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1	Manuscript full title: Biomechanical and physiological responses to 120 minutes of soccer-specific
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23	

24 Abstract

Purpose: To investigate biomechanical and physiological responses to soccer-specific exercise 25 incorporating an extra time period (ET) and assess the test-retest reliability of these responses. 26 Methods: Twelve soccer players performed 120 min of soccer-specific exercise. Tri-axial (PL_{Total}) and 27 uni-axial PlayerLoadTM in the vertical (PL_V), anterior-posterior (PL_{A-P}) and medial-lateral (PL_{M-L}) 28 planes were monitored using a portable accelerometer. Likewise, respiratory exchange ratio (RER) was 29 recorded throughout exercise. At the end of each 15 min period, players provided differential ratings of 30 perceived exertion ([d-RPE]) for legs [RPE-L], breathlessness [RPE-B] and overall [RPE-O]) and 31 capillary samples were taken to measure blood lactate (BLa) concentrations. The soccer-specific 32 exercise was completed twice within seven days to assess reliability. Results: A main effect for time 33 was identified for PL_{Total} (p = 0.045), PL_V (p = 0.002), PL_{A-P} (p = 0.011), RER (p = 0.001), RPE-L 34 35 0.001), RPE-O (p = 0.003) and CMJ (p = 0.020). A significant increase in PL_{Total} (234 ± 34 a.u) and decrease in RER (0.87 \pm 0.03) was evident during 105-120 min versus 0-15 min (215 \pm 25 a.u; p =36 0.002 and 0.92 \pm 0.02; p = 0.001). Coefficient of variations were <10% and Pearson's correlation 37 38 coefficient demonstrated moderate to very strong (0.33-0.99) reliability for all PL variables, RPE-B, 39 BLa and RER. Conclusions: These results suggest that mechanical efficiency is compromised and an 40 increased rate of lipolysis is observed as a function of exercise duration, particularly during ET. This 41 data has implications for practitioners interested in fatigue-induced changes during ET.

Keywords Football, PlayerLoadTM; Oxygen consumption; Perceived exertion; Fatigue; Reliability

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49 Introduction:

50 Professional soccer players are reported to cover a total distance of 9-12 km during a typical 90 min match (Mohr, Krustrup, & Bangsbo 2003). This activity involves frequent changes of direction, 51 periods of high-speed running (HS) and sprinting, as well as rapid accelerations and decelerations that 52 53 may result in temporary fatigue (Stølen, Chamari, Castagna & Wisløff, 2005). This is evidenced by 54 reductions in HS distances during the second half compared to the first half in Premier League 55 matches, both with (Bradley et al., 2009) and without the ball (Di Salvo, Gregson, Atkinson, Tordoff 56 & Drust, 2009). In recent years, researchers have investigated these responses during 120 min of 57 soccer-specific exercise (i.e., with the addition of a 30 min extra-time [ET] period). This period of match-play is required in domestic and international tournaments when matches are tied at the end of 58 59 90 min. Notably, at the previous four FIFA World Cups, 36% of knockout phase matches have proceeded to ET with 50% at the 2014 tournament requiring 120 min of match-play (Harper, 60 61 Fothergill, West, Stevenson & Russell, 2016a).

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63 To date, observations across 120 min of soccer match-play demonstrate that distances covered, and the number of accelerations, decelerations and number of HS sprint efforts performed are reduced in 64 65 professional players during ET versus 90 min (Russell, Sparkes, Northeast & Kilduff, 2015; Winder, Russell, Naughton & Harper, 2018). Moreover, a reduction in the number of dribbling and passing 66 actions in elite soccer players has been reported during the final 15 min of ET compared with a 67 68 number of other time points during matches (Harper, West, Stevenson & Russell, 2014). However, it 69 is unlikely that decreases in the number of actions are explained by a reduction in skill proficiency, 70 rather, a fatigue-related compromised ability to be involved with build-up play. In further support of 71 this notion, increased neuromuscular fatigue (i.e., reduced muscle force production) has been 72 observed during and following ET (Goodall et al., 2017). Furthermore, higher plasma glycerol, non-73 esterified fatty acids (NEFA) and adrenaline as well as lower blood lactate (BLa) and glucose has 74 been observed during ET, which is indicative of an increase rate of lipolysis (Stevenson et al., 2017). 75 This change in energy pathway potentially explains the reductions in HS and sprint performance

(Russell et al., 2015; Harper et al., 2016b) as evidence suggests that the favourable energy pathway to
fuel high-intensity exercise is glycogenolysis (Mohr et al., 2005). These changes are notable as ET is
typically played in important cup and tournaments, and indeed, matches often progress to or are
decided during this period.

80

Researchers have previously measured responses to 90 and 120 min of soccer using both match-play 81 and simulations (Page, Marrin, Brogden & Greig, 2015; Russell et al., 2015; Harper et al., 2016b). 82 83 Whilst match-play provides high ecological validity, between match physical performance is highly variable (Carling, Bradley, McCall & Dupont, 2016) and logistical constraints limit physiological 84 measurements during competitive games (Coutts et al., 2009). Therefore, simulating soccer match-85 86 play has been proposed as a feasible alternative which further reduces injury-risk by negating the 87 physical contact associated with match-play (Page et al., 2015), which is responsible for ~70% of 88 injuries (Aoki, O'Hata, Kohno, Morikawa & Seki, 2012). However to date, the Soccer Match 89 Simulation (Russell, Rees, Benton & Kingsley, 2011) is the only reliable soccer-exercise stimulus 90 over 120 min (Harper et al., 2016b). This free-running soccer-specific exercise incorporates skill 91 actions and changes of direction, although due to pacing, potentially fails to elicit a mechanistically 92 valid fatigue-response inherent within treadmill-based protocols (Page, Marrin, Brogden & Greig, 93 2019). Furthermore, treadmill simulations eliminate self-pacing as periods of workload are 94 standardised and thus, changes in a given variable are likely fatigue-induced (Page et al., 2015). A 95 validated motorised treadmill simulation has been used previously to investigate changes in physiological and biomechanical responses across 90 min of soccer-specific exercise (Page et al., 96 97 2015). This simulation has also shown to be valid over 120 min as distances covered (Winder et al., 2018) and number of sprints actions (Russell et al., 2015) are consistent with 120 min of actual-match 98 99 play. However, the test-retest reliability has not previously been assessed and thus there is scope to 100 further investigate reliability of these responses over 120 min.

101

Over recent years, assessments of within-match fatigue profiles have become increasingly common
 since International Football Association Board (IFAB) approved the use of wearable technologies

104 (e.g., accelerometers). A novel metric that has been proposed by Catapult Innovations as a measure of 105 movement efficiency is that of PlayerLoad[™] (PL) (Cormack, Mooney, Morgan & McGuigan, 2013). 106 This parameter is defined as the instantaneous rate of acceleration across three planes of motion (i.e., 107 vertical, anterior/posterior and medial/lateral) and otherwise referred to as 'jerk' (Nicolella, Torres-108 Ronda, Saylor & Schelling, 2018). Previously, changes in PL have been observed during the latter 109 stages of a contemporary 90 min soccer simulation, indicating a fatigue-induced deterioration in movement efficiency (Page et al., 2015). These fatigue patterns are analogous with those of a match 110 111 (Bradley et al., 2009) which can manifest through a reduced vertical stiffness in the lower extremities (detectable via changes in PL/jerk) (Oliver, Croix, Lloyd & Williams, 2014). The aetiology of such 112 changes may be a decreased neuromuscular control (Oliver et al., 2014), potentially compromising 113 dynamic joint stability (Hughes & Watkins, 2008). This could be owing to a reduced absorption 114 115 capacity and increased stress response to soft tissues (Hughes & Watkins, 2008), leading to impaired 116 movement efficiency which suggests an increased load per distance covered (Barrett et al., 2016) and 117 potential increased injury-risk. Furthermore, recent research suggests that exacerbated recovery 118 following matches that proceed to ET (Winder et al., 2018) may potentially predispose players to 119 injury, especially during fixture congested microcyles (Silva et al., 2018). Possible risk factors for 120 injury may involve lower limb muscle activity reductions and inhibited recovery kinetics, contributing 121 to subsequent impeded movement quality whilst performing explosive and unaccustomed activities (Silva et al., 2018). Therefore, investigating changes in PL responses over 120 min may help elucidate 122 123 whether recovery and injury-risk are impacted further by the additional duration and workload 124 associated with ET.

125

Whilst PL is a useful measure of external load, internal load (e.g., heart rate [HR] and rating of perceived
exertion [RPE]) can provide important information pertaining to the impact of external stressors
(Macpherson et al., 2019). Whilst increases in RPE have been observed during ET (Harper et al.,
2016b), differential RPE (d-RPE) responses have yet to be collected during this period. This method
comprises three discrete scores for breathlessness (RPE-B), legs (RPE-L) and overall (RPE-O) loads

131 (Borg et al., 2010). It has previously been employed as an approach to measuring exertional perceptions in soccer training (Macpherson et al., 2019) and 15-30 min post soccer match-play (Barrett, McLaren, 132 Spears, Ward, & Weston, 2018). Given the demands of soccer are multifactorial, d-RPE may highlight 133 distinct mechanisms of fatigue (Macpherson et al., 2019). For instance, RPE-L could quantify 134 135 peripheral and mechanical loads which in turn inform the need for strength, power or endurance work on specific working muscles (Weston et al., 2015). Whereas consistent high RPE-B could indicate a 136 higher central and physiological load imposed on players, highlighting the need for aerobic training. 137 138 Furthermore, as this measure is applied, sensitive and easily administered (Macpherson et al., 2019), investigating d-RPE responses may assist in capturing multiple dimensions of exertion to facilitate 139 understanding of players perceptions of load over 120 min of soccer-specific exercise. 140

141

142 As mentioned previously, ET may cause a change in the predominant energy source used (i.e., 143 endogenous carbohydrate [CHO] to fat)) (Stevenson et al., 2017). However, previous researchers have 144 used venous blood samples to estimate these changes in substrate utilisation over 120 min (Stevenson 145 et al., 2017), which is logistically difficult to measure during exercise. An alternative measure used to 146 estimate relative proportions of CHO and fat oxidation is the respiratory exchange ratio (RER). This measure calculates volumes of gas exhange (i.e., O₂ uptake and CO₂ production) through use of a breah-147 148 by-breath system (Goedecke et al., 2000). Indeed, treadmill-based similations offer a feasible approach to measuring RER as fixed periods of exercise intensity are required (Page et al., 2015). Likewise, using 149 150 treadmills to apply fixed workloads is essential to assess changes in substrate throughout exercise when using the Wasserman et al. (1987) equation to calculate RER. To date, researchers have yet to employ 151 152 treadmill-based soccer simulations for ET and have thus far failed to calculate RER over 120 min. Therefore, using RER to estimate substrate utilisation may provide practitioners and coaches with novel 153 insight into CHO usage during ET and whether CHO intake must be modulated to spare endogenous 154 CHO use and help maintain performance. 155

To the authors' knowledge, no study has aimed to quantify biomechanical (i.e., PL) responses and measure substrate utilisation (through RER) or collect d-RPE over 120 min of soccer-specific exercise. Therefore, this study aimed to investigate the biomechanical and physiological responses to 120 min of treadmill-based soccer-specific exercise. A secondary aim was to examine the test-retest reliability of these responses. We hypothesised that changes in PL would be indicative of a reduced efficiency and that a shift in RER values towards predominant use of fat oxidation would be observed during ET. In addition, it was postulated that d-RPE would increase as a function of exercise duration.

164

165 Materials and methods

166 Participants and experimental authorisation

167 University-standard (n=6) and semi-professional (n=6) outfield soccer players (age: 21.3 ± 2.9 years, stature: 178.5 ± 7.1 cm, mass: 70.42 ± 8.47 kg, maximal oxygen uptake [\dot{VO}_{2max}]: 56.58 ± 7.23 168 ml·kg·min⁻¹, max heart rate (HR): $199 \pm 3 \text{ b·min}^{-1}$) with two or more years of soccer experience were 169 recruited for participation. Exclusion criteria specified the diagnosis of lower-limb musculoskeletal 170 injury within the preceding six months, any medical condition affecting participation following 171 completion of a medical screening questionnaire and a $\dot{V}O2_{max} \leq 48.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, consistent with 172 previous ET literature (Stevenson et al., 2017). Furthermore, participants were asked to refrain from 173 174 strenuous exercise 48 h prior to testing. Institutional ethical approval was granted to allow the 175 undertaking of research and written informed consent was obtained from participants prior to data collection. 176

177

178 Preliminary visit and familiarisation trials

Participants attended the laboratory on four separate occasions. The preliminary visit (1st visit) was used to assess $\dot{V}O_{2max}$, once body mass and stature were determined (DPS-Promatic srl via Edison 21 47122, Forlì, Italy). A graded ramp test was completed until volitional exhaustion, which commenced at 10

km⁻¹, increasing by 1 km⁻¹ every 30 seconds, until 17 km⁻¹ was reached, at which point the gradient 182 was increased by 0.5% at each 30 second interval. A 72-hour period then separated the 1st visit from the 183 familiarisation trial (2nd visit). This visit involved a full habituation of all experimental procedures 184 including the full 120 min of soccer-specific exercise, and the completion of a standardised, treadmill-185 186 based warm-up (~10 min). This comprised intermittent changes in speeds and a dynamic stretching sequence as dynamic stretching has acute ergogenic effects on sprint and jump performance, versus 187 static stretching (Needham, Morse & Degens, 2009). Thereafter, main trial 1 (3rd visit) was performed, 188 followed by main trial two (4th visit). The familiarisation and both main trials were interspersed by 189 seven days to ensure full recovery. Identical procedures were followed for main trials one and two to 190 191 assess the reliability of the mechanical and physiological responses and all other data are presented from 192 main trial 2.

193

194 *Main trial procedures*

Participants were asked to record and replicate dietary intake for both main trials commencing 24 h 195 prior until completion of exercise. Both main trials were conducted at the same time of day to minimise 196 the effects of circadian variation. Upon arrival at the laboratory, a mid-flow urine sample was provided 197 198 to measure urine osmolality (Osmocheck, Vitech Scientific, West Sussex, UK). A blood sample was then taken at rest followed by an assessment of body mass and stature. The warm-up, as reported above, 199 was completed, and 200 ml of water consumed. Measurements of countermovement jump height (CMJ) 200 201 proceeded the warm-up, followed by a five min passive rest period. The quantity of water administered was standardised and provided at half-time (HT; 500 ml), full-time (FT; 300 ml) and during the two 202 203 min interval (200 ml) separating ET as per previous investigations (Harper et al., 2016b). Assessments 204 of CMJ and body mass were taken upon completion, immediately followed by an assessment of urine osmolality to determine hydration status; euhydration was accepted as <600 mOsm kg⁻¹ (Hillman et al., 205 206 2013). Urine-corrected mass changes were calculated for body mass assessment.

207

208 Soccer simulation

209 A modified version of 90 min of soccer-specific exercise (Page, Marrin, Brogden, Greig, et al., 2015) was performed on a motorised treadmill (h/p/ cosmos pulsar[®] 3p: h/p/cosmos sports & medical GmBH, 210 Nussdorf, Germany). It comprised of two 45 min halves interspersed by a 15 min passive recovery 211 break (HT), followed by a five min passive rest period, and a further two 15 min periods (ET), separated 212 213 by two min passive recovery. Similar to previous research (Harper et al., 2016b), the soccer-specific exercise was performed in 15 min blocks (with data assessed likewise). The changes in speed were set 214 at the treadmill limit (1.39 m·s⁻²) and players reached maximal speeds of 25 km·h⁻¹ during the soccer-215 specific exercise (Fig 1). This soccer-specific exercise was designed to simulate the durations, 216 intensities and the velocity profile of match-play based on previous notational analyses (Mohr et al., 217 2003). The participants covered a distance of 16.26 km (similar to actual match-play; (Winder et al., 218 219 2018) during the soccer-specific exercise with blood capillary samples, and d-RPE provided at the end 220 of each 15 min bout of activity. Mean values for RER, VO2 and HR, and PL data were taken as an 221 average across each 15 min block of exercise.

222

223 ***INSERT FIG 1***

224

225 *Experimental measures*

Respiratory flow volumes were recorded continuously throughout the trial using a volume transducer (Triple[®] V, Cortex, Germany) and breath-by-breath automated gas analysis system (Metamax® 3B-R2, Cortex, Germany). Thereafter, individual mean ($\dot{V}O2_{mean}$) values were expressed as average O₂ intake across each 15 min bout and the highest value reached was defined as $\dot{V}O2_{peak}$. Additionally, respiratory exchange ratio (RER) was calculated from the proportion of CO₂ produced and O₂ consumed through use of the manufacturer software equation (Metasoft®Studio Cortex, Germany; Wasserman et al., 1987) and defined as the mean over each 15 min bout of the trial.

233

The treadmill automatically paused at the end of each 15 min block of exercise and a finger-tip blood capillary sample was taken before being manually resumed and sample was analysed for BLa (Biosen C-Line; EKF-diagnostic GmBH, Cardiff, Wales). In addition, d-RPE was provided through use of the Borg 15-point (6-20) linear scale (Borg, 1998) and participants were asked to differentiate between RPE-L, RPE-B and RPE-O for each 15 min block of activity. To eliminate order effects, these values were collected in a counterbalanced order. CMJ height was measured using two portable photoelectric cells (Optojump, Italy) and was calculated using Optojump software. The jumps were separated by a 10 *s* passive rest interval for each of five respective time-points (rest, post HT, pre second-half, 90 min and 120 min). Participants were instructed to jump with hands on hips to nullify the influence of momentum; the mean of three jumps were presented for analysis.

244

A portable accelerometer (Kionix KX94, Kionix, Ithaca, New York, USA), housed inside a GPS unit 245 (OptimEye S5, Catapult Innovations, Scoresby, Australia) was used to measure tri-axial PL (PL_{Total}) at 246 a sampling frequency of 100 Hz. Participants were assigned the same unit for each trial to avoid inter-247 248 unit variation, as the Catapult OptimEye S5 device has been shown to possess high intra-unit reliability, 249 yet inconsistent inter-unit reliability (Nicolella et al., 2018). A tight-fitted neoprene garment was worn 250 to secure the device, housed in-between the scapulae to limit movement artefacts. A HR sensor (Polar 251 H10, Polar USA, United States) was synchronised with the GPS unit, attached directly to the vest via a 252 snap fastener and worn inferior to the sternum to quantify both mean (HR_{mean}) and max (HR_{max}) HR 253 values; HR_{max} was defined as the peak value recorded during a given 15 min exercise bout. Uni-axial PlayerLoadTM was determined for each movement plane (vertical [PL_V], anterior-posterior [PL_{A-P}] and 254 medial-lateral [PL_{M-L}]). Participant PL_{Total} values were derived from the summation of the 255 256 aforementioned planes of motion. The relative contributions of the uni-axial vectors were calculated (PLv%, PLA-P% and PLM-L%) for the percentage contributions to PLTotal. All PL data were expressed as 257 arbitrary units (au) and defined across each 15 min period of soccer exercise. 258

259

260 Statistical analyses

261 Data were analysed using both Statistical Package for the Social Sciences (IBM SPSS Statistics 24 for

windows; SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p \le 0.05$ prior to analyses.

All data were expressed as mean ± standard deviation, unless indicated otherwise. An *a priori* power

analysis (GPower v3.1; Germany) deemed a sufficient sample size of 11 based on ≥80% power

265 $(1 - \beta)$, an alpha (α) of 0.05 and a medium effect size (d = 0.5) based on previous PL_{Total} data (Page et 266 al., 2019). Normal distribution of data and variance homogeneity between each 15 min interval were 267 assessed using Shapiro–Wilk and a Levene's test, respectively.

268 The data were divided into the following epochs: E1 (00:00–14:59 min), E2 (15:00-29:59 min), E3

269 (30:00-44:59 min), E4 (45:00-59:59 min), E5 (60:00-74:59 min), E6 (75:00-89:59 min), E7 (90:00-

270 104:59 min) E8 (105:00-119:59 min). A repeated measures analysis of variance was used to

determine the effect of time for each outcome. To control for family-wise error, post-hoc pairwise

272 comparisons were applied using the Bonferroni correction method. In accordance with Hopkins

273 (2000), test-retest reliability was established using typical error (TE) and coefficient of variation (CV)

for absolute reliability, and Pearson's correlation coefficient (r) for relative reliability. For CV, <10%

275 was accepted as good absolute reliability (Atkinson & Nevill, 1998) and thresholds for *r* were

considered moderate (0.3–0.5), strong (0.5–0.7), and very strong (>0.7) (Hopkins, 2000). Effect sizes

(ES) were calculated using Cohen's d and were categorised as small (0.2), medium (0.5), and large

278 (0.8) (Fritz, Morris & Richler, 2012).

279

280 Results

All participants were presented for data analysis, except for one participant for ventilatory values (owing to claustrophobia) and another for the blood analyses (due to haemophobia), albeit the remainder of their data was used.

284

285 PlayerLoad responses throughout 120 minutes of soccer exercise

As highlighted in Table 1, a main effect for time was observed for PL_{Total} (p = 0.045) with post-hoc analyses revealing that values were higher during E8 compared to E1 (+9.9 ± 5.3%; p = 0.002; d = 0.8; Fig 2). Likewise, a main effect for time was found for PL_V (p = 0.002) with significant increases observed during E8 (118 ± 19 a.u) compared to E1 (111 ± 16 a.u). In addition, main effects for time were identified for PL_{A-P} (p = 0.011) with significance identified between E1 (53 ± 7 a.u) and E8 (60 ± 5 a.u). No time effects were established for PL_{M-L} (p = 0.074), $PL_{V\%}$ (p = 0.703), $PL_{A-P\%}$ (p = 0.835) or $PL_{M-L\%}$ (p = 0.463).

294 Physiological and physical responses throughout 120 minutes of soccer exercise

Significant main effects for time were evident for RER (p = 0.001) with decreases observed during E8 295 $(0.87 \pm 0.03 \text{ a. u})$ compared to E1 $(0.92 \pm 0.02 \text{ a.u})$. Additionally, HR_{mean} demonstrated a main effect 296 297 for time (p = 0.003), with differences identified between E3 ($159 \pm 16 \text{ b} \cdot \text{min}^{-1}$) and E4 ($152 \pm 16 \text{ b} \cdot \text{min}^{-1}$) ¹; $p \le 0.001$), as well as E4 and E5 (159 ± 16 b·min⁻¹; p = 0.003). No main effects for time were observed 298 for $\dot{V}O2_{\text{mean}}$ (p = 0.407), $\dot{V}O2_{\text{peak}}$ (p = 0.879), HR_{peak} (p = 0.959) or BLa (p = 0.203). As outlined in Table 299 300 2, a main effect of time for both RPE-L (p = 0.001) and RPE-O (p = 0.003) was found. For RPE-L, a significant increase was detected from E1 (11 ± 1) to E8 (17 ± 1; $p \le 0.001$) and a similar pattern was 301 302 evident for RPE-O between E1 (11 ± 1) and E8 (17 ± 2; $p \le 0.001$). No main effects were observed for 303 RPE-B (p = 0.076). Main effects for time were established for CMJ (p = 0.020), however, post-hoc 304 comparisons revealed no differences between time points.

305

No differences were detected for urine osmolality pre $(610 \pm 132 \text{ mOsmoL} \cdot \text{kg}^{-1})$ and post $(586 \pm 135 \text{ mOsmoL} \cdot \text{kg}^{-1}; p = 0.985)$ trial. Once urine-corrected, no changes in mass were identified from pre $(70.5 \pm 8.8 \text{ kg})$ to post $(69.4 \pm 8.5 \text{ kg}; p = 0.928)$ trial.

309

310 ***INSERT FIG 2***

311 ***INSERT TABLE 1***

312

313 Reliability of biomechanical, physiological and physical responses

All PL variables across all time points demonstrated a significant, very strong *r*, except for E1 and E2 for PL_{M-L%}. All CV's were <8% for all PL metrics across each time point (Table 1). For RER, moderate to very strong (r = 0.33-0.72) correlations were observed during each epoch of which E4, E6, E7 and E8 displayed significance ($p \le 0.05$). All CVs for RER were <4% irrespective of time point. All time points for $\dot{V}O2_{mean}$ and $\dot{V}O2_{peak}$ (except for E8 for $\dot{V}O2_{mean}$; E5 and E6 for $\dot{V}O2_{peak}$) were >0.70 (r), however, both demonstrated good (<7%) CVs. For all epochs, the CV values were lower than 10% and strong and very strong (r = 0.51-0.75) relationships were found between trials for HR_{mean}. Majority of 321 CVs were >10% for HR_{peak} except E1, E4 and E6, and r was less than 0.3 for all epochs excluding E1 and E6. All CVs were <3% for d-RPE values, and RPE-L and RPE-B demonstrated moderate to very 322 strong (0.39-0.88) relationships for all time points except for RPE-L during E2 (0.29) and RPE-B for 323 E4 (0.34). Although RPE-O demonstrated moderate to very strong relationships for six out of eight time 324 325 points (E1, E4-E8; r = 0.45-0.9), all CVs were <3% throughout the trial (refer to Table 2 for significance). Average CVs for CMJ were ~1%, and very strong, significant correlations were detected 326 (>0.9; r). CVs were <10% for BLa and only two time points for r values were identified as significant 327 328 $(p \le 0.05).$

329

330 ***INSERT TABLE 2 ***

331

332 Discussion

The aim of this study was to investigate the biomechanical and physiological responses to a 120 min of treadmill-based soccer-specific exercise. In accordance with our hypotheses, biomechanical performance was negatively impacted as a function of time, with further reductions in efficiency identified for PL_{Total} during the final 30 min of exercise. Furthermore, increases in d-RPE and the rate of fat oxidation were observed during ET. Furthermore, the test-retest reliability for most of the variables was moderate to very strong for relative reliability (i.e., *r*) and good for absolute reliability (i.e., CVs).

340

To date, evidence suggests that the physical capacity of players is reduced during ET matches (Peñas, Dellal, Owen & Gómez-Ruano, 2015; Russell et al., 2015; Winder et al., 2018). However caution should be applied when interpreting match-play observations as they do not account for contextual variables (Abbott, Brownlee, Harper, Naughton & Clifford, 2018) such as stoppages in play and altered tactical approaches. However, in the present study, the use of a treadmill simulation enabled the standardisation of workload, thus allowing for any observed changes to be attributable to changes in a player's capacity to cope with the work rate, and as such, any observed changes in PL can be 348 attributable to changes in movement efficiency rather than modifications to the activity and

349 locomotion profile performed (Page et al., 2015).

350 Page et al. (2015) assessed PL responses across 90 min of treadmill-based, soccer-specific bouts of 351 fixed, identical bouts of repeated activity. The authors reported increases in PL_{Total}, concurrent with 352 reductions in PL_{V%} as a function of exercise duration. Similarly, previous match-play observations 353 found a reduction in the vertical contribution of PL in Australian rules football players (Cormack et al., 354 2013). Consistent with the two studies above, our data suggests a similar temporal pattern, which, 355 although not directly measured in the current study, may be indicative of an attempt to conserve energy 356 by adjusting stride length or frequency, or both, which could in turn reduce running efficiency (Hobara et al., 2010). It appears that when fatigued, individual running patterns become biomechanically less 357 efficient, which can cause additional stress to joints, tendons and ligaments (Wilk, Nau & Valero, 2009). 358 Furthermore, the modifications in vertical movement as seen in the present research have previously 359 360 been associated with reductions in lower extremity stiffness (Buchheit, Gray & Morin, 2015).

361

Although the relationship between stiffness and injury is not well established, it is believed that 362 363 disproportionate levels of stiffness can lead to an increased injury-risk to the lower extremities (Butler, 364 Crowell, & Davis, 2003). From our data it can be speculated that participants displayed less vertical displacement as a function of exercise duration, indicative of a reduced vertical stiffness and increased 365 risk to soft tissues (Hughes & Watkins, 2008). However, during matches, it is likely that in a 366 367 subconscious attempt to avoid injury, players will reduce work rate, in turn impacting physical 368 performance. In further support of a potential reduction in performance, impaired movement efficiency 369 may also manifest in a reduced ability to maintain high intensity output, with this in turn reducing a 370 team's ability to score goals. To support this suggestion, it has been suggested previously by Faude et 371 al., (2012) that 45% of goals in the German Bundesliga were preceded with straight line sprints. 372 Therefore, a reduced capacity during ET may result in fewer matches decided during this period and as such, preparing players for the additional 30 min duration associated with ET may reduce injury-risk 373 374 and increase physical performance, ultimately increasing team success.

376 Another novel feature of this study was the measurement of substrate utilisation via gas analysis (i.e., RER) across 120 min of soccer-specific exercise. An RER of 1.0 indicates 100% CHO oxidation (i.e., 377 378 glycolysis), whilst a value of 0.70 indicates 100% fatty acid oxidation (i.e., lipolysis), with 0.85 a.u 379 indicating an equal contribution. According to the current data and corresponding with previous ET 380 research (Stevenson et al., 2017), players utilise both fat and CHO to fuel soccer exercise, however, fat 381 oxidation increases during ET. Despite observing a shift in the direction of lipolysis and a downregulation in glycolysis, we are cognizant that our RER values during ET (0.87 a.u) suggest that 382 383 CHO is still being utilised. Furthermore, as participants were less efficient (i.e., increased energy cost to produce the same activity) across bouts, it was surprising to find a ~29% reduction (although non-384 significant) in BLa concentrations between E6 and E7 (Fig 3). This further supports that glucose levels 385 386 were potentially depleted and less was available to undergo biotransformation during ET as under 387 anaerobic conditions, BLa is the final product of glycolysis.

388 ***INSERT FIG 3***

389 Prior to the main trials, participants in the current research were not required to consume a pre-match 390 meal as done in a previous investigation that has attempted to measure changes in substrate use 391 (Stevenson et al., 2017). Therefore, this methodological discrepancy limits comparison between studies 392 as participants in the current study may have completed exercise with lesser endogenous CHO at 393 baseline. Furthermore, within-match CHO consumption is customary for soccer players (Anderson et 394 al., 2016) and participants in the present research were limited to water intake. Evidence shows that 395 exercising without exogenous CHO ingestion results in inherent increases in the oxidation of plasma 396 FFA (Gribok et al., 2016), however this is largely influenced by exercise intensity and duration, among 397 other factors (van Loon, Greenhaff, Constantin-Teodosiu, Saris & Wagenmakers, 2001). Accordingly, further investigations examining the effect of CHO supplementation across 120 min of soccer-specific 398 exercise are warranted. 399

401 The present results indicate that the soccer-specific exercise imposed the most considerable load on RPE- L with scores reaching 17 ± 1 during ET, equating to the verbal anchor 'very hard' according to 402 the Borg scale. This finding is consistent with other work whereby RPE-L was highest during aerobic 403 404 exercise (Borg et al., 2010) and ~ 30 min post soccer match-play (Weston et al., 2015). However, given 405 this study is the first to employ a within soccer-specific exercise approach to measuring d-RPE, it is difficult to compare our findings directly to other literature; therefore, this research may be used to 406 407 inform future investigation that measures within-match changes in d-RPE. Furthermore, d-RPE typically increased as a function of exercise duration and was reliable between sessions ($CV \le 2.1\%$). 408 This suggests that d-RPE can be used to reliably assess internal load during soccer-specific exercise 409 410 and may be a feasible tool to differentiate player exertion to assist in tailoring training programmes 411 (Macpherson et al., 2019).

412

413 The test re-test responses to the current soccer-specific exercise simulation were moderate to strong. Previous convergent validity analysis by Berreira and colleagues assessing two sessions of a free-414 415 running soccer match simulation demonstrated higher CVs for PL (14.5-24.5%) (Barreira et al., 2017) 416 compared with the current study ($PL_{Total} = 2.8-5\%$.). Considering the increased reliability of responses 417 elicited during soccer exercise performed on treadmills, the current simulation may be preferable 418 compared with free-running protocols such as the Soccer Match Simulation (Harper et al., 2016b), 419 especially if researchers have logistical issues, such as limited space. That said, skill performance is 420 also impacted by exercise duration (Russell & Kingsley, 2011) and it must be noted that treadmills are limited concerning the incorporation of technical actions. Therefore, if researchers are interested in 421 422 assessing physical and physiological responses of players across 120 min of soccer-specific exercise, the current simulation may be desirable. 423

424

Within the scope of the limitations, the findings of the current study add to an expanding body of literature that has thus far, failed to quantify the biomechanical demands and substrate usage estimated through the use of gaseous exchange during ET. To conclude, our data show a temporal shift in predominant energy pathway utilisation (i.e., aerobic glycolysis to fat oxidation) during 120 min of soccer exercise. Likewise, changes in PL throughout 120 min of soccer-specific exercise were evident,
with further fatigue-induce changes identified during ET.

432 What does this article add?

This study took a novel approach to investigating soccer-specific exercise over 120 min, that is, utilising standardised periods of workload to assess fatigue responses. Based on these findings, we postulate that the reduced mechanical efficiency observed may hold fatigue-induced injury-risk implications for players during this period of match-play. However, the extent to which the additional demands associated with ET contribute to injury prevalence may be required through the longitudinal surveillance of soccer players. It is recommended that practitioners condition players to be able to cope with this additional workload associated with an additional 30 min to reduce potential injury occurrence, although the contemporary issue of fixture congestion may limit time to do so. Therefore, at which stage during the season this is developed and maintained must be considered. This research also confirms the importance of CHO consumption during soccer matches to maintain the glycolytic process, which may assist with offsetting the impact of fatigue. Finally, FIFA now authorise a fourth substitution during ET and as such, utilising replacements during this period may be efficacious to reduce the deleterious effect that fatigue may have on player performance.

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Fig 1 A schematic of an individual 15-minute bout of the soccer-specific exercise



Fig 2 This graph shows the time history changes in PL_{Total} response throughout 120 min of soccerspecific exercise. Data are expressed as mean \pm SD. a denotes significant difference from E7 for PLTotal b denotes significant difference from E8 for PLTotal (both $p \le 0.05$)





Fig 3 Mean RER and BLa concentrations across 120 min for each 15 min period of soccer-specific
exercise. Data are expressed as mean ± SD. a denotes significant difference from E7 for RER. b

658 denotes significant difference from E8 ($p \le 0.05$) for RER.

	Time										
Variable	E1	E2	E3	E4	E5	E6	E7	E8			
PL _{Total} (a.u)											
Trial 1	208 ± 27	213 ± 28	217 ± 28	217 ± 27	219 ± 26	220 ± 25	225 ± 22	227 ± 23			
Trial 2	215 ± 25^{gh}	$216\pm23^{\text{gh}}$	220 ± 22^{gh}	$226\pm27^{\rm h}$	$226\pm24^{\rm h}$	$229\pm25^{\rm h}$	230 ± 23^{abc}	234 ± 24^{abcdef}			
Mean	209 ± 25	212 ± 24	217 ± 24	220 ± 26	220 ± 24	222 ± 24	226 ± 22	229 ± 23			
CV (%)	5	4.7	4.3	4.1	3.7	2.8	3	3.2			
r	0.84*	0.86*	0.9*	0.9*	0.91*	0.95*	0.92*	0.91*			
TE	11.05	10.28	9.5	9.1	8.06	6.15	6.67	7.44			
$PL_{V}(a.u)$											
Trial 1	110 ± 19	112 ± 21	112 ± 21	113 ± 21	113 ± 21	113 ± 21	116 ± 19	116 ± 20			
Trial 2	$111\pm16^{\rm h}$	111 ± 16	113 ± 15	116 ± 20	$115\pm18^{\mathrm{g}}$	116 ± 19	117 ± 18	118 ± 19^{a}			
Mean	110 ± 17	111 ± 18	112 ± 18	115 ± 20	114 ± 19	115 ± 19	116 ± 18	117 ± 19			
CV (%)	4.9	5.5	5.7	4.3	4.1	2.1	2.2	2.4			
r	0.91*	0.9*	0.91*	0.95*	0.96*	0.98*	0.98*	0.97*			
TE	5.76	6.89	6.91	4.96	4.66	3.1	2.87	3.47			
PL _{A-P} (a.u)											
Trial 1	52 ± 8	54 ± 7	55 ± 7	55 ± 8	57 ± 7	58 ± 7	58 ± 7	60 ± 7			
Trial 2	53 ± 7^{gh}	53 ± 7^{gh}	55 ± 7^{gh}	56 ± 8	57 ± 6^{h}	58 ± 6^{h}	59 ± 6^{abc}	60 ± 5^{abcef}			
Mean	52 ± 7	54 ± 7	55 ± 7	56 ± 7	57 ± 6	58 ± 6	58 ± 6	60 ± 6			
CV (%)	6	4.8	3.3	5	4.6	4.3	4	4.9			
r	0.86*	0.88*	0.94*	0.9*	0.88*	0.84*	0.89*	0.9*			
TE	3.11	2.62	1.87	2.62	2.45	2.27	2.1	2.3			
$PL_{M-L}(a.u)$											
Trial 1	47 ± 6	47 ± 6	48 ± 6	49 ± 6	49 ± 6	49 ± 6	51 ± 6	51 ± 6			
Trial 2	$47\pm7^{\mathrm{fh}}$	48 ± 7^{gh}	$49\pm7^{\mathrm{gh}}$	50 ± 7	$50\pm7^{\mathrm{h}}$	50 ± 7^{a}	51 ± 7^{bc}	52 ± 8^{abce}			
Mean	47 ± 6	47 ± 6	49 ± 6	49 ± 6	49 ± 6	50 ± 6	51 ± 6	52 ± 7			
CV (%)	7.5	6.1	5.7	5.2	5.4	5.2	4.8	5.2			
r	0.75*	0.82*	0.84*	0.85*	0.85*	0.87*	0.89*	0.9*			
TE	3.5	2.85	2.79	2.58	2.6	2.61	2.44	2.65			
PL _{V%}								'			
Trial 1	52 ± 4	52 ± 4	52 ± 4	52 ± 4	52 ± 4	51 ± 4	51 ± 4	51 ± 4			
Trial 2	53 ± 3	52 ± 3	52 ± 3	52 ± 4	52 ± 4	52 ± 4	51 ± 4	51 ± 4			

Table 1. Biomechanical responses throughout 120 min of soccer-specific exercise (mean \pm SD)

Mean	52 ± 3	52 ± 3	52 ± 4	52 ± 4	52 ± 4	51 ± 4	51 ± 4	51 ± 4
CV (%)	1.8	2.5	1.9	1.5	2.1	1.4	0.9	1.6
r	0.94*	0.88*	0.95*	0.96*	0.95*	0.98*	0.99*	0.99*
TE	0.95	1.34	1.05	0.82	1.09	0.7	0.47	0.77
PL _{A-P%}								
Trial 1	25 ± 4	26 ± 3	26 ± 4	26 ± 4	26 ± 4	27 ± 4	26 ± 4	27 ± 4
Trial 2	$25\pm3^{\rm h}$	26 ± 3	26 ± 3^{a}					
Mean	25 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3
CV (%)	4.6	3.5	3.2	4.5	3.2	3.7	2.9	2.5
r	0.92*	0.95*	0.96*	0.95*	0.96*	0.97*	0.96*	0.98*
TE	1.05	0.85	0.79	1.02	0.82	0.87	0.79	0.68
PL _{M-L%}								
Trial 1	22 ± 2	22 ± 2	22 ± 2	22 ± 2	22 ± 2	22 ± 2	23 ± 2	$23 \pm \pm 2$
Trial 2	22 ± 1	22 ± 2	22 ± 2	22 ± 1	22 ± 1	22 ± 1	23 ± 1	23 ± 1
Mean	22 ± 1	22 ± 2	23 ± 2	23 ± 2				
CV (%)	5	4.9	4.1	3.5	3	2.8	2.3	1.9
r	0.46	0.56	0.71*	0.84*	0.88*	0.86*	0.94*	0.96*
TE	1.1	1.08	0.93	0.82	0.64	0.67	0.52	0.43

Note. PL_{Total} = tri- axial playerload; PL_{V} = vertical playerload; PL_{A-P} anterior/ posterior playerload; PL_{M-L} = medial/lateral playerload; $PL_{V\%}$ = Percentage contribution of vertical vector; $PL_{A-P\%}$ = percentage contribution of anterior/posterior vector; $PL_{M-L\%}$ = percentage contribution of medial/lateral vector; a.u = arbitrary units; CV = coefficient of variation (%); r = Pearson's correlation coefficient; TE = typical error; E1 = 00:00–14:59 min, E2 = 15:00–29:59 min, E3 = 30:00–44:59 min, E4 = 45:00–59:59 min, E5 = 60:00–74:59 min, E6 = 75:00–89:59 min, E7 = 90:00–104:59 min and E8 = 105:00–119:59 minutes; mean = mean of both trial 1 and 2. Significant differences are only reported for trial 2. ^{a-h} Denotes significance from E1-E8 ($p \le 0.05$), respectively.

* Denotes significance for Pearson's correlation coefficient for specified time points between trials ($p \le 0.05$).

669

		Time							
Variable	E1	E2	E3	E4	E5	E6	E7	E8	
RER (a.u)									
Trial 1	0.93 ± 0.03	0.92 ± 0.02	0.92 ± 0.02	0.90 ± 0.03	0.91 ± 0.02	0.89 ± 0.03	0.88 ± 0.02	0.88 ± 0.03	
Trial 2	0.92 ± 0.02^{gh}	0.92 ± 0.02^{gh}	0.91 ± 0.02^{gh}	$0.89\pm0.02^{\text{gh}}$	$0.90\pm0.02^{\text{gh}}$	$0.89\pm0.02~^{\rm h}$	0.87 ± 0.02^{abcde}	0.87 ± 0.02^{abcdef}	
Mean	0.93 ± 0.03	0.92 ± 0.02	0.91 ± 0.02	0.90 ± 0.02	0.91 ± 0.02	0.89 ± 0.02	0.88 ± 0.02	0.87 ± 0.03	
CV (%)	2.9	2.1	2.2	2.9	2.6	2.7	2.1	3.2	
r	0.33	0.47	0.42	0.65*	0.52	0.71*	0.72*	0.67*	
TE	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	
$\dot{V}O2_{mean}$ (ml·kg ⁻¹ ·min ⁻¹)									
Trial 1	35 ± 4	34 ± 5	35 ± 5	34 ± 5	34 ± 5	34 ± 5	34 ± 5	34 ± 5	
Trial 2	34 ± 4	34 ± 4	34 ± 5	34 ± 4	34 ± 4	34 ± 4	34 ± 4	33 ± 4	
Mean	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	
CV (%)	2.1	2.1	1.9	1.4	1.6	1.6	1.3	2.4	
r	0.78*	0.8*	0.85*	0.9*	0.88*	0.87*	0.91*	0.67*	
TE	2.07	2.06	1.88	1.41	1.59	1.63	1.3	2.35	
$\dot{V}O2_{peak}$ (ml·kg ⁻¹ ·min ⁻¹)									
Trial 1	35 ± 4	34 ± 5	35 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	
Trial 2	34 ± 4	34 ± 4	34 ± 5	34 ± 4	34 ± 4	34 ± 4	34 ± 4	33 ± 4	
Mean	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	34 ± 4	
CV (%)	4.4	4.1	5.7	5.4	5.5	6.3	3.2	4	
r	0.85*	0.9*	0.79*	0.79*	0.64*	0.29	0.85*	0.75*	
TE	4.29	4.06	5.58	5.22	5.32	6.15	3.61	3.9	
HR_{mean} (b·min ⁻¹)									
Trial 1	154 ± 17	157 ± 19	160 ± 21	152 ± 18	157 ± 22	157 ± 22	160 ± 20	163 ± 18	
Trial 2	150 ± 13	154 ± 16	159 ± 16^{d}	152 ± 16^{ce}	157 ± 16^{d}	157 ± 16	157 ± 15	159 ± 16	
Mean	152 ± 15	155 ± 17	159 ± 18	152 ± 17	157 ± 18	157 ± 19	158 ± 17	161 ± 17	
CV (%)	3.2	6.2	8.8	8.6	8.9	7.2	7.7	5.7	
r	0.91*	0.77*	0.56	0.53	0.59	0.75*	0.61*	0.77*	
TE	9.18	9.08	12.84	12.18	13.01	10.68	11.61	8.74	
HR_{peak} (b·min ⁻¹)									
Trial 1	186 ± 26	179 ± 21	179 ± 21	171 ± 22	173 ± 21	181 ± 24	178 ± 23	183 ± 24	
Trial 2	179 ± 18	176 ± 19	176 ± 20	175 ± 18	178 ± 23	175 ± 17	180 ± 16	185 ± 15	
Mean	182 ± 22	177 ± 19	177 ± 20	173 ± 19	175 ± 21	178 ± 20	179 ± 19	184 ± 20	

Table 2. Physiological responses throughout 120 min of soccer-specific exercise (mean \pm SD)

CV (%)	6	10.2	11	9.9	11.5	9.1	10.6	11.7
r	0.85	0.24	0.19	0.28	0.17	0.43	0.16	-0.02
TE	10.79	17.56	18.79	17	20.01	16.09	18.36	20.3
BLa (mmol \cdot L ⁻¹)								
Trial 1	2.4 ± 1.0	2.4 ± 1.0	2.1 ± 0.6	2.2 ± 1.2	2.5 ± 1.2	2.6 ± 1.2	2.2 ± 1.0	2.4 ± 1.0
Trial 2	2.4 ± 0.9	2.9 ± 1.5	2.7 ± 1.2	2.3 ± 0.9	3.0 ± 1.3	3.8 ± 2.7	2.7 ± 0.9	2.4 ± 1.1
Mean	$2.4\ \pm 0.9$	2.6 ± 1.2	$2.4\ \pm 0.9$	$2.4\ \pm 1.0$	$2.8 \hspace{0.1in} \pm 1.2 \hspace{0.1in}$	3.3 ± 2.1	$2.6\ \pm 0.9$	2.4 ± 1.0
CV (%)	0.8	0.9	0.7	0.5	0.7	1.8	0.6	0.6
r	0.6	0.41	0.12	0.46	0.89*	0.63*	0.32	0.59
TE	0.77	0.94	0.7	0.51	0.67	1.78	0.57	0.6
RPE-L (a.u)								
Trial 1	11 ± 1	11 ± 1	12 ± 1	13 ± 1	14 ± 1	15 ± 1	15 ± 1	17 ± 1
Trial 2	$11 \pm 1^{\text{gh}}$	$11 \pm 1^{\text{gh}}$	12 ± 1^{gh}	13 ± 1^{gh}	$14 \pm 1^{\text{gh}}$	15 ± 1^{gh}	15 ± 1^{abcdefh}	17 ± 1^{abcdefg}
Mean	11 ± 1	11 ± 1	12 ± 1	13 ± 1	14 ± 1	15 ± 1	15 ± 1	17 ± 1
CV (%)	0.7	2.1	1.9	1.8	0.7	0.7	1.6	1.5
r	0.64*	0.29	0.61*	0.39	0.61*	0.87*	0.64*	0.78*
TE	0.64	0.77	0.51	0.82	0.74	0.41	0.68	0.59
RPE-B (a.u)								
Trial 1	11 ± 1	12 ± 1	12 ± 1	13 ± 1	14 ± 1	14 ± 1	15 ± 2	16 ± 2
Trial 2	$11 \pm 1^{\mathrm{gh}}$	$12\pm1^{\text{gh}}$	$12\pm1^{\text{gh}}$	$13\pm1^{\text{gh}}$	14 ± 1	14 ± 1	15 ± 2^{abcd}	15 ± 2^{abcd}
Mean	11 ± 1	12 ± 1	12 ± 1	13 ± 1	14 ± 1	14 ± 1	15 ± 2	16 ± 2
CV (%)	0.7	1.8	0.7	0.7	0.7	0.7	1.5	0.7
r	0.75*	0.44	0.39	0.34	0.64*	0.68*	0.88*	0.86*
TE	0.55	0.84	0.77	0.96	0.82	0.84	0.76	0.86
RPE-O (a.u)								
Trial 1	11 ± 1	12 ± 1	13 ± 1	13 ± 1	14 ± 1	15 ± 1	14 ± 1	16 ± 1
Trial 2	$11 \pm 1^{\mathrm{gh}}$	$11 \pm 1^{\mathrm{gh}}$	12 ± 1^{gh}	13 ± 1^{gh}	$14 \pm 1^{\text{gh}}$	15 ± 1^{gh}	15 ± 2^{abcdefh}	17 ± 2^{abcdefg}
Mean	11 ± 1	12 ± 1	12 ± 1	13 ± 1	14 ± 1	15 ± 1	16 ± 1	17 ± 1
CV (%)	2.1	0.7	0.7	0.7	0.7	0.7	1.4	0.7
r	0.84	0.17	0.22	0.9	0.45	0.84*	0.87*	0.9*
TE	1.04	0.93	0.77	1.06	0.77	0.47	0.56	0.47
	Pre trial	Post HT	Pre 2 nd half	90 min	120 min	_		
CMJ (cm)						-		
Trial 1	35.2 ± 6.3	36.0 ± 6.4	33.9 ± 6.8	36.5 ± 7.2	34.2 ± 8.5			
Trial 2	35.4 ± 7.1	36.5 ± 7.4	34.6 ± 7.9	36.8 ± 7.7	34.3 ± 8.4			

Mean	35.3 ± 6.4	36.2 ± 6.6	34.3 ± 7.1	36.6 ± 7.1	34.2 ± 8.1
CV (%)	0.8	0.7	1.1	0.9	0.7
r	0.93*	0.94*	0.92*	0.99*	0.99*
TE	1.96	1.98	2.27	0.8	1.04

Note. $RER = respiratory exchange ratio; \dot{VO2} = oxygen consumption; HR = Heart rate; BLa = blood lactate; RPE = Ratings of perceived exertion; RPE-L = RPE legs; RPE-B = RPE breathlessness; RPE-O = RPE overall; CMJ = countermovement jump height; a.u = arbitrary units; <math>CV = coefficient of variation$ (%); r = Pearson's correlation coefficient; TE = typical error; E1 = 00:00-14:59 min, E2 = 15:00-29:59 min, E3 = 30:00-44:59 min, E4 = 45:00-59:59 min, E5 = 60:00-74:59 min, E6 = 75:00-89:59 min, E7 = 90:00-104:59 min and E8 = 105:00-119:59 minutes; mean = mean of both trial 1 and 2. Significant differences are only reported for trial 2. $<math>a^{-h}$ Denotes significant difference from E1-E8 ($p \le 0.05$), respectively.

* Denotes significance for Pearson's correlation coefficient for specified time points between trials ($p \le 0.05$).

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