Manuscript Title: The impact of 120 minutes of soccer-specific exercise on recovery

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Trial registration

The study was pre-registered on the Open Science Framework (DOI: 10.17605/OSF.IO/VGU6T Date: 10/06/2019).

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Abstract

Purpose: The extra-time (ET) period of soccer is competed during fixture congested schedules with often limited recovery time between matches. The aim of this study was to assess muscle damage recovery following 90- and 120-min (i.e., incorporation of ET) of simulated soccer match-play. **Methods:** Twelve semi-professional soccer players completed 90 and 120-min treadmill-based soccer-specific exercise in a counterbalanced order. Creatine kinase (CK), creatinine, urea, aspartate aminotransferase, perceived muscle soreness, pain pressure threshold, reactive strength index, countermovement jump height, and isokinetic strength assessments of eccentric knee flexors at 60, 180 and 270 deg·s^{-1} were taken at baseline and immediately-, 24, 48 and 72-hr post-exercise to assess recovery. **Results:** No significant between-trial interactions except for CK were found. Pairwise comparisons detected a 53% increase in CK at 24-hr (455 ± 29 μ·L^{-1}) following 120-min of simulated match-play vs. the corresponding post 90-min time-point (299 ± 29 μ·L^{-1}; p<0.01). The 120-min trial caused a 58% higher CK response at 72-hr (244 ± 25 μ·L^{-1}) vs. post 90-min comparisons (154 ± 29 μ·L^{-1}; p = 0.02). No interaction effects were detected for any other recovery variables. Creatine kinase and perceived muscle soreness remained elevated up to 72-hr in both trials (p<0.01). **Conclusions:** These data indicate that 120 min of simulated soccer match-play delays the time-course of CK recovery up to 72-hr post-match. However, 120 min of simulated soccer has no additional impact on functional recovery and perceived muscle soreness vs. 90 min. Recovery should be investigated following 90- and 120-min of actual match-play.

Keywords Extra-time • Football • Soccer simulation • Muscle damage

Introduction

The physical demands of soccer matches involve a myriad of eccentric muscular efforts including sprints, jumps, rapid accelerations, decelerations and changes of direction that induce fatigue (a debilitating symptom), perceptual stress, deplete endogenous substrates and impose structural damage within skeletal muscle (Harper et al., 2016; Mohr, Krustrup, & Bangsbo, 2005; Stevenson et al., 2017). Several mechanisms explain these detrimental effects at the local level including an increased inflammatory response, disturbance to calcium homeostasis and ultra-structural damage to muscle fibres and the surrounding connective tissues (Mohr et al., 2005; Nédélec et al., 2013; Proske & Morgan, 2001). Following these disturbances, recovery, defined as restoring homeostasis (i.e., returning players to pre-performance values), is paramount post-match (Nédélec et al., 2012b). Traditionally, soccer is contested over 90 min, and as such, there is a wealth of literature documenting fatigue and subsequent recovery post matches of this duration (Brownstein et al., 2017; Nedelec et al., 2014b; Nédélec et al., 2012b). However, when matches are tied, and an outright winner is required during the knockout phase of certain major tournaments and domestic cup competitions, an additional 30-min period is played, known as extra-time (ET) (Harper et al., 2016).

The prevelance of matches that progress to ET have become increasingly common over recent years, with 36% of the previous four FIFA World Cup knockout phase matches proceeding to this additional 30-min period (Field et al., 2020). For the first time in the tournament's history, a team competed in three consecutive ET matches (round 1/16, quarter- and semi-finals) in the knockout phase of the 2018 FIFA World Cup (Kołodziejczyk et al., 2021). Interestingly, players covered less distance across the 90 min duration in the final match compared to the previous knockout-phase matches, which could be related to fatigue from the accumulation of ET matches. Further evidence suggests that ET results in fatigue-induced decreases in relative ($m \cdot min^{-1}$) total distance and high-speed distance covered, as well as the number of sprints, accelerations and decelerations performed (Peñas, Dellal, Owen, & Gómez-Ruano, 2015; Russell et al., 2015a). Tournament matches are typically competed amid fixture congested scheduling, with an insufficient recovery time between matches (Julian, Page, & Harper, 2020), potentially predisposing players to injury/illness and impacting upon player availability and performance (Carling, McCall, Le Gall, & Dupont, 2015). Previous empirical research examining five English Premier League reserve team players found explosive power (i.e., countermovement jump [CMJ]) reduced and creatine kinase (CK) activity increased at 24 and 48 hr post 120 min matches (Russell, Sparkes, et al., 2015a). As players only competed in a single match that required ET and there were no comparisons to 90 min, it is difficult to determine the extent to which the additional 30-min duration further impacted recovery. Winder, Russell, Naughton, and Harper (2018)

monitored four professional players competing in a micro cycle, with the second of three matches progressing to ET (i.e., 90, 120, 90-min). The ET match negatively affected subjective measures of fatigue, muscle soreness, mood and CMJ height vs. the initial 90-min match. Although these findings are indicative of impeded recovery following ET matches, and notwithstanding the high ecological validity of these studies, limitations such as small sample sizes, insufficient control of confounders (Drust et al., 2013) and high inter-match variability associated with match-play reduce experimental rigour (Gregson, Drust, Atkinson, & Salvo, 2010). Therefore, there is a paucity of research attention afforded to investigating the impact of ET on recovery under controlled experimental conditions, utilising adequately powered trials and directly comparing the same group of subjects across both experimental conditions (i.e., 90 and 120-min exercise durations).

Researchers often use soccer-specific exercise protocols to replicate the demands of soccer match-play, while negating the influence of confounding factors to assess performance and recovery outcomes (Field et al., 2020). Free-running protocols are appealing for their increased ecological validity (implementation of technical actions, utility/multidirectional movements and attainment of maximum speeds) (Russell, Benton, & Kingsley, 2011; Uddin et al., 2020). However, these simulations appear to lack reproducibility/experimental control, fail to omit subconscious pacing approaches and are not reflective of the neuromechanical loading patterns of match-play (Field et al., 2020; Page, Marrin, Brogden, & Greig, 2019). Therefore, motorised treadmill protocols are preferred such that the activity profile is mechanistically valid and external running output can be standardised; thus, changes in a given variable are likely a fatigue-impaired physical capacity as opposed to player pacing and motivation (Field et al., 2020; Page et al., 2015). Therefore, utilising a treadmill-based model that elicits experimental control, integrating fixed bouts of activity appears appropriate since the magnitude of the physical output and the fatigue experienced whilst exercising, corresponds closely with the degree of muscle damage and the subsequent nature of the recovery response (Nedelec et al., 2014a).

The purpose of this study was to assess recovery following 90- and 120-min of treadmill-based simulated soccer. It was hypothesised that recovery of the primary outcome measure CMJ height, along with the other variables, would be impaired following 120 min vs. 90 min of simulated soccer.

Materials and Methods

Study design

Ten visits to the laboratory over a three-week period were required. The study was a single-blinded, within subjects, crossover design with trials completed in a counterbalanced order. The order was determined by a

number generator that randomly allocated participants to a sequence of trials. The two trials were performed 9 ± 2 days apart to ensure recovery. To eliminate the influence of self-pacing on running technique (Waldron & Highton, 2014), participants were told that both trials could last 120 min; however, the protocol was either terminated at 90 min or ET commenced. Recovery measures were measured in the following order: capillary blood sampling, perceived muscle soreness, pain pressure threshold (PPT), reactive strength index (RSI), CMJ and isokinetic peak torque, at baseline, immediately-, 24, 48 and 72 hr post-trial.

Participants

Following institutional ethical approval, 12 male semi-professional players (mass: 74 ± 8 kg; stature: 1.79 ± 0.3 m; age: 22 ± 3 years; estimated $\dot{V}O2_{max}$: 59 ± 7 ml·kg·min^{-1}) with 13 ± 3 years soccer experience provided informed consent prior to data collection. An *apriori difference between two dependant means (matched pairs)* power calculation was undertaken (GPower v3.1; Germany) which deemed a sample size of 12 sufficient based on 80% power ($1 - \beta$), an alpha (α) of 0.05, and a large effect size (Cohen's $d = 0.8$) to detect differences in the primary outcome variable, CMJ. Participants were included on the basis that they attained $\dot{V}O_{2max}$ ≥48.5 ml·min·kg^{-1} as per previous ET work (Field et al., 2020; Stevenson et al., 2017) and had no medical contraindications to exercise. Participants were asked to refrain from strenuous activity and prohibited from alcohol consumption, non-steroidal anti-inflammatory drugs and foods rich in polyphenols 72-hr prior and throughout testing. Players were also instructed to avoid any non-nutritional recovery interventions (massage, foam rolling, cryotherapy etc). Dietary intake was recorded throughout the five testing days (24-hr pre-trial to 72-hr post-trial) via weighed food diaries and later analysed to assess macronutrient composition and caloric intake. Dietary intake was replicated as closely as possible for the subsequent trial. In order to assess compliance and food wastage, pictures were sent to the lead researcher on two days (pre-selected) during data collection (-24 and +48-hr of trial) using a free smartphone messaging application (WhatsApp). This 'snap and send' method has been used in previous research (Costello, Deighton, Dyson, Mckenna, & Jones, 2017; Zhang et al., 2015).

Preliminary visits and main trials

Mass (SECA 875 electronic flat scale, SECA, Germany) and stature (SECA 213 portable stadiometer, SECA, Germany) were taken during the preliminary visit and $\dot{V}O_{2max}$ was estimated *via* an incremental treadmill protocol. Participants initially completed a standardised warm-up of intermittent speed changes and a dynamic stretching sequence. The incremental test then ensued, starting at a running speed of 10 km·h^{-1} and continued to increase by 1 km·h^{-1} every 30-s until 17 km·h^{-1}, whereby the protocol inclined by 0.5° every 30-s until volitional exhaustion (Field et al., 2020). A second session was used to fully habituate participants with trial day procedures and the

completion of a 120-min familiarisation trial. Upon arrival for the main trials, participants provided a mid-flow urine sample for measurement of urine osmolality (Osmocheck, Vitech Scientific, West Sussex, UK). Water and/or a carbohydrate electrolyte beverage was consumed *ad libitum*, recorded by the research team and replicated for the subsequent trial. Post-trial recovery measures were collected, and participants vacated the lab.

Soccer simulation

Participants completed the soccer-specific exercise protocol on a motorised treadmill (h/p/ cosmos pulsar® 3p: h/p/cosmos sports & medical GmBH, Germany). The protocol has previously been validated (Page et al., 2015), replicating the frequency, duration and speed of discrete locomotive phases, the distances covered, sprint quantities and low-to-high speed running ratios observed during 90-min of a professional soccer match (Mohr, Krustrup, & Bangsbo, 2003). Similar to ET match-play, participants perform 56 sprints and cover a distance of 16.26 km during the 120-min trial (Russell et al., 2015a; Winder et al., 2018). The protocol has also demonstrated moderate-to-very strong reliability (Field et al., 2020) and has shown to elicit a peripheral and central fatigue response comparable with soccer match-play (Field et al., 2020). Applying a 1% inclination at lower speeds and a 2% gradient at higher speeds, better reflects the energy cost of outdoor running (Jones & Doust, 1996); thus, these varying degrees of gradient were applied to account for the lack of air resistance indoors. The protocol was structured as two x 45-min halves (separated by a 15-min half-time break) and either terminated or continued following a 5-min rest period, with two additional 15-min periods (interspersed by a 2-min break). The same activity profile was repeated every 15-min (Figure 1). Maximum sprint velocities reached 25 $km \cdot h^{-1}$ with changes in velocity set at the treadmill threshold (1.39 $m \cdot s^{-2}$). Participants were asked to provide differential ratings of perceived exertion (d-RPE) in a counterbalanced order, for legs (RPE-L), breathlessness (RPE-B) and overall (RPE-O), through use of the 6-20 Borg scale (Borg, 1998). The treadmill paused briefly at the cessation of each 15-min bout, and d-RPE, and a blood capillary sample was taken and later analysed for blood lactate (BLa) (Biosen C-Line; EKF-diagnostic GmBH, Cardiff, Wales; CV both 1.5%). PlayerLoad™ (Catapult Innovations, Australia) data were continuously recorded throughout exercise and defined across each 15-min bout.

INSERT FIGURE 1

Recovery measures

Blood sampling procedures and biochemical analyses

Finger-prick capillary blood samples (200 µl) were taken once the foremost droplet of blood was discarded. This was collected in a lithium heparin coated microvette® and analysed for CK, creatinine, urea and aspartate aminotransferase (AST) activity using a colorimetric assay procedure (Reflotron® plus, Roch Diagnostics, Switzerland). All samples were analysed by a single researcher in duplicate to eliminate inter-assay variation.

Muscle soreness

A subjective assessment of perceived muscle soreness was undertaken using a visual analogue scale (VAS), which has previously demonstrated good reliability (intra-class correlation [ICC] = 0.65) (Rampinini et al., 2011). Once in a squat position (angle ~90°) with knees shoulder width apart, participants marked on a 100 mm horizontal line which comprised of "no pain whatsoever" (0 mm) and "maximum pain imaginable" (100 mm) (Chen, Lin, Chen, Lin, & Nosaka, 2011).

A PPT test was also performed at the biceps femoris (PPT_{BF}) on the dominant leg using a handheld Baseline Algometer (27.22 kg/ 60 lbs, Fabrication, Enterprises Inc., USA). The biceps femoris were identified as the most common site of injury in a recent epidemiological systematic review and meta-analysis (López-Valenciano et al., 2019). A mark was positioned central to the muscle belly and re-applied to the skin daily using indelible ink to ensure between-test consistency (Hermens et al., 2000). The lead researcher applied progressively increasing pressure with the circular flat tip (1-cm in diameter) perpendicular to the pre-marked site. Participants were instructed to signal at the point of shift from pressure-to-pain with the value recorded in pounds (lbs). Recordings were taken twice, separated by ~60-s, and the mean of both scores were presented for analyses. The use of PPT has previously demonstrated high inter- and intra-rater reliability (ICC = 0.63-0.97) (Binderup, Arendt-Nielsen, & Madeleine, 2010; Walton et al., 2011).

Muscle function

For RSI, participants were instructed to drop from a 0.3 m platform and upon landing, jump maximally, whilst minimising ground contact time and maximising vertical jump height. The RSI values were calculated automatically using manufacturer software (Optojump, Microgate, Italy) through the sum of jump height (cm) divided by contact time (ms). Following a 30-s rest period, participants performed a CMJ with hands on hips, feet shoulder width apart and when prompted, descended into a squat (~60°) and jumped vertically with maximal effort. Jump efforts were measured using a portable optical measurement system (Optojump, Microgate, Italy)

198 and were separated by a 30-s passive recovery period with the mean of three jump being used for analyses (Field
199 et al., 2020).

200 Isokinetic testing

201 Unilateral peak torque of the eccentric knee flexors (eccKF) were measured using a Cybex HUMAC Norm
202 isokinetic dynamometer with HUMAC2009 software version 0.8.4 (CSMI, USA). The eccKF were selected, given
203 that fatigue-induced strength deficits are commonly observed in the eccentric hamstrings, increasing stretch injury
204 susceptibility due to an impaired capacity to resist over lengthening whilst fatigued (Small, McNaughton, Greig,
205 & Lovell, 2010). Each of three sets were performed at respective angular velocities of 180, 270 and 60 deg·s^{-1};
206 order chosen to attenuate the possible fatiguing effect of slower speeds (Greig, 2008). The dynamometer was
207 setup specific to the participant for each session as per manufacturers guidelines. Whilst seated, the passive limb
208 was weighed at anatomical zero (defined as full knee extension) and the effects of gravity were applied to torque
209 data. Five reps of each speed were performed and each of the three sets were interspersed by a 30-s passive rest
210 period. The preferred kicking leg was assessed through a range of 0−90° (0° representing full extension and 90°
211 full flexion). Similar soccer research has reported ICCs of 0.78, 0.76 and 0.78 for eccentric knee flexion at 180,
212 300 and 60 deg·s $^{-1}$, respectively (Greig, 2008).

213 Dietary analysis

214 Participant food diaries were inputted into dietary analysis software (Nutrimen.co.uk, Dark Green Media Ltd,
215 ©2016). The estimated measurement error of inputting participant's dietary intake was calculated using the
216 standard error of measurement (SEM) with 95% confidence intervals (CI). The SEM was derived from the square
217 root of the mean square error from an ANOVA and expressed in the units of each given variable (Stratford &
218 Goldsmith, 1997). To examine intra-rater reliability, three participants were selected at random and a single
219 researcher inserted the same three food diaries into the analysis software on six separate occasions during a six-
220 week period. To assess inter-rater reliability, the same diaries were entered by three separate researchers twice in
221 two-weeks.

222 Statistical analysis

223 Exploratory data analysis was undertaken to evaluate the assumptions associated with the linear mixed model
224 (LMM). This statistical method was chosen as an appropriate test for a repeated measures design that involves
225 random and fixed level factors with missing data; assuming data are missing at random (Di Salvo, Gregson,

Atkinson, Tordoff, & Drust, 2009). A visual inspection of q-q plots, histograms and boxplots was undertaken to assess normality of residuals. Residuals > 3.0 SD from the mean values were removed prior to analyses in line with the assumptions of the LMM. A basic variance components assessment revealed the model Akaike's information criterion (AIC) was best fit for each recovery variable. Models were initially regarded as null and subsequently developed to more stringent models. A basic variance components model was utilised to calculate the intraclass correlation (ICC) of the random factors (i.e., *participant*) to establish if a significant variance contributed to the recovery variables. Wald Z statistics were employed to assess the null hypothesis (i.e., that zero variance existed between participants); if rejected, the random factor of participant ID was included in the successive hierarchical models. The covariance structure of the random factors was set to variance components in all models. The auto-regressive (AR-1) was established as the model most suitable for each recovery variable for the repeated measures of time. The fixed effects and their interactions included were *trial and time* for each model. All models estimated parameters using the maximum likelihood method. Least significant corrections were applied post-hoc with 95% CI of the difference reported. A paired samples T-test assessed differences between participant dietary intake across the five experimental days. Data are expressed as mean and ± SE unless otherwise stated and were processed using IBM SPSS Statistics 26 for windows (SPSS Inc., Chicago, IL, USA). Alpha was accepted at < 0.05 prior to analyses.

Results

Between trials measures

No differences were detected between trials for energy or macronutrient intake ($p > 0.35$; Table 1), urine osmolality, environmental conditions, cumulative PlayerLoad™, BLa and d-RPE (all $p > 0.33$; Table 2).

INSERT TABLE 1

INSERT TABLE 2

Variance calculations

Table 3 provides the ICC's (%) of the random factors accounted for in each of the LMMs. All recovery measures, except for CK, creatinine and AST, contributed significant variance to the dependant variables and were included as a random factor in the larger hierarchical models.

INSERT TABLE 3

253 Recovery responses following 90 and 120 minutes of simulated soccer

254 Interaction effects for trial and time were evident for CK (p = 0.01), with post-hoc comparisons revealing higher
255 values at 24-hr post 120-min (455.1 ± 29.4 U·L^{-1}; 95% CI = 396.7 to 513.7 U·L^{-1}) compared to 24-hr following
256 90-min (298.8 ± 29.3 U·L^{-1}; 95% CI = 240.6 to 356.9 U·L^{-1}; 95% CI for diff = 75.1 to 237.8 U·L^{-1}; p < 0.01).
257 Furthermore, trial interaction effects were observed at 72-hr post 120-min (244.1 ± 24.7 µ·L^{-1}; 95% CI = 194.7 to
258 293.4 U·L^{-1}) vs. 72-hr following the 90-min simulation (154.1 ± 29.3U·L^{-1}; 95% CI = 240.6 to 356.9 U·L^{-1}; 95%
259 CI for diff = 17.2 to 176.9 U·L^{-1}; p = 0.02). No interactions were identified for creatinine (p = 0.24), urea (p =
260 0.59), AST (p = 0.83), VAS (p = 0.22), PPT_{RF} (p = 0.99), PPT_{BF} (p = 0.78), RSI (p = 0.35), CMJ (p = 0.21),
261 $eccKF_{180}$ (p = 0.75), $eccKF_{270}$ (p = 0.67) and $eccKF_{60}$ (p = 0.42).

262 Main effects for time were observed for CK, urea, VAS, PPT_{BF}, RSI, CMJ, $eccKF_{180}$, $eccKF_{270}$ and $eccKF_{60}$ (all
263 p < 0.01; Table 4). No main effects for time were evident for creatinine (p = 0.24), AST (p = 0.23) and PPT_{RF} (p
264 = 0.46).

265 ***INSERT TABLE 4***

266 **Discussion**

267 The purpose of this study was to assess recovery in response to a 90- and 120-min (i.e., inclusion of an ET period)
268 simulated soccer match. No differences were established between trials for all variables aside from CK. Higher
269 CK activity was observed at 24 and 72 hr post 120 min of simulated soccer compared with a typical 90 min
270 duration. Contrary to the study hypothesis, little evidence that recovery is prolonged after 120 min vs. 90 min of
271 simulated soccer match-play was found.

272 Despite most variables demonstrating that recovery is not further impeded following ET, the current findings
273 demonstrate that compared to the 90-min trial, CK activity increased by a further 53 and 58% at 24 and 72-hr
274 following 120 min, respectively. The magnitude of change in CK far exceeds the intra- and inter-assay
275 measurement error (i.e., coefficient of variation = 1.4—2.1% and 1.5—4.2%, respectively), indicating the
276 variation is low enough not to obscure a true experimental effect. Considering the CK response to 120 min alone,
277 a 317% increase at 24-hr post-trial was observed vs. baseline. Previous ET match-play investigations observed a
278 236% increase in CK at 24-hr post 120 min of soccer match-play vs. baseline in professional players (Russell et
279 al., 2015a). The higher magnitude of change in the current study compared to previous research is potentially
280 explained by a wealth of factors such as standard of player and innate blood marker variability (Baird et al., 2012),
281 though we speculate most likely as a consequence of the differences in exercise modality (i.e., simulation vs

match-play). For instance, the match-play study identified reductions in total (-12%) and high-speed distance (-37.5%), as well as number of accelerations (-14%) and decelerations (-13%) during ET, relative to the preceding 90 min (Russell et al., 2015a). However, the activity was standardised for the current protocol, with players performing at the same intensity throughout the entire 120 min duration, unlike the players in the match-play study (Russell et al., 2015a). This also supports that the current treadmill protocol was sufficiently intense to induce a muscle damage response comparable with match-play and suggests recovery strategies may need to be modulated to restore the physiological perturbations of players competing for 120 min. Notwithstanding, it is important to note that CK has been criticised as a marker of muscle damage recovery (Twist & Highton, 2013), and therefore this finding should not be interpreted independent of the functional measures discussed below.

No between-trial interactions were present for eccKF peak torque in the current research, although the current soccer-specific exercise protocol reduced hamstring strength following both exercise durations. Due to the logistical complexities associated with collecting isokinetic peak torque data during match-play, comparable data are not available following 90 min or ET matches. However, a similar investigation also demonstrates that completing an ET match simulation on a treadmill, significantly reduces eccKF peak torque, at the same speeds to the present study, immediately post 120 min of exercise (Field et al., 2020). Therefore, the current research supports previous data and contributes new infromation that indicates the deficits in eccKF persist up to 24 hr and return to baseline at 72 hr following 120-min of soccer. It is importtant to note, however, that matches are occasionally interpersed by ~48 hr (Ranchordas, Dawson, & Russell, 2017), and thus, practitioners are advised to closely monitor recovery markers, before returning players to training and matches. The recovery of maximal strength should largely be considered on an indvidual basis, depsite the current research reporting an average response, since eccKF strength characteristics are susceptible to large individual variation following ET (Field et al., 2020). It is advised that replacements are used strategically during or before the additional 30 min ET period to attenuate the effects of fatigue, and maintain fitness and freshness since a fourth substitution is now permitted during ET (Hills et al., 2020). Furthermore, to support player welfare during the COVID-19 pandemic, the International Football Association Board approved a temporary amendment to the laws of the game, allowing teams the option of using five substitutions per match, with an additional replacement permitted during ET. This is especially important considering that the residual fatigue-induced decrements in eccKF in the intervening post match period, and may explain the increased injury incidence during ET (Aoki, O'Hata, Kohno, Morikawa, & Seki, 2012), especially if players are not entirely recovered prior to the next competitive encounter.

311 Epidemiological research, therefore, appears warranted to determine the extent to which ET matches exacerbate
312 injury incidence in subsequent training and matches.

313 Soccer-specific activity involves repetitive eccentric to concentric actions, that can induce neuromuscular fatigue
314 and reduce peak power output (Nédélec et al., 2012b). As such, given the functional relevance of such markers
315 and their ability to detect perturbations in muscle function and elastic energy usage (Oliver, Armstrong, &
316 Williams, 2008), RSI and CMJ were used as indicators of exercise-induced fatigue. Time-dependant reductions
317 in RSI and CMJ were evident for up to 48-hr following both trials in the current research, although no between-
318 trial differences were identified for these measures. Several studies have shown that the recovery of these markers
319 are prolonged following 90 and 120 min of simulated and actual match-play (Abbott, Brashill, Brett, & Clifford,
320 2019; Russell, Northeast, et al., 2015b; Thomas et al., 2017; Winder et al., 2018). Therefore, although the muscle
321 function data limit inference concerning how player recovery should be managed following ET, the results add to
322 an expanding body of literature that suggests recovery may need to be considered alongside the congested fixture
323 periods associated with major tournaments (Julian et al., 2020; Page et al., 2019). However, given that ET may
324 not further prolong recovery of muscle function, there appears to be little need to adapt practice and provide
325 additional measures to restore muscle function capacity irrespective of match duration.

326 An increased presence of blood urea was evident immediately post-exercise in both trials, and although differences
327 did not reach statistical significance, these values were further (~10%) increased immediately following the 120-
328 min exercise trial. Given macronutrient intake was similar between trials (Table 1), this finding, although
329 speculative, is indicative of an increased protein degradation following ET. Urea is strongly associated with
330 training volume potentially owing to the upregulation of gluconeogenesis during exercise that results in the
331 breakdown of structural proteins (Haralambie et al., 1976; Meyer & Meister, 2011). The rate of gluconeogenesis
332 is increased when glycogen stores are depleted and is stimulated by the secretion of glucagon, which can be linked
333 to an upregulation in lipolysis (de Sousa et al., 2019; Shephard, 1999). This finding may corroborate previous
334 research that suggests glycogen depletion and an increased rate of lipolysis is apparent during ET (Field et al.,
335 2020; Harper et al., 2016; Stevenson et al., 2017). This could potentially highlight the importance of modulating
336 fuelling strategies, including carbohydrate intake prior and during, as well as adapting protein intake post ET
337 matches. However, this research provides a foundation for future lines of exploration that are warranted to assess
338 substrate metabolism using invasive techniques such as a muscle biopsies following 120 min of soccer-specific
339 exercise.

Limitations are present within the current study that should be acknowledged. The cohort used within this study may not be representative of the population against which the soccer-specific protocol is based (Mohr et al., 2003), although semi-professional players were recruited with a $\dot{V}O_{2max}$ of 59 ± 7 ml·kg^{-1}·min^{-1}, which is similar to that of professional soccer players (Tønnessen, Hem, Leirstein, Haugen, & Seiler, 2013). Furthermore, the responses observed in the current study are likely conservative estimates; indeed, actual match-play involves contacts with other players and additional movements such as cutting manoeuvres that likely place a greater deal of stress on the musculoskeletal system, and therefore evoke a muscle damage response of a higher magnitude (Castellano, Blanco-Villaseñor, & Alvarez, 2011). It is well accepted that disparate physical loads, such as those experienced during match-play, can impact upon the muscle damage recovery response (Nédélec et al., 2012b). Therefore, a decision was taken to use a valid treadmill design that employs fixed running velocities of an equal exercise volume to ensure that differences in the recovery response occurred as a result of the additional 30-min duration rather than player pacing strategies to offset fatigue. Another limitation of the study was that the protocol elicited a physiological response (blood lactate: 2.4 ± 1.5 mmol·l^{-1}) near the lower end of the ranges previously reported during match-play (2—10 mmol·l^{-1}) (Bangsbo, Iaia, & Krustrup, 2007). This is a common observation with treadmill variants (Greig, Mc Naughton, & Lovell, 2006; Page et al., 2015), although, the mechanical strain emulates a match more closely, with the current protocol eliciting a comparable CK response to elite soccer match-play (Malone et al., 2018).

Practical applications

The findings demonstrate that although CK indices are higher following 120 min of soccer-specific exercise, functional measures of recovery are not exacerbated by the ET period versus 90-min markers. Therefore, according to the current data, it appears that recovery practices do not need to be adapted, and training can resume as normal following ET matches like traditional 90 min approaches. This finding conflicts with empirical research, which suggests that 82% of the professional soccer practitioners surveyed believe that practices should be adapted following ET compared with a typical 90 min match (Field et al., 2021). However, inter-individual variances in recovery potential are apparent (Nédélec et al., 2012), and as such, recovery protocols following ET matches should be individualised as opposed to generic for the entire team. Therefore, monitoring players following ET using physical, physiological, and subjective assessments may be required to identify individuals experiencing residual fatigue. If players are identified as needing an additional ‘rest’, then practitioners may need to consult the head coach to ensure training loads are sufficient to maintain optimal conditioning. Careful periodisation of

training is particularly key during fixture dense tournaments to ensure players are adequately conditioned for optimal performance and a reduced injury incidence during ET.

Conclusion

In summary, this study highlights that simulated soccer match-play of 120 min in duration is associated with increased CK compared with a typical 90-min duration. However, there were no other trial and time interactions, suggesting ET has no further effect on the muscle damage recovery response following simulated soccer. This suggests a disassociation between the CK response and functional variables. Collectively, these data might have implications for practitioners responsible for managing training and match loads during fixture dense tournaments that require ET matches. There appears to be little need (according to the current data) for adapting recovery practices in response to an ET match during fixture congested micro cycles and training can proceed as normal. Moving forward, recovery and subsequent performance should be investigated following 90- and 120-min of actual match-play.

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Disclosure of interest

The authors report no conflict of interest

Figure captions

Figure 1. A schematic of an individual 15-min bout of the soccer-specific exercise simulation

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Table 1. Mean energy and macro nutrient composition of participants diet across five days of testing for each trial and reliability of data input

	Mean dietary intake across 5 days			Inter-rater reliability		Intra-rater reliability	
Variable	90 min	120 min	*p* value	SEM	95% CI	SEM	95% CI
Energy (Kcal)	2008 ± 577	1985 ± 486	0.68	95	186	53	104
Carbohydrate (g)	237 ± 93	220 ± 67	0.35	12	23	4	7
Protein (g)	109 ± 40	109 ± 47	0.93	2	4	1	2
Fat (g)	75 ± 18	77 ± 18	0.76	3	6	4	8

Data are reported as mean ± SD

Table 2. Environmental conditions and responses across both trials.

Variable	90 min		120 min	*p* value
Urine osmolality (pre; mOsm·kg^{-1})	536 ± 120	————————	556 ± 167	0.79
Urine osmolality (post; mOsm·kg^{-1})	516 ± 104	————————	530 ± 160	0.83
Humidity (%)	33.8 ± 3.5	————————	33.3 ± 3.1	0.66
Barometric pressure (%)	29.2 ± 0.4	————————	29.4 ± 0.6	0.79
Ambient temperature (ºC)	20.1 ± 0.9	————————	19. 7 ± 1.1	0.33
Variable	90 min	90 min ($_{120\ min}$)	*p* value	120 min
PlayerLoad™ (a.u)	1359 ± 21	1347 ± 25	0.82	1817 ± 25
Blood lactate (mmol · L^{-1})	2.4 ± 1.5	2.4 ± 1.5	0.98	2.5 ± 1.5
RPE-L	12 ± 2	11 ± 2	0.65	12 ± 2
RPE-B	11 ±	11 ± 2	0.95	12 ± 2
RPE-O	12 ± 2	12 ± 2	0.83	12 ± 2

RPE-L = RPE legs; RPE-B = RPE breathlessness; RPE-O = RPE overall.
Urine and environmental significance values are reported as differences between both 90- and 120-min trials.
90 min ($_{120\ min}$) = 90 min duration for the 120 min trial.
Responses to exercise significance values are reported as differences between the 90 min duration of both trials.
PlayerLoad™ values are reported as the cumulative response across the specified duration.
Blood lactate and RPE values are reported as the mean response per epoch of exercise.
Data are reported as mean ± SD.

Table 3. The ICC's (%) of the random factor of participant ID for all recovery variables. Where significance was found, participant ID was included in the linear mixed model.

Variable	ICC (%)
CK	14.73
Creatinine	20.57
Urea	41.49*
AST	11.47
VAS	46.32*
PPT_{RF}	85.03*
PPT_{BF}	63.65*
RSI	86.09*
CMJ	95.14*
$eccKF_{180}$	57.77*
$eccKF_{270}$	64.27*
$eccKF_{60}$	66.24*

** Represents significant determinant of variance within the linear mixed model ($p < 0.05$).*

Table 4. Muscle damage recovery responses following 90 and 120 min of simulated soccer

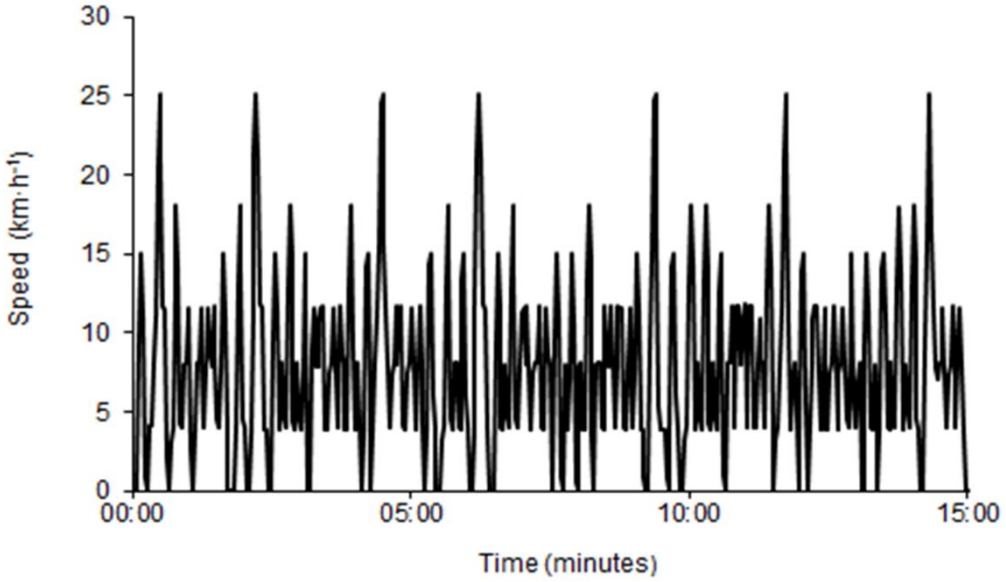
	Time				
Variable	Baseline	Post	24 hr	48 hr	72 hr
CK ($U \cdot L^{-1}$)					
90 min	102.7 ± 24.7	229.6 ± 25.6[a]	298.8 ± 29.3[ab]	258.6 ± 28.9[ac]	154.1 ± 29.3[acd]
120 min	109.4 ± 25.7	233.8 ± 26.8[a]	455.1 ± 29.4*[ab]	301.6 ± 30.8[ac]	244.1 ± 24.7*[acd]
Creatinine ($\mu mol \cdot L^{-1}$)					
90 min	77.6 ± 4.7	80.8 ± 4.9	79.9 ± 4.7	72.3 ± 4.7	74.2 ± 5.1
120 min	68.1 ± 4.9	89.6 ± 5.1	80.2 ± 5.1	77.1 ± 4.7	72.1 ± 4.9
Urea ($mmol \cdot L^{-1}$)					
90 min	6.64 ± 0.53	7.97 ± 0.53[a]	8.05 ± 0.53[a]	6.84 ± 0.52[bc]	6.52 ± 0.55[bc]
120 min	6.91 ± 0.52	9.01 ± 0.52[a]	8.27 ± 0.54[a]	7.48 ± 0.52[bc]	7.51 ± 0.53[bc]
AST ($U \cdot L^{-1}$)					
90 min	41.4 ± 3.2	44.01 ± 3.3	45.5 ± 3.2	39.6 ± 3.8	41.0 ± 3.4
120 min	38.0 ± 3.1	40.07 ± 3.2	44.5 ± 3.3	41.7 ± 3.4	37.9 ± 3.1
VAS					
90 min	2 ± 2	20 ± 2[a]	22 ± 2[a]	14 ± 3[ac]	6 ± 3[abcd]
120 min	1 ± 2	26 ± 3[a]	23 ± 3[a]	18 ± 2[ac]	5 ± 2[abcd]
PPT_{BF} (lbs)					
90 min	25 ± 1	25± 1	24 ± 1	24 ± 1	26 ± 1[cd]
120 min	22 ± 1	22 ± 1	22 ± 1	22 ± 1	24 ± 1[cd]
RSI (a.u)					
90 min	1.41 ± 0.12	1.31 ± 0.12[a]	1.30 ± 0.11[a]	1.31 ± 0.11[a]	1.37 ± 0.12[bcd]
120 min	1.33 ± 0.12	1.26 ± 0.12[a]	1.23 ± 0.12[a]	1.25 ± 0.12[a]	1.40 ± 0.12[bcd]
CMJ (cm)					
90 min	36.6 ± 2.2	35.4 ± 2.2	34.9 ± 2.2[ab]	35.3 ± 2.2[ac]	36.6 ± 2.2[ce]
120 min	36.4 ± 2.2	36.3 ± 2.2	34.9 ± 2.2[ab]	36.0 ± 2.2 [ac]	36.5 ± 2.2[ce]
$eccKF_{180}$ (Nm)					
90 min	160.4 ± 8.7	153.4 ± 8.7[a]	146.0 ± 8.7[a]	154.3 ± 8.8	163.8 ± 8.9[bcd]
120 min	162.3 ± 8.7	145.2 ± 8.7[a]	145.9 ± 8.9[a]	153.8 ± 8.7	163.7 ± 8.8[bcd]
$eccKF_{270}$ (Nm)					
90 min	159.4 ± 8.8	153.7 ± 8.8[a]	152.8 ± 8.8[a]	160.4 ± 8.9[bc]	164.5 ± 8.9[bc]
120 min	159.0 ± 8.8	147.1 ± 8.8[a]	145.5 ± 8.9[a]	161.8 ± 8.8[bc]	167.5 ± 8.8[bc]
$eccKF_{60}$ (Nm)					
90 min	164.1 ± 7.6	148.9 ± 7.6[a]	142.5 ± 7.5[ab]	148.0 ± 7.5[ac]	159.0 ± 7.5[c]
120 min	159.5 ± 7.6	147.2 ± 7.5[a]	137.9 ± 7.7[ab]	149.3 ± 7.5[ac]	160.7 ± 7.5[c]

Note. CK = creatine kinase = AST = aminotransferase, PPT = pain pressure threshold, $_{BF}$ = biceps femoris, RSI = reactive strength index, CMJ = countermovement jump height, eccKF = eccentric knee flexion, $_{180, 270, 60}$ denote isokinetic angular velocities ($deg \cdot s^{-1}$), VAS = Visual analogue scale.

**Represents significantly higher value for specified time point between trials.*

[a-e] Represents significant difference for time from baseline, post, 24, 48 and 72 hr, respectively.

Data are reported as mean ± SE.


30
25
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Speed (km·h⁻¹)
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Time (minutes)