Journal of Human Kinetics volume xx/xxxx, x–x DOI: 10.5114/jhk/224589 x
Section II – Exercise Physiology and Sports Medicine

From Pre-Season to In-Season: Variations in Fitness and Hematological Variables in Elite Soccer Players

by
Fioretta Silvestri [1], Ludovica Cardinali [2], Lorenzo Marcelli [1,], Matteo Campanella [3], Davide Sisti [4], Fabrizio Perroni [4], Alessandra Favoriti [5], Carlo Baldari [2,3], Laura Guidetti [1], Stefano Amatori [4], Davide Curzi [1]*

Several variables are commonly used to monitor soccer players during and off-training; however, how different phases of the season influence these variables and their relationship with performance requires further investigation. This study aimed to examine how pre-season and in-season soccer periods influence physical fitness as well as hematological and inflammatory markers in elite soccer players. Twenty-one soccer players of a second-highest division team in Italy (Ternana Football Club) were recruited and tested at three time points: before the pre-season, after the pre-season and in the middle of the season (in-season). Fitness tests included countermovement jump (CMJ), 10-meter sprint, repeated sprint ability (RSA), and shuttle-running (Yo-Yo IR-2) testing. Blood markers evaluated were hematocrit, C-reactive protein (CRP), creatine kinase (CK), lactate dehydrogenase (LDH), and interleukins 6 and 10 (IL-6, IL-10). Training and matches were monitored throughout the season via GPS tracking and load indices were derived. A repeated-measures MANOVA was used to test differences in the dependent variables among the three time points. Results showed decreased performance on the 10-m sprint and RSA, along with elevated inflammatory markers (CK, LDH, IL-6) following the pre-season. In contrast, in-season data revealed improved fitness performance accompanied by a reduction in IL-6 levels. Coherently, a negative correlation was observed between IL-6 variation and RSA performance. These results could help the teams' staff to select the most sensitive markers to investigate players' fatigue during the season, offering practical insights to optimize individualized recovery strategies and enhance performance.

***Keywords**: stress; recovery; soccer; training load; blood markers*

Introduction

In elite soccer leagues, players actively train for about 10–11 months per year (Meckel et al., 2018). During the pre-season, players are subject to physiologically stressful training programs aimed at rebuilding fitness levels after the off-season in order to sustain match demands in the competitive season (Nobari et al., 2022). However, excessive training loads combined with insufficient recovery may induce cumulative fatigue in athletes, thereby compromising performance during the competitive season (Ponce-Gonzalez et al., 2021). The competitive season comprises micro-cycles consisting of training, tapering, competition and recovery, to maintain and improve the specific fitness components developed during the pre-season, and during which one to three matches can be played within seven days (Nobari et al., 2022; Ponce-Gonzalez et al., 2021; Saidi et al., 2021). Recent evidence indicates that accumulated weekly training loads are directly associated with

[1] *Department of Economic, Psychological, Communication, Education and Movement Science, Niccolò Cusano University, Rome, Italy.*
[2] *Department of Life Sciences, Health, and Health Professions, Link Campus University, Rome, Italy.*
[3] *Department of Theoretical and Applied Sciences, eCampus University, Novedrate, Italy.*
[4] *Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy.*
[5] *Ternana Football Club, Terni, Italy.*
* Correspondence: lorenzo.marcelli@unicusano.it

CC BY

subsequent match high-intensity performance, reinforcing the importance of load distribution within the competitive micro-cycle (Asín-Izquierdo et al., 2026). Professional soccer competitions require athletes to perform at intensities reaching 80–90% of their maximum heart rate or approximately 70–75% VO_{2max}, involving numerous explosive movements and significant eccentric contractions that result in subsequent muscular tissue damage and elevated reactive oxygen species production within blood circulation and skeletal muscle mitochondrial structures (Ponce-Gonzalez et al., 2021).

These distinct training approaches across pre-competitive and competitive phases generate varying physiological requirements that, when inadequately monitored and controlled, may elevate injury susceptibility and performance deterioration through accumulated fatigue, tissue damage, inflammatory responses, and oxidative stress (Nobari et al., 2022; Ponce-Gonzalez et al., 2021). Therefore, continuous monitoring of physiological changes throughout the entire season provides useful information to maintain an optimal fitness level throughout the competitive season and to avoid non-functional overreaching or even overtraining (Saidi et al., 2021). In training load monitoring, both the external and internal load should be assessed to provide a greater understanding of the individual stress response (Haller et al., 2023; Nobari et al., 2022). To assess the internal load, subjective measures (e.g., rate of perceived exertion) or objective measures (e.g., heart rate) are often used (Haller et al., 2023). Both methods are valid and reliable tools for assessing training load in soccer players (Haddad et al., 2017; Impellizzeri et al., 2004, 2019). However, individual and contextual factors, such as daily fluctuations in physiological responses or accumulated fatigue, may still influence these measures and should be considered when interpreting internal load data.

Alternatively, physiological markers such as hematological variables have been used to objectively determine the internal load (Abderrahman et al., 2023; Bekris et al., 2015; Clemente et al., 2021; Heisterberg et al., 2013). Hematological variables (such as plasma volume, hemoglobin, hematocrit, red blood cell count) are crucial in regulating body fluids in response to physical exertion (Saidi et al., 2021). Their variation can be partly explained by variation in training load (Abderrahman et al., 2023), although there is inconsistent evidence regarding this dose-response relationship. In addition, several inflammatory and muscle damage markers (e.g., creatine kinase [CK]) have been used to assess inflammation after different training periods in elite soccer players. For example, Silva et al. (2014) showed significant increases in C-reactive protein (CRP) and CK activity at the end of the soccer season. Similarly, Huggins et al. (2019) and Walker et al. (2019) reported higher CK activity in-season compared to the beginning of the pre-season. Furthermore, Heisterberg and colleagues (2013) reported nearly doubled CK values two weeks after the beginning of the pre-season period compared to the competitive season, suggesting the high stress imposed by the resumption of training after the transition period. Conversely, Requena et al. (2017) found no significant difference in CK levels between the end of the previous competitive season and the beginning of the following pre-season period, while a significant reduction in lactate dehydrogenase (LDH) activity was reported.

Although the use of hematological and inflammatory markers to monitor exercise stress is a common practice in elite soccer, it is still necessary to determine how performance during the pre-season and in-season periods influences these physiological markers and what their relationship is. Therefore, this longitudinal study aimed to assess changes in players' physical performance, along with hematological and inflammatory markers at the beginning of pre-season training, at the end of pre-season training and in the middle of the competitive season to better understand the effect of training and match load on these variables.

Methods

Study Design

All athletes were tested before the pre-season (T0), after the pre-season (T1) and halfway through the competitive period (T2), twenty weeks after the beginning of the championship (Figure 1).

Participants

This investigation enrolled twenty-one professional athletes from a team competing in the second level of the national professional league

system. Participation eligibility required optimal health status without confounding physical or psychological conditions, pharmacological interventions, supplement usage, or therapeutic treatments that might affect measurement validity. Players who missed more than 10% of the training sessions during the competitive season were excluded from the study. Written informed consent from the athletes was obtained before the start of the study. Due to the player transfer market and exclusion criteria, seventeen players (age: 29.8 ± 3.9 years, body height: 180.0 ± 6.2 cm, body mass: 75.4 ± 5.4 kg) were finally considered for the study.

Ethics

Participant consent documentation was secured prior to the study's commencement. This study was conducted following the principles of the Declaration of Helsinki, and approved by the Ethics Committee of the University Niccolò Cusano, Rome, Italy (study protocol number MO3/22; approval date: 07 April 2022).

Measurements

On each testing day, blood samples were collected from the athletes and their fitness performance was measured, following the same protocol: blood withdrawal (7:00 a.m.), a general warm-up (around 9:00 a.m.), a countermovement jump test (CMJ), a 20-min specific warm-up, a 10-m sprint test (10-m sprint), 20-min recovery, a repeated sprint ability (RSA) test and a Yo-Yo intermittent recovery test level 2 (Yo-Yo IR2), performed at 5:00 p.m. after a specific warm-up. Physical test procedures were routinely used by the team's staff to assess athletes' fitness.

Hematological and Inflammatory Markers Assessment

Venous blood was drawn from the antecubital region using ethylenediaminetetraacetic acid-containing tubes, subsequently processed via centrifugation (3000 × g, 4°C, 10 min), with plasma aliquots preserved at −80°C pending analytical procedures. Complete blood counts were determined by the CELL-DYN Sapphire Automated Hematology Analyzer (Abbott). Following the manufacturer's instructions, plasma creatine kinase and lactate dehydrogenase activities were measured using commercial assay kits (Greiner Diagnostic GmbH, Bahlingen, Germany). C-reactive protein was quantified in serum using a clinical chemistry analyzer in order to obtain a measure of systemic inflammation (ILab 650; Instrumentation Laboratory, Lexington, Massachusetts, United States). Cytokines plasma levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) were both determined using ELISA kits commercially available (R&D systems, Minneapolis, Minnesota, USA).

Fitness Assessment

CMJ—No Arm Swing

From a vertical standing position, athletes performed a countermovement (90° of knee flexion) and executed a maximal vertical jump with arms held on the hips. Hip, knee and ankle joints were fully extended to avoid arm, trunk or leg strategies. All athletes performed a total of three correct attempts, recorded with an optical measurement system (Optojump Next, Microgate Srl, Italy), a valid tool for the assessment of CMJ-derived variables (Comyns et al., 2023). The maximum jump height in the three attempts was used as the final outcome.

10-m Linear Sprint

On the soccer field, athletes were asked to perform three trials of a 10-m sprint test. Sprint times [ms] were recorded by a photocell system (Witty—wireless training timer, Microgate Srl, Italy) (Haugen and Buchheit, 2016). Athletes performed three trials, with a complete rest interval between each attempt, and the best time from the three sprints was used for further analyses.

Repeated Sprint Ability

The repeated sprint ability (RSA) test was used to assess the capability to perform repeated sprints with incomplete recovery time, which had been reported as a relevant variable of soccer performance (Rampinini et al., 2009) and a reliable measure of sprinting ability in soccer (Wragg et al., 2000). RSA testing consisted of 7 repetitions of the RSA setting (Figure 2) for a total distance of 34.2 m for each repetition. A network of two couples of photocell systems (Witty— wireless training timer, Microgate Srl, Italy) was used to calculate the following variables: total time of the seven sprints, average sprint time in the seven repetitions (AST), the best sprint time among the seven repetitions, average change of direction time in the seven

Articles published in the Journal of Human Kinetics are licensed under an open access Creative Commons CC BY 4.0 license.

repetitions, the lowest change of direction time among the seven repetitions. Change of direction times referred to the time needed to cover the distance between the first and last change of direction for each repetition. In addition, the fatigue index (FI [%] = ((total sprint time/(lowest sprint time × 7)) × 100) − 100) was calculated to evaluate athletes' capability to respond to sprint-induced fatigue (Perroni et al., 2023).

Yo-Yo IR2

The Yo-Yo intermittent recovery test level 2 is a test performed to volitional exhaustion used to assess the aerobic capacity of athletes during intermittent exercise. It is considered a valid test for professional soccer players (Ingebrigtsen et al., 2012). The test consists of 20-m shuttle runs at increasing speed, with 10 s of active recovery every 40 m. The required speed for each shuttle (starting at 13 km/h and gradually increasing) was provided by an acoustic device. A heart rate device (K-shirt, K-Sport Srl, Italy) was used to ensure that players reached their maximal effort during the test. The final distance covered by each athlete was calculated to indirectly estimate VO_{2max} using the following equation (Bangsbo et al., 2008): VO_{2max} (mL/min/kg) = Yo-Yo IR2 distance (m) × 0.0136 + 45.3

Training Monitoring

External workload quantification employed GPS technology (K-50 system, K-Sport Srl, Italy, 50-Hz sampling rate) across all sessions and competitive matches, encompassing supplementary activities for non-starting players. Workload metrics encompassed temporal duration, locomotor coverage (total distance covered), velocity-specific movements (16–20, 20–24, >24 km·h^{-1} zones), metabolically demanding displacements (metabolic power exceeding 20 W·kg^{-1}), energetic output (mean metabolic power), and neuromuscular stress indicators (accelerations ≥2 m·s^{-2}, decelerations ≥−2 m·s^{-2}), providing comprehensive training stimulus assessment consistent with established methodologies (Castagna et al., 2017). During the data collection, players were asked to rate their perceived recovery each morning using the 11-point Perceived Recovery Status scale (Laurent et al., 2011). The descriptors were: "not at all recovered" at 0, "moderately recovered" at 5, and "fully recovered" at 10 points.

Statistical Analysis

Data were reported as mean and standard deviations, or median [q1–q3] where appropriate. Data distribution normality verification employed the Shapiro-Wilk assessment, while sphericity assumptions were evaluated through the Mauchly's procedure, with Greenhouse-Geisser adjustment implemented when assumption violations occurred.

Players' Training Load Data Analysis

To analyze each player's training load data (collected with GPS), morning and afternoon sessions were summed up (or averaged, where appropriate). A linear mixed model analysis was then used to compare GPS measures at three different occasions: training during the pre-season period, training during the competitive period and matches.

Hematological and Inflammatory Data Analysis

A multivariate analysis of variance (MANOVA) for repeated measures was used to test the differences in hematological variables among the three times (before the pre-season period, T0; after the pre-season period, T1; halfway through the competitive period, T2). Within-subjects contrasts were considered to explore the differences between T1 vs. T0 and T2 vs. T1.

Physical Fitness Data Analysis

The same MANOVA procedure, together with within-subject contrasts, was applied to assess changes in physical performance variables across the three time points (T0, T1, T2).

The partial eta squared (η^2_p) was considered as an effect size measure and interpreted as η^2_p = 0.01: small effect; η^2_p = 0.06: medium effect; η^2_p = 0.14: large effect. Relationships between variations in physical and hematological variables (for each time separately, and for delta T1–T0 and T2–T1) were assessed using Pearson's correlation coefficients (r), and interpreted as follows: $0.1 \le r < 0.3$ = small, $0.3 \le r < 0.5$ = moderate, $r \ge 0.5$ = large. A post-hoc power analysis was conducted using G*Power software (version 3.1.9.6, University of Kiel, Germany). The analysis was based on a predefined alpha level (two-tailed, $\alpha = 0.05$), a sample size of 17 participants (n = 17), and an estimated effect size f. A MANOVA model with time as a repeated measure (T0, T1, T2) and a

single group was used, representing a longitudinal design without between-group stratification. The analysis yielded a partial eta squared of 0.55 and an average correlation among repeated measures of 0.7. Based on these variables, the estimated statistical power (1-β) was approximately 1, indicating that the sample size was adequate to support the findings of this study. The analyses were conducted using the Statistical Package for Social Sciences (SPSS) v.29 or RStudio 2023.12.1, at the standard level of statistical significance set at $\alpha = 0.05$.

Results

Training load measures collected during pre-season and in-season periods are reported in Table 1.

All measured training variables demonstrated substantial variations when comparing pre-competitive and competitive phases, with competitive period sessions exhibiting reduced total training volumes. Matches showed lower total time and distance covered than training sessions, but greater high-intensity work (more distance over 16 km/h, high-metabolic intensity distance and higher average metabolic power). Figure 3 shows the external training load (expressed as total distance) and the recovery score, computed as the mean of the players, for each training day of the analyzed period.

Regarding hematological variables, significant increases in inflammatory (IL-6) and muscle damage (CK and LDH) markers were reported at T1 (end of the pre-season period) (Table 2). No major changes were reported for the other measures.

Several significant changes were detected in physical performance tests (Table 3). In particular, an improvement in maximal oxygen uptake estimated by the Yo-Yo IR2 test was reported, both at T1 and T2. Conversely, a reduction in performance in the 10-m sprint time and RSA indices was reported at T1, which was recovered at T2 only for the 10-m sprint time and the lowest RSA sprint time.

To explore whether variations in hematological variables could be associated with physical performance measures, correlations considering the delta (T1–T0 and T2–T1) data were computed. Significant correlations were found only at T2–T1 for IL-6, of which variation was positively associated with RSA measures (average sprint time: $r = 0.52$, $p = 0.032$; average change of direction time: $r = 0.58$, $p = 0.016$; lowest change of direction time: $r = 0.61$, $p = 0.010$). Although not significant, moderate correlations with IL-6 changes were also reported with variations in CMJ performance ($r = -0.46$, $p = 0.063$) and repeated sprint ability total time ($r = 0.47$, $p = 0.058$).

Discussion

The present longitudinal study aimed to assess physical performance as well as hematological and inflammatory variables of soccer players at the beginning of the pre-season (T0), at the end of the pre-season (T1) and halfway through the competitive period (T2). The main results showed a decrease in the 10-m sprint time and repeated sprint ability indices at the end of the pre-season period, and increases in inflammatory and muscle damage markers supported these findings. On the other hand, there was a significant progressive improvement in the estimated maximal oxygen uptake from the beginning of the pre-season period to the middle of the competitive period, with a trend towards a decrease in inflammatory and muscle damage markers from the end of the pre-season to the mid-season.

Inflammatory Markers

The results of this study showed a significant increase in creatine kinase (CK) and lactate dehydrogenase (LDH) activity at the end of the pre-season compared to the beginning of it, followed by a decrease at the mid-season, although not significant. It seems possible that these results are due to the intense training loads and a higher number of high-intensity accelerations and decelerations during the pre-season period, which may have caused muscle damage, resulting in increased activity of CK and LDH (Gastin et al., 2019).

Articles published in the Journal of Human Kinetics are licensed under an open access Creative Commons CC BY 4.0 license.

Table 1. Training load measures per day (if two training sessions were performed in a single day, data were summed/averaged). Data are reported as median [Q1–Q3].

Variable	Pre-season period	In-season period	
		Training	*Match*
Time (min)	67.5 [42.3–90.1]	59.0 [a] [48.3–73.6]	47.3 [a,b] [19.0–88.8]
Total distance (m)	6383.9 [3116.7–8446.8]	4369.1 [a] [3657.1–5739.4]	4959.5 [a,b] [2640.5–9497.7]
Distance 16–20 (m)	549.0 [220.5–937.0]	344.0 [a] [233.0–556.0]	676.0 [b] [373.5–888.8]
Distance 20–24 (m)	167.5 [56.0–323.5]	137.5 [a] [69.0–241.0]	347.5 [a,b] [204.0–556.3]
Distance >24 (m)	28.5 [1.0–98.0]	23.0 [a] [1.0–67.3]	98.0 [a,b] [17.8–194.3]
Distance MPHI (m)	1226.6 [643.1–2067.9]	951.0 [a] [688.6–1400.7]	1629.3 [b] [1011.7–2252.9]
High Intensity Accelerations (n)	79 [49–119]	75 [a] [54–99]	61 [a] [40–116]
High Intensity Decelerations (n)	64 [36–103]	60 [a] [41–80]	63 [17–118]
Avg Metabolic Power (w/kg)	8.1 [6.7–10.4]	7.6 [a] [6.3–9.5]	10.5 [a,b] [9.9–11.8]

MPHI: Metabolic Power High-Intensity; a: significantly different from "Pre-season period"; b: significantly different from "In-season Training"

Table 2. Physical fitness variables at the three time points.

Variable	T0	T1	T2	*p*	η^2_p
CMJ (cm)	40.84 ± 5.30	39.91 ± 4.85	39.81 ± 5.12	0.044	0.177
10-m sprint (s)	1.68 ± 0.05	1.78 ± 0.05 [a]	1.68 ± 0.05 [b]	<0.001	0.780
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	56.09 ± 2.36	57.89 ± 2.20 [a]	58.41 ± 1.90 [b]	<0.001	0.488
RSA: TT (s)	42.17 ± 1.32	43.28 ± 1.34 [a]	42.44 ± 1.43	<0.001	0.485
RSA: AST (s)	6.03 ± 0.18	6.18 ± 0.19 [a]	6.06 ± 0.19	<0.001	0.522
RSA: LST (s)	5.75 ± 0.17	5.98 ± 0.19 [a]	5.92 ± 0.24 [b]	<0.001	0.730
RSA: FI (%)	4.53 ± 2.50	3.28 ± 1.06	3.07 ± 1.04	0.044	0.177
RSA: ACDT (s)	2.46 ± 0.10	2.61 ± 0.10 [a]	2.60 ± 0.09	<0.001	0.766
RSA: LCDT (s)	2.35 ± 0.10	2.52 ± 0.11 [a]	2.53 ± 0.11	<0.001	0.859

CMJ: countermovement jump; RSA: repeated sprint ability; TT: total time; AST: average sprint time; LST: lowest sprint time; FI: fatigue index; ACDT: average change of direction time; LCDT: lowest change of direction time; [a] significantly different from T0; [b] significantly different from T1

Table 3. Hematological variables and inflammatory markers at the three time points.

Variable	T0	T1	T2	*p*	η^2_p
Ht (%)	43.75 ± 3.03	43.44 ± 2.77	43.85 ± 2.36	0.553	0.036
CRP (mgr/dl)	0.15 ± 0.02	0.13 ± 0.02	0.16 ± 0.02 [b]	0.012	0.278
CK (U/L)	253.35 ± 159.29	355.06 ± 173.69 [a]	286.04 ± 171.92	0.016	0.257
LDH (U/L)	163.50 ± 30.68	183.88 ± 26.49 [a]	171.66 ± 36.29	0.006	0.348
IL-6 (pg/ml)	17.77 ± 30.85	83.15 ± 33.08 [a]	19.99 ± 45.77 [b]	<0.001	0.530
IL-10 (pg/ml)	2.99 ± 1.04	3.41 ± 2.80	2.45 ± 1.22	0.330	0.063

CRP: c-reactive protein; CK: creatine kinase; Ht: hematocrit; LDH: lactate dehydrogenase; IL: interleukin; [a] significantly different from T0; [b] significantly different from T1



Figure 1. Timeline of the study.

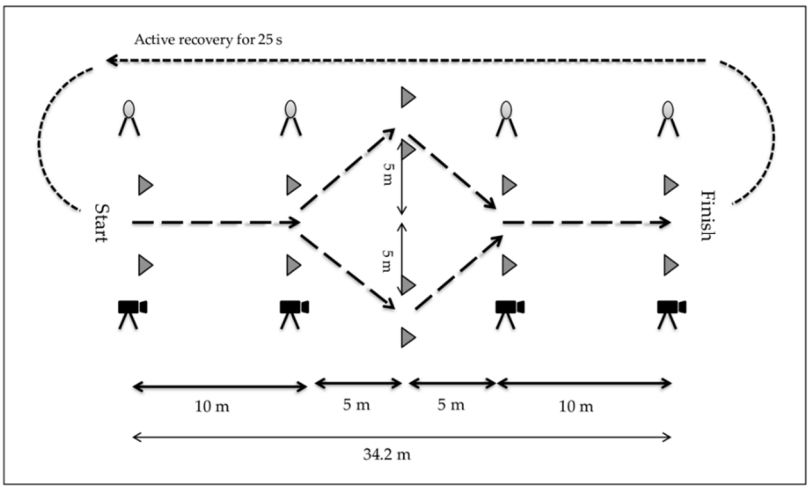


Figure 2. RSA protocol setting. Athletes performed 10 m of the linear sprint, changed direction (alternating directions at each sprint) for reaching a cone placed at the 5-m distance in the right or left direction for two times and finished the last 10 m sprinting linearly. After the finishing line, athletes must have reached the starting position in 25 s.

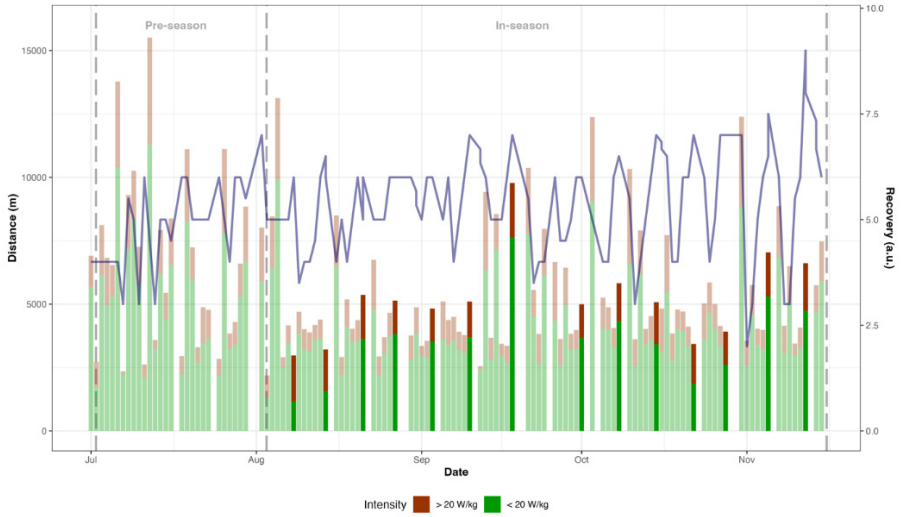


Figure 3. External training load (total distance at low [green] and high [red] metabolic power) and recovery scores for all the training days of the pre-season and competitive periods. The columns with more intense colors represent the match days. The recovery score is shown with the blue line.

Articles published in the Journal of Human Kinetics are licensed under an open access Creative Commons CC BY 4.0 license.

Our findings of increased CK activity parallel observations by Huggins and colleagues (2019), who documented similar increases following technically-focused, high-intensity pre-competitive training phases. Their investigation, however, revealed persistently elevated CK activity throughout subsequent competitive phases compared to normative values—contrasting with our mid-season reductions. Additionally, while our study demonstrated significant LDH activity fluctuations, their research indicated temporal stability of this enzyme within standard reference thresholds. Heisterberg et al. (2013) found a significant increase in CK activity after 14 days of pre-season training, which includes a large amount of running and resistance exercise. Unlike our study, Silva et al. (2014) found higher CK activity in the middle of the competitive season compared to the pre-season and the end of the season. However, the authors of that study explained that this increase could be associated with residual levels of muscle damage related to regular training sessions. Contrary to previous findings, Coppalle et al. (2019) found similar CK and LDH activity between the beginning and the end of the pre-season. However, this discrepancy was explained by the authors pointing to the timing of sample collection. The blood sample was taken one week after the end of the pre-season period, which was potentially enough time to allow for recovery.

In this study, we observed a significant increase in interleukin-6 (IL-6) levels at T1 compared to T0, followed by a significant decrease at T2. A similar trend was observed in interleukin-10 (IL-10) levels, which increased at T1 compared to T0, and then decreased at T2; however, these differences were not significant. In agreement with previous studies, the increase in the anti-inflammatory cytokine IL-10, although not significant, could be due to the stimulation of IL-6 (Ringleb et al., 2024). Huggins et al. (2019) found no significant differences in IL-6 during the soccer season, except between weeks 1 and 8 of the competitive period, concluding that athletes did not show any evidence of sustained inflammation. An opposite trend, compared to the above, was observed in C-reactive protein, which decreased at T1 compared to T0, although not significantly, followed by a significant increase at T2. Similar results were observed by Radziminski et al. (2020), who did not observe elevated C-reactive protein values in professional soccer players during 12 days of training in the pre-season period. In contrast, Clemente et al. (2021) found a significant increase in C-reactive protein values after the pre-season compared to the beginning of the season.

Sprinting, Repeated-Sprinting and Jumping Tests

The results of our study showed an increase (i.e., worse performance) in the 10-m sprint time (s) from T0 to T1, followed by a significant improvement at T2. Similarly, at the end of the pre-season period, we found an increase (i.e., worse performance) in repeated sprint ability total time, average sprint time, lowest sprint time, average change of direction time, and lowest change of direction time compared to the beginning of the pre-season period. Our RSA changes agree with Sal-de-Rellán et al. (2026), suggesting that repeated-sprint performance depends on acceleration, change-of-direction ability, and jump-related power, which are likely affected by seasonal fatigue. However, in the competitive period, we observed a significant improvement in the repeated sprint ability, lowest sprint time and an overall trend of improvement in all variables, although not significant. The observed decrease in performance seems to suggest signs of fatigue at the end of the pre-season period. Indeed, training load analysis clearly showed higher loads and a lower perceived level of recovery during the pre-season period. This performance reduction may be attributed to modifications in cellular contractile characteristics, as demonstrated by elevated muscle damage indicators (creatine kinase and lactate dehydrogenase activity) following pre-competitive training completion, which interferes with the optimal restoration of muscle glycogen stores, necessary to perform intermittent high-intensity exercise (Saidi et al., 2019). These findings corroborate the results obtained by Saidi et al. (2019), who also found a 2.6% decrease in repeated sprint ability after a 6-week match-congested period. In contrast to our study, Silva et al. (2022) showed an improvement in the 20-m linear sprint (5 m, 10 m, 15 m, 20 m) after the pre-season phase despite an increase in markers related to muscle damage and inflammation. Similarly, Meckel et al. (2018) demonstrated a significant improvement in the 4×10-m test at the end of the pre-season period,

with no further change at the mid-season.

A decrease in jumping performance is often considered a marker of neuromuscular fatigue, reflecting aspects of acute and chronic loading (Haller et al., 2023). In our study, no significant changes in CMJ performance were found among T0, T1 and T2. This stable CMJ profile is partially consistent with Nunes et al. (2026), although, unlike their late-season improvements, our players mainly preserved neuromuscular performance across the monitored phases. Similarly to our results, in the study of Saidi et al. (2019), CMJ performance did not change significantly after a 6-week match-congested period. However, the results are inconsistent, since previous studies had found improvements in CMJ performance in soccer players after the pre-season period (Perroni et al., 2019; Silva et al., 2022) and at the mid-season (Meckel et al., 2018), while others had even found decreases in jump height (Selmi et al., 2022; Silva et al., 2021).

Aerobic Performance

High levels of hemoglobin (Hb) and hematocrit (Ht) are generally associated with increased blood oxygen transport capacity and increased blood viscosity, respectively (Abderrahman et al., 2023; Bekris et al., 2015). In the current study, no significant changes in the Ht percentage were found between the pre-season and the competitive period. However, previous studies have shown a decrease in Ht levels after periods of intense soccer training (Huggins et al., 2019) or match congestion (Saidi et al., 2019) due to an increase in plasma volume. Anđelković et al. (2015) observed a significant increase in plasma volume of 7.5% and a decrease in Ht after a competitive half-season in nineteen elite soccer players. Similarly, Huggins et al. (2019) found a decrease in Ht from the start of the pre-season to week 12 of the season, although Yo-Yo IR2 estimated VO_{2max} remained unchanged. In contrast, Heisterberg et al. (2013) found an increase in Ht concentrations after the last competitive match of the season compared to PS, with a small but significant decrease ($\Delta\% = -2.26\%$) in VO_{2max} after the last competitive match of the season (4.75 ± 0.11 $L \cdot min^{-1}$) compared to the beginning of the pre-season period (4.86 ± 0.12 $L \cdot min^{-1}$). Contrary to those findings, in this study, a progressive improvement in maximal oxygen uptake estimated by the Yo-Yo IR2 test was observed from T0 to T1 and T2. It seems possible that these contradictory results are due to differences between studies in the duration of the training periods, workload (intensity, volume), and training characteristics. Therefore, the analysis of the variation in hematological and performance variables should be treated with caution.

Correlations between Variations in Hematological Variables, Inflammatory Markers and Physical Performance

The significant correlation found for IL-6, whose variation was positively associated with RSA measures only at T2–T1, highlights how a reduction in IL-6 value is associated with improved RSA performance. Similarly, a moderate negative correlation between variations in IL-6 value and CMJ performance was reported, although it was not statistically significant. Nash and colleagues (2023) recently suggested that IL-6 response could be considered a marker of the ability to tolerate training load in the athletic population. Therefore, since our players were subjected to similar training loads, it is possible that players who were better adapted to training loads in the competitive season showed a greater reduction in IL-6 (lower inflammation), thus leading to improved performance.

Limitations of the Study

A possible limitation of this study is related to the regular elite soccer season, which is characterized by player turnover due to injuries and the transfer market. As a result, only a limited number of players from the full roster could be evaluated at the three analyzed time points. Although all measurements followed a standardized schedule across testing days, the time gap between morning blood sampling and afternoon performance testing could have introduced variability due to diurnal fluctuations in both biomarkers and physical performance. Additionally, although testing and blood sampling were conducted using standardized procedures, both subjective (rate of perceived exertion) and objective (heart rate) indicators of the internal training load, as well as hematological and inflammatory markers, might be influenced by factors such as diurnal variation, previous training sessions, and individual physiological responses.

These aspects should be considered when interpreting the relationships observed in the present study.

Conclusions

The analysis of physical performance data as well as hematological and inflammatory markers revealed significant changes throughout a soccer season. At the end of the pre-season period, data showed decreases in the 10-m sprint and RSA performance and an increase in inflammatory cytokines IL-6 and markers of muscle damage (CK and LDH), while the opposite pattern occurred in the middle of the competitive season when performance improved, which was supported by reduced inflammation.

In-season, the reduced inflammatory markers could be due to the tapering and recovery strategies applied during the competitive period to promote recovery processes and maximize players' performance. These observations are supported by the analysis of training load, which showed the highest loads during the pre-season period, which could likely explain the observed physiological signs of fatigue at the end of this phase. Finally, given that previous studies have found elevated levels of IL-6 and other pro-inflammatory cytokines in individuals with overtraining syndrome, our results suggest a key role for IL-6 as a marker of the ability to tolerate training load. Adequate control of this marker could allow staff not only to monitor athletes' status but also to optimize personalized recovery and training strategies.

Author Contributions: Conceptualization: F.P., S.A. and D.C.; methodology: L.M. and F.P.; software: M.C. and D.S.; formal analysis: F.S. and L.C.; investigation: L.M., M.C. and A.F.; resources: A.F.; data curation: L.M., M.C. and D.S.; writing—original draft preparation: F.S. and L.C.; writing—review & editing: S.A. and D.C.; visualization: F.P., C.B. and L.G.; supervision: F.P., C.B. and L.G.; project administration: S.A. and D.C. Additionally, F.S. and L.C. contributed equally to this work and share the first authorship. S.A. and D.C. contributed equally to this work and share the last authorship. All authors have read and agreed to the published version of the manuscript.

ORCID iD:

Fioretta Silvestri: https://orcid.org/0000-0002-1997-7028

Ludovica Cardinali: https://orcid.org/0000-0003-1581-8027

Lorenzo Marcelli: https://orcid.org/0009-0003-7635-5131

Matteo Campanella: https://orcid.org/0000-0002-7222-7701

Davide Sisti: https://orcid.org/0000-0002-7925-7495

Fabrizio Perroni: https://orcid.org/0000-0003-2094-4351

Alessandra Favoriti: https://orcid.org/0009-0003-5859-8682

Carlo Baldari: https://orcid.org/0000-0001-6227-3571

Laura Guidetti: https://orcid.org/0000-0002-1419-4550

Stefano Amatori: https://orcid.org/0000-0001-7497-755X

Davide Curzi: https://orcid.org/0000-0003-2363-0956

Funding Information: This research received no external funding

Institutional Review Board Statement: This study was conducted following the principles of the Declaration of Helsinki, and approved by the Ethics Committee of the University Niccolò Cusano, Rome, Italy (study protocol number: MO3/22; approval date: 07 April 2022).

Articles published in the Journal of Human Kinetics are licensed under an open access Creative Commons CC BY 4.0 license.

Informed Consent: Informed consent was obtained from all participants included in the study.

Conflicts of Interest: The authors declare no conflict of interest.

Received: 10 March 2025

Accepted: 15 June 2026

References

Abderrahman, B. A., Jebabli, N., Rhibi, F., Rivière, F., Le Moal, E., Laher, I., & Zouhal, H. (2023). Plasma Volume Variations in Professional Soccer Players: Difference Between Pre- and Competitive Season. *International Journal of Sport Studies For Health*, *5*(2), e134030. https://doi.org/10.5812/intjssh-134030

Anđelković, M., Baralić, I., Đorđević, B., Stevuljević, J. K., Radivojević, N., Dikić, N., Škodrić, S. R., & Stojković, M. (2015). Hematological and biochemical parameters in elite soccer players during a competitive half season. *Journal of Medical Biochemistry*, *34*(4), 460. https://doi.org/10.2478/jomb-2014-0057

Asín-Izquierdo, I., Galiano, C., Nakamura, F. Y., Pareja-Blanco, F. H., & Jose A Asian-, C. (2026). You Play the Way You Train: The Influence of Accumulated Weekly Training Loads on Match Physical Performance in Soccer Players. *Journal of Human Kinetics*, *101*, 5–20. https://doi.org/10.5114/jhk/208874

Bangsbo, J., Iaia, F. M., & Krustrup, P. (2008). The Yo-Yo intermittent recovery test: a useful tool for evaluation of physical performance in intermittent sports. *Sports Medicine*, *38*(1), 37–51. https://doi.org/10.2165/00007256-200838010-00004

Bekris, E., Gioldasis, A., Gissis, I., Anagnostakos, K., & Eleftherios, M. (2015). From Preparation to Competitive Period in Soccer: Hematological Changes. *Sport Science Review*, *24*, 103–114. https://doi.org/10.1515/ssr-2015-0011

Castagna, C., Francini, L., Povoas, S. C. A., & D'Ottavio, S. (2017). Long-Sprint Abilities in Soccer: Ball Versus Running Drills. *International Journal of Sports Physiology and Performance*, *12*(9), 1256–1263. https://doi.org/10.1123/ijspp.2016-0565

Clemente, F. M., Gonzalez-Fernandez, F. T., Ceylan, H. I., Silva, R., Younesi, S., Chen, Y. S., Badicu, G., Wolanski, P., & Murawska-Cialowicz, E. (2021). Blood Biomarkers Variations across the Pre-Season and Interactions with Training Load: A Study in Professional Soccer Players. *Journal of Clinical Medicine*, *10*(23), 5576. https://doi.org/10.3390/jcm10235576

Comyns, T. M., Murphy, J., & O'Leary, D. (2023). Reliability, Usefulness, and Validity of Field-Based Vertical Jump Measuring Devices. *Journal of Strength and Conditioning Research*, *37*(8), 1594–1599. https://doi.org/10.1519/JSC.0000000000004436

Coppalle, S., Rave, G., Ben Abderrahman, A., Ali, A., Salhi, I., Zouita, S., Zouita, A., Brughelli, M., Granacher, U., & Zouhal, H. (2019). Relationship of pre-season training load with in-season biochemical markers, injuries and performance in professional soccer players. *Frontiers in Physiology*, *10*, 409. https://doi.org/10.3389/fphys.2019.00409

Gastin, P. B., Hunkin, S. L., Fahrner, B., & Robertson, S. (2019). Deceleration, Acceleration, and Impacts Are Strong Contributors to Muscle Damage in Professional Australian Football. *Journal of Strength and Conditioning Research*, *33*(12), 3374–3383. https://doi.org/10.1519/JSC.0000000000003023

Haddad M, Stylianides G, Djaoui L, Dellal A, Chamari K. (2017). Session-RPE Method for Training Load Monitoring: Validity, Ecological Usefulness, and Influencing Factors. *Frontiers in Neuroscience, 11*, 612. https://doi:10.3389/fnins.2017.00612

Haller, N., Kranzinger, S., Kranzinger, C., Blumkaitis, J. C., Strepp, T., Simon, P., Tomaskovic, A., O'Brien, J., During, M., & Stoggl, T. (2023). Predicting Injury and Illness with Machine Learning in Elite Youth Soccer: A Comprehensive Monitoring Approach over 3 Months. *Journal of Sports Science & Medicine*, *22*(3), 476–487. https://doi.org/10.52082/jssm.2023.476

Haugen, T., & Buchheit, M. (2016). Sprint Running Performance Monitoring: Methodological and Practical Considerations. *Sports Medicine*, *46*(5), 641–656. https://doi.org/10.1007/s40279-015-0446-0

Heisterberg, M. F., Fahrenkrug, J., Krustrup, P., Storskov, A., Kjaer, M., & Andersen, J. L. (2013). Extensive monitoring through multiple blood samples in professional soccer players. *Journal of Strength and Conditioning Research*, *27*(5), 1260–1271. https://doi.org/10.1519/JSC.0b013e3182653d17

Huggins, R. A., Fortunati, A. R., Curtis, R. M., Looney, D. P., West, C. A., Lee, E. C., Fragala, M. S., Hall, M. L., & Casa, D. J. (2019). Monitoring Blood Biomarkers and Training Load Throughout a Collegiate Soccer Season. *Journal of Strength and Conditioning Research*, *33*(11), 3065–3077. https://doi.org/10.1519/JSC.0000000000002622

Impellizzeri, F.M., Rampinini, E., Coutts, A.J., Sassi. A., Marcora. S.M. (2004). Use of RPE-based training load in soccer. *Medicine & Science in Sports & Exercise*, *36*(6), 1042–1047. https://doi.org/10.1249/01.mss.0000128199.23901.2f

Impellizzeri, F.M., Marcora, S.M., Coutts, A.J. (2019). Internal and External Training Load: 15 Years On. *International Journal of Sports Physiology and Performance*, *14*(2), 270–273. https://doi.org/10.1123/ijspp.2018-0935

Ingebrigtsen, J., Bendiksen, M., Randers, M. B., Castagna, C., Krustrup, P., & Holtermann, A. (2012). Yo-Yo IR2 testing of elite and sub-elite soccer players: performance, heart rate response and correlations to other interval tests. *Journal of Sports Sciences*, *30*(13), 1337–1345. https://doi.org/10.1080/02640414.2012.711484

Laurent, C. M., Green, J. M., Bishop, P. A., Sjokvist, J., Schumacker, R. E., Richardson, M. T., & Curtner-Smith, M. (2011). A practical approach to monitoring recovery: development of a perceived recovery status scale. *Journal of Strength and Conditioning Research*, *25*(3), 620–628. https://doi.org/10.1519/JSC.0b013e3181c69ec6

Meckel, Y., Doron, O., Eliakim, E., & Eliakim, A. (2018). Seasonal Variations in Physical Fitness and Performance Indices of Elite Soccer Players. *Sports (Basel)*, *6*(1), 14. https://doi.org/10.3390/sports6010014

Nash, D., Hughes, M. G., Butcher, L., Aicheler, R., Smith, P., Cullen, T., & Webb, R. (2023). IL-6 signaling in acute exercise and chronic training: Potential consequences for health and athletic performance. *Scandinavian Journal of Medicine & Science in Sports*, *33*(1), 4–19. https://doi.org/10.1111/sms.14241

Nobari, H., Ramachandran, A. K., Brito, J. P., & Oliveira, R. (2022). Quantification of Pre-Season and In-Season Training Intensity across an Entire Competitive Season of Asian Professional Soccer Players. *Healthcare (Basel)*, *10*(8), 1367. https://doi.org/10.3390/healthcare10081367

Nunes, R. F. H., Pereira, L. A., Fiorenza, J. P., Paes, C. A., Grazioli, R., Bryk, P. & Loturco, I. (2026). Strength and Power Responses to a Power-Oriented Resistance Training Model across an Entire Soccer Competitive Season. *Journal of Human Kinetics*, *101*, 245–255. https://doi.org/10.5114/jhk/218820

Perroni, F., Castagna, C., Amatori, S., Gobbi, E., Vetrano, M., Visco, V., Guidetti, L., Baldari, C., Rocchi, M. B. L., & Sisti, D. (2023). Use of Exploratory Factor Analysis to Assess the Fitness Performance of Youth Football Players. *Journal of Strength and Conditioning Research*, *37*(7), e430–e437. https://doi.org/10.1519/JSC.0000000000004414

Perroni, F., Fittipaldi, S., Falcioni, L., Ghizzoni, L., Borrione, P., Vetrano, M., Del Vescovo, R., Migliaccio, S., Guidetti, L., & Baldari, C. (2019). Effect of pre-season training phase on anthropometric, hormonal and fitness parameters in young soccer players. *PLoS One*, *14*(11), e0225471. https://doi.org/10.1371/journal.pone.0225471

Ponce-Gonzalez, J. G., Corral-Perez, J., de Villarreal, E. S., Gutierrez-Manzanedo, J. V., Castro-Maqueda, G., & Casals, C. (2021). Antioxidants Markers of Professional Soccer Players During the Season and their Relationship with Competitive Performance. *Journal of Human Kinetics*, *80*, 113–123. https://doi.org/10.2478/hukin-2021-0089

Radzimiński, L., Jastrzębski, Z., Lopez-Sanchez, G. F., Szwarc, A., Duda, H., Stuła, A., Paszulewicz, J., & Dragos, P. (2020). Relationships between Training Loads and Selected Blood Parameters in Professional Soccer Players during a 12-Day Sports Camp. *International Journal Environmental Research and Public Health*, *17*(22), 8580. https://doi.org/10.3390/ijerph17228580

Articles published in the Journal of Human Kinetics are licensed under an open access Creative Commons CC BY 4.0 license.

Rampinini, E., Sassi, A., Morelli, A., Mazzoni, S., Fanchini, M., & Coutts, A. J. (2009). Repeated-sprint ability in professional and amateur soccer players. *Applied Physiology, Nutrition, and Metabolism*, *34*(6), 1048–1054. https://doi.org/10.1139/H09-111

Requena, B., García, I., Suárez-Arrones, L., Sáez de Villarreal, E., Naranjo Orellana, J., & Santalla, A. (2017). Off-Season Effects on Functional Performance, Body Composition, and Blood Parameters in Top-Level Professional Soccer Players. *Journal of Strength and Conditioning Research*, *31*(4), 939–946. https://doi.org/10.1519/jsc.0000000000001568

Ringleb, M., Javelle, F., Haunhorst, S., Bloch, W., Fennen, L., Baumgart, S., Drube, S., Reuken, P. A., Pletz, M. W., Wagner, H., Gabriel, H. H. W., & Puta, C. (2024). Beyond muscles: Investigating immunoregulatory myokines in acute resistance exercise - A systematic review and meta-analysis. *FASEB Journal*, *38*(7), e23596. https://doi.org/10.1096/fj.202301619R

Saidi, K., Abderrahman, A. B., Hackney, A. C., Bideau, B., Zouita, S., Granacher, U., & Zouhal, H. (2021). Hematology, Hormones, Inflammation, and Muscle Damage in Elite and Professional Soccer Players: A Systematic Review with Implications for Exercise. *Sports Medicine*, *51*(12), 2607–2627. https://doi.org/10.1007/s40279-021-01522-w

Saidi, K., Zouhal, H., Rhibi, F., Tijani, J. M., Boullosa, D., Chebbi, A., Hackney, A. C., Granacher, U., Bideau, B., & Ben Abderrahman, A. (2019). Effects of a six-week period of congested match play on plasma volume variations, hematological parameters, training workload and physical fitness in elite soccer players. *PLoS One*, *14*(7), e0219692 https://doi.org/10.1371/journal.pone.0219692

Sal-de-Rellán, A., Ben Brahim, M., Martín, V., Zidi, N. & Hernaiz-Sánchez, A. (2026). How Different Physical Qualities Influence Repeated Sprint Ability Tests in Elite Youth Soccer Players?. *Journal of Human Kinetics*, *102*, 183–194. https://doi.org/10.5114/jhk/203832

Selmi, O., Ouergui, I., Levitt, D. E., Marzouki, H., Knechtle, B., Nikolaidis, P. T., & Bouassida, A. (2022). Training, psychometric status, biological markers and neuromuscular fatigue in soccer. *Biology of Sport*, *39*(2), 319–327. https://doi.org/10.5114/biolsport.2022.104065

Silva, A. F., Gonzalez-Fernandez, F. T., Ceylan, H. I., Silva, R., Younesi, S., Chen, Y. S., Badicu, G., Wolanski, P., Murawska-Cialowicz, E., & Clemente, F. M. (2022). Relationships between Fitness Status and Blood Biomarkers in Professional Soccer Players. *Journal of Healthcare Engineering*, *2022*, 5135817. https://doi.org/10.1155/2022/5135817

Silva, J. R., Rebelo, A., Marques, F., Pereira, L., Seabra, A., Ascensão, A., & Magalhães, J. (2014). Biochemical impact of soccer: an analysis of hormonal, muscle damage, and redox markers during the season. *Applied Physiology, Nutrition, and Metabolism*, *39*(4), 432–438. https://doi.org/10.1139/apnm-2013-0180

Silva, R., Lima, R., Camões, M., Leão, C., Matos, S., Pereira, J., Bezerra, P., & Clemente, F. M. (2021). Physical fitness changes among amateur soccer players: effects of the pre-season period. *Biomedical Human Kinetics*, *13*(1), 63–72. https://doi.org/doi:10.2478/bhk-2021-0009

Walker, A. J., McFadden, B. A., Sanders, D. J., Rabideau, M. M., Hofacker, M. L., & Arent, S. M. (2019). Biomarker Response to a Competitive Season in Division I Female Soccer Players. *Journal of Strength and Conditioning Research*, *33*(10), 2622–2628. https://doi.org/10.1519/JSC.0000000000003264

Wragg, C. B., Maxwell, N. S., & Doust, J. H. (2000). Evaluation of the reliability and validity of a soccer-specific field test of repeated sprint ability. *European Journal of Applied Physiology*, *83*(1), 77–83. https://doi.org/10.1007/s004210000246