

Research article

Adequate Interval between Matches in Elite Female Soccer Players

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Abstract

The present study compared four different intervals between three simulated soccer matches for changes in muscle damage and performance parameters. Thirteen well-trained female university soccer players performed three bouts of 90-min Loughborough Intermittent Shuttle Test (LIST) with four different intervals between bouts; one (1d), two (2d), three (3d) and four days (4d), with >12-weeks between conditions in a counterbalanced order. Heart rate, blood lactate, rating of perceived exertion and distance covered in each LIST were measured. Changes in several muscle damage markers (e.g., maximal voluntary isometric torque of the knee extensors: MVC-KE, muscle soreness), performance parameters (e.g., Yo-Yo intermittent recovery test level 1: Yo-Yo IR1), and blood measures (e.g., osmolality, high sensitivity cardiac troponin T) before the first LIST, 1 h after each LIST, and one to five days after the third LIST were compared among the conditions. The total distance covered during the first two LISTs was not different among the conditions, but that during the third LIST was shorter ($P < 0.05$) for the 1d ($9,416 \pm 885$ m) and 2d conditions ($9,737 \pm 246$ m) than the 3d ($10,052 \pm 490$ m) and 4d conditions ($10,432 \pm 538$ m). Changes in all measures were smaller ($P < 0.05$) in the 3d and 4d conditions (e.g., the decrease in MVC-KE at one day after the third LIST was $-13 \pm 4\%$ and $-10 \pm 3\%$, respectively) when compared with the 1d and 2d conditions ($-20 \pm 7\%$, $-18 \pm 5\%$). Performance parameters showed smaller ($P < 0.05$) changes in the 4d (e.g., the decrease in Yo-Yo IR1 at one day after the third LIST was $-9 \pm 3\%$) and 3d ($-13 \pm 6\%$) conditions when compared with the 1d ($-19 \pm 4\%$) and 2d ($-20 \pm 8\%$) conditions. These results suggest that muscle damage and fatigue accumulate when soccer matches are performed three consecutive days or every other day, but if more than three days are inserted between matches, this could be minimized.

Key words: 90-minute Loughborough intermittent shuttle test, muscle damage, counter movement jump, 30-m dash, Yo-Yo intermittent recovery test level 1.

Introduction

In official soccer (football) tournaments such as Fédération Internationale de Football Association (FIFA) World Cup and Asian Football Confederation (AFC) Asian Cup, matches of each team are generally arranged with at least two-day interval between matches (www.fifa.org). Fixture congestion is defined as a minimum of two successive match plays with an inter-match period of less than four days, and is a frequent and contemporary issue in professional soccer, because of increased commercialization and a rise in the number of domestic and international competitions (Julian et al., 2021; Page et al., 2020).

According to a recent systematic review article (Page et al., 2020), professional male soccer teams are often required to compete with less than four days between matches. The authors stated that injury risk was increased during fixture-congested periods, and more research was required to investigate injuries associated with congested match schedules. Soccer coaches and players in European professional football leagues complained the schedule of two matches per week before the 2022 World Cup (<https://theathletic.com/3330531/2022/05/26/world-cup-football-calendar/>). Bengtsson et al. (2013) documented in their epidemiological study that when matches were played less than four days apart, players were unable to recover fully in the period, which would decrease performance and increase injury risks. It has been documented in several studies (Ekstrand et al., 2011a; Howle et al., 2020; Mannino et al., 2023; McCall et al., 2018) that a match congestion increases non-contact injuries in professional soccer players. Dupont et al. (2010) reported that professional male soccer players could have two matches per week without affecting the distance covered and the numbers of sprint during matches, but it increased the injury rate 6 folds when compared with a match per week.

Muscle damage is induced in a soccer match even for well-trained players, resulting in symptoms such as decreased muscle function, delayed onset muscle soreness (DOMS), and impaired athletic performance (Chou et al., 2021; Draganidis et al., 2015; Hughes et al., 2018; Nedelec et al., 2012; Thomas et al., 2017). Such muscle damage is not serious, but could lead to more serious injuries. Thus, it is important to understand the time course of changes in muscle damage symptoms before and after a match or a training session. Chou et al. (2021) investigated muscle damage of elite female soccer players after a 90-minute Loughborough Intermittent Shuttle Test (LIST), which is considered to replicate the running activities in a soccer match. They reported that symptoms of muscle damage indicated by decreases in muscle function and DOMS lasted for three to five days after a single LIST, and the total distance covered during the LIST was decreased more than 5% in the second LIST that was performed in the next day.

These results appear to support the International Olympic Committee consensus that soccer matches should be interspersed by at least four days (Schwellnus et al., 2016; Soligard et al., 2016). Thus, less than four-day recovery time may not be adequate, which may lead to impairing performance and increasing an injury risk. However, a congested schedule with a shorter recovery time is

still often seen (Brito, 2017). Chou et al. (2021) investigated muscle damage after multiple 90-minute LISTs that were performed every day for three or six days, and showed that the total distance covered during the LIST was decreased in the second LIST onward, and decreases in muscle strength and other performance measures did not recover to the baseline even at five days after the sixth LIST. This is an extreme case of congested match examples, but to the best of our knowledge, no previous study has investigated the magnitude of effects of different recovery days between matches on muscle damage and performance parameters.

Fixture congestion or multiple soccer matches may also affect internal organs such as heart, liver and kidneys as shown by previous studies (Cirer-Sastre et al., 2020; Devrnja and Matković, 2018; Ekun et al., 2017; Hosseini et al., 2018). For example, Hosseini et al. (2018) reported elevations of serum cardiac troponin I (cTnI) concentration immediately after (+40%), and two (+60%) and 24 hours (+20%) following a soccer match that was played by 22 adolescent male soccer players. Cirer-Sastre et al. (2020) showed that serum cTnT concentration increased (+184%) at three hours after a football game in 12 adult male players. Ekun et al. (2017) showed that serum urea (+6%) and creatinine (+21%) concentration, aspartate aminotransaminase (AST: +10%), alanine aminotransaminase (ALT: +11%), alkaline phosphatase activity (ALP: +5%) all increased at 30 minutes after a football match performed by healthy young male university students. However, it has not been examined whether a different interval between matches affect heart, liver and kidney functions differently.

Moreover, dehydration is frequently observed during and after a soccer match or training session, and dehydration of >2% body mass has been demonstrated to impair football-specific performance (Devrnja and Matković, 2018). It is possible that these changes are exacerbated when multiple soccer matches are performed with a shorter rest interval between matches. However, this has not been investigated in previous studies.

Therefore, the present study compared four different intervals between LISTs; one, two, three and four days, for changes in several muscle damage markers, performance measures and blood measures associated with dehydration, liver, kidney and heart damage. It was hypothesized that the shorter interval (one and two days) between matches would induce greater extent of residual muscle fatigue and muscle damage, and impaired performance when compared with the longer interval (three and four days) between matches.

Methods

Participants and study design

The present study was approved by the Research Ethics Committee of National National Taiwan Normal University in Taiwan. The study was conducted in conformity with the policy statement regarding the use of human subjects by the Declaration of Helsinki. The participants were female university soccer players in a team that was the first place of the 2021 Taiwan University Football Tournament. They provided informed consents before participating in

the study.

The sample size was estimated using the data from our previous study in which university female soccer players performed one 90-minute Loughborough Intermittent Shuttle Test (LIST) or three 90-minute LISTs and with one day rest in-between (Chou et al., 2021). Based on the effect size of 1 for changes in maximal voluntary isometric contraction torque of the dominant knee extensors (MVC-KE) between one and three bout conditions, it was estimated that at least 12 participants were necessary for each condition, with an alpha level of 0.05 and power ($1-\beta$) of 0.80 (G*Power 3.1.9.2, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The data collection of the present study was performed in off-seasons between September 2021 and February 2023 over 18 months with four off-seasons in total. Initially, 20 players participated in the study, but seven players were not able to complete the four different conditions, because five players were chosen for the Taiwan national team (i.e., Chinese Taipei) and went abroad for Asian Cup and Qualify 2023 World Cup, and two players had an injury during in-seasons. Thus, 13 players completed the four conditions. Their (mean \pm SD; range) age (21.2 ± 1.2 y; 19-23 y), height (162.1 ± 5.4 cm; 152-173 cm), body mass (58.1 ± 6.6 kg; 48 - 69 kg), body mass index (22.1 ± 1.8 kg/m²; 18.8-24.7 kg/m²), percentage of body fat ($13.5 \pm 2.4\%$; 10.3-17.2%), and maximal oxygen consumption ($\dot{V}O_{2max}$, 52.4 ± 3.7 ml/kg/min; 46.7 - 59.7 ml/kg/min) were similar to elite level soccer players (Chou et al., 2021; Hsieh et al., 2022). The participants played 20 - 23 official matches during the experimental period of 40 weeks and had training sessions five days a week (~three hours per session). The present study was conducted during an off-season.

The participants performed three bouts of 90-minute LIST with four different intervals between bouts; one (1d), two (2d), three (3d) and four days (4d), with >12-weeks between conditions in a counterbalanced order (Figure 1). All muscle damage and performance measures were taken before, at one hour after each LIST, and one to five days after the last LIST for all conditions. Additionally, the muscle damage measures were taken between the first and second as well as the second and third LIST for each condition. There was no day of rest for one day (1d) condition, but there was one day, two days, and three days between bouts for the 2d, 3d, and 4d condition, respectively (Figure 1).

Familiarization session

In a familiarization session that was set at six to seven days before the first LIST, the participants experienced the measurements of muscle soreness, countermovement jump (CMJ), sub-maximal and MVC at 90° and 30° of knee flexion for knee extensors and flexors, respectively, on an isokinetic dynamometer (Biodex System S4; Biodex Medical Systems, Shirley, NY), 30-m dash, 30-m timed hop, agility t-test, 6 x 10-m shuttle run and Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1), and performed a 20-minute LIST. They also practiced the running corresponding to the velocity of 55% and 95% of their $\dot{V}O_{2max}$ for 5 minutes for each intensity (Chou et al., 2021; Hsieh et al., 2022).

	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1d	X	X	X	X	X	X	X	X	X						
2d	X	X	X	X	X	X	X	X	X	X	X	X			
3d	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
4d	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Figure 1. Experimental design and testing procedures of the study. Four conditions were based on the rest between three Loughborough Intermittent Shuttle Tests (LIST = indicated by \odot); one (1d), two (2d), three (3d) and 4 days (4d) interval rest between three Loughborough Intermittent Shuttle Tests performed consecutively by 13 participants, respectively. Time course of the measurements are indicated by X for each condition; B: baseline, one day before the first LIST, Day 1-14: days from the first LIST. The measurements in the LIST days were taken 1 hour after the LIST. All measurements were taken at 1, 2, 3, 4 and 5 days after the last LIST for all conditions. Muscle damage measurements were also taken between the first and second, and second and third LIST; days 2 and 4 for the 2d, days 2, 3, 4, and 5 for the 3d condition, and days 2, 3, 4, 6, 7, and 8 for the 4d conditions. The measurements included maximal voluntary isometric contraction torque of the knee extensors and flexors, muscle soreness, countermovement jump, 30-m dash, 30-m timed hop, agility T-test, 6 x 10-m shuttle run and Yo-Yo intermittent recovery test level 1 test, plasma creatine kinase activity, myoglobin concentration, glutamic oxaloacetate transaminase and glutamic pyruvic transaminase activity, uric acid, potassium phosphorus, high sensitivity cardiac troponin T concentrations and osmolality.

Maximal oxygen uptake (VO_{2max}) test

VO_{2max} was measured in a treadmill running test, and the details of the VO_{2max} were described elsewhere (Chou et al., 2021; Hsieh et al., 2022). The running speeds corresponding to 55% and 95% VO_{2max} were calculated for the LIST shown below.

The Loughborough intermittent shuttle test (LIST)

The present study used the 90-min LIST as a simulated soccer match, that had been designed to mimic the activities performed and the distance covered in a typical soccer match (Chou et al., 2021; Hsieh et al., 2022; Nicholas et al., 2000; Thomas et al., 2017). However, it should be noted that LIST does not replicate the musculoskeletal demands involved in playing with the ball, pivoting, cutting, tackling, jumping, and directional changes (Silva et al., 2018). A 90-minute LIST consisting of five sets of 15-minute intermittent running and intermittent shuttle run to exhaustion was performed in an indoor hall (Chou et al., 2021; Hsieh et al., 2022; Nicholas et al., 2000; Thomas et al., 2017). During each LIST, the distance covered, heart rate (HR), rating of perceived exertion (RPE), and blood lactate concentration were measured and recorded. The details of the LIST were described elsewhere (Chou et al., 2021; Hsieh et al., 2022; Nicholas et al., 2000). It should be noted that these participants occasionally performed LIST, so that all of them were familiar with the protocol.

Muscle damage markers

The muscle damage markers included MVC torque of the knee extensor and flexors, muscle soreness of the knee extensors and flexors, plasma CK activity and myoglobin (Mb) concentration. These markers were adopted from our previous studies, and the details were described elsewhere (Chen et al., 2020; Chou et al., 2022; Hsieh et al., 2022;

Lin et al., 2022).

Performance indices

The performance indices consisted of CMJ, 30-m dash, 30-m timed hop test, agility T-test, 6 x 10-m shuttle run and Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1). They were adopted from our previous studies, and the details were described elsewhere (Chou et al., 2021; Hsieh et al., 2022).

Biochemical markers

A 7-ml venous blood sample was withdrawn by standard venipuncture technique from the cubital fossa region of the arm and centrifuged for 10-minute to extract plasma. Plasma samples were stored at -80°C until analyses. Some selected markers of liver, kidney and heart damage as well as dehydration were measured including glutamic oxaloacetate transaminase (GOT) activity, glutamic pyruvic transaminase activity (GPT), potassium (K), phosphorus (P), uric acid (UA), high sensitivity cardiac troponin T (hsTnT) and osmolality (Osm). These markers were adopted from previous studies, and the details were described elsewhere (Chen and Hsieh, 2000; Chou et al., 2021; Clarkson et al., 2006; Nie et al., 2019).

Statistical analyses

A Shapiro–Wilk test was used to examine the normality assumption of the data, which demonstrated that all variables in the present study were normally distributed. All dependent variables before the first LIST were compared between the 1d, 2d, 3d and 4d conditions by a one-way analysis of variance (ANOVA). Changes in HR, RPE, blood lactate concentration, total distance covered during the LIST were compared between the conditions by two-way of repeated-measures ANOVA. Changes in each dependent variable before, one-hour after each LIST, and every 24-hour interval for five consecutive days after the last LIST were compared among conditions [condition (four) x time (nine)] by a two-way of repeated-measures ANOVA. When this showed a significant ($P < 0.05$) interaction effect, a two-way repeated-measures ANOVA [condition (two) x time (nine)] was run to compare between two conditions (i.e., 1d and 2d, 1d and 3d, 1d and 4d, 2d and 3d, 2d and 4d, and 3d and 4d) for the changes in the dependent variables. When a significant interaction effect ($P < 0.05$) was found, a Tukey's post-hoc test was performed. Changes in muscle damage measures between bouts for the 2d, 3d and 4d conditions were assessed by a one-way of repeated-measures ANOVA, respectively. When a significant main effect ($P < 0.05$) was found, a Tukey's post-hoc test was performed. Eta-squared values (η^2) were calculated as measures of effect size, and they were considered as ~ 0.02 : small effect; ~ 0.13 : medium effect; and > 0.26 : large effect (Bakeman, 2005). A significant level was set at $P \leq 0.05$. The data were presented as mean \pm SD.

Results

LIST

All participants completed four conditions over 18 months, and performed three LISTs in each interval condition as planned. Physical characteristics and fitness of the players

during the experimentation period did not significantly ($P > 0.05$) change, and no player had any injury in the study. As shown in Figure 2, no significant differences in the total distance covered ($F = 1.451$, $\eta^2 = 0.108$, $P = 0.244$), average heart rate ($F = 0.565$, $\eta^2 = 0.045$, $P = 0.642$), RPE ($F = 0.922$, $\eta^2 = 0.071$, $P = 0.440$), and post-LIST blood lactate concentration ($F = 0.134$, $\eta^2 = 0.011$, $P = 0.939$) were evident in the first LIST between conditions. No significant changes in the average heart rate (interaction effect: $F = 1.210$, $\eta^2 = 0.070$, $P = 0.308$), RPE ($F = 0.742$, $\eta^2 = 0.044$, $P = 0.617$), and post-LIST blood lactate concentration ($F = 0.054$, $\eta^2 = 0.003$, $P = 0.999$) were found over three LISTS for all conditions (Figure 2B-D). However, a significant interaction effect ($F = 10.666$, $\eta^2 = 0.400$, $P < 0.001$) was found for the total distance covered across the three LISTS among the conditions (Figure 2). The post-hoc tests revealed that the 1d and 2d conditions had significantly smaller distance than the 3d and 4d conditions in the third LIST (1d and 3d: $F = 12.867$, $\eta^2 = 0.349$, $P < 0.001$; 1d and 4d: $F = 18.088$, $\eta^2 = 0.430$, $P < 0.001$; 2d and 3d: $F = 10.593$, $\eta^2 = 0.306$, $P < 0.001$; 2d and 4d: $F = 20.894$, $\eta^2 = 0.465$, $P < 0.001$) (Figure 2A). Moreover, the average HR during the second LIST (1d: first vs second LIST: $P = 0.043$; 2d: first vs second LIST: $P = 0.001$) and third LIST (1d: first vs third LIST: $P = 0.049$; 2d: first vs third LIST: $P = 0.039$) was significantly higher than that of the first LIST for both 1d and 2d conditions (Figure 2C).

Baseline measurements

All variables at the baseline (before the first LIST) were not significantly ($P > 0.05$) different among the 1d, 2d, 3d and 4d conditions (Figure 3 and Figure 4, Table 1). When

comparing the measures taken before the first LIST over four conditions, no significant ($P > 0.05$) differences were found for any of the variables, indicating no order effect.

Muscle damage markers

All muscle damage markers changed significantly ($P < 0.05$) at 1 hour after the first LIST for all conditions without a significant difference among them (Figure 3). A significant interaction effect ($P < 0.001$) was evident for the changes in KE MVC torque ($F = 10.903$, $\eta^2 = 0.476$) and KF MVC torque ($F = 18.281$, $\eta^2 = 0.604$) among the four conditions. Comparing two conditions by a series of two-way repeated-measures ANOVA showed that the changes following the third LIST were greater ($P < 0.05$) for the 1d and 2d conditions than the 3d and 4d conditions. No significant difference between the 1d and 2d conditions, but the recovery of KE MVC and KF MVC was significantly ($P < 0.05$) faster for the 4d than 3d condition (Figure 3A-B). A significant interaction effect ($P < 0.001$) was found for changes in muscle soreness of KE ($F = 7.685$, $\eta^2 = 0.390$) and KF ($F = 12.663$, $\eta^2 = 0.513$), plasma CK activity ($F = 21.641$, $\eta^2 = 0.643$) and Mb concentration ($F = 16.672$, $\eta^2 = 0.581$) when the four conditions were compared. A series of two-way repeated-measures ANOVA identified that the changes were greater ($P < 0.05$) for the 1d and 2d conditions than the 3d and 4d conditions, without difference ($P > 0.05$) between the 1d and 2d conditions, but smaller ($P < 0.05$) changes for the 4d than 3d condition (Figure 3C-F). All muscle damage markers changed ($P < 0.001 - 0.024$) between the first and second LIST as well as the second and third LIST for the 2d, 3d and 4d conditions from the baseline level (Table 1).

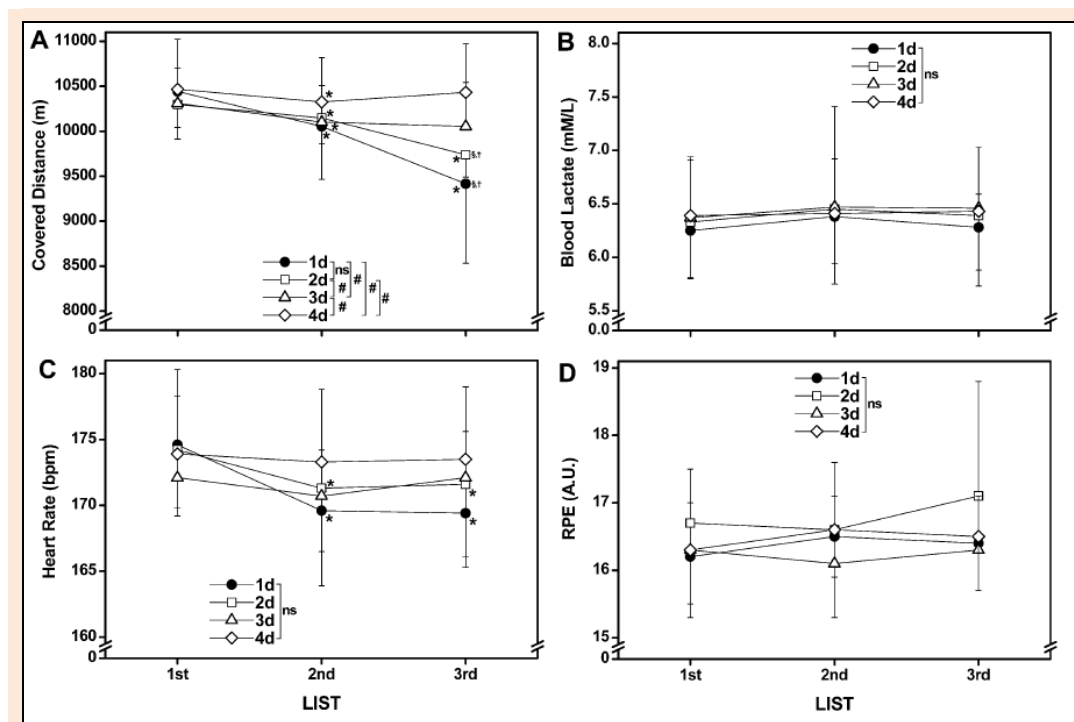


Figure 2. Changes (mean \pm SD) in the total distance covered (A), blood lactate concentration (B), heart rate (C) and ratings of perceived exertion (RPE, D) in the first to three (1st, 2nd, 3rd) Loughborough Intermittent Shuttle Test (LIST) for the 1 (1d), 2 (2d), 3 (3d) and 4 days (4d) interval rest conditions between LISTS (1st, 2nd, 3rd). #: a significant ($P < 0.05$) interaction effect by a two-way of repeated-measures ANOVA. *: a significant ($P < 0.05$) difference from the baseline (i.e., 1st) value. †: a significant ($P < 0.05$) difference from the 1d condition for the 3rd LIST based on the post hoc tests. ‡: a significant ($P < 0.05$) difference from the 2d condition for the 3rd LIST based on the post hoc tests.

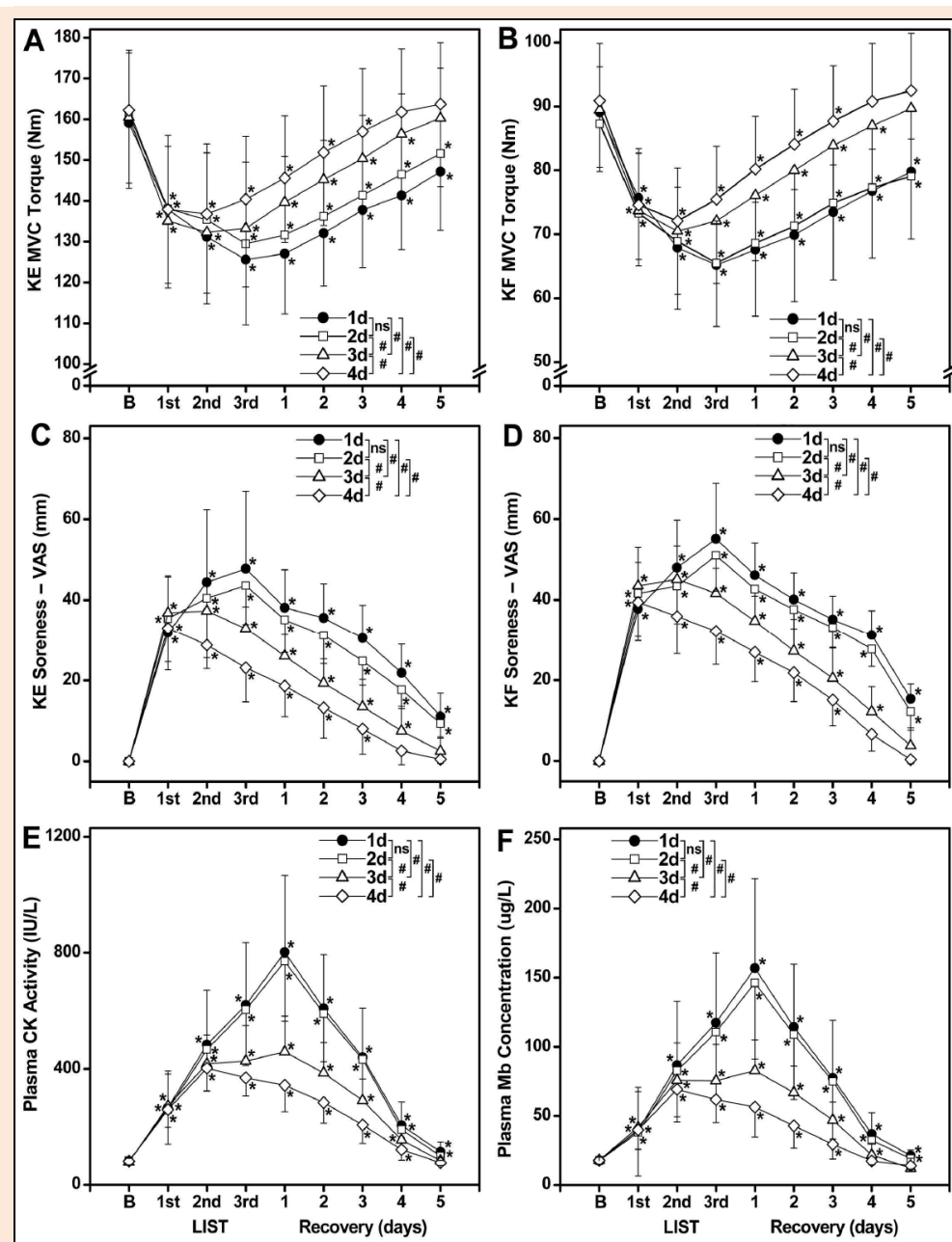


Figure 3. Changes (mean \pm SD) in maximal voluntary isometric contraction (MVC) torque of the knee extensors (KE, A) and flexors (KF, B), muscle soreness assessed by a 100-mm visual analog scale (VAS) of the knee extensors (C) and knee flexors (D), plasma creatine kinase (CK) activity (E) and myoglobin (Mb) concentration (F) at baseline (pre), 1 hour (1st, 2nd, 3rd) after each Loughborough Intermittent Shuttle Test (LIST) performed four different of rest interval between LISTs (1st, 2nd, 3rd), and 1, 2, 3, 4 and 5 days (1-5) after the last LIST for the one (1d), two (2d), three (3d) and four (4d) days of rest interval conditions. **B:** baseline measurements. #: a significant ($P < 0.05$) interaction effect by a two-way of repeated-measures ANOVA. ns: no significant ($P > 0.05$) interaction effect by a two-way of repeated-measures ANOVA. *: a significant ($P < 0.05$) difference from the baseline value.

Performance parameters

All performance measurements changed significantly over time for all conditions, and significant ($P < 0.001$) interaction effects were evident for all parameters; CMJ height ($F = 12.135$, $\eta^2 = 0.503$), 30-m dash ($F = 26.680$, $\eta^2 = 0.690$), 30-m timed hop ($F = 8.446$, $\eta^2 = 0.413$), agility T-test ($F = 6.925$, $\eta^2 = 0.366$), 6x10-m shuttle run ($F = 15.723$, $\eta^2 = 0.567$) and Yo-Yo IRI ($F = 6.059$, $\eta^2 = 0.336$). A series of two-way repeated-measures ANOVA showed that the changes in CMJ height and 30-m dash were significantly greater ($P < 0.05$) for the 1d and 2d conditions than the 3d

and 4d conditions without a difference ($P > 0.05$) between the 1d and 2d conditions, as well as the 3d and 4d conditions (Figure 4A-B). The changes in 30-m timed hop, agility T-test, 6x10-m shuttle run and Yo-Yo IRI were also significantly greater ($P < 0.05$) for the 1d and 2d conditions than the 3d and 4d conditions without difference between the 1d and 2d conditions, but the 3d condition was greater than the 4d condition (Figure 4C-F). The 6x10-m shuttle run returned ($P = 0.257$) to the baseline at 3 days after the third LIST for the 4d condition, but other performance measures did not return to the baseline at one, one to two

and one to three days after the second and third LIST for the 2d, 3d and 4d conditions, respectively, were significantly ($P \leq 0.001 - 0.005$) changed compared to their baseline level.

Biochemical measures

All biochemical markers increased significantly ($P < 0.05$) over time for all conditions, and significant interaction effects ($P < 0.001$) were found for all; plasma GOT ($F = 15.782, \eta^2 = 0.568$) and GPT activity ($F = 17.840, \eta^2 = 0.598$), K (interaction effect: $F = 14.394, \eta^2 = 0.545$), P ($F = 7.040, \eta^2 = 0.370$), UA ($F = 20.611, \eta^2 = 0.632$), hsTnT concentrations ($F = 4.900, \eta^2 = 0.290$) and Osm ($F = 5.719, \eta^2 = 0.323$) as shown in Table 2. After a series of two-way repeated-measures ANOVA was performed, it was found that the changes in all biochemical markers were greater ($P < 0.05$) for the 1d and 2d conditions than the 3d and 4d conditions without a difference ($P > 0.05$) between the 1d and 2d conditions. Also, the increases in plasma GOT and GPT activity, K, UA and hsTnT concentrations were greater ($P < 0.05$) for the 3d than 4d condition.

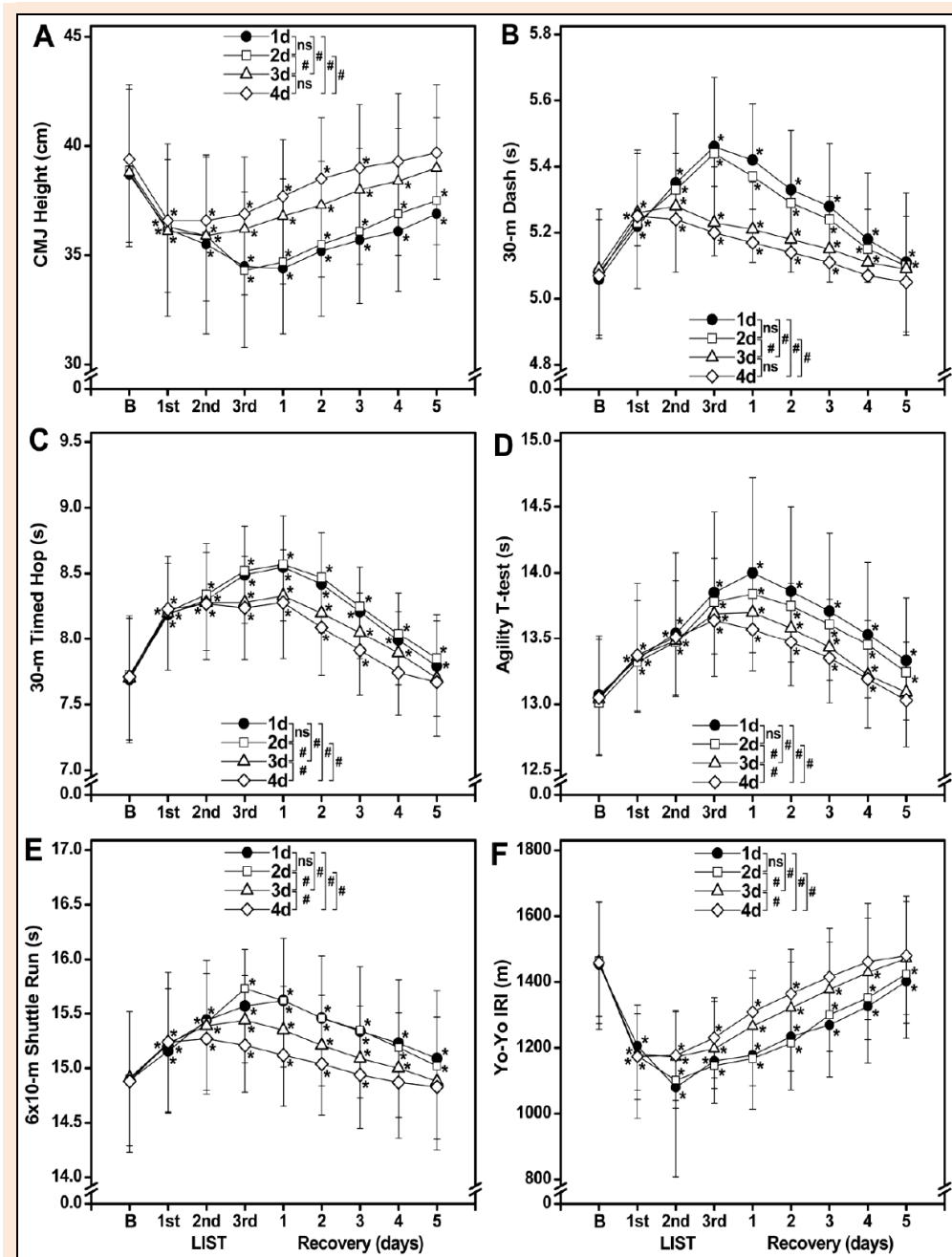


Figure 4. Changes (mean \pm SD) in countermovement jump (CMJ) height (A), 30-m dash (B), 30-m timed hop test (C), agility T-test (D), 6 x 10-m shuttle run (E) and Yo-Yo intermittent recovery test level 1 (Yo-Yo IRI, F) at baseline (pre), 1 hour (1st, 2nd, 3rd) after each Loughborough Intermittent Shuttle Test (LIST) performed four different rest interval between LISTs (1st, 2nd, 3rd), and 1, 2, 3, 4 and 5 days (1-5) after the last LIST for the one (1d), two (2d), three (3d) and four (4d) days of rest interval conditions. B: baseline measurements. #: a significant ($P < 0.05$) interaction effect by a two-way of repeated-measures ANOVA. ns: no significant ($P > 0.05$) interaction effect by a two-way of repeated-measures ANOVA. *: a significant ($P < 0.05$) difference from the baseline value.

Table 1. Mean (\pm standard deviation) values of maximal voluntary isometric contraction torque of the knee extensors (MVC-KE, Nm) and flexors (MVC-KF, Nm), muscle soreness assessed by a 100-mm visual analog scale for the knee extensors (SOR-KE, mm) and flexors (SOR-KF, mm), plasma creatine kinase (CK, IU/L) activity and myoglobin (Mb, μ g/L) concentration at baseline (B), and between the first and second, and second and third 90-minutes Loughborough Intermittent Shuttle Test (LIST) for the 2d (interval between LISTs was two days), 3d (three days), and 4d (four days) conditions.

C	Measures	Baseline	1	2	3	4	5	6	7	8	9
2d	MVC-KE	161 \pm 15	138 \pm 18*	142 \pm 18*	135 \pm 19*	140 \pm 19*	130 \pm 20*	□	□	□	□
	MVC-KF	88 \pm 9	73 \pm 10*	76 \pm 9*	70 \pm 9*	73 \pm 9*	65.4 \pm 8.1*	□	□	□	□
	SOR-KE	0.0 \pm 0.0	35 \pm 13*	40 \pm 19*	41 \pm 15*	39 \pm 16*	43.6 \pm 9.7*	□	□	□	□
	SOR-KF	0.0 \pm 0.0	42 \pm 11*	46 \pm 13*	43 \pm 9*	42 \pm 11*	51.0 \pm 8.8*	□	□	□	□
	CK	83 \pm 14*	259 \pm 120*	328 \pm 131*	467 \pm 144*	519 \pm 163*	604 \pm 193*	□	□	□	□
	Mb	18 \pm 3	38 \pm 32*	53 \pm 35*	83 \pm 37*	94 \pm 42*	111 \pm 49*	□	□	□	□
3d	MVC-KE	161 \pm 16	135 \pm 16*	138 \pm 17*	141 \pm 18*	132 \pm 15*	135 \pm 16*	138 \pm 17*	133 \pm 14*	□	□
	MVC-KF	90 \pm 9	74 \pm 9*	75 \pm 9*	77 \pm 9*	71 \pm 10*	72 \pm 11*	75 \pm 11*	72 \pm 10*	□	□
	SOR-KE	0 \pm 0	37 \pm 9*	38 \pm 7*	29 \pm 6*	37 \pm 7*	34 \pm 16*	22 \pm 12*	33 \pm 5*	□	□
	SOR-KF	0 \pm 0	44 \pm 10*	47 \pm 7*	37 \pm 5*	45 \pm 8*	41 \pm 13*	38 \pm 24*	42 \pm 6*	□	□
	CK	79 \pm 12*	272 \pm 110*	334 \pm 125*	350 \pm 67*	417 \pm 99*	472 \pm 108*	402 \pm 89*	427 \pm 122*	□	□
	Mb	18 \pm 2	42 \pm 26*	56 \pm 30*	60 \pm 18*	76 \pm 27*	88 \pm 29*	71 \pm 21*	76 \pm 26*	□	□
4d	MVC-KE	162 \pm 15	138 \pm 16*	142 \pm 16*	145 \pm 16*	150 \pm 15*	137 \pm 15*	139 \pm 15*	144 \pm 15*	148 \pm 16*	140 \pm 15*
	MVC-KF	91 \pm 9	75 \pm 9*	77 \pm 9*	79 \pm 9*	81 \pm 9*	72 \pm 8*	75 \pm 8*	78 \pm 8*	80.8 \pm 8.4*	76 \pm 8*
	SOR-KE	0 \pm 0	33 \pm 8*	38 \pm 12*	31 \pm 11*	22 \pm 9*	29 \pm 6*	26 \pm 8*	19 \pm 8*	14.5 \pm 7.3*	23 \pm 8*
	SOR-KF	0 \pm 0	39 \pm 10*	46 \pm 13*	39 \pm 12*	30 \pm 9*	36 \pm 9*	35 \pm 9*	29 \pm 11*	22 \pm 10*	32 \pm 8*
	CK	82 \pm 13*	259 \pm 61*	330 \pm 75*	356 \pm 67*	327 \pm 94*	402 \pm 77*	450 \pm 83*	355 \pm 69*	270 \pm 61*	369 \pm 63*
	Mb	18 \pm 3	40 \pm 14*	55 \pm 16*	61 \pm 15*	52 \pm 22*	69 \pm 20*	80 \pm 23*	59 \pm 18*	40 \pm 16*	62 \pm 17*

Baseline: baseline measurements, 1-9: days 1-9 shown in Figure 1. *: a significant ($P < 0.05$) difference from the baseline value. *Italic values* are the same as those shown in Figure 3.

Table 2. Changes (mean \pm SD) in plasma glutamic oxaloacetate transaminase activity (GOT, IU/L) and glutamic pyruvic transaminase activity (GPT, IU/L), potassium concentration (K, mEq/L), phosphorus (P, mg/dL), uric acid concentration (UA, mg/dL) and high sensitivity cardiac troponin T concentration (hsTnT, ng/L), and osmolality (Osm, mOsm/kg H₂O)) at baseline, 1 hour after the first (1st), second (2nd) and third (3rd) Loughborough Intermittent Shuttle Test (LIST), and 1, 2, 3, 4 and 5 days after the 3rd LIST for the one (1d), two (2d), three (3d) and four (4d) days of rest between LISTs.

C	Measures	Baseline	1	2	3	4	5	6	7	8	9
1d	GOT	15 \pm 4	26 \pm 15*	66 \pm 40*	94 \pm 47*	127 \pm 59*	93 \pm 44*	59 \pm 37*	28 \pm 12*	17 \pm 6*	17 \pm 6*
2d		16 \pm 4	25 \pm 12*	64 \pm 28*	87 \pm 38*	118 \pm 42*	86 \pm 37*	56 \pm 31*	24 \pm 9*	18 \pm 3*	18 \pm 3*
3d		14 \pm 3	27 \pm 21*	54 \pm 22*	53 \pm 20*#^	59 \pm 16*#^	46 \pm 15*#^	33 \pm 8*#^	18 \pm 8*#^	10 \pm 6*#^	10 \pm 6*#^
4d		15 \pm 4	26 \pm 13*	51 \pm 16*	45 \pm 13*#^	40 \pm 17*#^	31 \pm 10*#^	24 \pm 10*#^	14 \pm 4*#^	12 \pm 4*#^	12 \pm 4*#^
1d	GPT	16 \pm 5	25 \pm 13*	65 \pm 30*	89 \pm 37*	124 \pm 41*	87 \pm 31*	54 \pm 26*	32 \pm 11*	20 \pm 5*	20 \pm 5*
2d		16 \pm 6	23 \pm 0*	60 \pm 27*	84 \pm 38*	115 \pm 43*	85 \pm 33*	51 \pm 26*	26 \pm 12*	19 \pm 4*	19 \pm 4*
3d		16 \pm 4	27 \pm 6*	59 \pm 22*	63 \pm 21*#^	70 \pm 23*#^	56 \pm 20*#^	42 \pm 13*#^	20 \pm 9*#^	11 \pm 7*#^	11 \pm 7*#^
4d		17 \pm 5	28 \pm 11*	56 \pm 17*	50 \pm 14*#^	45 \pm 21*#^	32 \pm 14*#^	23 \pm 10*#^	15 \pm 5*#^	12 \pm 2*#^	12 \pm 2*#^
1d	K	4.3 \pm .2	4.6 \pm .3*	4.8 \pm .3*	5.1 \pm .3*	5.1 \pm .3*	4.8 \pm .3*	4.6 \pm .3*	4.5 \pm .3*	4.3 \pm .3*	4.3 \pm .3*
2d		4.2 \pm .3	4.6 \pm .3*	4.9 \pm .4*	5.1 \pm .5*	4.9 \pm .4*	4.7 \pm .3*	4.5 \pm .3*	4.3 \pm .3*	4.2 \pm .3	4.2 \pm .3
3d		4.2 \pm .2	4.7 \pm .4*	4.8 \pm .3*	4.6 \pm .3*#^	4.5 \pm .2*#^	4.3 \pm .2*#^	4.2 \pm .2*#^	4.0 \pm .1*#^	3.9 \pm .1*#^	3.9 \pm .1*#^
4d		4.2 \pm .2	4.8 \pm .5*	4.8 \pm .3*	4.6 \pm .2*#^	4.4 \pm .2*#^	4.2 \pm .2*#^	4.0 \pm .2*#^	3.9 \pm .2*#^	3.8 \pm .2*#^	3.8 \pm .2*#^
1d	P	3.5 \pm .4	3.9 \pm .4*	4.2 \pm .4*	4.6 \pm .4*	4.5 \pm .4*	4.2 \pm .4*	4.0 \pm .4*	3.8 \pm .4*	3.7 \pm .4*	3.7 \pm .4*
2d		3.5 \pm .3	3.9 \pm .3*	4.3 \pm .3*	4.6 \pm .4*	4.4 \pm .3*	4.1 \pm .2*	4.0 \pm .2*	3.7 \pm .3*	3.6 \pm .2*	3.6 \pm .2*
3d		3.5 \pm .3	4.0 \pm .3*	4.2 \pm .3*	4.2 \pm .0*#^	4.0 \pm .3*#^	3.8 \pm .3*#^	3.7 \pm .3*#^	3.6 \pm .3*#^	3.5 \pm .3	3.5 \pm .3
4d		3.4 \pm .4	4.0 \pm .3*	4.2 \pm .5*	4.2 \pm .4*#^	3.9 \pm .4*#^	3.7 \pm .4*#^	3.6 \pm .4*#^	3.5 \pm .4#	3.4 \pm .4	3.4 \pm .4
1d	UA	5.0 \pm .6	5.5 \pm .8*	5.6 \pm .7*	5.8 \pm .7*	5.7 \pm .7*	5.6 \pm .7*	5.4 \pm .7*	5.2 \pm .7*	5.1 \pm .7*	5.1 \pm .7*
2d		4.9 \pm .6	5.4 \pm .9*	5.7 \pm .9*	5.8 \pm .8*	5.6 \pm .7*	5.4 \pm .7*	5.3 \pm .7*	5.1 \pm .7*	5.0 \pm .6*	5.0 \pm .6*
3d		4.8 \pm .9	5.4 \pm .8*	5.6 \pm .8*	5.4 \pm .7*	5.3 \pm .7*	5.1 \pm .7*	4.9 \pm .8	4.7 \pm .8*#^	4.5 \pm .8*#^	4.5 \pm .8*#^
4d		4.8 \pm .7	5.4 \pm .9*	5.2 \pm .8*	5.0 \pm .7*#^	4.8 \pm .7*#^	4.6 \pm .7*#^	4.5 \pm .7*#^	4.3 \pm .7*#^	4.2 \pm .7*#^	4.2 \pm .7*#^
1d	hsTnT	2.1 \pm 1.0	7.4 \pm 6.4*	9.8 \pm 7.3*	15.7 \pm 15.5*	8.8 \pm 6.4*	6.2 \pm 4.4*	4.9 \pm 3.9*	3.6 \pm 2.1*	2.4 \pm .4*	2.4 \pm .4*
2d		1.7 \pm .5	5.9 \pm 5.1*	8.4 \pm 3.8*	10.2 \pm 3.0*	6.1 \pm 1.4*	4.5 \pm 1.1*	1.2 \pm 1.2*	2.0 \pm .9*	1.8 \pm .8	1.8 \pm .8
3d		2.0 \pm .7	6.8 \pm 1.2*	5.5 \pm 1.7*#^	5.0 \pm .9*#^	4.3 \pm 1.8*#^	2.6 \pm .9*#^	2.2 \pm .8#	2.0 \pm .8#	1.7 \pm .6#	1.7 \pm .6#
4d		1.8 \pm .6	5.6 \pm 2.5*	4.2 \pm 1.4*#^	3.7 \pm 2.0*#^	3.1 \pm 1.3*#^	2.3 \pm 1.2#	2.0 \pm 1.0#	1.9 \pm .9#	1.6 \pm .5#	1.6 \pm .5#
1d	Osm	282 \pm 5	298 \pm 5*	302 \pm 4*	305 \pm 7*	297 \pm 5*	289 \pm 3*	284 \pm 3*	282 \pm 5	282 \pm 5	282 \pm 5
2d		283 \pm 3	297 \pm 11*	300 \pm 9*	301 \pm 9*	291 \pm 2*	287 \pm 3*	284 \pm 3	283 \pm 3	283 \pm 3	283 \pm 3
3d		283 \pm 5	299 \pm 6*	298 \pm 6*#	294 \pm 6*#^	286 \pm 5*#^	285 \pm 5*#^	284 \pm 5	284 \pm 5	283 \pm 5	283 \pm 5
4d		283 \pm 6	301 \pm 9*	297 \pm 7*#	292 \pm 7*#^	285 \pm 5*#^	283 \pm 6#	282 \pm 6	282 \pm 6	281 \pm 6	281 \pm 6

*: a significant ($P < 0.05$) difference from the baseline value. #: significantly different from the 1d condition. ^: significantly different from the 2d condition. †: significantly different from the 3d condition.

Discussion

The results were in line with the hypothesis that the shorter

interval (one or two days) between matches would induce greater extent of residual muscle fatigue and changes in muscle damage and performance markers when compared

with the longer interval (three or four days) between matches. However, we did not see any injury in the present study based on observations and the reports from the players. The total distance covered during the first two LISTs was not different among the conditions, but that during the third LIST was significantly shorter for the 1d and 2d conditions than the 3d and 4d conditions (Figure 2A). Changes in the muscle damage, performance and biochemical parameters were significantly smaller in the 3d and 4d conditions than the 1d and 2d conditions without significant differences between the 1d and 2d conditions, but changes in most of the parameters were significantly smaller for the 4d than 3d condition (Figure 3 and Figure 4, Table 2). These results suggest that at least three days but ideally four days are necessary between matches for the female players to perform well in matches. However, it should be noted that some extent of muscle damage is inevitable in playing a match, which impairs performance and possibly taxes the body for several days.

A 90-min LIST has been used to replicate the physiological demands of soccer matches or used as simulated running activities in soccer matches (Nicholas et al., 2000). The present study adopted the LIST as a simulated match based on previous studies (Chou et al., 2021; Hsieh et al., 2022; Magalhes et al., 2010; Nedelec et al., 2012; Nicholas et al., 2000; Thomas et al., 2017). It is well known that the magnitude of muscle damage is attenuated in subsequent bouts of the same or similar eccentric exercise, which is referred to as the repeated bout effect (Chen et al., 2007; Chen et al., 2016; McHugh, 2003). Thus, the within-subject design used in the present study could have been affected by the repeated bout effect. However, the players had been already accustomed to the LIST before the study, and the order of the four resting conditions was randomized among the players. The baseline measures across the four conditions were similar (Figure 3 and Figure 4, Table 2), and the performance in the first LIST was also similar among the conditions (Figure 2). Thus, it is assumed that the study design was adequate to investigate the different resting intervals between matches.

The distance covered in the first and second LISTs was not significantly different among the four rest conditions, but the distance in the second LIST was significantly shorter than that in the first LIST for all conditions (Figure 2A). The shorter distance covered in the second than the first LIST may be associated with muscle damage induced by the first LIST. It is documented that intense eccentric contractions of lower limb muscles such as knee flexors and extensors are performed when decelerating, changing directions, keeping balance, and landing after jumps in soccer matches (Askling et al., 2007; Chumanov et al., 2011; Ekstrand et al., 2011b; Schuermans et al., 2016). Interestingly, the average HR during the second and third LISTs for the 1d and 2d conditions was significantly lower than that in the first LIST (Figure 2B). It is possible that the reduced running performance indicated by the reduced distance covered (Figure 2A) due to muscle damage from the previous LIST limited high-intensity running performance, resulting in lower HR.

Chou et al. (2021) showed that it took 4-5 days for muscle damage markers to return to pre-match levels after

a LIST performed by well-trained female soccer players. It should be noted that the distance in the second LIST gradually increased with increasing in the interval between the first and second LISTs (Figure 2A). This suggests that muscle damage after the first LIST affected the performance of the second LIST, but the longer the recovery time, the less effects on the performance, because of gradual recovery from muscle damage. In the third LIST, the distance was shorter by 10% and 6% in the 1d and 2d conditions, respectively when compared with their first LIST, while no such reduction was observed for the 3d and 4d conditions from the second to the third LIST (Figure 2A). This was in line with the findings of our previous study (Chou et al., 2021) reporting that the covered distance during the second ($10,277 \pm 220$ m, -5%) and third LISTs ($9,883 \pm 339$ m, -9%) was significantly shorter than that in the first LIST ($10,844 \pm 528$ m) in three consecutive days of the simulated soccer matches performed by elite female soccer players. These results suggest that female soccer players did not fully recover from a previous match, and their performance was reduced when the subsequent match is played within two days.

Significant changes in muscle damage markers (MVC, muscle soreness, plasma CK activity and Mb concentration) were found following the first LIST for all conditions without differences among the conditions (Figure 3). The magnitude of changes in the muscle damage markers were similar to that reported in the previous studies (Chou et al., 2021; Leeder et al., 2014; Magalhães et al., 2010; Thomas et al., 1999). Magalhães et al. (2010) compared changes in muscle damage markers following a LIST and an actual soccer match, and reported that the changes in muscle damage markers (MVC-KE and MVC-KF, DOMS, plasma myoglobin concentration and CK activity) and some performance measures (CMJ, 20-m sprint) were not different between them, although the soccer match induced greater changes in redox status, adenine nucleotide metabolism and lymphocyte counts than LIST. Thus, it seems likely that changes in the muscle damage and performance measures after the 90-minutes LIST represent the changes after an actual soccer match.

The changes in all muscle damage markers following three LISTs were significantly greater for the 1d and 2d conditions than the 3d and 4d conditions, and the 3d condition was significantly greater than the 4d condition (Figure 3). The muscle damage markers did not return to the baseline between LISTs for all conditions (Table 1). These indicate that muscle damage was induced after each LIST even for well-trained soccer players who were accustomed to LIST, and it appears that more muscle damage was accumulated with a shorter rest period between LISTs. Previous studies reported a similar finding in male and female soccer players (Leeder et al., 2014; Chou et al., 2021; Page et al., 2019). For example, Page et al. (2019) used three bouts of a 90-minute treadmill-based match simulation with 48 hours interval to investigate changes in physiological, perceptual, and mechanical measures. They reported that maximal voluntary eccentric contraction torque of the knee flexors decreased immediately after the second (-16%) and third (-19%) bouts, and DOMS increased after the first (100-mm visual analog scale: 42 mm) to the second (52

mm) and third bout (57 mm) in 10 male semi-professional soccer players. Chou et al. (2021) reported that changes in muscle damage and performance parameters were greater when female soccer players performed a 90-minute LIST (the same as that in the present study) for three consecutive days than one day only. It is assumed that physical and mental demand would be greater in an official match than in a LIST. Therefore, it seems likely that a fixture congestion reduces performance (Carling et al., 2012; Odetoynbo et al., 2007; Rollo et al., 2014) and increases muscle damage as well as non-contact injury risks (Dupont et al., 2010; Mannino et al., 2023).

In a systematic review and meta-analysis paper, Silva et al. (2018) showed that hamstring force production capacity (ES = -0.7), CK activity in the blood (ES = 0.4), well-being (fatigue: ES = 0.3 - 0.9; sleep: ES = 0.2 - 0.3; stress: ES = 0.2 - 0.3) and muscle soreness (ES = 0.6 - 1.3) did not return to the baseline levels at 72 hours after an official soccer match in male soccer players, and concluded that a period of 72 hours post-match rest would not be long enough. If players have a next match without a full recovery from a previous match, additional muscle damage appears to be induced, prolonging the recovery time as shown in the present study. In contrast, the muscle damage and performance measures returned closer to the baseline between bouts when the interval between LISTs was longer (e.g., four days) than shorter (e.g., one day) as shown in Table 1. It is also important to note that all muscle damage markers returned to the baseline level by 4 days after the third LIST in the 4d condition (Figure 3). This suggests that 4 days of recovery are necessary between matches.

Similar to the results of muscle damage, significant changes in performance parameters were observed after the first LIST for all conditions without differences among the conditions (Figure 4). The extent of changes in performance parameters following three LISTs for the 1d and 2d conditions were significantly greater than that of the 3d and 4d conditions. Changes in all performance parameters except for CMJ height and 30-m dash were significantly smaller for the 4d than 3d condition. However, it should be noted that the female players who performed LIST every four days for three times still showed impaired performance after the three LIST (Figure 4). This suggests that some impairment of performance is inevitable even for well-trained female soccer players with the four-day interval between matches.

Dupont et al. (2010) compared the effects of one versus two such as total distance covered, high-intensity distance, sprint distance, and number of sprints, was not significantly different between the conditions, but the injury rate was higher for the two matches per week than one match per week (25.6 vs 4.1 injuries per 1,000 hours of exposure; $P < 0.001$). They concluded that the recovery time between matches should be 72 to 96 hours, and suggested the need for player rotation and improved recovery strategies. Silva et al. (2018) showed from a systematic review and meta-analysis that physical performance such as CMJ (ES = -0.4 to -0.6), T-test (ES = -0.4 to 0.5), linear sprint time (ES = 0.4 to 0.6) and well-being (fatigue: ES = 0.3 - 0.9; sleep: ES = 0.2 - 0.3; stress: ES = 0.2 - 0.3) remained significantly impaired at 72 hours after an official soccer

match for male soccer players. Pvoas et al. (2022) compared lower-ranked team and higher-ranked team players for their technical performance in a tournament in which four matches were played with two to three days of rest in eight days, and found that the extent of muscle damage, perceived exertion, decline in technical performance markers were greater for the lower-ranked than higher-ranked team players. This was probably due to a better ability to for the higher-ranked team players to deal with the matches better to minimize fatigue and muscle damage by performing less number of accelerations, decelerations, and sprints during the matches when compared with the lower-ranked team players (Pvoas et al., 2022). Thus, it seems likely that a longer recovery time is required when matches are harder. In an epidemiological study, Bengtsson et al. (2013) showed that the total number of injuries and muscle injury rate were 8% and 24% greater, respectively when matches were played with less than four days than greater than six days between matches in a men's professional football league. Thus, it should be cognizant of the risk of more serious injuries than muscle damage by having a congestive match schedule. Further studies are warranted to identify markers of incomplete recovery and implement interventions to facilitate recovery to see if injury risk can be reduced.

A soccer match could induce dehydration and affect internal organs such as heart, liver and kidneys as shown by previous studies (Cirer-Sastre et al., 2020; Devrnja and Matković, 2018; Ekun et al., 2017; Hosseini et al., 2018). For example, Cirer-Sastre et al. (2020) showed that serum cTnT concentration increased (+184%) at three hours after a football game in 12 adult male players. Ekun et al. (2017) showed that serum urea (+5.6%) and creatinine (+20.8%) concentration, aspartate aminotransaminase (+10.0%), alanine aminotransaminase (+10.9%), alkaline phosphatase activity (+4.7%) all increased at 30 minutes after a football match performed by healthy young undergraduate male students. Dehydration of >2% body mass has been demonstrated to impair football-specific performance (Cheuvront and Kenefick, 2014). It is possible that these changes are exacerbated when multiple soccer matches are performed with a shorter rest interval between matches. As shown in Table 2, the increases in all blood measures were significantly smaller for the 3d and 4d conditions than the 1d and 2d conditions, and for the 4d condition than the 3d condition. This is the first study to show that multiple soccer matches induced significant changes in heart, liver, kidney and dehydration markers, suggesting that the cardiac, liver, kidney functions were affected more with a short rest interval between matches. These results highlight the need for player rotation and for improved recovery strategies to minimize muscle and internal organ damage, maintain a better performance and health, especially in an official tournament.

The present study has several limitations. First, the participants of the present study were female university soccer players, hence the results of the present study may not reflect male, youth or professional soccer players. To the best of our knowledge, two studies investigated sex-differences in changes in muscle damage markers after a soccer match (Souglis et al., 2015; 2018). They reported

smaller changes in tumor necrosis factor α for female than male players without a significant sex difference in C-reactive protein and plasma CK activity (Souglis et al., 2015). Souglis et al. (2018) also showed that average HR during a match was significantly greater for men players (166 bpm) than women players (160 bpm), and increases in plasma lactate dehydrogenase and CK activities, and changes in oxidative stress markers (e.g., protein carbonyl, catalase activity, glutathione, and uric acid) and inflammatory markers (e.g., interleukine-6, c-reactive protein, and fibrinogen) after an official soccer match were greater for men than female players. No previous study has compared male and female players for the effects of multiple soccer matches on muscle damage and/or performance measures, but it is possible that a shorter interval between matches affects male players more than female players. Second, the changes in ovarian hormone status of these female players during the study were not recorded, and the possible effects of the hormones on the outcome measures were not controlled. Third, the LIST does not include heading a ball, kicking a ball, tackles, maximal jumps, changes of directions, and direct contacts with opposing players, and the LIST was performed on the wooden floor of an indoor sport hall in the present study. Therefore, this may be different from actual matches played on a grass pitch. Fourth, the results of the present study cannot be generalized to real situations that soccer players may face in official soccer tournaments. In some tournaments, matches are scheduled with only one or two days of recovery, and players often play four to six matches in eight to 10 days in a competition (Pvoas et al., 2022). Fifth, the muscle damage and performance measures were not taken immediately before the second and third LIST. Sixth, mental fatigue and injury risk were not assessed in the present study. Future studies are warranted to be considered the above limitations to investigate these issues.

Page et al. (2020) have stated that players having insufficient time to fully recover before next match have greater injury risk and reduced physical performance in the subsequent match. The present study showed that muscle damage, impaired performance and changes in biochemical markers representing dehydration and heart, liver and kidney functions were smaller when the players had three to four days between matches. However, it should be noted that even with a four-day rest, the effects from the previous match still remained, since muscle damage markers did not return to the baseline at three days after the first and second LIST for the 4d condition.

We analyzed the schedule of the FIFA Women's World Cup 2023 for the interval between matches for each team and which team won in the matches (<https://www.fifa.com/fifaplus/en/tournaments/womens/womensworldcup/australia-new-zealand2023>). The interval between matches was three to seven days for the group rounds, and that for the quarter finals, semifinals, third place and final was three to six days. It is interesting that the teams who had a longer rest interval between matches (average: 5.0 days) won the matches more (5 out of 6 matches) than the teams with a shorter rest interval (average: 4.3 days) for the quarter finals, third place and final matches (in the two semifinal matches, all teams had four

days from the quarter final matches). It appears that the teams that had a longer interval between matches had some advantages. It is interesting to examine whether the number of injuries was affected by the interval between matches.

It is unlikely that soccer players can have a complete rest after a match, since they may prepare for the next match, thus, good recovery strategies are important (Ranchordas et al., 2017). Our recent study showed that far-infrared radiation lamp therapy significantly enhanced recovery from multiple soccer matches, and reduced muscle damage and performance impairment (Hsieh et al., 2022). A recent study (García-Aliaga et al., 2023) suggested that coaches, sports scientists, and medical teams should consider an increase in the number of substitutions (player rotation) to reduce the risk of injury and physical performance in addition to optimize the recovery protocol during congestion matches. The findings of the present study could be used to develop specific planning, preparation and training for tournaments.

Conclusion

The current study showed that muscle damage and fatigue accumulated when three simulated soccer matches were performed three consecutive days or every other day, but if more than three days were inserted between matches, this could be minimized. Therefore, it appears that more than three days should be provided between matches for the same team to play against other teams in a competitive soccer event with multiple matches.

Acknowledgements

This work was supported by the National Science and Technology Council (NSTC 112-2425-H-003-001 & 110-2918-I-003-004) and the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan. However, the funding body played no roles in the design of the study and collection, analysis, and interpretation of the data and in writing the manuscript. The authors acknowledge Miss Wan-Chen Yu, Chih-Yi Chuang and Yi-Ting Wang for their assistance in the data collection. The authors also would like to acknowledge the study participants. The authors declaration that the experiments comply with the current laws of the country (Taiwan) in which they were performed. There is no conflict of interest. The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author, who was an organizer of the study.

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Key points

- Changes in markers representing fatigue, muscle damage, performance and internal organ condition were greater over three simulated soccer matches when the interval between matches was one or two days than three or four days.
- Muscle damage and fatigue appear to accumulate more with a shorter interval of less than three days between matches.
- Less than three-day interval between matches impairs performance and increases injury risks greater, which should be considered in scheduling a soccer tournament.

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