

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

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28 **Abstract**

29 Few functional measures related to time-trial display diurnal variation. The diversity of
30 tests/protocols used to assess time-trials on diurnal effects and the lack of a standardised approach,
31 hinder agreement in the literature. Therefore, the aims of the present study were to investigate and
32 systematically review the evidence relating to diurnal differences in time-trial measures and to
33 examine the main aspects related to research design deemed specifically important for studies of
34 a chronobiological nature. The entire content of PubMed (MEDLINE), Scopus, Web of Science
35 and multiple electronic libraries were searched. Research studies published in peer reviewed
36 journals and non-peer reviewed studies, conducted in male adult participants aged ≥ 18 yrs before
37 November 2021 were screened. Studies assessing tests related to time-trials in cycling, rowing,
38 running and/or swimming between a minimum of 2 time-points during the day (morning [06:30-
39 10:30 h] vs. evening [14:30-20:00 h]) were deemed eligible. The primary search revealed that a
40 total of 10 from 40 articles were considered eligible and subsequently included. From these the
41 mode of exercise was either cycling (6), running (2) or swimming (2). Events ranged from 1 to
42 16.1-km, or 15 to 20-min time in the cycling and running time-trials; and 50-m to 200-m in the
43 swimming time-trials. Only 4 studies found one or more of their performance variables to display
44 daily variations, with significantly better values in the evening than the morning; while 6 studies
45 found no time-of-day significance in any of the variables assessed. There was a significant diurnal
46 variation for time to complete the event observed in 2 cycling time-trials (from 2.9 to 7.1 %). Work
47 rate during a 16.1-km time trial in cycling was 10 % higher in the evening than the morning. The
48 only other observed differences were stroke rate and stroke length during a swimming time-trial
49 and cycling stroke rate (cadence; revolutions per minute) during a mountain bike 20-min time-trial.
50 The magnitude of difference is dependent on the modality of the exercise, the chronotype of the
51 individual, the training status of the individual and sample size. The lack of diurnal variation in
52 most studies, can in-part be explained by the methodological limitations and issues present related
53 to quality and control. Therefore, it is paramount that research assessing diurnal variation in
54 performance uses appropriate timing of sessions around the core body temperature minimum (~
55 05:00 h) and maximum (~ 17:00 h) in the morning and evening, respectively. Although, differences
56 in motivation/arousal, habitual training times, chronotype and genotype could provide an
57 explanation as to why some research/variables did not display time-of-day variation, more work is

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

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

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78 **Introduction**

79 It has previously been established that most of the research related to physical and physiological
80 performance variables display diurnal variation in a temperate environment (around 17–22°C) in
81 healthy adolescent males (18 + years of age; Pullinger et al. 2020; Ravindrakumar et al. 2022). It
82 is currently believed that in the absence of external cues, levels of cortisol, core and/or muscle
83 temperatures and melatonin levels play a major role in circadian regulation through signals
84 directed by the suprachiasmatic nucleus (body clock), located in the anterior part of the
85 hypothalamus (Reilly 1990; Van Drunen and Eckel-Mahan 2021). A large body of research has
86 shown cortisol levels (Reilly and Waterhouse 2009), core body temperatures (Pullinger et al. 2018a,
87 2019) and muscle temperatures (Pullinger et al. 2014, 2018b) to be higher in the mid-afternoon
88 and/or early evening, while melatonin levels display values which are higher during the nocturnal
89 period (Edwards et al. 2000; Zawilska et al. 2009). Similarly, regardless of muscle group measured,
90 both muscle force production and power output also display an evening superiority (Atkinson and
91 Reilly 1996; Edwards et al. 2013; Robertson et al. 2018). In addition, most measures related to
92 repeated sprint performance and anaerobic power/capacity (Pullinger et al. 2020; Ravindrakumar
93 et al. 2022) are also time dependent with higher values, ranging from 1.8 to 13.1 %, in the afternoon
94 (16:00 and 19:30 h) compared to the morning (05:30 to 11:00 h).

95 To the best of our knowledge, the current research assessing diurnal variation on performance
96 measures related to time-trial which has been conducted yields inconclusive results. Time-trials
97 can further be defined as a “race against time or distance”, in which an athlete tries to complete
98 the race as fast as possible (Edwards et al. 2005). The mode of exercise utilised in time-trials can
99 range from cycling (Gough et al. 2021) to running (Boukelia 2018) to swimming (de Salles Painelli
100 et al. 2013) to rowing (Mujika et al. 2012). Findings have suggested that circadian rhythmicity has
101 an impact on aerobic activities, such as time-trials (Drust et al. 2005). Yet, most current research
102 on time-trial is diurnal of nature using two time-points (morning and evening). Currently, there is
103 a lack of agreement concerning the presence of diurnal variation in time-trials. It has been found
104 that a 16.1-km cycling time trial performance established a small and statistically significant
105 improvement in the morning vs. the afternoon (Atkinson et al. 2005). However, a time-trial cycling
106 performance of 15-min duration found no significant differences for any of the measured variables
107 when comparing morning, afternoon and evening sessions (Dalton et al. 1997). Nevertheless, the

108 majority of factors related to cycling performance such as strength, power and work are higher in
109 the afternoon and/or evening than in the morning (Atkinson and Reilly 1995 1996; Ravindrakumar
110 et al. 2022). Large differences in methods and procedures such as training status of participants,
111 familiarisation (number of times completed to be deemed that further learning was minimal),
112 counterbalancing of participants into groups to statistically distribute any additional learning,
113 randomisation of sessions, mode of exercise, time-trial distances, time-trial duration are some of
114 the main issues which make it difficult to compare between studies and affect current findings
115 (Drust et al. 2005; Pullinger et al. 2020). In addition, measurement error and sample size influence
116 research related to circadian variation, and play an important role on the discovery of variation
117 (Drust et al. 2005).

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

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

145

146 **Methods**

147 *Reporting Standard*

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

152

153 *Eligibility Criteria*

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

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

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

165 2. Time-of-day – compared the effects of morning versus evening in performance variables
166 related to time-trials (a minimum of two time-points).

167 3. Time-trials – individual time-trial, team time-trial, distance time-trial or track time-trial
168 tests.

169 4. Modality – cycling, running, swimming or rowing.

170 5. Design – Randomised and/or counterbalanced trials.

171

172 *Literature Search Strategy and Information Sources*

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

191 ***Study Selection***

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

201

202 ***Data Extraction***

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

219 specific reference to any of these primary areas were considered to indicate a negative response
220 and ‘no’ was marked against the area in question.

221

222 ***Quality Assessment***

223 A modified 27-item methodological quality assessment checklist on each included article using
224 the Downs and Black (1998) scale was conducted. The checklist consisted of 27 “yes”-or- “no”
225 questions which were scored totalling up to a possible 28 points. Item 27: “*Did the study have*
226 *sufficient power to detect a clinically important effect where the probability value for a difference*
227 *being due to chance is less than 5%?”* to a yes (1-point) or no/unable to determine (0 points)
228 scoring. The questions were categorized under 5 sections: Reporting (10 items; 1-10), External
229 validity (3 items; 11-13), Internal validity study bias (7 items; 14-20), internal validity confounding
230 selection bias (7 items; 21-26) and power (1 item; 27). The quality assessment of the articles was
231 conducted by two reviewers (AR and TB) independently with disagreement on 6 items across the
232 10 manuscripts (2.2 %). The observed differences were resolved by a third reviewer (SP).

233

234 **Results**

235 ***Search Results***

236 The literature search ended on 19 November 2021 and the primary database search revealed 766
237 articles and an additional 1138 via other methods. Figure 1 presents the number of articles found
238 in each electronic database or through other methods, and a detailed flow chart of the literature
239 search, including all the steps performed. Once duplicates were removed, 657 titles obtained via
240 databases remained in the reference manager (Mendeley, Elsevier, Amsterdam, The Netherlands).
241 Following the examination of titles, abstracts and keywords of all these manuscripts, 40 academic
242 studies were deemed eligible and retained for full text-analysis. After additional full-text analysis,
243 20 studies were assessed for eligibility, of which 10 were deemed eligible and included in the
244 systematic review. Reasons for exclusion can be found in Figure 1. Upon further inspection of all
245 articles in their bibliographical references and through organisations, 13 were assessed for

246 eligibility, but none met the inclusion criteria and hence were deemed ineligible. Therefore, a total
247 of 10 studies were used in the systematic review.

248

249 **Study Characteristics**

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

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

270 Only four studies found one or more of their performance variables to display time-of-day effects,
271 with values between the morning and evening significantly different, while six studies found no
272 significant differences between morning and evening in any of the variables assessed. Cycling
273 time-trial to complete a 16.1-km was found to be significantly better in the evening compared to

274 the morning by 3.5 % (Atkinson et al. 2005) and by 7.1 % in a 1-km time-trial (Fernandes et al.
275 2014). Both the 3-km (Boyett et al. 2016) and 4-km (Zadow et al. 2020) cycling time trial found
276 no significant differences in performance time. Both 10-km running time-trials also displayed no
277 significant differences in performance time (Boukelia et al. 2016; 2018), as did both swimming
278 time-trials (Lisbôa et al. 2021; Rae et al. 2015). The only other significant differences observed
279 were work rate during a 16-1-km cycling time-trial (10 %; Atkinson et al. 2005), stroke rate and
280 stroke length during a swimming time trial (2.0 to 3.3 %; Lisbôa et al. 2021), and stroke rate in a
281 20-min mountain bike time trial (2.9 %; Silveira et al. 2020). However, Rae et al. (2015) did
282 establish significant differences in time-of-day for 200-m swim time-trial when participants were
283 grouped according to habitual training time or chronotype.

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

291

292 **Quality of work**

293 Table 2 provides detailed information related to randomisation, counterbalancing, record of light
294 intensity, control of meals, control of room temperature, control of sleep and fitness, to quantify
295 for the control of aspects relating to research design deemed specifically important in
296 investigations of a chronobiological nature. None of the studies met all 7 criteria required for an
297 investigation of chronobiological nature. All the studies provided information related to fitness of
298 participants. A total of 3 counterbalanced the order of administration to minimise learning effects
299 and 7 studies performed the time-of-day session in a randomised order. From these, 2 studies
300 (Boyett et al. 2016 and Zadow et al. 2020) used counterbalancing and randomisation within their
301 protocol. The majority of studies controlled for meals (n=7) and controlled for room temperature

302 (n=6). However, less than half the studies controlled for sleep (n=4), while no study recorded light
303 intensity. None of the studies quantified all four of the 4 aforementioned criteria.

304

305 **Methodological quality control and publication bias**

306 Based on a modified 27-item Downs and Black (1998) checklist, the results of the methodological
307 quality assessment of the included studies ranged from 17 to 24. Reporting (10 items; items 1-10)
308 showed 6 items to be fully met by all studies (Items 1-4, 6 and 7). External validity (3 items; items
309 11-13) displayed all three items to be met by 9 studies. Internal validity study bias (7 items; items
310 14-20) reported 5 items out of 7 items (items 16-20) to be fully met, with one study fully meeting
311 all criteria for internal validity study bias (Boyett et al. 2016). Confounding selection bias (6 items;
312 items 21-26) were fully met by none of the studies, while half the studies used power to determine
313 sample size and/or whether the study had sufficient power (1 item; Item 27). Detailed
314 methodological quality assessment scores can be found in Table 3.

315

316 **Discussion**

317 The present study analysed data from studies that compared the effects of diurnal variation on
318 time-trial measures and determined the quality of evidence that reports a “peak” time for
319 performance. The main findings of this review were: 1) few of the variables assessed (23.1 %)
320 displayed diurnal variation, with 6 studies (60 %) displaying no differences between the afternoon
321 (14:00 – 20:00 h) and morning sessions (06:00 – 10:30 h) in any of the time-trial variables assessed;
322 2) methodological limitations and issues present related to quality and control affect observations
323 of diurnal variation in time-trial.

324

325 ***Time Trials***

326 Previous research has established diurnal variation to be present in many different human

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

353 However, the majority of results established within this review do not support the notion that time-
354 trial variables display diurnal variation in a temperate environment (around 17–22°C) in healthy
355 adolescent males (18 + years of age). Several factors have been put forward to explain the lack of
356 diurnal variation observed in time-trials. Several studies suggested that diurnal variation in time-

357 trial performance would be observed as a result of its causal link with core body temperature.
358 Several studies found core body temperature to vary with time-of-day, nevertheless time-trial was
359 unaffected (Boukelia et al. 2018; Dalton et al. 1997; Zadow et al. 2020). Adaptive responses to
360 training, competition times, the motivation of participants and habitual training patterns of athletes
361 were suggested as masking the effect of diurnal variation in time-trials in these studies. Although
362 diurnal variation in core body temperature was suggested as the main cause of diurnal variation
363 established in cycling stroke rate (cadence) during a 20-min time trial, no other performance
364 variables displayed diurnal variation (Silveira et al. 2020). They did not provide any information
365 as to why no diurnal variation was established in other measures. Nevertheless, other suggestions
366 have been put forward such as; unfamiliar testing conditions (cold/hot environment; Boukelia et
367 al. 2016; 2018), warm-up effect, single intraday study design (Lisbôa et al. 2021), and participant
368 chronotype (Rae et al. 2015). Interestingly, the study performed by Rae et al. (2015) did find 200-
369 m swim time-trial displayed diurnal variation when grouping athletes by chronotype, with
370 morning-types significantly faster in the morning (0.5 %), and intermediate-types significantly
371 faster in the evening (1.2 %). In addition, swimmers who consistently trained in the morning were
372 faster in the morning, while swimmers who consistently trained in the evening, were faster in the
373 evening. Nevertheless, the current literature is contradictory regarding chronotype effects on
374 diurnal variation and performance (Atkinson et al. 2005; Brown et al. 2008) and it is not clear
375 whether this diurnal variation in performance is due to solely endogenous factors or habitual
376 training times or a combination of both (Chtourou et al. 2012; Martin et al. 2007). Results observed
377 in elite male and female swimmers found athletic performance to be influenced by individual
378 circadian behavioural phenotype and to be closely associated to physiological and molecular
379 differences (Anderson et al. 2018).

380 Current findings on diurnal variation and time-trial are contradictory and present several
381 methodological issues. Some important primary weaknesses are discussed in different studies,
382 such as relatively small sample sizes utilised, the lack of mechanistic assessments and/or insight
383 and issues surrounding the nutrition timing/intake. All aforementioned aspects can highly
384 influence the observation of diurnal variation and are a necessity for creating rigorous laboratory-
385 based protocols (Drust et al. 2005). Before providing a conclusion surrounding diurnal variation
386 and time trials, better methodological quality and control is required, with main factors discussed

387 below. Nevertheless, based on current findings related to diurnal variation in performance, a
388 controlled laboratory-based investigation with scientific rigour in experimental design and data
389 collection with minimal measurement error would yield the same results. It is well established that
390 both endogenous and exogenous components influence performance, with motivational aspects,
391 subjective arousal, sleepiness, ionic changes and hormonal fluctuations (cortisol ratio, thyroid
392 secretion and testosterone ratio) playing a role (Edwards et al. 2013; Zhang et al. 2009). Recent
393 observations have suggested differences in the phosphorylation of proteins within or close to the
394 muscle M-band that could relate to the well-established morning versus evening differences in
395 performance might well provide a better explanation to time-of-day observations (Ab Malik et al.
396 2020). Gene (circadian clock gene PER3 variants) and environment (time of day) interactions
397 suggest that diurnal variation performance is associated with circadian phenotype and PER3
398 genotype. Diurnal variation in performance is complex and involves multiple components and
399 mechanisms which require further research. Even though direct evidence has been established
400 regarding a large endogenous component related to the daily variation in muscle force production
401 (from the body clock and peripheral clocks: Zhang et al. 2009), it is presently still unproven
402 (Sargent et al. 2010). To fully explore this internal component, complex, time consuming, and
403 challenging chronobiological protocols (for both researchers and participants) are required.
404 Protocols which attempt to reduce or standardize the exogenous component of the rhythm using
405 constant routines, forced desynchronization, or ultrashort sleep-wake-cycle protocols remain to
406 be performed (Kline et al. 2007; Reilly and Waterhouse 2009), adding to our understanding
407 regarding which diurnal variation factors might play a major role.

408

409 ***Methodological quality and control***

410 As far as we are aware, only two reviews have looked into aspects related to chronobiology study
411 design (Pullinger et al. 2020; Ravindrakumar et al. 2022). In agreement, an apparent lack of control
412 was also established within this review. Considering the periodicity of the body clock in human
413 beings is affected by external environmental rhythmic cues which affect the continual adjustment
414 of the body clock (zeitgebers), and ultimately act as circadian time cues, several rhythmic cues,
415 such feeding-fasting cycle (control of meals), the activity-inactivity cycle (fitness) and light-dark

416 cycle (recording of light intensity; Aschoff 1965; Aschoff and Wever 1980; Dunlap et al. 2004)
417 require control. Surprisingly, no studies reported information related to light and/or dark exposure
418 through recording of light intensity (Table 2), or even basic information on the time of year the
419 research was conducted (hence timing of sun rise and sun set). Light exposure influences mood
420 and alertness (Bedrosian and Nelson 2017; Souman et al. 2018) and have also shown to improve
421 time-trial performance (Kantermann et al. 2012; Thompson et al. 2015), although the extent is
422 associated to light intensity, wavelength, time-of-day (Knaier et al. 2017). Most studies (70 %) did
423 control for meals, a factor previously stressed to play a vital role in chronobiology studies (Table
424 2; Bougard et al. 2009). In order to limit the variability in results, intake and/or timing of meals
425 need precaution and standardisation across the testing protocol. It has been suggested participants
426 only have a glass of water (Atkinson & Reilly 1995; Moussay et al. 2002) or in a fasted state prior
427 to a morning session (Ab Malik et al. 2020; Pullinger et al. 2014), and not to consume food for \geq
428 4 h prior to an evening session (Ab Malik et al. 2020; Brotherton et al. 2019). In studies where
429 aspects related to nutrition timing/intake are not mentioned at all, could potentially mask or
430 increase morning-evening difference in many physiological variables (Bougard et al. 2009). All
431 studies did report information related to participant background and fitness levels, thus unlikely
432 negatively influencing findings (Guette et al. 2005; Häkkinen 1989). Nevertheless, training status
433 (trained vs. untrained) does influence performance in different modalities (Bishop and Spencer
434 2004; Hopker et al. 2013; Riboli et al. 2021). Diurnal variation in performance is linked to training
435 status, mode of exercise specificity and participant familiarisation and therefore needs to be well-
436 controlled (Bambaeichi et al. 2005; Giacomoni et al. 2006; Reilly et al. 1997).

437 When looking at chronotype assessment and distribution, only three studies (30 %) assessed their
438 participant's chronotype scores. Previous observations have found differences in time-trial (Brown
439 et al. 2008; Rae et al. 2015), $\dot{V}O_2\text{max}$, cortical and spinal excitability levels (Roden et al. 2017),
440 thus suggesting the importance of providing detailed information related to chronotype. Similarly,
441 only four studies (40 %) controlled for sleep, such as keeping similar sleeping habits to “normal
442 life”, not staying up late, habitual rising and waking times, and whether any prevalence of insomnia
443 or sleep deprivation is present. Sleep is essential for the human brain and body to function and a
444 lack of sleep and/or sleep deprivation is closely associated with impairment in time-trial
445 performance (Chase et al. 2017; Souissi et al. 2020; Walsh et al. 2021). Findings related to time-

446 trial performance are in agreement with previous research which has examined the effects of sleep
447 (deprivation) on central fatigue and performance (Edwards and Waterhouse 2009; Kirschen et al.
448 2020; Waterhouse et al. 2011), also establishing that a lack of sleep, sleep deprivation and disturbed
449 sleeping patterns negatively affect performance. Increased levels of fatigue are closely associated
450 with time-since-last-sleep and as time-awake increases, cognitive performance, central arousal and
451 the restorative influences of sleep wane (Ball et al. 1999).

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

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

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

480 There is a need for more rigorous laboratory-based protocols with better methodological quality
481 and control, which uses appropriate timing of sessions around the core body temperature minimum
482 (~ 05:00 h) and maximum (~ 17:00 h) in the morning and evening, respectively. Current studies
483 assessing time-trial and diurnal variation use a testing range from 06:30-10:30 h in the morning
484 and 14:30-20:00 h in the evening. Some of these timings are not within the appropriate time-frame
485 to establish diurnal variation as they do not maximise the peaks and troughs of the rhythm, which
486 might explain the lack of observation. Factors affecting the interpretation of a diurnal variation in
487 maximal performance in the current literature are the willingness of participants to undertake
488 sessions early in the morning and the opening times of laboratories within research “buildings”. In
489 addition, there is a further need to investigate and establish the circadian variation of time-trial
490 performance, by using several time-points (4-6) equally spaced over a 24-h period. When such
491 studies have been conducted, only then can accurate conclusions be provided.

492

493 **Strength and weaknesses**

494 The main strength of the present review is that it was performed using a structured analysis
495 according to the PRISMA guidelines (Page et al. 2021) and is the first and only review to provide
496 an in-depth overview of all the literature considering time-of-day and time-trial performance.
497 Further, as far as we are aware, this is only the third review providing in-depth analysis relating
498 chronobiological factors and how these factors may influence time-trial performance (Pullinger et
499 al. 2020; Ravindrakumar et al. 2022). A further strength of this systematic review is the diversity
500 of databases that have been used within the search strategy and the strong method created and
501 adopted to incorporate search terms that are specific and important to the review topic. Importantly,
502 the current review focused solely on the time-trial paradigm and only included studies designed to
503 assess diurnal variation, where all inclusion criteria were met. It is worth noting that when age was

504 set at ≥ 18 years old and only males were included, many studies normally highlighted in narrative
505 reviews were removed as evidence for a daily variation in time-trial performance (Drust et al.
506 2005).

507 The primary limitation of the present systematic review is associated to several methodological
508 limitations, with considerable differences in methodological and clinical heterogeneity among the
509 10 studies meant we were unable to conduct a meta-analysis and pool the observed data-sets to
510 evaluate the evidence related to findings in time-trial performance (Borenstein et al. 2009). Not
511 only did our findings observe considerable inconsistencies with reference to chronobiological
512 study design perspectives in the methods and scientific rigor of the past research, there was also
513 disagreement as to whether time-trial performance displays time-of-day or diurnal variation.
514 Future studies ought to consider stricter protocols which take into account these factors to reduce
515 external influences on time-trial performance and additional research is required to provide up to
516 date findings.

517

518 **Conclusion**

519 The present systematic review shows that time-trial performance currently yields inconclusive
520 findings as to whether it is time-of-day dependent, with less than half the studies displaying at least
521 one variable to present higher values in the afternoon (14:00 – 20:00 h) compared to the morning
522 (06:00 – 10:30 h). Time-of-day variations ranged from 2 to 10 % and were dependant on factors
523 such as chronotype of the individual, training status and mode of exercise, although the current
524 literature available surrounding other measures of human performance would suggest it is rather
525 more complex than this. Many suggestions were provided as to why no diurnal variation in time-
526 trial performance was established. Differences in motivation/arousal, habitual training times,
527 chronotypes and genotypes could provide an explanation as to why some studies/variables did not
528 display time-of-day variation. However, many methodological limitations and issues with quality
529 and control were present. There is an apparent lack of control for important factors which
530 specifically relate to investigations of chronobiological nature in current research of time-trial
531 performance, with a severe lack of standardisation of the methodology. Therefore, there is a need

532 to conduct more rigorous studies of diurnal variation/time-of-day and time-trial performance that
533 utilise appropriate testing times, as close to the time-points of the core body temperature minimum
534 and maximum values as possible, whilst taking into account effects of sleep inertia and restriction
535 and all factors important for investigations of chronobiological nature.

536

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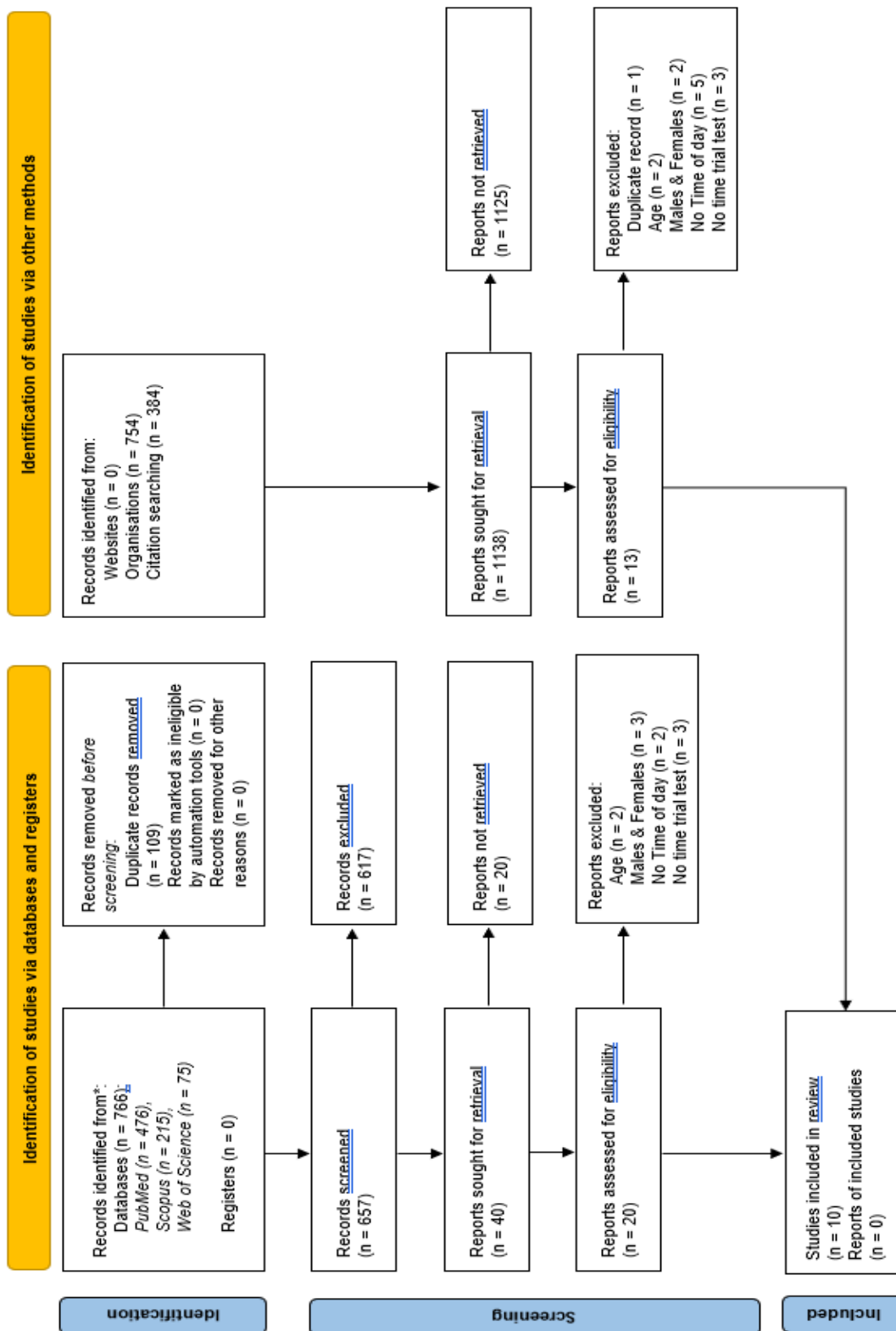


Figure 1. PRISMA 2020 flow diagram (Page et al. 2021) of the study selection process.

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. (2008)	8 cyclists 24.9 ± 3.3 60% , 1.76 ± 0.06 m, 72.3 ± 4.7 kg	Compass Scale of Chronotypes (Smith et al. 1989) 1-M type, 6-N types, 1-E type	M = 07:30 h E = 17:30 h	16.1-km time trial	Cycling	Performance Time	P = 0.0002	Performance time was significantly better in E as compared to M, 3.4 %; M = 1426 ± 104 s vs. E = 1370 ± 99 s
Chollet et al. (2016)	8 endurance runners 32 ± 5 60% , 1.78 ± 0.08 m, 69 ± 4 kg	Not assessed	M = 09:00 h E = 16:00 h	10-km time trial	Running Treadmill (Woodway, et al. ILLUS, Well run Rhein, Germany)	Mean time Mean speed	na na	Work rate was significantly higher in E as compared to M, 10 %; M = 227 ± 47 W vs. E = 252 ± 48 W No significant difference between E and M No significant difference between E and M
Chollet et al. (2018)	15 highly trained runners 35 ± 5 60% , 1.80 ± 0.05 m, 71 ± 7 kg	Not assessed	M = 09:00 h E = 18:00 h	10-km time trial	Running Treadmill	Running Time Performance Time	na na	No significant difference between E and M No significant difference between E and M
Boyet et al. (2016)	11 trained and 9 untrained cyclists 22 (18–44) 60% , 1.75 ± 0.07 m, 73.6 ± 10.9 kg	Not assessed	M = 06:00–06:00 h E = 16:00–20:00 h	5-km time trial	Cycling	Performance Time	na	Performance time was "very likely" better in the E as compared to M, 2.9 %
Dalton et al. (1997)	7 competitive cyclists or triathletes 31.0 ± 7.3 60% , 1.75 ± 0.08 m, 73.8 ± 11.6 kg	Not assessed	M = 08:00–10:00 h E = 14:00–16:00 h N = 20:00–22:00 h	15-min time trial	Cycling Cycle Ergometer (Valhalla, Valhalla Inc., Seattle, WA, USA)	Average Power Output Total Work Accumulated	na na	No significant difference between M, E and N No significant difference between M, E and N
Fernandes et al. (2014)	9 recreational cyclists 31.0 ± 7.3 60% , 1.75 ± 0.08 m, 73.8 ± 11.6 kg	Chronotypes questionnaire (Horne & Reyner 1976) 4-M types, 3-N types	M = 08:00 h E = 18:00 h	1000-m cycling time trial	Cycle ergometer Cycle ergometer (Jolba, T1680 Flow, Netherlands)	Performance Time Power Output	P = 0.05 P < 0.10	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 No significant difference between E and M
Lubbe et al. (2021)	11 competitive swimmers 20 ± 3 60% , 1.82 ± 0.08 m, 77 ± 5 kg	Chronotypes questionnaire (Horne & Reyner 1976) 1-M type, 10-N types	M = 10:00 h E = 17:00 h	50-m swim time trial	Swimming	Performance Time	P = 0.76	No significant difference between E and M
						Block Time Velocity Stroke Rate	P = 0.12 P = 0.11 P = 0.04	No significant difference between E and M No significant difference between E and M Stroke rate was higher in the M as compared to the E condition, 2 %; M = 1426 ± 104 Hz vs. E = 1370 ± 99 Hz
Rae et al. (2015)	18 trained male swimmers 1.79 ± 0.10 m, 78.9 ± 11.4 kg	Chronotypes questionnaire (Horne & Reyner 1976) 9-M types, 9-N types	M = 06:30 h E = 17:00 h	200-m swim time-trial	Swimming	Stroke Length Time	P = 0.03 P = 0.002	Stroke rate was higher in the E as compared to the M condition, 3.3 %; M = 2 ± 0.1 beats vs. E = 2.04 ± 0.1 m/cycle No significant difference between E and M
Silveira et al. (2020)	16 cycling male practitioners 34.8 ± 3.8 60% , 1.77 ± 0.04 m, 70.2 ± 2.4 kg	Not assessed	M = 06:30–10:30 h E = 14:30–18:30 h	20-minute time trial	Cycling Personal mountain bikes	Mean Power Maximal Power	P = 0.005 P = 0.061	No significant difference between E and M No significant difference between E and M

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

