

The Neuromuscular, Endocrine and Mood Responses to a Single Versus Double Training Session Day in Soccer Players.

Abstract

25 Objectives: This study profiled the 24 hour (h) neuromuscular, endocrine and mood responses to a
26 single **versus a double** training day in soccer players.

27

28 Design: Repeated measures

29

30 Methods

31 Twelve semi-professional soccer players performed small-sided-games (SSG's; 4vs4 + goalkeepers;
32 6x7-min, 2-min inter-set recovery) with neuromuscular (peak-power output, PPO; jump height, JH),
33 endocrine (salivary testosterone, cortisol), and mood measures collected before (pre) and after (0h,
34 +24h). The following week, the same SSG protocol was performed with an additional lower body
35 strength training session (back-squat, Romanian deadlift, barbell hip thrust; 4x4 repetitions, 4-min inter-
36 set recovery; 85% 1 rep-max) added at 2h after the SSG's.

37

38 Results

39 Between-trial comparisons revealed possible to likely small impairments in PPO ($2.5 \pm 2.2 \text{ W}\cdot\text{kg}^{-1}$; 90%
40 Confidence Limits: $\pm 2.2 \text{ W}\cdot\text{kg}^{-1}$), JH (-1.3 ; $\pm 2.0 \text{ cm}$) and mood (4.6 ; $\pm 6.1 \text{ AU}$) in response to the double
41 versus single sessions at +24h. Likely to very likely small favourable responses occurred following the
42 single session for testosterone (-15.2 ; $\pm 6.1 \text{ pg}\cdot\text{ml}^{-1}$), cortisol (0.072 ; $\pm 0.034 \text{ ug}\cdot\text{dl}^{-1}$) and
43 testosterone/cortisol ratio (-96.6 ; $\pm 36.7 \text{ AU}$) at +24 h compared to the double session trial.

44

45 Conclusions

46 These data highlight that performance of two training sessions within a day resulted in possible to very
47 likely small impairments of neuromuscular performance, mood score and endocrine markers at +24h
48 relative to a single training session day. **A strategy of alternating high intensity explosive training days
49 containing multiple sessions with days emphasising submaximal technical/tactical activities may be
50 beneficial for those responsible for the design and delivery of soccer training programs.**

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56 **Key Words:** Fatigue, recovery, concurrent training, training prescription.

Introduction

57 Soccer players are required to maintain and develop multiple physical qualities aligned to successful
58 soccer performance, including but not limited to: strength, power, speed, agility, aerobic capacity,
59 repeated sprint ability, as well as engaging with technical and tactical training.^{1,2} As limited training
60 time separates fixtures, the ability to simultaneously develop such physical, technical, and tactical
61 qualities is desirable.^{1,2,3} Accordingly, concurrent training methods with the aim of maintaining and
62 developing multiple physical qualities often occur, with multiple sessions often undertaken on the same
63 day and within 24 h of each other.^{1,2,3} Indeed, professional players often perform soccer-specific and
64 resistance training sessions on the same day, before training again 24 h later.^{1,2,3} However, for
65 adaptation to occur, the training stimulus should be applied in an order that facilitates recovery to a
66 point where players are able to meet the demands of each training session.⁴

67

68 The recruitment of high threshold motor units has been reported necessary for inducing neural
69 adaptations associated with speed, agility and power.⁵ However, if fatigue and muscle damage is
70 present then athletes may be unable to perform the high intensity explosive movements required to
71 recruit these fast twitch muscle fibers. It is therefore recommended that training sessions aimed at
72 maximizing these neural adaptations should be performed when the athletes are not fatigued and in an
73 optimal condition. Recent work has shown that whilst there is an impairment of neuromuscular function
74 immediately after a small sided games (SSG) session in soccer (countermovement jump height $-3.2 \pm$
75 1.9 cm; peak power output -1.1 ± 0.9 W·kg⁻¹), there may be a temporary recovery at 2 h post, before
76 further impairment after 24 h (countermovement jump height -2.5 ± 1.2 cm; peak power output $-0.9 \pm$
77 0.8 W·kg⁻¹).² Therefore, it seems that the performance of a second intense neuromuscular training
78 session after 2 h of passive recovery may not be dampened. However it is unclear whether the addition
79 of a second intense session may further impair performance and recovery status at 24 h post. This may
80 be worthy of investigation, given that professional team sport players often train on consecutive days,
81 and also on the day following a double session.^{3,6} Having this information would better allow the coach
82 to make informed decisions about the use of twice daily training and the placement and type of sessions
83 they wish to have the athlete perform during the rest of the training week.

84

85 The majority of research looking at the responses to multiple training sessions in a day has examined
86 the combined effects of similar training modalities (e.g. resistance training twice daily)^{7,8,9}, and it is
87 unclear how neuromuscular function was affected in the 24 h following the double session training day,
88 and whether these changes differed according to the number of sessions performed. Additionally, whilst
89 multiple daily resistance training sessions are often undertaken by weightlifters,⁹ team sport players are
90 often required to undertake both on-field training and weightlifting sessions on the same day.^{3,6}

91 Johnston et al. ⁶ compared the 24 h responses from a single (speed) session to a double (speed & weights)
92 session training day in Rugby players. Their data indicates that the addition of a weight training session
93 2 h after a speed session did not influence endocrine responses or neuromuscular capability 24 h post
94 stimulus, despite the participants reporting a higher perception of soreness.

95
96 Intense dynamic exercises containing repeated eccentric and/or stretch shortening cycle actions are
97 likely to result in inflammatory processes; more specifically muscle damage ^{10,11}, muscle soreness ^{10,11},
98 and reduced neuromuscular performance. ² In addition, these types of exercise may induce changes in
99 testosterone ⁸, and cortisol ¹² release, which could influence both neuromuscular function and adaptation
100 to training. ¹³ Therefore, it is important to consider the combined effect of two sessions performed within
101 close proximity to each other, to determine if the addition of a second training session results in elevated
102 fatigue in the 24 h that follow. Considering that team sport players regularly train on consecutive days,
103 this data would be valuable to those responsible for the design of programming in team sports.

104
105 To date, no studies have reported the fatigue and recovery profiles of combined soccer and weight
106 training sessions on the same day. This is somewhat surprising given the prevalence of such practices
107 in applied scenarios. It has been suggested that variation in exercise stimuli and muscle contraction are
108 factors that may exacerbate the inflammatory response. ¹⁴ The addition of a second training session may
109 therefore result in greater metabolic stress and reduced neuromuscular performance in the 24 h
110 following a double training day. This information would allow coaches to make informed decisions on
111 where to structure double training sessions throughout the week, and the impact this may have on the
112 subsequent days training. Therefore the aim of this study was to compare the 24 h fatigue response from
113 a single versus a double training session day on neuromuscular function, endocrine and mood responses.

114 **Methods**

115 Each experimental protocol was completed over two days on consecutive weeks. The study took place
116 midway through the 2017-2018 competitive season with players being given 72 h rest before test
117 involvement. Countermovement jump (CMJ; peak power output: PPO; jump height: JH), saliva
118 (testosterone and cortisol concentrations), and brief assessment of mood (BAM+) responses were
119 collected before (pre), and after (0 h, +24 h) SSG training. **These measures have been used extensively**
120 **in previous research to measure neuromuscular function of the lower body (CMJ), anabolic/ catabolic**
121 **activity (salivary testosterone and cortisol) and for monitoring the fatigue and recovery cycles in elite**
122 **athletes (BAM+).** ^{2, 6 15} The following week, players returned and completed the exact same protocol,
123 with the inclusion of a lower body weight training session 2 h after the SSG session. The 0 h time-point
124 remained immediately post the completion of the SSG's on week 2.

127 Data are presented from 12 male semi-professional soccer players (age: 21 ± 2 years, mass: 74.8 ± 5
128 kg, height: 1.81 ± 0.06 m). Despite the involvement of goalkeepers in the SSG protocol, only data
129 from outfield players was included in the current study from a range of playing positions. All players
130 were considered healthy and injury-free at the time of the study and were in part-time training.
131 Players were in the maintenance phase of their training season, undertaking resistance training
132 programs, team-based conditioning sessions, and technical and tactical training. On a typical
133 microcycle which consisted of 1 game per week, players were completing two on-field training
134 sessions (1.5-2 h) and one resistance training session (1 h). The training week was structured so that
135 the team performed both on-field training and resistance training on match day -2 and a single on-
136 field training session on match day -1. Ethical approval was granted by the ethics advisory board of
137 Swansea University. Players were also informed of the risks and benefits and provided written
138 informed consent prior to participation in the study.

139
140 On arrival at the training centre (~17:00 h), pre salivary samples, BAM+ mood questionnaire scores
141 and CMJ performance was assessed. Prior to CMJ testing, players completed a 5-min standardized
142 warm up consisting of jogging and dynamic stretching. The SSG training session began at 17:30 h.
143 Follow up measures (saliva, BAM+, CMJ's) were collected at 0 h and +24 h post-training. The
144 following week, players performed the same procedure but with the inclusion of a lower body strength
145 training session 2 h after the completion of the SSG's (~20:30 h). Immediately after the 0 h testing on
146 both trials, players were provided with water, a banana and a protein bar (Energy: 171 kcal, Fats: 3.7 g,
147 Carbohydrate: 20 g, Sugars: 9.3 g, Protein: 14 g).

148
149 After a five-min warm-up, which consisted of dynamic stretching and short sprints, players were split
150 into four teams of five by coaching staff. The teams were organized such that playing positions were
151 balanced within each team (e.g., one goalkeeper, one defender, one winger, one midfielder, and one
152 striker). The sport surface was a modern third generation artificial grass pitch and players wore their
153 normal soccer boots during the SSG's. Players were instructed to play against another team for six
154 blocks of seven-min (overall work: 42-min) with two-min between each game being allowed for players
155 to drink water and passively rest before the next repetition. Pitch size was 24 x 29 m and full-sized
156 goals with goalkeepers were used. Further, players were allowed unlimited touches of the ball and the
157 aim was to score as many goals as possible. This SSG format complemented the player's training
158 regimes and was similar to previous literature.^{2,16} The total time that the participants were on the field
159 from the beginning of the warm-up to the end of the SSG's was 59-min.

160
161 The physical demands of the SSG's were collected via 10 Hz global positioning system (GPS) units
162 embedded with 100 Hz tri-axial accelerometers (OptimEye X4, Catapult Innovations, Melbourne,
163 Australia), which have shown to hold an acceptable level of reliability and validity when tracking player

164 movements.¹⁷ Each unit was attached to the upper back of players using a specifically designed vest
165 garment. The data was downloaded and processed automatically using Catapult Sports software
166 (Openfield, Catapult Innovations, Melbourne, Australia). The high speed running (HSR) threshold was
167 defined as the total distance (m) covered at a velocity >5.5 m·s⁻¹, and was set in line with previous work
168 in soccer time-motion analysis.^{18,19} Player load [Playerload™] is defined as the sum of gravitational
169 forces on the accelerometer in each individual axial plane (anteroposterior, mediolateral and vertical),
170 and has been reported previously in soccer time-motion analysis.^{2,20}

171
172 The lower body strength training content was selected to both match the exercises that the players were
173 familiar with in their normal routines whilst also being within the guidelines for the development of
174 strength.⁶ Players were only included who had a minimum of 1 year of strength training experience.
175 Specifically, the session consisted of four sets of four repetitions of the parallel back squat, Romanian
176 dead lift, and the barbell hip thrust, all at 85% of current 1 repetition maximum (RM) with four-min
177 recovery between sets and exercises. Each exercise was preceded by two sets of four at 50% and 70%
178 1RM. The strength training session lasted approximately one hour. Prior to test involvement, each
179 participant was required to perform a 3RM testing session of all three exercises, which occurred exactly
180 a week prior to testing. Using the 3RM data, 1RM was estimated using an equation which accurately
181 predicts 1RM.²¹ The session was supervised by an accredited strength and conditioning coach (United
182 Kingdom Strength and Conditioning Association; UKSCA) to ensure appropriate technique was
183 maintained throughout.

184
185 A portable force platform (Type 92866AA, Kistler) was used to measure performance of the lower
186 body. This required CMJ's to be performed at maximum effort, with arms akimbo to isolate the lower
187 body musculature. Two CMJ's were completed after a standardized warm-up at each timepoint. The
188 vertical ground reaction forces from the jumps were used to assess PPO from previously reported
189 methods.^{2, 6, 22} This data was converted into relative peak power (W·kg⁻¹) by dividing PPO by the
190 player's body mass in kilograms. Additionally, JH was calculated by multiplying the velocity at each
191 sampling point by the time (0.005 s). It was then defined as the difference between vertical displacement
192 at take-off and maximal vertical displacement. Test-retest reliability (intraclass correlation coefficient)
193 for PPO, and JH were 0.89 and 0.84, respectively. The coefficient of variation (CV) for PPO and JH
194 were 2.3% and 3.2%, respectively.

195
196 At all timepoints, 2 ml of saliva was collected by passive drool into sterile containers. Saliva samples
197 were stored at -20 °C for seven days until assay. After thawing and centrifugation (2000
198 revolutions·min⁻¹ x 10 min), the saliva samples were analyzed in duplicate for testosterone and cortisol
199 concentrations using commercial kits (Salimetrics LLC, USA). The minimum detection limit for the

200 testosterone assay was 6.1 pg·ml⁻¹ with an inter-assay CV of 5.8%. The cortisol assay had a detection
201 limit of 0.12 ng·ml⁻¹ with inter-assay CV of 5.5%

202

203 Mood state was assessed using a modified version of the brief assessment of mood questionnaire
204 (BAM+).¹⁵ This 10-item questionnaire is based on the Profile of Mood State assessment and consists
205 of a scale where players mark on a 10 cm scale about how they feel at that moment in time. Scale
206 anchors ranged from 'not at all' to 'extremely'. The questions assess the following mood adjectives:
207 anger, confusion, depression, fatigue, tension, alertness, confidence, muscle soreness, motivation and
208 sleep quality. Players completed the questionnaires in isolation of teammates and it took approximately
209 two minutes to complete. The BAM+ questionnaire has been shown to be an effective tool for
210 monitoring the fatigue and recovery cycles in elite athletes.^{2,15} The scores were totalled up by giving
211 the 6 unfavourable questions (anger, confusion, depression, fatigue, tension and muscle soreness) a
212 positive value, and the 4 favourable questions (alertness, confidence, motivation and sleep quality) a
213 negative value. The original total mood score ranged from -40 – 60, before adding 40 to each score so
214 that the scale ranged from 0 – 100, with 0 indicating the best mood and 100 indicating the worst.^{2,15}

215

216 Data are reported as mean ± SD. Visual inspection of the residual plots revealed no clear evidence of
217 heteroscedasticity, so all analyses were performed on the raw untransformed data. Custom-made
218 spreadsheets were used to analyze the effect of training session (single, double) on our measures of
219 neuromuscular function, endocrine and mood responses.²³ The analysis of within training session
220 effects was made using the post-only crossover spreadsheet, with the analysis of between-group
221 changes (single training session vs double training session) made using the before and after parallel-
222 group spreadsheet. Here, we used the pre value of the dependent variable as a covariate to control for
223 pre imbalances between the single and double training sessions. The uncertainty of our estimates is
224 expressed as 90% confidence limits (CL). Standardised thresholds for small, moderate and large
225 effects derived from between-player standard deviations of the pre values (0.2, 0.6 and 1.2,
226 respectively) were used to assess the magnitude of all effects and effect probability of the effect was
227 interpreted using the following scale: 25–75%, possibly; 75–95%, likely; 95–99.5%, very likely;
228 >99.5%, most likely.²⁴ We classified the magnitude of effects mechanistically, whereby if the 90%
229 CL overlapped the thresholds for the smallest worthwhile positive and negative effects, the effect was
230 deemed unclear.²⁵ A paired samples T-test was used to determine if there were any differences
231 between the GPS metrics on week 1 (single session) and week 2 (double session). GPS metrics for
232 total distance (single, 4475 ± 397 m; double, 4315 ± 641 m), HSR (single, 21 ± 22 m; double, 30 ± 35
233 m) and Playerload™ (single, 452 ± 59 AU; double, 443 ± 85 AU) were similar between trials (p >
234 0.05).

235

236 **Results**

237

238 Between trial (single vs double) comparisons revealed that the double session day resulted in a *possibly*
239 *small* compromised mood score between pre and +24 h and 0 h and +24 h (see Figure 1A & Table 1).
240 Within-session effects revealed a *very likely moderate* decrease (0 h and +24 h) and a *likely moderate*
241 increase (pre and 0 h) for the single session, with a *likely moderate* increase (pre and 0 h) and a *possibly*
242 *moderate* decrease (0 h and +24 h) for the double session.

243

244 There was a *possibly small* impairment in CMJ height following the double training session day in
245 comparison to the single session, both between pre and +24 h and 0 h and +24 h (see Figure 1B & Table
246 1). Within-trial analyses revealed predominantly *small* changes between timepoints for the single and
247 double sessions (see table 1). A *likely small* impairment in relative PPO was observed after the double
248 session compared to the single session between time points +24 h and baseline and 0 h and +24 h (see
249 Figure 1C & Table 1). Within-trial effects for the single and double sessions revealed predominantly
250 *small* changes across timepoints (see table 1).

251

252 ***** TABLE 1*****

253

254 Between-trial comparisons revealed *likely small* higher testosterone concentrations following the single
255 compared to the double training session between time points +24 h and baseline and 0 h and +24 h (see
256 Figure 1D & Table 2). Within-trial analyses revealed that despite no changes in testosterone following
257 the single session, after the double session there were *possible small* decreases (pre and +24 h, 0 h and
258 +24 h). There were higher concentrations (*likely small*) in cortisol following the double compared to
259 the single session (pre and +24 h, 0 h and +24 h) (see Figure 1E & Table 2). Following the single
260 session, there were *possible small* decreases between all timepoints (see table 2). Between-session
261 comparisons revealed *very likely small* (pre and +24 h) and *possibly small* (pre and 0 h) decreases in
262 the testosterone to cortisol (T/C) ratio following the double compared to the single session (see Figure
263 1F & Table 2). Within-trial comparisons revealed *possible* to *very likely small* changes across all
264 timepoints following for the single and double sessions (see table 2).

265

266 *****TABLE 2*****

267

268 *****FIGURE 1*****

269

270

271

272 **Discussion**

273

274 The primary aim of this study was to compare the 24 h responses of neuromuscular, endocrine and
275 mood markers following a single session training day consisting of small-sided games, to a double
276 training session day consisting of small-sided games and a weight training session performed 2 h later.

277 On both trials, the SSG training (6 x 7 min; 42 min total playing time) induced immediate fatigue as
278 evidenced by *moderate* disturbances in mood (single, 9.8 ± 11.2 AU; double, 10.4 ± 7.2 AU) and *small*
279 decreases in jump height (single, -1.6 ± 2.7 cm; double, 1.7 ± 3.8 cm). The addition of a weights training
280 session 2 h after SSG's resulted in *small* impairments in neuromuscular performance (PPO; -2.5 ± 2.2
281 $\text{W}\cdot\text{Kg}^{-1}$; JH; -1.3 ± 2.0 cm), mood (4.6 ± 6.1 AU), endocrine markers (testosterone; 15.2 ± 6.1 $\text{pg}\cdot\text{ml}^{-1}$;
282 cortisol; 0.072 ± 0.034 $\text{ug}\cdot\text{dl}^{-1}$; T/C ratio; -96.6 ± 36.7 AU) at +24 h, indicating additive fatigue effects.
283

284 It is unsurprising that immediately after the SSG's on both trials, there were *likely moderate*
285 disturbances in mood score combined with *possible to likely small* impairments of neuromuscular
286 performance. An explanation for the initial impairment in neuromuscular performance at 0 h may relate
287 to a reduced functioning of the muscle fiber contractile mechanisms in the presence of metabolites (i.e.,
288 hydrogen ions, adenosine diphosphate, inorganic phosphate) accumulated during exercise.²⁶ Moreover,
289 a decreased calcium ion release from the sarcoplasmic reticulum, resulting in less calcium ion binding
290 to troponin and a negative influence on actin-myosin interactions during cross-bridge cycling may also
291 have contributed.²⁶ Interestingly, the single session did not result in impaired neuromuscular
292 performance or mood at +24 h; a finding which contradicts previous data in response to the same SSG
293 protocol in professional players², whereby higher playing intensities may have resulted from full-time
294 professional players as opposed to semi-professional players. Nevertheless, comparisons between the
295 present data and that from the professional players appeared broadly similar for total distance (semi-
296 professional, 4475 ± 397 m; professional, 4388 ± 231 m), high speed running (> 5.5 $\text{m}\cdot\text{s}^{-1}$; semi-
297 professional, 21 ± 22 m; professional, 41 ± 30 m), and PlayerloadTM (semi-professional, 452 ± 59 AU;
298 professional, 483 ± 38 AU).² For the pitch size used, it may be that the common and comparable metrics
299 collected across both studies were not sensitive enough to measure the discrepancies in playing intensity
300 that may have occurred.

301
302 The endocrine markers measured in the current study showed similar pattern to both neuromuscular
303 function and mood, with between trial comparisons (single vs double) revealing no immediate
304 differences at 0 h for both testosterone and cortisol. However, at +24 h there were *likely small* lower
305 concentrations of testosterone (-15.2 ± 6.1 $\text{pg}\cdot\text{ml}^{-1}$) and *likely small* higher concentrations of cortisol
306 (0.072 ± 0.034 $\text{ug}\cdot\text{dl}^{-1}$) following the single session in comparison to the double. This resulted in a *very*
307 *likely small* beneficial response for the T/C ratio following the single session versus the double training
308 session day (-96.6 ± 36.7 AU). With respect to their opposing roles in the regulation of protein
309 metabolism, it has been suggested that testosterone and cortisol may differentially respond to metabolic
310 stress.²⁷ Therefore, the ratio between the two hormones has been reported as a balance of anabolic and
311 catabolic activity.²⁷ It is thought that a decreased T/C ratio may result in impaired physical performance.

312 ²⁸ It could be that the added metabolic stress of the weights session in the current study resulted in a
313 lower T/C ratio, which has been suggested to reduce neuromuscular performance and mood at +24 h.

314
315 Overall, our results suggest that performance of a double training session day resulted in *possible to*
316 *very likely small* impairments of neuromuscular performance, mood score and endocrine markers in
317 comparison to a single training session day at +24 h; possibly suggesting that a heavy lower body
318 training session further exacerbated the fatigue response to the SSG protocol used here. The most likely
319 explanation for this is that the added eccentric stress of the lower body resistance training resulted in
320 further muscle inflammation and hydrogen ion accumulation. ⁶ While these mechanisms have been
321 extensively studied across sessions separated by several days or weeks ²⁹, limited research has examined
322 the effects of multiple sessions performed in the same day. Our results are conflicting with previous
323 findings on the effects of multiple daily resistance sessions, cycling sessions, and combined speed and
324 weights training sessions ^{6,9,30}. However, the authors acknowledge that the small differences may be
325 due to variations in the training history of the participants involved, exercises selected, and intensity of
326 the protocols. Nevertheless, our study is the first to examine the combined response from SSG's and
327 weight training in soccer players.

328
329 There are a number of limitations to the current study that should be acknowledged. Firstly, some teams
330 and practitioners may schedule a rest day at 24 h post a double training session, therefore the inclusion
331 of a 48 h post timepoint to the study design may have been useful to investigate whether fatigue still
332 persisted. In addition, collection of internal load and/or other GPS metrics such as acceleration and
333 deceleration activity during the SSG's may have given a better indication of the playing intensity.
334 Finally, we did not measure player's aerobic fitness prior to the start of this study, which is a factor that
335 may influence fatigue and recovery profiles.

336 **Conclusion**

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338
339 This study shows that 42 min of SSG's combined with lower body weight training resulted in small to
340 moderate disturbances in neuromuscular performance, mood, and endocrine markers over a 24 h period
341 in comparison to the SSG's alone. As soccer players are often required to concurrently train multiple
342 physical qualities in the same day (i.e. strength and soccer), this data may be of use to those responsible
343 for the design of soccer training programs. More specifically, consideration of the added 24 h fatigue
344 response from a double training session should be considered when programming into the training
345 week, as players may require longer to recover and adapt.

346 **Practical Implications**

- 347 • Consideration of the added 24 h fatigue response from a double training session should be
348 considered when programming into the training week, as players may require longer to
349 recover and adapt.
- 350 • A strategy of alternating high intensity explosive training days containing multiple sessions
351 with days emphasising submaximal technical/tactical activities may be beneficial.

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354

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Figure Legends

357 Figures 1 A-F. Mean±SD mood (A), CMJ height (B), relative peak power output (C),
358 testosterone (D), cortisol (E) and mood (F) responses to a single vs double training session.
359 Qualitative inferences are shown above the figure for the between trial differences between
360 each timepoint. (*25-75 %, *possibly*; **75-95 %, *likely*; ***95-99.5 %, *very likely*).

Table 1. Mean (\pm SD) fatigue marker changes between timepoints. Qualitative inferences are shown for both the within and between trial differences (single vs double session).

Variable	Trial	Comparison		
		Pre – 0 h	Pre – 24 h	0 h – 24 h
Mood Score (AU)	Single \pm SD	9.8 \pm 11.2 (<i>Moderate**</i>)	-2.1 \pm 7.7 (<i>Trivial*</i>)	-11.9 \pm 14.6 (<i>Moderate***</i>)
	Double \pm SD	10.4 \pm 7.2 (<i>Moderate**</i>)	2.5 \pm 6.8 (<i>Trivial*</i>)	-7.9 \pm 8.8 (<i>Moderate*</i>)
	Between trial difference \pm 90% CL	0.7 \pm 7.5 (<i>Trivial*</i>)	4.6 \pm 6.1 (<i>Small*</i>)	4.0 \pm 7.3 (<i>Small*</i>)
CMJ Height (cm)	Single \pm SD	-1.6 \pm 2.7 (<i>Small*</i>)	0.5 \pm 2.7 (<i>Trivial**</i>)	2.1 \pm 2.5 (<i>Small**</i>)
	Double \pm SD	-1.7 \pm 3.8 (<i>Small**</i>)	-0.8 \pm 1.9 (<i>Small*</i>)	0.9 \pm 3.9 (<i>Small*</i>)
	Between trial difference \pm 90% CL	-0.1 \pm 1.3 (<i>Trivial**</i>)	-1.3 \pm 2.0 (<i>Small*</i>)	-1.2 \pm 2.1 (<i>Small*</i>)
CMJ Relative PPO (W·Kg ⁻¹)	Single \pm SD	-0.3 \pm 3.6 (<i>Trivial*</i>)	1.0 \pm 3.9 (<i>Small*</i>)	1.2 \pm 3.9 (<i>Small*</i>)
	Double \pm SD	-0.9 \pm 3.3 (<i>Small*</i>)	-1.5 \pm 1.9 (<i>Small*</i>)	-0.6 \pm 3.9 (<i>Trivial*</i>)
	Between trial difference \pm 90% CL	-0.6 \pm 1.5 (<i>Trivial*</i>)	-2.5 \pm 2.2 (<i>Small**</i>)	-1.8 \pm 2.2 (<i>Small**</i>)

SD, standard deviation; SSG, small-sided game; AU, arbitrary units.

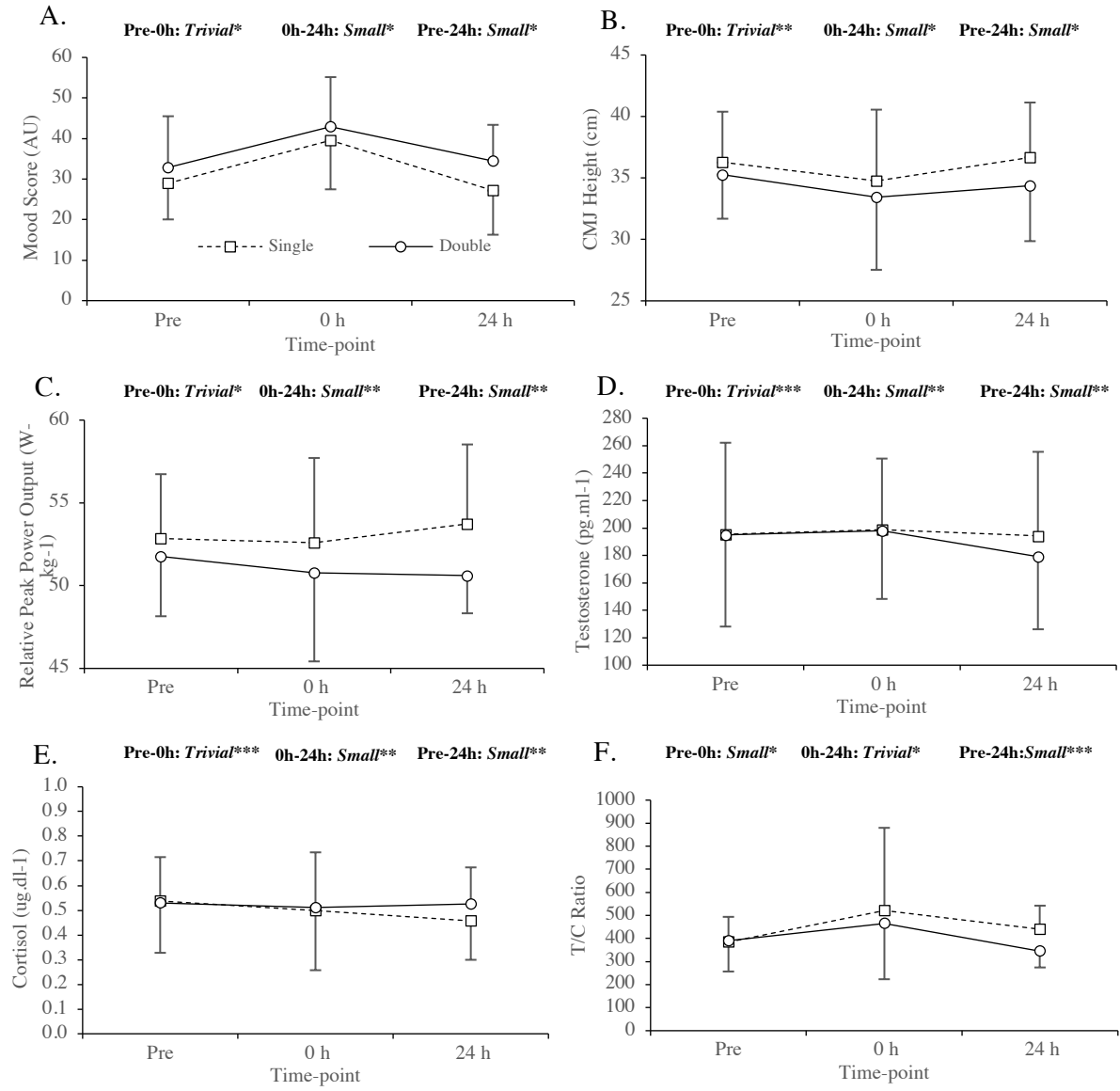
*25-75 %, possibly; **75-95 %, likely; ***95-99.5 %, very likely.

Table 2. Mean (\pm SD) endocrine marker changes between timepoints. Qualitative inferences are shown for both the within and between trial differences (single vs double session).

Variable	Trial	Comparison		
		Pre – 0 h	Pre – 24 h	0 h – 24 h
Testosterone (pg·ml ⁻¹)	Single \pm SD	+3.6 \pm 26.3 (<i>Trivial**</i>)	-0.8 \pm 61.7 (<i>Trivial***</i>)	-4.4 \pm 65.5 (<i>Trivial**</i>)
	Double \pm SD	+3.0 \pm 27.9 (<i>Trivial**</i>)	-16.0 \pm 54.8 (<i>Small*</i>)	-19.0 \pm 59.9 (<i>Small*</i>)
	Between trial difference \pm 90% CL	-0.6 \pm 5.7 (<i>Trivial***</i>)	-15.2 \pm 6.1 (<i>Small**</i>)	14.6 \pm 6.2 (<i>Small**</i>)
Cortisol (ug·dl ⁻¹)	Single \pm SD	-0.035 \pm 0.251 (<i>Small*</i>)	-0.078 \pm 0.152 (<i>Small*</i>)	-0.043 \pm 0.240 (<i>Small*</i>)
	Double \pm SD	-0.022 \pm 0.234 (<i>Trivial*</i>)	-0.006 \pm 0.143 (<i>Trivial***</i>)	+0.016 \pm 0.244 (<i>Trivial**</i>)
	Between trial difference \pm 90% CL	0.013 \pm 0.020 (<i>Trivial***</i>)	0.072 \pm 0.034 (<i>Small**</i>)	0.059 \pm 0.045 (<i>Small**</i>)
T/C Ratio (AU)	Single \pm SD	+138.3 \pm 336.8 (<i>Small**</i>)	+53.6 \pm 93.9 (<i>Small*</i>)	-84.8 \pm 294.2 (<i>Small*</i>)
	Double \pm SD	+74.0 \pm 190.2 (<i>Small*</i>)	-43.0 \pm 69.5 (<i>Small*</i>)	-117.0 \pm 194.4 (<i>Small**</i>)
	Between trial difference \pm 90% CL	-64.3 \pm 85.0 (<i>Small*</i>)	-96.6 \pm 36.7 (<i>Small***</i>)	-32.3 \pm 71.3 (<i>Trivial*</i>)

SD, standard deviation; SSG, small-sided game; AU, arbitrary units.

*25-75 %, possibly; **75-95 %, likely; ***95-99.5 %, very likely.



Figures 1 A-F. Mean±SD mood (A), CMJ height (B), relative peak power output (C), testosterone (D), cortisol (E) and mood (F) responses to a single vs double training session. Qualitative inferences are shown above the figure for the between trial differences between each time point. (*25-75 %, possibly; **75-95 %, likely; ***95-99.5 %, very likely).