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To cite this article: Stuart J. Hesketh , Kazi D. Jahan , Mariam S. Luvna , Rojan Pakka , Subash Sing Lo & Ismat J. Talukder (07 Sep 2026): Time-of-day variation in endurance and strength performance, and physiological state across the waking day, Chronobiology International, DOI: 10.1080/07420528.2026.2730559

To link to this article: <https://doi.org/10.1080/07420528.2026.2730559>



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Published online: 07 Sep 2026.



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ORIGINAL ARTICLE



Time-of-day variation in endurance and strength performance, and physiological state across the waking day

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ABSTRACT

The goal of this study was to characterise time-of-day variation in short-duration cycling performance, handgrip strength, and associated physiological and perceptual responses under standardised laboratory conditions. Fifteen healthy recreationally active adults (8 males, 7 females; aged 26.9 ± 2.9 y) completed five experimental trials at 06:00, 10:00, 14:00, 18:00, and 22:00 h in a randomised, counterbalanced repeated-measures design. At each visit, participants completed a 10-min self-paced cycling time trial and assessments of handgrip strength, tympanic temperature, blood glucose, blood lactate, haemoglobin concentration, subjective sleepiness, and perceived exertion. Linear mixed-effects models were used to assess time-of-day effects, and mixed-effects 24-h cosinor analyses were performed as a secondary analysis to estimate peak timing of performance outcomes. Mean cycling power output varied significantly across the day ($p < 0.001$, $\eta^2 = 0.507$), increasing by 8.4% from 06:00 h to a peak at 18:00 h. Handgrip strength also exhibited significant temporal variation ($p < 0.001$, $\eta^2 = 0.495$), increasing by 9.0% between 06:00 h and 18:00 h. Cosinor analyses estimated similar acrophases for cycling performance (17:51 h) and handgrip strength (17:27 h). Pre-exercise tympanic temperature ($p < 0.001$, $\eta^2 = 0.698$), sleepiness ($p < 0.001$, $\eta^2 = 0.437$), blood glucose ($p < 0.001$, $\eta^2 = 0.435$), and blood lactate concentrations ($p = 0.019$, $\eta^2 = 0.186$) varied significantly across the day. Exercise-induced changes in blood glucose ($p < 0.001$, $\eta^2 = 0.347$) and blood lactate ($p < 0.001$, $\eta^2 = 0.295$) were also influenced by testing time, whereas post-exercise perceived exertion ($p = 0.270$, $\eta^2 = 0.070$) and exercise-induced temperature responses ($p = 0.140$, $\eta^2 = 0.114$) were unaffected. Short-duration cycling performance and handgrip strength are enhanced during the late afternoon and early evening and occur alongside coordinated temporal variation in physiological and perceptual state. These findings highlight time-of-day as a meaningful source of variation in exercise capacity and an important consideration in exercise testing and research design.

ARTICLE HISTORY

Received 18 June 2026
Revised 27 August 2026
Accepted 29 August 2026

KEYWORDS

Circadian rhythm; exercise performance; time of day; handgrip strength; chronotype; exercise metabolism

Introduction

Human physiological function exhibits pronounced daily variation driven by the interaction of endogenous circadian rhythms and behavioural factors associated with the sleep–wake cycle (Hesketh and Esser 2024). These temporal processes influence numerous systems relevant to exercise performance, including isometric strength (Douglas et al. 2021; Singh and Hesketh 2026), metabolism (van Moorsel et al. 2016), thermo-regulation (Edwards et al. 2008), cognition, and perception of effort (Schmidt et al. 2007). Consequently, physical performance is increasingly recognised not as a static characteristic, but as a dynamic phenotype that varies according to biological time.

A substantial body of evidence indicates that many measures of physical performance are enhanced later in the day compared with the morning (Hesketh and Esser

2024; Martin-López et al. 2024). Recent systematic reviews and meta-analyses have demonstrated that maximal strength, power output, and endurance performance are generally superior during the late afternoon or early evening, with performance differences often ranging from 3–10% depending on the exercise modality and population studied (Knaier et al. 2022; Bruggisser et al. 2023; Martin-López et al. 2024). These observations have practical implications for athletic performance, exercise testing and rehabilitation, as well as experimental research where uncontrolled variation in testing time may contribute substantially to outcome variability.

Despite growing consensus that exercise capacity varies across the day, the mechanisms underpinning these responses remain incompletely understood. Daily variation in physical performance occurs alongside

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coordinated rhythms in physiological systems known to influence exercise capacity (Hesketh and Esser 2024). Core body temperature typically rises throughout the waking day and reaches peak values during the late afternoon, coinciding with periods of enhanced neuromuscular performance (Reilly and Waterhouse 2009b; Knaier et al. 2022). Similarly, glucose regulation, substrate metabolism, hormonal secretion, and autonomic function exhibit strong temporal organisation (Van Cauter et al. 1997; Aoyama and Shibata 2020; Dighriri et al. 2024), while alertness, vigilance, and subjective sleepiness vary according to both circadian and homeostatic influences (Schmidt et al. 2007). These observations suggest that time-of-day differences in exercise performance likely reflect the integration of multiple physiological and behavioural processes rather than variation in any single factor.

Recent advances in chronobiology have further shifted the field beyond simple morning-versus-evening comparisons (Edwards et al. 2024). Experimental and molecular studies now demonstrate that exercise capacity itself exhibits circadian regulation. In both animal and human models, biological timing influences exercise performance, substrate utilisation, metabolic flexibility, and exercise-responsive molecular signalling pathways (Ezagouri et al. 2019; Sato et al. 2019; Hesketh and Esser 2024). Furthermore, chronotype has emerged as an important moderator of exercise responses, with individuals exhibiting different performance profiles according to their behavioural circadian phenotype (Facer-Childs and Brandstaetter 2015; Forster et al. 2025). These findings highlight the need to consider biological timing as a fundamental determinant of exercise physiology rather than simply a source of experimental variability. Circadian phenotype encompasses inter-individual differences in circadian and sleep–wake characteristics, including diurnal preference, habitual sleep timing and the temporal alignment of behaviour with the endogenous circadian system (Hesketh 2026). Within this broader construct, questionnaire-derived morningness–eveningness provides a practical measure of diurnal preference and can be used to classify individuals according to morning, intermediate, or evening orientation.

Nevertheless, important gaps remain within the literature. Much of the existing evidence is derived from studies comparing only two testing windows, typically morning and evening, which provides limited insight into how exercise capacity changes across the waking day. Furthermore, considerable methodological heterogeneity exists between studies, including differences in feeding status, sleep control, chronotype, environmental conditions, familiarisation procedures, and

exercise protocols (Bommasamudram et al. 2022; Bruggisser et al. 2023). Recent investigations employing rigorous standardisation procedures have reported clearer temporal patterns in endurance performance (Edwards et al. 2024, 2025), suggesting that methodological control may be critical for accurately characterising daily variation in exercise capacity. However, relatively few studies have examined performance across multiple evenly distributed time points while simultaneously assessing physiological and perceptual variables that may contribute to these responses.

Therefore, the aim of the present study was to characterise time-of-day variation in short-duration cycling performance, handgrip strength, and associated physiological and perceptual responses under standardised laboratory conditions. Healthy recreationally active adults completed standardised exercise trials at five evenly distributed time points across the waking day (06:00, 10:00, 14:00, 18:00, and 22:00 h). In addition to exercise performance, measures of handgrip strength, body temperature, blood glucose, blood lactate, cardiovascular responses, and subjective sleepiness were assessed to provide a broader evaluation of physiological state across the day. We hypothesised that cycling performance and handgrip strength would be enhanced during the late afternoon and early evening and that these responses would occur alongside coordinated temporal variation in thermoregulatory, metabolic, and perceptual measures.

Methods

Participants

Fifteen healthy recreationally active adults consisting of 8 males (aged 26.0 ± 1.9 y, body mass 71.2 ± 11.3 kg, and height 175.4 ± 7.4 cm) and 7 females (aged 28.0 ± 3.7 y, body mass 59.3 ± 7.6 kg, and height 166.2 ± 3.2 cm) participated in this study. Participants were free from diagnosed cardiovascular, metabolic, respiratory, neurological, or sleep disorders and were not taking medications known to influence cardiovascular function, metabolism, sleep, thermoregulation, or circadian rhythms. Exclusion criteria included current smoking, habitual night-shift work within the previous three months, transmeridian travel across more than two time zones within four weeks of testing, current musculoskeletal injury limiting exercise participation, and participation in structured endurance training exceeding five hours per week.

The night shift and transmeridian travel criteria were applied to minimise potential disruption of habitual sleep–wake and circadian timing before participation.

A longer exclusion window was applied to habitual night-shift work because repeated exposure to work and sleep at atypical biological times can produce sustained alterations in sleep–wake behaviour and circadian alignment, whereas the four-week travel criterion was intended to exclude recent acute circadian disruption associated with crossing multiple time zones. The threshold of more than two time zones was selected to distinguish transmeridian travel likely to meaningfully shift environmental time cues from minor changes in local clock time. Participants were not recruited on the basis of competitive performance level, and detailed training history or performance calibre data required for retrospective classification according to the framework proposed by (McKay et al. 2022) were not collected. Accordingly, participants are described throughout as recreationally active adults rather than being retrospectively assigned to a specific training calibre tier. Consistent with our previous work Singh and Hesketh (2026) habitual caffeine consumption was assessed using the Caffeine Consumption Questionnaire (CCQ) (Landrum 1988). To minimise the potential influence of caffeine withdrawal on exercise performance and physiological responses during morning testing sessions, individuals reporting habitual caffeine consumption of $>150 \text{ mg} \cdot \text{day}^{-1}$ were excluded from participation.

To minimise between-participant variability associated with circadian phenotype, recruitment was restricted to individuals classified as intermediate chronotypes. Chronotype was assessed using the Composite Morningness Questionnaire (CMQ) (Shahid et al. 2011a), with scores of 22–44 the established criteria for classification as an intermediate chronotype. The CMQ was selected because the purpose of chronotype assessment was to characterise morningness–eveningness preference and exclude individuals with extreme diurnal preference, rather than estimate habitual timing or circadian phase, and was consistent with our previous experimental approach (Singh et al. 2026). Of 26 individuals screened, 19 met the predefined intermediate chronotype criterion and were enrolled in the study; seven individuals were excluded because their CMQ scores fell outside this range. Four enrolled participants subsequently withdrew during the study, leaving 15 participants who completed all five experimental conditions. Individuals reporting a strong preference for either morning or evening exercise training were excluded. To reduce variability associated with hormonal fluctuations, all female participants were tested between days 10–15 following the onset of menstruation, based on self-report of the first day of their most recent menstrual cycle and typical cycle length, which

were verified at each laboratory visit, as done previously (Singh and Hesketh 2026). None of the female participants were using hormonal contraceptives at the time of the study. All participants provided written informed consent prior to participation. The study was approved by the University of Lancashire School of Medicine and Dentistry Ethics Committee and conducted in accordance with the Declaration of Helsinki.

No formal a priori sample-size calculation was undertaken. The study therefore employed a repeated-measures design in which each participant completed all five experimental conditions, thereby reducing between-participant variability. However, the final sample size should be considered when interpreting the precision and generalisability of the observed effects, particularly for secondary outcomes.

Experimental Design

A randomised, counterbalanced repeated-measures design was employed (Figure 1). Participants attended the laboratory on six occasions, consisting of one familiarisation visit followed by five experimental trials performed at 06:00, 10:00, 14:00, 18:00, and 22:00 h. During the familiarisation visit, participants were accustomed to all testing procedures and completed a practice 10-min cycling time trial on a Wattbike Pro cycle ergometer (Wattbike Ltd., Nottingham, UK), which has demonstrated acceptable validity and reliability for the measurement of cycling power output (Hopker et al. 2010; Wainwright et al. 2017). Ergometer configuration, including saddle height, handlebar position, and resistance settings, was standardised and replicated across all subsequent visits.

All testing was conducted within an environmentally controlled laboratory maintained at $21 \pm 1^\circ\text{C}$ and 45–55% relative humidity. Environmental conditions were monitored throughout the study and remained consistent between visits. Experimental trial order was randomised using a computer-generated sequence and counterbalanced between participants to minimise potential order and learning effects. Participants were allocated to one of five testing orders to ensure an even distribution of trial sequences across the study (Figure 1). Consecutive trials were separated by a minimum of seven days to minimise residual fatigue and behavioural carryover effects.

Participants were instructed to maintain their habitual sleep–wake schedules throughout the study period, except where earlier waking was required for the 06:00 h trial, as well instructed to avoid daytime napping on testing days. Habitual sleep behaviour was monitored using wrist-worn actigraphy throughout the study

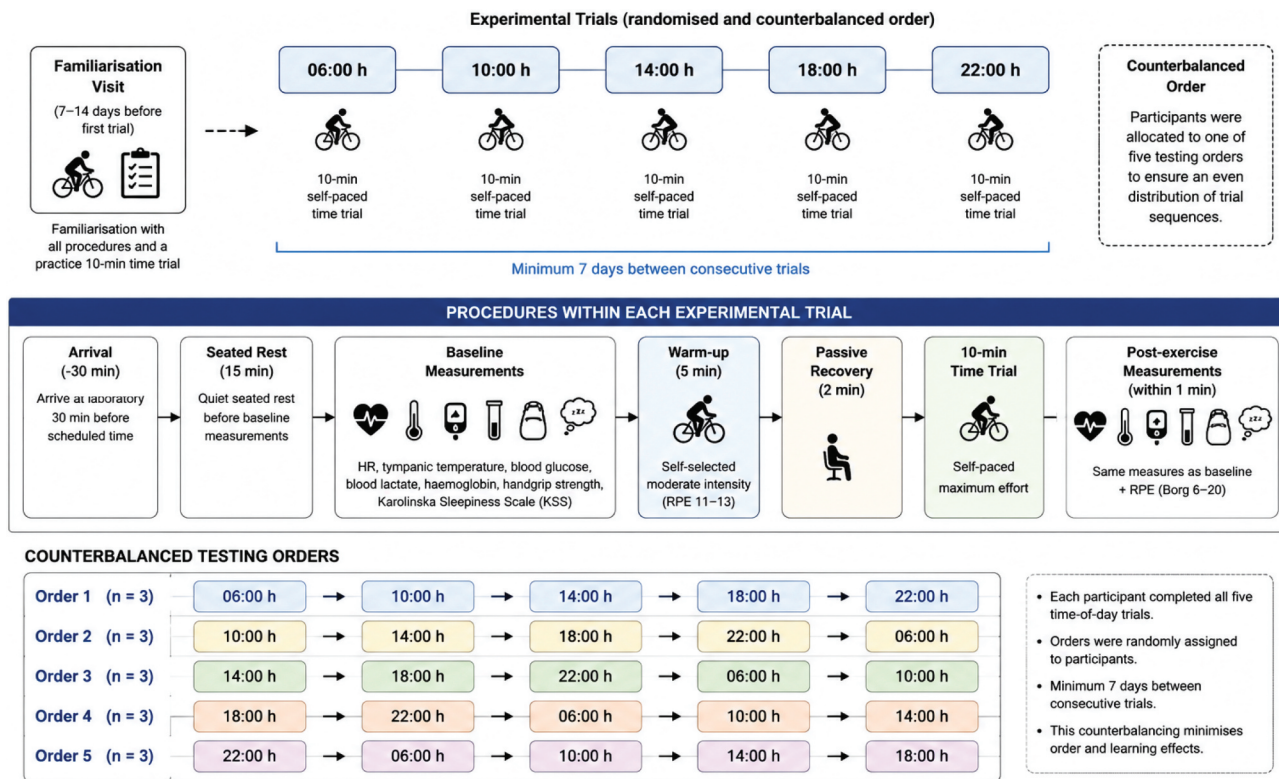


Figure 1. Experimental design and study protocol. Schematic overview of the study design and experimental procedures, showing familiarisation visit and subsequent five experimental trials at 06:00, 10:00, 14:00, 18:00, and 22:00 h in a randomised, counterbalanced order. At each experimental visit, participants completed baseline assessments, including heart rate (HR), tympanic temperature, blood glucose concentration, blood lactate concentration, haemoglobin concentration, maximal handgrip strength, and subjective sleepiness assessed using the Karolinska Sleepiness Scale (KSS). Participants then performed a standardised warm-up, followed by cycling time trial. Post-exercise measurements were then obtained. Participants were allocated to one of five counterbalanced testing orders ($n = 3$ per order) to minimise potential order and learning effects.

(Haghighayegh et al. 2019; Stucky et al. 2021; Park et al. 2024). Additional controls relating to sleep, physical activity, dietary intake, and caffeine consumption were implemented before each experimental trial and are described below. Experimental sessions were conducted under standardised indoor lighting conditions, with participants proceeding directly to the laboratory upon arrival for morning testing sessions.

Sleep Monitoring and Standardisation

Habitual sleep–wake behaviour was monitored continuously using a wrist-worn activity monitor (Fitbit Inspire 3, Fitbit Inc., USA) for seven consecutive days prior to the first experimental trial and throughout the study period. Actigraphy-derived measures included sleep onset time, wake time, total sleep duration, and sleep efficiency. Validation studies comparing Fitbit-derived sleep measures with polysomnography have demonstrated high sensitivity for sleep detection (0.95–0.96 for sleep staging models), although specificity is more moderate (0.58–0.69) (Haghighayegh et al. 2019). In

a naturalistic validation study, Stucky et al. (2021) reported mean biases of -1.6 min for sleep onset, -5.6 min for sleep offset, and -4.0 min for total sleep time compared with polysomnography, although limits of agreement were relatively wide. Collectively, these findings support the use of Fitbit devices for characterising habitual sleep timing and duration under free-living conditions, while recognising that individual estimates are less precise than polysomnography and that performance varies according to device model and sleep metric (Haghighayegh et al. 2019; Stucky et al. 2021; Park et al. 2024).

Participants were instructed to maintain consistent bedtimes and wake times throughout the study and to obtain a minimum of six hours of sleep prior to each testing session. Participants were instructed to maintain a consistent sleep opportunity throughout the study period and were contacted before each testing session to confirm compliance with pre-trial sleep requirements. Daytime napping was prohibited on testing days. Actigraphy data were reviewed throughout the study to verify compliance with sleep standardisation

procedures and identify any substantial deviations from habitual sleep behaviour. Participants were also asked to report any factors that may have influenced sleep quality or duration prior to each experimental visit.

Familiarisation Session

Participants attended a familiarisation session 7–14 days prior to the first experimental trial. During this visit, anthropometric measurements including stature and body mass were recorded, and participants were familiarised with all testing procedures, equipment, and outcome measures.

Cycle ergometer setup, including saddle height, handlebar position, and resistance settings, was individually adjusted and recorded for replication during all subsequent testing sessions. Participants were fitted with the heart rate monitor and familiarised with the procedures used for capillary blood sampling, tympanic temperature assessment, maximal handgrip strength testing, ratings of perceived exertion (RPE) (Borg and Borg 1982), and the Karolinska Sleepiness Scale (KSS) (Shahid et al. 2011b). Participants performed three practice maximal handgrip contractions to ensure familiarity with the testing procedures and to minimise potential learning effects during subsequent experimental visits.

Following baseline measurements, participants completed a standardised warm-up consisting of 5 min of cycling at a self-selected moderate intensity corresponding to an RPE of approximately 11–13 on the Borg 6–20 scale, followed by 2 min of passive recovery. Participants then completed a 10-min self-paced cycling time trial on a Wattbike Pro cycle ergometer (Wattbike Ltd., Nottingham, UK) to familiarise themselves with the pacing strategy, perceptual demands, and performance characteristics of the protocol. Standardised instructions, verbal encouragement, and feedback procedures used during the familiarisation session were replicated during all subsequent experimental trials to minimise potential learning effects.

Pre-Trial Standardisation

Participants were instructed to maintain their habitual sleep–wake schedule throughout the study period and to avoid daytime napping on testing days. During the 24 h preceding each experimental trial, participants were required to refrain from strenuous physical activity and alcohol consumption. Caffeine consumption was prohibited for at least 12 h before testing.

Dietary intake was standardised by using a 24 h dietary record and replication approach. Participants

recorded all food and fluid consumed during the 24 h preceding their first experimental trial, including the approximate timing and composition of meals, snacks, and beverages, and were instructed to replicate this intake as closely as possible during the 24 h preceding each subsequent trial. For the 06:00 h condition, participants arrived at the laboratory following an overnight fast. For all remaining testing sessions, participants consumed their final meal approximately 3–4 h before arrival and replicated both the timing and composition of this meal before each trial. Participants were instructed not to consume any food or caloric beverages between their final standardised meal and arrival at the laboratory.

Participants were instructed to obtain a minimum of six hours of sleep prior to each experimental visit and to maintain consistent bedtimes and wake times throughout the study, except where earlier waking was required for the 06:00 h trial. Compliance with sleep standardisation procedures was monitored using wrist-worn actigraphy and confirmed verbally upon arrival at each testing session. Participants were also required to report any illness, medication use, unusual physical activity, or deviations from habitual sleep or dietary behaviour that occurred during the 24 h preceding testing. To minimise variability associated with time elapsed since waking, participants were instructed to maintain normal daily activities prior to laboratory attendance while adhering to the standardisation procedures described above.

Experimental Protocol

Participants arrived at the laboratory 30 min before the scheduled testing time. Following arrival, participants rested quietly in a seated position for 15 min before baseline measurements were obtained. Baseline assessments included resting heart rate, tympanic temperature, capillary blood glucose concentration, blood lactate concentration, haemoglobin concentration, maximal handgrip strength, and subjective sleepiness assessed using the KSS. Heart rate was monitored continuously throughout each testing session using a Polar H10 chest-strap monitor (Polar Electro Oy, Kempele, Finland).

All exercise trials were performed on a commercially available air-braked cycle ergometer (Wattbike Pro; Wattbike Ltd., Nottingham, UK). Individual bike setup, including saddle height, handlebar position, and resistance settings, was standardised during the familiarisation session and replicated for all subsequent experimental trials. Participants completed a standardised warm-up consisting of 5 min of cycling at a self-selected moderate intensity corresponding to a rating of perceived exertion (RPE) of

approximately 11–13 on the Borg 6–20 scale, followed by 2 min of passive recovery. Standardised instructions were provided prior to each trial, emphasising that the objective was to achieve the highest possible average power output over the duration of the test.

Following the warm-up, participants completed a 10-min self-paced cycling time trial. Visual feedback was restricted to elapsed time only, with no information provided regarding power output, cadence, or distance covered in order to minimise pacing bias. The Wattbike performance monitor was configured accordingly for all trials. Standardised verbal encouragement was provided at regular intervals throughout the test. Power output (W), cadence (rpm), distance covered (km), and heart rate were recorded continuously throughout the time trial.

Immediately following completion of the time trial, capillary blood glucose concentration, blood lactate concentration, haemoglobin concentration, tympanic temperature, maximal handgrip strength, subjective sleepiness (KSS), and RPE were reassessed using the same procedures employed at baseline. Exercise-induced changes (Δ) in blood glucose, blood lactate, haemoglobin concentration, tympanic temperature, handgrip strength, and KSS score were subsequently calculated as the difference between post-exercise and pre-exercise values.

Physiological and Perceptual Measurements

Mean power output achieved during the 10-min cycling time trial was designated a priori as the primary performance outcome. Secondary performance outcomes included peak power output and total distance covered. Heart rate was recorded continuously throughout exercise using a Polar H10 chest-strap monitor (Polar Electro Oy, Kempele, Finland) and expressed as mean and peak values.

Tympanic temperature was measured using a calibrated infrared tympanic thermometer (Braun ThermoScan 7, IRT6520, Braun GmbH, Germany) according to manufacturer guidelines. Capillary blood samples were collected via fingertip puncture immediately before exercise and within one minute following exercise cessation. Blood glucose concentration was determined using a portable glucose analyser (Accu-Chek Guide, Roche Diagnostics, Germany), blood lactate concentration using a Lactate Pro 2 analyser (Arkay Inc., Kyoto, Japan), and haemoglobin concentration using a HemoCue Hb 201+ analyser (HemoCue AB, Sweden). Exercise-induced changes (Δ) in blood glucose, blood lactate, and haemoglobin concentration

were calculated as the difference between post-exercise and pre-exercise values.

Maximal handgrip strength was assessed using a digital handgrip dynamometer (Takei Scientific Instruments, Niigata, Japan). Participants performed three maximal contractions with their dominant hand separated by 30 s of recovery, with the highest value retained for analysis. Exercise-induced changes in handgrip strength were calculated as the difference between post-exercise and pre-exercise values.

Ratings of perceived exertion (RPE) were assessed immediately following exercise using the Borg 6–20 scale. Subjective sleepiness was assessed before and after exercise using the KSS. Exercise-induced changes in KSS score were calculated as the difference between post-exercise and pre-exercise values.

For variables measured before and after exercise, pre-exercise values and exercise-induced changes (Δ ; post-exercise minus pre-exercise) were calculated where appropriate and included in subsequent analyses to determine whether time-of-day influenced resting status, the response to exercise, or both.

Statistical Analysis

All statistical analyses were performed in R (version 4.3.1). Linear mixed-effects models were fitted using the *lme4* package, with statistical inference performed using *lmerTest* and estimated marginal means generated using the *emmeans* package. Pairwise comparisons were adjusted for multiple testing using the Šidák method. All statistical figures were generated in R using *ggplot2*. Data are presented as mean $\pm$ standard deviation (SD) unless otherwise stated. Data distributions were assessed using Shapiro–Wilk tests and visual inspection of Q–Q plots. Statistical significance was accepted at $p < 0.05$.

The effect of time-of-day on performance, physiological, and perceptual outcomes was assessed using linear mixed-effects models, with participant included as a random intercept and time-of-day (06:00, 10:00, 14:00, 18:00, and 22:00 h) included as a fixed effect. Significance of fixed effects was evaluated using F-tests obtained from analysis of variance (ANOVA) tables generated from the mixed-effects models. Where significant main effects were identified, post hoc pairwise comparisons were performed using estimated marginal means with Šidák-adjusted correction for multiple comparisons. Significance groupings displayed in figures were derived from these adjusted comparisons.

Mean cycling power output was designated a priori as the primary outcome. All remaining performance, physiological, and perceptual outcomes were considered

secondary and were analysed to characterise the broader temporal profile of performance and physiological state across the waking day. Given the sample size and number of secondary outcomes examined, findings from these analyses were considered exploratory and interpreted accordingly. For variables measured before and after exercise, analyses were conducted separately for pre-exercise values and exercise-induced changes (Δ), calculated as post-exercise minus pre-exercise values. Post-exercise ratings of perceived exertion (RPE) were analysed as absolute values.

As a secondary analysis, mixed-effects cosinor models were fitted using a predefined 24-h period to further characterise temporal variation in mean cycling power output and body mass-normalised handgrip strength. Sine and cosine terms corresponding to a 24-h period were included as fixed effects, with participant included as a random intercept to account for repeated measurements within individuals. Mesor, amplitude, and acrophase were derived from the fitted coefficients and are reported with 95% confidence intervals. The joint contribution of the sine and cosine terms was used to assess the presence of a significant 24-h sinusoidal component. Given that measurements were obtained at five time points across the waking day and no direct marker of circadian phase was obtained, these analyses were considered secondary to the primary mixed-effects comparisons across testing times and were not interpreted as definitive evidence of endogenous circadian rhythmicity. Actigraphy-derived sleep variables, including sleep duration, sleep onset time, and wake time, were analysed using the same mixed-effects model approach to assess pre-trial standardisation procedures and characterise habitual sleep timing and duration across the study period.

Effect sizes for omnibus time-of-day effects are reported as partial eta-squared (η^2) with 95% confidence intervals derived from the corresponding

F distributions. Estimated mean differences and 95% confidence intervals are additionally reported for key pairwise comparisons of the prespecified primary outcome.

As an exploratory sensitivity analysis, the primary mixed effects model for mean cycling power output was repeated with pre-trial sleep duration included as a covariate to determine whether variation in sleep duration influenced the observed time-of-day effect. Time since awakening was not included as an additional covariate because it was intrinsically coupled to scheduled testing time within the normally entrained study design and therefore could not be meaningfully separated from the fixed effect time-of-day.

Results

Participant Characteristics and Trial Standardisation

Fifteen recreationally active adults completed all experimental trials. Participant characteristics are presented in Table 1. Participants were classified as intermediate chronotypes according to the Composite Morningness Questionnaire (CMQ score between 22 and 44) (Shahid et al. 2011a) and demonstrated consistent habitual sleep patterns throughout the study period.

Compliance with the pre-trial standardisation procedures was high across all experimental conditions (Table 2). Sleep duration, sleep onset time, and wake time differed significantly across testing conditions (all $p < 0.05$), reflecting the earlier bedtime and wake time required for participation in the 06:00 h trial. In contrast, no effect of testing time was observed for pre-exercise haemoglobin concentration ($p = 0.52$), indicating comparable hydration status across all experimental sessions. Despite the expected differences in sleep timing associated with the early-morning condition,

Table 1. Participant characteristics.

Variable	Total cohort ($n = 15$)	Male ($n = 8$)	Female ($n = 7$)
Age (years)	26.9 $\pm$ 2.9	26.0 $\pm$ 1.9	28.0 $\pm$ 3.7
Body mass (kg)	65.6 $\pm$ 11.2	71.2 $\pm$ 11.3	59.3 $\pm$ 7.6
Height (cm)	171.1 $\pm$ 7.4	175.4 $\pm$ 7.4	166.2 $\pm$ 3.2
CMQ score	34.6 $\pm$ 2.6	34.6 $\pm$ 3.0	34.6 $\pm$ 2.4
Habitual caffeine intake (mg·day ⁻¹)	77.3 $\pm$ 41.8	65.0 $\pm$ 42.7	91.4 $\pm$ 38.8
Habitual sleep onset time	23:13 $\pm$ 15 min	23:12 $\pm$ 18 min	23:14 $\pm$ 10 min
Habitual wake time	07:08 $\pm$ 17 min	07:02 $\pm$ 11 min	07:15 $\pm$ 21 min
Sleep duration (h·night ⁻¹)	7.92 $\pm$ 0.39	7.83 $\pm$ 0.39	8.02 $\pm$ 0.39
Sleep efficiency (%)	88.7 $\pm$ 2.9	88.6 $\pm$ 2.0	88.7 $\pm$ 3.8

Values are presented as mean $\pm$ standard deviation (SD). CMQ = Composite Morningness Questionnaire. Sleep onset time, wake time, sleep duration, and sleep efficiency were derived from wrist-worn actigraphy collected over seven consecutive days prior to the first experimental trial and maintained throughout the study period. All participants met the established criteria for classification as intermediate chronotypes and reported habitual caffeine consumption < 150 mg·day⁻¹. Male and female value are presented descriptively; no inferential comparisons between sexes were performed because sex was not a prespecified grouping factor and the study was not powered for sex-stratified analyses.

Table 2. Pre-trial standardisation variables across experimental conditions.

Variable	06:00 h	10:00 h	14:00 h	18:00 h	22:00 h	<i>p</i> value
Sleep duration (h)	6.6 ± 0.4	7.8 ± 0.5	7.8 ± 0.4	7.9 ± 0.4	7.9 ± 0.7	<0.001
Sleep onset time (hh:mm)	22:36 ± 0:13	23:19 ± 0:16	23:16 ± 0:21	23:17 ± 0:18	23:12 ± 0:18	<0.001
Wake time (hh:mm)	05:11 ± 0:16	07:12 ± 0:20	07:08 ± 0:21	07:10 ± 0:21	07:11 ± 0:22	<0.001
Time awake before testing (h)	0.8 ± 0.3	2.8 ± 0.3	6.9 ± 0.3	10.8 ± 0.3	14.8 ± 0.4	—
Pre-exercise haemoglobin (g·dL ⁻¹)	14.5 ± 1.0	14.5 ± 0.8	14.4 ± 1.0	14.4 ± 1.0	14.5 ± 0.9	0.52

Values are presented as mean ± standard deviation (SD). Sleep variables were derived from wrist-worn actigraphy and are presented to demonstrate compliance with study standardisation procedures. Time awake before testing was calculated from actigraphy-derived wake time and scheduled testing time. Pre-exercise haemoglobin values are shown as an indicator of hydration status prior to exercise. *p* values represent the main effect of testing time obtained from linear mixed-effects models.

participants remained compliant with the sleep-standardisation procedures and maintained consistent sleep behaviour across the remaining testing sessions. Collectively, these findings indicate that participants arrived at each trial in a comparable hydration state and generally adhered to the prescribed pre-trial standardisation procedures.

Time-Of-Day Variation in Cycling Performance and Handgrip Strength

A significant effect of time-of-day was observed for mean power output during the 10-min cycling time trial ($F_{4,56} = 14.42$, $p < 0.0001$, $\eta^2 = 0.507$, 95% CI 0.300–0.632; **Figure 2(A)**). Mean power output increased from 173.9 ± 37.6 W at 06:00 h to 188.5 ± 40.4 W at 18:00 h, representing an increase of approximately 8.4%. Post hoc analyses demonstrated that performance was significantly lower during the morning trials (06:00 and 10:00 h) compared with the afternoon and evening trials (14:00, 18:00, and 22:00 h; all $p \leq 0.004$). Relative to 06:00 h, estimated mean power output was 13.68 W greater at 14:00 h (95% CI 8.39–18.97), 14.67 W greater at 18:00 h (95% CI 9.37–19.96), and 10.63 W greater at 22:00 h (95% CI 5.33–15.92). No significant differences were observed within the morning or afternoon/ evening time points. The highest mean power output was recorded at 18:00 h, although values at 14:00, 18:00, and 22:00 h did not differ significantly from one another. These findings indicate a clear diurnal variation in short-duration endurance performance, characterised by reduced morning performance and enhanced afternoon-evening performance. Body mass-normalised handgrip strength also demonstrated a significant effect of time-of-day ($F_{4,56} = 13.72$, $p < 0.0001$, $\eta^2 = 0.495$, 95% CI 0.285–0.622; **Figure 2(B)**). Handgrip strength increased from 0.575 ± 0.128 kg·kg⁻¹ at 06:00 h to 0.626 ± 0.134 kg·kg⁻¹ at 18:00 h, corresponding to an increase of approximately 9.0%. Post hoc analyses revealed that handgrip strength at 14:00 h and 18:00 h was significantly greater than that observed at both 06:00 h and 10:00 h (all $p \leq 0.005$), while values at 22:00 h were greater than 06:00 h ($p = 0.006$) but did

not differ significantly from 10:00 h, 14:00 h, or 18:00 h. Collectively, these findings demonstrate parallel time-of-day effects on both whole-body exercise performance and handgrip strength, with peak values occurring during the afternoon to early evening.

Secondary Mixed-Effects Cosinor Analysis

To further characterise temporal variation across the waking day, mixed-effects cosinor models with a predefined 24-h period were fitted for mean cycling power output and body mass-normalised handgrip strength (**Figure 3**). A significant 24-h sinusoidal component was identified for mean cycling power output ($p < 0.0001$). The estimated mesor was 180.9 W (95% CI 162.4–199.4), with an amplitude of 8.4 W (95% CI 6.3–10.8) and an estimated acrophase of 17:51 h (95% CI 16:46–19:04 h) (**Figure 3(A)**). Body mass-normalised handgrip strength similarly demonstrated a significant 24-h sinusoidal component ($p < 0.0001$), with an estimated mesor of 0.597 kg·kg⁻¹ (95% CI 0.531–0.663), amplitude of 0.026 kg·kg⁻¹ (95% CI 0.020–0.034), and estimated acrophase of 17:27 h (95% CI 16:27–18:41 h) (**Figure 3(B)**). The closely aligned acrophases indicate similar fitted temporal profiles for endurance and strength performance. Given that measurements were obtained at five time points across the waking day without a direct marker of circadian phase, these analyses were interpreted as secondary characterisation of temporal variation rather than evidence of an endogenous circadian rhythm.

Pre-Exercise Physiological and Perceptual Variables

Pre-exercise physiological and perceptual measures exhibited significant time-of-day variation (**Figure 4**). Pre-exercise tympanic temperature demonstrated the largest temporal effect ($F_{4,70} = 40.38$, $p < 0.0001$, $\eta^2 = 0.698$, 95% CI 0.572–0.773; **Figure 4(A)**). Tympanic temperature increased progressively throughout the day, rising from $36.25 \pm 0.13^\circ\text{C}$ at 06:00 h to a peak of $36.84 \pm 0.17^\circ\text{C}$ at 18:00 h, representing an increase of approximately 1.6%. Post hoc analyses revealed that

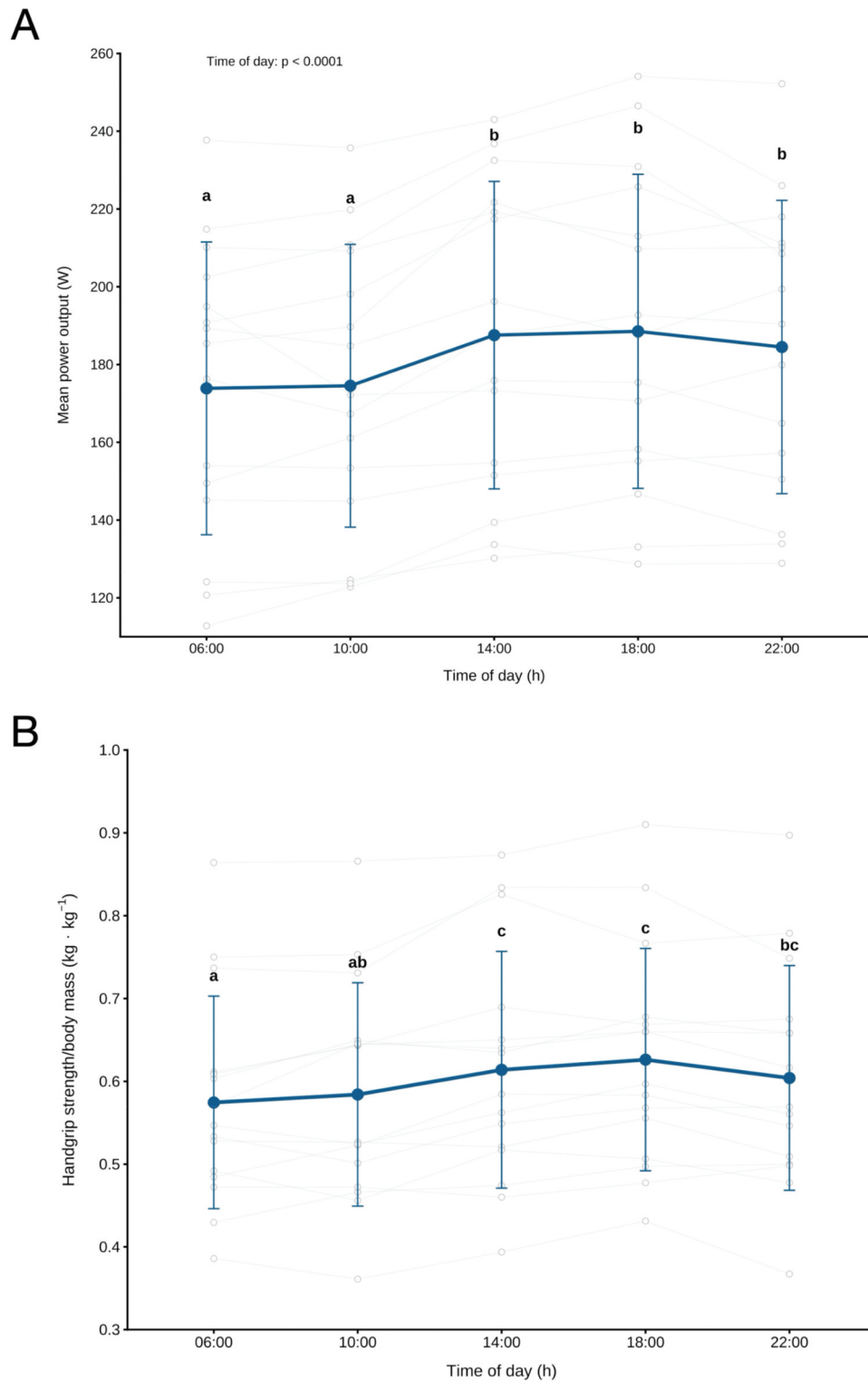


Figure 2. Time-of-day variation in cycling performance and handgrip strength. (A) Mean power output (W) achieved during a 10-min self-paced cycling time trial performed at 06:00, 10:00, 14:00, 18:00, and 22:00 h. (B) Body mass-normalised handgrip strength ($\text{kg} \cdot \text{kg}^{-1}$) assessed immediately prior to exercise at each corresponding time point. Grey lines and open circles represent individual participant responses ($n = 15$), while blue symbols and error bars represent group mean $\pm$ standard deviation (SD). Time-of-day effects were assessed using linear mixed-effects models with participant included as a random effect and time of day included as a fixed effect. A significant main effect of time of day was observed for mean power output ($F_{4,56} = 14.42$, $p < 0.0001$) and body mass-normalised handgrip strength ($F_{4,56} = 13.72$, $p < 0.0001$). Different letters indicate significant differences between time points following Šidák-adjusted post hoc comparisons ($p < 0.05$).

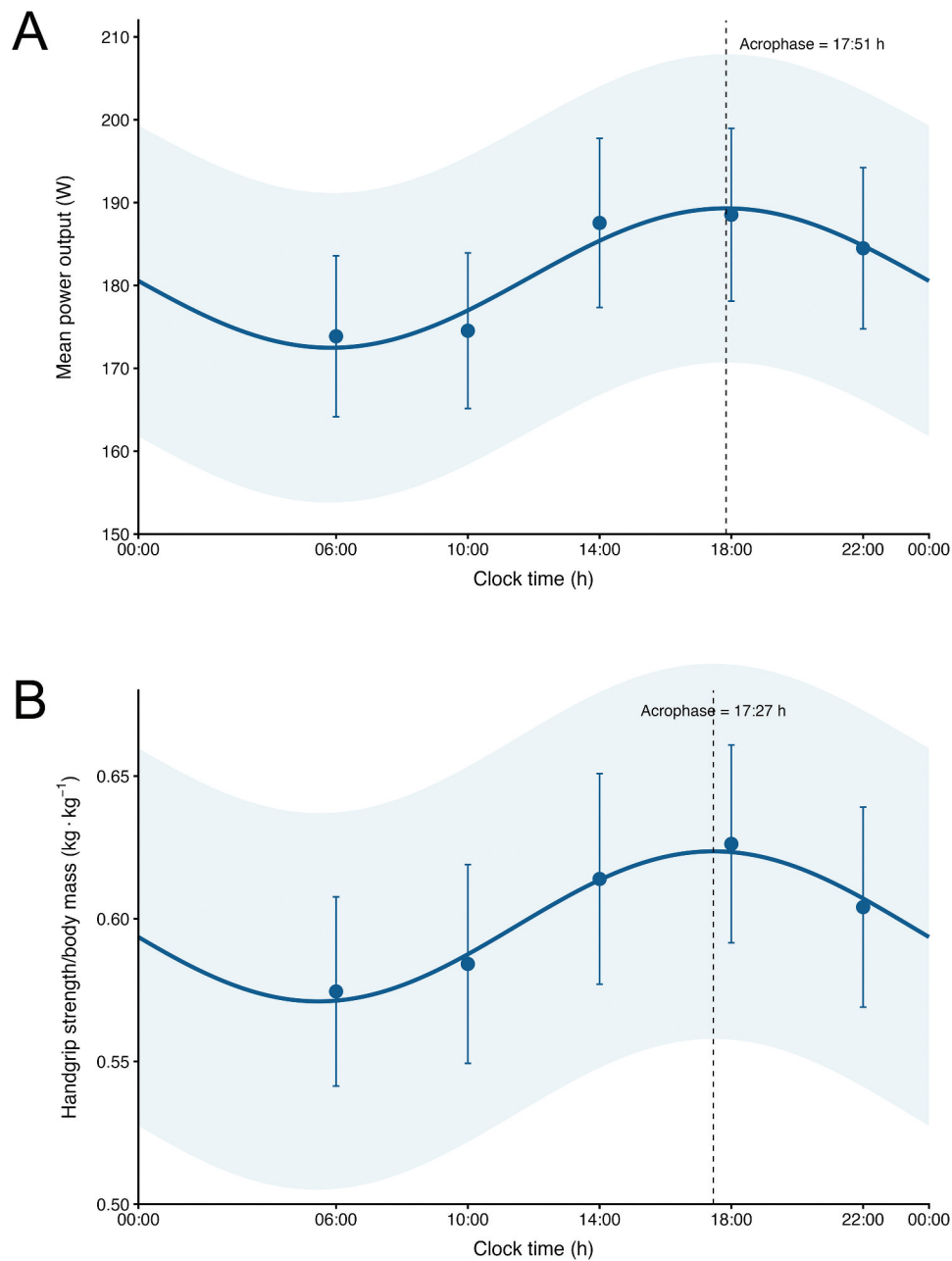


Figure 3. Mixed-effects cosinor profiles of mean power output and handgrip strength. (A) Mean power output (W) achieved during 10-min self-paced cycling time trials performed at 06:00, 10:00, 14:00, 18:00, and 22:00 h. (B) Body mass-normalised maximal handgrip strength ($\text{kg} \cdot \text{kg}^{-1}$) measured immediately prior to exercise at the corresponding time points in healthy recreationally active adults ($n = 15$). Symbols represent observed group means and error bars represent standard error of the mean (SEM). Solid blue lines represent population-level fitted values from the mixed-effects 24-h cosinor models, while shaded regions denote the corresponding 95% confidence intervals. Vertical dashed lines indicate the estimated acrophase (time of peak fitted value), occurring at 17:51 h for mean power output and 17:27 h for body mass-normalised handgrip strength. Cosinor modelling was performed using a fixed 24-h period to characterise temporal variation across the waking day and estimate the timing of peak fitted values for each outcome. The joint sine and cosine terms indicated a significant 24-h sinusoidal component for both mean power output and body mass-normalised handgrip strength (both $p = 0.0001$).

temperature at 06:00 h was significantly lower than all subsequent time points (all $p \leq 0.014$). Furthermore, temperature at 18:00 h was significantly greater than both 14:00 h and 22:00 h, indicating a clear late-afternoon peak.

Karolinska Sleepiness Scale (KSS) scores also varied significantly across the day ($F_{4,56} = 10.89$, $p < 0.0001$, $\eta^2 = 0.437$, 95% CI 0.219–0.575; [Figure 4\(B\)](#)). Participants reported the highest sleepiness at 06:00 h (5.0 ± 0.9 AU), with scores progressively declining to

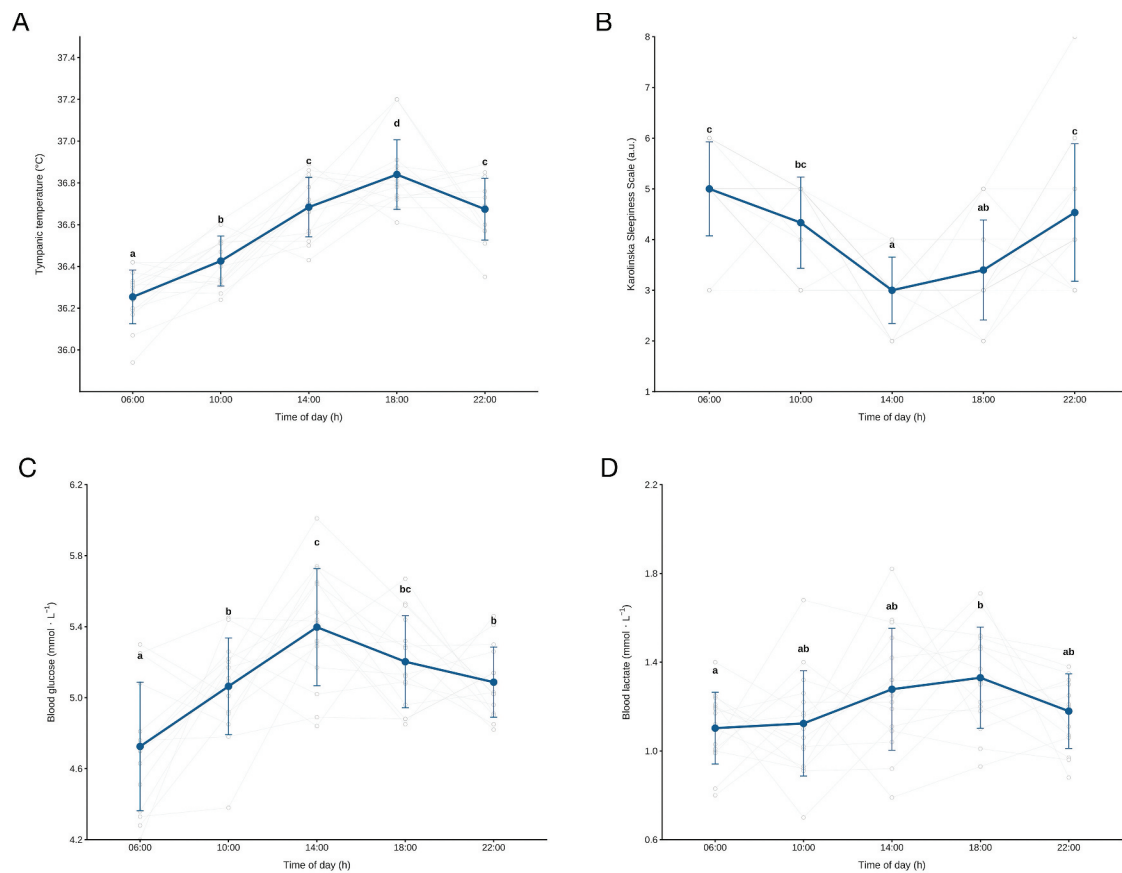


Figure 4. Time-of-day variation in physiological and perceptual status prior to exercise. Pre-exercise physiological and perceptual measures obtained immediately before the 10-min self-paced cycling time trial performed at 06:00, 10:00, 14:00, 18:00, and 22:00 h in healthy recreationally active adults ($n = 15$). (A) Tympanic temperature (°C), (B) Karolinska Sleepiness Scale (KSS) score, (C) blood glucose concentration (mmol·L⁻¹), and (D) blood lactate concentration (mmol·L⁻¹). Grey lines and open circles represent individual participant responses, while blue symbols and error bars represent group mean $\pm$ standard deviation (SD). Time-of-day effects were assessed using linear mixed-effects models with participant included as a random effect and time-of-day included as a fixed effect. Significant main effects of time-of-day were observed for tympanic temperature ($F_{4,56} = 37.33, p < 0.0001$), KSS score ($F_{4,56} = 10.89, p < 0.0001$), blood glucose concentration ($F_{4,56} = 10.77, p < 0.0001$), and blood lactate concentration ($F_{4,56} = 3.21, p = 0.019$). Different letters indicate significant differences between time points following Šidák-adjusted post hoc comparisons ($p < 0.05$).

a nadir at 14:00 h (3.0 ± 0.7 AU), corresponding to a reduction of approximately 40%. KSS scores at 06:00 h were significantly greater than those observed at 14:00 h and 18:00 h (both $p < 0.05$), while sleepiness increased again during the late evening trial, resulting in values at 22:00 h that did not differ from those observed at 06:00 h.

A significant effect of time-of-day was also observed for pre-exercise blood glucose concentration ($F_{4,56} = 10.77, p < 0.0001, \eta^2 = 0.435, 95\% \text{ CI } 0.216\text{--}0.572$; Figure 4(C)). Blood glucose increased from 4.73 ± 0.36 mmol·L⁻¹ at 06:00 h to 5.40 ± 0.34 mmol·L⁻¹ at 14:00 h, representing an increase of approximately 14%. Glucose concentrations at 06:00 h were significantly lower than those measured at 10:00 h, 14:00 h, 18:00 h, and 22:00 h

(all $p \leq 0.022$). In addition, values at 14:00 h remained significantly greater than those observed at both 10:00 h and 22:00 h, indicating a distinct afternoon elevation in circulating glucose concentration.

Pre-exercise blood lactate concentration exhibited a smaller but statistically significant effect of time-of-day ($F_{4,56} = 3.21, p = 0.019, \eta^2 = 0.186, 95\% \text{ CI } 0.004\text{--}0.334$; Figure 4(D)). Lactate concentration increased from 1.10 ± 0.16 mmol·L⁻¹ at 06:00 h to 1.32 ± 0.22 mmol·L⁻¹ at 18:00 h, corresponding to an increase of approximately 20%. Post hoc analyses demonstrated that lactate concentration at 18:00 h was significantly greater than that observed at 06:00 h ($p < 0.05$), whereas no other pairwise comparisons reached statistical significance.

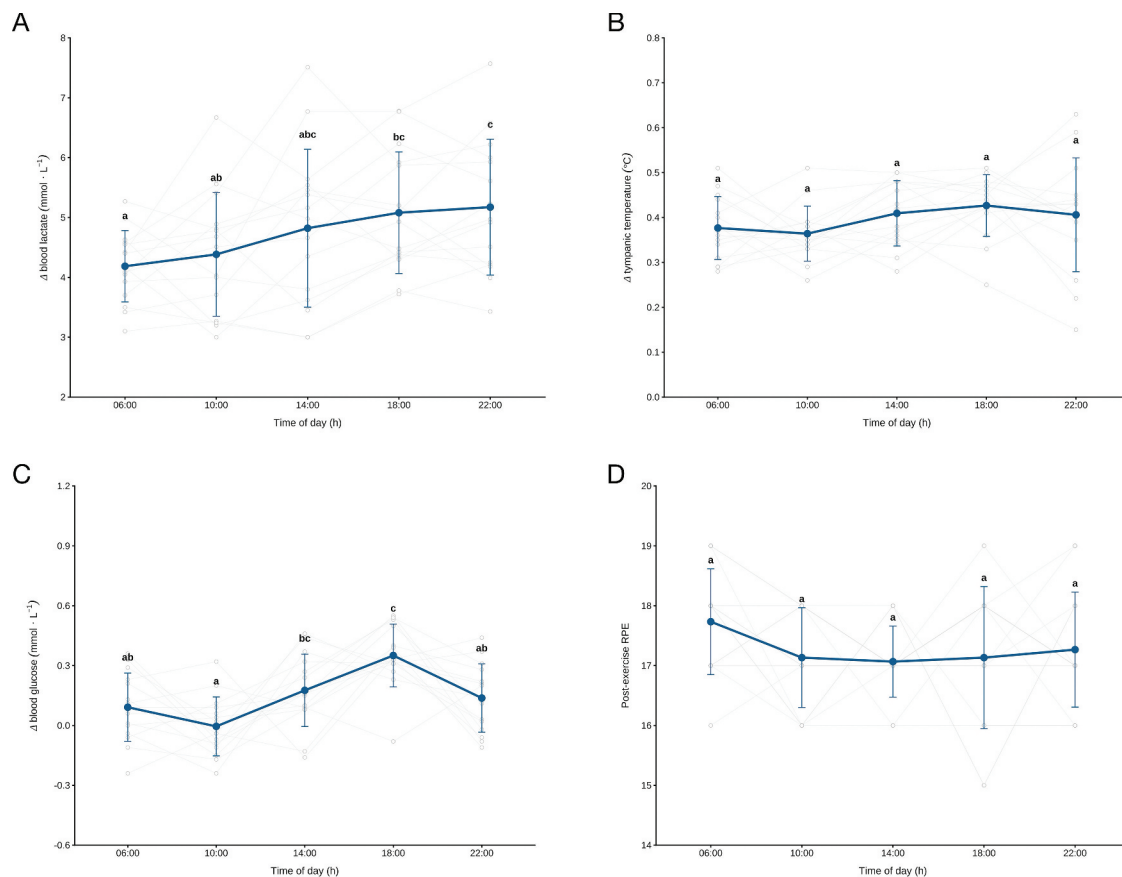


Figure 5. Time-of-day variation in physiological and perceptual responses to a 10-min cycling time trial. (A) Change in blood lactate concentration (Δ blood lactate; post-exercise minus pre-exercise), (B) change in tympanic temperature (Δ tympanic temperature; post-exercise minus pre-exercise), (C) change in blood glucose concentration (Δ blood glucose; post-exercise minus pre-exercise), and (D) post-exercise rating of perceived exertion (RPE) measured following 10-min self-paced cycling time trials performed at 06:00, 10:00, 14:00, 18:00, and 22:00 h in healthy recreationally active adults ($n = 15$). Grey lines and open circles represent individual participant responses, while blue symbols and error bars represent group mean $\pm$ standard deviation (SD). Time-of-day effects were assessed using linear mixed-effects models with participant included as a random effect and time of day included as a fixed effect. Significant main effects of time of day were observed for Δ blood lactate ($F_{4,56} = 5.86$, $p = 0.0005$) and Δ blood glucose ($F_{4,56} = 8.90$, $p < 0.0001$), whereas no significant effects were observed for Δ tympanic temperature ($F_{4,56} = 1.72$, $p = 0.16$) or post-exercise RPE ($F_{4,56} = 1.32$, $p = 0.27$). Different letters indicate significant differences between time points following Šidák-adjusted post hoc comparisons ($p < 0.05$).

Physiological and Perceptual Responses to Exercise

The exercise-induced increase in blood lactate concentration (Δ blood lactate) differed significantly according to time-of-day ($F_{4,56} = 5.86$, $p = 0.0005$, $\eta^2 = 0.295$, 95% CI 0.079–0.447; Figure 5(A)). The lactate response increased progressively across the day, rising from 4.19 ± 0.60 mmol·L⁻¹ at 06:00 h to 5.17 ± 1.14 mmol·L⁻¹ at 22:00 h, representing an increase of approximately 24%. Post hoc analyses revealed that the lactate response at 22:00 h was significantly greater than that observed at 06:00 h ($p < 0.05$), while responses at 14:00 h and 18:00 h were intermediate and did not differ significantly from either extreme. These findings indicate greater exercise-induced lactate responses during the afternoon and evening. However, these responses occurred during

a self-paced performance test in which greater power output, and consequently greater total work, was achieved later in the day. In contrast, the exercise-induced increase in tympanic temperature (Δ tympanic temperature) did not differ significantly across the day ($F_{4,56} = 1.81$, $p = 0.140$, $\eta^2 = 0.114$, 95% CI 0.000–0.245; Figure 5(B)). Participants demonstrated a relatively consistent increase in tympanic temperature following exercise, ranging from 0.36 ± 0.06 °C at 10:00 h to 0.43 ± 0.07 °C at 18:00 h, with no significant pairwise differences observed between testing times. A significant effect of time-of-day was observed for the exercise-induced change in blood glucose concentration (Δ blood glucose; $F_{4,70} = 9.30$, $p < 0.0001$, $\eta^2 = 0.347$, 95% CI 0.152–0.483; Figure 5(C)). The largest increase was

observed at 18:00 h ($0.35 \pm 0.16 \text{ mmol}\cdot\text{L}^{-1}$), whereas glucose concentration changed minimally during the 10:00 h trial ($-0.01 \pm 0.15 \text{ mmol}\cdot\text{L}^{-1}$). Post hoc analyses demonstrated that the glucose response at 18:00 h was significantly greater than that observed at 10:00 h ($p < 0.05$), while responses at 14:00 h were greater than those observed at 10:00 h and approached those recorded at 18:00 h. Responses at 06:00 h and 22:00 h were intermediate and did not differ significantly from the afternoon values. Post-exercise ratings of perceived exertion did not vary according to time-of-day ($F_{4,70} = 1.32$, $p = 0.27$, $\eta^2 = 0.070$, 95% CI 0.000–0.169; [Figure 5\(D\)](#)). Mean post-exercise RPE ranged from 17.1 ± 0.6 to 17.7 ± 0.9 AU across the five testing sessions, indicating that participants perceived the exercise bout to be similarly demanding irrespective of testing time. Notably, these findings occurred despite the significant enhancement in cycling performance observed during the afternoon and evening trials.

Sensitivity Analysis

In the exploratory sensitivity analysis, inclusion of pre-trial sleep duration as a covariate did not materially alter time-of-day pattern in mean cycling power output. Sleep duration was not independently associated with mean power output ($\beta = 1.43 \text{ W}\cdot\text{h}^{-1}$, 95% CI -3.36 to 6.21 , $p = 0.560$), while adjusted mean power remained greater at 14:00 h ($\beta = 11.97 \text{ W}$, 95% CI 4.37 to 19.57 , $p = 0.002$), 18:00 h ($\beta = 12.86 \text{ W}$, 95% CI 5.01 to 20.71 , $p = 0.001$), and 22:00 h ($\beta = 8.80 \text{ W}$, 95% CI 0.88 to 16.71 , $p = 0.029$) compared with 06:00 h.

Discussion

The present study investigated time-of-day variation in short-duration cycling performance, handgrip strength, and associated physiological and perceptual responses in healthy recreationally active adults under standardised experimental conditions. The principal findings were that both mean cycling power output and body mass-normalised handgrip strength exhibited significant time-of-day variation ([Figure 2](#)), with peak values occurring during the late afternoon to early evening. Specifically, mean power output increased by approximately 8.4% between 06:00 h and 18:00 h, while body mass-normalised handgrip strength increased by approximately 9.0% over the same period, demonstrating a similar temporal profile for physical performance. In contrast, post-exercise ratings of perceived exertion and exercise-induced changes in tympanic temperature

remained unchanged across the day ([Figure 5](#)), despite the significant enhancement in cycling performance and associated metabolic responses observed during the afternoon and evening trials.

Pre-exercise tympanic temperature, subjective sleepiness, blood glucose concentration, and blood lactate concentration also exhibited significant time-of-day variation ([Figure 4](#)), while exercise-induced blood glucose and lactate responses demonstrated clear temporal dependence ([Figure 5](#)). These findings are consistent with the well-established influence of biological time on thermoregulatory (Reilly and Waterhouse 2009b), metabolic (Van Cauter et al. 1991, 1997), and behavioural processes (Schmidt et al. 2007), suggesting that the observed performance differences occurred within a broader context of coordinated physiological variation across the waking day. Furthermore, population-mean cosinor modelling identified closely aligned acrophases for cycling performance and handgrip strength, occurring at 17:51 h and 17:27 h, respectively ([Figure 3](#)). Although these models were interpreted as secondary characterisation of temporal variation, the estimated peak timings were highly consistent with the afternoon-to-evening elevations identified by the mixed-effects analyses ([Figures 2 and 3](#)). These data demonstrate a coherent temporal pattern in which maximal physical performance occurs during the late afternoon to early evening and is accompanied by concurrent variation in thermoregulatory, metabolic, and perceptual state, consistent with established circadian regulation of human physiology and exercise capacity (Reilly et al. 2007; Chtourou and Souissi 2012; Vitale and Weydahl 2017; Vitale et al. 2017).

The observed 8.4% increase in mean power output observed between 06:00 h and 18:00 h indicates a substantial effect of time-of-day on short-duration cycling performance. This finding is consistent with recent endurance and time-trial literature showing superior performance later in the day (Edwards et al. 2025; Forster et al. 2025). In a recent cycling-specific study, Forster et al. (2025) reported significantly better 20-km cycling time-trial performance in the evening compared with the morning, with chronotype also influencing the magnitude of the response. Similarly, recent systematic reviews and meta-analyses indicate that endurance and time-trial performance are generally enhanced during late-afternoon or evening exercise compared with morning assessments (Bruggisser et al. 2023; Kang et al. 2023), although the magnitude and consistency of these effects vary according to exercise modality and outcome measure (Knaier et al. 2022; Kang et al. 2023), participant characteristics and

training status (Bruggisser et al. 2023), chronotype (Facer-Childs and Brandstaetter 2015; Forster et al. 2025), and the degree of experimental control and standardisation employed within the study design (Edwards et al. 2024, 2025). The present findings therefore add to contemporary evidence that time-of-day is an important determinant of endurance exercise capacity, while extending previous work by demonstrating a clear performance profile across five evenly distributed time points across the waking-day, rather than a single morning-evening comparison.

It is important to note that not all cycling time-trial studies have observed a time-of-day effect. For example, Zadow et al. (2020) reported no significant differences in overall mean power output or pacing during a 4-km cycling time trial performed at five time points between 08:30 and 20:30 h, despite observing a significant time-of-day effect for pre-exercise tympanic temperature. Such discrepancies are consistent with systematic review evidence demonstrating that time-of-day effects on endurance and time trial performance are less consistent than those observed for maximal strength and anaerobic performance, with considerable heterogeneity between studies in exercise modality and duration, participant characteristics and training status, chronotype, testing times, and experimental standardisation (Bommasamudram et al. 2022; Knaier et al. 2022; Bruggisser et al. 2023; Kang et al. 2023). Chronotype may be particularly relevant because the magnitude and timing of diurnal variation in performance can differ according to circadian phenotype (Facer-Childs and Brandstaetter 2015; Forster et al. 2025; Singh et al. 2026). Furthermore, studies employing more rigorous control of sleep, dietary intake, environmental conditions, and pre-trial behaviour have reported significant time-of-day effects in cycling and running performance (Edwards et al. 2024, 2025). In the present study, restriction to intermediate chronotypes, standardisation of sleep, diet, environmental conditions and recent activity, and assessment at five evenly distributed time points may therefore have reduced sources of inter-individual and methodological variability that could otherwise obscure temporal differences in performance.

These methodological considerations are particularly important when interpreting studies assessing endurance performance across the day. Recent investigations employing rigorous chronobiological standardisation have reported clear temporal effects. For example, Edwards et al. (2024) demonstrated significant diurnal variation in 4-km cycling time-trial performance under tightly controlled laboratory conditions, while Edwards et al. (2025) subsequently reported a significant circadian rhythm in 10-km running performance, with peak

performance occurring during the early evening. The present findings align closely with these observations and extend this emerging literature by characterising performance across five evenly distributed waking-day time points. Although the protocol did not provide complete 24-h coverage, the inclusion of assessments at 06:00, 10:00, 14:00, 18:00, and 22:00 h enabled a more detailed characterisation of the daily performance profile than traditional morning-versus-evening comparisons. Thus, suggesting that endurance performance is not simply different between two arbitrary testing windows, but instead follows a progressive waking-day trajectory culminating in a late-afternoon to early-evening performance optimum.

Handgrip strength demonstrated a temporal profile that closely paralleled cycling performance, increasing by approximately 9.0% between 06:00 h and 18:00 h. This finding is consistent with contemporary evidence showing that maximal strength and neuromuscular performance are generally enhanced later in the day, with peak values often occurring during the late afternoon or early evening (Knaier et al. 2022; Bruggisser et al. 2023; Del Val-Manzano et al. 2025). Notably, the estimated acrophase for handgrip strength in the present study occurred at 17:27 h, closely aligning with the acrophase for mean cycling power output at 17:51 h. A similar temporal organisation was reported by Edwards et al. (2025), who observed a significant rhythm in right-hand grip strength with an acrophase at 17:30 h alongside peak 10-km running performance in the evening. The agreement between cycling performance and handgrip strength in the present study suggests that the observed time-of-day effect was not limited to a task-specific cycling response but reflected broader temporal variation in physical performance capacity.

Pre-exercise tympanic temperature also increased progressively across the day, peaking at 18:00 h, closely coinciding with the highest observed cycling power output and handgrip strength. This temporal alignment is consistent with previous work suggesting that daily variation in body temperature contributes to, but does not fully explain, time-of-day differences in physical performance (Reilly and Waterhouse 2009a; Edwards et al. 2025). Higher body and muscle temperature may facilitate performance through several mechanisms, including improved muscle contractile properties and faster nerve conduction velocity (Racinais et al. 2005, 2010). Increased muscle temperature may also enhance enzyme activity and processes involved in calcium handling and excitation-contraction coupling (Knaier et al. 2022). However, the present data should not be interpreted as demonstrating a causal relationship between tympanic temperature and performance.

Rather, the parallel timing of peak temperature, cycling performance, and handgrip strength supports the interpretation that thermal state formed part of a broader physiological environment that was more favourable to high-intensity self-paced exercise during the late afternoon and early evening.

Pre-exercise blood glucose concentration was lowest at 06:00 h and highest at 14:00 h, with values at 06:00 h significantly lower than all subsequent time points. This likely reflects, at least in part, the overnight fast imposed before the 06:00 h condition and the greater opportunity for feeding before later trials, despite standardisation of meal timing and composition. This is an important consideration when interpreting the reduced morning performance, as carbohydrate availability and pre-exercise metabolic state can influence self-paced exercise capacity, particularly during protocols requiring sustained high-intensity work (Jeukendrup 2017; Hawley et al. 2020). However, the observed glucose profile should not be interpreted solely as a nutritional artefact. Glucose regulation is strongly time-of-day dependent, reflecting the combined influence of sleep–wake timing, feeding behaviour, insulin sensitivity, hepatic glucose output, and circadian regulation of metabolic tissues (Aoyama and Shibata 2020; Cauter et al. 1997; Dighriri et al. 2024). Therefore, although the lower pre-exercise glucose concentration at 06:00 h may have contributed to reduced morning performance, the present design cannot separate the relative contribution of feeding state, behavioural timing, and endogenous circadian phase.

Importantly, the 06:00 h trial also required participants to wake earlier than habitual, resulting in shorter sleep duration and substantially shorter interval between waking and exercise compared with the later testing conditions. Consequently, greater subjective sleepiness, residual sleep inertia, and reduced prior wakefulness may also have contributed to the lower performance observed at 06:00 h. The morning disadvantage should therefore be interpreted as a time-of-day effect occurring under the behavioural conditions associated with early morning testing, rather than as evidence of an isolated endogenous circadian effect.

Exercise-induced changes in blood lactate and glucose also varied according to time-of-day (Figure 5). The lactate response increased progressively across the day, with the largest response observed at 22:00 h, while the greatest exercise-induced increase in blood glucose occurred at 18:00 h, coinciding with peak mean power output and peak pre-exercise tympanic temperature. These findings suggest that the physiological response to a fixed-duration self-paced exercise bout was not uniform across the day. Accordingly,

because exercise was self-paced and neither external work nor exercise intensity was matched between conditions, the present data cannot establish time-of-day effect on metabolic responses independent of the greater exercise performance achieved later in the day. Nevertheless, the temporal pattern is consistent with previous observations that metabolic responses to exercise vary according to biological time. Edwards et al. (2025) reported lower post-exercise blood lactate values when running performance was slower, while Ezagouri et al. (2019) demonstrated time-of-day-dependent variation in exercise capacity and metabolic responses in both mice and humans. More recently, systematic review evidence has highlighted that the metabolic, glucose, and insulin responses to exercise may differ according to the time of day at which exercise is performed, although findings remain heterogeneous and dependent on study design, population, exercise mode, and feeding status (Dighriri et al. 2024).

The present data therefore support the concept that time-of-day differences in performance occur alongside variation in metabolic state and exercise-induced metabolic responses, but they do not establish a direct mechanistic pathway. The post-exercise glucose response may reflect hepatic glucose production (Wasserman 2008), altered peripheral glucose uptake (Richter and Hargreaves 2013), or differences in glucose flux during higher-intensity exercise performed later in the day (Ezagouri et al. 2019). Similarly, the larger lactate response may reflect increased glycolytic contribution, greater muscle recruitment, higher absolute power output (Gladden 2004; Brooks 2018), or a combination of these factors. This interpretation aligns with emerging molecular and metabolomic evidence indicating that the timing of exercise can shape tissue-specific metabolic responses, including skeletal muscle and liver-derived signalling pathways (Ezagouri et al. 2019; Sato et al. 2019; Hesketh and Esser 2024). However, because the present study measured capillary glucose and lactate only immediately before and after exercise, it cannot determine whether the observed responses reflect differences in substrate availability, production and/or utilisation, or recovery kinetics. Future studies incorporating indirect calorimetry, continuous glucose monitoring, stable isotope tracers, or targeted metabolomic approaches would be required to determine whether the enhanced afternoon–evening performance profile is driven by altered substrate use, metabolic flexibility, or coordinated circadian regulation of skeletal muscle and systemic metabolism.

Subjective sleepiness exhibited a pronounced time-of-day effect, with the highest KSS scores observed at 06:00 h and the lowest values occurring during the

afternoon. This temporal profile broadly paralleled the observed changes in cycling performance (Figure 2), suggesting that fluctuations in alertness may have contributed to the reduced morning performance and enhanced afternoon–evening exercise capacity. Previous laboratory studies have demonstrated that alertness, vigilance, and cognitive performance vary systematically across the day under the influence of circadian and homeostatic processes (Wright et al. 2002; Schmidt et al. 2007), and these factors may influence pacing decisions, motivation, and central motor drive during self-paced exercise. However, sleepiness alone is unlikely to fully explain the present findings. Although KSS scores increased again during the 22:00 h trial, mean power output remained substantially greater than during the morning sessions, indicating that factors beyond subjective alertness contributed to the observed performance rhythm. Consequently, sleepiness may represent one component of a broader network of physiological and behavioural processes influencing exercise capacity across the waking day.

Despite these differences in performance and physiological status, post-exercise RPE remained remarkably stable across all testing times (Figure 5). Participants therefore produced substantially greater power output and exhibited larger exercise-induced metabolic responses during the afternoon and evening without perceiving the exercise bout as more demanding. This observation is particularly interesting within the context of self-paced exercise, where perception of effort is considered an important regulator of pacing strategy and exercise tolerance (Marcora 2009; De Paula Caraça Smirmaul 2012). One interpretation is that the physiological cost associated with producing a given external workload was reduced later in the day, allowing participants to sustain higher power outputs while reaching a similar perceptual endpoint. Alternatively, participants may have selected a higher work rate during the afternoon and evening to maintain a comparable perception of effort across trials. Regardless of the precise mechanism, the dissociation between objective performance and subjective effort suggests that biological time influences not only physiological capacity but also the relationship between physiological strain and exercise perception.

Practical Implications

The present findings also have important practical implications for both research and applied practice. From a methodological perspective, the observed ~8–9% variation in cycling performance and handgrip

strength across the day highlights the importance of carefully controlling, reporting, and where possible standardising testing time in exercise physiology research. Failure to account for time-of-day may introduce substantial variability and potentially obscure or exaggerate the effects of training, nutritional, pharmacological, or rehabilitation interventions. Indeed, the magnitude of performance variation observed in the present study exceeds the improvements frequently reported following many short-term exercise interventions, emphasising that time-of-day should be considered an important experimental variable rather than a source of uncontrolled noise.

These findings also have relevance for applied settings. Performance testing, athlete monitoring, rehabilitation assessment, and evaluations of physical function are often performed at different times of day for logistical convenience. However, the present data suggest that testing conducted in the morning and late afternoon may yield meaningfully different outcomes, even when sleep, diet, environmental conditions, and recent activity are controlled. Consequently, practitioners should consider maintaining consistent testing times when monitoring changes in performance or physical function over time. More broadly, the identification of a late-afternoon performance optimum is consistent with growing evidence that exercise capacity varies systematically across the waking day and suggests that biological timing may represent an underutilised factor when interpreting physical performance assessments and designing future exercise interventions (Hesketh 2026).

Limitations

Several limitations should be acknowledged when interpreting the present findings. First, although performance and physiological responses were assessed at five evenly distributed time points across the waking day, measurements were not obtained continuously across a complete 24-h cycle. Consequently, the present findings should be interpreted as evidence of time-of-day variation under controlled but normally entrained conditions rather than definitive evidence of endogenous circadian rhythmicity. Consistent with this, mixed-effects cosinor analyses were included as secondary analyses to characterise the timing and magnitude of temporal variation across the waking day. Although significant 24-h sinusoidal components were identified for both cycling performance and handgrip strength, the absence of complete 24-h sampling and a direct marker of circadian phase precludes interpretation of these findings as evidence of endogenous circadian

rhythmicity. Accordingly, the mixed-effects comparisons across testing times remain the primary evidence for time-of-day differences.

Second, no direct markers of circadian phase were obtained. Measures such as dim-light melatonin onset (DLMO), melatonin concentration, or continuous core body temperature monitoring were not assessed. Therefore, it is not possible to distinguish the relative contributions of endogenous circadian regulation and behavioural or environmental influences to the observed temporal variation. Future studies incorporating established circadian phase markers and controlled laboratory protocols will be required to determine the extent to which the observed performance rhythms are driven by endogenous circadian mechanisms.

Third, the 06:00 h trial differed from the later conditions in several behavioural factors intrinsically associated with early morning testing. Participants completed this trial following an overnight fast, with shorter sleep duration and substantially less time awake before exercise than during subsequent conditions. These differences may have contributed to the lower pre-exercise blood glucose, greater subjective sleepiness, and reduced exercise performance observed at 06:00 h. Consequently, the present design cannot fully separate the effects of clock time from feeding status, sleep duration, sleep inertia, or time since awakening. The observed morning disadvantage should therefore be interpreted as time-of-day variation under normally entrained behavioural conditions rather than evidence of an isolated endogenous circadian effect. Similarly, the greater exercise-induced glucose and lactate responses observed later in the day may partly reflect both temporal variation in physiological regulation and/or higher absolute work performed during those trials rather than independent time-of-day effect on metabolic responses.

Additional methodological considerations should also be recognised. Tympanic temperature was used as a practical, non-invasive estimate of body temperature but does not provide the same precision as rectal, gastrointestinal, or continuously monitored core temperature measurements. Sleep behaviour was monitored using a consumer-grade wearable device rather than research-grade actigraphy or polysomnography. Although Fitbit-derived sleep timing measures have demonstrated acceptable agreement with validated actigraphy for monitoring habitual sleep–wake behaviour (Haghighat et al. 2019; Stucky et al. 2021; Park et al. 2024), measurement precision is inherently lower. Furthermore, menstrual cycle phase was controlled by scheduling testing during days 10–15 following the onset of menstruation; however, circulating ovarian hormones were not measured directly.

Finally, no formal a priori sample-size calculation was undertaken and the sample size was relatively small ($n = 15$). Although the repeated-measures design provided substantial within-participant information across the five testing conditions, estimates for secondary outcomes should be interpreted cautiously and require confirmation in larger cohorts. Furthermore, the sample consisted exclusively of healthy recreationally active adults classified as intermediate chronotypes. Restricting recruitment to intermediate chronotypes was intended to reduce between-participant variability in circadian phenotype and facilitate interpretation of group level time-of-day responses. However, this necessarily limits generalisability to individuals with morning or evening chronotypes. This may be particularly relevant to cycling and endurance populations, in which relatively early sleep–wake and rest–activity profiles have been reported (Vitale, Banfi et al. 2019, Vitale, La Torre, et al. 2019). Consequently, the exclusion of morning oriented individuals may have influenced the magnitude of the observed early morning performance decrement, and the present findings should not be assumed to generalise to individuals whose habitual circadian phenotype is better aligned with early morning exercise. Future studies should examine time-of-day variation across a broader range of circadian phenotypes and determine whether the magnitude and timing of performance differences vary according to chronotype.

Conclusions

In conclusion, the present study demonstrates clear time-of-day variation in short-duration cycling performance and handgrip strength under controlled repeated-measures conditions. Mean cycling power output and body mass-normalised handgrip strength were enhanced during the afternoon and evening compared with the morning, with both outcomes exhibiting peak values during the late afternoon to early evening. These performance differences occurred alongside coordinated variation in thermoregulatory, metabolic, and perceptual measures, including pre-exercise tympanic temperature, subjective sleepiness, blood glucose and lactate concentration, as well as exercise-induced glucose and lactate responses. In contrast, post-exercise ratings of perceived exertion remained unchanged despite the greater power outputs achieved later in the day.

Collectively, these findings suggest that daily variation in physical performance reflects the integration of multiple physiological and behavioural processes rather than changes in any single factor alone. Importantly, the magnitude of the observed performance differences

highlights time-of-day as a meaningful source of variability in exercise testing and performance assessment. However, the lower performance observed during the early morning condition may reflect the combined influence of clock time and behavioural factors associated with early testing, including shorter sleep duration, reduced time awake, and overnight fasting. Accordingly, time-of-day should be carefully considered and, where possible, standardised in exercise physiology research and applied practice. More broadly, these findings support the concept that human exercise capacity follows a distinct waking-day profile, with peak short-duration endurance and strength performance occurring during the late afternoon to early evening in healthy recreationally active adults of intermediate chronotype.

Acknowledgments

SJH conceived and designed the study. KDJ, MSL, RP, SSL, IJT, and SJH collected the data. SJH analysed the data and drafted the manuscript. KDJ, MSL and SSL contributed to manuscript preparation and revision. All authors read and approved the final manuscript.

Author Contributions

CRedit: **Stuart J. Hesketh**: Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing; **Kazi D. Jahan**: Data curation, Investigation, Project administration, Writing – review & editing; **Mariam S. Luvna**: Data curation, Investigation, Project administration, Writing – review & editing; **Rojan Pakka**: Data curation, Investigation, Project administration; **Subash Sing Lo**: Data curation, Investigation, Project administration, Writing – review & editing; **Ismat J. Talukder**: Data curation, Investigation, Project administration.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

The authors report generative AI was not used in their research or preparation of this manuscript.

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Data Availability Statement

The data supporting the findings of this study are available in the Figshare repository, DOI: <https://doi.org/10.6084/m9.figshare.32731137>

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