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The Impact of 120 Minutes of Soccer-Specific Exercise on Recovery

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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 semiprofessional 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}; $p < .01$). 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}; $p = .02$). 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 ($p < .01$). **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.

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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Extra-time; football; muscle damage; soccer simulation

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 et al., 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 fibers 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 . 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). 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 has 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, Naughton 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 semifinals) 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·min^{-1}) total distance and high-speed distance covered, as well as the number of sprints, accelerations and decelerations performed (Peñas et al., 2015; Russell, Sparkes et al., 2015). Tournament matches are typically competed amid fixture congested scheduling, with an insufficient recovery time between matches (Julian et al., 2021), potentially predisposing players to injury/illness and impacting upon player availability and performance (Carling et al., 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., 2015). 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 et al. (2018) monitored four professional players competing in a micro-cycle, with the second of three matches progressing to

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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 and high inter-match variability associated with match-play reduce experimental rigor (Gregson et al., 2010). Therefore, there is a paucity of research attention afforded to investigating the impact of ET on recovery under controlled experimental conditions, utilizing adequately powered trials and directly comparing the same group of participants 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, Page 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 et al., 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, Page et al., 2020; Page et al., 2019). Therefore, motorized treadmill protocols are preferred such that the activity profile is mechanistically valid and external running output can be standardized; thus, changes in a given variable are likely a fatigue-impaired physical capacity as opposed to player pacing and motivation (Field, Corr et al., 2020; Page et al., 2015). Therefore, utilizing 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., 2014).

The purpose of this study was to assess recovery following 90- and 120-min of treadmill-based simulated soccer. It was hypothesized 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 3-week period were required. The study was a single-blinded, within participants, 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 semiprofessional 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 dependent 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, Page 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 5 testing days (24-hr pre-trial to 72-hr post-trial) via weighed food diaries and later analyzed 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 2 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 et al., 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 standardized 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, Page et al., 2020). A second session was used to fully habituate participants with trial day procedures and the completion of a 120-min familiarization 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 were 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 motorized 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 et al., 2003). Similar to ET match-play, participants perform 56 sprints and cover a distance of 16.26 km during the 120-min trial (Russell, Sparkes et al., 2015; Winder et al., 2018). The protocol has also demonstrated moderate-to-very strong reliability (Field, Page et al., 2020) and has shown to elicit a peripheral and central fatigue response comparable with soccer match-play (Field, Page 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 × 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 $km.h^{-1}$ 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 analyzed 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.

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 analyzed for CK, creatinine, urea and aspartate aminotransferase (AST) activity using a colorimetric assay procedure (Reflotron® plus, Roch Diagnostics, Switzerland). All samples were analyzed 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 comprises "no pain whatsoever" (0 mm) and "maximum pain imaginable" (100 mm; Chen et al., 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., 2020). 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 was presented for analyses. The use of PPT has previously demonstrated high inter- and intra-rater reliability (ICC = 0.63–0.97; Binderup et al., 2010; Walton et al., 2011).

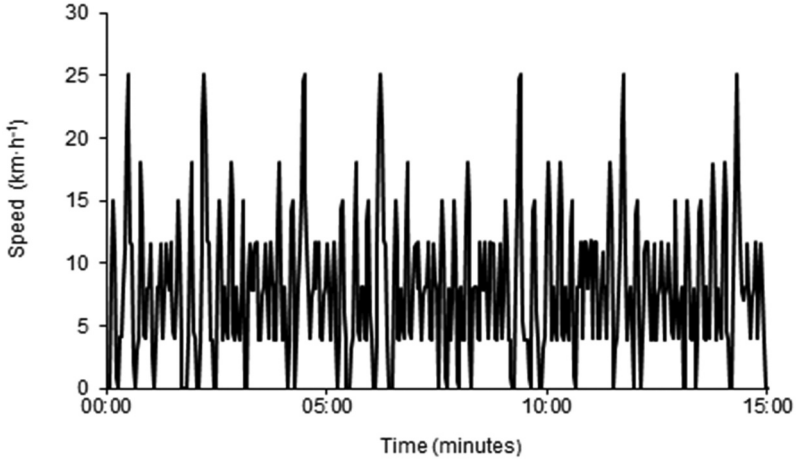

Figure 1. A schematic of an individual 15-min bout of the soccer-specific exercise simulation.

Muscle function

For RSI, participants were instructed to drop from a 0.3 m platform and upon landing, jump maximally, whilst minimizing ground contact time and maximizing 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) and were separated by a 30-s passive recovery period with the mean of three jump being used for analyses (Field, Page et al., 2020).

Isokinetic testing

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

Dietary analysis

Participant food diaries were inputted into dietary analysis software (Nutrimen.co.uk, Dark Green Media Ltd, ©2016). The estimated measurement error of inputting participant's dietary intake was calculated using the standard error of measurement (SEM) with 95% confidence intervals (CI). The SEM was derived from the square root of the mean square error from an ANOVA and expressed in the units of each given variable (Stratford & Goldsmith, 1997). To examine intra-rater reliability, three participants were selected at random and a single researcher inserted the same three food diaries into the analysis software on six separate occasions during a 6-week period. To assess inter-rater reliability, the same diaries were entered by three researchers twice in 2 weeks.

Statistical analysis

Exploratory data analysis was undertaken to evaluate the assumptions associated with the linear mixed model (LMM). This statistical method was chosen as an appropriate test for a repeated measures design that involves random and fixed level factors with missing data; assuming data are missing at random (Di Salvo et al., 2009). A visual inspection of q-q plots, histograms and boxplots was undertaken to assess the 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 using Akaike's information criterion (AIC) was the 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 utilized 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 5 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 > .35; Table 1), urine osmolality, environmental conditions, cumulative PlayerLoad™, BLa and d-RPE (all p > .33; Table 2).

Table 1. Mean energy and macronutrient composition of participants' diet across 5 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	.68	95	186	53	104
Carbohydrate (g)	237 ± 93	220 ± 67	.35	12	23	4	7
Protein (g)	109 ± 40	109 ± 47	.93	2	4	1	2
Fat (g)	75 ± 18	77 ± 18	.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	.79
Urine osmolality (post; mOsm·kg^{-1})	516 ± 104	————————	530 ± 160	.83
Humidity (%)	33.8 ± 3.5	————————	33.3 ± 3.1	.66
Barometric pressure (%)	29.2 ± 0.4	————————	29.4 ± 0.6	.79
Ambient temperature (°C)	20.1 ± 0.9	————————	19. 7 ± 1.1	.33
Variable	**90 min**	**90 min ($_{120\ min}$)**	***p* Value**	**120 min**
PlayerLoad™ (a.u)	1359 ± 21	1347 ± 25	.82	1817 ± 25
Blood lactate (mmol · L^{-1})	2.4 ± 1.5	2.4 ± 1.5	.98	2.5 ± 1.5
RPE-L	12 ± 2	11 ± 2	.65	12 ± 2
RPE-B	11 ±	11 ± 2	.95	12 ± 2
RPE-O	12 ± 2	12 ± 2	.83	12 ± 2

Notes: 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 ICCs (%) 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 ($p < .05$).

Variance calculations

Table 3 provides the ICCs (%) 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 dependent variables and were included as a random factor in the larger hierarchical models.

Recovery responses following 90 and 120 min of simulated soccer

Interaction effects for trial and time were evident for CK ($p = .01$), with post hoc comparisons revealing higher 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 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}; $p < .01$). Furthermore, trial interaction effects were observed at 72-hr post 120-min (244.1 ± 24.7 μ·L^{-1}; 95% CI = 194.7 to 293.4 U·L^{-1}) vs. 72-hr following the 90-min simulation (154.1 ± 29.3 U·L^{-1}; 95% CI = 240.6 to 356.9 U·L^{-1}; 95% CI for diff = 17.2 to 176.9 U·L^{-1}; $p = .02$). No interactions were identified for creatinine ($p = .24$), urea ($p = .59$), AST ($p = .83$), VAS ($p = .22$), PPT_{RF} ($p = .99$), PPT_{BF} ($p = .78$), RSI ($p = .35$), CMJ ($p = .21$), $eccKF_{180}$ ($p = .75$), $eccKF_{270}$ ($p = .67$) and $eccKF_{60}$ ($p = .42$).

Main effects for time were observed for CK, urea, VAS, PPT_{BF}, RSI, CMJ, $eccKF_{180}$, $eccKF_{270}$ and $eccKF_{60}$ (all $p < .01$; Table 4). No main effects for time were evident for creatinine ($p = .24$), AST ($p = .23$) and PPT_{RF} ($p = .46$).

Discussion

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

Despite most variables demonstrating that recovery is not further impeded following ET, the current findings demonstrate that compared to the 90-min trial, CK activity increased by a further 53 and 58% at 24 and 72 hr following 120 min, respectively. The magnitude of change in CK far exceeds the intra- and inter-assay measurement error (i.e., coefficient of variation = 1.4-2.1% and 1.5–4.2%, respectively), indicating the variation is low enough not to obscure a true experimental effect. Considering the CK response to 120 min alone, a 317% increase at 24-hr post-trial was observed vs. baseline. Previous ET match-play investigations observed a 236% increase in CK at 24-hr post 120 min of soccer match-play vs. baseline in professional players (Russell, Sparkes et al., 2015). The higher magnitude of change in the current study compared to previous research is potentially explained by a wealth of factors such as standard of player and innate blood marker variability (Baird et al., 2012), 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, Sparkes et al., 2015). However, the activity was standardized for the current protocol, with players performing at the same

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]

Notes: 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.

intensity throughout the entire 120 min duration, unlike the players in the match-play study (Russell, Sparkes et al., 2015). 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 criticized 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, Page 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 et al., 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 individual basis, depsite the current research reporting an average response, since eccKF strength characteristics are susceptible to large individual variation following ET (Field, Page 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 et al., 2012), especially if players are not entirely recovered prior to the next competitive encounter. Epidemiological research, therefore, appears warranted to determine the extent to which ET matches exacerbate injury incidence in subsequent training and matches.

Soccer-specific activity involves repetitive eccentric to concentric actions, that can induce neuromuscular fatigue and reduce peak power output (Nédélec et al., 2012b). As such, given the functional relevance of such markers and their ability to detect perturbations in muscle function and elastic energy usage (Oliver et al., 2008), RSI and CMJ were used as indicators of exercise-induced fatigue. Time-dependent reductions in RSI and CMJ were evident for up to 48-hr following both trials in the current research, although no between-trial differences were identified for these measures. Several studies have shown that the recovery of these markers are prolonged following 90 and 120 min of simulated and actual match-play (Abbott et al., 2020; Russell, Northeast et al., 2015; Thomas et al., 2017; Winder et al., 2018). Therefore, although the muscle function data limit inference concerning how player recovery should be managed following ET, the results add to an expanding body of literature that suggests recovery may need to be considered alongside the congested fixture periods associated with major tournaments (Julian et al., 2021; Page et al., 2019). However, given that ET may not further prolong recovery of muscle function, there appears to be little need to adapt practice and provide additional measures to restore muscle function capacity irrespective of match duration.

An increased presence of blood urea was evident immediately post-exercise in both trials, and although differences did not reach statistical significance, these values were further (~10%) increased immediately following the 120-min exercise trial. Given macronutrient intake was similar between trials (Table 1), this finding, although speculative, is indicative of an increased protein degradation following ET. Urea is strongly associated with training volume potentially owing to the upregulation of gluconeogenesis during exercise that results in the breakdown of structural proteins (Haralambie & Berg, 1976; Meyer & Meister, 2011). The rate of gluconeogenesis is increased when glycogen stores are depleted and is stimulated by the secretion of glucagon, which can be linked to an upregulation in lipolysis (Shephard, 1999; De Sousa et al., 2019). This finding may corroborate previous research that suggests glycogen depletion and an increased rate of lipolysis is apparent during ET (Field, Page et al., 2020; Harper et al., 2016; Stevenson et al., 2017). This could potentially highlight the importance of modulating fueling strategies, including carbohydrate intake prior and during, as well as adapting protein intake post ET matches. However, this research provides a foundation for future lines of exploration that are warranted to assess substrate metabolism using invasive techniques such as a muscle biopsies following 120 min of soccer-specific 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 semiprofessional 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 et al., 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 maneuvers that likely place a greater deal of stress on the musculoskeletal system, and therefore evoke a muscle damage response of a higher magnitude (Castellano et al., 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., 2013). 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 et al., 2007). This is a common observation with treadmill variants (Greig et al., 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., 2022). However, inter-individual variances in recovery potential are apparent (Nédélec et al., 2012), and as such, recovery protocols following ET matches should be individualized 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 periodization 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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