload in thermoneutral (8, 15, 17) and hot (24) environments. Hobson et al. (24) recently reported that, during exercise at 65% $V_{O_{2}}$ peak in the heat (35°C), $V_{E}$ measured after 15 min of exercise was similar in the morning and evening. We have extended that finding by showing that the time course of the change in $V_{E}$ during prolonged submaximal exercise in the heat is unaffected by time of day. The reason for the absence of a time-of-day effect on $V_{E}$ during exercise may be related to the observed change in the control of ventilation in response to rising core temperature, as discussed below.

**Time-of-Day Effect on Control of Ventilation in Response to Rising Core Temperature**

Determining threshold and sensitivity by plotting $V_{E}$ as an output response against $T_{es}$ as thermal input enables one to quantitatively compare the control of ventilation in response to rising core temperature (4, 13, 14, 20, 46, 47, 50). Our main finding of a higher $T_{es}$ threshold for increases in $V_{E}$ in the evening (Fig. 2A) indicates the ventilatory response to the same core temperature is smaller in the evening than in the morning. We speculate that this reduction in the ventilatory response to
temperature in the evening is responsible for the absence of a time-of-day effect on the time course of the change in $V_E$ during exercise, despite the higher core temperature in the evening. In support of the diurnal variation in the threshold for hyperventilation in humans, Graf (16) and Heller et al. (21) found that, when the spinal cord in diurnally active pigeons was passively heated during the day and at midnight, the spinal cord showed that, when the spinal cord in diurnally active pigeons hyperventilation in humans, Graf (16) and Heller et al. (21) even evening. In support of the diurnal variation in the threshold for hyperventilation during exercise, despite the higher core temperature in the evening, it is likely that absolute core temperature rather than relative increases in core temperature is an important factor driving the observed heat-induced hyperventilation. In addition, our observation that the time course of changes in $V_E$ and $f$ were lower during exercise in the evening than morning, although the $T_{es}$ were similar at the two times (Fig. 3), strongly supports the aforementioned suggestion that the ventilatory response to the same core temperature is smaller in the evening.

Several factors may contribute to the core temperature threshold for hyperventilation during exercise in the heat. The possible mechanisms underlying hyperventilation induced by progressive hyperthermia include 1) increased activity of respiratory pacemaker neurons in the medulla oblongata due to the rising temperature (9, 45), 2) increased afferent input from group III and IV due to rising muscle temperature (23, 26), and 3) increased efferent output from the cerebral cortex (central command) (3). Perhaps one or more of these responses exhibit circadian variation, which could contribute to the difference between the core temperature threshold for hyperventilation in the morning and evening.

The sensitivity of $V_E$ to rising $T_{es}$ was greater in the evening (Fig. 2A), suggesting that, contrary to the effect of change in threshold, the ventilatory response to thermal input is greater in the evening than in the morning. Therefore, we speculate that the time course of the change in $V_E$ during exercise would be greater in the evening than in the morning under conditions where core temperature is above $38.5^\circ C$, which we could not adequately evaluate. In this regard, we analyzed $V_E$ at the same $T_{es}$ level [three levels: 37.4°C (range 37.2–37.6°C), 37.8°C (37.6–38.0°C), and 38.2°C (38.0–38.4°C)] in the morning and evening. We found that $V_E$ at 37.4°C tended to be lower in the evening (morning vs. evening 65.6 vs. 60.7 l/min, $P = 0.13$), but the difference in $V_E$ became smaller at 37.8°C (69.3 vs. 67.5 l/min, $P = 0.66$), and $V_E$ at 38.2°C was higher in the evening, on average (71.4 vs. 74.1 l/min, $P = 0.54$). These results may indirectly support the aforementioned specula-
Tsk. We previously found that the effect of Tsk on the slope of had an effect on the difference in the sensitivity of V˙E time of day. Therefore, it is unlikely that metabolic factors ventilatory responses (49), the sensitivities of V˙E/V˙O2 and the other hand, although V˙O2 and V˙CO2 are known to affect diurnal variation such that the ventilatory response to hy-

Therefore, we cannot conclude that V˙E during exercise is

the cutaneous thermoreceptors over the entire body surface.

the preexercise resting value used to calculate Tsk in this study, which is based on relative surface areas, may not precisely reflect the activity of the cutaneous thermoreceptors over the entire body surface. Therefore, we cannot conclude that V˙E during exercise is unaffected by thermal input from the skin. On the other hand, we also think that the effect of skin temperature on the ventilatory response with rising core temperature may differ between exercising and resting subjects, based on the earlier finding that decreasing Tsk from 35.3 to 33.8°C through acute cooling alleviated the ventilatory response during passive heating at rest (28).

Perspectives and Significance

The present study is the first to suggest that the absence of a time-of-day effect on the time course of the increases in V˙E during prolonged exercise in the heat is due to diurnal variation in the control of ventilation in response to rising core temperature, as reflected by the higher Tes threshold for hyperventilation in the evening than in the morning. An understanding of the time-of-day effect on hyperthermic hyperventilation is important because hyperventilation-induced decreases in PacO2 contribute to cerebral hypoperfusion during hyperthermia (6, 11, 19, 32, 36), which can sometimes lead to elevated brain temperature (34). Given the similar increases in V˙E and decreases in PETCO2 with exercise time in this study, as well as the absence of a time-of-day effect on cerebrovascular reactivity to decreases in PETCO2 (1), we speculate that time of day has little effect on cerebral blood flow during hyperthermic exercise.

Conclusion

In the present study, time of day had no effect on the time course of the hyperventilatory response and hypoxia, even though core temperature, the thermal stimulus to increase V˙E, was higher in the evening than in the morning. By contrast, the ventilatory response to the same core temperature was smaller in the evening than in the morning, as reflected by higher core temperature threshold for hyperventilation in the evening. We suggest that this diurnal variation in the control of ventilation in response to rising core temperature is responsible for the absence of a time-of-day effect on the time course of the ventilatory response during prolonged exercise in the heat.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

B.T., N.K., and T.N. conception and design of research; B.T. performed experiments; B.T. and Y.H. analyzed data; B.T., N.K., and T.N. interpreted results of experiments; B.T. prepared figures; B.T. drafted manuscript; B.T., Y.H., N.K., and T.N. edited and revised manuscript; B.T., Y.H., N.K., and T.N. approved final version of manuscript.

Table 3. Core temperature thresholds and sensitivities calculated after plotting the indicated variables against esophageal temperature in protocol 2

<table>
<thead>
<tr>
<th></th>
<th>Morning</th>
<th>Evening</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold, °C</td>
<td>36.5 ± 0.7 (36.0–37.1)</td>
<td>37.2 ± 0.7 (36.7–37.7)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Minute ventilation</td>
<td>11.4 ± 5.8 (6.9–15.9)</td>
<td>17.7 ± 13.1 (7.6–27.8)*</td>
<td>0.04</td>
</tr>
<tr>
<td>Tidal volume</td>
<td>120.8 ± 62.2 (-175.8 to -80.1)</td>
<td>106.3 ± 63.6 (-155.2 to -57.4)</td>
<td>0.43</td>
</tr>
<tr>
<td>Respiratory frequency, breaths·min⁻¹·°C⁻¹</td>
<td>15.4 ± 6.1 (10.7–20.1)</td>
<td>20.5 ± 12.0 (11.3–29.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>End-tidal CO2 pressure, Torr/°C</td>
<td>-4.2 ± 2.3 (-6.0 to -2.4)</td>
<td>-7.6 ± 7.8 (-13.6 to -1.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Ventilatory equivalent for VO₂, units/°C</td>
<td>3.9 ± 2.8 (1.7–6.0)</td>
<td>7.6 ± 9.2 (0.6–14.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Ventilatory equivalent for VCO₂, units/°C</td>
<td>5.0 ± 2.9 (2.8–7.3)</td>
<td>8.0 ± 8.2 (1.6–14.3)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values are means ± SD (95% confidence interval); n = 9. *Significant difference from morning. P < 0.05.
REFERENCES


