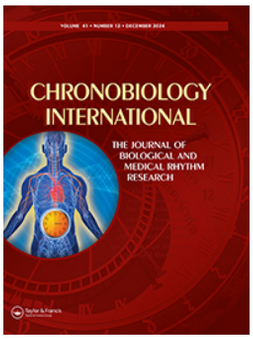

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ORIGINAL ARTICLE

Evidence of a circadian variation in 10-km laboratory running time-trial performance, where a standardised approach has been employed

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ABSTRACT

Diurnal variations in time-trial performance have been shown in people living normally, where a "standardised protocol" has been employed to reduce bias. We tested the hypothesis that a circadian variation exists for a 10-km running laboratory-based time-trial, where such a standardised approach is used. Twelve recreationally active adult males were recruited. The participants completed three familiarisation time-trials to the best of their ability at a self-selected pace and six 10-km time-trials at 06:00, 10:00, 14:00, 18:00, 22:00 and 02:00 h. Each session was separated by 7-days. Participants were allocated into 6 groups due to finish times (FT); sessions were counterbalanced in order of administration. A cosine fit for resting intra-aural temperature and FT both showed a significant circadian rhythm ($p < 0.05$) with mesor, amplitude and acrophases of 36.61°C vs 2994 s, 0.34°C vs 149 s; and 17:29 vs 18:44 h:min, respectively. The parallelism of temperature and FT agrees with previously published research. The finding of a 24-h rhythm in 10-km FT (5.0%, d = 0.80; power = 100%) concurs with that of a shorter distance where "standardised protocols" have been employed (4-km, 2.6%, d = 0.34). This finding has implications for scheduling of competition and training. Whether this variation is apparent in other populations, however, is unclear.

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Daily-variation; time-of-day; core body temperature; design-checklist

Introduction

In healthy males' physiological variables associated with sporting performance in a temperate environment (around 17–22°C), at rest and during sub-maximal exercise have been shown to exhibit daily variations in line with the temperature rhythm; with relatively lower levels of the measured variable in the morning and peaks in the afternoon (Drust et al. 2005; Reilly et al. 2000). A daily variation in maximal exercise exists, such that greater muscle force production (force, torque, power and velocity) in single-joint isometric and isokinetic ergometry dynamometers occurs in the evening compared to the morning (Edwards et al. 2013; Pullinger et al. 2019). But also, in complex multi-joint movements (using a linear encoder), which is more directly transferable to the world of athletic and sports performance. Where time-to-peak velocity and time-to-peak torque occur in the morning compared to the evening (Robertson et al. 2018, 2024). Similar temporal pattern occurs for repeated sprint performance (Ravindrakumar et al. 2022) and is observed in 30-s all out supramaximal Wingate cycle ergometer if the warm-up duration and intensity is not prolonged and of high intensity respectively (Hill and Smith 1991; Souissi et al. 2007, 2010). However, maximal aerobic ability or $\dot{V}O_2$ max/peak is a stable function whether observed transversely within the same day or over multiple days (longitudinally), in respect of time of day (Drust et al. 2005).

Time-of-day variation in performance in participants who have not undertaken rhythm disturbance (jet-lag and night shift work) hence are entrained (synchronised using zeitgebers [time-givers] such as light, feeding, and social activity) to a 24-h day is attributed to two main factors. Firstly, the feedback mechanisms associated with the internal controller of rhythmicity (the biological clock), which is evolutionary pre-set to keep the human active and awake during the solar day and asleep and inactive during the night (endogenous input to the rhythm; Reilly and Waterhouse 2009). And, secondary the general nychthemeral lifestyle, with a noisy, exciting environment in the daytime and a time-of-day temporal preference to train, etc. (exogenous input to the rhythm; Edwards et al. 2023). To the best of our knowledge there has only been one study investigating if a circadian rhythm (where 6 equally spaced time points were

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measured over the solar day) exists in sporting time-trial performance. Kline et al. (2007) employed an ultra-short-sleep wake cycle protocol to standardise the exogenous input and enable the investigation of the internal component. This type of protocol can remove the "environmental" component and effects of time-since-sleep are greatly decreased but cannot remove the direct effects of sleep (Reilly and Waterhouse 2009; Edwards et al. 2023. Kline et al. (2007) reported that for a 200 m swimming time-trial, fastest finishing times were in the evening; with group cosinor analysis showing an acrophase/peak at 18:03 h:min, hence minimum at 02:03 h:min (as difference between peak and minimum is 12 h).

Research investigating daily effects on time-trial performance itself (time-to-completion of a set distance) other than Kline's work, have use a diurnal approach - where experimental times are taken during the daylight hours of the day rather than throughout the 24 h of the solar day. Normally in the morning (06:00–09:00 h) and evening (17:00–21:00 h) are undertaken. This morning time-of-day is chosen pragmatically, to be to as close to (body temperature minimum, 04:00–05:00 h Waterhouse et al. 2005) but without compromising performance by sleep loss (Edwards et al. 2024; Reilly and Edwards 2007; Walsh et al. 2021). However, such a research design where only two or more times of day are employed (during the daylight hours) risks missing the true peak of the variation. Hence a circadian approach like that of Kline et al. (2007) where several equally spaced sessions (normally 6, at 4 h spacings) around the solar day is recommenced Reilly and Waterhouse (2009). The findings of diurnal variation type studies investigating time-trial are equivocal (Bommasamudram et al. 2022). It might be that daily rhythms that contribute to such performance are lost in maximal activity (like $\dot{V}O_2$ max/peak) as they reach a "ceiling," hence negating a time-of-day effect (Edwards et al. 2024). This may be more prevalent in an elite population whose pacing strategy may be less variable, however, this has not been systematically investigated (Drust et al. 2005; Edwards et al. 2024). Another explanation in laboratory-based chronobiological research could be due to methodological issues, because of a lack of rigor and standardisation in method employed reduce the chance of finding a variation. As well as a lack of control for important factors which specifically relate to investigations of chronobiological nature and other considerations (Reilly et al. 2000; Winget et al. 1985; Youngstedt and O'Connor 1999).

Although a recent systematic review highlighted the need for a rigorous, standardised approach to be adopted by future investigations into a possible daily variation in time-trial performance (Bommasamudram et al. 2022). Presently, there is no consensus on what exactly a standardised approach should entail, as well as no universally adopted practical guidelines for researchers to consider exist (Bruggisser et al. 2023; Drust et al. 2005; Knaier et al. 2022; Winget et al. 1985; Youngstedt and O'Connor 1999; Yousefzadehfard et al. 2022). However, an attempt to consider some of these concerns and standardise this approach has recently been presented (Edwards et al. 2024). This involves checklist of considerations in chronobiological studies on humans and sporting performance with participant, environmental, as well as methodological and equipment considered. Therefore, the aim of the present study was to investigate whether a circadian rhythm in time-trial performance was present in 10-km time-trial treadmill running, using a standardised protocol. Where bias is reduced by inclusion/exclusion criteria, participant, methodological, equipment and environment considerations. We hypothesis that finishing times will be lower ~18 h (better performance), with a corresponding peak in core temperature.

Methods

Participants

Twelve "recreationally active" adult males as classified by the "Participant Classification Framework" (McKay et al. 2021) took part in the study (mean ± SD): age = 21.8 ± 2.9 years, body mass = 77.6 ± 9.6 kg, height = 180 ± 7.0 cm, $\dot{V}O_2$ peak = 63.2 ± 3.7 $mL^{-1}{\cdot}kg.min^{-1}$ and habitual total sleep time = 8.4 ± 0.9 h. Participants were required to arrive fasted and abstain from alcohol, caffeine, and exercise 24 h preceding a testing session, with no napping between sessions. *Inclusion criteria*: Participants had to be healthy males (18–30 years), free from musculoskeletal injury and agree to retire to bed at 22:30 h and rise at 06:30 h. *Exclusion criteria*: None of the participants could be receiving any pharmacological treatment throughout the study period (Yousefzadehfard et al. 2022). Habitual caffeine consumption was assessed using the caffeine consumption questionnaire (CCQ) and those with <150 mg per day were excluded (Landrum 1992). Further, all participants expressed no preference to training regarding time of day by a weekly self-reported 2-week training diary. Lastly, only "intermediate chronotypes types" were recruited. Where the circadian chronotype of the participants was assessed using a composite "morningness questionnaire" by Smith et al. (1989). The participants' mean "chronotype" score on a 13–52 scale was 34.1 ± 5.4 (intermediate types). A circadian-type inventory questionnaire of Folkard et al. (1979) determining languidness/vigorous, and flexibility/rigidity of participants was also utilized. The participants' mean scores were 41.4 ± 8.7 and

47.0 ± 7.1 for languidness/vigour and flexibility/rigidity respectively. Further considerations are given in Table 1. All the participants gave their written informed consent and were found to be flexible and languid – according to the definitions of the questionnaire. The study was conducted in accordance with the Local Ethics committee (Ethics code U06SECO22), the ethical standards of the journal and complied with the Declaration of Helsinki.

Experimental Design

The participants attended the environmentally controlled chamber (Edge, Nottingham, UK) on nine occasions, the chamber was held at a dry temperature of 19°C, 35–45% humidity and a barometric pressure of 750–760 mmHg, respectively. Each participant completed i) three 10-km time-trial (TT) familiarisation sessions at a self-selected pace (detailed below) and thereafter each participant completed, ii) a $\dot{V}O_2$ peak/max test on a treadmill (H/P/cosmos Pulsar 4.0 treadmill, Nussdorf- Traunstein, Germany). This treadmill was used for all running measured in this research and will just be reference to as a "treadmill" in later text. Individuals then completed six 10 km time-trials at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00 h (Figure 1). The design of the study was such that the sessions were counterbalanced in order of administration to minimise any potential learning effects, with volunteers allocated into six groups based on finishing times (Edwards et al. 2024). There was a minimum of 72 h to ensure recovery between trials, during this time participant did not engage in in heavy exercise between sessions with specific requirements for rising and retiring and food intake given in the *Protocol for the experimental sessions* section. All experiments were completed between the months of October and December (Autumn to Winter in the UK) to ensure the individual's exposure to sunlight in the mornings when entering the laboratories was < 80 Lux (Physically measured using a Lightwatch, Neurotechnologies, Cambridge, UK), with sunrise and sunset range from start to the end of the experiment being 07:29 to 08:01 h and 18:01 to 19:50 h respectively.

Protocol and Measurements

Familiarisation

Participants completed three familiarisation tests at 12:00 h. A standardised 5-min warm-up (WU) at 10 $km.h^{-1}$ was completed prior to each time-trial on a treadmill (h/p cosmos, pulsar® 3p, Germany). Heart rate (HR, Polar FT2), ratings of perceived exertion (RPE, Borg 1973) and split times were measured every 1-km during the time-trial. Together with the participants' perception of the pacing required to finish as quickly as possible (−5, "too slow," 0, "optimal" to + 5, "too fast," Bridget Optimal Pacing scale; BOP). Fingertip blood lactate samples (Biosen C-Line Glucose and Lactate Analyzers, EKF Diagnostics, Cardiff, UK) and intra-aural (T_{IA}) temperature measurement (Genius 3 Tympanic Thermometer, Cardinal Health, Leeds, UK) were taken at rest and immediately after the time-trial. The random error between the two initial reliability trials was heteroscedastic and the data were therefore expressed as 95% ratio limits of agreement. For simplicity, only the 95% ratio limits of agreement for the first and second 10-km time-trial performance were investigated for the 12 participants; these were */÷ 1.028 (about 2.8%) and coefficient of variance of 1.5%, indicating little measurement error (Atkinson and Nevill 1998). Paired *t*-test showed no difference ($t_{11} = 1.493$, $p = 0.164$). One week after these preparatory tests, volunteers underwent the experimental data collection sessions.

Subsequently, participants completed a $\dot{V}O_2$ peak/max test using the Metalyzer 3B (Cortex GmbH, Leipzig, Germany). Tests were conducted on a HP cosmos treadmill with reusable masks (7450 Series Silicone V2, Hans-Rudolph Inc., KS, USA) following the BASES guidelines (Hale et al. 1988). Five min of resting data

Table 1. Mean values for parameters of the circadian rhythm of resting TIA, finishing time, grip strength and stroop tests.

Variable	Mesor	Amplitude	Acrophase (h:min)	Significance (*p*-value)	d, Observed power (%), % Rhythm
Resting core temperature T_{IA} (°C)	**36.61**	**0.34**	**17:29**	$p < 0.0001$	**2.65, 70.1, 0.93**
Finishing time (s)	**2994**	**149**	**18:44**	$p = 0.0147$	**0.80, 100, 4.98**
Grip strength					
Right grip strength ($N.m^{-1}$)	**42.2**	**3.4**	**17:30**	$p = 0.038$	**0.27, 100, 8.06**
Left grip strength ($N.m^{-1}$)	37.9	1.9	17:55	$p = 0.237$	0.20, 61.7. 5.01
Stroop					
Word-not-colour Total Score	107	4 .4	16:37	$p = 0.526$	0.53, 76.7, 4.11
Word-not-colour Errors	*1*	*0.6*	*03:54*	$p = 0.08$	*0.77, 56.5, 60.00*
Colour-not-words Total Score	56	2.4	14:19	$p = 0.527$	0.15, 7.3, 4.29
Colour-not-words Errors	1	0.1	20:44	$p = 0.819$	0.34, 90.7, 10.00
Mental toughness	67.7	0.4	23:45	$p = 0.921$	1.11, 68.4, 0.59

Statistical significance ($p < 0.05$) is indicated in **bold**, those where $0.10 \geq p \geq 0.05$ are shown in *italics*. Effect size (d) from within-factor ANOVA for each group as well as observed power (%) and % rhythm (Amplitude/Mesor *100) values are given.

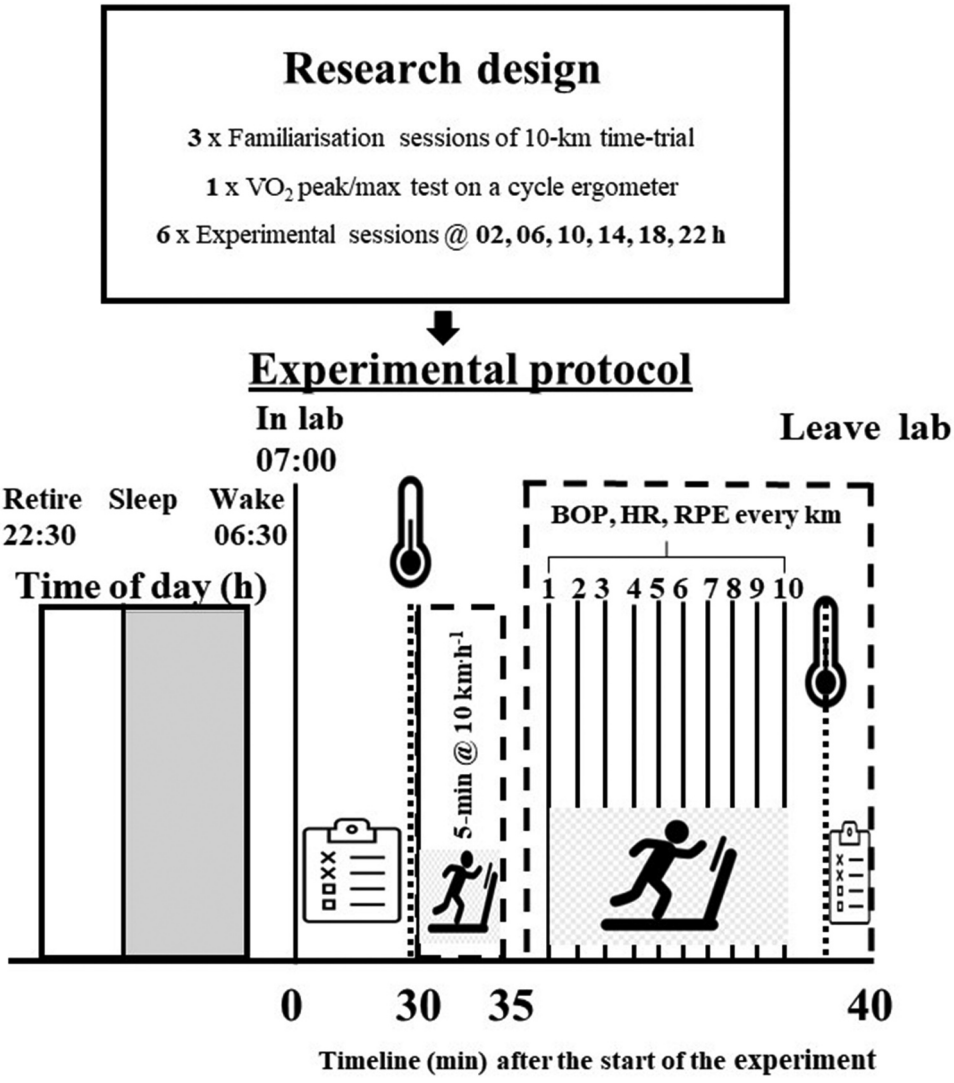

Figure 1. Schematic of the research design and protocol for six conditions, for most conditions participants upon waking came to the lab for 07:00 h and filled out the sleep questionnaires to return at the designated time. The only exception was in the 06:00 h session, where the participants retired at 22:00 h to rise at 05:30 h. Intra-aural (T_{IA}) temperature and ratings of perceived exertion (RPE) were measured after the participants had reclined for 30-in at the start of the protocol and after the warm-up. RPE and HR values were also measured prior to taken every 1-km and the 10 km time-trial in the environmental chamber; with pacing asked every 1-km. Black dotted lines indicate blood taken.

were collected before participants completed a 5 min WU at 10 km.h^{-1}, which also served as the first testing stage. Speed was then increased by 2 km.h^{-1} every 3 min until 16 km.h^{-1} was reached, there after each stage consisted of increasing the gradient by 2% until volitional exhaustion. The test will be referred to a "peak" rather than "max" if the participants did not reach the criterial of a plateau in the $\dot{V}O_2$ data between the last and penultimate stage.

Protocol for the Experimental Sessions

The main experiment consisted of six visits: at 06:00, 10:00, 14:00, 18:00, 22:00 and 02:00 h in the environmental chamber (Edge, Nottingham). Participants came into the morning session having fasted, were given a glass of water, and were asked to refrain from consuming food 3–4 h before the other sessions (Drust et al. 2005; See Figure 1). In most sessions the participants retired at around 22:30 h and rose around 06:30 h (opportunity for 8 h sleep). The only exceptions were in the 06:00 and 02:00 h sessions, where the participants either retired at 22:00 h to rise at 05:30 h (opportunity for 7.5 h; 06:00 h condition) or stayed up until the start of the 02:00 h condition respectively (Baxter and Reilly 1983). When retiring or rising participants were sent an automated text message which they had to

respond to within 5 minutes (min). Compliance with retiring and rising was high, with participants confirming they had adhered to the schedule as assessed by the text response or by verbal check in the morning and during the experimental sessions. The volunteers were free to live a "normal life," between sessions sleeping at home at night, attending lectures and doing light office work in the day. They were asked to refrain from any other training or heavy exertion for the 48 h before the experiments or during them. Participants recorded the type, amount, and timing of the food they ate for the period of 24 h before the day of the first session and were asked to replicate this diet for the days before the other experimental conditions. Water and non-caffeinated/non-alcoholic, calorie-free beverages were allowed ad libitum up to a total volume of 3 L per day.

Measurement Procedures

On arrival to the laboratory, 30 min before the tests individuals rested in a supine position to measure resting intra-aural temperature (T_{IA}). Like familiarisation T_{IA} temperatures were recorded as the average of the last two min of the 30 min of rest. At this time fingertip blood lactate samples were also taken, both T_{IA} temperature and blood lactate measures were taken immediately after the time-trial (Edwards et al. 2002). Participants provided urine samples upon entering the laboratory and after the TT. To determine volunteer's hydration status urine samples were analysed using an osmometer (Pocket PAL-OSMO, Vitech Scientific, Japan). During the resting period participants completed questionnaires on profile of mood states (POMS; Shacham 1983) and their sleep (adapted from Waterhouse et al. 2005). Thereafter they undertook the Stroop test (Stroop 1935), this test was completed again after the time-trial; where participants were asked to read out their responses to words or colours for 45 s. This was filmed and the number of errors and total amount completed were recorded and analysed. The first sheet had text (red, blue, yellow, black and green) in black ink (Naming word test), the second sheet blocks of colour corresponding to the text on the first sheet (Naming colour test). The third sheet the participants had to read out the word (which was coloured differently to the word e.g. the word was yellow and the colour red; referred to as Naming colour of word test) and the 4th sheet the participants had to read out the colour (which was wrongly named e.g. the colour was yellow but the word was red referred to as Naming of colour test), in this 4th sheet the words were printed in the reverse order to the 3rd sheet.

Participants were also asked to put on a heart rate (HR) monitor. Following the rest period, participants completed a 5 min WU at 10 $km.h^{-1}$, on a treadmill. After the WU participants completed a 10 km TT. RPE and HR were recorded every 1-km completed. Further participants were asked to verbally rate their pacing corresponding for that 1 km, relative to that required to finish as quickly as possible (−5, "too slow," 0, "optimal" to + 5, "too fast"). Strong and consistent verbal encouragement was provided throughout the TT. Participants were blinded from the clock but were provided with feedback on distance covered and speed ($m.s^{-1}$) via the display (Edwards et al. 2005; Gough et al. 2017).

Statistical Analysis

Our primary outcome was the time to complete the 10 km time-trial. Such protocols have been found to be highly reliable with test-retest coefficients of variation (CV) < 2% - like our study (Batterham and Atkinson 2005). Using the NQuery software (Cork, Ireland), it was estimated that eight participants would facilitate the detection of a statistically significant difference between trials of 3.3% assuming a CV of 2%, $p < 0.05$ and 80% power with a two-tailed paired *t*-test. We recruited 12 participants to account for dropouts. Data were analysed using the Statistical Package for Social Sciences version 29 (SPSS, Chicago, IL, USA). All data were checked for normality using the Shapiro-Wilk test. General linear models were used for all measurements collected and this was supplemented by single and group cosinor analyses using a period of Tau = 24 h (Nelson et al. (1979). The parameters derived from this analysis were: mesor (M: a time series mean), the amplitude (A: half the distance from the peak to the trough of the fitted curve) and the acrophase (Φ: time-of-peak of the fitted curve). The significance of the fitted curve was determined by comparing its variance with the total variance using a F-test. The bathyphase (time of minimum of the fitted curve, 12 h difference from the acrophase) was derived. To correct sphericity violations the degrees of freedom were corrected using Greenhouse-Geisser (ε<0.75) or Huynh-Feldt (ε>0.75). Bonferroni pairwise and graphical comparisons were made where main effects were found. Results are presented as mean ± standard deviation (SD) unless otherwise stated. Significance was set at $p < 0.05$. Effect sizes (Cohens d) were calculated from the ratio of the mean difference to the pooled standard deviation. The magnitude of "d" was classified as trivial (≤0.2), small

(>0.2–0.6), moderate (>0.6–1.2), large (>1.2–2.0) and very large (>2.0) based on guidelines from Batterham and Hopkins (2006). The results are presented as the mean ± the standard deviation throughout the text unless otherwise stated. Ninety-five percent confidence intervals are presented where appropriate as well as the mean difference between pairwise comparisons.

Results

Intra-Aural Temperature

Resting T_{IA} temperature showed a significant circadian rhythm ($p < 0.05$), the values were lower in the morning and higher in the evening (mesor, amplitude and acrophase of 36.61°C, 0.34°C and 17:29 h:min, respectively; d = 2.65, Figure 2 and Table 1). In agreement, general linear models showed a significant time-of-day effect with higher T_{IA} values (mean difference = 0.74°C, $p < 0.001$, 95% CI: 0.47–1.01°C; Table 2) at 06:00 than 22:00 h:min. A significant rise in temperature values from rest to immediately after the time-trial was evident (mean difference = 0.93°C, $p < 0.0005$, 95% CI: 0.64–1.22°C; d = 0.82), however there was an interaction for TOD x Pre-post time-trial T_{IA} where profiles for pre and post differ. Between 02:00–06:00 h pre values ride and post declines; and between 18:00–22:00 h pre-values are flat and post T_{IA} rises ($p > 0.05$). *The presence of a diurnal difference in Intra-aural temperature agrees with other work.*

Time-Trial Performance

Group cosinor analysis on the rhythm parameters for each participant showed a significant circadian rhythm ($p < 0.0001$), with mesor, amplitude and acrophase (peak) of 2994 s, 149 s and 18:43 h:min respectively (d = 0.80, Table 1). There was a significant time-of-day effect for finishing time ($p = 0.013$), wherein performance (as indicated by faster completion time) was worse in the morning and better in the evening (mean difference = 346 s, $p < 0.0005$, 95% CI: 121–813 s; see Figure 2 and Table 2). *The presence of a circadian variation in finishing time addresses a principle aim of this research project.*

Grip Strength (Right and Left Hand)

Group cosinor analysis on the rhythm parameters for each participant showed a significant circadian rhythm ($p = 0.038$, d = 0.27) for right grip strength, with mesor, amplitude and acrophase of 42.2, 3.4 $N.m^{-1}$ and 17:30 h:min respectively. In agreement there was a significant time-of-day effect for right grip strength with lower strength values at 02:00 and higher at 20:00 h ($p < 0.001$; Tables 1 and 2). There was no significant rhythm or time-of-day effect for left grip strength ($p > 0.05$).

Responses to TT Performance (Split Times/Km), HR, RPE, BOP, Urine Osmolarity, HCT HB and Blood Lactate)

There was a main effect for distance such that during the time-trial RPE and HR values increased from the first to the last km ($p < 0.05$; See Table 2 and Figure 3). Split times were relatively even throughout the TT with a decrease in the last Km ($p = 0.004$), with participants perceptions of pacing expressed per km moving from zero (optimal) for the first 5-km to, too fast after 5 km (a positive score $p < 0.001$). This agreed when perception of pacing expressed for the first half and second, respectively (Table 2). Blood lactate (Δ5 mmol/L) and urine osmolarity values (Δ11.8 mmol/L; $p < 0.05$) increased post time-trial compared to rest, Hct and Hb measures did not change. There was a time-of-day effect for split times and heart rate, with heart rate reflecting the faster speeds (hence, lower split times) during the 18:00–22:00 h time-trials. Further, when perception of pacing is expressed for the first half and second, respectively, participants rated their pacing as below or around optimal for the morning 02:00–10:00 h and as too fast to complete the time-trial as fast as possible from 14:00–-22:00 h (Table 2, Figure 2). Lactate values showed an interaction where the post TT values were lower at 02:00 than 14:00 h (2.5 mmol/L, CI = 0.24 to 4.8, $p = 0.025$; Table 2).

Resting POMS, Mental Toughness, Sleep Questionnaire Variables and Resting and Post Stroop Responses

Stroop showed a significant worsening of performance (decrease in total and increase in errors) pre-post time-trial where participants concentrated on black ink, words not the colours of the text for total score and errors. With an increase in errors in colours not the words of the text ($p < 0.05$, see Table 2). There was a significant TOD effect where lower ratings of the mood state fatigue (mean difference = 4.4, $p = 0.006$, 95% CI: 0.1–8.8; d = 0.33) and higher rating of vigour values (mean difference = 4.5, $p = 0.010$, 95% CI: 0.4–8.6; d = 0.25) between 14:00–18:00 h compared to the 06:00 h condition (Table 3). There were no main effects for TOD for any other mood responses. Self-reported ratings of Alertness 30 min after waking, tiredness and waking time were worst and earlier

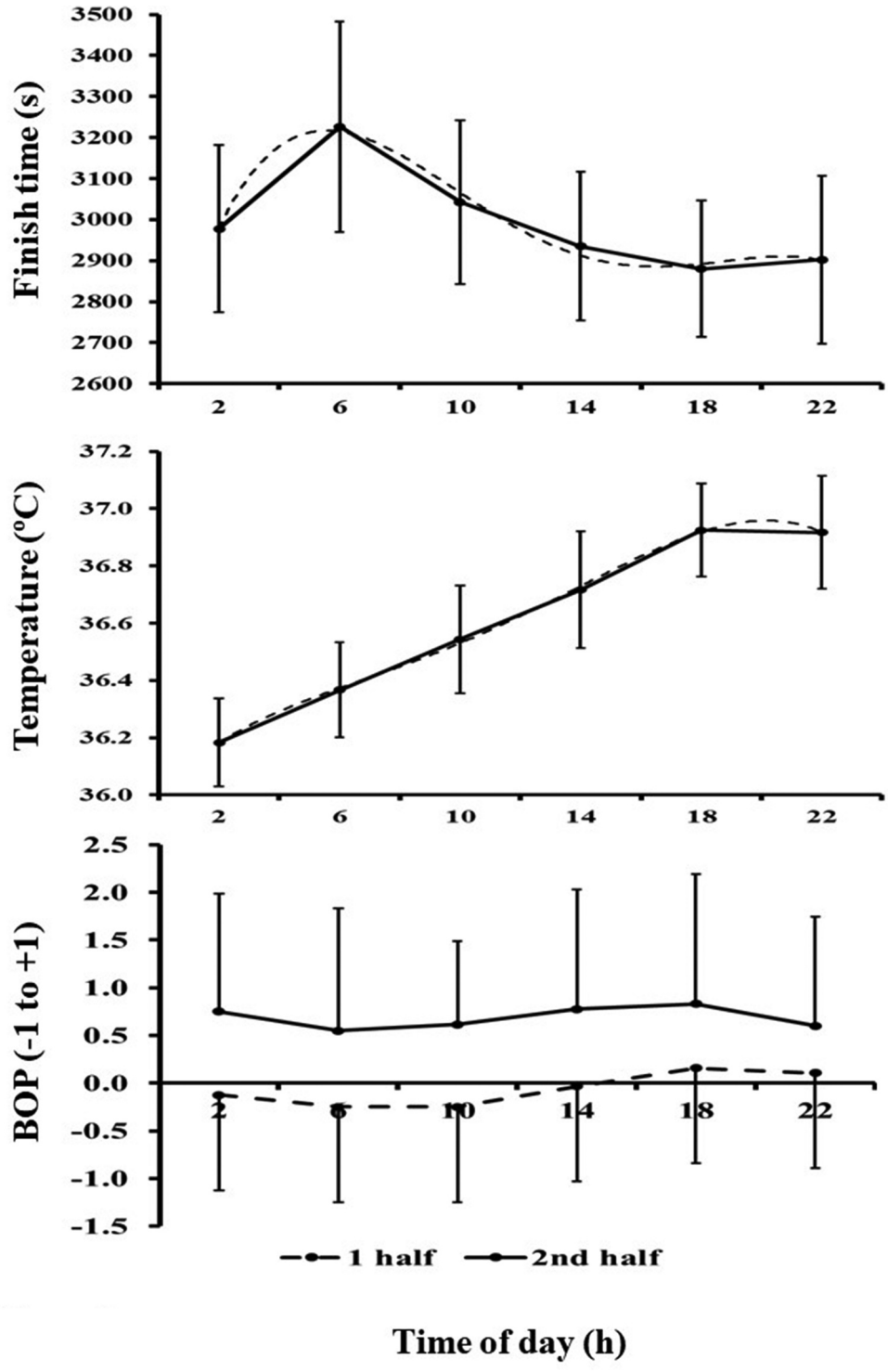

Figure 2. Circadian rhythms for finishing time, T_{IA} temperature and perception of pacing (for the first 5-km and second, ZERO = optimal strategy) for six times-of-day.

respectively, at 02:00 and 06:00 h; reflecting the imposition of the schedule compared to normal sleep/wake cycle of the participant (Table 3). Mental toughness values were lower at 02:00 and 14:00 h than the other TODs ($p = 0.024$). There was a TOD effect for Colours-Not Word Total Score where responses were worst at 02:00 h and highest at 10:00 h ($p < 0.05$). An interaction for pre-post by TOD was found for Word-Not Colours Total Score, where differences between pre-post values are greatest from 06:00–10:00 h (Table 3).

Table 2. Significance (*p*-value) for main effects for time-of-day (DV), distance or pre-post (PP) and the interaction of (DV x distance or DV x PP) for variables are given.

Variable	*p*-value Distance or PP	*p*-value TOD	*p*-value TOD x distance (or PP)
Rest and post TT core temperature T_{IA} (°C)	**$F_{1, 11}$ = 50.099, $p < 0.001$**	**$F_{3.0, 32.5}$ = 14.535, $p < 0.001$**	**$F_{3.1, 34.68}$ = 5.386, $p = 0.003$**
Grip Strength (N.m^{-1})			
Right		**$F_{2.8, 30.7}$ = 26.941, $p < 0.001$**	
Left		*$F_{3.1, 34.4}$ = 2.704, $p = 0.059$*	
Time-trial			
Split times (s)	**$F_{4.0, 43.6}$ = 4.424, $p = 0.004$**	**$F_{2.4, 26.8}$ = 4.698, $p = 0.013$**	$F_{3.9, 4.2} = 1.073$, $p = 0.381$
RPE (6–20)	**$F_{3.0, 32.9}$ = 134.063, $p < 0.001$**	$F_{3.2, 34.8} = 1.897$, $p = 0.146$	$F_{6.1, 67.3} = 1.146$, $p = 0.346$
Heart rate (BPM)	**$F_{2.2, 23.8}$ = 91.828, $p < 0.001$**	**$F_{2.3, 25.2}$ = 5.412, $p = 0.009$**	$F_{5.9, 64.7} = 1.444$, $p = 0.213$
Pacing (BOP)	**$F_{2.0, 21.7}$ = 11.740, $p < 0.001$**	$F_{3.1, 34.4} = 0.858$, $p = 0.476$	$F_{5.5, 60.6} = 0.823$, $p = 0.548$
Pacing (BOP) 5 and 10 km	$F_{1, 11} = 0.118$, $p = 0.737$	**$F_{2.8, 31.2}$ = 6.941, $p = 0.001$**	$F_{5.0, 55.0} = 0.720$, $p = 0.611$
Lactate (mmol/L)	**$F_{1, 11}$ = 106.101, $p < 0.001$**	$F_{3.4, 37.1} = 1.630$, $p = 0.195$	**$F_{2.5, 27.3}$ = 3.623, $p = 0.032$**
Haematocrit (%)	$F_{1, 11} = 0.883$, $p = 0.368$	$F_{3.3, 36.4} = 2.194$, $p = 0.100$	$F_{2.6, 29.4} = 1.270$, $p = 0.290$
Haemoglobin (mmol/L)	$F_{1, 11} = 0.302$, $p = 0.593$	*$F_{3.7, 40.7}$ = 2.194, $p = 0.069$*	*$F_{2.5, 36.7}$ = 2.332, $p = 0.084$*
Urine osmolarity (mmol/L)	**$F_{1, 11}$ = 90.117, $p < 0.001$**	$F_{3.1, 34.0} = 0.154$, $p = 0.930$	$F_{2.8, 31.3} = 1.337$, $p = 0.280$
Stroop			
Black Ink Total Score	**$F_{1, 11}$ = 5.765, $p = 0.035$**	$F_{2.0, 21.7} = 2.039$, $p = 0.155$	$F_{2.0, 22.4} = 1.666$, $p = 0.211$
Black Ink Errors	**$F_{1, 11}$ = 5.500, $p = 0.039$**	$F_{2.9, 32.0} = 0.551$, $p = 0.646$	$F_{2.3, 29.4} = 1.735$, $p = 0.192$
Coloured Squares Total Score	$F_{1, 11} = 1.719$, $p = 0.216$	$F_{2.9, 32.2} = 0.821$, $p = 0.489$	$F_{2.5, 30.0} = 0.276$, $p = 0.810$
Coloured Squares Error	*$F_{1, 11}$ = 3.920, $p = 0.073$*	$F_{3.3, 58.5} = 0.627$, $p = 0.613$	$F_{3.1, 33.9} = 0.300$, $p = 0.830$
Word-Not Colours Total Score	**$F_{1, 11}$ = 8.719, $p = 0.013$**	$F_{2.8, 30.9} = 1.804$, $p = 0.170$	**$F_{2.9, 32.2}$ = 3.865, $p = 0.012$**
Word-Not Colours Errors	**$F_{1, 11}$ = 5.427, $p = 0.040$**	*$F_{3.0, 33.2}$ = 2.553, $p = 0.072$*	$F_{2.7, 30.6} = 1.105$, $p = 0.359$
Colours-Not Word Total Score	$F_{1, 11} = 0.234$, $p = 0.638$	**$F_{3.2, 35.0}$ = 2.827, $p = 0.050$**	$F_{3.2, 34.9} = 1.680$, $p = 0.187$
Colours-Not Word Errors	**$F_{1, 11}$ = 13.039, $p = 0.004$**	$F_{3.5, 38.1} = 1.212$, $p = 0.321$	$F_{3.6, 40.0} = 0.955$, $p = 0.436$

Statistical significance ($p < 0.05$) is indicated in **bold**, those where $0.10 \geq p \geq 0.05$ are shown in *italics*. *indicates a significant DV. Where RPE = rating of perceived exertion and BOP = pacing performance.

Discussion

Summary of Main Observations

To date our understanding of the daily variation in time-trial performance is largely informed by diurnal studies, where two or more times of day during the solar day are measured – rather than the full 24 h period. The current investigation found that in a population of recreational runners, a circadian rhythm in 10-km TT running finishing times. With acrophase at 18:44 h, mesor of 2994 s and amplitude of the rhythms of 149 s respectively (Δ 5.0% in performance from the cosinor analysis of amplitude divided by mesor or Δ 10.0% in performance from 02:00 and 18:00 h data), in agreement with our hypothesis. These individuals followed a standardised protocol to address the main concerns in the literature and reduce the effects of bias and testing (Table 4). This circadian rhythm was accompanied by a decrease in split times per km with heart rate reflecting the faster speeds (hence, lower split times) during the 18:00–22:00 h time-trials than the 06:00 h. T_{IA} temperature values were significantly higher at rest in the evening than the morning as expected and in agreement with the literature (Edwards et al. 2023). With lower post blood lactate values corresponding to slower finishing times (Atkinson et al. 2005). Heart rate and subjective rating of perceived exertion increased with distance, but there was no interaction such that the profile of values increased similarly at all times-of-day from 1 to 10 km. The lack of sensitivity of heart rate and subjective rating of perceive exertion to split times reported in diurnal studies are not new (Drust et al. 2005).

Direct comparisons between our result and others are challenging, even those that are most like our study – where multiple time points over some of the solar-day have been used. As others have either used populations of both males and females (Baxter and Reilly 1983; Kline et al. 2007) and/or adolescence individuals and did not collect data from 22:00 to 06:30 h so missed the night portion of the rhythm (average 14 years old; Baxter and Reilly 1983). Or employed an ultra-short-sleep wake cycle protocol to standardise the exogenous input and enable the investigation of the internal component, where the protocol itself may have induced fatigue (Kline et al. 2007). That said, our finding of a faster finishing time in the evening agrees with other studies in which the distances were shorter such as 200 and 400-m swimming time-trial where ~ 20:00 h gave better performances (Baxter and Reilly 1983) and, 100-m swimming time-trial peaking at 18:02 h:min (Kline et al. 2007). If we consider only diurnal research that employed time-trials in a thermoneutral laboratory (~19–21°C), in adult males, where the distance is fixed and the time to finish is variable – our findings agree with five out of nine of the manuscripts in the literature see review by Bommasamudram et al.

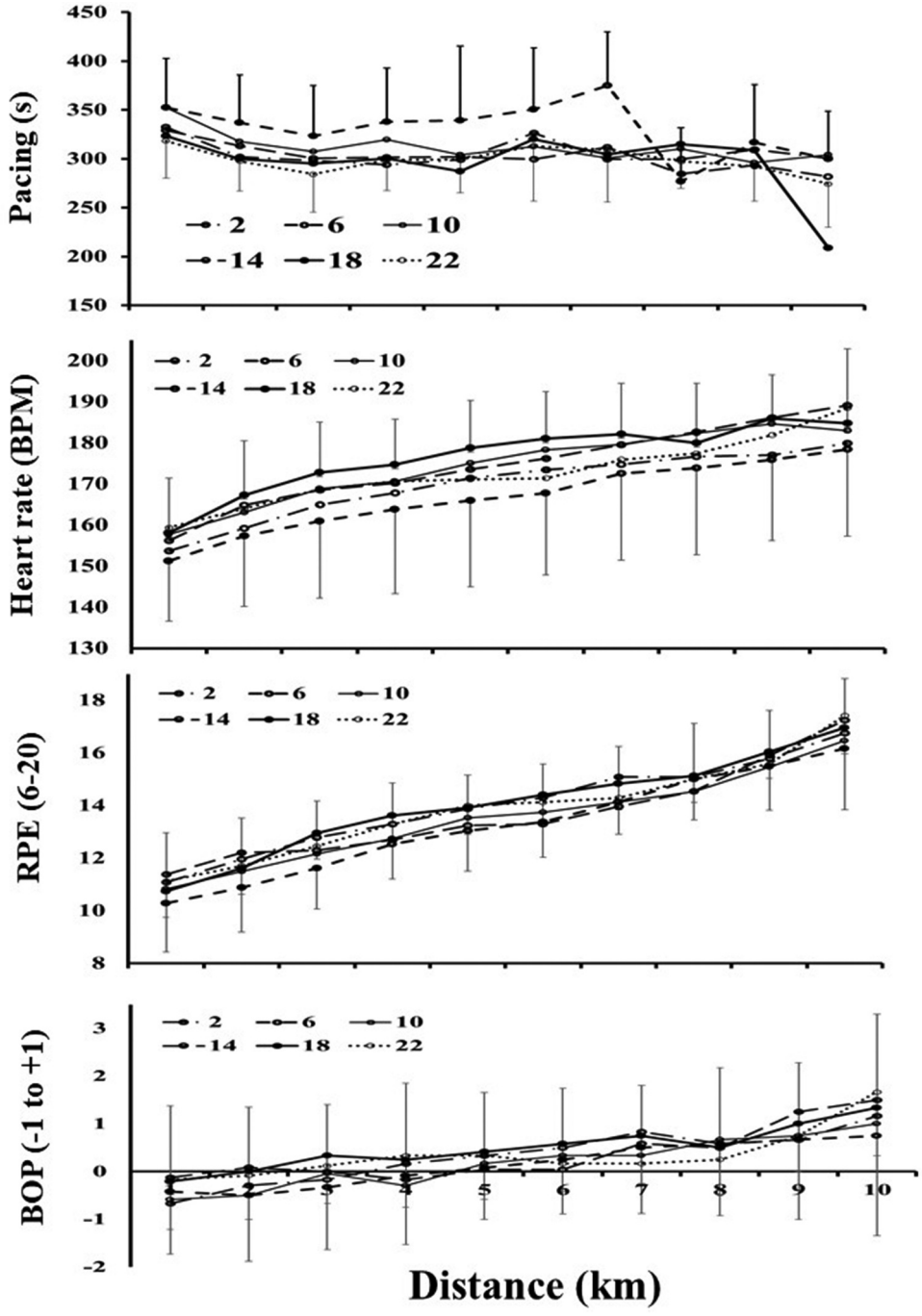

Figure 3. Mean (95%CI) values for Pacing (s), heart rate (BPM), RPE (6–20) and pacing (BOP −1 to = 1) every 1-km for 6 times-of-day for a 10-km time-trial.

(2022). Where faster swimming (100 m Δ 2.6%, Martin et al. 2007), 1 km cycling (Δ 6.9%, Fernandes et al. 2014), 4 km cycling (Δ 2.6%, Edwards et al. 2024), 10 km cycling (Δ 2.3%, Souissi et al. 2021), or 16.1 km cycling time-trial (Δ 3.6%, Atkinson et al. 2005) was found in the evening than in the morning and disagree with others (Boyett et al. 2016; Fiedler et al. 2022; Rae et al. 2015; Zadow et al. 2020). Discrepancies between the approaches or rather a lack of standardisation adopted in the literature between research teams further compromise the comparison of findings.

Methodological Considerations

Edwards et al. (2024) suggested a checklist of considerations in chronobiological studies on humans and

Table 3. Mean (SD) values for six times-of-day.

	Time (h)						
Variable	02:00	06:00	10:00	14:00	18:00	22:00	(*p*-value) DV
Mental toughness	**64.0 ± 5.7**	69.2 ± 6.9	69.8 ± 4.8	63.5 ± 6.2	69.0 ± 5.0	**70.8 ± 6.2**	**$F_{1.6,\ 17.2} = 5.118$, $p = 0.024$**
POMS							
Mood state – Vigour	10.5 ± 5.0	9.6 ± 3.3	12.3 ± 5.3	**14.1 ± 4.2**	13.4 ± 6.1	**14.2 ± 4.5**	**$F_{5,\ 55} = 3.749$, $p = 0.010$**
Mood state – Anger	8.3 ± 2.4	7.7 ± 1.0	7.3 ± 0.9	7.1 ± 0.3	7.4 ± 1.2	8.0 ± 3.5	$F_{2.0,\ 21.7} = 0.688$, $p = 0.511$
Mood state – Tension	7.5 ± 4.0	6.8 ± 1.5	6.2 ± 0.6	6.0 ± 0.0	6.8 ± 1.7	6.0 ± 0.0	$F_{1.7,\ 18.5} = 1.156$, $p = 0.328$
Mood state – Confusion	7.2 ± 3.7	6.4 ± 2.2	5.4 ± 0.7	5.1 ± 0.3	5.3 ± 0.8	5.1 ± 0.3	$F_{1.5,\ 17.0} = 3.289$, $p = 0.072$
Mood state – Depression	9.7 ± 4.1	9.2 ± 1.9	8.8 ± 1.8	7.8 ± 0.6	8.1 ± 0.3	8.0 ± 0.0	$F_{1.6,\ 17.2} = 1.738$, $p = 0.208$
Mood state – Fatigue	**10.6 ± 5.4**	**10.3 ± 4.4**	7.6 ± 3.9	6.2 ± 2.1	5.9 ± 1.4	7.0 ± 2.1	**$F_{2.7,\ 29.5} = 5.385$, $p = 0.006$**
Sleep questions							
Ease to sleep	0.0 ± 1.4	−0.4 ± 2.1	−0.4 ± 1.8	0.3 ± 0.7	0.5 ± 1.1	0.2 ± 1.3	$F_{2.6,\ 28.7} = 1.002$, $p = 0.397$
Waking time	−0.1 ± 1.5	**−2.3 ± 0.9**	0.1 ± 1.5	−0.1 ± 1.8	0.1 ± 1.1	−0.1 ± 1.6	**$F_{2.4,\ 26.6} = 6.358$, $p = 0.004$**
How well slept	−0.3 ± 1.4	0.1 ± 1.5	0.1 ± 1.5	0.1 ± 1.5	0.1 ± 1.5	0.1 ± 1.5	$F_{2.6,\ 29.0} = 1.043$, $p = 0.381$
Time to sleep	0.7 ± 1.3	0.8 ± 1.3	0.6 ± 1.4	0.5 ± 1.4	0.5 ± 1.2	0.6 ± 1.0	$F_{3.6,\ 39.2} = 0.138$, $p = 0.957$
Alertness 30-min of waking	0.0 ± 0.9	**−1.4 ± 1.8**	0.7 ± 1.3	0.1 ± 0.6	−0.3 ± 0.9	−0.1 ± 1.2	**$F_{2.5,\ 27.1} = 4.589$, $p = 0.014$**
Feelings of tiredness now	**1.5 ± 1.6**	−0.3 ± 2.2	0.1 ± 1.2	0.0 ± 0.8	0.3 ± 1.2	0.0 ± 1.4	**$F_{2.6,\ 28.6} = 3.113$, $p = 0.048$**

Significance (*p*-value) for main effects for time-of-day (DV) are given. Statistical significance ($p < 0.05$) is indicated in **bold**, those where $0.10 \geq p \geq 0.05$ are shown in *italics*. POMS = perceived onset of mood states.

sporting performance to reduce bias (of measurement, and reported result) for participant, methodological and equipment and environmental considerations. We applied this advice to the design of the current study, to best reduce the signal to noise ratio, to allow a diurnal variation should it exist to be found.

For some recommendations, we have been pragmatic like previous work (Edwards et al. 2024), so we did not attempt to bind the researchers as well as the participants to the conditions as suggested by others (Knaier et al. 2022). We realise that for the 02:00 and 06:00 h conditions may cause sleep loss resulting in an increase in homeostatic pressure to sleep. This sleep loss was reflected in the mood state and sleep questions where self-reported vigour and tiredness were lower or higher, respectively at 02:00 and 10:00 h than the other conditions. And participants reported earlier wake-time than normal, with a corresponding drop in alertness 30-mins after waking in the at 06:00 h condition. With greater self-rated tiredness values at the 02:00 h condition (Table 3). The effect of 0.5 h sleep loss as incurred in the 06:00 h condition on time-trial performance, is probably negligible (Edwards et al. 2023; Lopes et al. 2023; Walsh et al. 2021). However, as any observed rhythm need not be due to the clock, but it might reflect lifestyle, staying up from 06:30 to complete the time-trial at 02:00 h (~19.5 h) may have been a contributing factor to the higher values for finishing time.

The main finding of a circadian rhythm in finishing time is underpinned by findings of key variables, such as a significant circadian rhythm in resting T_{IA} temperature values ($p < 0.05$). With circadian characteristics similar to previously published values (Acrophase 19:31 h:min; Edwards et al. 2002) but not others (15:57 h:min; Kline et al. 2007), this difference could be attributed to factors associated with inclusion of female and male participants data without consideration of menstrual phase or contraceptive use, as well as the masking effects of the temperature site due to the participants swimming and hence having water in the ear, facial cooling or protocol differences. The circadian rhythm of core body temperature has substantial effects throughout the body and increased temperature values in the evening produce an increase in neural function to the muscle (reduced twitch time-course or increase in speed of contraction), an increase in the force-generating capacity of the muscle and result in an elevation in strength and power output (Bernard et al. 1998; Giacomoni et al. 2005; Melhim 1993). The causal link between core temperature and performance is complex, due to a multiplicity of components and mechanisms which require further research (Edwards et al. 2013). At present, the exact mechanisms for the observed diurnal variation in time-trial performance have been attributed to input from the body clock and proteins and peripheral clocks, hence an endogenous component to the daily variation in time-trial performance has been suggested to be important (Atkinson and Reilly 1996; Edwards et al. 2009; Reilly and Waterhouse 2009). The evidence relating to this internal input from the body clock to time-trial performance is limited to the work of Kline et al. (2007), who reported that a 200 m time-trial in swimming has been shown to have a strong internal component, independent of environmental and behavioural masking effects which peak in performance at 18:03 h:min. There are two phase-response-curves by independent groups investigating exercise as a zeitgeber that show that timing of exercise relative

Table 4. Comparison of current study to the Edwards et al. (2024) checklist for consideration for diurnal variation (DV) studies.

Participant considerations				Methodological and equipment considerations				Environment considerations	
Sample size/Sex/ Age a priori sample test/	Training status/ $\dot{V}O_2$peak/ Performance info/	Inclusion/Exclusion criteria to reduce confounding variables in studies.	Habitual sleep times/ daily preferences for exercise/ Chronotype/	Distance and mode of TT/ Time between sessions/TOD of sessions/	Familiarisation to TT/ Allocation of IDs to sessions/	Diet (timing/content) on day of testing/	Core temp site (CT)/kit Ergometers chosen for the time-trial/	Time of year/Sunrise and sunset from start to study end/	Laboratory Dry temp/Humidity/ BP/
12/Males/26 ± 6y/ n-8 given *p*ower levels of 80%, $p = 0.05$ and meaningful effect size given.	Recreational runners (McKay et al. 2021), 3×Week@3-4 h/ day/63.2 ± 3.7 mL.kg.min^{-1}.	Given – No diagnosed sleep disorders; shift work/Jet lag; injury-free; habitual total sleep time and retiring and protocol waking times within that of the participants normal hygiene; not receiving pharmacological treatment (including NSAIDs); a habitual caffeine "low" consumption.	23:30–07:30 h/No preference/ Intermediate types.	10-km treadmill time-trial/>48 h/ 02, 06, 10, 14, 18, 22 h.	3 × sessions/ Allocated to groups due to familiarisation finish time then Counterbalanced.	Fasting before 06 or 07 h when entering the lab, eat immediately after, then at 12 and/or 17 h or 3 h before the condition (such as 14, 18 h session). Replicated previous 24 h intake.	Intra-aural (ear), Covidien Genius 2 UK/ Woodway, ergo ELG 55, Germany.	October to December (UK Autumn to Winter)/Sunrise-set from start to end of experiment = 07:29 to 08:01 h and 18:01 to 19:50 h.	19°C/ 35–45%/ 750–760 mmHg.

to a marker rhythm (core temperature or melatonin) can shift the clock (Edwards et al. 2002, 2009; Youngstedt et al. 2019). Lastly, central, or neurological factors (central nervous system command, alertness, motivation and mood have been suggested (Castaingts et al. 2004; Giacomoni et al. 2005; Racinais and Oksa 2010).

Time-of-Day and Pacing

Participants in this study adopted a relatively even pacing strategy (see Figure 3), with a marked decrease in split time towards the end of the TT indicative of a sprint finish (Abbiss and Laursen 2008; Foster et al. 1993). It is thought that a diurnal difference in pacing strategy of endurance performance may be due to altered feedback mechanisms from factors previously covered (such as body clock input, core body and muscle temperature etc) and through perception of effort being easier for any given workload in the evening than the morning (Reilly and Garrett 1995). In our case the adopted pacing strategy was the same regardless of time-of-day (p = 0.381) in agreement with others (Edwards et al. 2024; Fernandes et al. 2014; Zadow et al. 2020), only in the current study the participants put in 5% faster at 22:00 h than 02:00–06:00 h. Further, participants perception of how well they had subjectively paced themselves to finish as quickly as they could, did not reflect their pacing as was subject to distance covered (perceived as going to fast after 5 km) and time-of-day (perception of going too fast for optimal from 14:00–22:00 h.

Time-of-Day and Stroop Findings

Stroop variables showed to be sensitive to pre-post time-trial and showed a diurnal variation in colours-not-words. This daily variation peaked ~ 14:00 h in agreement with the subjective alertness rhythm and the peak in cognitive performance (Edwards et al. 2023). We have previously shown sensitivity of the Stroop test for show intervention effectiveness, for "naps" and sleep supplements after partial sleep restriction of 4-h (Gallagher et al. 2023, 2024).

Limitations

Participants in this study were not elite, rather recreational runners ($\dot{V}O_2$ peak = 63.2 ± 3.7 $mL^{-1}{\cdot}kg.min^{-1}$), although we familiarised them three times and all ran 10-km distances routinely. Future research should consider testing with a cohort of recreational up to elite performers ($\dot{V}O_2$ peak >70 $mL^{-1}{\cdot}kg.min^{-1}$), to see if this effects the occurrence of rhythmicity in time-trial performance. It should be noted that intra-aural temperature is high susceptible to confounding variables including environment producing facial heating or cooling. Although we had inclusion criteria and protocol to reduce these effects, masking may still be present (Edwards et al. 2002).

Conclusion

This study is the first to employ a standardised approach to research (participants did not have time-of-day preference to exercise nor and were intermediate types). We found a circadian variation in rectal temperature of 0.9% (0.74°C) and 10-km TT performance (5.0%) with peaks in the evening. The parallelism of T_{IA} and rhythm in time-trial finishing times agree with previously published research with a delay in peak of 1.24 h in performance. The finding of a circadian rhythm in 10 km time-trial performance (duration 2994 s) agrees with that of a shorter distance (100 m swimming, duration 169.5 s). These findings of a circadian rhythm in performance in parallel with temperature, provide a rational for the need for appropriate adaption strategies for jet-lag in athletes; to aid in recovery for competition after rapid time-zone transition.

Practical Implications of the Findings

Circadian rhythms in time-trial performance are not consistently reported, this lack of agreement may be explained by a lack of rigor and standardisation in method employed, population chosen and a lack of control. Here we show the usefulness in a practical tick list for consideration to "standardise" research investigating diurnal variation in performance.

Resultant finishing times after a standardised protocol were faster in the evening by 2.6% than the morning. Discrepancies in past findings of a daily difference in time-trial performance could be related to fitness and level of athleticism of the population rather than methodological differences. We used the practical tick list for consideration for research investigating diurnal variation in performance, to inform research choices (Table 4). This information aids in consideration of method and study design, as well as interpretation of results and discussion – whilst not overburden the method section of manuscript.

Peaks in running performance in the evening in intermediate types, adds weight that the choice of time-of-day to investigate time-trial performance should be 17–19 h. And has implications for scheduling of competition and finals.

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