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Daily variation in time-trial sporting performance: A systematic review

Daily variation on performance measures related to time-trials: A systematic review.

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Abstract

Few functional measures related to time-trial display diurnal variation. The diversity of tests/protocols used to assess time-trials on diurnal effects and the lack of a standardised approach, hinder agreement in the literature. Therefore, the aims of the present study were to investigate and systematically review the evidence relating to diurnal differences in time-trial measures and to examine the main aspects related to research design deemed specifically important for studies of a chronobiological nature. The entire content of PubMed (MEDLINE), Scopus, Web of Science and multiple electronic libraries were searched. Research studies published in peer reviewed journals and non-peer reviewed studies, conducted in male adult participants aged ≥ 18yrs before November 2021 were screened. Studies assessing tests related to time-trials in cycling, rowing, running and/or swimming between a minimum of 2 time-points during the day (morning [06:30-10:30 h] *vs.* evening [14:30-20:00 h]) were deemed eligible. The primary search revealed that a total of 10 from 40 articles were considered eligible and subsequently included. From these the mode of exercise was either cycling (6), running (2) or swimming (2). Events ranged from 1 to 16.1-km, or 15 to 20-min time in the cycling and running time-trials; and 50-m to 200-m in the swimming time-trials. Only 4 studies found one or more of their performance variables to display daily variations, with significantly better values in the evening than the morning; while 6 studies found no time-of-day significance in any of the variables assessed. There was a significant diurnal variation for time to complete the event observed in 2 cycling time-trials (from 2.9 to 7.1 %). Work rate during a 16.1-km time trial in cycling was 10 % higher in the evening than the morning. The only other observed differences were stroke rate and stroke length during a swimming time-trial and cycling stroke rate (cadence; revolutions per minute) during a mountain bike 20-min time-trial. The magnitude of difference is dependent on the modality of the exercise, the chronotype of the individual, the training status of the individual and sample size. The lack of diurnal variation in most studies, can in-part be explained by the methodological limitations and issues present related to quality and control. Therefore, it is paramount that research assessing diurnal variation in performance uses appropriate timing of sessions around the core body temperature minimum (~ 05:00 h) and maximum (~ 17:00 h) in the morning and evening, respectively. Although, differences in motivation/arousal, habitual training times, chronotype and genotype could provide an explanation as to why some research/variables did not display time-of-day variation, more work is

needed to provide an accurate conclusion. There is a clear demand for a rigorous, standardised approach to be adopted by future investigations which control factors that specifically relate to investigations of time-of-day, such as appropriate familiarisation, counterbalancing the order of administration of tests, providing sufficient recovery time between sessions and testing within a controlled environment.

Keywords: Time-of-day, circadian rhythms; diurnal variation, time-trial; review.

Introduction

It has previously been established that most of the research related to physical and physiological performance variables display diurnal variation in a temperate environment (around 17–22°C) in healthy adolescent males (18 + years of age; Pullinger et al. 2020; Ravindrakumar et al. 2022). It is currently believed that in the absence of external cues, levels of cortisol, core and/or muscle temperatures and melatonin levels play a major role in circadian regulation through signals directed by the suprachiasmatic nucleus (body clock), located in the anterior part of the hypothalamus (Reilly 1990; Van Drunen and Eckel-Mahan 2021). A large body of research has shown cortisol levels (Reilly and Waterhouse 2009), core body temperatures (Pullinger et al. 2018a, 2019) and muscle temperatures (Pullinger et al. 2014, 2018b) to be higher in the mid-afternoon and/or early evening, while melatonin levels display values which are higher during the nocturnal period (Edwards et al. 2000; Zawilska et al. 2009). Similarly, regardless of muscle group measured, both muscle force production and power output also display an evening superiority (Atkinson and Reilly 1996; Edwards et al. 2013; Robertson et al. 2018). In addition, most measures related to repeated sprint performance and anaerobic power/capacity (Pullinger et al. 2020; Ravindrakumar et al. 2022) are also time dependent with higher values, ranging from 1.8 to 13.1 %, in the afternoon (16:00 and 19:30 h) compared to the morning (05:30 to 11:00 h).

To the best of our knowledge, the current research assessing diurnal variation on performance measures related to time-trial which has been conducted yields inconclusive results. Time-trials can further be defined as a "race against time or distance", in which an athlete tries to complete the race as fast as possible (Edwards et al. 2005). The mode of exercise utilised in time-trials can range from cycling (Gough et al. 2021) to running (Boukelia 2018) to swimming (de Salles Painelli et al. 2013) to rowing (Mujika et al. 2012). Findings have suggested that circadian rhythmicity has an impact on aerobic activities, such as time-trials (Drust et al. 2005). Yet, most current research on time-trial is diurnal of nature using two time-points (morning and evening). Currently, there is a lack of agreement concerning the presence of diurnal variation in time-trials. It has been found that a 16.1-km cycling time trial performance established a small and statistically significant improvement in the morning *vs.* the afternoon (Atkinson et al. 2005). However, a time-trial cycling performance of 15-min duration found no signficant differences for any of the measured variables when comparing morning, afternoon and evening sessions (Dalton et al. 1997). Nevertheless, the majority of factors related to cycling performance such as strength, power and work are higher in the afternoon and/or evening than in the morning (Atkinson and Reilly 1995 1996; Ravindrakumar et al. 2022). Large differences in methods and procedures such as training status of participants, familiarisation (number of times completed to be deemed that further learning was minimal), counterbalancing of participants into groups to statistically distribute any additional learning, randomisation of sessions, mode of exercise, time-trial distances, time-trial duration are some of the main issues which make it difficult to compare between studies and affect current findings (Drust et al. 2005; Pullinger et al. 2020). In addition, measurement error and sample size influence research related to circadian variation, and play an important role on the discovery of variation (Drust et al. 2005).

It has previously been highlighted that there is a lack of standardisation of aspects related to research design deemed specifically important for studies of a chronobiological (time-of-day) nature (Drust et al. 2005; Pullinger et al. 2020; Ravindrakumar et al. 2022; Youngstedt and O'Connor 1999). Lack of methodological quality and adherence to these aspects hinder agreement on time-of-day effects and performance. Therefore, considering the large differences between findings and methodologies currently used to assess time-of-day and time-trial measures, providing a clear and comprehensive review on this topic will help identify the current research gaps in our understanding within the area. In addition, highlighting the methodological concerns and other findings will help improve future studies related to time-trial measures and time-of-day. Previous observations suggest notable changes in diurnal variation are still unknown but involve several potential contributing factors (Edwards et al. 2013; Pullinger et al. 2018a, 2018b), with the evening superiority in muscle force production and power output attributed to a causal link between core body/muscle temperatures and performance (Robinson et al. 2013). In addition, other suggestions put forward to explain diurnal variation in muscle performance are central/neurological factors (central nervous system command, alertness, motivation, and mood: Castaingts et al. 2004; Giacomoni et al. 2005; Racinais 2010; Racinais et al. 2005), peripheral or muscle-related variables (contractibility, metabolism, and morphology of muscle fibres) influenced by hormonal and ionic muscle process variations (Reilly and Waterhouse 2009; Tamm et al. 2009) and more recently greater phosphorylation of M-band-associated proteins (Ab Malik et al. 2020).

Therefore, given the current equivocal evidence presented in the literature, the aim of the present manuscript was to examine the following research question: "In healthy adolescent males, what is the magnitude of diurnal (morning session *vs.* evening session) differences in performance variables related to time-trial?" In addition, in-depth information will be provided in relation to aspects related to research design deemed specifically important for studies of a chronobiological (time-of-day) nature to ensure information is available for more rigorous research to be conducted that control these factors.

Methods

Reporting Standard

This systematic review conforms to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines (Page et al. 2021). The PRISMA 2020 checklist is presented in Appendix 1, indicating the page numbers where items of information are present in the current manuscript.

Eligibility Criteria

The inclusion criteria were based on the Cochrane guidelines for conducting systematic reviews (Higgins 2021). The criteria for inclusion and exclusion were set and agreed by all seven authors. Following the initial selection process of studies, three authors (AR, IG & TB) independently completed the eligibility assessment in a blinded standardized way by screening the titles and abstracts. To be considered eligible, the manuscript had to meet the following inclusion criteria:

1. Population – healthy males and adult participants (18+ years of age) only. Females were excluded due to the impact of hormonal fluctuations on performance parameters thereby rendering it difficult to interpret findings. Female sex hormones have displayed substantial physiological effects related to altering fluid regulation, and modifications in

thermoregulatory, muscular and metabolic responses all of which have been shown to affect performance (Meignie et al. 2021).

2. Time-of-day – compared the effects of morning versus evening in performance variables related to time-trials (a minimum of two time-points).
3. Time-trials – individual time-trial, team time-trial, distance time-trial or track time-trial tests.
4. Modality – cycling, running, swimming or rowing.
5. Design – Randomised and/or counterbalanced trials.

Literature Search Strategy and Information Sources

A computerised English-language literature search of the grey literature (SP & TB): Manipal Academy of Higher Education electronic library and Qatar National Library; and electronic databases: PubMed (MEDLINE), Scopus and Web of Science were conducted (July 2021 – November 2021). A search for relevant content related to time-trials and time-of-day variation using the following search syntax using Boolean operators in titles, abstracts, and keywords of indexed documents: ("time of day" OR "time-of-day" OR "daily rhythm" OR "daily variation" OR "daily fluctuation" OR "diurnal rhythm" OR "diurnal variation" OR "diurnal fluctuation" OR "circadian rhythm" OR "circadian variation" OR "circadian fluctuation") AND ("time trial" OR "time trial performance" OR "team time trial" OR "individual time trial" OR "swimming time trial" OR "running time trial" OR "cycling time trial" OR "swimming performance" OR "running performance" OR "cycling performance" OR "track cycling" OR "prologue") was conducted. Additional advanced search techniques using wildcards, truncation and proximity searching were incorporated to widen the search. Secondary searches consisting of the reference lists of all papers included were screened manually for additional relevant papers, as part of the secondary search (AR & TB). In addition, forward reference searching was conducted to explore potential follow-up studies through citations and authors. One author (SP) independently carried out the searches for study selection to minimise potential selection bias. Figure 1 presents the flow of papers through the study selection process using the PRISMA 2020 flow diagram (Page et al. 2021).

Study Selection

Where both male and female participants took part in a research study, the article was included if the data from male participants could be independently identified. In instances where the title and abstract did not contain enough detail to indicate whether an article was relevant to the review, the complete article was obtained and read. This enabled the authors to determine whether the paper met the primary inclusion criteria. In instances where the primary purpose of the article was not an investigation looking at the effects of time-of-day, meaning a minimum of two time-points were not assessed (morning and evening), the papers were excluded from the review. Letters to the editor, conference abstracts and literature reviews were excluded as these studies were not found to be methodologically-quality-assessable and/or critically appraisable.

Data Extraction

Data extraction was performed by two authors (AR & IG) independently and a data check performed by a third author (SP) with the following data extracted from the included studies: 1) the study authors and date; 2) the number of participants and their characteristics (e.g. age, body mass, stature); 3) the circadian chronotype questionnaire used to assess the participants (and their scores); 4) the time-of-day testing sessions took place (e.g. morning, afternoon, evening); 5) time-trial test used; 6) equipment used (e.g. cycle ergometer, treadmill); 7) performance variables assessed (e.g. velocity, time, power output), along with numerical results; 8) the significance established with P values; and 9) % difference between testing time-points (if results were provided), the mean ± SD values between time-of-day conditions (for significant variables) and information as to whether diurnal variation was established. In addition, analysis regarding aspects relating to research design and factors deemed specifically important in investigations of chronobiological nature were quantified; randomisation, counterbalancing, record of light intensity, control of meals, control of room temperature, control of sleep and fitness of participants, as previously used by Pullinger et al. (2019) and Ravindrakumar et al. (2022). In most instances, a simple ‘yes’ or ‘no’ was recorded against each of the included studies, other than ‘fitness’ (when the studies were classified as having ‘trained’ or ‘untrained’ participants). All articles that made no specific reference to any of these primary areas were considered to indicate a negative response and ‘no’ was marked against the area in question.

Quality Assessment

A modified 27-item methodological quality assessment checklist on each included article using the Downs and Black (1998) scale was conducted. The checklist consisted of 27 “yes”-or- “no” questions which were scored totalling up to a possible 28 points. Item 27: “*Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?”* to a yes (1-point) or no/unable to determine (0 points) scoring. The questions were categorized under 5 sections: Reporting (10 items; 1-10), External validity (3 items; 11-13), Internal validity study bias (7 items; 14-20), internal validity confounding selection bias (7 items; 21-26) and power (1 item; 27). The quality assessment of the articles was conducted by two reviewers (AR and TB) independently with disagreement on 6 items across the 10 manuscripts (2.2 %). The observed differences were resolved by a third reviewer (SP).

Results

Search Results

The literature search ended on 19 November 2021 and the primary database search revealed 766 articles and an additional 1138 via other methods. Figure 1 presents the number of articles found in each electronic database or through other methods, and a detailed flow chart of the literature search, including all the steps performed. Once duplicates were removed, 657 titles obtained via databases remained in the reference manager (Mendeley, Elsevier, Amsterdam, The Netherlands). Following the examination of titles, abstracts and keywords of all these manuscripts, 40 academic studies were deemed eligible and retained for full text-analysis. After additional full-text analysis, 20 studies were assessed for eligibility, of which 10 were deemed eligible and included in the systematic review. Reasons for exclusion can be found in Figure 1. Upon further inspection of all articles in their bibliographical references and through organisations, 13 were assessed for eligibility, but none met the inclusion criteria and hence were deemed ineligible. Therefore, a total of 10 studies were used in the systematic review.

Study Characteristics

The detailed participant characteristics are shown in Table 1. A total of 120 male participants were included across the 10 studies (mean number of participants per study = 12), ranging from a total of 7 to 19 participants. Four studies (40 %) assessed circadian chronotype of participants, with three studies using the morningness-eveningness questionnaire (Horne and Ostberg 1976) and one used the modified Smith's Composite Scale of Morningness (Smith et al. 1989). From the 46 participants assessed, 30 of the participants belonged to the intermediate chronotype (65.3 %), 15 to the morning chronotype (32.6 %) and 1 to the evening chronotype (2.2 %). A total of six studies failed to report any information related to chronotype for their participants.

The time-of-day during which morning sessions took place ranged from 06:00 to 10:30 h and evening sessions between 14:00 to 20:00 h. Two studies used additional time-points to assess diurnal variation; Dalton et al. (1997) 20:00 to 22:00 h; Zadow et al. (2020) 11:30, 14:30 and 20:30 h. A total of seven studies used cycling as the mode of exercise, while two used running and two used swimming. The studies that used cycling to assess time-trials used an air-braked ergometer (n=1), road bikes with training ergometer (n=4) or mountain bike (n=1). In the running studies, both studies used a motorised treadmill. Numerous performance variables were examined in each study, with time to complete the time trial (distance) used in 7 studies and set time to complete the time trial (minutes) used in the other 2 studies. Distances ranged from 1-km to 16.1-km during cycling time trials, with both running time-trials conducted over 10-km and the swim over a shorter distance (50-m and 200-m). The time-based time-trials were 15-min (Dalton et al. 1997) and 20-min (Silveira et al. 2020) in duration, respectively.

Only four studies found one or more of their performance variables to display time-of-day effects, with values between the morning and evening significantly different, while six studies found no significant differences between morning and evening in any of the variables assessed. Cycling time-trial to complete a 16.1-km was found to be significantly better in the evening compared to the morning by 3.5 % (Atkinson et al. 2005) and by 7.1 % in a 1-km time-trial (Fernandes et al. 2014). Both the 3-km (Boyett et al. 2016) and 4-km (Zadow et al. 2020) cycling time trial found no significant differences in performance time. Both 10-km running time-trials also displayed no significant differences in performance time (Boukelia et al. 2016; 2018), as did both swimming time-trials (Lisbôa et al. 2021; Rae et al. 2015). The only other significant differences observed were work rate during a 16-1-km cycling time-trial (10 %; Atkinson et al. 2005), stroke rate and stroke length during a swimming time trial (2.0 to 3.3 %; Lisbôa et al. 2021), and stroke rate in a 20-min mountain bike time trial (2.9 %; Silveira et al. 2020). However, Rae et al. (2015) did establish significant differences in time-of-day for 200-m swim time-trial when participants were grouped according to habitual training time or chronotype.

The substantial differences in methodological and clinical heterogeneity among studies meant we were unable to conduct a meaningful meta-analysis and pool the observed datasets to evaluate the evidence related to findings in anaerobic performance and therefore provided in-depth information related to unweighted results. Missing data information, differences in populations, metrics, outcomes and designs were the main reasons for a meta-analysis not to be pursued. Conducting a meta-analysis will simply compound the errors and produce an inappropriate set of results and summary.

Quality of work

Table 2 provides detailed information related to randomisation, counterbalancing, record of light intensity, control of meals, control of room temperature, control of sleep and fitness, to quantify for the control of aspects relating to research design deemed specifically important in investigations of a chronobiological nature. None of the studies met all 7 criteria required for an investigation of chronobiological nature. All the studies provided information related to fitness of participants. A total of 3 counterbalanced the order of administration to minimise learning effects and 7 studies performed the time-of-day session in a randomised order. From these, 2 studies (Boyett et al. 2016 and Zadow et al. 2020) used counterbalancing and randomisation within their protocol. The majority of studies controlled for meals (n=7) and controlled for room temperature (n=6). However, less than half the studies controlled for sleep (n=4), while no study recorded light intensity. None of the studies quantified all four of the 4 aforementioned criteria.

Methodological quality control and publication bias

Based on a modified 27-item Downs and Black (1998) checklist, the results of the methodological quality assessment of the included studies ranged from 17 to 24. Reporting (10 items; items 1-10) showed 6 items to be fully met by all studies (Items 1-4, 6 and 7). External validity (3 items; items 11-13) displayed all three items to be met by 9 studies. Internal validity study bias (7 items; items 14-20) reported 5 items out of 7 items (items 16-20) to be fully met, with one study fully meeting all criteria for internal validity study bias (Boyett et al. 2016). Confounding selection bias (6 items; items 21-26) were fully met by none of the studies, while half the studies used power to determine sample size and/or whether the study had sufficient power (1 item; Item 27). Detailed methodological quality assessment scores can be found in Table 3.

Discussion

The present study analysed data from studies that compared the effects of diurnal variation on time-trial measures and determined the quality of evidence that reports a "peak" time for performance. The main findings of this review were: 1) few of the variables assessed (23.1 %) displayed diurnal variation, with 6 studies (60 %) displaying no differences between the afternoon (14:00 – 20:00 h) and morning sessions (06:00 – 10:30 h) in any of the time-trial variables assessed; 2) methodological limitations and issues present related to quality and control affect observations of diurnal variation in time-trial.

Time Trials

Previous research has established diurnal variation to be present in many different human performance variables (Robertson et al. 2018; Pullinger et al. 2018a, b). In agreement, time-of-day variation was observed in some studies and ranged from 2 to 10 %. The only study to display diurnal variation in all performance variables aimed to assess whether morning to evening differences could be negated through an adequate active warm-up (25-min) in time-trials (Atkinson et al. 2005). Nevertheless, evening values for performance time and power were both higher compared to the morning irrespective of whether a warm-up was administered or not. Participants in the study were fully familiarised to the 16.1-km protocol, meaning that further learning was minimal and not the cause for observed diurnal variation. Unsurprisingly, intra-aural temperature was found to be significantly higher in the evening compared to the morning across all sessions in this study, but Atkinson et al. (2005) suggested that diurnal variation in performance is not completely controlled by body temperature variation but potentially attributable to time-of-day training preference as opposed to any external/endogenous mechanism. The individuals within the study had a slight "morning preference", meaning sleep-wake and training habits preferences tend to be earlier than intermediate types. Nevertheless, they still performed significantly better in the afternoon, thus discarding the possibility of chronotype or training preference being attributed to diurnal variation in time-trials. Further, the effects of sleep inertia and a lack of flexibility which takes place after a night's sleep were well controlled and could not have explained the superior time-trial values in the afternoon. The only aspect which was not well controlled was dietary timing and intake and require more focus to understand its influence on time-trial performance. Fernandes et al. (2014) also found performance time to improve, although power output was no different between morning and evening in a 1-km cycling time-trial. The improvement in evening performance is associated with a maintained increase in both anaerobic and aerobic contributions throughout the time trial and hormonal/metabolic differences between morning and evening conditions. It is suggested that the "optimal" hormonal and metabolic environment may explain these observed differences in time-trial performance (Hammouda et al. 2012; Romijn et al. 1995; Teo et al. 2011). However, findings are specifically relevant to amateur, recreational cyclists.

However, the majority of results established within this review do not support the notion that time-trial variables display diurnal variation in a temperate environment (around 17–22°C) in healthy adolescent males (18 + years of age). Several factors have been put forward to explain the lack of diurnal variation observed in time-trials. Several studies suggested that diurnal variation in time-trial performance would be observed as a result of its causal link with core body temperature. Several studies found core body temperature to vary with time-of-day, nevertheless time-trial was unaffected (Boukelia et al. 2018; Dalton et al. 1997; Zadow et al. 2020). Adaptive responses to training, competition times, the motivation of participants and habitual training patterns of athletes were suggested as masking the effect of diurnal variation in time-trials in these studies. Although diurnal variation in core body temperature was suggested as the main cause of diurnal variation established in cycling stroke rate (cadence) during a 20-min time trial, no other performance variables displayed diurnal variation (Silveira et al. 2020). They did not provide any information as to why no diurnal variation was established in other measures. Nevertheless, other suggestions have been put forward such as; unfamiliar testing conditions (cold/hot environment; Boukelia et al. 2016; 2018), warm-up effect, single intraday study design (Lisbôa et al. 2021), and participant chronotype (Rae et al. 2015). Interestingly, the study performed by Rae et al. (2015) did find 200-m swim time-trial displayed diurnal variation when grouping athletes by chronotype, with morning-types significantly faster in the morning (0.5 %), and intermediate-types significantly faster in the evening (1.2 %). In addition, swimmers who consistently trained in the morning were faster in the morning, while swimmers who consistently trained in the evening, were faster in the evening. Nevertheless, the current literature is contradictory regarding chronotype effects on diurnal variation and performance (Atkinson et al. 2005; Brown et al. 2008) and it is not clear whether this diurnal variation in performance is due to solely endogenous factors or habitual training times or a combination of both (Chtourou et al. 2012; Martin et al. 2007). Results observed in elite male and female swimmers found athletic performance to be influenced by individual circadian behavioural phenotype and to be closely associated to physiological and molecular differences (Anderson et al. 2018).

Current findings on diurnal variation and time-trial are contradictory and present several methodological issues. Some important primary weaknesses are discussed in different studies, such as relatively small sample sizes utilised, the lack of mechanistic assessments and/or insight and issues surrounding the nutrition timing/intake. All aforementioned aspects can highly influence the observation of diurnal variation and are a necessity for creating rigorous laboratory-based protocols (Drust et al. 2005). Before providing a conclusion surrounding diurnal variation and time trials, better methodological quality and control is required, with main factors discussed below. Nevertheless, based on current findings related to diurnal variation in performance, a controlled laboratory-based investigation with scientific rigour in experimental design and data collection with minimal measurement error would yield the same results. It is well established that both endogenous and exogenous components influence performance, with motivational aspects, subjective arousal, sleepiness, ionic changes and hormonal fluctuations (cortisol ratio, thyroid secretion and testosterone ratio) playing a role (Edwards et al. 2013; Zhang et al. 2009). Recent observations have suggested differences in the phosphorylation of proteins within or close to the muscle M-band that could relate to the well-established morning versus evening differences in performance might well provide a better explanation to time-of-day observations (Ab Malik et al. 2020). Gene (circadian clock gene PER3 variants) and environment (time of day) interactions suggest that diurnal variation performance is associated with circadian phenotype and PER3 genotype. Diurnal variation in performance is complex and involves multiple components and mechanisms which require further research. Even though direct evidence has been established regarding a large endogenous component related to the daily variation in muscle force production (from the body clock and peripheral clocks: Zhang et al. 2009), it is presently still unproven (Sargent et al. 2010). To fully explore this internal component, complex, time consuming, and challenging chronobiological protocols (for both researchers and participants) are required. Protocols which attempt to reduce or standardize the exogenous component of the rhythm using constant routines, forced desynchronization, or ultrashort sleep–wake-cycle protocols remain to be performed (Kline et al. 2007; Reilly and Waterhouse 2009), adding to our understanding regarding which diurnal variation factors might play a major role.

Methodological quality and control

As far as we are aware, only two reviews have looked into aspects related to chronobiology study design (Pullinger et al. 2020; Ravindrakumar et al. 2022). In agreement, an apparent lack of control was also established within this review. Considering the periodicity of the body clock in human beings is affected by external environmental rhythmic cues which affect the continual adjustment of the body clock (zeitgebers), and ultimately act as circadian time cues, several rhythmic cues, such feeding-fasting cycle (control of meals), the activity-inactivity cycle (fitness) and light-dark cycle (recording of light intensity; Aschoff 1965; Aschoff and Wever 1980; Dunlap et al. 2004) require control. Surprisingly, no studies reported information related to light and/or dark exposure through recording of light intensity (Table 2), or even basic information on the time of year the research was conducted (hence timing of sun rise and sun set). Light exposure influences mood and alertness (Bedrosian and Nelson 2017; Souman et al. 2018) and have also shown to improve time-trial performance (Kantermann et al. 2012; Thompson et al. 2015), although the extent is associated to light intensity, wavelength, time-of-day (Knaier et al. 2017). Most studies (70 %) did control for meals, a factor previously stressed to play a vital role in chronobiology studies (Table 2; Bougard et al. 2009). In order to limit the variability in results, intake and/or timing of meals need precaution and standardisation across the testing protocol. It has been suggested participants only have a glass of water (Atkinson & Reilly 1995; Moussay et al. 2002) or in a fasted state prior to a morning session (Ab Malik et al. 2020; Pullinger et al. 2014), and not to consume food for $\geqslant$ 4 h prior to an evening session (Ab Malik et al. 2020; Brotherton et al. 2019). In studies where aspects related to nutrition timing/intake are not mentioned at all, could potentially mask or increase morning-evening difference in many physiological variables (Bougard et al. 2009). All studies did report information related to participant background and fitness levels, thus unlikely negatively influencing findings (Guette et al. 2005; Häkkinen 1989). Nevertheless, training status (trained *vs*. untrained) does influence performance in different modalities (Bishop and Spencer 2004; Hopker et al. 2013; Riboli et al. 2021). Diurnal variation in performance is linked to training status, mode of exercise specificity and participant familiarisation and therefore needs to be well-controlled (Bambaeichi et al. 2005; Giacomoni et al. 2006; Reilly et al. 1997).

When looking at chronotype assessment and distribution, only three studies (30 %) assessed their participant's chronotype scores. Previous observations have found differences in time-trial (Brown et al. 2008; Rae et al. 2015), $\dot{V}O_2$max, cortical and spinal excitability levels (Roden et al. 2017), thus suggesting the importance of providing detailed information related to chronotype. Similarly, only four studies (40 %) controlled for sleep, such as keeping similar sleeping habits to "normal life", not staying up late, habitual rising and waking times, and whether any prevalence of insomnia or sleep deprivation is present. Sleep is essential for the human brain and body to function and a lack of sleep and/or sleep deprivation is closely associated with impairment in time-trial performance (Chase et al. 2017; Souissi et al. 2020; Walsh et al. 2021). Findings related to time-trial performance are in agreement with previous research which has examined the effects of sleep (deprivation) on central fatigue and performance (Edwards and Waterhouse 2009; Kirschen et al. 2020; Waterhouse et al. 2011), also establishing that a lack of sleep, sleep deprivation and disturbed sleeping patterns negatively affect performance. Increased levels of fatigue are closely associated with time-since-last-sleep and as time-awake increases, cognitive performance, central arousal and the restorative influences of sleep wane (Ball et al. 1999).

Other important factors to control are related to the time of day and number of familiarisation sessions. A lack of familiarisation results in neuromuscular adaptations still taking place within the experimental sessions. The familiarisation sessions should be at a time of day dissimilar to that of the experimental sessions and if possible, between the morning and evening experimental sessions such as 12:00 h. This would then limit any effects of habit on performance (Edwards et al. 2005). The number of sessions required to "familiarise" the participants depends on the performance task they have to do, the task complexity and the individual's level of expertise of the task. If the last and penultimate finishing times of the familiarisation sessions were analysed the random variation and systemic bias of the population of the task for the research could be quantified and the level of learning provided. Counterbalancing and randomisation (if possible) of sessions provides a guarantee of internal validity, eliminates selection bias and the balance of known and unknown confounding factors. In this systematic review, around two-thirds of studies (70 %) randomised their sessions and less than a third (30 %) counterbalanced their sessions. Lack of familiarisation, counterbalancing and randomisation will result in acute neuromuscular adaptations through the initial learning of motor recruitment pathways to take place during testing sessions as opposed to any endogenously driven diurnal rhythm.

Finally, room temperature also needs close control, with changes in core body and muscle temperature affecting performance. The higher local muscle temperatures (~0.3 to 0.6 °C in vastus lateralis; Edwards et al. 2013; Pullinger et al. 2014; Robinson et al. 2013) and core body temperatures (~0.6 to 0.8 °C in rectal and gut sites; Edwards et al. 2002; Edwards et al. 2013; Pullinger et al. 2019) present in the evening have shown to increase both force-generating capacities of the muscles (Bernard et al. 1998; Coldwells et al. 1994; Giacomoni et al. 2005; Melhim 1993) and neural function (Martin et al. 1999). Every 1 °C increase in resting core temperature (Bergh and Ekblom 1979) or through the passive warming of the musculature

(Asmussen and Bøje 1945; Ball et al. 1999), muscle force development increases by ~ 5 %. Although recent findings suggest that diurnal variation in performance can be partially attributed to core and/or local muscle temperatures (Robinson et al. 2014; Pullinger et al. 2018b), it is more complex, but still requires close control.

There is a need for more rigorous laboratory-based protocols with better methodological quality and control, which uses appropriate timing of sessions around the core body temperature minimum (~ 05:00 h) and maximum (~ 17:00 h) in the morning and evening, respectively. Current studies assessing time-trial and diurnal variation use a testing range from 06:30-10:30 h in the morning and 14:30-20:00 h in the evening. Some of these timings are not within the appropriate time-frame to establish diurnal variation as they do not maximise the peaks and troughs of the rhythm, which might explain the lack of observation. Factors affecting the interpretation of a diurnal variation in maximal performance in the current literature are the willingness of participants to undertake sessions early in the morning and the opening times of laboratories within research "buildings". In addition, there is a further need to investigate and establish the circadian variation of time-trial performance, by using several time-points (4-6) equally spaced over a 24-h period. When such studies have been conducted, only then can accurate conclusions be provided.

Strength and weaknesses

The main strength of the present review is that it was performed using a structured analysis according to the PRISMA guidelines (Page et al. 2021) and is the first and only review to provide an in-depth overview of all the literature considering time-of-day and time-trial performance. Further, as far as we are aware, this is only the third review providing in-depth analysis relating chronobiological factors and how these factors may influence time-trial performance (Pullinger et al. 2020; Ravindrakumar et al. 2022). A further strength of this systematic review is the diversity of databases that have been used within the search strategy and the strong method created and adopted to incorporate search terms that are specific and important to the review topic. Importantly, the current review focused solely on the time-trial paradigm and only included studies designed to assess diurnal variation, where all inclusion criteria were met. It is worth noting that when age was set at ≥18 years old and only males were included, many studies normally highlighted in narrative reviews were removed as evidence for a daily variation in time-trial performance (Drust et al. 2005).

The primary limitation of the present systematic review is associated to several methodological limitations, with considerable differences in methodological and clinical heterogeneity among the 10 studies meant we were unable to conduct a meta-analysis and pool the observed data-sets to evaluate the evidence related to findings in time-trial performance (Borenstein et al. 2009). Not only did our findings observe considerable inconsistencies with reference to chronobiological study design perspectives in the methods and scientific rigor of the past research, there was also disagreement as to whether time-trial performance displays time-of-day or diurnal variation. Future studies ought to consider stricter protocols which take into account these factors to reduce external influences on time-trial performance and additional research is required to provide up to date findings.

Conclusion

The present systematic review shows that time-trial performance currently yields inconclusive findings as to whether it is time-of-day dependent, with less than half the studies displaying at least one variable to present higher values in the afternoon (14:00 – 20:00 h) compared to the morning (06:00 – 10:30 h). Time-of-day variations ranged from 2 to 10 % and were dependant on factors such as chronotype of the individual, training status and mode of exercise, although the current literature available surrounding other measures of human performance would suggest it is rather more complex than this. Many suggestions were provided as to why no diurnal variation in time-trial performance was established. Differences in motivation/arousal, habitual training times, chronotypes and genotypes could provide an explanation as to why some studies/variables did not display time-of-day variation. However, many methodological limitations and issues with quality and control were present. There is an apparent lack of control for important factors which specifically relate to investigations of chronobiological nature in current research of time-trial performance, with a severe lack of standardisation of the methodology. Therefore, there is a need to conduct more rigorous studies of diurnal variation/time-of-day and time-trial performance that utilise appropriate testing times, as close to the time-points of the core body temperature minimum and maximum values as possible, whilst taking into account effects of sleep inertia and restriction and all factors important for investigations of chronobiological nature.

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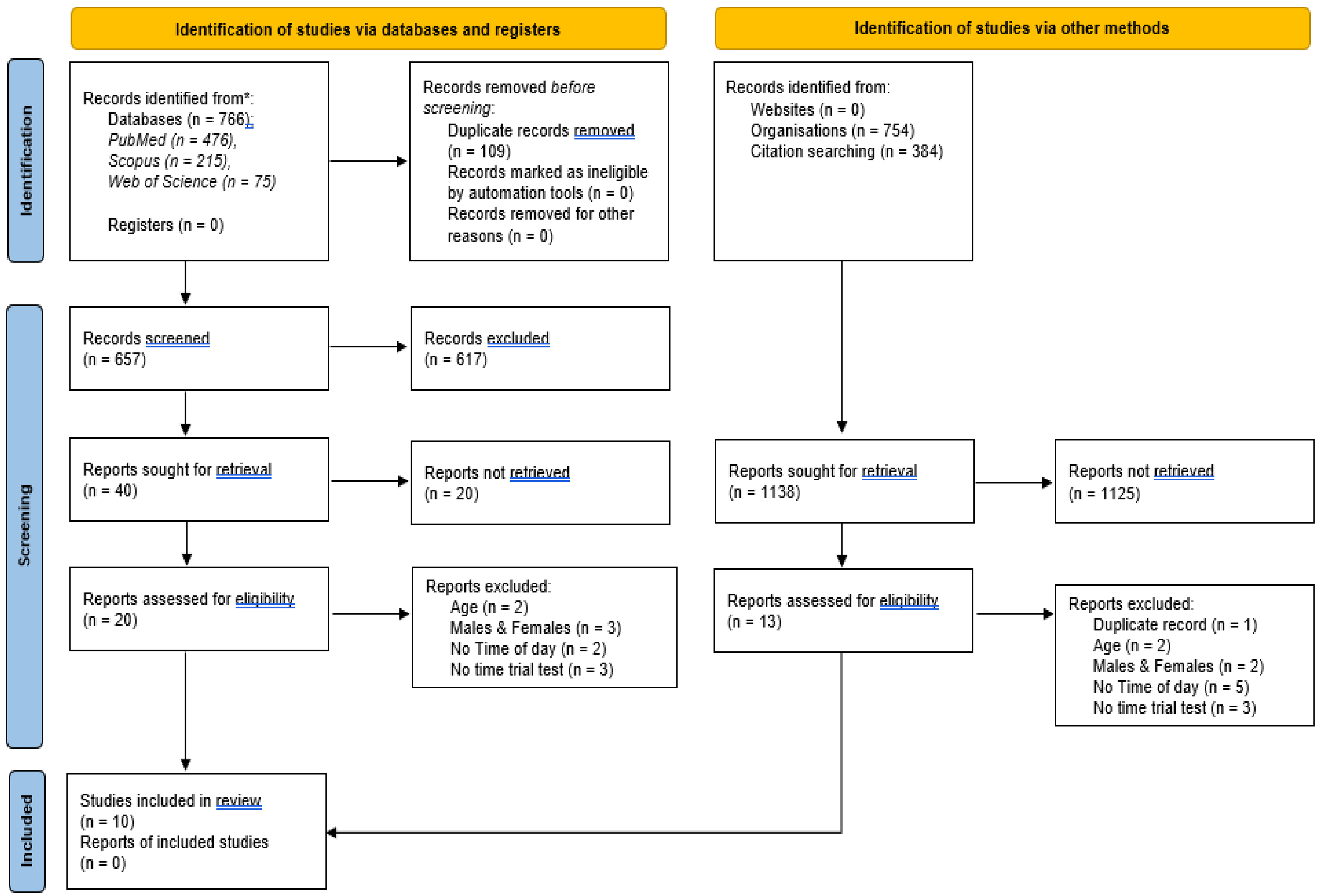

Figure 1. PRISMA 2020 flow diagram (Page et al. 2021) of the study selection process.

Table 1. Summary of the articles reviewed for time-trial performance (n = 10) with an overview of the participants, the experimental protocols with the time-of-day, exercise mode, performance test, the variables examined, and the main findings related to time-of-day in relation to each variable.

Author and Date	Participants	Chronotype assessment and distribution	Testing time-of-day	Test	Mode of exercise	Performance variables examined	Significance of main effects between condition	Main findings
Atkinson et al. (2005)	8 cyclists	Composite Scale of Morningness (Smith et al. 1989)	M = 07:30 h	16.1-km time trial	Cycling	Performance Time	**P = 0.0002**	Performance time was significantly better in E as compared to M; 3.5 %; M = 1426 ± 104 s vs. E = 1370 ± 99 s
	24.9 ± 3.5 yrs, 1.76 ± 0.06 m, 72.5 ± 4.7 kg	1-M type, 6-N types, 1-E type	E = 17:30 h		Kingcycle Ergometer (EDS Portaprompt Ltd, High Wycombe, UK)	Work Rate	**P = 0.003**	Work rate was significantly higher in E as compared to M; 10 %; M = 227 ± 47 W vs. E = 252 ± 48 W
Boukelia et al. (2016)	8 endurance runners	Not assessed	M = 09:00 h	10-km time trial	Running	Mean time	n/a	No significant difference between E and M
	32 ± 5 yrs, 1.78 ± 0.06 m, 69 ± 4 kg		E = 16:00 h		Treadmill (Woodway, ergo ELG 55, Weil am Rhein, Germany)	Mean speed	n/a	No significant difference between E and M
Boukelia et al. (2018)	15 highly trained runners	Not assessed	M = 09:00 h	10-km time trial	Running	Running Time	n/a	No significant difference between E and M
	33 ± 5 yrs, 1.80 ± 0.05 m, 71 ± 7 kg		E = 18:00 h		Treadmill	Performance Time	n/a	No significant difference between E and M
Boyett et al. (2016)	11 trained and 9 untrained cyclists	Not assessed	M = 06:00 - 10:00 h	3-km time trial	Cycling	Performance Time	n/a	Performance time was "very likely" better in the E as compared to M; 2.9 %.
	22 (18 – 44) yrs, 1.75 ± 0.07 m, 73.6 ± 10.9 kg		E = 16:00 - 20:00 h		Cycle Ergometer (Velotron, Racermate Inc., Seattle, WA, USA)			
Dalton et al. (1997)	7 competitive cyclists or triathletes	Not assessed	M = 08:00 - 10:00 h	15-min time trial	Cycling	Average Power Output	n/a	No significant difference between M, E and N
			E = 14:00 – 16:00 h		Hayes Dual Ring Cycle Ergometer	Total Work Accumulated	n/a	No significant difference between M, E and N
			N = 20:00 – 22:00 h					
Fernandes et al. (2014)	9 recreational cyclists	Morningness-eveningness questionnaire (Horne & Ostberg 1976)	M = 08:00 h	1000-m cycling time trial	Cycle ergometer	Performance Time	**P < 0.05**	Performance time was significantly better in E as compared to M; 7.1%; M = 94.7 ± 10.9 s vs. E = 88.2 ± 8.7 s
	31.0 ± 7.5 yrs, 1.75 ± 0.08 m, 73.5 ± 11.6 kg	4-M types, 5-N types	E = 18:00 h		Cycle ergotrainer (Tacx T1680 Flow, Netherlands)	Power Output	P > 0.10	No significant difference between E and M
Lisbôa et al. (2021)	11 competitive swimmers	Morningness-eveningness questionnaire (Horne & Ostberg 1976)	M = 10:00 h	50-m swim time trial	Swimming	Performance Time	P = 0.76	No significant difference between E and M
	20 ± 3 yrs, 1.82 ± 0.05 m, 77 ± 5 kg	1-M type, 10-N types	E = 17:00 h			Block Time	P = 0.12	No significant difference between E and M
						Velocity	P = 0.11	No significant difference between E and M
						Stroke Rate	**P = 0.04**	Stroke rate was higher in the M as compared to the E condition; 2 %; M = 1426 ± 104 Hz vs. E = 1370 ± 99 Hz
						Stroke Length	**P = 0.03**	Stroke rate was higher in the E as compared to the M condition; 3.3 %; M = 2 ± 0.1 m.cycle^{-1} vs. E = 2.04 ± 0.1 m.cycle^{-1}
Rae et al. (2015)	18 trained male swimmers	Morningness-eveningness questionnaire (Horne & Ostberg 1976)	M = 06:30 h	200-m swim time-trial	Swimming	Time	P = 0.902	No significant difference between E and M
	32.6 ± 5.7 yrs, 1.79 ± 0.10 m, 78.9 ± 11.4 kg	9-M types, 9-N types	E = 17:00 h					
Silveira et al. (2020)	16 cycling male practitioners of MTB	Not assessed	M = 06:30 - 10:30 h	20-minute time trial	Cycling	Mean Power	P = 0.305	No significant difference between E and M
	34.8 ± 3.8 yrs, 1.72 ± 0.04 m, 70.2 ± 5.4 kg		E = 14:50 – 18:50 h		Personal mountain bikes	Maximal Power	P = 0.761	No significant difference between E and M

						Relative Mean Power	P = 0.625	No significant difference between E and M
						Mean Power/Cycle	P = 0.429	No significant difference between E and M
						Relative Mean Power/Cycle	P = 0.430	No significant difference between E and M
						Stroke Rate (Cadence)	**P = 0.044**	Stroke Rate was higher in the M as compared to E condition; 2.9 %; M = 85.1 ± 7.6 cycle.m^{-1} vs. E = 82.6 ± 7.4 cycle.min^{-1}
Zadow et al. (2020)	19 Trained cyclists	Not assessed	M = 08:30 h	4-km time trial	Cycling	Performance Time	P > 0.05	No significant difference between any conditions
	39 ± 10.7 yrs, 1.8 ± 0.1 m, 78 ± 9.4 kg		LM = 11:30 h		Cycle Ergometer (Wahoo Kickr)	Power Output	P = 0.78	No significant difference between any conditions
			A = 14:30 h			Cadence	P = 0.46	No significant difference between any conditions
			E = 17:30 h					
			N = 20:30 h					

M = Morning, LM = Late morning, A = Afternoon, E = Evening, N = Night, h = hours, m = metre, km = kilometre, min = minute, s = seconds, yrs = years, kg = kilogram, W = Watts, Hz = Hertz, MTB = mountain bike, n/a = not available

Statistical significance ($P < 0.05$) is indicated in bold.

Table 2. Detailed information related to randomisation, counterbalancing, record of light intensity, control of meals, control of room temperature, control of sleep and fitness for articles related to chronobiology (time-of-day).

Date	Author	Randomisation	Counterbalancing	Record of light intensity	Control of meals	Control of room temperature	Control of sleep	Fitness
2005	Atkinson et al.	No	Yes	No	Yes	Yes	No	Trained Cyclists
2016	Boukelia et al.	Yes	No	No	No	Yes	No	Endurance Runners
2018	Boukelia et al.	Yes	No	No	No	Yes	No	Highly Trained Runners
2016	Boyett et al.	Yes	Yes	No	Yes	No	No	Healthy males
1997	Dalton et al.	No	No	No	Yes	No	Yes	Competitive Athletes or Triathletes
2014	Fernandes et al.	Yes	No	No	Yes	Yes	No	Recreational Cyclists
2021	Lisbôa et al.	No	No	No	No	Yes	No	Competitive Swimmers
2015	Rae et al.	Yes	No	No	Yes	No	Yes	Trained Swimmers
2020	Silveira et al.	Yes	No	No	Yes	Yes	Yes	Mountain Bike Practitioners
2020	Zadow et al.	Yes	Yes	No	Yes	No	Yes	Trained Cyclists

Table 3. Results of the detailed methodological quality assessment scores based on a modified 27-item Downs and Black (1998) checklist.

		Reporting (Items 1-10)										External validity (Items 11-13)			Internal validity: Study bias (Items 14-20)							Internal validity: Confounding selection bias (Items 21-26)						Power (Item 27)		
Date	**Study Author**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**Total Score**	**Rating**
2005	Atkinson et al.	1	1	1	1	2	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	20	71
2016	Boukelia et al.	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	0	1	17	61
2018	Boukelia et al.	1	1	1	1	2	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	19	68
2016	Boyett et al.	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	24	86
1997	Dalton et al.	1	1	1	1	2	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	0	0	20	71
2014	Fernandes et al.	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	0	1	21	75
2021	Lisbôa et al.	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	0	0	17	61
2015	Rae et al.	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	22	79
2020	Silveira et al.	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	0	0	1	20	71
2020	Zadow et al.	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	0	1	22	79
	Total	10	10	10	10	13	10	10	5	2	9	9	9	9	1	1	10	10	10	10	10	8	2	7	7	4	1	5		72.1
	Maximum	10	10	10	10	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10		
	% of lost points	0	0	0	0	35	0	0	50	80	10	10	10	10	90	90	0	0	0	0	0	20	80	30	30	60	90	50		

1 = criteria was met; 0 = criteria was not met.