Manuscript full title: Biomechanical and physiological responses to 120 minutes of soccer-specific exercise.

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Abstract

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

Keywords Football, PlayerLoad™; Oxygen consumption; Perceived exertion; Fatigue; Reliability

Introduction:

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, periods of high-speed running (HS) and sprinting, as well as rapid accelerations and decelerations that may result in temporary fatigue (Stølen, Chamari, Castagna & Wisløff, 2005). This is evidenced by reductions in HS distances during the second half compared to the first half in Premier League matches, both with (Bradley et al., 2009) and without the ball (Di Salvo, Gregson, Atkinson, Tordoff & Drust, 2009). In recent years, researchers have investigated these responses during 120 min of 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 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, Fothergill, West, Stevenson & Russell, 2016a).

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 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 actions in elite soccer players has been reported during the final 15 min of ET compared with a number of other time points during matches (Harper, West, Stevenson & Russell, 2014). However, it is unlikely that decreases in the number of actions are explained by a reduction in skill proficiency, rather, a fatigue-related compromised ability to be involved with build-up play. In further support of this notion, increased neuromuscular fatigue (i.e., reduced muscle force production) has been observed during and following ET (Goodall et al., 2017). Furthermore, higher plasma glycerol, non-esterified fatty acids (NEFA) and adrenaline as well as lower blood lactate (BLa) and glucose has been observed during ET, which is indicative of an increase rate of lipolysis (Stevenson et al., 2017). 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.

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

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 (e.g., accelerometers). A novel metric that has been proposed by Catapult Innovations as a measure of movement efficiency is that of PlayerLoad™ (PL) (Cormack, Mooney, Morgan & McGuigan, 2013). This parameter is defined as the instantaneous rate of acceleration across three planes of motion (i.e., vertical, anterior/posterior and medial/lateral) and otherwise referred to as 'jerk' (Nicolella, Torres-Ronda, Saylor & Schelling, 2018). Previously, changes in PL have been observed during the latter 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 (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 changes may be a decreased neuromuscular control (Oliver et al., 2014), potentially compromising dynamic joint stability (Hughes & Watkins, 2008). This could be owing to a reduced absorption capacity and increased stress response to soft tissues (Hughes & Watkins, 2008), leading to impaired movement efficiency which suggests an increased load per distance covered (Barrett et al., 2016) and potential increased injury-risk. Furthermore, recent research suggests that exacerbated recovery following matches that proceed to ET (Winder et al., 2018) may potentially predispose players to injury, especially during fixture congested microcyles (Silva et al., 2018). Possible risk factors for injury may involve lower limb muscle activity reductions and inhibited recovery kinetics, contributing 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 whether recovery and injury-risk are impacted further by the additional duration and workload associated with ET.

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

(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, Spears, Ward, & Weston, 2018). Given the demands of soccer are multifactorial, d-RPE may highlight distinct mechanisms of fatigue (Macpherson et al., 2019). For instance, RPE-L could quantify 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 higher central and physiological load imposed on players, highlighting the need for aerobic training. 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 understanding of players perceptions of load over 120 min of soccer-specific exercise.

As mentioned previously, ET may cause a change in the predominant energy source used (i.e., endogenous carbohydrate [CHO] to fat)) (Stevenson et al., 2017). However, previous researchers have used venous blood samples to estimate these changes in substrate utilisation over 120 min (Stevenson et al., 2017), which is logistically difficult to measure during exercise. An alternative measure used to estimate relative proportions of CHO and fat oxidation is the respiratory exchange ratio (RER). This measure calculates volumes of gas exhange (i.e., O_2 uptake and CO_2 production) through use of a breah-by-breath system (Goedecke et al., 2000). Indeed, treadmill-based simlations offer a feasible approach to measuring RER as fixed periods of exercise intensity are required (Page et al., 2015). Likewise, using 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 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 insight into CHO usage during ET and whether CHO intake must be modulated to spare endogenous CHO use and help maintain performance.

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.

Materials and methods

Participants and experimental authorisation

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 [$\dot{V}O_{2max}$]: 56.58 ± 7.23 $ml \cdot kg \cdot min^{-1}$, max heart rate (HR): 199 ± 3 $b \cdot min^{-1}$) with two or more years of soccer experience were recruited for participation. Exclusion criteria specified the diagnosis of lower-limb musculoskeletal injury within the preceding six months, any medical condition affecting participation following completion of a medical screening questionnaire and a $\dot{V}O2_{max} \leq 48.5\ ml \cdot kg^{-1} \cdot min^{-1}$, consistent with previous ET literature (Stevenson et al., 2017). Furthermore, participants were asked to refrain from strenuous exercise 48 h prior to testing. Institutional ethical approval was granted to allow the undertaking of research and written informed consent was obtained from participants prior to data collection.

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·h^{-1}, increasing by 1 km·h^{-1} every 30 seconds, until 17 km·h^{-1} was reached, at which point the gradient was increased by 0.5% at each 30 second interval. A 72-hour period then separated the 1st visit from the familiarisation trial (2nd visit). This visit involved a full habituation of all experimental procedures including the full 120 min of soccer-specific exercise, and the completion of a standardised, treadmill-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 static stretching (Needham, Morse & Degens, 2009). Thereafter, main trial 1 (3rd visit) was performed, followed by main trial two (4th visit). The familiarisation and both main trials were interspersed by seven days to ensure full recovery. Identical procedures were followed for main trials one and two to assess the reliability of the mechanical and physiological responses and all other data are presented from main trial 2.

Main trial procedures

Participants were asked to record and replicate dietary intake for both main trials commencing 24 h prior until completion of exercise. Both main trials were conducted at the same time of day to minimise the effects of circadian variation. Upon arrival at the laboratory, a mid-flow urine sample was provided 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, was completed, and 200 ml of water consumed. Measurements of countermovement jump height (CMJ) 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 min interval (200 ml) separating ET as per previous investigations (Harper et al., 2016b). Assessments 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., 2013). Urine-corrected mass changes were calculated for body mass assessment.

Soccer simulation

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, Nussdorf, Germany). It comprised of two 45 min halves interspersed by a 15 min passive recovery break (HT), followed by a five min passive rest period, and a further two 15 min periods (ET), separated 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 at the treadmill limit (1.39 $m \cdot s^{-2}$) and players reached maximal speeds of 25 $km \cdot h^{-1}$ during the soccer-specific exercise (Fig 1). This soccer-specific exercise was designed to simulate the durations, intensities and the velocity profile of match-play based on previous notational analyses (Mohr et al., 2003). The participants covered a distance of 16.26 km (similar to actual match-play; (Winder et al., 2018) during the soccer-specific exercise with blood capillary samples, and d-RPE provided at the end of each 15 min bout of activity. Mean values for RER, $\dot{V}O2$ and HR, and PL data were taken as an average across each 15 min block of exercise.

*****INSERT FIG 1*****

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 O_2 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 CO_2 produced and O_2 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.

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.

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

Statistical analyses

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 $p \leq 0.05$ 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

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

The data were divided into the following epochs: 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) 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 comparisons were applied using the Bonferroni correction method. In accordance with Hopkins (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% 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 (0.8) (Fritz, Morris & Richler, 2012).

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.

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 $PL_{A\text{-}P}$ (p = 0.011) with significance identified between E1 (53 ± 7 a.u) and E8 (60 ± 5 a.u). No time effects were established for $PL_{M\text{-}L}$ (p = 0.074), $PL_{V\%}$ (p = 0.703), $PL_{A\text{-}P\%}$ (p = 0.835) or $PL_{M\text{-}L\%}$ (p = 0.463).

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 (0.87 ± 0.03 a. u) compared to E1 (0.92 ± 0.02 a.u). Additionally, HR_{mean} demonstrated a main effect for time (p = 0.003), with differences identified between E3 (159 ± 16 $b \cdot min^{-1}$) and E4 (152 ± 16 $b \cdot min^{-1}$; $p \leq 0.001$), as well as E4 and E5 (159 ± 16 $b \cdot min^{-1}$; p = 0.003). No main effects for time were observed for $\dot{V}O2_{mean}$ (p = 0.407), $\dot{V}O2_{peak}$ (p = 0.879), HR_{peak} (p = 0.959) or BLa (p = 0.203). As outlined in Table 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; $p \leq 0.001$) and a similar pattern was evident for RPE-O between E1 (11 ± 1) and E8 (17 ± 2; $p \leq 0.001$). No main effects were observed for RPE-B (p = 0.076). Main effects for time were established for CMJ (p = 0.020), however, post-hoc comparisons revealed no differences between time points.

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

*****INSERT FIG 2*****

*****INSERT TABLE 1*****

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 $PL_{M\text{-}L\%}$. 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 ($p \leq 0.05$). 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

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 strong (0.39-0.88) relationships for all time points except for RPE-L during E2 (0.29) and RPE-B for E4 (0.34). Although RPE-O demonstrated moderate to very strong relationships for six out of eight time 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 (>0.9; *r*). CVs were <10% for BLa and only two time points for *r* values were identified as significant ($p \leq 0.05$).

*****INSERT TABLE 2 *****

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).

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 attributable to changes in movement efficiency rather than modifications to the activity and locomotion profile performed (Page et al., 2015).

Page et al. (2015) assessed PL responses across 90 min of treadmill-based, soccer-specific bouts of fixed, identical bouts of repeated activity. The authors reported increases in PL_{Total}, concurrent with reductions in $PL_{V\%}$ as a function of exercise duration. Similarly, previous match-play observations found a reduction in the vertical contribution of PL in Australian rules football players (Cormack et al., 2013). Consistent with the two studies above, our data suggests a similar temporal pattern, which, although not directly measured in the current study, may be indicative of an attempt to conserve energy 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 efficient, which can cause additional stress to joints, tendons and ligaments (Wilk, Nau & Valero, 2009). Furthermore, the modifications in vertical movement as seen in the present research have previously been associated with reductions in lower extremity stiffness (Buchheit, Gray & Morin, 2015).

Although the relationship between stiffness and injury is not well established, it is believed that disproportionate levels of stiffness can lead to an increased injury-risk to the lower extremities (Butler, 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 risk to soft tissues (Hughes & Watkins, 2008). However, during matches, it is likely that in a subconscious attempt to avoid injury, players will reduce work rate, in turn impacting physical performance. In further support of a potential reduction in performance, impaired movement efficiency may also manifest in a reduced ability to maintain high intensity output, with this in turn reducing a team's ability to score goals. To support this suggestion, it has been suggested previously by Faude et al., (2012) that 45% of goals in the German Bundesliga were preceded with straight line sprints. 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 and increase physical performance, ultimately increasing team success.

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., glycolysis), whilst a value of 0.70 indicates 100% fatty acid oxidation (i.e., lipolysis), with 0.85 a.u indicating an equal contribution. According to the current data and corresponding with previous ET research (Stevenson et al., 2017), players utilise both fat and CHO to fuel soccer exercise, however, fat 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 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-significant) in BLa concentrations between E6 and E7 (Fig 3). This further supports that glucose levels were potentially depleted and less was available to undergo biotransformation during ET as under anaerobic conditions, BLa is the final product of glycolysis.

*****INSERT FIG 3*****

Prior to the main trials, participants in the current research were not required to consume a pre-match meal as done in a previous investigation that has attempted to measure changes in substrate use (Stevenson et al., 2017). Therefore, this methodological discrepancy limits comparison between studies as participants in the current study may have completed exercise with lesser endogenous CHO at baseline. Furthermore, within-match CHO consumption is customary for soccer players (Anderson et al., 2016) and participants in the present research were limited to water intake. Evidence shows that exercising without exogenous CHO ingestion results in inherent increases in the oxidation of plasma FFA (Gribok et al., 2016), however this is largely influenced by exercise intensity and duration, among 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 exercise are warranted.

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 the Borg scale. This finding is consistent with other work whereby RPE-L was highest during aerobic exercise (Borg et al., 2010) and ~ 30 min post soccer match-play (Weston et al., 2015). However, given 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 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 ≤ 2.1%). This suggests that d-RPE can be used to reliably assess internal load during soccer-specific exercise and may be a feasible tool to differentiate player exertion to assist in tailoring training programmes (Macpherson et al., 2019).

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-running soccer match simulation demonstrated higher CVs for PL (14.5-24.5%) (Barreira et al., 2017) compared with the current study (PL_{Total} = 2.8-5%.). Considering the increased reliability of responses elicited during soccer exercise performed on treadmills, the current simulation may be preferable compared with free-running protocols such as the Soccer Match Simulation (Harper et al., 2016b), especially if researchers have logistical issues, such as limited space. That said, skill performance is 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 assessing physical and physiological responses of players across 120 min of soccer-specific exercise, the current simulation may be desirable.

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.

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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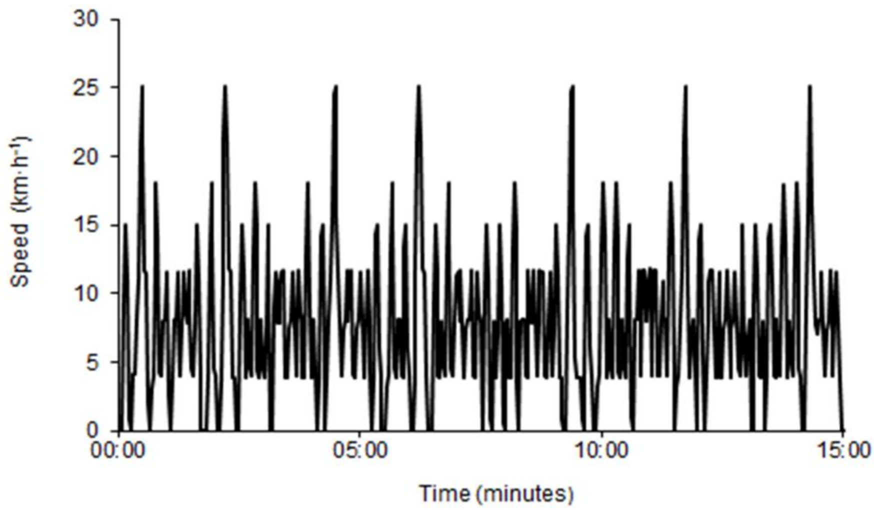

Fig 1 A schematic of an individual 15-minute bout of the soccer-specific exercise

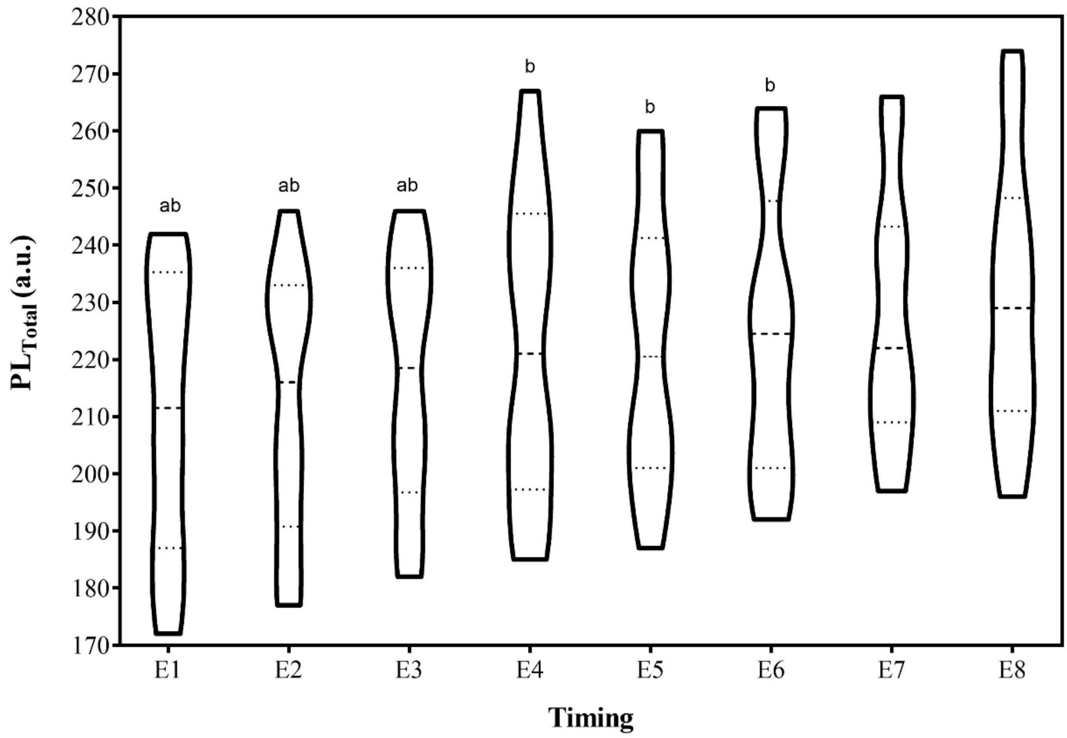

Fig 2 This graph shows the time history changes in PL_{Total} response throughout 120 min of soccer-specific exercise. Data are expressed as mean ± SD. a denotes significant difference from E7 for PLTotal b denotes significant difference from E8 for PLTotal (both $p \leq 0.05$)

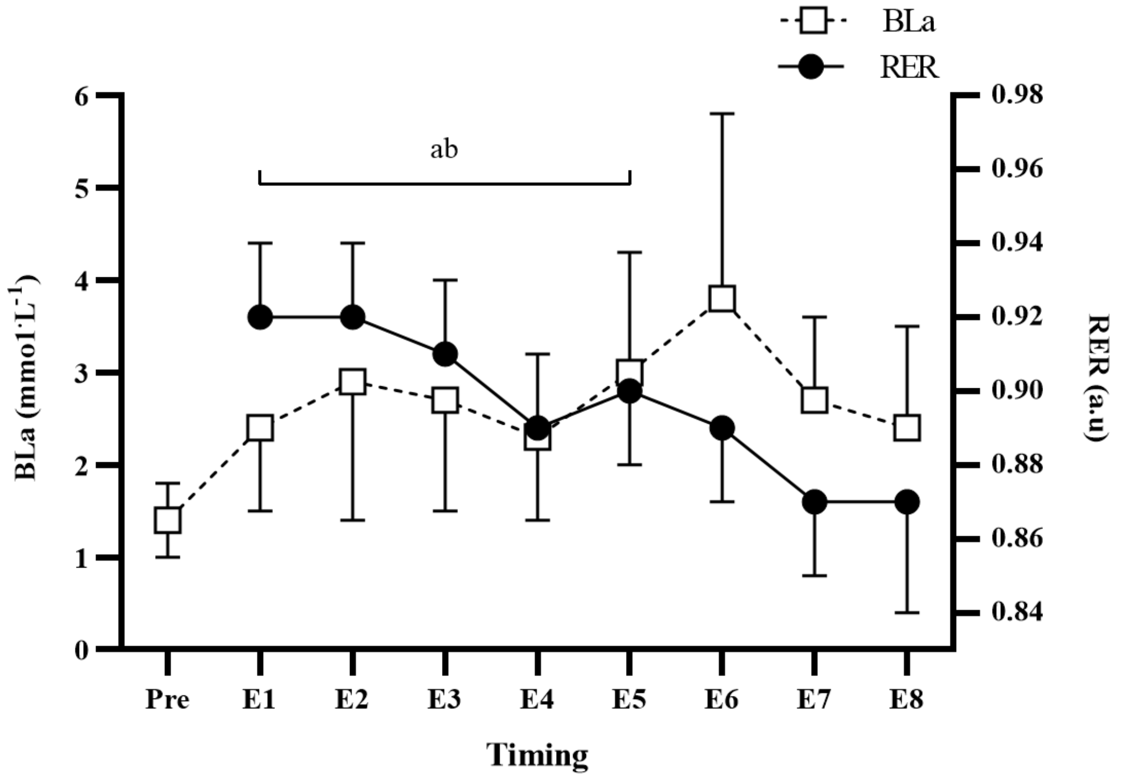

Fig 3 Mean RER and BLa concentrations across 120 min for each 15 min period of soccer-specific exercise. Data are expressed as mean ± SD. a denotes significant difference from E7 for RER. b denotes significant difference from E8 ($p \leq 0.05$) for RER.

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

	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 ± 23[gh]	220 ± 22[gh]	226 ± 27[h]	226 ± 24[h]	229 ± 25[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 ± 16[h]	111 ± 16	113 ± 15	116 ± 20	115 ± 18[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 ± 7[fh]	48 ± 7[gh]	49 ± 7[gh]	50 ± 7	50 ± 7[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

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
$PL_{A\text{-}P\%}$								
Trial 1	25 ± 4	26 ± 3	26 ± 4	26 ± 4	26 ± 4	27 ± 4	26 ± 4	27 ± 4
Trial 2	25 ± 3[h]	26 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3	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
$PL_{M\text{-}L\%}$								
Trial 1	22 ± 2	22 ± 2	22 ± 2	22 ± 2	22 ± 2	22 ± 2	23 ± 2	23 ± ± 2
Trial 2	22 ± 1	22 ± 2	22 ± 2	22 ± 1	22 ± 1	22 ± 1	23 ± 1	23 ± 1
Mean	22 ± 1	22 ± 2	22 ± 2	22 ± 2	22 ± 2	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; $PL_{A\text{-}P}$ anterior/ posterior playerload; $PL_{M\text{-}L}$ = medial/ lateral playerload; $PL_{V\%}$ = Percentage contribution of vertical vector; $PL_{A\text{-}P\%}$ = percentage contribution of anterior/posterior vector; $PL_{M\text{-}L\%}$ = 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 ($p \leq 0.05$), respectively.

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

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

	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 ± 0.02^{gh}	0.90 ± 0.02^{gh}	0.89 ± 0.02 ^{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

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 · 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 ± 0.9	2.6 ± 1.2	2.4 ± 0.9	2.4 ± 1.0	2.8 ± 1.2	3.3 ± 2.1	2.6 ± 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 ± 1[gh]	11 ± 1[gh]	12 ± 1[gh]	13 ± 1[gh]	14 ± 1[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 ± 1[gh]	12 ± 1[gh]	12 ± 1[gh]	13 ± 1[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 ± 1[gh]	11 ± 1[gh]	12 ± 1[gh]	13 ± 1[gh]	14 ± 1[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 2nd 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; $\dot{V}O2$ = 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; 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 significant difference from E1-E8 ($p \leq 0.05$), respectively.

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