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Chronobiological effects on exercise performance and selected physiological responses

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Abstract Previous studies investigating the impact of circadian rhythms on physiological variables during exercise have yielded conflicting results. The purpose of the present investigation was to examine maximal aerobic exercise performance, as well as the physiological and psychophysiological responses to exercise, at four different intervals (0800 hours, 1200 hours, 1600 hours, and 2000 hours) within the segment of the 24-h day in which strenuous physical activity is typically performed. Ten physically fit, but untrained, male university students served as subjects. The results revealed that exercise performance was unaffected by chronobiological effects. Similarly, oxygen uptake, minute ventilation and heart rate showed no time of day influences under pre-, submaximal, and maximal exercise conditions. Ratings of perceived exertion were unaffected by time of day effects during submaximal and maximal exercise. In contrast, rectal temperature exhibited a significant chronobiological rhythm under all three conditions. Under pre- and submaximal exercise conditions, significant time of day effects were noted for respiratory exchange ratio, while a significant rhythmicity of blood pressure was evident during maximal exercise. However, none of these physiological variables exhibited significant differential responses (percent change from pre-exercise values) to the exercise stimulus at any of the four time points selected for study. Conversely, resting plasma lactate levels and lactate responses to maximal exercise were found to be significantly sensitive to chronobiological influences. Absolute post-exercise plasma norepinephrine values, and norepinephrine responses to exercise (percent change from pre-exercise values), also fluctuated significantly among the time points studied. In summary, these data suggest that aerobic exercise performance does not vary during the time frame within which exercise is normally conducted, despite the fact that some important physiological responses to exercise do fluctuate within that time period.

Key words Circadian · Catecholamines · Lactate · Metabolism · Respiration

Introduction

Numerous investigators have found that under resting conditions most physiological parameters demonstrate cyclic variations that correspond roughly with the 24-h day. These circadian rhythms have been established for resting heart rate (Faria and Drummond 1982; Reilly and Brooks 1990), rectal temperature (Cabri et al. 1988; Reilly and Brooks 1986), blood pressure (Millar-Craig et al. 1978; Reilly et al. 1984), metabolic variables (Reilly and Brooks 1982, 1990), and circulating hormones (Akerstedt and Levi 1978; Katz et al. 1975), including catecholamines (Stene et al. 1980). Several investigations have revealed that physical or athletic performance may also be influenced by circadian fluctuations. For example, isometric strength appears to be greater in the afternoon than in the morning (Coldwells et al. 1994; Rutenfranz and Colquhoun 1979). More complex athletic skills such as swimming performance also appear to be affected by the time of day at which they are performed (Baxter and Reilly 1983).

Studies reporting the physiological responses to exercise performed at different times of the day appear to be equivocal. For example, maximal oxygen consump-

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tion ($\dot{V}O_2$) has been reported by some to vary according to the time of day at which exercise is performed (Hill et al. 1989; Wojtczak-Jaroszowa and Banaszkiewicz 1974), while other investigations fail to confirm any biorhythmic variation in peak $\dot{V}O_2$ (Faria and Drummond 1982; Reilly and Brooks 1990; Torii et al. 1992). Similarly, the data concerning diurnal effects upon heart rate during maximal exercise are conflicting. A positive time of day effect was noted by Reilly et al. (1984), but not by Cohen (1980). Minute ventilation ($\dot{V}_E$) during maximal aerobic exercise may (Hill et al. 1989), or may not (Reilly and Brooks 1990) demonstrate circadian rhythmicity. Even the psychophysiological parameter of perceived exertion (Borg 1970) may be sensitive (Faria and Drummond 1982; Hill et al. 1989), or resistant (Reilly et al. 1984) to circadian variability.

Some of the contradictions observed in these previous investigations may be related to the times of day selected for study. For instance, the rhythmicity in heart rate appears to be confirmed only when exercise testing is performed in the early hours of the morning (i.e., 0200 hours–0600 hours), when heart rate values are at their nadir (Reilly and Brooks 1990; Reilly et al. 1984). Similar results have been observed for perception of exertion during exercise (Faria and Drummond 1982). The purpose of the present study was therefore to determine whether the physiological and psychophysiological responses to exercise are differentially affected by time of day during the segment of the solar day that most individuals participate in strenuous physical activity.

Methods

Subjects

Ten healthy male university students [mean (SD) age 21.1 (3.0) years, height 1.8 (0.1) m, body mass 80.2 (8.3) kg, body fat 11.6 (3.4)%] volunteered to participate in the study. These individuals were active, but were not involved in any formal exercise training program. After receiving a verbal description of the purpose of the study and the experimental procedures to be employed, the subjects provided written consent. In addition, a physician reviewed each subject's medical records before approving his inclusion into the study. All experimental procedures were approved by the Committee for the Protection of Human Subjects at The College of William and Mary.

Experimental design

Each subject performed four exhaustive aerobic power tests on an electrically braked cycle ergometer (Excalibur Unit, Groningen, The Netherlands). For each volunteer, tests were performed at 0800 hours, 1200 hours, 1600 hours, and 2000 hours in a randomized fashion. These times were selected because they represent the time frame in which people typically engage in physical activity. At least 48 h separated any two consecutive tests and subjects refrained from exercise for a minimum of 24 h prior to testing. Volunteers consumed only water for at least 8 h prior to arriving at the laboratory and reported normal sleeping habits for the duration of the study. Each subject completed all test sessions within a 4-week interval.

Upon arrival for their first test session, each subject's body mass, height, and estimated body composition (Jackson and Pollock 1978) were determined and recorded. During every test session the subjects arrived in the laboratory 30 min prior to the scheduled time for the cycle ergometer test. The volunteer then inserted a rectal temperature probe to ≈150 mm beyond the external anal sphincter (Saltin and Hermansen 1966), and a 20-gauge Teflon catheter fitted with a male adapter was placed into an antecubital vein and kept patent with an isotonic saline solution. Subjects then sat quietly in a chair for 20 min preceding the cycle ergometer test. At the end of this equilibration period a pre-exercise blood sample was drawn.

Exercise testing

At each test session, an exercise protocol featuring progressively increasing intensity was performed to the point of volitional exhaustion: verbal encouragement was provided during all test sessions. Initially the subject sat on the ergometer for 5 min without performing exercise so that pre-exercise cardiorespiratory data could be collected. Following this, a 2-min cycling warm-up was performed at a resistance of 40 W. Stage 1 of the actual test consisted of 2 min of cycling against 80 W of resistance. Each subsequent stage was 2 min in duration with an increased resistance of 30 W. During the last 30 s of each stage heart rate, rectal temperature, blood pressure, and ratings of perceived exertion (RPE) were recorded. Metabolic data were recorded during the last 15 s of each exercise intensity, and immediately before exhaustion. A second blood sample was taken immediately (<20 s) following the cessation of exercise.

Quantitation

Heart rates were determined via a portable telemetry unit (Cardiochamp, Sensor Dynamics, Sacramento, Calif., USA) that was secured around the subject's chest. Rectal temperature was monitored using a thermistor connected to a digital thermometer (model 400, VWR Scientific, Bridgeport, N.J., USA). Blood pressure was assessed via a sphygmomanometer and stethoscope (Moore Medical, New Britain, Conn., USA). Mean arterial blood pressure was calculated as the diastolic pressure plus 33% of the difference between the systolic and diastolic pressures. This value represents the average pressure driving blood into the tissue over the entire cardiac cycle (Vander et al. 1994).

To determine metabolic variables ($\dot{V}_E$, $\dot{V}O_2$, and respiratory exchange ratio, *R*), expired air samples were analyzed using an open-circuit, on-line system (Q-plex I, Quinton Instrument, Seattle, Wash., USA). Blood samples were collected into ethylenediaminetetraacetic acid- treated tubes. Aliquots of whole blood were used immediately for hemoglobin and hematocrit measurements. Hematocrit was measured in triplicate using microcapillary tubes following centrifugation at 4000 *g* for 5 min, while hemoglobin values were determined via the cyanmethemoglobin method. Changes in plasma volume were calculated from hematocrit and hemoglobin values, according to Dill and Costill (1974). The remaining blood was centrifuged at 3000 *g* for 15 min at 5°C. The plasma fraction was stored at −75°C until analyses for lactate and catecholamine values were performed. Plasma lactate was measured in duplicate using an automated analyzer (YSI model 23L, Yellow Springs Instruments, Yellow Springs, Ohio, USA). To determine plasma catecholamine concentrations, a high performance liquid chromatography instrument with an electrochemical detector and automated injection system (Millipore, Milford, Mass, USA) was utilized. Samples were analyzed in duplicate with a sensitivity of 0.01 $ng \cdot ml^{-1}$. The intra-assay coefficients of variation for epinephrine and norepinephrine were 9.4% and 10.1%, respectively, while the inter-assay coefficients of variation were 23.7% and 16.6%, respectively. All samples for any one subject were analyzed in the same run to control for inter-assay variation. Catecholamine data were analyzed using a Maxima 820 data module (Millipore). Hormone values were not corrected for plasma volume shifts.

Statistical analysis

Repeated measures analyses of variance were used to compare variables across the four time points (0800 hours, 1200 hours, 1600 hours, 2000 hours) measured. When significant *F* ratios were established, a Fisher PLSD post-hoc analysis was used to determine pairwise differences. Statistical significance was set at $P < 0.05$.

Results

Pre-exercise data

Pre-exercise data are given in Table 1. In the present investigation, there were no significant differences established in pre-exercise $\dot{V}O_2$ or $\dot{V}_E$ during the times of day that testing was performed. Neither of the cardiovascular variables of mean arterial blood pressure nor heart rate demonstrated significant rhythmicity before exercise. While most of the blood variables measured (i.e., hematocrit, hemoglobin and catecholamines) failed to show significant chronobiological effects, pre-exercise lactate values at 0800 hours were significantly greater ($P = 0.04$) than those values recorded at 1600 hours and 2000 hours. In addition, significant time of day effects were detected for rectal temperature ($P = 0.001$) and *R* ($P = 0.01$). Rectal temperature was found to be less at 0800 hours than at all other time points measured. Also noteworthy was the trend for rectal temperature to increase progressively from 0800 hours to 2000 hours. Conversely, *R* was determined to be greatest at 0800 hours, and lowest at 1600 hours.

Submaximal exercise data

Data gathered during submaximal exercise are given in Table 2. These data are values recorded during the 2-min exercise stage representing the midpoint [7.6 (1.5) min; mean (SD)] of the duration of each exercise test, and thus were not taken under steady-state conditions.

As with pre-exercise conditions, the only physiological variables to show significant time of day effects at submaximal exercise were *R* ($P = 0.03$) and rectal temperature ($P = 0.001$), and their patterns of variation emulated those seen at rest. That is, *R* values were lowest at 1600 hours while temperature during submaximal exercise was lowest at 0800 hours. It is important to note, however, that the exercise-induced response (i.e., change from pre- to exercise values) of *R* and rectal temperature were not differentially affected at the time points studied. While mean arterial blood pressure exhibited no significant time of day effect during submaximal exercise, a nonsignificant degree of rhythmicity ($P = 0.10$) was observed, with a nadir recorded at 0800 hours.

RPE during short-duration submaximal exercise did not vary significantly among the four time periods examined in this study. These results are consistent with

Table 1 Data recorded under pre-exercise conditions at the time points investigated. Values are given as the mean (SD) of $n = 10$ subjects. ($\dot{V}O_2$ Oxygen uptake, $\dot{V}_E$ minute ventilation, *R* respiratory exchange ratio, *HR* heart rate, *MABP* mean arterial blood pressure)

Variable	0800 hours	1200 hours	1600 hours	2000 hours
$\dot{V}O_2$ (ml · kg^{-1} · min^{-1})	5.0 (1.2)	5.5 (0.9)	5.8 (3.9)	5.5 (0.8)
$\dot{V}_E$ (l · min^{-1})	11.7 (3.4)	14.0 (6.1)	13.3 (7.1)	12.4 (4.1)
R	0.81 (0.08)*	0.75 (0.06)	0.70 (0.05)	0.72 (0.07)
HR (beats · min^{-1})	80.6 (12.1)	77.3 (8.6)	78.9 (12.6)	78.4 (10.6)
MABP (mm/Hg)	90.5 (4.7)	93.8 (6.8)	95.8 (10.8)	92.7 (7.4)
Rectal temperature (°C)	36.6 (0.5)**	37.0 (0.4)	37.2 (0.3)	37.4 (0.2)
Plasma lactate (mmol · l^{-1})	1.1 (0.2)***	0.9 (0.2)	0.8 (0.2)	0.9 (0.1)
Epinephrine (pmol · l^{-1})	469.4 (720.5)	125.5 (120.1)	693.2 (655.0)	611.3 (562.2)
Norepinephrine (nmol · l^{-1})	1.5 (0.5)	1.7 (0.8)	1.7 (0.7)	1.4 (0.4)

* Significant difference from *R* at 1600 hours and 2000 hours

** Significant difference from rectal temperature at 1200 hours, 1600 hours and 2000 hours

*** Significant difference from plasma lactate at 1600 hours and 2000 hours

Table 2 Data recorded under submaximal exercise conditions at the time points investigated. Values given as the mean (SD) of $n = 10$ subjects. (*RPE* rating of perceived exertion)

Variable	0800 hours	1200 hours	1600 hours	2000 hours
$\dot{V}O_2$ (ml · kg^{-1} · min^{-1})	31.7 (3.9)	32.7 (5.5)	33.8 (6.8)	32.6 (5.5)
$\dot{V}_E$ (l · min^{-1})	65.5 (10.9)	66.4 (13.9)	64.4 (14.8)	64.8 (12.5)
R	0.93 (0.05)	0.93 (0.04)	0.88 (0.05)*	0.91 (0.04)
HR (beats · min^{-1})	156.8 (11.3)	159.2 (13.5)	158.4 (12.7)	157.8 (13.2)
MABP (mm/Hg)	101.6 (4.8)	107.6 (6.7)	109.3 (8.4)	106.0 (8.0)
Rectal temperature (°C)	36.7 (0.5)**	37.2 (0.4)***	37.4 (0.3)	37.6 (0.1)
RPE	14.4 (1.5)	14.4 (1.8)	15.1 (1.2)	15.1 (1.3)

* Significant difference from *R* at 0800 hours and 1200 hours

** Significant difference from rectal temperature at 1200 hours, 1600 hours and 2000 hours

*** Significant difference from rectal temperature at 2000 hours

Table 3 Data recorded under maximal exercise conditions at the time points investigated. Values are given as the mean (SD) of $n = 10$ subjects

Variable	0800 hours	1200 hours	1600 hours	2000 hours
Test duration (min)	13.7 (2.9)	14.2 (2.8)	14.1 (2.8)	14.4 (2.7)
$\dot{V}O_2$ (ml · kg^{-1} · min^{-1})	52.0 (7.0)	55.1 (10.2)	56.7 (9.5)	56.9 (10.2)
$\dot{V}_E$ (l · min^{-1})	140.3 (15.3)	150.9 (30.7)	152.7 (23.3)	155.4 (26.8)
R	0.97 (0.05)	0.96 (0.06)	0.92 (0.07)	0.95 (0.08)
HR (beats · min^{-1})	192.8 (9.5)	195.5 (8.3)	193.8 (9.4)	196.3 (7.9)
MABP (mm/Hg)	109.6 (4.1)*	116.4 (5.3)	118.1 (5.8)	115.1 (8.0)
Rectal temperature (°C)	36.9 (0.4)**	37.5 (0.4)***	37.6 (0.2)	37.9 (0.1)
RPE	19.2 (1.0)	19.8 (0.6)	19.7 (0.7)	19.6 (0.7)
Plasma volume shift (%)	−21.8 (5.3)	−22.4 (8.4)	−22.9 (4.9)	−21.2 (4.9)
Plasma lactate (mmol · l^{-1})	11.9 (2.4)	13.2 (3.4)	12.7 (2.3)	14.0 (3.1)
Epinephrine (pmol · l^{-1})	3951.6 (2958.2)	3198.4 (1484.6)	4180.8 (2887.3)	4382.8 (4033.5)
Norepinephrine (nmol · l^{-1})	25.2 (9.3)†	34.8 (16.8)	30.3 (11.3)	29.7 (13.9)

* Significant difference from MABP at 1200 hours and 1600 hours
** Significant difference from rectal temperature at 1200 hours, 1600 hours and 2000 hours
*** Significant difference from rectal temperature at 2000 hours
† Significant difference from norepinephrine concentration at 1200 hours

previously reported data (O'Connor and Davis 1992; Reilly et al. 1984).

Maximal exercise data

Data collected during maximal exercise are given in Table 3. No differences in time to exhaustion during maximal exercise testing were found among the four time points selected for study ($P = 0.96$). Similar to the results obtained during submaximal exercise, no statistically significant time of day effects were noted for peak $\dot{V}O_2$, and $\dot{V}_E$ values. However, unlike the data gathered during submaximal exercise, $\dot{V}O_2$ and $\dot{V}_E$ at maximal exercise demonstrated progressive increases (≈9% and ≈11%, respectively) over the four chosen time points. Of the ten subjects studied, six demonstrated greater peak $\dot{V}O_2$ values, and seven experienced higher maximal $\dot{V}_E$ values at 2000 hours compared with 0800 hours.

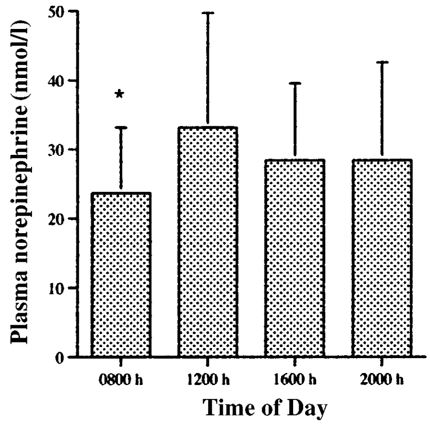

Fig. 1 Exercise induced responses (pre- to post-exercise differences) of plasma norepinephrine concentrations at time points investigated. Values are mean ± (SD). $n = 10$. *Indicates significant difference from value at 1200 h

As noted during pre-exercise, no significant rhythmicity in the blood variables of epinephrine, hematocrit, and hemoglobin was determined. The absence of time of day effects on hematocrit and hemoglobin values led to comparable exercise-related plasma volume shifts among the time points investigated ($P = 0.94$). Yet other blood-borne variables did demonstrate a significant sensitivity to biorhythmic influences. For example, unlike plasma epinephrine, plasma norepinephrine concentrations did reveal a significant ($P = 0.03$) biorhythmic response to maximal exercise; these values being lowest at 0800 hours (Fig. 1). In addition, post-exercise plasma lactate values revealed chronobiological effects that approached statistical significance ($P = 0.07$). Due to the significant differences in pre-exercise lactate concentrations, the elevations in plasma lactate from resting to post-exercise conditions demonstrated significant ($P = 0.03$) differences among the time points selected for study. Specifically, the lactate response at 0800 hours was significantly less than those detected at 1200 hours, 1600 hours, and 2000 hours (Fig. 2).

While *R* had exhibited significant time of day variation at pre- and submaximal exercise, no such effect was detected during maximal exercise. Conversely, the significant variation noted for rectal temperature across the four time points during rest and submaximal exercise persisted during exhaustive exercise. However, similar to the results observed during submaximal exercise, the actual increase in rectal temperature in response to exhaustive exercise demonstrated no significant rhythmicity. Thus, time of day did not affect the temperature response to exercise.

During maximal exercise, heart rate continued to be impervious to time of day influences. In contrast, the non-significant ($P = 0.10$) sensitivity of mean arterial blood pressure to the chronobiological effects observed during submaximal exercise was amplified during maximal exercise ($P = 0.02$). This significant difference in

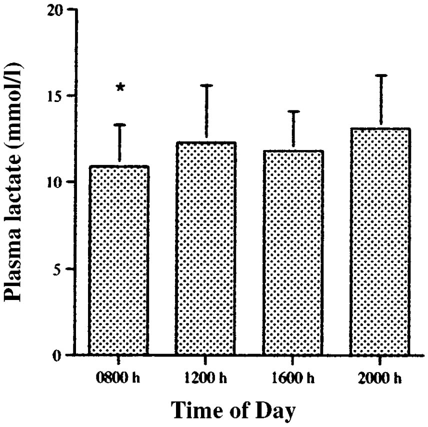

Fig. 2 Exercise induced responses (pre- to post-exercise differences) of plasma lactate concentrations at time points investigated. Values are means ± (SD). $n = 10$. *Indicates significant difference from value at 2000 h

mean arterial blood pressure was related primarily to a significant ($P = 0.04$) time of day impact on systolic blood pressure that was not evident during pre- or submaximal exercise. However, the actual response of mean arterial blood pressure to maximal exercise failed to display any significant sensitivity to chronobiological rhythms.

As with submaximal exercise, the RPE values recorded at maximal exercise intensity did not reveal any significant time of day effect. These data are supported by the findings of Reilly et al. (1984).

Discussion

In general, the literature concerning circadian or time of day effects on exercise performance and physiological responses to exercise yields inconclusive results. In part, these conflicting data may be explained by the selection of time points during the day used to investigate exercise performance and physiological responses to the stimulus of exercise. "Circadian" refers to the 24-h cycle that comprises a day. Thus, to accurately assess the circadian effects on physical performance, tests should be conducted at time points that are separated by equal increments encompassing the solar day. Such studies (Cohen 1980; Millar-Craig et al. 1978; Reilly and Brooks 1986; Reilly et al. 1984) typically report significant circadian effects on variables such as heart rate and rectal temperature during rest and exercise. In contrast, other investigators (Hill et al. 1989, 1992; Stephenson et al. 1989) have not studied exercise performance and physiological responses at regular intervals throughout the solar day. Rather, single time points before and after 1200 hours were chosen to study what are most accurately described as the time of day effects on functional capacity and on the physiological responses to exercise. In the present study, four equally dispersed time points (0800 hours, 1200 hours, 1600 hours, 2000 hours) were selected. These time points span the portion of the day during which people typically participate in physical activity. It was postulated that the data gathered at these time points would provide meaningful and useful information for practitioners and researchers. Time of day effects on selected physiological variables were studied under pre-exercise, submaximal exercise, and maximal exercise conditions.

Pre-exercise conditions

The data presented here demonstrate a significant time of day effect on pre-exercise rectal temperature, which increased progressively at each of the four time periods selected for study. These results were not surprising since gradual increases in resting temperature during waking hours have been reported consistently (Faria and Drummond 1982; Reilly and Brooks 1990; Stephenson et al. 1989). *R*, which was found to be greatest at 0800 hours, also exhibited a significant time of day effect. This result was somewhat surprising as it suggests a greater reliance on non-fat energy substrate utilization in the morning following a night's sleep. To our knowledge, no previous study has documented circadian or time of day effects on *R*. The chronobiological oscillation of pre-exercise plasma lactate levels was found to mirror that of *R*. That is, both pre-exercise lactate and *R* values were significantly higher at 0800 hours than their corresponding values at 1600 hours and 2000 hours. However, this similarity appears to be purely coincidental since correlational analyses have revealed a virtually complete absence of shared variance. Indeed, while pre-exercise plasma lactate concentrations were found to vary significantly among the times of day investigated, the actual differences in the values were so slight that they were probably not physiologically meaningful.

Submaximal exercise conditions

As with pre-exercise conditions, most physiological variables were resistant to biorhythmic influences during submaximal exercise. These data are consistent with earlier reports in which it is suggested that $\dot{V}O_2$ (McMurray et al. 1990; Reilly and Brooks 1982), $\dot{V}_E$ (Reilly and Brooks 1990), and heart rate (Cohen 1980; O'Connor and Davis 1992) do not display variability according to the time of day when exercise of submaximal intensity is performed. This is true whether data are obtained under steady-state exercise conditions (McMurray et al. 1990; Reilly and Brooks 1982), or during submaximal workloads of progressive exercise tests brought to maximal intensity (Cohen 1980; Reilly and Brooks 1990). The submaximal exercise data of the present study were collected at the midpoint of progressive maximal exercise protocols that showed no time of day variation in test duration.

Rectal temperature did demonstrate significant rhythmicity during submaximal exercise performed at different times of the day. This variation emulated precisely that observed at rest. Specifically, rectal temperature was at its nadir at 0800 hours and increased progressively at 1200 hours, 1600 hours and 2000 hours. However, the responsiveness (i.e., change from the resting value at the same time point) of rectal temperature failed to show any time of day effects. Both the chronobiological variation in temperature during submaximal exercise and the absence of a time of day impact in the responsiveness of this variable support the results of previous studies (Reilly and Brooks 1986, 1990; Stephenson et al. 1989).

Similar to rectal temperature, the time of day differences in R detected at rest persisted during submaximal exercise, exhibiting peaks and troughs that were identical to those observed before exercise. As a result, the actual responses of R to submaximal exercise did not exhibit significant variation during the four time periods studied. The lack of any time of day influences on the responses of rectal temperature and R to submaximal exercise suggests that this stimulus exerts a constant impact on these variables that simply amplifies their resting state rhythmicity. Submaximal exercise alone does not alter the time of day effects observed in R and rectal temperature under resting conditions.

Maximal exercise conditions

Interestingly, the present investigation has revealed that despite the lack of statistically significant differences, peak $\dot{V}O_2$ and $\dot{V}_E$ were ≈10% greater at 2000 hours than at 0800 hours. Faria and Drummond (1982) documented a similar, nonsignificant trend in enhanced peak $\dot{V}O_2$ values from the early morning to early evening hours. The considerable inter-individual variation demonstrated in the data reported here accounts for the failure of these differences to reach statistical significance. Other variables, however, did demonstrate significant biorhythmic sensitivity. For example, as evident during submaximal exercise, a significant time of day variation in rectal temperature during maximal exercise was established, as was the lack of any rhythmic responsiveness of this variable to the exercise stimulus. These data are in agreement with those of previous studies (Reilly and Brooks 1986, 1990). Again, it appears that while this variable is subject to time of day effects, and that exercise does increase rectal temperature, this exercise-induced elevation is not itself sensitive to chronobiological variation. It was also determined that maximal exercise thoroughly obviated the time of day impact on R that was observed during pre- and submaximal exercise. This suggests that at maximal intensity the stimulus of exercise no longer simply enhances the time of day effects seen at rest. Rather, during all-out efforts, R is constant throughout the time periods studied, reflecting adjustments in substrate utilization necessary to provide the energy required to sustain such elevated metabolic rates.

While exercise at peak intensity negated the biorhythmicity of R observed under resting conditions, this was not true of plasma lactate values, which continued to show a tendency ($P = 0.07$) for significant time of day effects immediately following exercise. Closer inspection of the data, however, reveals that maximal exercise evokes a chronobiological effect on lactate that is opposite to that seen under pre-exercise conditions. That is, post-exercise plasma lactate values were greatest at 2000 hours, while pre-exercise lactate values were highest at 0800 hours. The responsiveness of plasma lactate to strenuous exercise was thus affected by biorhythmic influences so that lactate responses were significantly greater at 2000 hours than at 0800 hours. These data are consistent with those of previous reports which indicate that plasma lactate responses to high intensity exercise are sharpest later in the day and blunted during the morning hours (Hill et al. 1992; Reilly and Baxter 1983).

The data presented here indicate that the time of day variation in plasma lactate responses to exhaustive aerobic exercise may be related to similar fluctuations in plasma catecholamine responses to that stimulus. It is well established that catecholamines, epinephrine and norepinephrine, stimulate glycogenolysis in skeletal muscle, leading to increased lactate production (Hedge et al. 1987). This study documents a nadir in exercise-induced elevations in the circulating levels of both lactate and norepinephrine at 0800 hours. Closer inspection, however, reveals that the greatest exercise-induced responses of plasma lactate and norepinephrine were not well coupled, occurring at 2000 hours and 1200 hours, respectively. Nonetheless, a moderately high correlation ($r = 0.61$) in the chronobiological rhythmicity of these variables was established. Interestingly, a much milder correlation ($r = 0.25$) was found between time of day fluctuations in plasma lactate and epinephrine responses to the exercise challenge. This was not unexpected since the responsiveness of blood-borne epinephrine to maximal exercise was found to be unaffected by biorythmic influences ($P = 0.95$).

Another observation may help to explain the significant chronobiological variability in plasma lactate response to maximal exercise. While no significant time of day differences were detected for maximal test duration, a very high correlation ($r = 0.98$) was displayed between that variable and lactate response to exercise across the time points studied. It appears that even though the differences in exercise duration at 0800, 1200, 1600, and 2000 hours were slight, they may have contributed to the significant biorhythmicity of plasma lactate responses to all-out exertion.

The significant variation in mean arterial blood pressure observed at peak exercise intensity was accounted for mainly by a similar and significant time of day impact on systolic blood pressure: No significant oscillation occurred in diastolic blood pressure. The data reported here indicate that at the four time points examined, no significant variation in mean arterial blood pressure occurred pre-exercise, but chronobiological effects became increasingly apparent as the stress of exercise was magnified. For example, during submaximal exercise a trend ($P = 0.10$) for a variation in blood pressure was established that was enhanced with maximal exercise, resulting in a significant chronobiological effect. As with the plasma lactate response, time of day differences in blood pressure may be accounted for by similar variations in circulating norepinephrine concentrations. Catecholamines are known to increase the contractility of the myocardium (Hedge et al. 1987). This inotropic effect would alter systolic blood pressure as well as mean arterial blood pressure. These data confirm that both post-exercise systolic and mean arterial blood pressure ebbed at the same time point, 0800 hours, as plasma norepinephrine. Given the impact of catecholamines on myocardial contractility, it is likely that the observed time of day variation in systolic and mean arterial blood pressure that followed maximal exercise was related to a similar pattern in the post-exercise plasma levels of norepinephrine. Indeed, a moderately high correlation ($r = 0.78$) was observed between post-exercise values of plasma norepinephrine and mean arterial blood pressure. However, unlike norepinephrine, no significant time of day effect was established for post-exercise plasma epinephrine concentrations. Moreover, the correlation between the chronobiological fluctuations in circulating epinephrine and mean arterial blood pressure was virtually nonexistent ($r = 0.06$). The present results concerning time of day effects on catecholamine values, in conjunction with lactate and blood pressure responses to maximal exercise, suggest that under stressful physiological conditions, norepinephrine may play a greater role in regulating glycogenolysis and myocardial contractility than epinephrine. This would seem reasonable since plasma levels of norepinephrine are several-fold greater than those of epinephrine, both at rest and following stimulation. Indeed, it has been found that the responsiveness of norepinephrine to exercise is greater than that of epinephrine, and that this difference in responsiveness increases with increasing exercise intensity (Howley 1976; Schwarz and Kindermann 1990). This difference is caused mainly by the "spill-over" of norepinephrine released as a neurotransmitter by the sympathetic nervous system into the bloodstream, rather than a greater release from the adrenal gland (Hedge et al. 1987).

In summary, this investigation has shown that despite the rhythmicity of some physiological variables such as blood pressure, plasma lactate, and rectal temperature, other important variables, such as $\dot{V}O_2$ and $\dot{V}_E$, fail to demonstrate any significant sensitivity to chronobiological influences either before or during exercise. This may help to explain why maximal exercise performance, measured as time to exhaustion, remained remarkably constant during the time points examined. These data are consistent with those of previous studies (Reilly and Brooks 1986; Reilly et al. 1984; Wahlberg and Astrand 1973) which also demonstrate that despite the presence of circadian variation in some physiological parameters, maximal aerobic performance remains insensitive to such variation. The results presented here have important practical and scientific implications. From an applied or practical standpoint, it appears that maximal aerobic performance remains constant throughout the segment of the day when most physical activity, such as athletic training and contests, takes place. Despite this consistency in performance, it appears that during that same time span certain important physiological parameters are subject to chronobiological variation both at rest and during exercise. In view of this, accurate scientific investigations into human physiology and its responses to stress must consider, and adequately control for, the effects of naturally occurring biorhythmic influences.

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