

1 **TITLE: EVIDENCE OF DISTURBED SLEEP AND INCREASED ILLNESS IN**
2 **OVERREACHED ENDURANCE ATHLETES.**

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25 **Running title:** Overreaching, sleep and illness

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27 **Disclosure funding received for this work:** We received funding for research on which this article is
28 based from the French Anti Doping Agency. The authors report no conflict of interest. The results of
29 the present study do not constitute endorsement by the American College of Sports Medicine.

30 **ABSTRACT**

31

32 **Purpose:** To examine whether i) objective markers of sleep quantity and quality are altered in
33 endurance athletes experiencing overreaching in response to an overload training program and ii)
34 whether potential reduced sleep quality would be accompanied with higher prevalence of upper
35 respiratory tract infections in this population.

36 **Methods:** Twenty seven trained male triathletes were randomly assigned to either overload ($n=18$) or
37 normal (CTL, $n=9$) training groups. Respective training programs included a 1-week moderate training
38 phase, followed by a 3-week period of overload or normal training, respectively and then a subsequent
39 2-week taper. Maximal aerobic power and oxygen uptake ($\dot{V}O_{2max}$) from incremental cycle ergometry
40 were measured after each phase, whilst mood states and incidences of illness were determined from
41 questionnaires. Sleep was monitored every night of the 6 weeks using wristwatch actigraphy.

42 **Results:** Nine of the 18 overload training group subjects were diagnosed as functionally overreached
43 (F-OR) after the overload period, as based on declines in performance and $\dot{V}O_{2max}$ with concomitant
44 high perceived fatigue ($p<0.05$), whilst the nine other overload subjects showed no decline in
45 performance (AF, $p>0.05$). There was a significant time \times group interaction for sleep duration (SD),
46 sleep efficiency (SE) and immobile time (IT). Only the F-OR group demonstrated a decrease in these
47 three parameters ($-5.4\pm 5.3\%$, $-2.1\pm 2.0\%$ and $-5.7\pm 5.0\%$, for SD, SE and IT, respectively, $p<0.05$),
48 which was reversed during the subsequent taper phase. Higher prevalence of upper respiratory tract
49 infections were also reported in F-OR (67%, 22%, 11% incidence rate, for F-OR, AF and CTL,
50 respectively).

51 **Conclusion:** This study confirms sleep disturbances and increased illness in endurance athletes who
52 present with symptoms of F-OR during periods of high volume training.

53

54 **Keywords:** fatigue; overtraining; endurance training; recovery; immunity

55 **INTRODUCTION**

56

57 *Paragraph 1.* Increases in training intensity or volume are typically undertaken by athletes in
58 an attempt to enhance physiological adaptation and improve physical performance. However, when
59 the balance between appropriate training stress and adequate recovery is disrupted, an abnormal
60 training response may occur and a state of short-term "overreaching" (functional OR, F-OR) (23) may
61 develop, resulting in a decline in performance. Even though the F-OR state is generally reversed when
62 an appropriate period of recovery is provided (~1-3 weeks) (18, 23), it can compromise competition
63 outcomes in the short term, particularly when insufficient recovery is available prior to competition.
64 However, critical reviews of existing scientific literature continue to conclude that the underlying
65 causes of F-OR in endurance athletes remain uncertain (11, 23, 26, 36).

66

67 *Paragraph 2.* One of the most commonly reported methods for managing fatigue and
68 enhancing recovery is obtaining adequate passive rest and sufficient sleep (25, 31). The restorative
69 qualities of sleep for maintaining optimal bodily function are well recognized. The recovery of
70 cognitive processes and metabolic functions, both of which are important contributors to exercise
71 performance, can be affected by the quality and quantity of sleep (31). Despite health-based survey
72 research reporting associations between regular moderate physical activity and better sleep (5), few
73 studies have reported alterations in sleep quality in response to highly demanding training programs
74 (19, 35). Taylor et al. (35) measured sleep via polysomnography during the 'onset of training', 'heavy
75 training' and during the 'pre-competition taper' in elite female swimmers. Sleep onset latency, time
76 awake after sleep onset, total sleep time, rapid eye movement and sleep times were similar at all three
77 training phases but the number of movements during sleep was significantly higher (6%) during
78 higher training volumes, suggesting some alteration to sleep. Nevertheless, the improvement in
79 performance time and low levels of tension and anger at peak training suggests that the swimmers
80 were not F-OR. Recently, Fietze et al. (7) used wrist actigraphy during a 67-day period of high
81 physical and mental stress to study sleep patterns in 24 classical ballet dancers before a ballet premiere
82 performance. They found small but significant reduction in sleep duration (-6%), in sleep efficiency (-

83 2%), in time in bed (-3%) and an increase in wakefulness after sleep onset (+3%). Sleep onset latency
84 did not change. Nevertheless, these authors did not report changes in physical performance in response
85 to the prescribed overload program, making clear conclusions for sleep disruption in OR athletes
86 difficult. However, studies during which sleep was monitored in athletes who demonstrated clear signs
87 of OR (i.e. high perceived fatigue and decreased performance), remain few and involve self-reporting
88 of reduced perceived subjective sleep quality (13, 14). Given such equivocal findings, a recent joint
89 consensus statement led Meeusen et al. (23) to recommend additional research to determine the
90 relationship between F-OR and altered sleep patterns.

91

92 *Paragraph 3.* Past research showed that aspects of both innate and adaptive immunity are
93 depressed during sustained periods of heavy training (for review, (38)). An imbalance between
94 training loads and recovery has been shown as a major contributor to illness (38). These abnormalities
95 share similarities with impairment in immune function observed after moderate sleep deprivation (33).
96 Vgontzas et al. studied the effects of modest sleep restriction from 8 to 6h per night for 1 week in 25
97 young, healthy, normal sleepers for 12 consecutive nights in a sleep laboratory (37). Their results
98 showed that modest sleep loss is associated with significant increased secretion of pro-inflammatory
99 cytokines, suggesting a link between the recuperative processes of sleep and the immune system.
100 Similarly, Cohen et al. (4) showed that insufficient sleep volume over consecutive days can impair
101 immune function, and increase the risk of developing upper respiratory tract infections. These authors
102 reported that participants with less than 7 hours of sleep were 2.94 times more likely to develop a
103 'cold' than those with 8 hours or more of sleep once administered nasal drops containing a rhinovirus
104 and monitored for ensuing development of a clinical cold. The association with sleep efficiency was
105 also graded, with participants reporting <92% sleep efficiency 5.5 times more likely to develop a cold
106 than those with >98% efficiency. Taken together, these results have led some authors to suggest that
107 the potential immunosuppressive effects of overreaching may act through sleep disturbances (38).
108 However, no scientific investigation to date provides evidence of this relationship to substantiate this
109 argument.

110

111 *Paragraph 4.* The aim of the present study was therefore to determine whether changes in
112 objective sleep parameters were evident between an experimental group of triathletes developing F-
113 OR compared to a control group. After one week of light training (baseline), the experimental group
114 completed a 3-week overload period followed by a 2-week taper. By programming intensified training
115 over a large population of endurance athletes ($n = 28$), we hypothesized that some participants would
116 demonstrated signs of F-OR (*i.e.* transient reduced performance). By this way, the present research
117 gave us also the opportunity to determine whether sleep disturbances would be observed during an
118 overload period in participants led to F-OR. In the light of past literature, we hypothesized that the
119 development of F-OR would be accompanied by a decline in sleep quality and sleep quantity.
120 Furthermore, we investigated whether potential presence of F-OR and reduced sleep quality in F-OR
121 athletes would be accompanied with higher prevalence of upper respiratory tract infections (URTI).

122

123

124 **MATERIAL AND METHODS**

125

126 *Subjects*

127

128 *Paragraph 5.* Forty well-trained triathletes volunteered to participate in this study. All subjects
129 had been competing for 3 years and were training a minimum of 7 times per week. During the
130 experimental period, 7 subjects did not follow the protocol due to injury or personal obligations and
131 were excluded from subsequent analyzes. Additionally, 5 participants, who worked at night with
132 irregular schedules were excluded from subsequent analyzes. One subject was also excluded due to
133 technical problems with equipment. The final sample size included in analysis was $n = 27$. The
134 experimental design of the study was approved by the Ethical Committee of Saint-Germain-en-Laye
135 (acceptance no. 12048) and was conducted in accordance with the Declaration of Helsinki. Prior to
136 participation, subjects underwent medical assessment with a cardiologist to ensure normal
137 electrocardiograph patterns and obtain a general medical clearance. All subjects were free from
138 chronic diseases and were not taking prescribed medication at the commencement of the study. After

139 comprehensive verbal and written explanations of the study, all subjects gave their written informed
140 consent to participate.

141

142

143 *Study design*

144

145 **Paragraph 6.** An overview of the study design is shown in Figure 1. The subjects were
146 randomly assigned to either the control group ($n = 9$) or the overload training group ($n = 18$)
147 according to a matched group experimental design based on maximal aerobic power, habitual training
148 volume and years of past experience in endurance sports. All subjects had regularly competed in
149 triathlons for at least three years and were training a minimum of $10 \text{ h}\cdot\text{wk}^{-1}$. The training of each
150 triathlete was monitored for a period of 9 weeks in total, which was divided into a pre-testing phase
151 and then three distinct experimental phases. Both the pre-testing phase and the first phase were the
152 same for for all groups. The pre-testing phase consisted of 3 weeks during which the subjects
153 completed their usual training regime without any study intervention (*i.e.* normal training load). The
154 first experimental phase (Baseline) consisted of one week of moderate training load during which the
155 subjects were asked to reduce their habitual training volume by ~50% while maintaining the training
156 intensity. This mini-taper was selected according to the guidelines for optimal tapering in endurance
157 sports (2). During the second experimental period (overloading phase), the overload group completed
158 a 3-week overload program designed to deliberately overreach the subjects. The duration of each
159 training session of the normal (pre-testing) training period was increased by 30% (*e.g.* a 1 hour run
160 including 8 repetitions of 400 m at the maximal aerobic running speed was converted into a 80 min
161 run including 11 repetitions of 400 m at the maximal aerobic running speed). As particualr subjects
162 were unable to accommodate such prolonged -duration cycling sessions (≥ 5 hours) into their routines,
163 these specific sessions were split (*e.g.* a 5 h cycling session was converted into two sessions of 2h30).
164 The participants reproduced the same training program during each week of the overload period, so
165 that both the content and the weekly distribution of the training sessions remained consistent. The
166 control group repeated its habitual training program during this period. Next, all the participants

167 completed a two-week taper period (third experimental phase, Taper), where their normal training load
168 was decreased by 50% each week (e.g. a 1 hour run including 8 repetitions of 400 m at the maximal
169 aerobic running speed was converted into a ~30 min run including 4 repetitions of 400 m at the
170 maximal aerobic running speed). All training sessions were performed by the triathletes in their own
171 training structure according to the training program established by the same sport scientist.
172 Throughout the entire study, the same sport scientist was responsible for coaching and controlling the
173 training loads of all subjects. To avoid injuries, particular attention was devoted to daily feedback
174 obtained from the triathletes. Before the beginning of the experimental period, the subject reported
175 once to the laboratory to become familiarized with the maximal incremental cycling test (described
176 below) and the daily testing used during the protocol (sleep actigraphy and questionnaires). Testing
177 was performed on 3 occasions (Figure 1), including Pre (ie. after baseline phase), Post (after overload
178 phase) and Taper (after taper phase), respectively. To ensure that performance variations during the
179 maximal incremental cycling tests were due to the training regimen and not to the training session(s)
180 performed the day before each test, the subjects respected a 24 h rest period before each laboratory
181 session.

182

183

184 ***Training monitoring***

185

186 ***Paragraph 7.*** Training volume and intensity were calculated and controlled on the basis of
187 heart rate (HR) measurement (Polar, Kempele, Finland). For all subjects, HR was measured every 5 s
188 during each training session over the entire protocol. The distribution of HR into training zones was
189 subsequently calculated using three HR zones: (1) \leq HR at 2 mmol.L⁻¹, (2) between HR at 2 mmol.L⁻¹
190 and HR at lactate threshold and (3) HR values superior to HR at lactate threshold. Given that the
191 relationship between blood lactate accumulation and HR values at exercise can be influenced by a
192 heavy training load program (17), these reference HR values were reassessed after each maximal
193 incremental cycling test.

194

195 **Laboratory testing**

196 **Paragraph 8.** During the 48 h prior to each maximal incremental cycling test, the subjects
197 received specific nutritional guidelines in order to ensure muscle glycogen stores were replenished.
198 Specifically, they were instructed to eat until satiety was reached during each lunch. Breakfast
199 consisted of a variety of macronutrients from both solid and liquid energy sources. The selected foods
200 included an assortment of cereals, bread, fruit, yogurt, milk, juice, ham and cheese. For lunch and
201 dinner, the subjects consumed a mixed salad as starter, then white meat during lunch and fish during
202 dinner. The side plate consisted of a mixed of 50% carbohydrates (i.e., pasta, rice, noodles) and 50%
203 of vegetables (i.e., green beans, broccoli, tomatoes). One piece of fruit and tub of yogurt were added as
204 dessert, at both lunch and dinner. To ensure the subjects were well-hydrated on each testing day, they
205 were instructed to ensure the maintenance of a well-hydrated state.

206

207 **Paragraph 9. Profile of mood state.** Before exercise testing, subjects were asked to complete
208 the profile of mood state (POMS) questionnaire to assess overall mood disturbance (21). The POMS
209 questionnaire is a 65-item Likert scale questionnaire, which provides measures of six specific mood
210 states: vigor, depression, fatigue, anger, anxiety, and confusion.

211

212 **Paragraph 10. Performance and $\dot{V}O_{2max}$.** Maximum oxygen uptake ($\dot{V}O_{2max}$) was assessed
213 on an electronically braked cycle ergometer (Excalibur Sport, Lode®, Groningen, The Netherlands)
214 equipped with standard 170-mm cranks and the athletes used their own shoes. Positions of the
215 handlebars and seat height were adjusted to the measures used by the athletes on their own bike and
216 replicated between sessions. The test was performed until complete exhaustion to estimate $\dot{V}O_{2max}$
217 and maximal aerobic power. This exercise protocol started with a warm-up of 5 min at a workload of
218 100 W, followed by 5 min at 150 W and 5 min at 200 W. Thereafter, further increments of 25 W were
219 added every 2 min until volitional exhaustion. Subjects wore a face mask covering their mouth and
220 nose for breath collection (Hans Rudolph, Kansas City, MO, USA), and oxygen and carbon dioxide
221 concentration in the expired gas was continuously measured and monitored as breath-by-breath values

222 (Quark, Cosmed®, Rome, Italy). The gas analyzers and the flowmeter of the applied spirometer were
223 calibrated prior to each test.

224

225 **Paragraph 11.** After the test, breath-by-breath values were visually inspected and averaged
226 over 30 s. The highest 30-s average value was used as $\dot{V}O_{2max}$. Performance (W_{max}) was calculated as
227 $W_{max} = W_{compl} + 25 \times (t/120)$; W_{compl} is the last completed workload, and t is the number of
228 seconds in W_{compl} .

229

230 **Paragraph 12. Perceived exertion.** The rating of perceived exertion (RPE) was measured
231 verbally using the Borg 6-20 scale (1) immediately at the end of the maximal incremental cycling test.
232 The subjects completed standard anchoring procedures with the scale at the start of the study and were
233 reminded of its correct use before each maximal incremental cycling test throughout the experiment.

234

235

236 ***Sleep monitoring***

237

238 **Paragraph 13.** All subjects were monitored continuously using an Actiwatch worn on the non-
239 dominant wrist (Cambridge Neurotechnology Ltd. UK), with the epoch length set to 1 minute.
240 Athletes were monitored in the home environment every day at baseline (7 days), during overloading
241 (21 days) and during the taper (14 days) (see Figure 1). Mean behavioural activity over the whole
242 recording period was automatically calculated using the Sleepwatch software (Actiwatch activity and
243 sleep analysis version 5.28, Cambridge Neurotechnology Ltd., UK). Wristwatch Actigraphy is a non-
244 intrusive, cost-effective tool used to estimate sleep quantity and quality which has been compared to
245 polysomnography, showing an accuracy of up to 80% in sleep disordered patients for total sleep time
246 and sleep efficiency (16) and as such is widely used in the sleep literature (24, 34). In a recent review
247 on the role and the validity of actigraphy in sleep medicine, Sadeh concluded that according to most
248 studies, actigraphy has reasonable validity and reliability in normal individuals with relatively good
249 sleep patterns (29).

250

251 **Paragraph 14.** Eloquent sleep-wake scoring can be reliably obtained only with additional
252 information provided in manually completed sleep logs (7). All participants were therefore requested
253 to complete daily sleep diaries. The subjects were asked to record the times of going to bed, falling
254 asleep, waking up, and leaving the bed. Additionally, the subjects were asked to mark the time of
255 switching off the light to sleep and wake-up time with a push of the button on the face of the
256 actiwatch.

257

258 **Paragraph 15.** Individual nights of sleep were analyzed for the following range of variables: time
259 in bed, bedtime, get-up time, sleep latency, actual sleep time, percent time sleeping whilst in bed
260 (sleep efficiency), and sleep restlessness (fragmentation index) and immobile minutes. The following
261 dependent variables were derived from the sleep diary and activity monitor data:

- 262 • Time in bed (h): the amount of time spent in bed attempting to sleep between bedtime and
263 get-up time.
- 264 • Bedtime (hh:mm): the self-reported clock time at which a participant went to bed to attempt
265 to sleep.
- 266 • Get-up time (hh:mm): the self-reported clock time at which a participant got out of bed and
267 stopped attempting to sleep.
- 268 • Sleep onset latency (min): the period of time between bedtime and sleep start.
- 269 • Actual sleep time (h:min): The time asleep from sleep start to sleep end.
- 270 • Sleep efficiency (%): sleep duration expressed as a percentage of time in bed.
- 271 • Fragmentation index: A measure of restlessness during sleep, using the percentage of epochs
272 where activity is > 0 .
- 273 • Immobile time (min): The actual time spent immobile during time in bed.

274

275 **Paragraph 16.** The term ‘sleep quality’ in this investigation is determined by wrist actigraphy by
276 measures of sleep efficiency and fragmentation index, however this is different from ascertaining sleep
277 quality from sleep stages measured by polysomnography.

278 **Paragraph 17.** In order to quantify how the training weeks affected the perceived sleep
279 quality, the participants reported their perceived feelings on a 7-point scale, ranging from ‘very very
280 good’ to ‘very very poor’ after waking up each morning. Additionally, the effect of the training
281 regimen on perceived fatigue?? was recorded each week and? on the morning before the maximal
282 incremental cycling test via a visual 0-100 analog scale (from "no fatigue" to "maximum fatigue").

283

284

285 *Illness symptoms*

286 **Paragraph 18.** During the 6-week experimental period, the subjects were required to complete
287 a health questionnaire (URTI symptoms and gastrointestinal-discomfort symptoms) on a weekly basis,
288 as performed in previous studies (8, 9). They were not required to abstain from medication when they
289 were suffering from illness symptoms, but they were required, on a weekly basis, to report any
290 unprescribed medication taken, visits to the doctor, and any prescribed medications. The illness
291 symptoms listed on the questionnaire were sore throat, inflammation in the throat, runny nose, cough,
292 repetitive sneezing, fever, joint aches and pains and headache. Two usual items of URTI diagnosis (*i.e.*
293 muscle soreness and loss of sleep) were not included given that they could be potentially influenced by
294 training overloading and not necessarily the signs of illness. The numerical ratings of light, moderate
295 and severe (L, M, or S, respectively) were scored as 1, 2, and 3, respectively. In any given week of
296 total symptom score ≥ 12 was taken to indicate that a URTI was present. This score was chosen in
297 previous studies (8, 9) because to achieve it a subject would have to record at least three moderate
298 symptoms lasting for 2 days or two moderate symptoms lasting for at least 3 days in a given week. A
299 single URTI episode was defined as a period during which the weekly total symptom score was ≥ 12
300 and separated by at least 1 week from another week with a total symptom score ≥ 12 . Subjects were
301 also asked to rate the impact of illness symptoms on their ability to train (normal training maintained,
302 training reduced, or training discontinued; L, M, or S, respectively). The gastrointestinal-discomfort

303 symptoms listed on the questionnaire were loss of appetite, stomach upset, vomiting, abdominal pain,
304 and diarrhea. These symptoms were rated and scored the same way as the illness symptoms (8, 9).

305

306

307 **Data analysis**

308

309 *Paragraph 19.* As per the methods of previous research (17), the subjects in the OR group
310 were distributed into two subgroups according to their response to the overload period and during the
311 subsequent taper. The triathletes who demonstrated decreased performance (vs. Pre) and high
312 perceived fatigue ("very tired" to "extremely tired" on the POMS scale) at Post with subsequent
313 performance restoration or supercompensation were diagnosed as functionally overreached (F-OR
314 group). The remaining subjects in the overload group who maintained or increased their performance
315 after the overload period, despite increased perceived fatigue, were considered acutely fatigued (AF,
316 21). Additionally, because extended monitoring reduces the inherent measurement errors in actigraphy
317 and increases reliability (29), subsequent analyses were conducted using the mean value of each sleep
318 parameter over each training phase (*i.e.* baseline, overload, taper).

319

320

321 **Statistical analysis**

322

323 *Paragraph 20.* Normality of data was tested using a Kolmogorov–Smirnov test. Values at
324 baseline for age, weight, height, past experience in endurance sport, MAP and $\dot{V}O_{2\max}$ were compared
325 between groups (*i.e.*, CTL, AF, F-OR) using a one-way analysis of variance (ANOVA). Two-way
326 (group \times time) ANOVAs were used to examine differences in dependent variables (*i.e.*, $\dot{V}O_{2\max}$, RPE,
327 POMS items, perceived sleep quality and actimetry data during sleep) between group means at each
328 time point. When the sphericity assumption in repeated-measures ANOVAs was violated (Mauchly's
329 test), a Geisser–Greenhouse correction was used. If a significant main effect was found, pairwise
330 comparisons were conducted using Duncan's *post hoc* analysis. These statistical tests were conducted

331 using Statistica (Version 7.0, StatSoft, Tulsa, OK) and the data are presented as means and standard
332 deviation (SD).

333

334 *Paragraph 21.* The magnitude of the within-group changes in performance were
335 expressed as standardized mean differences (Cohen's *d*), which were calculated using the pooled
336 standard deviations (3) in modified statistical spreadsheets (12). The magnitude of the percentage
337 change in time was interpreted by using values of 0.3, 0.9, 1.6, 2.5 and 4.0 of the within-athlete
338 variation (coefficient of variation) as thresholds for *small, moderate, large, very large* and *extremely*
339 *large* differences in the change in performance between the trials. The typical variation (CV) for
340 performance during the maximal incremental cycling test was established using the values collected in
341 CTL group during the protocol.

342

343 **RESULTS**

344

345 *Paragraph 22.* Changes in weekly mean training volume, the distribution of the relative
346 training time spent in the intensity zones and the number of training sessions per week in the three
347 groups during each respective training phase are presented in Table 1. The results demonstrated that
348 the three experimental groups successfully adhered to the prescribed training program, and that the
349 respective OR groups increased training volume substantially more than the CTL group ($p < 0.001$).

350

351

352 *Assessment of the OR syndrome.*

353 *Paragraph 23.* At Pre, all subjects reported low perceived fatigue at rest (*i.e.* all subjects
354 responded "not at all" or "a little" on the POMS fatigue item), confirming that they were not already in
355 an OR state. Nine of the 18 overloaded subjects demonstrated a *moderate* to *very large* decrease in
356 performance (-10 ± 4 W) at Post followed by a *small* to *large* performance restoration or
357 supercompensation effect at the end of the Taper (5 ± 5 W, Table 2). This reduced performance was
358 systematically associated with a concomitant high fatigue score at Post (*i.e.* "quite bit" to "extremely"

359 on the POMS fatigue item, Table 2). On the basis of this analysis, these 9 triathletes were considered
360 as "functionally OR", the short term form of OR (F-OR) (23). The 9 other subjects in the overload
361 training group demonstrated higher perceived fatigued but no reduction in performance. According to
362 the nomenclature of Meeusen et al. (21), they were not diagnosed as OR and instead considered
363 acutely fatigued (AF). Thus, the subsequent results are presented for 9 F-OR subjects (F-OR group), 9
364 AF subjects (AF group) and 9 control subjects (CTL group). Mean (\pm SD) age, height and weight were
365 37 ± 6 years, 182 ± 6 cm, 72 ± 9 kg for the CTL group, 35 ± 8 years, 179 ± 9 cm, 73 ± 8 kg for the AF
366 group 35 ± 5 years, 180 ± 5 cm, 72 ± 9 kg for the F-OR group. There were no differences between
367 groups for these descriptive parameters ($P > 0.05$)???

368

369 *Perceived sleep quality and sleep actigraphy data*

370 *Paragraph 24.* Measures of sleep data for the three experimental groups are presented in
371 Tables 3. There was no significant time \times group interaction in perceived sleep quality ($p = 0.11$), time
372 in bed ($p = 0.09$), bedtime ($p = 0.77$), get-up time ($p = 0.42$), sleep latency ($p = 0.33$) and
373 fragmentation index ($p = 0.35$). A significant interaction effect was observed for actual sleep time ($p =$
374 0.006), sleep efficiency ($p = 0.003$) and immobile time ($p = 0.004$). A progressive decrease of these
375 three parameters was systematically observed only in the F-OR group during the overload period
376 compared to baseline (actual sleep time, $p = 0.01$, sleep efficiency, $p = 0.05$; immobile time, $p = 0.005$,
377 during the last week of the overload period). All of these parameters were progressively restored to
378 baseline values during the ensuing taper.

379

380 *Infection-Symptom Incidence*

381 *Paragraph 25.* Analysis of illness questionnaires indicated that 8 subjects reported at least one
382 episode of URTI during the training overload and/or tapering periods. The occurrence of URTI
383 symptoms during the protocol is presented in Table 4. The proportion of subjects who experienced
384 symptoms of infection was higher in the F-OR group ($n = 6$, 67% of total infection cases) than in AF
385 ($n = 2$, 22%) and CTL groups ($n = 1$, 11%). No subjects reported symptoms of gastrointestinal-
386 discomfort during any phase of the training program.

387

388

389 **DISCUSSION**

390 *Paragraph 26.* In the present study, we studied nocturnal actimetry in a group of trained
391 triathletes who completed an overload training program followed by a two week taper period and
392 developed symptoms of F-OR in comparison to control counterparts without signs of training
393 intolerance. The most important finding indicated a progressive decrease in the indices of sleep
394 quality, alongside small reductions in sleep quantity, during the overload period in the F-OR athletes,
395 which was progressively reversed during the subsequent taper. Furthermore, higher prevalence of
396 upper respiratory tract infections was also reported in this F-OR group.

397

398 *Paragraph 27.* Signs of decreased sleep quality in overreached or overtrained endurance
399 athletes have been reported by previous researches. Jurimaä et al. (13) monitored the recovery-stress
400 state in competitive male rowers over a six-day training camp in response to an average increase in
401 training load by approximately 100% compared to average weekly loads. Using the Recovery-Stress-
402 Questionnaire for Athletes (RESTQ-Sport) (15), these authors showed decreased levels of perceived
403 sleep quality, suggesting that recovery may not have been adequate during this training camp, leading
404 to performance impairment and genesis of high perceived fatigue (*i.e.* overreaching). However, given
405 the lack of objective markers of sleep actimetry, the reliance on perception of sleep quality is
406 problematic to then associate sleep disruption with overloading. In a similar vein, Matos et al. (22)
407 recently reported frequent perceived sleep problems was one of the most reported physical symptoms
408 by athletes who had experienced persistent daily fatigue and a significant decrement in performance
409 that lasted for long periods of time. Altogether, these results suggest that heavy load training may exert
410 a negative effect on sleep quality, but to date limited literature provides objectively measured changes
411 in sleep characteristics during periods of confirmed F-OR.

412 *Paragraph 28.* The novelty of the present research provides objective measures of sleep in a
413 group of trained athletes demonstrating differentiated performance responses (*i.e.* acute fatigue vs.
414 functional overreaching, 21) during a 6-week training program involving periodic high training load.

415 Time in bed and sleep latency were not different at any period of the training program in the F-OR
416 group; however, these subjects demonstrated a progressive decrease in actual sleep duration, sleep
417 efficiency and immobile time during the overload period, suggesting substantial sleep disturbances.
418 This finding was reported in at least 7 of the 9 F-OR athletes for each parameter. During the same
419 period, mean sleep values remained unchanged in the control and in the AF groups. To the best of our
420 knowledge, this study is the first to show such alterations in objective markers of sleep quality during
421 a period of intensified training that resulted in overreaching. Whilst causation is not inferred, it is
422 possible the sleep disturbance may have been related to mild muscle fatigue or soreness resulting from
423 the high training loads. Certainly given neither bed time, sleep latency or time spent in bed were
424 altered, the reduction in sleep duration may result from the lowered efficiency due to difficulty in
425 remaining immobile during sleep. However, despite such measured sleep disruption, subjective quality
426 of sleep remained unaltered; suggesting some disconnect in actual and perceived measures of sleep.
427 Indeed, despite 8 of the F-OR athletes reporting reduced scores for perceived sleep quality, the
428 magnitude in change was insufficient to reach statistical significance.

429

430 **Paragraph 29.** Although the results suggest that F-OR athletes demonstrated a modest
431 decrease in quality and quantity of sleep during the overload period, it remained considerably better
432 than that experienced by sleep disorder patients (30), extreme sleep deprivation (32) or by athletes in
433 response to jet lag (20) or hypoxic exposure (27). Additionally, Halson et al. (10) reported larger sleep
434 deficiency during the period leading to overtraining (< 6 hours per night) in a talented female sprint
435 cyclist who developed signs of overtraining (*i.e.* persistent feeling fatigued and underperforming over
436 months). The actual sleep time in the F-OR subjects during the overload training period remained
437 higher than the values reported by Leeder et al. (19) in a cohort of elite athletes under normal training
438 conditions. Nevertheless, we cannot exclude that the moderate changes observed in the F-OR during
439 the present experiment would not unduly affect performance in elite athletes, where very small
440 differences in performance can have large impact on the competition issue. It remains also unclear
441 during the present study whether sleep disturbance was an etiological mechanism of overreaching, or
442 simply just a symptom. Regardless, it is acknowledged that the reduction in sleep duration during F-

443 OR was small in the spectrum of sleep disturbances, and may suggest that sleep monitoring to detect
444 F-OR may require extended periods of data collection. Further research is required to determine the
445 relationship of sleep with training tolerance and adaptation, especially in athletes developing training
446 maladaptations (*i.e.* overreaching, overtraining).

447

448 **Paragraph 30.** Given the purported relationship between prolonged reductions in sleep quality
449 and quantity with increased risk of illness, it was interesting to observe a higher infection rate in the F-
450 OR subjects during the present experiment. Of the 9 F-OR athletes, 5 reported increased URTI
451 symptoms during the overload period and concurrent to the noted sleep disturbances, while only two
452 cases were observed in the AF and CTL groups during the same period. Interestingly, this illness
453 prevalence was the highest during the last week of the overload period, which is temporally aligned to
454 when sleep disturbances reached their highest magnitude during the study, perhaps implying an
455 accumulative effect. The observed decrease in sleep and increase in URTI during F-OR is in
456 accordance with several previous studies reporting both innate and adaptive immunity are depressed
457 during sustained periods of heavy training (38). This association suggests there is a link between the
458 recuperative processes of sleep and the immune system (28). The current study provides supporting
459 evidence that high volume training periods may result in increased URTI, alongside reduced sleep
460 quality. However, again whether sleep disturbance was an etiological mechanism of URTI
461 development as a result of overreaching, or simply coincidental symptoms remains to be elucidated,
462 particularly given the relatively small reductions in sleep durations reported.

463

464 **Paragraph 31.** In conclusion, F-OR athletes showed objective signs of moderate sleep
465 disturbances and higher prevalence of infections in the present study. These results were in contrast
466 with control counterparts, who did not demonstrate any symptoms of training intolerance during the
467 protocol. Whether poor sleep was a consequence of increased training causing the development of
468 overreaching, or whether sleep disturbances were simply symptoms of OR remain unclear. Whatever
469 the causative link between F-OR and sleep, we suggest that endurance athletes should be encouraged
470 to ensure ideal sleeping environment (quiet, cool, and dark) and to implement short post-lunchtime

471 naps, when they are exposed to high training load (6). The ability to nap for short periods during the
472 day may be a useful skill for athletes to compensate the potential decline in sleep quality associated
473 with development of F-OR. Further investigations are required to investigate the importance of sleep
474 and its relationship with overreaching.

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478 **ACKNOWLEDGMENTS**

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481 This study was made possible by technical support from the French Federation of Triathlon. The
482 authors are especially grateful to Frank Bignet and Benjamin Maze for their help and cooperation. We
483 received funding for research on which this article is based from the French Ministry of Sport and the
484 French National Institute of Sport, Expertise and Performance (Paris). The authors report no conflict
485 of interest.

486

487 The authors report no conflict of interest. The results of the present study do not constitute
488 endorsement by the American College of Sports Medicine.

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FIGURE 1. Schematic representation of the experimental protocol. Bicycle symbols represent maximal incremental cycling tests. Note that 9 subjects of the overload group developed symptoms of functional overreaching at Post (decreased performance vs. Pre associated with high perceived fatigue, F-OR group). The nine other overloaded subjects were only acutely fatigued (AF group).

FIGURE 2. Change in mean weekly sleep parameters from baseline values during the overload and taper phase in the three experimental groups. CTL: control; AF: acute fatigue; F-OR: functionally overreached. Gray areas and dashed lines represent 1xCV and 2xCV of the considered parameter during the 6-week protocol in the control group. *, significantly different from Baseline at $P < 0.05$; †, significantly different from the third week of the Overload period at $P < 0.05$.