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TITLE: Acute pre- and post-competitive soccer match-play changes in neuromuscular factors, physical performance, and muscle integrity in youth male players

RUNNING HEAD: Soccer match effects on neuromuscular measures

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Acute pre- and post-competitive soccer match-play changes in neuromuscular factors, physical performance, and muscle response in youth male players

Abstract

The main purpose was to analyze the acute pre- and post-competitive soccer match-play changes in measures of landing mechanics, stretch-shortening cycle capability, physical performance, muscle damage, and match intensity. A secondary purpose was to explore whether the players' maturity status and chronological age were associated with post-soccer match-play responses. Thirty-two male youth outfield soccer players from two chronological competition age groups (U14 and U16) were assessed pre-and post-competitive soccer match-play for 2D dynamic knee valgus at landing (DKV), leg stiffness, reactive strength index, 20m sprint time, CMJ-Abalakov jump height, creatine kinase and urea activity and visual analogue scale for muscle soreness (VAS). Players' maturity status was also estimated using a previously validated regression equation. There were statistically significant ($p < 0.05$) post-competitive soccer match-play impairments in all measures of stretch-shortening cycle capability and muscle damage markers assessed irrespective of the age group. Likewise, significant post-match play alterations in landing mechanics (DKV of the dominant leg) and physical performance (sprint time and jump height) scores were documented for the U14 players. Significant interactions between maturity and landing mechanics and VAS responses to competitive soccer match-play were also found whereby more mature players demonstrated larger post-match changes. Therefore, the findings of this study suggest the existence of a negative influence of competitive match-play on neuromuscular function and muscle damage in youth soccer.

Keywords: Adolescence, football, fatigue, maturation, team sports

Introduction

During competitive soccer match-play, youth players are required to repeatedly perform high-intensity, intermittent, and multidirectional actions (e.g., sprints, jumps, and changes of direction) in unpredictable environments [1]. These high physical demands may cause youth players to experience significant levels of transient and accumulated fatigue throughout a match, which might place them in a more injury-prone situation and in a sub-optimal state to re-perform [2]. In youth soccer, it has been documented that the lower extremity, mainly the knee and ankle joints, is the anatomical region most frequently injured [3]. Furthermore, muscle/tendon strains and ligament tears and sprains are the types of injuries with the highest incidence rates in youth male players [3]. Recently developed machine learning models have identified altered movement patterns and neuromuscular control strategies, such as excessive dynamic valgus (DKV) at the knee joint, as key contributors to lower extremity soft tissue injuries in young male soccer players, although other factors also play a role [4,5]. Leg stiffness (LS) and reactive strength index (RSI) are indicators of stretch-shortening cycle (SSC) capability in stabilizing the knee joint and absorption of tensile forces exerted on the muscle-tendon complex respectively [6]. Deficiencies in these neuromuscular parameters are not direct causes of injury; however, injuries may result when they lead to compromised joint stability under high-intensity tasks, imposing excessive stress on the soft tissues beyond their load capacity and causing mechanical failure [7]. Similarly, movements involving a high proportion of intense eccentric muscle actions, such as most of the ones that are repetitively performed during the game of soccer (e.g., sprints, rapid changes of direction, sudden accelerations and decelerations, jump and landing actions), may induce considerable acute and accumulative muscle damage [8,9]. Furthermore, physical contact, as occurs during collisions when players are involved in tackle situations to keep/win possession of the ball, has also been identified as a further source of muscle damage [10]. It has been previously indicated that muscle damage can affect SSC function [11] and a muscle's ability to produce force [12]. Accordingly, following Kalkhoven et al.'s injury model [7], significant muscle damage may

reduce the muscle's resilience to stress and strain, potentially raising the risk of injury and diminishing a player's performance in subsequent training and matches.

In youth soccer, a significant proportion of injuries often occur towards the end of match-play, when fatigue is most likely to be present [13,14]. The increased susceptibility to injury in the final 15-20 minutes of a match may be due, in part, to the substantial neuromuscular fatigue accumulated by that time, leading to both heightened neuromuscular activity for the same workload and decreased dynamic joint stability [15]. Furthermore, time motion analysis has also reported that in competitive soccer match-play a noticeable drop in players' work rate is usually observed during the second half, partially attributed to accumulated fatigue, which may adversely affect several performance-related components, such as sprinting and jumping [16]. As most soccer matches are often decided by just one goal, a drop in physical performance could make the difference between winning and losing. Based on these findings, some studies have explored the acute effects of soccer match-play on certain neuromuscular factors, including isokinetic knee strength [17,18], LS [17-23] and RSI [17-19,21], and physical performance measures, mainly jump height [21,23] in youth soccer players. However, the results reported in these studies are often contradictory, whereby for the same risk factor (e.g., LS) and physical performance measure (e.g., jump height) some studies do not exhibit statistically significant ($p < 0.05$) effects [19-22] whilst others demonstrate significant fatigue-induced impairments [17,18,23]. Few studies have considered if the varying ability of adolescent players to resist fatigue could partly explain these differing results [19-22]. In particular, four studies have investigated the interaction between maturity status and post-soccer match-play fatigue on two neuromuscular injury risk factors (LS and RSI) [20-22] whereas just one study has described this interaction on a measure of physical performance (CMJ) [21] in youth players. Most of these studies [19-21], but not all [22], reported that individual differences in biological maturation did not influence the fatigue-related responses to soccer match-play. Despite having been identified as a significant risk factor for the most burdensome injuries in youth players, no studies appear to have explored the acute effects of soccer match-play on DKV motion during drop landing, nor whether the maturity status of the

players modulates these responses. Most studies investigating soccer's impact on injury risk physical performance in youth players have predominantly used simulated [17,18,20-23] rather than competitive soccer match-play [19], potentially limiting the external validity of their findings [24].

Several studies have investigated the immediate impact of soccer matches on muscle damage indicators, like creatine kinase (CK) and urea (UR) blood concentrations, and muscle soreness in adult players [24]. However, only two studies appear to have explored the acute effects of competitive match-play on muscle damage and perceived muscle soreness in youth male [19] and female [25] soccer players showing increases in CK and muscle soreness scores that were not mediated by the maturational status and sex of the players.

The main purpose of this study was to analyze the acute pre- and post-competitive soccer match-play changes in measures of drop landing mechanism (DKV), SSC capability (RSI and LS), physical performance (sprint time and jump height), muscle damage (CK and UR), perceived muscle soreness and match intensity in male youth soccer players. A secondary purpose was to explore associations between the players' maturity status, chronological age, and post-soccer match-play responses. We hypothesized that substantial post-soccer match-play impairments would be found in all the measures, and more so in the older and more mature players.

Materials and Methods

Participants

Forty-eight male youth outfield soccer players from two chronological competition age groups (U14 [n = 31] and U16 [n = 17]) who were considered injury-free participated in this study. Players were recruited from a professional soccer club academy. Participants trained three to four times per week and played one competitive match per week (usually at the weekend) during the season. Players were excluded if they did not play a full match (U14 = 70 min and U16 = 80 min). Before any participation, experimental procedures and potential risks were fully

explained to both parents and children in verbal and written form. Written informed consent to the testing procedures and the use of the data for further research was obtained from the parents and children. The study was approved by the Institution's ethics committee and conformed to the Declaration of Helsinki regarding the use of human subjects [26]. The final sample was 32 male youth soccer players (U14 = 22 and U16 = 10) as 10 players (U14 = 9 and U16 = 7) were excluded as they did not play a full competitive match.

Procedure

All players were tested an hour prior to and immediately after two competitive soccer matches for sprint (20 m sprint) and jump (CMJ-Abalakov) performance, SSC capability (RSI and LS), landing mechanics (DKV), muscle damage (CK and UR), perceived soreness (VAS) and perceived match intensity. The two competitive matches from which data were extracted were separated by a seven-day interval. Within this seven-day interval, all players completed four training sessions (from Monday to Thursday). Soccer matches were played on outdoor artificial grass pitches during the 2016 season (May). Matches were played on the same day (Saturday) in a structured league setting so the opposition was deemed to be of the same standard as the team being measured. The day before and after testing, the players were not exposed to any high-intensity exercise. During each pre-match testing, participants began by completing their regular warm-up under the coaches' supervision. All dependent variables were assessed 2–3 minutes after the warm-up program was completed using a randomized circuit-style approach. Each outcome variable station was run by an experienced researcher and this approach was used to minimize testing time, especially post-competitive match-play. The same testing battery and procedures were used in all pre- and post-match testing sessions. All testing methods selected have been widely used in previous applied studies carried out on youth athletes (including soccer players) due to their moderate to good validity and reliability scores, high portability, and ease of being conducted in a team under time-constrained situations [27,28].

Test protocols

Anthropometrics and maturity status

Body mass (kg) was measured on a calibrated physician scale (Seca 786 Culta, Milan, Italy). Standing and sitting height (cm) were recorded to the nearest 0.1 cm on a measurement platform (Seca 274, Milan, Italy) with seated height measured using a box. Leg length was calculated as the difference between the players' height in both standing and seated conditions.

Chronological age was determined using the date of birth and the date of the testing session. Biological maturity was calculated in a noninvasive manner using a regression equation comprising measures of age, body mass, standing height and sitting height taken during the familiarization session conducted a week before the match-play [29]. Using this method, maturity offset (calculation of years from PHV) was determined. The following equation to calculate maturity offset was used:

$$- 9.236 + [0.0002708*\text{leg length and sitting-height interaction}] - [0.001663*\text{age and leg-length interaction}] + [0.007216*\text{age and sitting-height interaction}] + [0.02292*\text{weight by height ratio}]$$

Dynamic knee valgus

Two-dimensional frontal plane knee kinematic data were captured with high-speed video cameras (Casio Exilim, Tokyo, Japan) during a single-legged countermovement jump (SL CMJ) with arm swing. Cameras were positioned 2 m from the capture area to reduce potential perspective errors and were focused and zoomed toward the capture location. Participants stood two steps behind a landing mat and were instructed to perform an SL CMJ off their dominant leg following a 2-step run-up, aiming to then land on both feet on the landing mat. This jump-landing sequence has previously been referred to as a vertical stop-jump task [30,31]. Participants were instructed to perform the SL CMJ aiming to jump maximally to replicate heading a soccer. This jump-landing task is suggested to have more sports specificity, replicating a soccer-specific action, as compared with a previous methodology where participants performed a drop-jump task off a box [30,31]. Before testing, participants

were allowed to familiarize themselves with the SL CMJ landing, performing 2-3 practice trials. Once participants were able to perform the jump-landing sequence, they performed three test trials, and feedback between trials was provided, ensuring that participants jumped maximally in all trials. Two-dimensional valgus and knee flexion ROM were determined from each trial and the greatest values were used in subsequent analysis. Frontal and sagittal videos were imported into the Quintic biomechanics' software (v 26) package to measure knee valgus motion and knee flexion ROM. Initial video calibration for the x-axis was achieved with the known distance of the landing mat. The knee valgus motion was calculated with the frontal view by drawing a line on the knee joint center at the frame before initial contact (L1) and at the maximum medial position (L2), with the displacement measured between the line's representative of knee valgus motion during the SL CMJ landing task. With the sagittal view knee, flexion angles were measured at the frame before initial contact and at maximum knee flexion from the greater trochanter, lateral knee joint line, and lateral malleolus. Knee flexion ROM was defined as the difference between the angles ($Q2 - Q1$) before initial contact and maximum knee flexion.

Stretch-shortening cycle capability

RSI was determined during a 5-maximum hop test which was assessed using a portable optical timing system Opto jump Next (Microgate, Bolzano, Italy) with a manufacturer-declared accuracy of 0.001s. Participants were instructed to maximize jump height and minimize ground contact time [32] with the RSI calculated as the ratio between jump height and contact time on the floor [33]. The first hop served as a CMJ and was consequently excluded from analysis, with the 4 remaining hops averaged for analysis of RSI. To familiarize the players with the testing procedure, two trials were performed followed by three measured attempts with a 30s rest interval between the sets. The greatest value of the three attempts was used in further analyses.

LS was calculated from contact time data obtained during a sub-maximal bilateral hopping protocol and a coefficient of variation for male youth soccer players has been reported to be 8.6% [34]. The procedures were repeated twice, and an average stiffness value was reported.

For each trial, participants were instructed to perform 20 consecutive hops using a portable optical timing system Opto jump Next (Microgate, Bolzano, Italy) at a frequency of 2.5 Hz to reflect the typical behavior of a spring model [34]. The hopping frequency was maintained via an audio signal from a quartz Wittner metronome (GmbH & Co. KG, Isny, Germany). Participants were instructed to: a) keep hands on the hips at all times to avoid upper body interference; b) jump and land on the same spot; c) land with legs fully extended and to look forward at a fixed position to aid balance. For data analysis, the first 4 hops were discounted and the next 10 consecutive hops closest to the hopping frequency were used. Absolute leg stiffness (kN m^{-1}) was calculated using the equation proposed by Dalleau et al. [32] and relative leg stiffness was determined by dividing absolute leg stiffness by body mass and limb length to provide a dimensionless value [34].

Physical performance measures

The time during a 20 m sprint in a straight line was measured utilizing single beam photocell gates placed 1 m above the ground level (Time It; Eleiko Sport, Halmstad, Sweden). Each sprint was initiated from an individually chosen standing position, 50 cm behind the photocell gate, which started a digital timer. Each player performed three maximal 20 m sprints interspersed with 1 min of passive recovery, and the fastest time achieved was determined for the statistical analysis.

To determine jump height, participants performed three CMJ-Abalakov on an infrared contact platform (Optojump, Microgate, Bolzano, Italy) from an upright position with the depth of the countermovement self-selected. Arm swing was allowed during the jump. Jump height in the CMJ-Abalakov was calculated from the flight time ($h=ft^2 \cdot g \cdot 8^{-1}$). The best jump performed was used for the subsequent statistical analysis.

Muscle damage markers

Approximately 2 x 30 μ L of capillary blood was collected from a finger via a prick made with a spring-loaded disposable lancet (Accu-Check, Roche Diagnostics, Germany). The blood sample was immediately analyzed using a colorimetric assay procedure using a Reflotron®

systems spectrophotometer (F. Hoffman-La Roche Ltd, Basel, Switzerland) for plasma CK activity and plasma UR concentrations.

Perceived muscle soreness

Using a visual analogue scale (VAS) participants gave an indication of their current level of perceived muscle soreness on a subjective scale. The scale was 10 cm in length, with 0 (no soreness) and 10 (very, very sore) representing the extreme ends of the scale [35]. Participants were instructed to mark a cross along the line that relates to the amount of muscle soreness that they felt at that current time. To avoid potential bias from previous measurements a blank VAS scale was provided at each testing session. This method has been validated for being used with paediatric populations [35].

Perceived match intensity

Within half an hour post-match, participants were asked to rate their perceived level of exertion during the match. Each team had differing match durations in accordance with Spanish Soccer Association rules (U14 = 2 x 35 min halves and U16 = 2 x 40 min). The Borg scale was used as it has been previously reported to be a reliable measure of rate of perceived exertion (RPE) regardless of age [36] or sex and easily learned by older children and adolescents [37]. The simplified fixed ten-point Borg CR-10 Scale [38] was used to allow an easy method of measuring the level of intensity on a scale ranging from zero to ten, zero representing low intensity/rest up to ten representing maximal effort.

Statistical analysis

Statistical analyses were performed using JASP software version 0.13.01 (Amsterdam, Netherlands) and the Statistical Package for Social Sciences (SPSS, v. 25.0 for Mac; SPSS Inc, Chicago).

A descriptive statistic (mean \pm standard deviation) was calculated for each measure separately by age group. The distribution of raw data sets was checked for homogeneity and skewness using the Shapiro-Wilk expanded test.

Separate two-way (time [pre-match vs. post-match] x age group [U14 vs. U16]) repeated measures analyses of covariance (RM-ANCOVAs) were conducted in order to explore both intra and inter-subject differences in the possible effects elicited by soccer match-play on the dependent variables previously described. For those non-normally distributed variables, the non-parametric alternative technique to the RM-ANCOVA was performed. While time and age group were added as within and between-subject factors respectively in each RM-ANCOVA, the maturity offset was included as covariable. Individual “player code” was treated as a random factor for all analyses [39]. Each RM-ANCOVA run was adjusted to the dependent variable pre-match (i.e., baseline) values to avoid regression to the mean phenomenon [40]. The homogeneity of variance across groups was verified using Levene’s test. Mauchly’s test was inspected for each variable of interest to test its assumption of sphericity. For cases in which the assumption of sphericity was violated, the Greenhouse-Geisser correction was used to reduce the likelihood of a Type I Error. As a measure of effect size, the partial eta squared (η_p^2) was calculated for the RM-ANOVAs and values of <0.01, from 0.01 to 0.06, from >0.06 to 0.15, and above 0.15 were considered trivial, small, medium and large, respectively. Where a significant F-ratio was revealed, a Bonferroni post hoc test was used to determine the level of significance. Cohen’s d was also calculated for each of the paired comparisons carried out. Magnitudes of the Cohen’s effect sizes were interpreted as: trivial (<0.2), small (>0.2 – 0.6), moderate (>0.6 – 1.2), large (>1.2 – 2.0), and very large (>2.0 – 4.0) [41]. Statistical significance was set at $p < 0.05$ for all tests. Whether statistically significant two-way interactions were found for time x maturity offset and/or for time x pre-match values in a dependent variable, we conducted subsequent Pearson correlation analyses between the pre-and post-match differences (factor time) and the respective covariables (maturity offset and pre-match values) to explore the direction and magnitude of the associations. The correlation coefficients were classified according to Hopkins [42]: trivial [<0.1], small [0.1–0.3], moderate [>0.3–0.5], large [>0.5–0.7], very large [>0.7–0.9], and nearly perfect [>0.9]. To consider that either maturity offset or pre-match values were meaningfully associated with acute pre- and post-competitive soccer match-play changes in any of the dependent variables, the Pearson correlation analysis

had to show statistical significance ($p < 0.05$) with an r score ≥ 0.3 (at least moderate). Participants' chronological age was not included as covariable in the different RM-ANCOVAs conducted but as nominal between subject fixed factor due to the fact that a very homogeneous range of age was observed for the participants belonging to each of the two age categories (table 1) that allowed carrying out a clear discretization (no borderline cases) into two different groups (U14 and U16).

An independent sample t-test was conducted to explore possible inter-group differences in post-match-play rates of perceived exertion (RPE scores). Statistical significance was set at $p < 0.05$. Finally, a Pearson correlation analysis was also conducted to assess whether players with higher RPE scores had larger pre- and post-match-play absolute differences (fatigability) in the measures of landing mechanism, SCC capability, physical performance, and muscle damage.

Results

Descriptive data for each age group can be found in table 1 with the U16 group being significantly older, taller, heavier, and more mature than the U14 group. Table 2 displays descriptive pre- and post-match-play data of landing mechanics, SCC capability, sprint and jump performances, muscle damage, and perceived muscle soreness measures separately by age group. All dependent variables presented a normal distribution (except for the VAS measure).

The RM-ANCOVA analysis conducted with the DKV of the dominant leg as the dependent variable showed two-way interactions for time x age group ($p = 0.04$, $\rho n^2 = 0.05$), time x pre-match scores ($p = 0.03$, $\rho n^2 = 0.6$, $r = -0.25$ [$p = 0.03$]) and time x maturity offset ($p = 0.004$, $\rho n^2 = 0.11$). However, no statistically significant main effects for the factor time ($p = 0.49$, $\rho n^2 = 0.01$) were found for this variable in contrast to the factor age group ($p = 0.04$, $\rho n^2 = 0.05$). Post hoc comparisons reported statistically significant ($p = 0.04$) post-match impairments in the DKV scores of the dominant leg only for the U14 players (pre- and post-match-play differences = 3.6° [95%CI = 0 to 7.3] and $d = 0.48$). Likewise, U16 players exhibited statistically lower DKV

scores ($p = 0.04$) than their younger U14 counterparts (inter-group differences = -4.7° [95%CI = -9.5 to -0.1] and $d = -0.63$). Finally, players who were more mature ($r = 0.30$ and $p = 0.01$) reported larger post-match impairments in this variable. For the DKV of the non-dominant leg, no two-way interactions for time x age group ($p = 0.05$, $\rho n^2 = 0.05$) nor for time x maturity offset ($p = 0.05$, $\rho n^2 = 0.05$) were observed but a time x pre-match value interaction was evident ($p < 0.01$, $\rho n^2 = 0.17$, $r = -0.41$ [$p < 0.01$]). Additionally, no main effects were shown for the factors time ($p = 0.14$) and age group ($p = 0.05$).

For both LS and RSI, the RM-ANCOVA analysis reported no two-way interactions for time x maturity offset ($p = 0.31$ [LS] and 0.05 [RSI], $\rho n^2 = 0.01$ [LS] and 0.05 [RSI]) unlike for time x LS ($p < 0.01$, $\rho n^2 = 0.26$, $r = -0.51$ [$p < 0.01$]) and RSI ($p < 0.01$, $\rho n^2 = 0.21$, $r = -0.42$ [$p < 0.01$]) pre-match values. In addition, there were main effects for the factor time ($p < 0.05$, $\rho n^2 = 0.23$ [LS] and 0.08 [RSI]) in both variables so that LS (pre- and post-match-play differences = -1.5 $\text{kN}\cdot\text{m}^{-1}$ [95%CI = -2.4 to -0.6] and $d = -0.7$) and RSI (pre- and post-match-play differences = -0.11 [95%CI = -1.64 to -0.06] and $d = -0.88$) were significantly impaired ($p < 0.01$) post-match-play. Unlike LS, statistically significant two-way interactions for time x age group ($p = 0.02$, $\rho n^2 = 0.07$) and main effects for the factor age group ($p = 0.02$ and $\rho n^2 = 0.07$) were observed in RSI whereby the U14 group exhibited larger post-match-play impairments and older players (U16) obtained lower values (inter-group differences = -0.09 [95%CI = -0.17 to -0.01] and $d = -0.74$).

For 20 m sprint time, there were significant two-way interactions for time x age group ($p < 0.01$, $\rho n^2 = 0.11$) and for time x maturity offset ($p = 0.02$, $\rho n^2 = 0.08$, $r = 0.15$ [$p = 0.2$]) unlike for time x pre-match scores ($p = 0.08$, $\rho n^2 = 0.04$). Moreover, significant effects were observed for the factors time ($p = 0.04$, $\rho n^2 = 0.05$) and age group ($p = 0.004$ and $\rho n^2 = 0.11$). In particular, the average time spent covering 20 m was only longer post-match-play (pre- and post-match-play differences = 0.12 s [95%CI = 0.06 to 0.17] and $d = 1.4$) in the U14 group. Likewise, U16 players needed less time to cover 20 m sprinting than their U14 counterparts (inter-group differences = -0.08 s [95%CI = -0.13 to 0.03] and $d = -0.95$). Statistically significant two-way interactions for time x age group ($p = 0.01$, $\rho n^2 = 0.08$) and for time x pre-match scores ($p =$

0.03, $\rho n^2 = 0.06$, $r = -0.13$ [$p = 0.24$]) were found for CMJ-Abalakov height but not for time x maturity offset ($p = 0.29$, $\rho n^2 = 0.01$). There were no main effects for the factor time but for the factor age group ($p = 0.01$, $\rho n^2 = 0.08$). Subsequent post hoc analyses indicated that the U16 group jumped higher than the U14 group (inter-group differences = 1.7 cm [95%CI = 0.4 to 3] and $d = 0.84$) with the U14s demonstrating a significant decrease in jump height post-match-play (pre- and post-match-play differences = -2.7 cm [95%CI = -4 to -1.3] and $d = -1.32$).

For both CK and UR, the RM-ANCOVA reported no statistically significant two-way interactions for time x age group ($p = 0.62$ [CK] and 0.63 [UR], $\rho n^2 < 0.01$ [CK and UR]) nor for time x maturity offset ($p = 0.08$ [CK] and 0.37 [UR], $\rho n^2 = 0.04$ [CK] and 0.01 [UR]) in contrast to time x pre-match scores ($p = 0.01$ [CK] and 0.03 [UR], $\rho n^2 = 0.08$ [CK] and 0.06 [UR], $r = 0.36$ [$p < 0.01$] [CK] and -0.26 [$p = 0.02$] [UR]). In addition, main effects were found for the factor time ($p < 0.01$, $\rho n^2 = 0.8$ [CK] and 0.12 [UR]) but not for the factor age group ($p = 0.67$ [CK] and 0.63 [UR], $\rho n^2 < 0.01$ [CK and UR]) in both variables. Subsequent post hoc analysis demonstrated significant post-match increases in CK (164.9 I·UI-1 [95%CI = 97.6 to 232.1] and $d = 1.02$) and UR (1 mmol/l [95%CI = 0.56 to 1.44] and $d = 0.96$) compared to pre-match play.

Finally, for VAS the analysis revealed no two-way way interactions for time x age group ($p = 0.85$, $\rho n^2 < 0.01$) unlike time x maturity offset ($p = 0.02$, $\rho n^2 = 0.07$, $r = 0.30$ [$p = 0.01$]) and time x pre-match scores ($p = 0.03$, $\rho n^2 = 0.06$, $r = -0.1$ [$p = 0.37$]). Additionally, main effects for time ($p < 0.001$ and $\rho n^2 = 0.37$) were found in contrast to the factor age group ($p = 0.85$, $\rho n^2 < 0.01$). In particular, post-match-play scores were higher than pre-match-play scores (pre- and post-match-play differences = 0.9 cm [95%CI = 0.55 to 1.27] and $d = 1.07$) and more mature players reported higher pre- and post-match-play differences.

Contingency analyses reported no age group-related differences in the percentages of soccer players who demonstrated (or did not), clinically relevant post-match-play changes in their measures of landing mechanic, SSC capability (apart from the RSI [$p = 0.04$]), physical performance (with the exception of CMJ-Abalakov [$p = 0.02$]) and muscle damage. As shown

in figure 1, there were large inter-player variability responses to soccer-induced fatigue in most of the neuromuscular measures analyzed.

There was no statistically significant difference ($p = 0.05$) in game intensity RPE between age groups (U14 = 6.8 ± 1.2 ; U16 = 7.4 ± 1). Furthermore, the post-match-play RPE scores had no significant associations (table 3) with the magnitude of the post-match play changes in landing mechanism, SSC capability, physical performance and muscle damage measures (correlation plots are displayed in supplementary file 1).

Discussion

This study shows that competitive soccer match-play is physically demanding based on raised post-match-play perceived levels of exertion (RPE from 3 [light] to 9 [very strong]) in youth male players, independent of chronological age (U14 and U16). However, it should be highlighted that individual responses to match-play were diverse with some players describing their feeling of fatigue as light in contrast to others that described it as very strong. This variability in players' perceptions of fatigue could offer some explanatory reasoning for why post-match-play perceived levels of exertion were independent (not associated) of the acute effects elicited by a soccer match-play on measures of landing mechanics, SSC capability, physical performance, and muscle damage. In other words, player' perception of fatigue seems to not mirror underlying changes in the just mentioned parameters in this cohort of soccer players. These data suggest that perceived exertion as a marker of fatigue-related responses in youth soccer players should be used with a degree of caution.

The findings of this study also demonstrate that the fatigue elicited by competitive soccer match-play did substantially alter the landing mechanics of U14 male players, especially of those who were in a period of maximal rate of growth and maturation (i.e., peak height velocity). On the contrary, in U16 male players, soccer-induced fatigue was not large enough to impair their movement patterns and motor control strategies in the frontal plane during the execution of a single-leg landing task. It has been documented that during periods of rapid physical development, adolescents often experience a temporary disruption in motor control which is

characterized by a reduced ability to effectively control limb motion and complete athletic tasks [43]. This phenomenon is referred to as “adolescent awkwardness” and appears to be mitigated with progression towards a more mature state as strength and neuromuscular control increase and body mass and limb length decrease their rates of growth and full maturity is approached [44]. Thus, circum-pubertal adolescents may be especially vulnerable to impairments in movement mechanics from fatigue during demanding sports like soccer. This susceptibility, akin to adolescent awkwardness, could diminish as they mature. This hypothesis is corroborated by our findings in U16 players and the work of Smeets et al. [45] and Wright et al. [46], who reported no significant changes in landing kinematics (hip, knee, and ankle angles) in adults after simulated soccer matches. This scenario may partially explain the high susceptibility growth and overuse injuries in circum-pubertal players compared to prepubertal players [47,48]. This change over the developmental period (2 chronological years), may also be attributed to a greater training age, however, this hypothesis requires further investigation. Therefore, training interventions aimed at consolidating movement skills and increasing resistance to fatigue in soccer players should be delivered early in adolescence or late childhood before they progress through the growth spurt [49].

On the other hand, moderate and statistically significant post-competitive soccer match-play decreases in LS (-3.8%) and RSI (-14.9%) values were found in our population of U14 and U16 male soccer players. Similar post (competitive and simulated) soccer match-play percentage differences to the ones reported in our study were found in previous youth studies [17-19,21,22] for RSI (Δ ranged from -6.4 to -13%) and LS (Δ ranged from -4 to -10%). As both LS and RSI measures represent the SSC capability, it could be suggested that fatigue induced from soccer match-play may be sufficient to alter the muscle-tendon unit functions to stabilize the knee joint and to absorb and re-utilize the high tensile forces generated during the execution of the explosive movements that are repeatedly performed by players. Furthermore, the results of our study also revealed that post-match-play decreases in RSI scores were larger in the U14 group, which might increase injury risk in this cohort of soccer players [47,48]. This

finding further supports the idea that pre-adolescence may represent an optimal time to implement programs aimed at developing neuromuscular control [49].

Post-competitive soccer match-play inhibition of SSC function can partly explain the responses to sprint and CMJ-Abalakov observed in the U14 group. The results indicate an impaired 20 m sprint time of 2.4% and a reduced CMJ-Abalakov height of 5.2% in this age group. These reductions in sprint and jump performances when fatigued are likely to have practical and meaningful effects on U14 players' physical performance during match-play. Indeed, these two actions are associated with crucial moments of match-play such as heading the ball either defensively or offensively and when sprinting to kick or catch the ball before the opponents during intense periods of play. On the contrary, no statistically significant post-match-play effects in sprint time and jump height were observed in the U16 group. These latter findings are not aligned with the results reported by Oliver et al. [23] who did find statistically significant post-simulated soccer match-play decreases in CMJ height in U16 soccer players. Consequently, future studies are needed to elucidate whether there may be age-group-related differences in the post-soccer match-play responses for measures of physical performance.

Competitive soccer match-play induces significant increases in biochemical markers of muscle damage and perceived soreness. Recent studies have shown that there are significant alterations in CK activity in youth soccer players post-competitive match-play [19,25]. It has previously been suggested that pre-pubertal children are CK non-responders, showing minor CK activity levels following fatiguing exercise [50,51]. The data from the present study support previous findings, with younger and less mature players exhibiting lower post-match CK elevations (U14 = 70% vs. U16 = 95%). Although the existence of a dose-response relationship between CK levels and exercise-induced muscle damage has not been proven, it seems plausible that less mature individuals, who are likely to have a lower proportion of Type II fibers and less developed anaerobic metabolisms, may be less susceptible to muscle damage following high-intensity exercise [52,53]. Although no direct link between elevated CK and muscle strain has been established in youth athletes, given that muscle strain injury in U15 age groups represents the greatest injury incidence [3], we might hypothesise that this may be

a contributing factor that requires further investigation. Regarding the perceived muscle soreness, statistically significant post-competitive match-play increases in VAS scores were observed in both age groups, irrespective of the maturity offset of the players. While the effect sizes of the post-competitive match-play response to VAS were similar in both age groups ($d < 0.6$ [small]), pre-and post-match VAS scores were higher in the more mature players, which is aligned with what was found for the CK. For its part, UR has been used as a workload marker, mainly for long-lasting high-intensity exercises [54]. The most frequently given reason for increased UR values in elite athletes is their training volume associated with some degree of glyconeogenesis that leads to degradation of structural or functional proteins [55]. Previous studies have shown that serum urea levels increase after training in male soccer players regardless of age and maturity status [56]. All players in this study showed an acute increase in UR levels post-competitive match-play. Whilst the values are in the normal physiological range a pronounced increase in levels could be indicative of strenuous efforts and therefore could be used as a marker to indicate overreaching in youth players during maturational periods of development. Longer-term assessment of biochemical markers might provide an indication of within-season fluctuations, showing patterns of fatigue-related effects and readiness to re-perform.

There are some limitations in this study that should be mentioned. Due to including only players who completed the whole match our sample size in each age group was relatively small. Despite significant main effects for various parameters, the results from this study, which only included U14 and U16 male youth players, should not be generalized to other soccer player cohorts. Future research should assess players across a broader spectrum of maturity offsets. The observed differences between groups suggest that playing duration and load variations are associated with the fatigue responses of the measured outcomes. No studies to date have explored the relationship between fatigue-related match loads and RPE in youth players, especially across different maturity stages, and this, therefore, requires further investigation. Due to the number of variables assessed post-match play we used a randomized 'circuit' approach to try and reduce data collection time. However, we acknowledge that this inevitably

resulted in differing time gaps between the end of the play and the assessment of the outcome variables, which may have influenced the findings despite our randomization technique.

Conclusions

Competitive match play does appear to compromise SSC capability, sprint and jump performance as well as to increase perceived muscle soreness and muscle damage, irrespective of age in youth soccer players. Furthermore, in the youngest players (U14) landing mechanics (in the frontal plane) experienced significant impairments post-soccer match-play. Therefore, the increased risk of injury documented during the last 15-20 minutes of a soccer match-play, when fatigue is present, might be due to alterations in neuromuscular capability parameters. The magnitude of the acute neuromuscular and muscle damage responses to competitive soccer match-play seems (to some extent) to be associated with players' maturation and chronological age. Consequently, soccer-induced fatigue responses should be examined within players, and interventions appropriately prescribed on an individual basis to make sure each young player is ready for the next training session and competitive match.

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Author contributions

Conceptualization – Imanol Martin-Garetxana, Jonathan Hughes, José A. Lekue.

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Writing review and editing – All the authors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are available from the corresponding author, FA, upon reasonable request.

Code availability statement

Not applicable

Ethics approval and informed consent

The University of Murcia's Ethics Committee approved the study (ID: 1551/2017) that conformed to the Declaration of Helsinki regarding the use of human subjects. Before any participation, experimental procedures and potential risks were fully explained to both parents and children in verbal and written form. Written informed consent to the testing procedures and the use of the data for further research was obtained from the parents and children.

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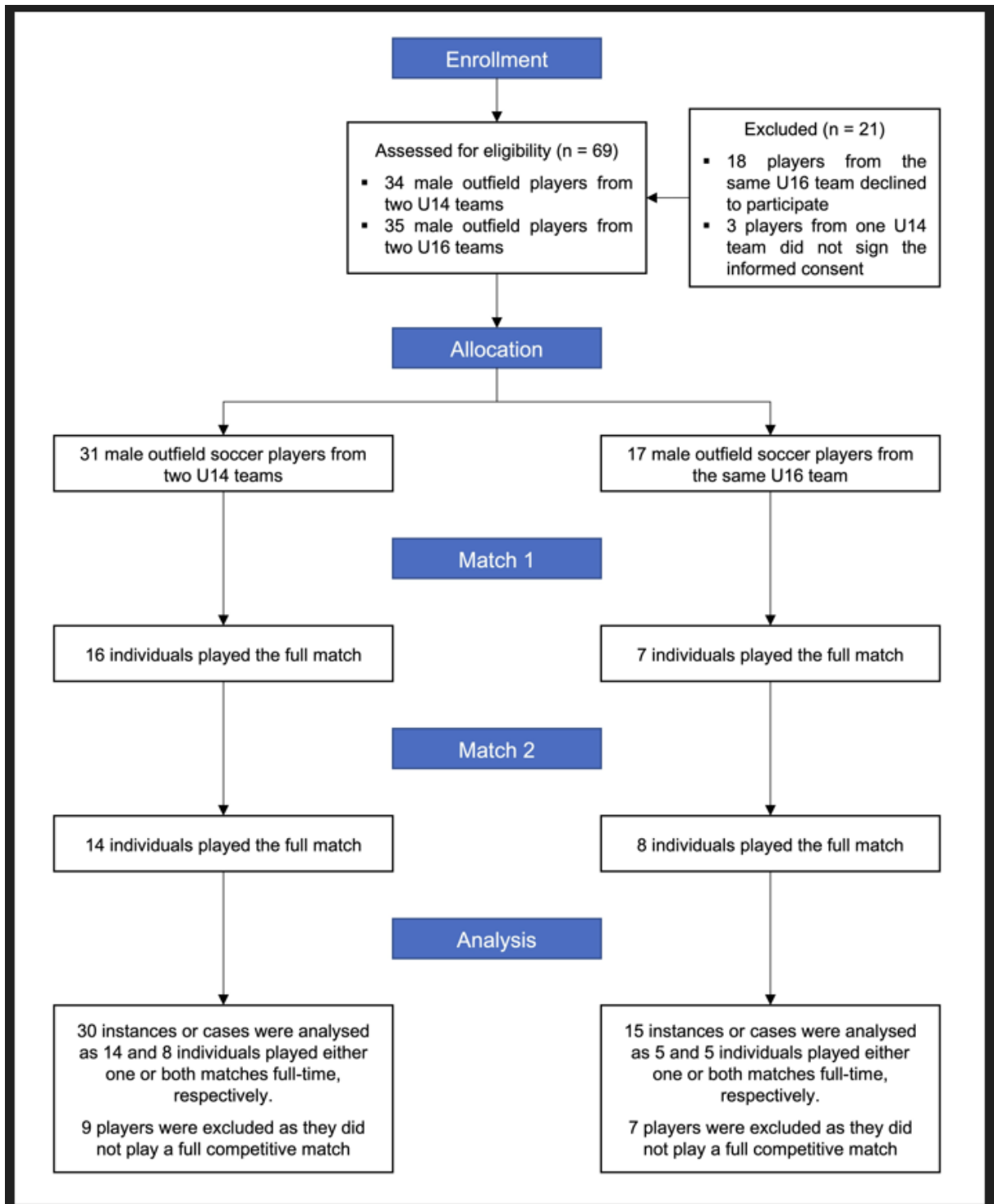
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FIGURE CAPTIONS

Figure 1. Participants flow chart



Supplementary file 1 Correlation plots

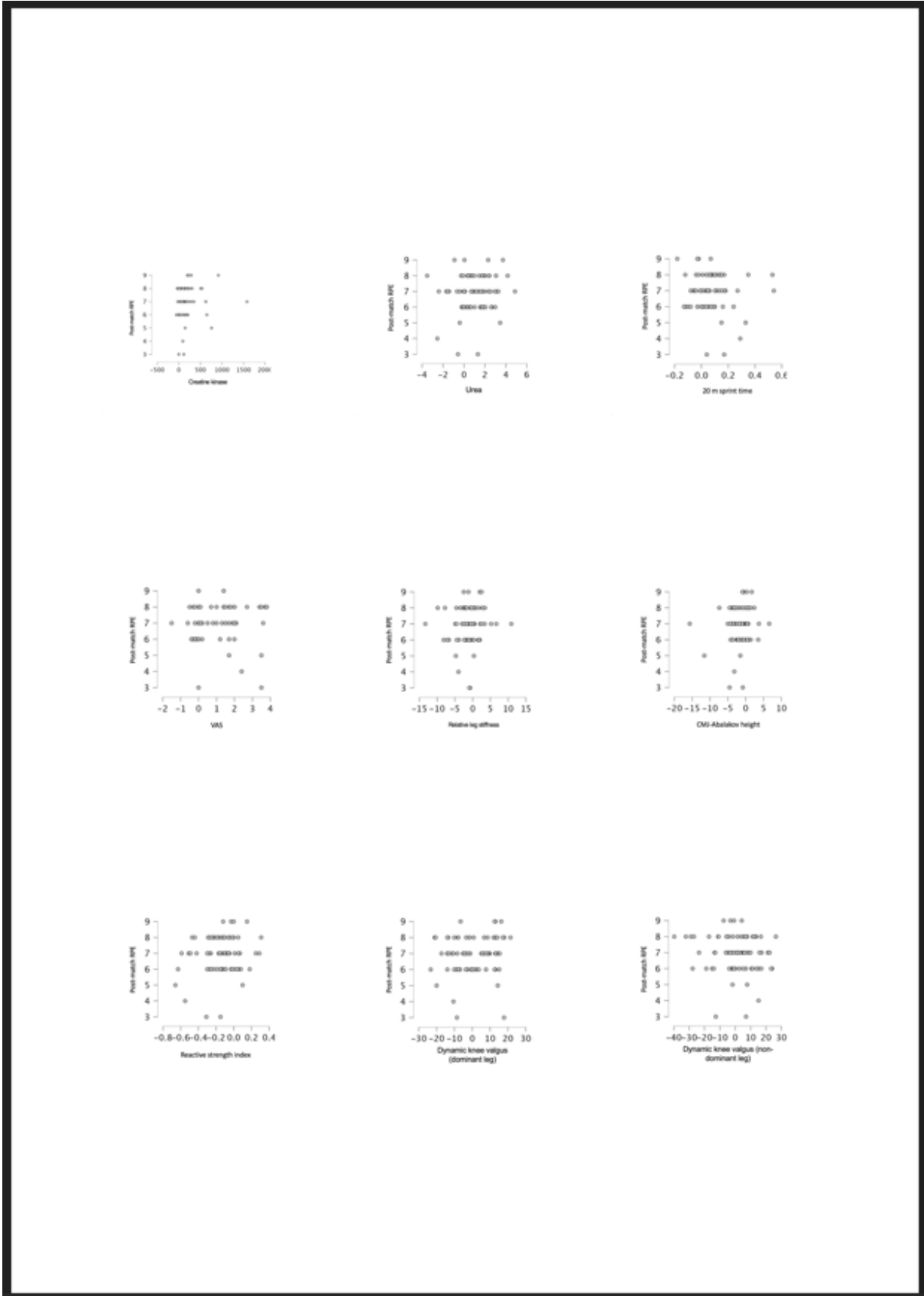


Table 1. Participant's characteristics by age group (mean \pm standard deviation)

Variable*	Under 14 (n = 27)	Under 16 (n = 15)
Age (y)	13.2 \pm 0.7	15.2 \pm 0.3
Stature (cm)	160.5 \pm 7.3	175.9 \pm 4.5
Body mass (kg)	47.1 \pm 7.6	63.2 \pm 3.7
Maturity offset	-0.87 \pm 0.54	0.7 \pm 0.32

*: there were inter-age group differences in all descriptive variables

Table 2. Pre and post-match-play descriptive statistic (mean \pm standard deviation) of the sprint performance, landing mechanic, SCC capability, muscle damage and perceived muscle soreness measures separately by age group

Measures	Age group	
	Under 14	Under 16
Landing mechanics		
Dynamic knee valgus (dominant leg) ($^{\circ}$)		
▪ Pre-match	16.5 \pm 13.3	10.1 \pm 12.7
▪ Post-match	16.5 \pm 16.4	13.6 \pm 12
Dynamic knee valgus (non-dominant leg) ($^{\circ}$)		
▪ Pre-match	16.7 \pm 15.6	14.2 \pm 12.7
▪ Post-match	17.6 \pm 15.6	14.7 \pm 12.7
SCC capability		
Relative leg stiffness (kN·m-1)		
▪ Pre-match	28 \pm 3.5	27.4 \pm 4.6
▪ Post-match	27.2 \pm 3.3	25.8 \pm 4.3
Reactive strength index		
▪ Pre-match	1.21 \pm 0.33	1.08 \pm 0.26
▪ Post-match	1.03 \pm 0.31	0.99 \pm 0.27
Physical performance		
20 m sprint time (s)		
▪ Pre-match	3.27 \pm 0.12	3.12 \pm 0.09
▪ Post-match	3.35 \pm 0.13	3.19 \pm 0.16
CMJ-Abalakov height (cm)		
▪ Pre-match	36.6 \pm 3.4	42.8 \pm 3.2
▪ Post-match	34.7 \pm 4	41.7 \pm 3.2
Muscle damage		
Creatine kinase (I·UI ⁻¹)		
▪ Pre-match	191.1 \pm 125.9	291.8 \pm 257
▪ Post-match	323.5 \pm 324.7	570.1 \pm 368.7
Urea (mmol/l)		
▪ Pre-match	4.88 \pm 1.24	5.1 \pm 1.43
▪ Post-match	6.2 \pm 1.76	5.55 \pm 1.68
VAS (cm)		
▪ Pre-match	0.38 \pm 0.63	1.52 \pm 1.11
▪ Post-match	1.17 \pm 1.27	2.79 \pm 1.43

Table 3. Pearson correlation results

Pre- and post-match play differences	Post-match RPE	
	Pearson's r scores	p-values
Landing mechanics		
▪ → Dynamic knee valgus (dominant leg)	0.16 (-0.62 to 0.37)	0.15
▪ → Dynamic knee valgus (non-dominant leg)	-0.13 (-0.34 to 0.09)	0.25
SCC capability		
▪ → Relative leg stiffness	0.1 (-0.12 to 0.32)	0.37
▪ → Reactive strength index	0.17 (-0.06 to 0.38)	0.14
Physical performance		
▪ → 20-m sprint time	-0.07 (-0.29 to 0.16)	0.54
▪ → CMJ-Abalakov height	0.09 (-0.13 to 0.31)	0.41
Muscle damage		
▪ → Creatine kinase	0.12 (-0.11 to 0.33)	0.3
▪ → Urea	0.11 (-0.12 to 0.33)	0.34
▪ → VAS	0.08 (-0.14 to 0.3)	0.46