In Athletes, the Diurnal Variations in Maximum Oxygen Uptake Are More Than Twice as Large as the Day-to-Day Variations

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In competitive sports any substantial individual differences in diurnal variations in maximal performance are highly relevant. Previous studies have exclusively focused on how the time of day affects performance and disregarded the maximal individual diurnal variation of performance. Thus, the aims of this study were (1) to investigate the maximum diurnal variation in maximum oxygen uptake (VO$_2$max), (2) to compare the diurnal variation of VO$_2$max during the day to the day-to-day variation in VO$_2$max, and (3) to investigate if there is a time-of-day effect on VO$_2$max. Ten male and seven female athletes (mean VO$_2$max: 58.2 ± 6.9 ml/kg/min) performed six maximal cardiopulmonary exercise tests including a verification-phase at six different times of the day (i.e., diurnal variation) and a seventh test at the same time the sixth test took place (i.e., day-to-day variation). The test times were 7:00, 10:00, 13:00, 16:00, 19:00, and 21:00. The order of exercise tests was the same for all participants to ensure sufficient recovery but the time of day of the first exercise test was randomized. We used paired t-tests to compare the nadir and peak of diurnal variations, day-to-day variations and the difference between diurnal and day-to-day variations. The mean difference in VO$_2$max was 5.0 ± 1.9 ml/kg/min (95% CI: 4.1, 6.0) for the diurnal variation and 2.0 ± 1.0 ml/kg/min (95% CI: 1.5, 2.5) for the day-to-day variation. The diurnal variation was significantly higher than the day-to-day variation with a mean difference of 3.0 ± 2.1 ml/kg/min (95% CI: 1.9, 4.1). The linear mixed effects model revealed no significant differences in VO$_2$max for any pairwise comparison between the different times of the day (all $p > 0.11$). This absence of a time-of-day effect is explained by the fact that peak VO$_2$max was achieved at different times of the day by different athletes. The diurnal variations have meaningful implications for competitive sports and need to be considered by athletes. However, the results are also relevant to research. To increase signal-to-noise-ratio in intervention studies it is necessary to conduct cardiopulmonary exercise testing at the same time of the day for pre- and post-intervention exercise tests.

Keywords: circadian, chronotype, time of day, habitual, performance
INTRODUCTION

In competitive sports any substantial individual differences in diurnal variations in maximal performance are highly relevant. As shown by several reviews (Chtourou and Souissi, 2012), the diurnal variation of short-duration performance has been examined considerably more extensively than that of long-duration endurance performance. Our systematic literature search (see Supplemental Methods) in Pubmed and Web of Science revealed 15 studies (Reilly and Baxter, 1983; Hill et al., 1988; Burgoon et al., 1992; Dalton et al., 1997; Deschenes et al., 1998; Reilly and Garrett, 1998; Atkinson et al., 2005; Brown et al., 2008; Chtourou et al., 2012; Souissi et al., 2012; Hill, 2014; Chin et al., 2015; Facer-Children and Brandstaetter, 2015; Rae et al., 2015; Aloui et al., 2017), that have examined the effect of the time of day on endurance performance with inconclusive results (Table 1). In detail, five studies showed significant differences (Atkinson et al., 2005; Chtourou et al., 2012; Hill, 2014; Facer-Children and Brandstaetter, 2015; Aloui et al., 2017), three studies showed differences in subgroups (i.e., different chronotypes) (Hill et al., 1988; Brown et al., 2008; Chin et al., 2015), and seven studies showed no significant differences (Reilly and Baxter, 1983; Dalton et al., 1997; Burgoon et al., 1992; Reilly and Garrett, 1998; Souissi et al., 2012; Rae et al., 2015) in maximum performance at different times of the day.

However, the main issue is that most studies measured performance only at two times of the day (Reilly and Baxter, 1983; Hill et al., 1988; Burgoon et al., 1992; Reilly and Garrett, 1998; Atkinson et al., 2005; Brown et al., 2008; Chtourou et al., 2012; Souissi et al., 2012; Hill, 2014; Rae et al., 2015; Aloui et al., 2017) with 07:00 and 17:00 being the most frequently used times. Such a large measurement interval carries a high risk of missing the peak and nadir of performance during the day. Consequently, these studies are unable to describe the full extent of diurnal variation in performance. Furthermore, all studies show several methodological shortcomings, including missing sample size calculations in all studies, sample sizes ≤12 in half of the studies (Reilly and Baxter, 1983; Dalton et al., 1997; Deschenes et al., 1998; Reilly and Garrett, 1998; Atkinson et al., 2005; Souissi et al., 2012; Aloui et al., 2017), not reporting the absolute data for the primary outcome (Brown et al., 2008; Souissi et al., 2012; Chin et al., 2015; Facer-Children and Brandstaetter, 2015), and no information regarding the sequence of the investigated times of day or regarding a performed randomization. Additionally, some studies tested untrained or moderately trained participants (Hill et al., 1988; Burgoon et al., 1992; Deschenes et al., 1998), making the results not generalizable for athletes. Moreover, the majority of studies used more time efficient but also less precise methods such as shuttle-run tests. The measurement of maximum oxygen uptake (VO₂max) in a cardiopulmonary exercise test (CPET), which is supposed to represent the gold standard to determine aerobic performance, was only measured in studies with a small sample size (Deschenes et al., 1998) or with large measurement intervals (Hill et al., 1988; Burgoon et al., 1992; Hill, 2014). Previous studies also do not consider exhaustion criteria, leaving room for speculation whether lower performance at a certain time of day is due to physiological (i.e., no higher performance possible) or psychophysiological (i.e., not motivated to perform with maximum effort) reasons.

In addition to the study design, data analysis in previous studies is debatable as these studies solely investigated the effects of daytime on maximum performance by comparing performances achieved at different times of the day on a group level. Because several factors such as habitual training time (Torii et al., 1992; Rae et al., 2015) or chronotype (Hill et al., 1988; Brown et al., 2008; Facer-Children and Brandstaetter, 2015) seem to influence the time of day when peak performance is achieved, we expected it to be unlikely that all participants reach their peak and nadir of performance at the same time of day. If an athlete performs better in the morning and another athlete performs better in the afternoon, the differences would be canceled out on a group level leading to the false conclusion that there are no diurnal variations in maximum performance. Therefore, we additionally compared each participant’s VO₂max at the peak and nadir of the day to describe the diurnal variations in VO₂max. Furthermore, we aimed to determine the day-to-day variation in athletes’ VO₂max to be able to put the possible diurnal variation into context.

The aims of this study, therefore, were to (1) investigate the maximum diurnal variation in VO₂max, (2) to compare the diurnal variation of VO₂max during the day to the day-to-day variation in VO₂max, and (3) to investigate if there is a time of peak VO₂max on a group level. A further aim was (4) to investigate to which extent chronotype and habitual training time contribute to the diurnal variation of VO₂max during the day.

To address methodological shortcomings from previous studies, we used a measurement interval of 3 h (i.e., six times of the day) in our study, tested an adequate number of trained athletes in a randomized sequence using the gold standard for cardiopulmonary exercise testing, and considered exhaustion criteria.

MATERIALS AND METHODS

Study Design

This study was conducted between December 2016 and May 2018 in the laboratory of the Department of Sport, Exercise and Health of the University of Basel, Switzerland. Participants gave written informed consent before inclusion, and the study was approved by the local ethics committee “Ethikkommission Nordwest- und Zentralschweiz” (EKNZ 2016-01572). To investigate the diurnal variation, participants performed CPET at 7:00, 10:00, 13:00, 16:00, 19:00, and 21:00. The order of CPET was the same for all participants to ensure sufficient recovery of at least 24 h. However, the time of day of the first CPET was randomized. To investigate the day-to-day variation, we performed a seventh CPET at the same time of day as the sixth CPET. 21:00 instead of 22:00 was chosen because this time represented the dimmed light melatonin onset in a study with athletes of comparable age (Knaier et al., 2017).

Abbreviations: VO₂max, maximum oxygen uptake; CPET, cardiopulmonary exercise test; MSFsc, midpoint of sleep on free days corrected for oversleeping due to sleep debt on workdays.
TABLE 1 | Time of the day effects on maximum aerobic performance.

| References                  | Participants                          | Time points (hh:mm) | Test                                   | Outcome         | Chronotype | Training time | Total | Variation (%)/significancea |
|-----------------------------|---------------------------------------|---------------------|----------------------------------------|-----------------|-------------|---------------|-------|---------------------------|
| Aloui et al., 2017          | 11 (not reported) Physical education students | 07:00, 17:00        | Shuttle-run test                       | Total distance (m) | Yes         | Not reported  | 07:00: 891 ± 76   | p < 0.05 |
| Atkinson et al., 2005       | 8 (male) Cyclists                      | 07:30, 17:30        | 16.1 km bicycle ergometer time trial  | Duration (s)   | Yes         | Not reported  | 07:30: 1,426 ± 104 | p < 0.05 |
| Brown et al., 2008          | 8 (male) 8 (female) Trained rowers     | 05:00–07:00, 16:30–18:00 | 2000 m rowing ergometer time trial  | Duration (s)   | Yes         | Not reported  | 17:30: 1,370 ± 99  |              |
| Burgooon et al., 1992       | 26 (male) Untrained                   | 07:30–08:30, 19:30–20:30 | Treadmill incremental test (Bruce protocol) | VO₂max (ml/kg/min) | Yes         | Not reported  | 07:30: 43.6 ± 5.6 | n.s.        |
| Chin et al., 2015           | 35 (male) Athlete students             | 09:00–10:00, 12:00–13:00, 16:00–17:00 | Shuttle-run test                     | Total distance (m) | Not reported | Not reported  | 19:30: 43.4 ± 4.8 |              |
| Chitourou et al., 2012      | 20 (male) Soccer players              | 07:00, 17:00        | Shuttle-run test                       | Total distance (m) | Not reported | Not reported  | 07:00: 1,765 ± 485 | p < 0.05 |
| Dalton et al., 1997         | 7 (male) Competitive cyclists          | 08:00–10:00, 14:00–16:00, 20:00–22:00 | 15 min bicycle ergometer time trial | Total work (kJ) | Not reported | Not reported  | 17:00: 2,046 ± 535 |              |
| Deschenes et al., 1998      | 10 (male) Untrained                   | 08:00, 12:00, 16:00–20:00 | Bicycle ergometer ramp test (30W/2min) | VO₂max (ml/kg/min) | Not reported | Not reported  | 20:00: 56.9 ± 10.2 | n.s.        |
| Facer-Childs and Brandstaetter, 2015 | 20 (not reported) Trained hockey players | 07:00, 10:00, 13:00, 16:00, 19:00, 22:00 | Shuttle-run test                     | Total distance (m) | Yes         | Yes           | Not reported | ECT: 7.6% ± 1.2 | n.s.        |
| Hill et al., 1988           | 8 (male) 24 (female) Not reported      | 06:00–08:30, 15:30–18:00 | Bicycle ergometer incremental test (20W/min) | VO₂max (l/min) | Yes         | Not reported  | 15:30: 2.75b | n.s.        |
| Hill, 2014                  | 20 (male) Physically active           | 06:30–09:30, 17:00–20:00 | Bicycle ergometer step test (50W/2min) constant-power test (100% of peak power) | VO₂max (ml/kg/min) | Not reported | ECT: 06:00: 2.75b | 18:30: 2.75b | < 0.05     |
| Rae et al., 2015            | 18 (male) 8 (female) Trained swimmers | 06:30, 18:30        | 200 m swimming time trial             | Duration (s)   | Yes         | Yes           | 17:00: 329 ± 35 | p < 0.05 |
| Reilly and Baxter, 1983     | 8 (female) Not reported                | 06:30, 22:00        | Bicycle ergometer constant load test (95% of VO₂max) | Time to exhaustion (s) | Not reported | Not reported  | 06:30: 260 ± 150 | n.s.        |
| Reilly and Garrett, 1998    | 7 (male) Not reported                  | 08:30, 17:30        | Bicycle ergometer constant load test (70% of VO₂max) | Time to exhaustion (min) | Not reported | Not reported  | 17:30: 60.9 ± 6.6 |              |

(Continued)
Participants

Inclusion criteria were physical health, age between 18 and 40 years, no shift-work in the last 3 months, and no travel across time zones in the 4 weeks prior to the study. To avoid training effects resulting from performing multiple CPET and to be able to generalize the results for athletes, the inclusion criterion for VO$_2$max was $\geq$ 50 ml/kg/min for males and $\geq$ 45 ml/kg/min for females achieved during the first CPET. This criterion was based on the 95th percentile of The American College of Sports Medicine reference values for VO$_2$max (i.e., 56 ml/kg/min for males and 50 ml/kg/min for females). Because it may be possible that a participant performs his/her first CPET at the nadir of performance, we reduced the criterion for VO$_2$max by 10% based on the expected maximum diurnal variation of VO$_2$max during the day.

Testing Procedure

The Munich Chronotype Questionnaire (Roenneberg et al., 2004) was used to determine individuals’ midpoint of sleep on free days corrected for oversleeping due to sleep debt on workdays (MSFsc). Further, participants filled out a questionnaire about their habitual training times, and a questionnaire asking the athletes at which of the six times of the day they would expect to have the best VO$_2$max. The same questionnaire was filled out again after the sixth CPET when participants had performed one CPET at each of the predefined times of the day. Subsequently, sex and age were recorded and body height and blood pressure were measured. Then, participants filled out the Physical Activity Readiness Questionnaire (Shephard, 1988) and underwent a physical examination by a physician that included 12-channel resting electrocardiography and assessment of medical history.

Cardiopulmonary Exercise Test

Immediately before each CPET, body mass (kg) and body fat mass (kg) were measured with four-segment bioelectrical impedance analyses (Inbody 720, Biospace, Seoul, South Korea). Subjective sleepiness was assessed before CPET with the Karolinska Sleepiness Scale (Kaida et al., 2006) ranging from 1 (“extremely alert”) to 9 (“very sleepy, great effort to keep alert”). CPET was performed on a bicycle ergometer (Sport Excalibur, Lode Medical Technology, Groningen, The Netherlands) under standardized laboratory conditions (air humidity 40–55%, room temperature 20–22°C). For male/female participants the exercise protocol consisted of a warm-up of 5 min at 75/50 W, a linear increase of workload with 25/20 W/min up to exhaustion, and a 10 min cool-down phase at 75/50 W. Throughout the entire CPET, participants were verbally encouraged by the test supervisors to perform with maximum effort in all tests. Gas exchange was measured breath-by-breath (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Germany) with the highest 30 consecutive seconds of VO$_2$ being determined as VO$_2$max. After the cool-down phase a VO$_2$max verification test was performed. Therefore, workload was set to 50% of maximum power output achieved during CPET for 2 min, then increased to 70% for 1 min and in the final stage of the verification test to 105% of maximum power output until exhaustion. VO$_2$max verification was accepted if the verification-VO$_2$ was $\pm$ 3% of the initially
measured \( \text{VO}_2\text{max} \) (Nolan et al., 2014). Because \( \text{VO}_2\text{-plateau} \) was not reached in all tests and \( \text{VO}_2\text{-verification} \) data from this study showed low agreement for diurnal variations (data not shown—under review), secondary exhaustion criteria were used to verify \( \text{VO}_2\text{max} \). During the first CPET, heart rate was measured with 12-channel electrocardiography and in the subsequent CPETs via 3-channel electrocardiography (Customed GmbH, Ottobrunn, Germany). In all tests, heart rate was additionally measured with a heart rate belt (Polar T-34, Polar Electro Europe AG, Zug, Switzerland). Maximum heart rate was determined by the values recorded with the heart rate belt. Ratings of perceived exertion was assessed according to the 6–20 Borg scale (Borg, 1982). Blood lactate concentration was measured at rest, immediately after exhaustion, and at minutes one, three, and five of the cool-down phase. Blood samples were analyzed immediately after the exercise test (SuperGL Ambulance, Hitado Diagnostic Systems, Moeheense, Germany). All participants reached all of the following exhaustion criteria in every test: respiratory exchange ratio \( \geq 1.1 \), heart rate \( \geq 95\% \) of predicted maximum heart rate [210—age (years)], ratings of perceived exertion \( \geq 19 \), and blood lactate concentration \( \geq 8 \text{ mmol/l} \). These cut-off values were based on a previous study with athletes (Knaier et al., 2017) and proved to strongly reduce type I errors without increasing the chance of type II errors (Knaier et al., 2018).

### Observational Phase

Participants were advised to restrain from alcohol and sport during the entire study duration and to keep a constant sleeping routine. To monitor the compliance, participants filled out a diary recording bedtime, sleep time, wake-up time, and subjective sleep quality. Compliance to restrain from sport was monitored objectively by wGT3X+ ActiGraphs (Pensacola, United States) throughout the entire study.

### Statistical Analysis

The primary outcome of this study was the difference in \( \text{VO}_2\text{max} \) (in ml/kg/min) between the CPET with the lowest (i.e., nadir) and the CPET with the highest (i.e., peak) \( \text{VO}_2\text{max} \) (i.e., maximum diurnal variation). We used paired \( t \)-tests to compare the nadir and peak of diurnal variations, day-to-day variations and the difference between diurnal and day-to-day variations. To calculate the day-to-day variations we compared the highest and lowest value from the sixth and the seventh test irrespective of which of these values was recorded in the first or second test. To investigate if there is a time of peak \( \text{VO}_2\text{max} \) on a group level, we used descriptive statistics (i.e., boxplots) and a linear mixed effects model with post-hoc tests to compare the \( \text{VO}_2\text{max} \) achieved at different times of the day. Habitual training time was coded as four different dummy variables, which were set to “1” if a participant trained during one of the predefined timeframes on any day during a usual week and to “0” if not. The timeframes were (Table 2): 04:00–08:59 (morning), 09:00–13:59 (noon), 14:00–18:59 (afternoon/evening), and 19:00–23:59 (evening/night). We used proportional odds logistic regression models to model the probability of achieving the peak \( \text{VO}_2\text{max} \) in a certain time frame (Hosmer et al., 2013). The midpoint of

### Sample Size Calculation

For the sample size calculation, we assumed that the \( \text{VO}_2\text{max} \) achieved at the time of peak \( \text{VO}_2\text{max} \) was \( 60 \pm 6 \text{ ml/kg/min} \) (Knaier et al., 2017). Based on the previous reported difference of 10% in intermediate chronotypes and of 11.6% in all participants, we expected the \( \text{VO}_2\text{max} \) achieved at the time of nadir \( \text{VO}_2\text{max} \) to be \( 54 \pm 6 \text{ ml/kg/min} \). Furthermore, we expected to reduce error variability by conservatively assuming a correlation of 0.5 between the \( \text{VO}_2\text{max} \) from the peak and \( \text{VO}_2\text{max} \) from the nadir of the day. With a 2-sided significance level of 0.05, the

### TABLE 2 | Participant characteristics—median (interquartile range).

| Characteristic | Males \((n = 10)\) | Females \((n = 7)\) |
|---|---|---|
| Age (years) | 26 (23; 33) | 27 (23; 34) |
| Height (cm) | 179 (173; 184) | 168 (166; 174) |
| Body mass (kg) | 73 (68; 82) | 64.9 (60.6; 68.6) |
| Body fat content (%) | 12 (10; 14) | 22 (17; 28) |
| **CPET** | | |
| Pmax (W) | 375 (352; 408) | 293 (261; 318) |
| VO2max L/min | 4.38 (4.23; 4.77) | 3.29 (3.11; 2.75) |
| VO2max mL/kg/min | 61.5 (57.5; 67.5) | 54.3 (46.8; 56.7) |
| Sleepiness on KSS | 2.5 (1.8; 3.3) | 3.0 (2.0; 5.0) |
| **CHRONOTYPE** | | |
| MSFsc (h) | 3.61 (3.11; 5.07) | 3.55 (2.52; 3.77) |
| **PARTICIPANTS’ TRAINING HABITS [% TRAINED BETWEEN ...]** | | |
| 04:00–08:59 | 30% | 14% |
| 09:00–13:59 | 90% | 71% |
| 14:00–18:59 | 70% | 71% |
| 19:00–23:59 | 80% | 71% |

*Multiple answers possible.*

CPET, cardiopulmonary exercise test; Pmax, maximum power output; VO2max, maximum oxygen uptake; KSS, Karolinska Sleepiness Scale (range: 1 [extremely alert] to 9 [very sleepy]); MSFsc, midpoint of sleep on free days corrected for oversleep due to sleep debt on workdays (MSFsc).

### Participants’ Training Habits

| % Trained between times | Males \((n = 10)\) | Females \((n = 7)\) |
|---|---|---|
| 04:00–08:59 | 30% | 14% |
| 09:00–13:59 | 90% | 71% |
| 14:00–18:59 | 70% | 71% |
| 19:00–23:59 | 80% | 71% |

*Multiple answers possible.*

[^1]: Nolan et al., 2014.
[^2]: Knaier et al., 2017.
[^3]: Hosmer et al., 2013.
[^4]: March 2019 | Volume 10 | Article 219
[^5]: Knaier et al., 2018.
sample size needed to attain a targeted power of 90% to show a significant difference between the peak and nadir of VO$_2$max was 13 participants. We increased this sample size to 18 participants to ensure an equal number of three participants in each of the six groups (i.e., the six different starting times for the first CPET). For our sample size calculation, we used G*Power (University of Kiel, Kiel, Germany).

Randomization
We used Friedman’s Urn Model [UD(1, 0, 2)] to allocate the participants to the different starting times (Wei, 1978; Smith, 2014). This randomization procedure is known to lead to more balanced groups if the number of groups is large compared to the number of participants while still being free of selection or accidental bias (Wei, 1978). Participant recruitment, therefore, continued until three participants were allocated to each of the six starting times for CPET.

RESULTS
Participant Flow and Characteristics
Twenty-seven participants were assessed for eligibility, whereby six participants did not meet the inclusion criteria for VO$_2$max. One participant was excluded due to medical reasons. The remaining 20 participants were randomly allocated to the six different starting times (Figure 1). Two participants were excluded due to technical problems of measuring VO$_2$ and one participant due to medical reasons. Participants’ characteristics from the CPET with the highest VO$_2$max are presented in Table 2. None of the participants was an exceptionally early or late chorotype. Participants followed the instructions regarding sleeping routine and physical activity.

Diurnal and Day-to-Day Variation in VO$_2$max
Mean and standard deviation of VO$_2$max in ml/kg/min, VO$_2$max in L/min and Pmax at the time of the peak and the time of the nadir are presented in Table 3. There were significant diurnal variations for all three parameters as indicated by the mean differences. Furthermore, for all parameters there were significant correlations between the values from the peak and the nadir of the day. These correlations were higher than the conservatively assumed 0.5 from the sample size calculation.

There were also significant day-to-day variations for all three performance related parameters (Table 3). For all three performance parameters the diurnal variations were significantly higher than the day-to-day variations with a mean difference of 5.0 ± 2.0 ml/kg/min (95% CI: 4.1, 6.0), 0.21 ± 0.17 L/min (95% CI: 0.12, 0.30), and 15.1 ± 11.8 W (95% CI: 12.2, 18.0).

| TABLE 3 | Diurnal variation and day-to-day variation for different performance parameters. |
|---|---|---|---|---|---|
| Characteristic | Peak (n = 17) | Nadir (n = 17) | Mean difference ± SD (95% CI) | Pearson’s correlation r (p) |
| **DIURNAL VARIATION** |  |  |  |  |
| VO$_2$max (mL/kg/min) | 58.2 ± 6.9 | 53.2 ± 6.5 | 5.0 ± 2.0 (4.1, 6.0) | 0.961 (p ≤ 0.001) |
| VO$_2$max (L/min) | 4.06 ± 0.72 | 3.72 ± 0.71 | 0.34 ± 0.14 (0.27, 0.41) | 0.989 (p ≤ 0.001) |
| Pmax (W) | 348.7 ± 61.3 | 328.7 ± 56.1 | 20.0 ± 9.4 (15.2, 24.8) | 0.981 (p ≤ 0.001) |
| **DAY-TO-DAY VARIATION** |  |  |  |  |
| VO$_2$max mL/kg/min | 56.7 ± 7.1 | 54.7 ± 7.0 | 2.0 ± 1.0 (1.5, 2.5) |  |
| VO$_2$max L/min | 3.94 ± 0.75 | 3.81 ± 0.74 | 0.13 ± 0.09 (0.08, 0.17) |  |
| Pmax (W) | 343.5 ± 61.7 | 338.6 ± 61.5 | 4.9 ± 5.3 (2.2, 7.6) |  |

Pmax, maximum power output; VO$_2$max, maximum oxygen uptake; SD, standard deviation; 95% CI, 95% confidence interval.
Differences between the cardiopulmonary exercise test with the highest and the exercise test with the lowest maximum oxygen uptake (VO\textsubscript{2max}) during the day (i.e., diurnal variation) and between the two exercise test taking place at the same time of the day (i.e., day-to-day variation). Values are presented for VO\textsubscript{2max} in ml/kg/min (A) and l/min (B). Square, mean difference; triangles, males; circles, females.}

9.0, 21.1), respectively. The diurnal variations were 2.5 to 4 times greater than the day-to-day variations for all three parameters. Figure 2 shows the contrast in diurnal and day-to-day variations in VO\textsubscript{2max} in ml/kg/min. Pearson’s correlation showed no relationship between the diurnal variation in VO\textsubscript{2max} and the day-to-day variation in VO\textsubscript{2max} between the participants (r = 0.050, p = 0.848).

**Influence of Time of Day on VO\textsubscript{2max}**

The linear mixed effects model revealed no significant differences in VO\textsubscript{2max} between the different times of day (Figure 3). Neither the fixed effect of time (\(\chi^2 = 8.00, p = 0.156\)) nor any pairwise comparison between the time points reached statistical significance (all \(p > 0.11\)). Time of day when peak VO\textsubscript{2max} was achieved was nearly equally distributed between the times 10:00 (n = 4), 16:00 (n = 3), 19:00 (n = 5), and 21:00 (n = 4). Interestingly, no participant achieved his/her peak VO\textsubscript{2max} at 13:00. The individual profiles for the diurnal variation differed between participants (Figure 4).

**Influence of Chronotype and Habitual Training Time on the Time of Peak VO\textsubscript{2max}**

There was little evidence (\(p = 0.129\)) that the habitual training time influenced the time of day at which participants reached their peak VO\textsubscript{2max} since no effect reached statistical significance. Chronotype had a significant influence on the time of day when peak VO\textsubscript{2max} was achieved (OR = 0.34, 95%-CI: 0.11, 0.88, \(p = 0.035\)). Participants with later MSFsc had a higher probability to reach their peak VO\textsubscript{2max} earlier during the day than participants with earlier MSFsc. Figure 5 shows the percentage of participants reaching their peak VO\textsubscript{2max} earlier during the day (i.e., at 16:00 and earlier) and reaching it later during the day (i.e., at 19:00 or later) for the two groups “morning types” and “evening types.”

**Training Effects, Participants’ Expectation, Adverse Events, and Exhaustion Criteria**

The trial when peak VO\textsubscript{2max} (ml/kg/min) was achieved was nearly equally distributed between the trials: T1 (n = 1), T2 (n = 2), T3 (n = 4), T4 (n = 3), T5 (n = 3), T6 (n = 4). The linear regression analysis showed little evidence for training effects from the first to the last CPET (\(\chi^2 = 1.80, p = 0.179\)). None of
the participants expected to reach their peak VO$_2$\textsubscript{max} at 07:00, which proved to be true. Seven out of 17 participants anticipated their time of peak VO$_2$\textsubscript{max} correctly. Except for one participant that was excluded because of cramps during the CPET, no further severe adverse events occurred during the study. The median (IQR) for the secondary exhaustion criteria at the time of peak VO$_2$\textsubscript{max} (ml/kg/min) were maximum respiratory exchange ratio 1.23 (1.18, 1.26), maximum heart rate 187 (183, 197) and maximum rating of perceived exertion 20 (20, 20), respectively. At the time of the nadir of VO$_2$\textsubscript{max} the values were maximum.
respiratory exchange ratio 1.23 (1.16, 1.26), maximum heart rate 186 (180, 192) and maximum rating of perceived exertion 20 (20, 20), respectively.

**DISCUSSION**

This is the first study showing that diurnal variations in VO$_{2\text{max}}$ can be present without time-of-day effects. In line with several previous studies (Reilly and Baxter, 1983; Hill et al., 1988; Burgoon et al., 1992; Dalton et al., 1997; Deschenes et al., 1998; Reilly and Garrett, 1998; Brown et al., 2008; Souissi et al., 2012; Rae et al., 2015), we found no significant effect of the time of day on peak aerobic power. However, this was not due to the absence of diurnal variations, but rather to the fact that peak VO$_{2\text{max}}$ is achieved at different times of the day by different athletes and therefore masks the time-of-day effect. Furthermore, we were able to show that the presented diurnal variation in VO$_{2\text{max}}$ of 5.0 ± 1.9 ml/kg/min is 2.5 times greater than the day-to-day variation in VO$_{2\text{max}}$ of 2.0 ± 1.0 ml/kg/min, which is highly likely to have a striking impact on athletes. Similarly, for VO$_{2\text{max}}$ in l/min and Pmax the diurnal variations were 2.5 and 4 times greater than the day-to-day variations. Interestingly, only three participants (18%) showed their nadir of VO$_{2\text{max}}$ at 07:00—the time of day of the fewest competitions. Thus, the reported relevant diurnal variation is present at times when most competitions take place.

Previous studies reported an influence of habitual training time on the time of day when peak performance is achieved (Rae et al., 2015) and that training at a specific time of day can reduce diurnal variations (Tordo et al., 1992). However, we chose a realistic free-living setting with trained athletes and found that most endurance athletes train at several times during the day to balance their training load with other obligations. Because athletes already train at different times of the day, this, therefore, may reduce the relevance of training habits on the time of peak VO$_{2\text{max}}$ and diurnal variations.

In contrast to Facer-Childs and Brandstaetter (2015), we did not find the association of early chronotypes reaching peak performance earlier during the day and late chronotypes reaching it later during the day. In fact, we found the opposite. Participants with later MSFsc had a higher probability to achieve peak VO$_{2\text{max}}$ at 10:00 or 16:00 than 19:00 or 21:00 (Figure 5). Several methodological differences between the two studies may explain the inconclusive results. Facer-Childs and Brandstaetter (2015) did not use MSFsc as a continuous measure for chronotype, but grouped athletes into the three categories early, intermediate, and late chronotype. This grouping resulted in an inadequate sample size of five participants in both the early and the late group. Furthermore, in Facer-Childs and Brandstaetter (2015), participants had to perform only five tests out of the six tests taking place at the different times of the day and the sequence of test times was not reported to be randomized. Finally, the authors did not explain the high differences in time of day when peak performance was reached between the three chronotype groups. Early and intermediate chronotypes were reported to reach peak performance 5.6 and 6.5 h after an entrained wake-up time, respectively, while late chronotypes needed 11.2 h to reach peak performance.

We are aware that this study was not powered to show the association between chronotype and diurnal variation. However, the results from our well-controlled study suggest that the association between time of peak VO$_{2\text{max}}$ and habitual training time or chronotype seems to be more complicated and not as simple and linear as reported by Facer-Childs and Brandstätter (2015). Similarly, it is most likely that physiological factors, which are supposed to explain the time of day effects in physical performance, are also not simple and linear. Changes in core body temperature is the most frequently used explanation for the peak of performance in the late afternoon and early evening. However, Atkinson et al. (2005) demonstrated more than a decade ago that body temperature before an exercise test cannot solely explain the time of day differences in performance. In detail, athletes still performed better in a time trial at 17:30 following a short warm-up than at 07:30 even after a vigorous warm-up for 25 min, although post warm-up temperatures did not differ between the two times of the day. Our study was planned as a proof of concept to show actual diurnal variations independent of time of day effects, and we, therefore, refrained to measure any physiological parameters such as body temperature or melatonin. However, to explain the underlying physiological mechanism of diurnal variation in performance, further studies are necessary. These studies should include the measurement of a broad range of possible and expected confounders such as body temperature, melatonin, testosterone, chronotype, training time, and sleep times. The knowledge about the interaction of these factors with the time of peak VO$_{2\text{max}}$ might help to predict the time of peak performance and shift it to the time of competition. Furthermore, we observed high differences in VO$_{2\text{max}}$ between two neighboring measurement points in some athletes indicating a rapid increase or decrease in performance. These athletes seem to be most vulnerable to changing competition times. In a previous study, evening bright light exposure showed a trend to increase maximum cycling performance through reduced melatonin levels (Knaier et al., 2017). This method might be most beneficial for athletes with rapid decreases in performance in the evening.

**CONCLUSIONS**

Athletes show significant diurnal variations in VO$_{2\text{max}}$, which are more than twice as large as the day-to-day variations. In competitive sports, these diurnal variations are highly relevant, because there are substantial differences in the time of the day when individuals achieve their peak performance. However, the direct application of our results for competitive sports is limited. We were able to show that some athletes have clear disadvantages if their time of peak performance does not comply with the time of competition, but further studies are required to investigate the underlying physiological mechanism causing these diurnal variations and to demonstrate methods to shift
an athlete’s time of peak performance. However, the results are directly applicable to exercise testing in research, athletes, and in a clinical setting. We could clearly show that it is necessary to conduct cardiopulmonary exercise testing at the same time of the day for pre- and post-intervention exercise tests to increase signal-to-noise-ratio. VO

\( \text{max} \) was not significantly higher at a specific time of the day compared to other times of the day. This absence of a time-of-day effect is explained by the fact that peak VO

\( \text{max} \) was achieved at different times of the day by different athletes. Habitual training times seem to have no influence on the time of day when peak VO

\( \text{max} \) is achieved, whereas the participants’ chronotype may have an impact.

### DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

### AUTHOR CONTRIBUTIONS

RK, CC, AS-T: concept and design; RK: data acquisition; RK, DI, and MN: data analysis and interpretation; DI: statistical expertise; RK: writing manuscript; DI, MN, CC, and AS-T: writing—review and editing.

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### SUPPLEMENTARY MATERIAL

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00219/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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