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Seasonal Variations of Haematological, Biochemical and Physical Performance Indices in Elite Beach Soccer Players

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Abstract

Purpose: Haematological, hormonal, biochemical and physical performance parameters were altered after long-term soccer training in professional soccer players. These alterations can be influenced by different contextual factors such as playing surface, training load, duration of training and competition. The purpose of this study was to investigate the changes in the haematological, inflammatory, antioxidant and physical performance of beach soccer players during the competitive season of the beach soccer. **Method:** The study examined 15 elite beach soccer players in Iranian beach soccer primer league (age 24.64 ± 4.01 y, weight 75.08 ± 8.15 kg, height 181.00 ± 5.17 cm, body mass index 22.76 ± 2.36) from the pre-season, mid-season and end-season. Measurements of haematological, inflammatory, antioxidants indices and aerobic / anaerobic power were repeated in the pre-season, mid-season and end-season. Repeated-measures analysis of variance was used to examine indicators change during league season. **Results:** Significant decrease in SOD ($p = 0.001$), TAC ($p = 0.043$) and anaerobic power ($p \leq 0.048$) and significant increase in GPx ($p = 0.001$), ALT ($p = 0.022$) was observed from pre-season to the end of the season. LDH levels showed a significant increase in the mid-season compared to the pre-season ($p = 0.042$) and

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a significant decrease at the end of the season compared to the mid-season(p = 0.014). However, no significant changes were observed in other indices during the competition season(p ≥ 0.05). **Conclusions:** It is suggested that some physical and physiological fatigue markers ar increase during mid-season. Therefore, beach soccer players may be monitored continuously during the competition season in order to be able to provide the best training, nutrition and recovery systems for performance optimization. Coaches may use the interplay between biomarker alterations and physical performance changes to better manage workload and monitor fatigue during beach soccer training and competition.

***Keywords**:* Inflammation, Oxidative stress, Physical Performance, Beach soccer.

INTRODUCTION

Beach soccer has been considered a sport since the 1990s and FIFA has promoted it through qualifying and international program. The first tournament was hold in Miami Beach and the first beach soccer world cup was hosted by Brazil in 1995 (Leite, 2016a). Beach soccer has certain differences in size and type of field and numbers of players from that of official soccer and futsal. Activity intensity in beach soccer is altering between high (90% of max HR) and low to moderate (50% to 70% of max HR) that's why a great deal of energy is needed for the game in additive ،playing on sand apart from increasing the intensity of acivity itself require high physical and muscle strength (Castellano & Casamichana, 2010). The sandy surface slows down the player during the game and makes it difficult to move the unstable and low resistance surface of sand put an extra load on ankle joint and all a consequence it cause move effort at a moment which necessarily causes more bending of the pelvis and knees joints (Leite, 2016b). At the same line, the beach soccer players experience a higher intensity of exercise achieve the same speed of a grass and futsal players. On average, a beach soccer player approximately run 100 m/min. The ratio of high speed to low-speed activity is 1:1.4 (for every minute of "low speed" there is 1.4 minutes of "high speed"), which indicates the very high intensity of beach soccer playing (Castellano & Casamichana, 2010). Also, during beach soccer season, the team play every weekend and totally each team plays about 30 league and cup games. (Lastella et al., 2020).

During a beach soccer season, muscle tissue may be damaged by multiple metabolic and mechanical factors. Serum levels of protein and enzymes of skeletal muscle are indicators of muscle tissue function and could be changed in both pathological and physiological condition (Miri et al., 2013). Apoptosis in muscle tissue following strenuous exercise may also occur with increased oxidative stress. Therefore, total antioxidant status (TAC) along with other markers such as malondialdehyde (MDA), glutathione peroxidase (GPX), superoxide dismutase (SOD) and others can be used to assess muscle stress levels (MIRANDA NETO et al., 2020). Multiple indicators provide a complete picture of muscle condition that's why Barbacio et al. (2010) recommended using more indicators to better evaluate muscle condition. In this context, it has been shown that muscle damage after exercise leads to a significant rise in same level of muscle cell proteins in the blood (Muazu Musa et al., 2019). It has been shown that long term exercise cause a transient increase in serum level of biochemical markers of muscle damage such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and lactate dehydrogenase (LDH) (Brancaccio, Lippi, & Maffulli, 2010). Also, In this regard, over the last decades the cellular immune infammation markers neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-infammation index (SII=NLR×platelets) have emerged in clinical context as markers of disease-related infammation and are now widely appreciated due to their integrative character (Joisten et al., 2019). Transferring these clinically established inflammation markers into exercise physiology seems highly benefcial, especially due to the low temporal, financial and infrastructural resources needed for assessment and calculation (Walzik, Joisten, Zacher, & Zimmer, 2021). Despite sparse evidence, multiple investigations revealed responsiveness of the markers to acute and chronic exercise, thereby opening promising avenues in the feld of exercise physiology. In performance settings, they might help to infer information for exercise programming by refecting exercise strain and recovery status or periods of overtraining and increased infection risk (Schlagheck et al., 2020). In health settings, application involves the depiction of anti-infammatory efects of chronic exercise in patients exhibiting chronic inflammation (Walzik et al., 2021).

On the other hand, beach soccer players have special physical demand for blasting movement, speed, agility, balance, body stability, flexibility and endurance (Castellano & Casamichana, 2010; Leite, 2016a, 2016b). Aerobic capacity has a significant effect on parameters such as total time spent for strenuous activity amount of sprinting and number of hit to the ball during the match (Brancaccio et al., 2010). Sprinting, acceleration, deceleration, rotation, jumping, hitting the ball and stopping opponent are designed physical activity during the race (Ramírez-Vélez et al., 2017), and performing them depend on the explosive muscle strength the player. It has been shown that exercising on the sand improves jumping, sprinting, and it also reduces muscle soreness. Exercising on the sand prevents the re-use of elastic energy and energy loss due to slipping of the foot during introverted operation (Leite, 2016a). Also, much of the energy produced by the muscle may not be returned (ie. energy will be absorbed) and as a result, muscle function in the sand will be less compared to a hard surface. Thus, mechanical and subsequent physiological stress during beach soccer season may reduce player performance (Binnie, Dawson, Pinnington, Landers, & Peeling, 2014). In fact, the metabolic and mechanical stress exerted on beach soccer players may lead to physical diserde, that might be growing over a long period of time (Mirzaei, Asghar Norasteh, Saez de Villarreal, & Asadi, 2014). So far, the level of hormonal, biochemical and inflammatory markers of beach soccer players during the tournament haven't been studied yet. Therefor, the aim of this study was to monitoring the resting level of heamatological, inflammatory, biochemical and oxidative stress markers as wellas physical performance of beach soccer players during the 2019-2020 Iran beach soccer premier league.

METHOD

Selection and Description of Participants

This study was a quasi-experimental study with repeated measures design. The study was conducted according to the Declaration of Helsinki and approved by research ethics committee of Shahrekord University. The statistical population of this study was all beach soccer players of the Premier League 2019-2020, of which 13 male palyers (age 24.64 ± 4.01, weight 75.8 ± 8.15, height 181.00 ± 5.17, body mass index 22.76 ± 2.36 and VO2max 38.60 ±5.48) were selected through convenience sampling. Inclusion criteria included regular playing experience in the previous league, at least 3 years of official activity in beach soccer and the exclusion criteria were as followed any disease, taking injury, medication or deity complement special diet.

After the essential coordination with the club management and explaining the purpose of the study, written informed consent was obtained from subjects to participate in the study. A past medical history and previous injuries were recorded using a medical screeing questionnaire. All measurements, including anthropometric measurements, physical function and blood samples were repeated at three times at pre-season (T1), mid-season (T2) and the end-season (T3). At least 48h before the assessment subject while being fasted prepared for morning blood sampling followed by an overnight fasting. Blood sampling were performed in the morning, while the subject were fasted since the night before blood samples were taken from the anterior brachial vein by laboratory expert at rest and in a sitting position after centrifugation and serum separation, blood sample is stored at -80° C for analyzing.

After blood sampling, the subjects ate the same breakfast with the same calories and volume, and one hour after breakfast, the body composition indices including height, weight, waist and hips (WHR) ratio of the players were measured. Players height was measured by a wall mounted height gauge with a sensitivity of 0.1 cm. This device is mounted vertically on the wall and usually includes a ruler and a movable horizontal bar. To determine the height, the bar is placed just above the head so that the number of the ruler is where the bar is located record the height of the person. Without shoes and with least clothing subject weight were measured by a digital scale with a sensitivity of 0.01 kg. Waist circumference was measured at the level of the navel, without upper body clothing. The player hip circumference was also measured at the level of the iliac spine using tape measure. Then, anaerobic performance functional tests (RAST test) (Kalva-Filho et al., 2013), and aerobic performance tests (shuttle run test) (Stojanovic et al., 2016) were performed.

Biochemical measurement

To measure the levels of TAC indices, complete antioxidant kit made in Iran, BYREX Fars Company, unit of measurement of micromole / liter, durability at 2 to 8 ° C, SOD superoxide dismutase kit made in Iran, BYREX Fars Company, Branch International unit measurement / ml, shelf life at 2 to 8 ° C, GPX glutathione peroxidase kit made in Iran, BYREX Fars company, unit of measurement international unit / ml, shelf life at 2 to 8 ° C, LDH Lactate dehydrogenase kit made in Iran, Pars Azmoun company, international unit of measurement / liter, shelf life at minus 20 ° C for about 6 weeks, spectrophotometric method was used. Also, ALT levels of alanine aminotransferase kit, made in Iran, Pars Azmoun company, international unit / liter unit, shelf life at 2 to 8 ° C for one week and 15 to 25 ° C in one day, AST aspartate aminotransferase kit Made in Iran, Pars Azmoun Company, International Unit / Liter, Shelf life at 2 to 8 ° C for one week and 15 to 25 ° C in one day, CPK Creatine Phosphokinase Kit Made in Iran, Pars Azmoun Company, Unit of measurement mg / dl, shelf life was measured at 2 to 8 ° C by auto-analysis.

Measurements of hematology indices were analyzed using Counter Cell device. NLR, SII and PLR were calculated from haemograms using an automated haematology analyser (Sysmex XN-1000, Norderstedt, Germany). SII was calculated as platelets×neutrophils×lymphocytes−1.

Anaerobic power measurement

Anaerobic capacity was assessed using RAST test. RAST test (anaerobic sprinting test based on continuous running) is one of the valid tests to evaluate the repetitive anaerobic power with maximum intensity including 6 repetitions of sprinting over 30 meters with maximum intensity with a passive rest interval of 25 seconds between each Repetition. The validity and reliability of this test have been confirmed by WINGITE anaerobic power test in domestic and foreign researches. There is a significant correlation between RAST test and WINGITE anaerobic test about maximum, mean and minimum power (Kalva-Filho et al., 2013). In this study, a 30-meter path was identified inside the hall and the start and end lines were marked with obstacles and light-colored tape. In order to eliminate the reaction time, 65 cm before the starting line was marked and the subjects were asked to start running at max power hearing the first referee whistle. The second referee stationed at the finish line, in coordination with the first referee stationed at the starting line, activates the digital chronometer as soon as the subject crosses the starting point and the raise of the first referee flog, and stops the chronometer at the same time as the subject crosses the finish line and with the rise of second referee. At the end, if there is a time difference between to referee chronometer, the average of the two times is calculated and the some of the means is recorded as the final record for all 6 paths to get better result of RAST test, subject was asked to perform every repetition with their maximum power. Finally, peak power, minimum power, average power and also fatigue index were calculated according to the test instructions (Faramarzi, (2010)):

Force = weight × distance^2 / time^3

Force = Weight × 1225 ÷ Time^3

Power peak = weight × (35)^3 / (fastest repetition time)^3

Minimum anaerobic power = weight × (35)^3 / (slowest repetition time)^3

Average anaerobic power = total of 6 repetitions of two speeds / 6

Fatigue index = (minimum power _ peak power) / total time 6 repetitions

Aerobic power measurement

Endurance performance was evaluated by shuttle run test. This test consists of 21 levels, each of which has 10 time periods, and the subjects in each period must run a distance of 20 meters in a reciprocal way. Performing the test requires a space of at least 20 meters in length and a device for playing songs. Subjects are stationed at the end of one of the marked 20-meter lines. With hearing the first beep, they move at a slow speed towards the end of the 20-meter path so that when they hear the second beep, they reach the end of the 20-meter path. If a person reaches the end of the 20-meter path before hearing the second beep, he must wait until the next beep is heard, and then return to the other side and the 20-meter line. If the subject is not able to reach the 20-meter line before hearing the horn, he will be asked not to continue the test after two consecutive rounds or three non-consecutive rounds. The last record that has reached the 20-meter line for him (the number of complete round trips) is immediately recorded on the sheet. In order to evaluate the

VO2max of the subjects, the following formula was used (Ramírez-Vélez et al., 2017):
Maximum oxygen consumption (Ml / min / kg) =
31.025+3.238×speed×age+0.1536×speed×age
In this formula, age, year and speed, the last speed of the test is considered. In the Shuttle Run test, the first level is running at a speed of 8.5 km / h, and in the next levels, the running speed is increased by 0.5 km / min per level (Gibson, Wagner, & Heyward, 2018).

Statistical analysis

Shapiro-Wilk test was used to investigate the natural distribution of indices and Mauchlys test was used to examine the homogeneity of variances. Also, Repeated measures analysis of variance test was used to examine the changes in research variables in the pre-season, half-season and end of the season of beach soccer premier league matches. If the test was significant, Bonferroni post-hoc test was used. SPSS software version 25 was used. A significance level of $P \leq 0.05$ was considered. Graph Pad Prism-7 software was used to draw the graphs.

RESULTS

Table 1 shows the changes in anthropometric indices during the tournament season. There was no significant change in the indices of height, weight ($p = 0.283$), body mass index ($p = 0.596$) and WHR ratio ($p = 0.875$) during the tournament season.

As well as, change in blood CBC indicators shows in Table 2. The result showed significant difference between pre-season, mid- season and end- season at HCT (p=0.010), MCV (p=0.034), MCHC (p=0.001), PLT (p=0.015), PLR (p=0.011), NLR (p=0.019), and SII (p=0.25), but not ither indices ($p \geq 0.05$). Post hoc comparisons showed significant increase in HCT (p=0.008) at mid- season compared to pre-season. Also, there were significant deccrease in MCHC at mid-season (p=0.001) and end- season (p=0.001) compared to pre- season. MCV (p=0.041) and PLT (p=0.016) significantly increased at end- season compared to pre season (p=0.016). PLR and SII significantly indresed at mid-season (resoectively, p=0.013, p=0.012) and end-season (resoectively, p=0.026, p=0.042) compared to pre-season. NLR significantly increased at mid-season compared to pre-season (p=0.022) and significantly decreased at end-season compared to mid-season (p=0.019).

Table 1: Anthropometric indices of alite beach soccer players during league.

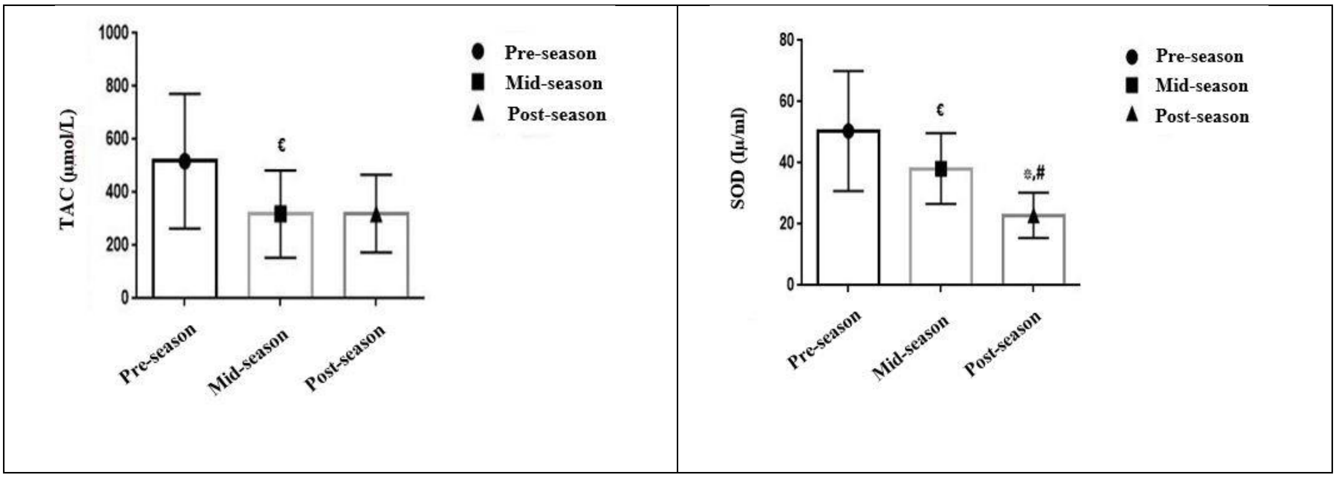
Indicator	Pre- season	Middle of the season	End of the season	F	P-value
Height (cm)	181.00±5.17	181.00±5.17	181.00±5.17	------	------
Weight (kg)	75.08±8.15	70.75±5.59	71.30±5.35	1.324	0.283
BMI (kg/m^2)	22.76±2.36	21.81±1.98	22.11±1.95	0.528	0.596
WHR	0.825±0.039	0.829±0.042	0.820±0.029	0.134	0.875

Table 2. Haematological parameters of alite beach soccer player during league.

FACTOR	Pre- season	Mid- season	End- season	F,T	P-value
WBC	5.52±1.03	5.60±1.35	5.39±0.84	0.106	0.900
RBC	5.36±0.41	5.47±0.40	5.17±0.63	1.150	0.329
HBG	15.95±0.84	16.15±0.91	15.33±0.94	2.529	0.096
HCT	46.22±1.99	49.32±2.43*	47.86±2.57	5.290	0.010
MCV	86.46±3.92	90.32±4.92	93.41±8.68#	3.758	0.034
MCH	29.85±1.57	29.57±1.97	29.92±2.73	0.088	0.916
MCHC	34.53±0.78	32.75±0.85 $	31.90±1.37 &	19.81	0.001
PLT	211.83±47.04	250.33±45.43	265.09±34.14 #	4.801	0.015
LYM#	2.20±0.59	2.08±0.45	2.20±0.56	0.184	0.833
NEUT#	2.70±0.55	2.94±1.10	2.60±0.40	0.557	0.579
PLR	96.28±11.15	120.35±12.47*	120.49±10.22#	5.296	0.011
NLR	1.23±0.43	1.41±0.45*	1.18±0.38 &	4.318	0.019
SII	259.97±112.55	353.83±125.36*	313.29±108.93#	3.799	0.025

*indicated significant increase in mid-season compared to pre-season. #indicated significant increase in end-season compared to pre-season. $ indicated significant decreased in mid-season compared to pre-season, & indicated significant decreased in end-season compared to pre-season.

The results of oxidative stress indices during the tournament season showed a significant change in the TAC (p = 0.021). As a result of post-hoc test, a significant decrease in TAC was observed in mid-season compared to the pre-season (p = 0.043). Also, a significant change in SOD was observed during the season (p = 0.001). As a result of post-hoc test, a significant decrease in SOD was observed in mid-season compared to the pre-season (p = 0.009) and at the end-season compared to the pre-season (p = 0.001) and mid-season (p = 0.003). In addition, a significant change in GPX levels was observed during the season (p = 0.001). The results of post-hoc test showed a significant increase in GPX in the mid-season (p = 0.001) and the end-season (p = 0.001) compared to the pre-season. However, there was no significant changes for MDA during the season (p = 0.192) (fig. 1).



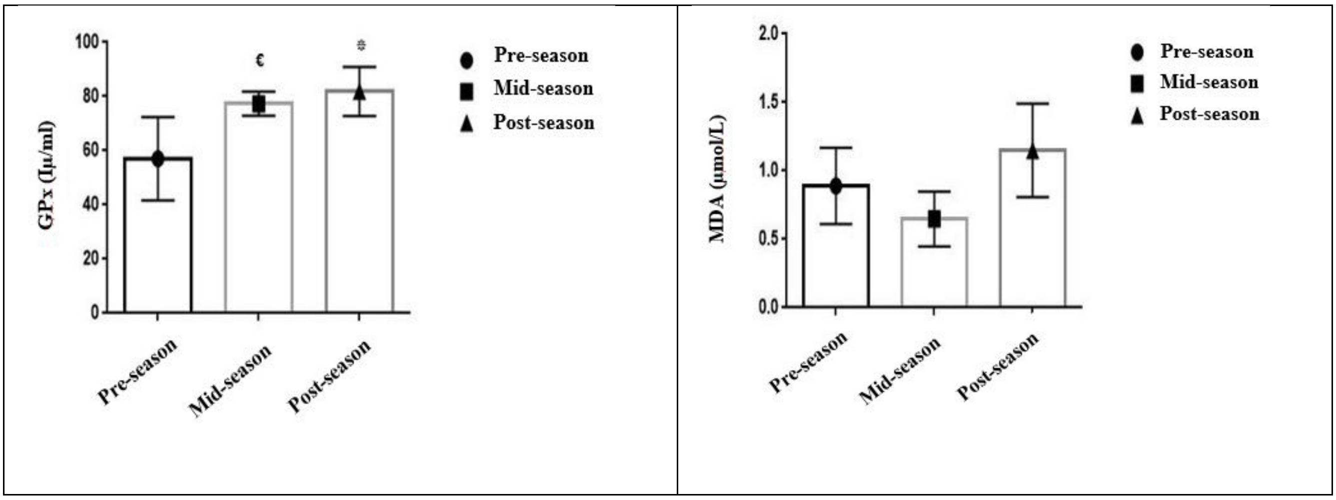


Figure 1: Changes in the oxidative stress indices of beach soccer players during the Premier League season. € Significant change in half of the season compared to pre-season. *Significant change at the end-season compared to the pre-season. #Significant change at the end-season compared to the mid-season.

Also, the results showed a significant change in ALT ($p = 0.043$). As a result of post-hoc test, a significant increase in ALT level was observed at the end-season compared to the pre-season ($p = 0.022$). A significant change in LDH levels was observed during the season ($p = 0.009$). As a result of post-hoc test, a significant increase in LDH levels was observed in the mid-season compared to the pre-season ($p = 0.042$) and a significant decrease at the end-season compared to the pre-season ($p = 0.014$). However, there were no significant changes for AST ($p = 0.500$) and CPK ($p = 0.151$) levels during the season (fig.2).

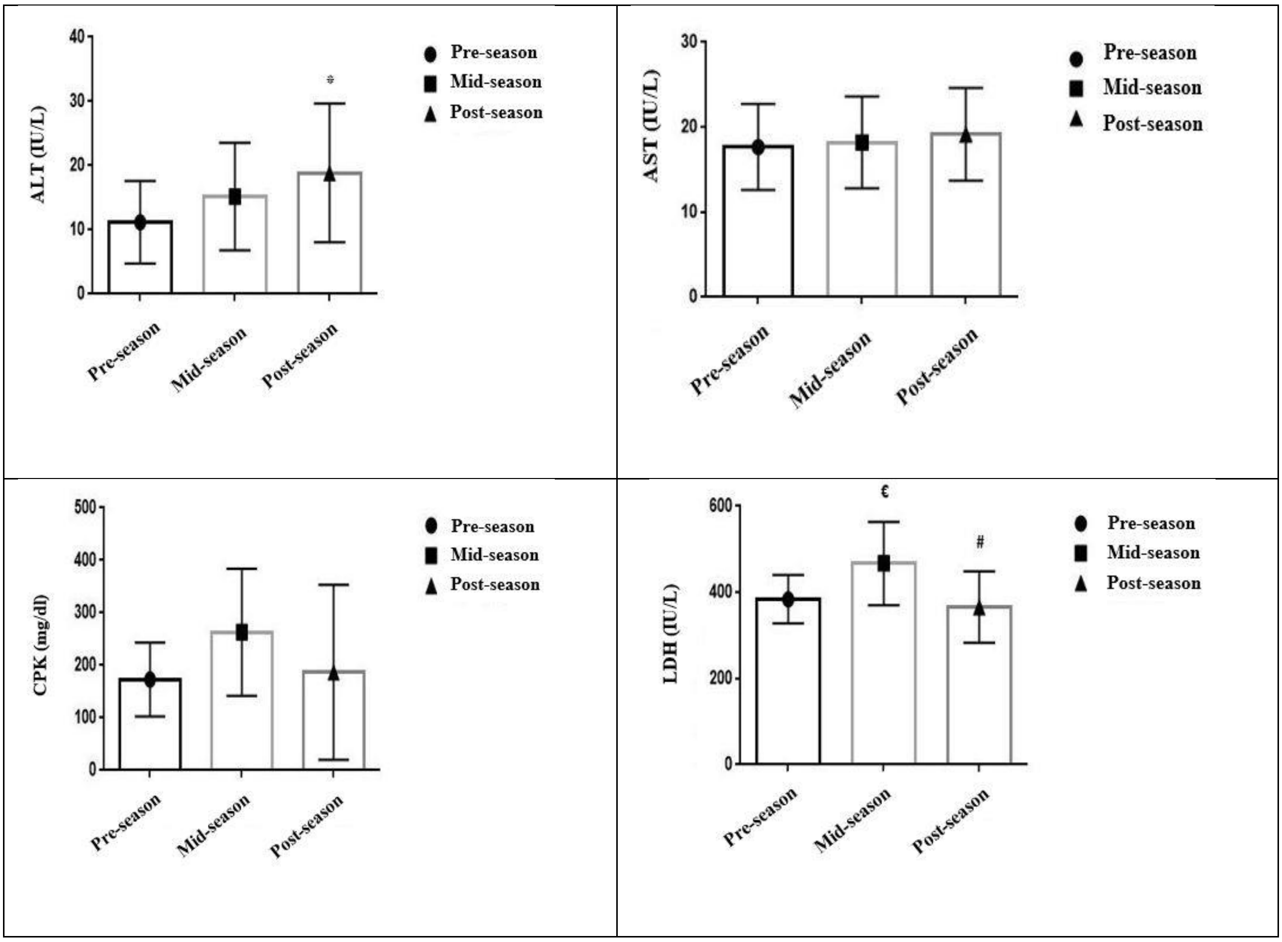


Figure 2: Changes in the inflammatory indices of beach soccer players during the Premier League season. € Significant change in mid-season compared to pre-season. * Significant change at the end-season compared to the pre-season. # Significant change at the end-season compared to the mid-season.

In addition, the results showed no significant change in aerobic power ($p = 0.117$). However, significant changes were observed in the minimum anaerobic power ($p = 0.002$), maximum anaerobic power ($p = 0.001$) and average anaerobic power ($p = 0.001$). As a result of post-hoc test, there was a significant decrease in minimum, maximum and average power in mid-season (respectively, $p = 0.003$, $p = 0.001$, $p = 0.001$) and the end-season (respectively, p = 0.020, p = 0.048, P = 0.010) was observed in comparison with the pre-season (fig.3).

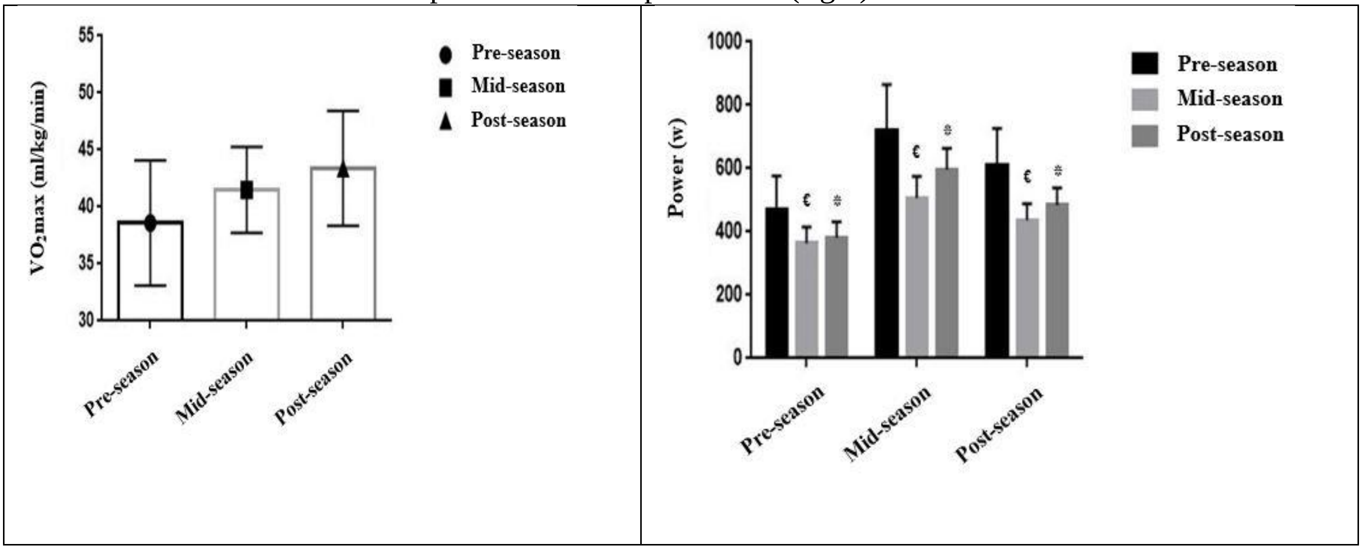


Figure 3: Changes in the aerobic and anaerobic capacity of beach soccer players during the Premier League season. € Significant change in mid-season compared to pre-season. * Significant change at the end-season compared to the pre-season.

DISCUSSION

The results of the present study show a significant decrease in TAC and SOD, a significant increase in GPX and a non-significant increase in MDA as indicators of oxidative stress during the competition season from pre-season to the end of the season. Also, in the case of inflammatory indices, the results showed a significant increase in ALT levels of beach soccer players at the end of the season compared to pre-season. The LDH index also showed a significant increase in the middle of the season and a significant decrease at the end of the season. There wasn't a significant difference during the season for other inflammatory markers such as CPK and AST.

Interestingly, we found significant increase in HCT at mid-season compared to pre-season, in MCHC at mid-season and end- season compared to pre- season. For MCV and PLT we found significant increase at end- season compared to pre season. PLR and SII significantly indresed at mid-season and end-season compared to pre-season. NLR significantly increased at mid-season compared to pre-season and significantly decreased at end-season compared to mid-season. In addition, a non-significant increase in aerobic capacity and a significant decrease in anaerobic capacity were observed from pre-season to the end of the season.

Changes in antioxidants depend not only on the type, duration, and intensity of exercise but also on the state of antioxidant defense from one organ to another, as well as the amount of these enzymes under various conditions (ERDEMLI, TaySi, & TaraKÇioGlu, 2018). Oxidative stress caused by physical activity under the influence of factors such as personal health status, age, sex, race, genetics, level of physical fitness, individual differences, different tissue responses, muscle fibers and tendons, and various types and reducing the intake of antioxidants is in the daily diet of people (Aktaş & Çelik, 2019). Also, the variety of lipid peroxidation indices and their measurement methods and sensitivities in different studies can lead to inconsistent results (Volek et al., 2016).

In the present study, a significant decrease in TAC and SOD was observed during the season from pre-season to the end of the season, and on the other hand, a significant increase in GPX and a non-significant increase in MDA was observed. Numerous mechanisms have been proposed for the response of antioxidant enzymes to exercise. It has been well shown that the production of free radicals increases following intense physical activity, especially in soccer (Saidi et al., 2021). In most sports exercises, including endurance and anaerobic exercises, the amount of oxidative and antioxidant indices is increased in order to prevent the activity of free radicals (Kawamura & Muraoka, 2018). Thus, the decrease in TAC and SOD seems to be due to their greater use against free radicals and on the other hand due to the restriction of these enzymes by reactive oxygen species. Also, increased GPX and non-significant change in MDA can be related to the adaptations in elite athletes (Kawamura & Muraoka, 2018). On the other hand, MDA increases after performing anaerobic and intense endurance activities that may affect the lipid peroxidation of red blood cell membranes. Following the increase of oxidative stress in the body, the cell defense system is stimulated and activated like antioxidant enzymes to deal with the oxidative stress produced (Leaf, Kleinman, Hamilton, & Barstow, 1997). Frequent and continuous exposure to exercise stress and high-intensity exercise can be one of the reasons for the increase in malondialdehyde, which can be a good reason for this. In addition, endurance activity increases the activity of both SOD and GPX erythrocytes in skeletal muscle, and high-intensity exercise is likely to play a greater role in regulating the activity of superoxide dismutase and glutathione peroxidase enzymes than low-intensity exercise. Because elite athletes cannot abstain from training for more than 24 hours during the in-season period, suggesting that additional recovery may be needed for full recuperation of the athletes (Fatouros et al., 2010).

Exhaustive exercise decreases liver and muscle GSH concentration probably because acute exercise decreases cystine in the liver, which is the rate-limiting precursor for glutathione synthesis (Somani, Frank, & Rybak, 1995). Exercise-induced decreases in GSH and cystine may account for the increased MDA and PC seen in the present study. MDA and PC are negatively correlated with GSH and cystine following acute exercise, respectively (Liu et al., 2000).

Increased GPX activity seen after a soccer game in the present study may be dependent on scavenger cell migration into damaged muscle tissue. Macrophage migration in damaged muscle fibers has been observed 24 to 72 hours after exercise (Uchiyama, Tsukamoto, Yoshimura, & Tamaki, 2006). Exhaustive exercise increased GPX activity by 87% in lymphocytes, indicating blood cell damage. Soccer engages large muscle groups during intermittent types of activity at varying intensities and types of muscular contractions. Catalase increased only immediately post-game in agreement with previous findings (Sureda et al., 2005). However, catalase has no apparent function in serum because it is an intracellular enzyme. Therefore, its increased activity after exercise probably indicates increased damage of erythrocyte membranes, which results in its increased leakage into the circulation (Fatouros et al., 2010).

Regarding the inflammatory indices, the results of the present study are in line with the results of MELO et al. (2017), which showed that moderate-intensity soccer training does not cause a significant change in AST and CPK, but causes a significant increase in ALT (Mello et al., 2017). The study of Gomez et al. (2018), which showed that football training does not cause significant changes in AST and CPK levels (Viana-Gomes et al., 2018), that is consistent with our result. Muscle activity can change the blood concentration of some cellular enzymes such as CK and LDH (Leite, 2016b). In most studies, exercise has been shown to increase plasma CPK and LDH, so that more intense exercise leads to a greater increase in enzyme activity, and if these exercises are accompanied by increased muscle damage, the amount of enzyme secretion it will intensify into the blood (Vafaee, Soori, Hedayati, Ainy, & Hatamabadi, 2019). Another point is the duration of exercise so that in studies that examined one session or one race, there is generally an increase in the levels of inflammatory factors (Ghahfarrokhi, Habibi, & Nasab, 2019; Mardaniyan Ghahfarrokhi, Habibi, & Rezaei Nasab, 2018; Vafaee et al., 2019). In contrast, long-term exercise seems to lead to beneficial adaptations and shows a decrease or no change in inflammatory markers (Beavers, Brinkley, & Nicklas, 2010). Studies have shown that participating in intense and increasing physical activities causes delayed damage to the muscle fiber membrane. If physical activities also include eccentric areas, such as some beach soccer moves, the damage may be more severe (Castellano & Casamichana, 2010). The excessive mechanical force produced during exercise, especially intense intermittent exercise, can destroy or break down the structural proteins of muscle fibers and connective tissue (Leite, 2016b). Muscle injury usually reduces athletic performance; therefore, reducing the rate of muscle damage and its aftermath, subsequent protein breakdown associated with an intense and long-term activity, can be beneficial (Withee et al., 2017). Recently, there is evidence which supports the application results of sand in a sports venue. These studies showed that for a specific training session, the use of sand in front of solid training ground surfaces can create more relative training intensity without causing muscle damage in the next day (24 hours after exercise) (Meckel, Doron, Eliakim, & Eliakim, 2018). In addition, recent studies have shown well that running on sand requires 1.2 to 1.6 times the cost of running on solid surfaces such as grass at similar speeds. Several factors may contribute to this increase, including decreased elastic energy recovery, decreased tendon efficiency, and increased workload. In addition, it has been shown that due to the increased stabilizing needs around the ankle joints, knee joints, and hip joints, the electromyography activation of the lower limb muscles when running on sand is significantly higher than on grass (João Renato Silva et al., 2014). According to this point, when the foot contact with the surface is coordination, the lower level of muscle damage caused by exercise was recorded after sand training, compared to firm levels muscle. As a result, it reduces the degree of pain and reduces muscle soreness in the next day (João R Silva et al., 2011). Despite a 1.2 to 1.6-fold increase in exercise intensity (based on HRmax) on sand, the concentration of C-reactive protein did not show a significant increase compared to grass. These results may in fact indicate that at the same training intensity muscle damage and inflammation are far lower in activity on the sand surface because the C-reactive protein is a known indicator of muscle damage and systemic inflammation (Filaire, Lac, & Pequignot, 2003). A common belief among researchers is that regular exercise prevents damage and increases enzymes. Some studies have shown that long-term exercise reduces damage and CPK levels, and in the same it has been mentioned that different types of exercise cause different effects of adaptation and regeneration with serum enzymes and lowering CPK is a response to the stimulus of permanent exercise (Porsesh, Habibi, Ahmadi Barati, & Mardaniyan Ghahfarokhi, 2018). DOMS, CK, and leukocyte elevations reveal a muscle damage response following a soccer game. Muscle microtrauma seen following a soccer game may be attributed, at least partially, to intermittent repetitions of intense eccentric actions such as running, jumping, and rapid acceleration and deceleration movements (e.g., hamstrings act eccentrically to decelerate hip flexion and knee extension during running's landing phase) that represent an integral part of this sport (Fatouros et al., 2010). Eccentric contractions induce higher tension per cross-sectional area of active muscle mass compared to concentric actions, resulting in significant structural muscle damage. Similar findings have been shown for other field sports that involve prolonged high-intensity shuttle running (rugby and hockey) (Thompson, Nicholas, & Williams, 1999).

The results of the present study on heamatological indices significant increase in HCT at mid-season compared to pre-season, in MCHC at mid-season and end- season compared to pre- season. For MCV and PLT we found significant increase at end- season compared to pre season. Also, functional inflammatory indices PLR and SII significantly indresed at mid-season and end-season compared to pre-season. NLR significantly increased at mid-season compared to pre-season and significantly decreased at end-season compared to mid-season. To the best of our knowledge, no study has examined the changes in hematologic markers and functional inflammatory markers based on white blood cells. Most studies have examined the acute changes in these indicators following training methods (Schlagheck et al., 2020), and different training intensities (Pourrahim Ghouroghchi & Pahlevani, 2019). In line with our result, studies showed that after acute aerobic and resistance training the levels of NLR, PLR and SII significantly increased in healthy men. However, the increase was more pronounced in the aerobic group (Schlagheck et al., 2020). Another study showed that after 30 minutes aqua cycling NLR, PLR and SII significantly increased and after 48 hours of recovery, return to resting levels (Joisten et al., 2019). Also, Exercise intensity has been shown to be an important factor in increasing the levels of inflammatory markers associated with white blood cells as well as the hematological index (Pourrahim Ghouroghchi & Pahlevani, 2019). Reently, a review study examined the markers of NLR, PLR, and SII cellular immune inflammation in exercise physiology. In response to acute exercise, markers of cellular immune inflammation may be useful for showing exercise stress and recovery processes in competitive exercise. Also, in chronic sports interventions, they may portray periods of overexertion and an increased risk of infection in athletes (Walzik et al., 2021). The results of the present study are consistent with limited previous studies showing that cellular inflammatory markers can be evaluated in sports monitoring. Examining these indicators during the competition season as a cheap, simple, accessible but reliable tool can provide a good view of the athlete's inflammatory and infectious status.

The results of the present study on performance indices showed a non-significant increase in aerobic capacity and a significant decrease in anaerobic capacity from pre-season to the end of the season. Most sports scientists and coaches believe that high levels of aerobic fitness are a prerequisite for high anaerobic performance during long-term intermittent activity. In addition, athletes in intermittent sports often use long endurance runs to improve aerobic capacity during pre-season training (Bangsbo, Iaia, & Krustrup, 2007). Speed and power are very important in beach soccer, especially when attacking, beating and returning to defense. In addition to having higher power, as we get closer to the end of a race, it can help to better execute the shooting technique and affect the outcome of the race. Knowledge of physiological and motor characteristics opens a new window on the condition of individuals for football coaches, supervisors and those involved (football and beach) (Bangsbo et al., 2007). Beach soccer players must have the necessary adaptations to repeat the fast-paced activities supported by anaerobic glycolysis; because high anaerobic capacity is very important in football (Murtagh et al., 2018). Today, the success of football teams in various competitions is due to their good physical condition. Research evidence shows that new competitions are held at higher levels than in the past. So,the current players in a match run almost 10% more than the players before them (Smpokos, Mourikis, Tsikakis, Katsikostas, & Linardakis, 2020). Therefore, body size and composition, aerobic and anaerobic fitness of football players are the most important factors that have changed (Smpokos et al., 2020). In some intermediate studies, the maximum oxygen uptake for endurance capacity and vertical jump was maintained as an indicator of anaerobic power without lactic acid during the competition season. The present study is consistent because the amount of aerobic power in the present study has not changed significantly (Silvestre, West, Maresh, & Kraemer, 2006). Also, the findings of Hadi Miri et al. (2013) showed that there is no significant difference between the aerobic capacity of the national grass football team and beach soccer (Miri et al., 2013). In Impliceri et al.'s (2008) study, 37 amateur soccer players performed three weeks of plyometric training four times a week on a surface of artificial sand or turf. Both training interventions in sand and grass resulted in similar improvements in two-speed performance (Impellizzeri et al., 2008).

CONCLUSIONS

The results of the present study for the first time indicates that cellular inflammatory markers can be used for monitoring of elite soccer players in the field of inflammatory and infectious conditions. Also, this study indicate an increase in inflammation, a decrease in antioxidant status and a decrease in the anaerobic capacity of beach soccer players during the tournament season. Therefore, it is necessary for athletes to be continuously evaluated in terms of hormonal, inflammatory, oxidative and functional indicators during the tournament season in order to plan the best training, nutrition and recovery systems for athletes. Coaches may use the interplay between biomarker alterations and physical performance changes to better manage workload and monitor fatigue during beach soccer training and competition.

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Conflict of Interests
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REFERENCES

Aktaş, Y., & Çelik, H. (2019). Investigation of Oxidative Stress Levels of Male Football Players. *Journal of Education and Training Studies, 7*(8).

Bangsbo, J., Iaia, F. M., & Krustrup, P. (2007). Metabolic response and fatigue in soccer. *International journal of sports physiology and performance, 2*(2), 111-127.

Beavers, K. M., Brinkley, T. E., & Nicklas, B. J. (2010). Effect of exercise training on chronic inflammation. *Clinica chimica acta, 411*(11-12), 785-793.

Binnie, M. J., Dawson, B., Pinnington, H., Landers, G., & Peeling, P. (2014). Sand training: a review of current research and practical applications. *Journal of sports sciences, 32*(1), 8-15.

Brancaccio, P., Lippi, G., & Maffulli, N. (2010). Biochemical markers of muscular damage. *Clinical chemistry and laboratory medicine, 48*(6), 757-767.

Castellano, J., & Casamichana, D. (2010). Heart rate and motion analysis by GPS in beach soccer. *Journal of sports science & medicine, 9*(1), 98.

ERDEMLI, H. K., TaySi, S., & TaraKÇioGlu, M. (2018). The acute effects of interval exercise on oxidative stress and antioxidant status in volleyball players. *The Journal of Sports Medicine and physical fitness, 58*(4), 421-427.

Faramarzi, M., Gharakhanlou, R., & Chuobineh, S. (2010). Physical fitness profile of Iranian elite soccer players. *Olympic, 18*(1), 127-140.

Fatouros, I. G., Chatzinikolaou, A., Douroudos, I. I., Nikolaidis, M. G., Kyparos, A., Margonis, K., . . . Jamurtas, A. Z. (2010). Time-Course of Changes in Oxidative Stress and Antioxidant Status Responses Following a Soccer Game. *The Journal of Strength & Conditioning Research, 24*(12), 3278-3286. doi:10.1519/JSC.0b013e3181b60444

Filaire, E., Lac, G., & Pequignot, J.-M. (2003). Biological, hormonal, and psychological parameters in professional soccer players throughout a competitive season. *Perceptual and Motor Skills, 97*(3_suppl), 1061-1072.

Ghahfarrokhi, M. M., Habibi, A., & Nasab, H. R. (2019). Effect of acute aerobic exercise in different times of day on iron status and hematological factors in professional football players. *Journal of Shahrekord University of Medical Sciences, 21*(3), 125-130.

Gibson, A. L., Wagner, D., & Heyward, V. (2018). *Advanced Fitness Assessment and Exercise Prescription, 8E*: Human kinetics.

Impellizzeri, F. M., Rampinini, E., Castagna, C., Martino, F., Fiorini, S., & Wisloff, U. (2008). Effect of plyometric training on sand versus grass on muscle soreness and jumping and sprinting ability in soccer players. *British journal of sports medicine, 42*(1), 42-46.

Joisten, N., Walzik, D., Schenk, A., Bloch, W., Zimmer, P., & Wahl, P. (2019). Aqua cycling for immunological recovery after intensive, eccentric exercise. *European journal of applied physiology, 119*(6), 1369-1375.

Kalva-Filho, C. A., Loures, J. P., Franco, V. H., Kaminagakura, E. I., Zagatto, A. M., & Papoti, M. (2013). Comparação da potência anaeróbia mensurada pelo teste de Rast em diferentes condições de calçado e superfícies. *Revista Brasileira de Medicina do Esporte, 19*, 139-142.

Kawamura, T., & Muraoka, I. (2018). Exercise-induced oxidative stress and the effects of antioxidant intake from a physiological viewpoint. *Antioxidants, 7*(9), 119.

Lastella, M., Escobar, L., Lovell, G. P., Scanlan, A., Ellison, E., McGowan, L., & Guy, J. H. (2020). An exploration of goal scoring strategies in an elite beach soccer tournament. *Science and Medicine in Football, 4*(3), 192-195.

Leaf, D. A., Kleinman, M. T., Hamilton, M., & Barstow, T. J. (1997). The effect of exercise intensity on lipid peroxidation. *Medicine and science in sports and exercise, 29*(8), 1036-1039.

Leite, W. S. (2016a). Beach soccer: Analysis of the goals scored and its relation to the game physiology. *International Journal of Physical Education, Fitness and Sports, 5*(1), 12-17.

Leite, W. S. (2016b). Physiological demands in football, futsal and beach soccer: a brief review. *European Journal of Physical Education and Sport Science*.

Liu, J., Yeo, H. C., Overvik-Douki, E., Hagen, T., Doniger, S. J., Chu, D. W., . . . Ames, B. N. (2000). Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *Journal of Applied Physiology, 89*(1), 21-28.

Mardaniyan Ghahfarrokhi, M., Habibi, A., & Rezaei Nasab, H. (2018). Effect of acute aerobic activity after a period of taking vitamin C supplement on Iron status and hematological indices of professional football players. *Research in Sport Medicine and Technology, 16*(16), 29-38.

Meckel, Y., Doron, O., Eliakim, E., & Eliakim, A. (2018). Seasonal variations in physical fitness and performance indices of elite soccer players. *Sports, 6*(1), 14.

Mello, R., Mello, R., Gomes, D., Paz, G. A., Nasser, I., Miranda, H., & Salerno, V. P. (2017). Oxidative stress and antioxidant biomarker responses after a moderate-intensity soccer training session. *Research in Sports Medicine, 25*(3), 322-332.

Miranda Neto, M., Toscano, L. L., Tavares, R. L., Toscano, L. T., Padilhas, O. P., Silva, C. S. D., . . . Silva, A. S. (2020). Whole purple grape juice increases nitric oxide production after training session in high level beach handball athletes. *Anais da Academia Brasileira de Ciências, 92*.

Miri, H., HEIDARI, M. R., EBRAHIM, K., Ahanjan, S., MAHDILOO, M., & GHASEMALIPUR, H. (2013). Comparison of the selected physiological and functional variables of the National team players of the grassy and beach soccer.

Mirzaei, B., Asghar Norasteh, A., Saez de Villarreal, E., & Asadi, A. (2014). Effects of six weeks of depth jump vs. countermovement jump training on sand on muscle soreness and performance. *Kinesiology, 46*(1.), 97-108.

Muazu Musa, R., PP Abdul Majeed, A., Abdullah, M. R., Ab. Nasir, A. F., Arif Hassan, M. H., & Mohd Razman, M. A. (2019). Technical and tactical performance indicators discriminating winning and losing team in elite Asian beach soccer tournament. *PloS one, 14*(6), e0219138.

Murtagh, C. F., Brownlee, T. E., O'Boyle, A., Morgans, R., Drust, B., & Erskine, R. M. (2018). Importance of speed and power in elite youth soccer depends on maturation status. *The Journal of Strength & Conditioning Research, 32*(2), 297-303.

Porsesh, M., Habibi, A., Ahmadi Barati, S., & Mardaniyan Ghahfarokhi, M. (2018). Comparison of the effect of a selected resistance exercise course with two different intensities on some metabolic indices in active young girls. *Jundishapur Scientific Medical Journal, 17*(2), 115-130.

Pourrahim Ghouroghchi, A., & Pahlevani, M. (2019). The effect of resistance training with two maximum and sub-maximal intensities on leukocytes and blood coagulation factors in non-athlete men: A clinical trial study. *Journal of Rafsanjan University of Medical Sciences, 18*(7), 637-656.

Ramírez-Vélez, R., Palacios-López, A., Humberto Prieto-Benavides, D., Enrique Correa-Bautista, J., Izquierdo, M., Alonso-Martínez, A., & Lobelo, F. (2017). Normative reference values for the 20 m shuttle-run test in a population-based sample of school-aged youth in Bogota, Colombia: the FUPRECOL study. *American Journal of Human Biology, 29*(1), e22902.

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.

Schlagheck, M. L., Walzik, D., Joisten, N., Koliamitra, C., Hardt, L., Metcalfe, A. J., . . . Zimmer, P. (2020). Cellular immune response to acute exercise: Comparison of endurance and resistance exercise. *European Journal of Haematology, 105*(1), 75-84.

Silva, J. R., Magalhães, J. F., Ascensão, A. A., Oliveira, E. M., Seabra, A. F., & Rebelo, A. N. (2011). Individual match playing time during the season affects fitness-related parameters of male professional soccer players. *The Journal of Strength & Conditioning Research, 25*(10), 2729-2739.

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.

Silvestre, R., West, C., Maresh, C. M., & Kraemer, W. J. (2006). Body Composition And Physical Performance In Men's Soccer: Astudy Of A National Collegiate Athletic Association Division Iteam. *The Journal of Strength & Conditioning Research, 20*(1), 177-183.

Smpokos, E., Mourikis, C., Tsikakis, A., Katsikostas, N., & Linardakis, M. (2020). Reference performance values of pre-seasonal physical fitness in elite youth male football players in Greece. *Journal of Public Health*, 1-12.

Somani, S., Frank, S., & Rybak, L. (1995). Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacology Biochemistry and Behavior*, *51*(4), 627-634.

Stojanovic, M. D., Calleja-Gonzalez, J., Mikic, M., Madic, D. M., Drid, P., Vuckovic, I., & Ostojic, S. M. (2016). Accuracy and criterion-related validity of the 20-m shuttle run test in well-trained young basketball players. *Montenegrin Journal of Sports Science and Medicine*, *5*(2), 5.

Sureda, A., Tauler, P., Aguiló, A., Cases, N., Fuentespina, E., Córdova, A., . . . Pons, A. (2005). Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise. *Free radical research*, *39*(12), 1317-1324.

Thompson, D., Nicholas, C., & Williams, C. (1999). Muscular soreness following prolonged intermittent high-intensity shuttle running. *Journal of sports sciences*, *17*(5), 387-395.

Uchiyama, S., Tsukamoto, H., Yoshimura, S., & Tamaki, T. (2006). Relationship between oxidative stress in muscle tissue and weight-lifting-induced muscle damage. *Pflügers Archiv*, *452*(1), 109-116.

Vafaee, R., Soori, H., Hedayati, M., Ainy, E., & Hatamabadi, H. (2019). Effects of resveratrol supplementation in male Wistar rats undergoing an endurance exercise and acute exercise training. *Human Antibodies*, *27*(4), 257-264.

Viana-Gomes, D., Rosa, F., Mello, R., Paz, G., Miranda, H., & Salerno, V. (2018). Oxidative stress, muscle and liver cell damage in professional soccer players during a 2-game week schedule. *Science & Sports*, *33*(5), e221-e228.

Volek, J. S., Freidenreich, D. J., Saenz, C., Kunces, L. J., Creighton, B. C., Bartley, J. M., . . . Maresh, C. M. (2016). Metabolic characteristics of keto-adapted ultra-endurance runners. *Metabolism*, *65*(3), 100-110.

Walzik, D., Joisten, N., Zacher, J., & Zimmer, P. (2021). Transferring clinically established immune inflammation markers into exercise physiology: focus on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. *European journal of applied physiology*, *121*(7), 1803-1814.

Withee, E. D., Tippens, K. M., Dehen, R., Tibbitts, D., Hanes, D., & Zwickey, H. (2017). Effects of Methylsulfonylmethane (MSM) on exercise-induced oxidative stress, muscle damage, and pain following a half-marathon: a double-blind, randomized, placebo-controlled trial. *Journal of the international society of sports nutrition*, *14*(1), 24.