

1 **Morning priming exercise strategy to enhance afternoon performance in young elite soccer**
2 **players**

3

4 Submission Type: **Original Investigations**

5 Preferred running head: **Priming morning exercises in soccer's match day**

6

7 **Authors:**

8 Federico Donghi¹, Ermanno Rampinini¹, Andrea Bosio¹, Maurizio Fanchini², Domenico
9 Carlomagno¹, Nicola A. Maffiuletti³

10

11 **Affiliations:**

12 1, Human Performance Laboratory, MAPEI Sport Research Centre, Olgiate Olona, Varese, Italy.

13 2, US Sassuolo Calcio, Sassuolo, Italy.

14 3, Human Performance Lab, Schulthess Clinic, Zurich, Switzerland.

15

16 Corresponding author: Federico Donghi

17 Human Performance Laboratory, MAPEI Sport Research Centre, Olgiate Olona, Varese, Italy

18 federico.donghi@mapeisport.it

19 Telephone: +39 0331 575757

20

21

22 Abstract word count: **250 words**

23 Manuscript word count: **3695 words**

24 Number of figures and tables: 3 figures and 2 tables

25 **Abstract**

26 **Purpose:** To compare the effects of different modalities of morning (AM) priming exercise on
27 afternoon (PM) physical performance with the associated hormonal and psychophysiological
28 responses in young soccer players.

29 **Methods:** In a randomized counterbalanced crossover-design, twelve young soccer players
30 completed three different AM conditions on three different days: repeated sprint running (RSR,
31 6x40-m), easy exercise (EASY, 4x12 fast half squats, 6 speed ladder drills, 20-m sprints) and
32 control (CON, no exercise). Blood testosterone and cortisol concentrations were assessed upon
33 arrival (~08:30) and ~5h 30min later. Body temperature, self-reported mood, quadriceps
34 neuromuscular function (maximal voluntary contraction, voluntary activation, rate of torque
35 development and twitch contractile properties), jump and sprint performance were evaluated twice a
36 day, while rate of perceived exertion, motivation and the Yo-Yo intermittent recovery Level 2 test
37 (Yo-Yo IR2) were assessed once in a day.

38 **Results:** Compared to CON, RSR induced a possible positive effect on testosterone (+11.6%) but a
39 possible to very likely negative effect on twitch contractile properties (-13.0%), jump height (-
40 1.4%) and Yo-Yo IR2 (-7.1%). On the other hand, EASY had an unclear effect on testosterone (-
41 3.3%), resulted in lower self-reported fatigue (-31.0%) and cortisol (-12.9%) together with possible
42 positive effect on rate of torque development (+4.3%) and Yo-Yo IR2 (+6.5%) compared to CON.

43 **Conclusions:** Players' testosterone levels were positively influenced by RSR, but this did not
44 translate into better physical function as both muscular and endurance performance were reduced.
45 EASY seemed to be suitable to optimize physical performance and psychophysiological state in
46 young soccer players.

47 **Key words:** priming exercise, delayed potentiation, soccer match day

48 **Introduction**

49 Soccer's match days anecdotally include light activities in the morning when the kick off
50 occurs in the late afternoon or evening. Indeed, due to the reluctance of coaches and athletes to
51 perform high-intensity exercise on a game day light physical and technical-tactical exercises are
52 usually preferred to avoid fatigue¹. Despite this common practice, there seems to be lack of
53 evidence regarding its usefulness and benefits on physical performance during the match, which is
54 characterized by a multitude of energy-demanding events (high-intensity running, accelerations,
55 decelerations, sprints, tackles, changes of direction and jumps) interspersed with less intense
56 actions².

57 The effect of these so-called priming activities performed some hours before performance
58 has already been investigated in several sports, with contradictory results regarding effectiveness
59 and suitable exercise type^{1,3-8}. Semi-professional rugby players had better sprint, jump and strength
60 performance 6 hours after heavy strength training exercises conducted in the morning⁴.
61 Furthermore, a study on elite rugby players reported that repeated sprint running (RSR: 6 x 40 m
62 with 20 s of recovery) was an effective priming strategy to improve afternoon sprint performance⁵.
63 In contrast, young elite rugby sevens players showed no improvement in sprint ability 2 hours after
64 a 30-min session consisting of accelerations, small-sided games and 2 x 50 m maximal sprints⁷.
65 Similarly, amateur soccer players who completed small-sided games and repeated sprints at
66 different intensities in the morning showed no differences in a football-specific endurance test
67 performed in the afternoon⁸. However, to our knowledge, no studies have examined the effect of
68 morning activity on afternoon performance in young elite soccer players.

69 In these previous studies, performance enhancements following high-intensity morning
70 exercise were mainly ascribed to an attenuated circadian decline of testosterone^{1,4,5}. The level of
71 testosterone in the blood normally displays a circadian profile with an early morning peak and a
72 subsequent decrease during the day⁹. Testosterone has a substantial influence on skeletal muscle
73 contractile function¹⁰ and motor cortex output facilitation¹¹, but also on mental aspects such as
74 motivation¹² and confidence to compete⁶. As a result, the level of testosterone was found to be
75 related with different expressions of strength and power and also with physical performance^{1,6}.
76 However, factors other than testosterone concentration could also be responsible for the
77 performance improvements detected in the afternoon following morning priming exercise⁵, and thus
78 strict monitoring of psycho-physiological variables such as mood, motivation, body temperature⁶,
79 cortisol levels⁹ and neuromuscular function⁹ is warranted.

80 The purpose of this study was to investigate the priming effect induced by different exercise
81 protocols in young elite soccer players. More specifically, we aimed to determine the type of
82 exercise that could best fit the need of the pre-match period, while also investigating the psycho-
83 physiological mechanisms potentially underlying the priming effect. Beside the RSR protocol⁵,
84 **which has already been shown to be effective in other team sports but somehow** difficult to
85 propose in a real soccer pre-game scenario, we investigated the effect of an easy (EASY) exercise
86 protocol that is mainly focused on explosive strength/power with no/light loads and minimal
87 equipment. Anecdotally, EASY exercise is already used by some strength and conditioning coaches
88 in the pre-game morning, as it is believed to promote the priming effect with no resulting fatigue,
89 **however this speculation still needs to be confirmed with controlled research studies.**
90 Considering its applicability and the absence of information on its effects, we included this
91 condition in the present research and we hypothesized that EASY exercise performed in the
92 morning, **besides being less demanding than RSR**, could positively affect afternoon performance
93 in young elite soccer players.

94

95 **Methods**

96 *Design*

97 A randomized, counterbalanced crossover design was implemented for this study, which
98 was conducted immediately after the end of the first half of the season. Familiarization with all the
99 test procedures took place before the beginning of data collection. Players completed three different
100 morning protocols (RSR, EASY and control [CON]) on three different days separated by at least 48
101 h of recovery. The sequence of the exercise was established a priori using a randomization plan
102 (<http://www.randomization.com>). Participants underwent the same test battery to assess hormone
103 levels, body temperature, mood, quadriceps neuromuscular function, jump and sprint performance
104 both in the morning and in the afternoon of each day, while rate of perceived exertion (RPE),
105 motivation and endurance performance were assessed only once a day (figure 1). All the
106 evaluations were conducted in the same indoor gym at the same time of the day to minimize
107 possible circadian-related effects. Moreover, the same controlled dietary intake was adopted during
108 the days of the tests and subjects were asked to avoid physical activity and caffeine intake in the 24
109 h preceding each condition and during data collection.

110

111 *****Figure 1*****

112

113 *Subjects*

114 Twelve young male soccer players (age 17 ± 1 years, height: 1.78 ± 0.06 m, mass: 69 ± 4 kg,
115 playing position: 3 defenders, 4 midfielders, 5 strikers) from an Italian Serie A club participated in
116 this study. All of them had at least 6 years of experience in playing soccer at competitive level.
117 They were used to train five times and to play one match per week during the competitive season.
118 Before starting data collection, players were asked to complete the questionnaire of Horne and
119 Östberg Self-Assessment version¹³ to assess if their chronotypes were “intermediate” (n=10),
120 “moderate morning” (n=1) or “moderate evening” (n=1). The players or the parents of the underage
121 players provided written informed consent before participating in the study. The study was
122 approved by the Independent Institutional Review Board of Mapei Sport Research Centre according
123 to the Declaration of Helsinki. All the subjects were free from injury and illness at the time of the
124 study.

125

126 *Exercise conditions*

127 In the RSR condition, players performed 6 all-out shuttle sprints over a distance of 20+20 m
128 with a 180° change of direction and 20 s of recovery between each sprint^{5,14}. In the EASY
129 condition, players completed 4x12 fast half squats with an overload of 10 kg (weight disc plate,
130 Technogym, Italy), performed as fast as possible with 20 s of recovery between each set.
131 Immediately after, they performed 6x5 m speed ladder drills followed by all-out shuttle sprints over
132 a distance of 10+10 m with a 180° change of direction and 20 s of recovery. No morning exercise
133 was undertaken in the CON condition, and players were instructed to avoid physical activity after
134 the tests conducted in the morning.

135

136 *Measurements*

137 *Hormone levels and body temperature*

138 Blood testosterone and cortisol concentrations were assessed upon arrival. Blood collection
139 and sample management was carried out following laboratory practice for the pre-analytical phase
140 of sports biochemistry and hematology tests¹⁵. Blood was drawn from athletes via venipuncture in

141 the antecubital vein, early in the morning just after wake-up (~08:30 AM) and later in the afternoon.
142 Samples were collected into plastic 8-mL serum gel tubes (Vacutest Kima, Arzegrande, Padua,
143 Italy) that were allowed to clot for 30 min, and then spun at 3000 g for 10 min at room temperature.
144 Within 2 hours, serum was aliquoted into 1.5 mL plastic cryogenic tubes and frozen at -20° C for no
145 more than 3 months, until analysis. After thawing, all samples were assayed in the same analytical
146 run on the Centaur XP automated analyzer (Siemens Healthineers AG, Munich, Germany) using the
147 dedicated chemiluminescence immunoassay kits. Siemens Centaur cortisol and testosterone assays
148 are competitive immunoassays that use direct chemiluminescent technology (coefficient of variation
149 were 4.0% for cortisol and 4.7% for testosterone).

150 Body temperature was assessed with an infrared aural thermometer (Braun Thermoscan IRT
151 4520; Braun, South Boston, MA)¹⁶. Three measurements per side were conducted and the mean
152 temperature of the six measurements was retained.

153

154 *Quadriceps neuromuscular function*

155 Quadriceps neuromuscular function (right side) was evaluated in isometric conditions with
156 the subject comfortably seated on the chair of a custom-developed dynamometer. The dynamometer
157 consists of a modified leg extension machine equipped with a load cell (range: 0-500 daN,
158 sensitivity: 2.008 mV/V; S500, Studio A.I.P., Varese, Italy) connected to a data acquisition system
159 (BIOPAC MP 100; BIOPAC System, Inc., Santa Barbara, CA, USA). The force signal was
160 amplified, filtered, converted to torque using the lever arm length (0.4 m) and subsequently stored
161 into a personal computer (sampling rate: 1000 Hz) using a dedicated software. The knee joint was
162 positioned at 90° of flexion and the trunk-thigh angle was approximately 100°. The lever arm of the
163 dynamometer was strapped to the leg above the lateral malleolus via Velcro® straps.

164 The assessments consisted in a series of voluntary and/or electrically-stimulated contractions
165 of the knee extensor muscles for the quantification of maximal voluntary contraction (MVC) torque,
166 voluntary activation (VA), voluntary rate of torque development (RTD) and doublet contractile
167 properties. Subjects were first familiarized with electrical stimulation procedures and supramaximal
168 current intensity was carefully and individually determined using a common procedure¹⁷.
169 Transcutaneous electrical stimuli (pulse duration: 1 ms) were delivered using a constant-current
170 stimulator (Digitimer DS7AH, Hertfordshire, UK) connected to two self-adhesive electrodes
171 (femoral triangle-gluteal fold configuration). Players completed a standardized warm up consisting
172 of three voluntary contractions of 5 s at 25, 50 and 75% of their estimated MVC torque. They were

173 then asked to perform two 5-s MVC separated by 2-min rest periods, without any concern for the
174 rate of torque development. Supramaximal paired stimuli were manually delivered 2-3 s after the
175 onset of the contraction to evoke a superimposed doublet response, and then at rest, 1-2 s after each
176 MVC, to evoke a potentiated doublet response¹⁸. MVC torque was defined as the highest torque
177 attained either before or after the superimposed response. The peak torque (PT) associated to the
178 superimposed and resting doublet responses were used to estimate VA according to the formula of
179 Allen et al.¹⁹. We also quantified the following contractile properties from the resting doublet
180 response: doublet PT, RTD and rate of torque relaxation (RTR). RTD and RTR were calculated as
181 the highest slope of the torque-time curve during the contraction and relaxation phase, respectively.
182 For all the variables, the best of the two trials was retained. Finally, voluntary RTD was quantified
183 according to recent guidelines²⁰. Briefly, subjects were asked to perform five “as fast and hard as
184 possible” short contractions (~1 s) separated by ~30 s, with strong verbal encouragement. Any
185 contraction not attaining at least 80% of the MVC torque or with an evident countermovement was
186 discarded²⁰. The average RTD of the three best contractions was retained.

187

188 *Rate of perceived exertion, self-reported mood and motivation*

189 The RPE was assessed with the Borg’s CR10 scale at the end of the morning sessions, as an
190 indicator of overall load²¹. The Brunel Mood Scale (BRUMS)²² was used to assess mood both in the
191 morning and in the afternoon before the beginning of the assessments and after the blood samples.
192 Even if the BRUMS contains six subscales (anger, confusion, depression, fatigue, tension and
193 vigor), with four items per subscale, in the present study we only considered the fatigue and vigor
194 subscales. Items were answered on a 5-point Likert-type scale (0: not at all, 1: a little, 2:
195 moderately, 3: quite a bit, 4: extremely). Motivation was measured only in the afternoon
196 immediately after the BRUMS, using the validated scales for intrinsic and extrinsic motivation²³.
197 Each subscale consists of seven items on a 5-point Likert-type scale with the same anchor described
198 for the BRUMS. At the end of the last day of data collection, players were also asked to indicate
199 their preferred protocol (EASY, RSR, CON). More specifically, they were asked to choose the
200 condition that allowed them to achieve the best performance in the tests of the afternoon, according
201 to a subjective and general feeling.

202

203 *Jump and sprint performance*

204 After a standardized ~5-min warm-up consisting of low-intensity running, dynamic mobility
205 drills and three countermovement jumps (CMJ) while keeping arms akimbo, the players performed
206 six maximal CMJ arms akimbo separated by ~30 s on a force platform (sampling rate: 500 Hz,
207 Type 2822A1-1, Kistler, Winterthur, Switzerland). The mean of the three best values of jump height
208 and peak power output (PPO) were retained.

209 After 2 sub-maximal trials as a warm-up, the players completed 2 all-out shuttle sprints of
210 20 + 20 m interspersed with 2 min of rest². Running time was measured with a photocells system
211 (Polifemo, Microgate, Bolzano, Italy) with the best sprint time considered as the main outcome. The
212 players were asked to adopt the split standing starting technique.

213

214 *Endurance performance*

215 During the afternoon, players performed the Yo-Yo Intermittent Recovery Test level 2 (Yo-
216 Yo IR2)²⁴. This test consists of repeated 20-m shuttle runs at a progressively increasing speed
217 controlled by an audio track, with 10 s of recovery between each shuttle run. When the participant
218 failed to reach the finishing line twice, the distance covered was recorded. Heart rate data were also
219 collected throughout the test (Polar Team², Kempele, Finland), and the maximal heart rate was
220 retained.

221

222 *Statistical analysis*

223 Descriptive results are reported as means \pm standard deviations (SD). Data analysis was
224 conducted using the magnitude-based decision (MBD) method with the Hopkins' spreadsheet²⁵. All
225 data were first log-transformed to reduce bias due to non-uniformity error²⁵. Post-only crossover
226 and pre-post crossover were the two Excel spreadsheets used to run the statistical analyses²⁶. The
227 first spreadsheet was used to compare the data obtained both in the morning and in the afternoon
228 within every experimental condition and the data collected only once in every experimental
229 condition, while the second spreadsheet was used to compare the differences in the changes
230 between pairs of conditions. Practical significance of changes was also assessed by calculating the
231 Cohen's d effect size (ES)²⁷. The following threshold values were considered for ES: 0-0.2 trivial,
232 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large, >2.0 very large. The MBD analyses were conducted
233 using the smallest worthwhile change (SWC), which was obtained by multiplying the between-
234 subjects SD by 0.2. The qualitative probabilistic terms were assigned using the following scale:

235 <0.5%, *almost certainly not*; <5%, *very unlikely*; <25%, *unlikely*; 25-75%, *possibly*; >75%, *likely*;
236 >95, *very likely*; >99.5%, *almost certainly*. The magnitude was considered *unclear* if the confidence
237 intervals overlapped the positive and negative thresholds^{25,26}.

238

239 **Results**

240 *Hormone levels and body temperature*

241 Within-condition variations are reported in Table 1 while standardized differences in the
242 changes between conditions are shown in Figure 2. In all the conditions, a substantial reduction in
243 hormones concentrations was observed from morning to afternoon. The within-day decrease in
244 blood testosterone concentration was *possibly* attenuated in RSR compared to CON and *likely*
245 attenuated in RSR compared to EASY. On the other hand, blood cortisol concentration was *likely*
246 more reduced in EASY compared to CON. Body temperature naturally increased during the day
247 with no clear differences between the three conditions.

248

249 *****Table 1 *****

250

251 *Quadriceps neuromuscular function*

252 Within-condition variations and standardized differences in the changes between conditions
253 are reported in Table 1 and Figure 2, respectively. Neither MVC torque nor VA showed any
254 differences within and between conditions. Voluntary RTD was *possibly* improved after EASY and
255 *possibly* decreased after RSR. Voluntary RTD changes were *likely* negative when comparing RSR
256 with EASY. Doublet PT was *possibly* improved after CON, while it remained stable following RSR
257 and EASY. Moreover, CON had a *possibly* positive effect on doublet RTD, whereas it remained
258 stable after EASY and was *likely* negatively reduced following RSR. As a consequence, doublet
259 RTD changes were, *likely* compared to EASY and *very likely* compared to CON, negatively reduced
260 after RSR. No substantial changes were detected for doublet RTR within different conditions and
261 only a *possibly* negative effect was identified after RSR compared to CON.

262

263 *****Figure 2 *****

264

265 *Rate of perceived exertion, self-reported mood and motivation*

266 The RPEs at the end of the morning sessions were *almost certain* different in the two
267 exercise conditions (RSR: 5.03 ± 1.24 ; EASY: 4.29 ± 0.69) compared to CON (2.92 ± 0.79) and
268 RPE was also *likely* higher after RSR compared to EASY.

269 When comparing afternoon to morning BRUMS data, the level of fatigue was *likely* lower
270 only following EASY, while vigor was *possibly* and *likely* lower after CON and EASY, respectively
271 (table 1). Task-dependent motivation was *likely* lower after RSR compared to CON and EASY,
272 while task-independent motivation was *possibly* higher only following EASY compared to CON
273 (table 2). Most of the players (n=9) subjectively rated EASY as the condition they preferred (n=2
274 for RSR and n=1 for CON).

275

276 *****Table 2 *****

277

278 *Jump, sprint and endurance performance*

279 Jump height and PPO were *possibly to likely* increased following CON and EASY (table 1).
280 However, differences between conditions were trivial to small (figure 3). Sprint performance was
281 *possibly to likely* improved in all the experimental conditions (table 1) with trivial differences
282 between pairs of conditions (Figure 3).

283 A *possibly* longer distance was reached in Yo-Yo IR2 following EASY compared to CON
284 with a *possibly* higher maximal heart rate recorded during the test (table 2). Furthermore, the Yo-Yo
285 IR2 distance after RSR was *possibly* lower compared to CON and *very likely* lower compared to
286 EASY (table 2).

287

288 *****Figure 3 *****

289

290 **Discussion**

291 The main findings of the present study were that RSR exercise performed in the morning
292 had a somehow detrimental effect on afternoon physical performance in young elite soccer players
293 while EASY priming exercise seemed to positively influence afternoon performance.

294 RSR priming exercise was previously found to attenuate the circadian testosterone decline,
295 which typically occurs in the afternoon⁵. However, the effect in the present group of young soccer
296 players was less notable compared to this previous study⁵ (testosterone decline was ~31% vs. ~12%
297 in our study). This can be partially explained by inter-study differences in mean age and training
298 level of the participants⁵. In fact, testosterone levels have already been found to be better related
299 with performance in subjects with relatively high strength levels²⁸. Even if maximal voluntary
300 strength of the knee extensor muscles was not affected in our present study, RSR negatively
301 impacted voluntary RTD (i.e., explosive strength) and led to some signs of peripheral fatigue (as
302 doublet RTD was impaired), at least for the quadriceps muscle. Furthermore, it is possible that
303 neuromuscular fatigue of the knee extensors caused by RSR has prevailed over the positive effects
304 resulting from the attenuated reduction in testosterone levels. However, it is difficult to discuss
305 these findings in relation with other studies because no neuromuscular evaluations were previously
306 conducted.

307 Although rugby players showed an increase in vertical jump height (~4%) following RSR⁵, we did
308 not observe the same result in our group of young soccer players, perhaps due to fatigue.
309 Conversely, the lack of decrement in sprint performance following RSR could be ascribed to the
310 specificity of the task, since priming activity has been suggested to be specific to the characteristics
311 of the subsequent performance^{1,3,4}. In fact, RSR negatively impacted vertical jump and intermittent
312 running endurance (Yo-Yo IR2) performance but not the sprinting task. The shorter Yo-Yo IR2
313 distance detected following RSR was likely due to neuromuscular fatigue of the knee extensors²⁹
314 and/or low motivational levels¹².

315 The longer Yo-Yo IR2 distance covered in the EASY condition might be ascribed to higher
316 motivational levels, reduced cortisol levels (likely indicating lower stress levels⁹) and enhanced
317 quadriceps contractile properties (as witnessed by doublet RTD results). All together these changes,
318 accompanied by the absence of neuromuscular fatigue of the knee extensors, may have contributed
319 to a better muscular and overall attitude to perform. **As suggested by the differences in RPEs
320 between the conditions, the effort associated to EASY could not have not been intense enough
321 to elicit an effect on testosterone, whose levels have already been shown to increase following
322 high-intensity exercise¹.** It is therefore remarkable that, subjectively, players preferred the EASY
323 exercise modality, suggesting a potential and powerful placebo effect affecting afternoon physical

324 activity. Taken as a whole, the results of our current study suggest that an exercise protocol like the
325 EASY, performed in the morning, could be a good strategy to optimize afternoon performance in
326 young elite soccer players, as it resulted in better overall outcomes than no exercise. On the other
327 hand, RSR exercise seemed to be too demanding to promote specific performance enhancements in
328 the afternoon.

329 The present study has some limitations that merit consideration. In this research,
330 **testosterone was not measured through saliva samples, which have already been shown to be**
331 **more sensitive to exercise, but with the use of blood samples³⁰. The different measurement**
332 **techniques (saliva vs. blood) and forms of testosterone (free vs. total) could well have**
333 **produced different testosterone variations following the same exercise protocol (RSR) in the**
334 **different studies⁵**. However, because total and free testosterone variations have been found to be
335 similar following sprint exercise, similar results might have been expected with these two
336 measurements³¹. The performance in the Yo-Yo IR2 test is mainly related to the peak distance of
337 high intensity running in a 5-min period during the match²⁴. As a consequence, this endurance
338 performance index could not reflect the actual overall demands of a 90-min football match.
339 Moreover, training sessions and official competitions have different psychological demands.
340 Additionally, the daily schedule selected for this research was similar to previous investigations³⁻⁵
341 as it could well represent the real scenario of the soccer match day, but we acknowledge that
342 different time intervals could bring to different results. Finally, the psychological outcomes of the
343 present study should be carefully considered when preparing a real competition because aspects
344 such as self-reported fatigue, vigor and motivation may be different on the day of an official match.

345

346 **Practical Implications**

347 In young elite soccer players, priming exercise performed in the morning could represent a
348 strategy to optimize afternoon performance. In particular, an exercise combination like EASY
349 seems to be effective to enhance physical performance and also players' motivation while avoiding
350 the occurrence of neuromuscular fatigue. It appears particularly important to avoid excessively
351 demanding exercise sessions (such as RSR) on a day game to prevent the occurrence of
352 neuromuscular fatigue and a concomitant impact on self-reported motivation. Further studies should
353 determine whether these results can be extended to other cohorts of soccer players, including
354 professional and female players.

355

356 **Conclusions**

357 We investigated the effects of different types of morning exercises on afternoon
358 performance in young elite soccer players. With a time interval of ~5 h 30 min between morning
359 priming activity and afternoon performance evaluation, the exercise combination that best improved
360 both physical and psychological variables was EASY, since it was less demanding and subjectively
361 better accepted by young soccer players than RSR.

362

363 **Acknowledgments**

364 This study was founded by Fondazione Giuseppina Mai (Confindustria) and Mapei S.p.A.

365

- 367 1. Harrison PW, James LP, McGuigan MR, Jenkins DG, Kelly VG. Resistance Priming to
368 Enhance Neuromuscular Performance in Sport: Evidence, Potential Mechanisms and
369 Directions for Future Research. *Sports Medicine*. 2019.
- 370 2. Rampinini E, Bosio A, Ferraresi I, Petruolo A, Morelli A, Sassi A. Match-related fatigue in
371 soccer players. *Med Sci Sports Exerc*. 2011;43(11):2161-2170.
- 372 3. Ekstrand LG, Battaglini CL, McMurray RG, Shields EW. Assessing explosive power
373 production using the backward overhead shot throw and the effects of morning resistance
374 exercise on afternoon performance. *J Strength Cond Res*. 2013;27(1):101-106.
- 375 4. Cook CJ, Kilduff LP, Crewther BT, Beaven M, West DJ. Morning based strength training
376 improves afternoon physical performance in rugby union players. *J Sci Med Sport*.
377 2014;17(3):317-321.
- 378 5. Russell M, King A, Bracken RM, Cook CJ, Giroud T, Kilduff LP. A Comparison of
379 Different Modes of Morning Priming Exercise on Afternoon Performance. *Int J Sports
380 Physiol Perform*. 2016;11(6):763-767.
- 381 6. Kilduff LP, Finn CV, Baker JS, Cook CJ, West DJ. Preconditioning strategies to enhance
382 physical performance on the day of competition. *Int J Sports Physiol Perform*.
383 2013;8(6):677-681.
- 384 7. Marrier B, Durguerian A, Robineau J, et al. Preconditioning Strategy in Rugby-7s Players:
385 Beneficial or Detrimental? *Int J Sports Physiol Perform*. 2019;14(7):918-926.
- 386 8. Oh SH, Mierau A, Thevis M, Thomas A, Schneider C, Ferrauti A. Effects of different
387 exercise intensities in the morning on football performance components in the afternoon.
388 *German Journal of Exercise and Sport Research*. 2018;48(2):235-244.
- 389 9. Teo W, Newton MJ, McGuigan MR. Circadian rhythms in exercise performance:
390 implications for hormonal and muscular adaptation. *J Sports Sci Med*. 2011;10(4):600-606.
- 391 10. Curl CL, Delbridge LM, Canny BJ, Wendt IR. Testosterone modulates cardiomyocyte
392 Ca(2+) handling and contractile function. *Physiol Res*. 2009;58(2):293-297.
- 393 11. Bonifazi M, Ginanneschi F, della Volpe R, Rossi A. Effects of gonadal steroids on the
394 input-output relationship of the corticospinal pathway in humans. *Brain Res*.
395 2004;1011(2):187-194.
- 396 12. Crewther BT, Carruthers J, Kilduff LP, Sanctuary CE, Cook CJ. Temporal associations
397 between individual changes in hormones, training motivation and physical performance in
398 elite and non-elite trained men. *Biol Sport*. 2016;33(3):215-221.
- 399 13. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-
400 eveningness in human circadian rhythms. *Int J Chronobiol*. 1976;4(2):97-110.
- 401 14. Rampinini E, Bishop D, Marcora SM, Ferrari Bravo D, Sassi R, Impellizzeri FM. Validity
402 of simple field tests as indicators of match-related physical performance in top-level
403 professional soccer players. *Int J Sports Med*. 2007;28(3):228-235.
- 404 15. Banfi G, Dolci A. Preanalytical phase of sport biochemistry and haematology. *J Sports Med
405 Phys Fitness*. 2003;43(2):223-230.
- 406 16. Purssell E, While A, Coomber B. Tympanic thermometry--normal temperature and
407 reliability. *Paediatr Nurs*. 2009;21(6):40-43.
- 408 17. Maffiuletti NA, Barbero M, Cescon C, et al. Validity of the twitch interpolation technique
409 for the assessment of quadriceps neuromuscular asymmetries. *J Electromyogr Kinesiol*.
410 2016;28:31-36.
- 411 18. Place N, Maffiuletti NA, Martin A, Lepers R. Assessment of the reliability of central and
412 peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle.
413 *Muscle Nerve*. 2007;35(4):486-495.
- 414 19. Allen GM, Gandevia SC, McKenzie DK. Reliability of measurements of muscle strength
415 and voluntary activation using twitch interpolation. *Muscle Nerve*. 1995;18(6):593-600.

- 416 20. Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force
417 development: physiological and methodological considerations. *Eur J Appl Physiol.*
418 2016;116(6):1091-1116.
- 419 21. Impellizzeri FM, Rampinini E, Coutts AJ, Sassi A, Marcora SM. Use of RPE-based training
420 load in soccer. *Med Sci Sports Exerc.* 2004;36(6):1042-1047.
- 421 22. Terry PC, Lane AM, Lane HJ, Keohane L. Development and validation of a mood measure
422 for adolescents. *J Sports Sci.* 1999;17(11):861-872.
- 423 23. Matthews G, Campbell SE, Falconer S. Assessment of Motivational States in Performance
424 Environments. *Proceedings of the Human Factors and Ergonomics Society Annual Meeting.*
425 2001;45(13):906-910.
- 426 24. Krustrup P, Mohr M, Nybo L, Jensen JM, Nielsen JJ, Bangsbo J. The Yo-Yo IR2 test:
427 physiological response, reliability, and application to elite soccer. *Med Sci Sports Exerc.*
428 2006;38(9):1666-1673.
- 429 25. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in
430 sports medicine and exercise science. *Med Sci Sports Exerc.* 2009;41(1):3-13.
- 431 26. Hopkins WG. Spreadsheets for analysis of controlled trials, with adjustment for a subject
432 characteristic. *Sportscience.* 2006;10(1):46-50.
- 433 27. Cohen, J. Statistical power analysis for the behavioral sciences. In: L. Erlbaum Associates;
434 1988.
- 435 28. Crewther BT, Cook CJ, Gaviglio CM, Kilduff LP, Drawer S. Baseline strength can
436 influence the ability of salivary free testosterone to predict squat and sprinting performance.
437 *J Strength Cond Res.* 2012;26(1):261-268.
- 438 29. Marcora SM, Bosio A, de Morree HM. Locomotor muscle fatigue increases
439 cardiorespiratory responses and reduces performance during intense cycling exercise
440 independently from metabolic stress. *Am J Physiol Regul Integr Comp Physiol.*
441 2008;294(3):R874-883.
- 442 30. Crewther BT, Lowe TE, Ingram J, Weatherby RP. Validating the salivary testosterone and
443 cortisol concentration measures in response to short high-intensity exercise. *J Sports Med*
444 *Phys Fitness.* 2010;50(1):85-92.
- 445 31. Smith AA, Toone R, Peacock O, Drawer S, Stokes KA, Cook CJ. Dihydrotestosterone is
446 elevated following sprint exercise in healthy young men. *J Appl Physiol (1985).*
447 2013;114(10):1435-1440.

449 **TITLES OF TABLES:**

450 **Table 1.** Variables measured in the morning and in the afternoon by condition.







451

452 **Table 2.** Variables measured in the afternoon by condition.

453

454

455 **TITLES OF FIGURES:**

456 **Figure 1.** Schematic representation of the study protocol.  blood samples,  ear temperature,   counter-
457 movement jump,   sprint, CON control, EASY easy exercise, RSR repeated sprint
458 running, RPE rating of perceived exertion, Yo-Yo IR2 intermittent recovery level 2 test.

459

460 **Figure 2.** Standardized differences between afternoon vs morning changes among the different
461 conditions and relative 90% confidence limits for blood parameters, temperature and self-reported
462 mood (fatigue and vigor).

463

464 **Figure 3.** Standardized differences between afternoon vs morning changes among the different
465 conditions and relative 90% confidence limits for MVC maximal voluntary contraction, VA
466 voluntary activation, PT peak torque, RTD rate of torque development, RTR rate of torque
467 relaxation, CMJ counter-
468 movement jump, PPO peak power output.

469 **Table 1.** Variables measured in the morning and in the afternoon by condition.

	CON		ECO		RSR	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
<i>Hormone levels and body temperature</i>						
Testosterone (ng·mL ⁻¹)	6.5 ± 1.4	4.2 ± 1.5****↓	6.7 ± 1.3	4.1 ± 1.1****↓	6.1 ± 1.7	4.2 ± 0.9****↓
Cortisol (ng·mL ⁻¹)	154 ± 36	91 ± 22****↓	159 ± 46	84 ± 34****↓	157 ± 46	81 ± 40****↓
Ear temperature (°C)	36.4 ± 0.3	36.7 ± 0.2****↑	36.2 ± 0.3	36.7 ± 0.4****↑	36.5 ± 0.3	36.7 ± 0.3****↑
<i>Quadriceps neuromuscular function</i>						
MVC torque (N·m)	283 ± 70	276 ± 58	288 ± 69	287 ± 65	280 ± 74	279 ± 78
VA (%)	87.8 ± 7.5	87.1 ± 8.8	86.9 ± 11.1	85.4 ± 11.4	85.8 ± 9.3	85.4 ± 11.2
Voluntary RTD (N·m·s ⁻¹)	4780 ± 1386	4801 ± 1258	4786 ± 1224	5089 ± 1419*↑	4735 ± 1177	4508 ± 1396*↓
Doublet PT (N·m)	68.0 ± 6.9	69.3 ± 6.6*↑	71.3 ± 7.5	70.9 ± 7.9	70.1 ± 8.3	69.4 ± 8.4
Doublet RTD (N·m·s ⁻¹)	1602 ± 331	1673 ± 264*↑	1675 ± 272	1765 ± 503	1677 ± 246	1549 ± 275**↓
Doublet RTR (N·m·s ⁻¹)	1029 ± 314	1039 ± 308	1048 ± 355	1036 ± 362	1037 ± 355	994 ± 310
<i>Self-reported mood</i>						
Fatigue (au)	2.83 ± 2.12	2.75 ± 1.36	3.50 ± 2.47	2.17 ± 1.80**↓	4.17 ± 3.71	4.17 ± 3.13
Vigor (au)	8.42 ± 2.75	7.58 ± 3.06*↓	7.92 ± 2.57	7.08 ± 3.82**↓	7.42 ± 2.94	7.17 ± 3.16
<i>Jump and sprint performance</i>						
CMJ height (cm)	45.7 ± 2.8	46.5 ± 3.5*↑	45.7 ± 3.6	46.3 ± 4.4*↑	45.2 ± 4.0	45.4 ± 3.9
CMJ PPO (W)	3815 ± 420	3946 ± 449**↑	3825 ± 418	3913 ± 413*↑	3853 ± 494	3869 ± 484
Sprint time (s)	7.10 ± 0.17	7.04 ± 0.22*↓	7.13 ± 0.19	7.05 ± 0.20**↓	7.15 ± 0.20	7.06 ± 0.18**↓

471 Mean data \pm SD. According to Magnitude Based Inference, differences to am were rated as follow: *possibly, **likely, ***very likely, ****almost certain, \uparrow
472 higher, \downarrow lower, compared to the same condition in the am. CON: control, ECO: ecological, RSR: repeated sprint running, MVC: maximal voluntary
473 contraction, VA: voluntary activation, RTD: rate of torque development, PT: peak torque, RTR: rate of torque relaxation, CMJ: countermovement jump, PPO:
474 peak power output.

475

476 **Table 2.** Variables measured in the afternoon by condition.

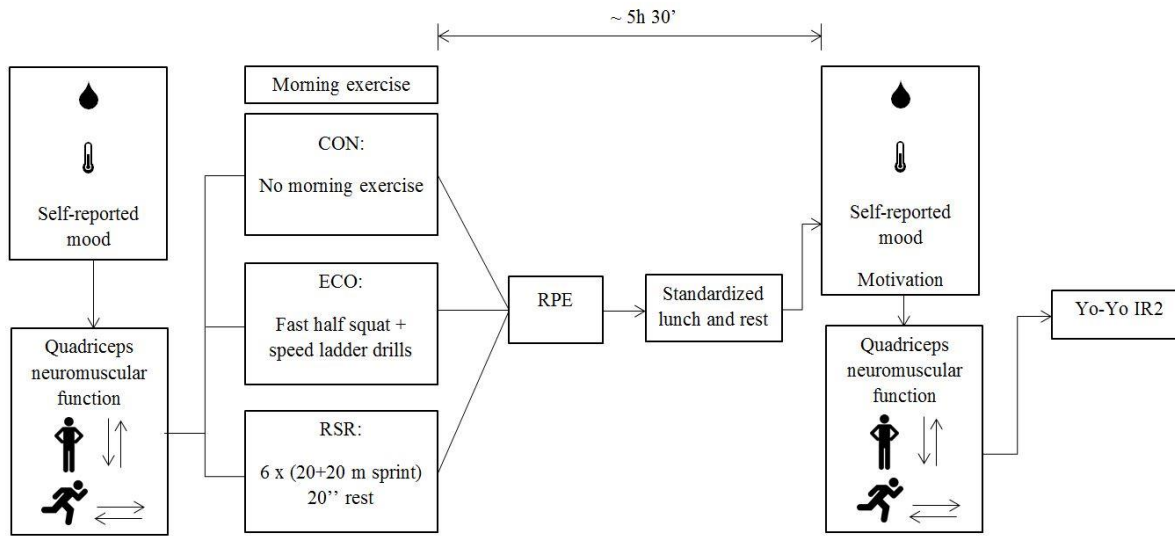
	CON			ECO			RSR			RSR vs CON	ECO vs CON	RSR vs ECO
<i>Motivation</i>												
Task-dependent motivation (au)	11.8	±	6.4	12.2	±	7.0	10.0	±	7.5	**↓ -0.26 ± 0.30	unclear 0.05 ± 0.33	**↓ -0.31 ± 0.35
Task-independent motivation (au)	20.3	±	4.5	21.0	±	4.8	20.5	±	3.7	unclear 0.05 ± 0.26	*↑ 0.16 ± 0.15	unclear -0.10 ± 0.30
<i>Endurance performance</i>												
Distance covered (m)	693	±	162	740	±	162	650	±	173	*↓ -0.25 ± 0.39	*↑ 0.27 ± 0.35	***↓ -0.52 ± 0.33
Maximal heart rate (bpm)	191	±	9	192	±	9	189	±	10	unclear -0.12 ± 0.56	*↑ 0.17 ± 0.14	*↓ -0.29 ± 0.46

477

478 Mean data ± SD. According to Magnitude Based Inference, differences were rated as follow: *possibly, **likely, ***very likely, ****almost
 479 certain, ↑ higher, ↓ lower. Effects sizes are reported with 90% confidence limits. RPE: rate of perceived exertion, CON: control, ECO: ecological
 480 exercise, RSR: repeated sprint running.

481

482



483

484

