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Biomarkers of Muscle Damage and Physical Performance After Soccer Matches for Women's Soccer Teams

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Abstract: *Soccer is the most famous sport in the world. Soccer is a sport with moderate to high intensity, which can cause physical and physiological fatigue in soccer athletes. Limited recovery time can also increase muscle tissue damage. Therefore, this study aimed to examine the time interval for performance recovery in female amateur soccer athletes as measured by checking physical performance and through biomarkers after a soccer match. Quantitative research is used. The type of experimental research is pre-experimental with a one-group pretest-posttest design. Sampling in this study used an accidental sampling technique. The respondents were 20 female soccer athletes who competed in the u17 women's soccer competition. The variables examined in the research included vertical jump, CPK, LDH, and Il-6. Examinations are carried out before the match, immediately after the match, 24 hours after, and 48 hours after. Next, the results were analyzed using the one-way ANOVA and post-hoc follow-up tests via the SPSS application. Doing soccer can reduce physical performance and increase CPK, LDH, and IL-6 levels due to fatigue. Fatigue peaks significantly after 24 hours after the match. However, 48 hours after the match, the fatigue gradually disappeared and decreased. Based on this research, female athletes' soccer matches must be accompanied by a sufficient recovery time of at least 48 hours to avoid muscle damage and an increased decline in muscle performance.*

Keyword: *Biomarkers, Muscle Damage, Muscle Performance, Women Soccer.*

Introduction

The numerous soccer teams from different parts of Indonesia are proof of the country's high interest in soccer (Fuller, 2015). Both Indonesia's women's and men's soccer teams are highly intriguing. Indonesia has 1,300 female soccer players registered with FIFA, and the number of female soccer players in Asia reached 784 thousand in 2019 (Doewes et al., 2021). Only a small percentage of Indonesian female soccer players are registered with FIFA (Destrian et al., 2022).

The players' physical strength is required to withstand frequent changes in the direction of the ball, sprinting, and acceleration in the high-intensity soccer game (Hisdal et al., 2013). Soccer players will have a lot of matches to attend. These loads are composed of psychological, physiological, and physical loads (Hisdal et al., 2013). Soccer players also expect that they will win each game they play. This may have an impact on soccer players' decisions to give their all in each game (Pollard & Gómez, 2014). The physiological and physical reactions after this match might have an impact on physical performance in the following one. If it keeps happening, this can lead to an acute and persistent fatigue response (Montgomery et al., 2008).

A process that occurs in tandem with an increase in muscle temperature leads to high rates of reactive oxygen species (ROS) and an inflammatory response from the muscles mediated by neutrophil xanthin oxidase (Dewangga & Irianto, 2023). High-intensity exercise causes oxidative stress, which alters cell structure and disturbs intracellular homeostasis, allowing intracellular molecules like enzymes to migrate to extracellular regions and altering function (Dewangga et al., 2021).

Physical stressors that are severe and ongoing are the primary cause of fatigue (Poenaru et al., 2021). Damage to micromuscles can result from prolonged stress. Furthermore, a decrease in muscle glycogen stores is linked to the fatigue process following a soccer game (Krustrup et al., 2022). It has been demonstrated that there is a 48–72-hour recovery phase following competition that is responsible for restoring muscle glycogen and performance (Marqués-Jiménez et al., 2022). Both the extent of the inflammatory response and the degree of muscle damage affect how long recovery takes. When muscle damage happens, a number of biomarkers of muscle damage, including interleukin-6, creatine kinase, and lactate dehydrogenase, appear at the site of injury (Dewangga et al., 2022). As these molecules accumulate, cell necrosis, elevated body temperature, and delayed-onset muscle soreness (DOMS) result (dos Santos et al., 2020).

Giving athletes enough time to recuperate can help them in a number of ways. For example, it can accelerate metabolic processes like protein synthesis, ensuring that their bodies are balanced with protein when they return to the field (de Sousa et al., 2022). Athletes can compete with enjoyment if they have a good recovery, which can enhance sleep quality, decrease fatigue, and reduce DOMS (Rahman et al., 2022). The bulk of earlier research on the recovery aspects of soccer games only looked at male athletes' game responses; however, women could also be the subjects of this research to find out how changes in biomarkers of muscle damage and physical performance occur after soccer games (Pérez-Castillo et al., 2023). The fact that estrogen, for instance, has been demonstrated to have a protective effect on reactive oxygen species (ROS) is another reason for conducting this research. This suggests that gender differences may influence recovery patterns (Iorga et al., 2017).

Research on female players is also important, considering that the impact of the menstrual cycle on performance parameters in elite athletes is still inconclusive. However, recent literature suggests a possible influence of the menstrual cycle phase on the recovery process and health (Goulart et al., 2022). Therefore, it is still being determined whether applying information regarding male players' post-match recovery can accurately understand female players' responses, which is important for adequate training and recovery planning (Doeven et al., 2018). This study aimed to examine the time interval for performance recovery in female amateur soccer athletes as measured by checking physical performance and through biomarkers after a soccer match.

Method

The type of research used is quantitative research. The type of experimental research is pre-experimental with a one-group pretest-posttest design. Sampling in this study used an accidental sampling technique. Incidental sampling is a method of determining samples based on chance, meaning anyone who coincidentally or accidentally meets the researcher can be used as a sample. The total number of samples obtained in this research was 20. The research is carried out after the sample agrees to fill in the consent information.

Four women's soccer teams participated in a tournament between representatives of the West Java and Central Java Provinces. The women's soccer teams include the Persib Women's Soccer Team, the Princess Wijaya Subang Soccer Team, the Surakarta Women's Soccer Team, and the Semarang Ratanika Soccer Team. Female soccer players under the age of 17 (U-17) are competing in this tournament. This tournament, held for one week, requires one team to play three matches so that one team will compete every two days. From each team, 5 people will be randomly selected to be respondents for this research.

The Faculty of Medicine, Sultan Agung Islamic University, Semarang, Central Java, research ethics committee has approved this study No. 436/X/2023/Bioethics Committee. Before conducting the research, the respondents agreed and signed their consent to become research respondents after reading the experimental methods. Researchers conducted research in the city of Surakarta in November 2023.

The research data is a general examination, such as age, heart rate, and body mass index. Then, physical performance data was taken from vertical jump results. Muscle damage biomarker research data was examined using creatine kinase, lactate dehydrogenase, and interleukin-6 results. Physical performance data and muscle damage biomarkers were taken 2 hours before the first match, 24 hours after the match, and 48 hours after the first match. A general examination is carried out by asking for your name and age, then continuing by calculating your heart rate in one minute (BPM). The HR examination is manual: palpating the radial artery and counting for 1 minute. Then, the BMI examination was carried out by measuring the height and weight of the research respondents. Then, after the height and weight data is obtained, it is calculated using a formula. BMI = Body Weight (kg): [Height (m) x Body Height (m)] (Syamsuryadin et al., 2022).

The athlete performs the vertical jump test by extending the hand that is closest to the wall while standing sideways to the wall. Keep feet flat on the ground, toe tips marked or noted. This is called the standing-to-reach height. The athlete then stands away from the wall and jumps vertically as high as possible, using both arms and legs to help protect the body upwards. The jumping technique may or may not use a counter-movement (see vertical jump technique). Try to touch the wall at the highest jumping point. The difference in distance between standing reach height and jumping height is called the score. Sampling was carried out in the brachial artery.

The blood sample was then put into a vacutainer tube and centrifuge machine. centrifuge at 3000 rpm for 30 minutes to obtain blood serum. Then, the blood sample is put into a microtube, placed, and stored in a cooler with a temperature of -20^{o}C (Dewangga & Irianto, 2023). Then, blood samples were taken to be examined for the biomarkers creatine phosphokinase, lactic acid dehydrogenase, and interleukin-6 using basic biomedical research techniques with ELISA at the Clinical Pathology Laboratory of Dr. Hospital Sardjito Yogyakarta.

The process of examining creatine phosphokinase consists of several stages. First, prepare all the tools and muscle blood samples. Next, make solutions of monoreagents 1 and 2 by adding 2.5 ml of monoreagent 1 to the monoreagent. CPK activity was measured at 25^{o}C. Next, 40 µL of homogenate was mixed with 1.0 mL of mono reagent Incubate for 3 minutes. Read the absorbance at 1, 2, and 3 minutes at a wavelength of 340 nm (Bernat-Adell et al., 2021).

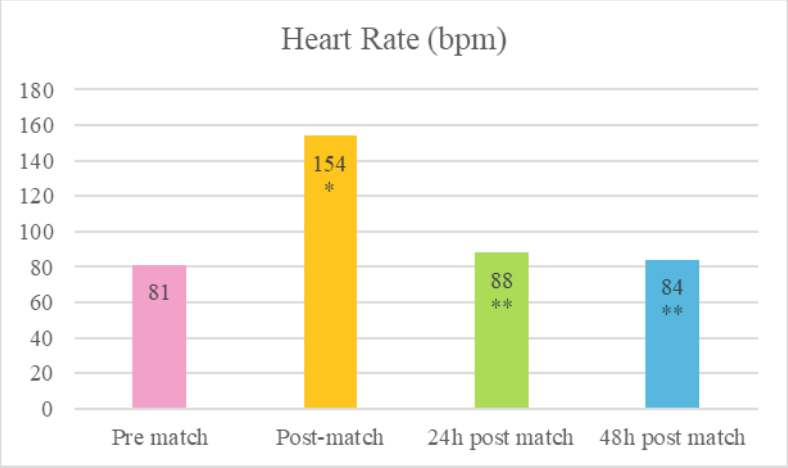
Examination of serum interleukin-6 levels was carried out using an ELISA tool (Human Interleukin 6 ELISA Kit Cat. No. E0090Hu and Elisa Reader PHO MO Autobio), which is an immunoserological examination tool at the Immunology Laboratory, Department of Medical Laboratory Technology, Muhammadiyah University, Semarang. The interleukin-6 examination procedure was carried out using the SOP Bioassay Technology Laboratory's Human Interleukin 6 ELISA kit, Cat. No. E0090Hu, with a sensitivity of 1.03 ng/L and a 96-well size. The principle of the examination is a microplate coated with IL-6 antibodies. Interleukin-6 contained in the sample will bind to antibodies coated on the microplate. When biotinylated IL-6 antibodies are added, they bind to the IL-6 in the sample. Biotin binds covalently to proteins, nucleic acids, or other molecules. Then streptavadin-HRP is added and binds to the biotinylated IL-6 antibody. After incubation, unbound Streptavidin-HRP was removed during a washing step. The substrate is then added, and the color changes proportionally to the IL-6 level. The reaction will end by adding a stop solution, and absorbance will be measured at a wavelength of 450 nm. The work procedure is as follows:

Results

Variable	Mean (SD)	P-value
HR Pre-match (bpm)	81±9.06	0.000
HR Post-match(bpm)	120±7.94	
HR 24h after match (bpm)	88±9.4	
HR 48h after match (bpm)	84±4.4	
Vertical Jump Pre-match (cm)	44.7±5.88	0.000
Vertical Jump Post-match (cm)	38.2±6.01	
Vertical Jump 24h after match (cm)	43.4±4.82	
Vertical Jump 48h after match (cm)	44.3±3.38	
CK Pre-match (U/mL)	153.4±51.04	0.000
CK Post-match (U/mL)	165.5±57.48	
CK 24h after match (U/mL)	233.7±81.93	
CK 48h after match (U/mL)	173.2±48.1	
LDH Pre-match (mg/dL)	201.7±26.44	0.000
LDH Post-match (mg/dL)	207.05±16.11	
LDH 24h after match (mg/dL)	273.3±32.62	
LDH 48h after match (mg/dL)	228.8±22.41	
IL-6 Pre-match (pg/mL)	2.7±0.33	0.000
IL-6 Post-match (pg/mL)	3.5±0.52	
IL-6 24h after match (pg/mL)	5.4±0.71	
IL-6 48h after match (pg/mL)	3.7±0.55	

Rate (HR)

The normality test results using the Shapiro-Wilk test for measuring HR before the match, after the match, 24 hours after the match, and 48 hours after the match proved that all groups had a normal distribution. This is indicated by the results of the Shapiro-Wilk test, with a significance value of <0.05. Then, the data was tested for influence using the one-way ANOVA test. The results of the one-way ANOVA test obtained a significance value of 0.00. This proves that there is a significant difference in influence between before the match, post-match, 24 hours after, and 48 hours after. In this study, the soccer match was held in the afternoon with a 2 x 45-minute playing time. The soccer match will certainly increase HR significantly ($p<0.05$), but after 24 hours and 48 hours, the heart rate will drop significantly and return to normal. Before the game, the average HR was 81 bpm. Then, after the match, the average HR increased to 154 bpm. 24 hours after the match, the average HR decreased to 88 bpm, and 48 hours after the match, the average heart rate decreased to 84 bpm. The difference in average HR can be seen in Graph 1.

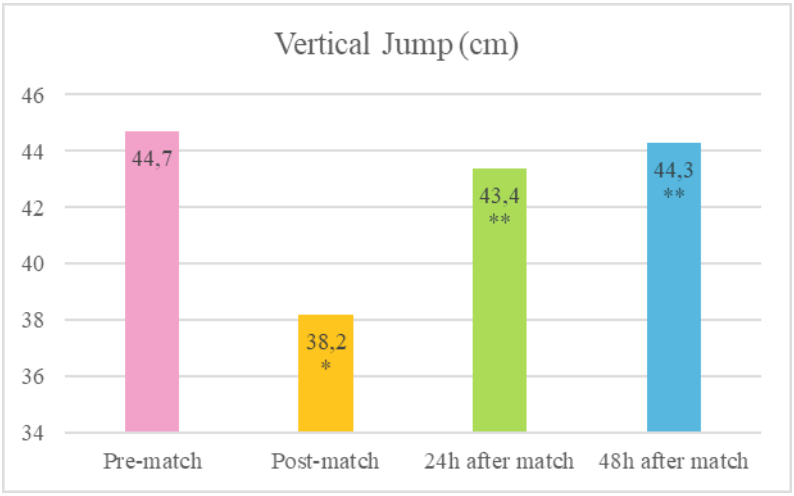


Graph 1. *The difference in average HR*

*ANOVA followed by Post Hoc Analysis by SPSS 26. *P < 0.05 has significant diferent Il-6 levels with pre-match. **P < 0.05 has significant diferent Il-6 with post-match. ***p<0.05 has significant diferent Il-6 with 24h after match*

Vertical Jump

The normality test results using the Shapiro-Wilk test for vertical jump measurements before, after, 24 hours after, and 48 hours after the match proved that all groups had a normal distribution. This is indicated by the results of the Shapiro-Wilk test, with a significance value of <0.05. Then, the data was tested for influence using the one-way ANOVA test. The results of the one-way ANOVA test obtained a significance value of 0.00. This proves that there is a significant difference in influence between before, post-match, 24 hours after, and 48 hours after. In this study, soccer matches were held in the afternoon, with a playing time of 2 x 45 minutes. Soccer matches will certainly reduce the results of vertical jumps caused by fatigue significantly ($p<0.05$). Before the match, the average vertical jump result was 44.7 cm. Then, after the match, the average vertical jump result decreased to 38.2 cm. 24 hours after the match, the average vertical jump result increased to 43.4 cm, and 48 hours after the match, the average vertical jump result increased to 44.3cm. The difference in average vertical jump results can be seen in graph 2.

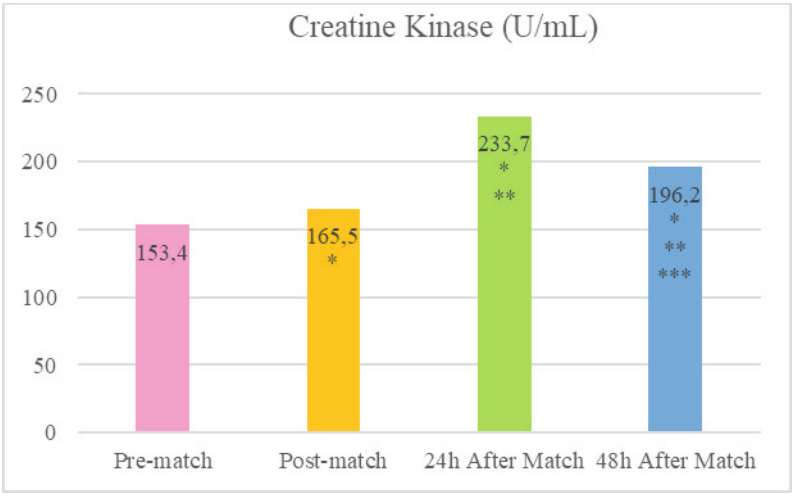


Graph 2. *The difference in average vertical jump results*

*ANOVA followed by Post Hoc Analysis by SPSS 26. *P < 0.05 has significant diferent HR with pre-match. **P < 0.05 has significant diferent HR with post-match.*

Creatine Kinase (CK)

The normality test results using the Shapiro-Wilk test for measuring creatine kinase before the match, after the match, 24 hours after the match, and 48 hours after the match proved that all groups had a normal distribution. This is indicated by the results of the Shapiro-Wilk test, with a significance value of <0.05. Then, the data was tested for influence using the one-way ANOVA test. The results of the one-way ANOVA test obtained a significance value of 0.00. This proves that there is a significant difference in influence between before the match, post-match, 24 hours after, and 48 hours after. In this study, soccer matches were held in the afternoon with a 2 x 45-minute game time. Soccer matches will certainly increase the results of creatine kinase, which is caused by damage to tired muscles, significantly (p<0.05). Before the competition, the average creatine kinase result was 153.4 U/mL. Then, after the competition, the average creatine kinase result increased to 165.5 U/mL. 24 hours after the match, the average creatine kinase result increased to 233.7 U/mL 48 hours after the match, and the average creatine kinase result decreased to 196.2 U/mL. The average difference in creatine kinase results can be seen in graph 3.

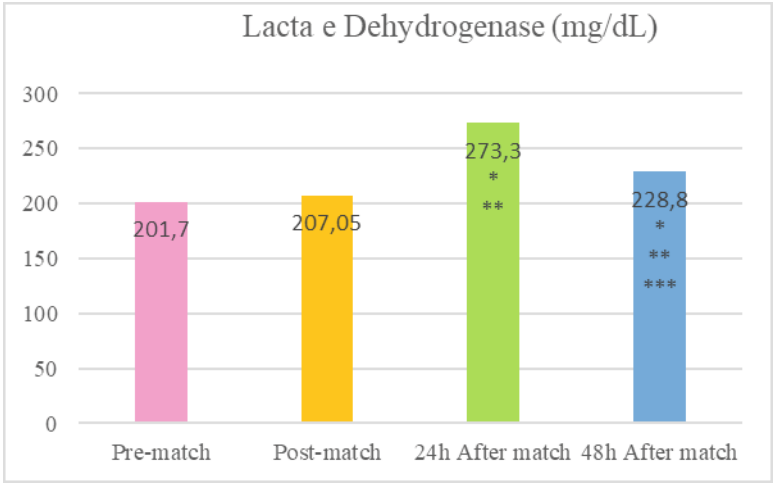


Graph 3. *The average difference in creatine kinase results*

*ANOVA followed by Post Hoc Analysis by SPSS 26. *P < 0.05 has significant diferent CK levels with pre-match. **P < 0.05 has significant diferent CK with post-match. ***p<0.05 has significant diferent CK with 24h after match*

Lactate Dehydrogenase (LDH)

The normality test results using the Shapiro-Wilk test for measuring lactate dehydrogenase before the match, after the match, 24 hours after the match, and 48 hours after the match proved that all groups had a normal distribution. This is indicated by the results of the Shapiro-Wilk test, with a significance value of <0.05. Then, the data was tested for influence using the one-way ANOVA test. The results of the one-way ANOVA test obtained a significance value of 0.00. This proves that there is a significant difference in influence between before, post-match, 24 hours after, and 48 hours after. In this study, soccer matches were held in the afternoon, with 2 x 45 minutes of playing time. Soccer matches will certainly increase lactate dehydrogenase results, which are significantly caused by damage to tired muscles (p<0.05). Before the match, the average lactate dehydrogenase result was 201.7 mg/dL. Then, after the match, the average lactate dehydrogenase result increased to 207.05 mg/dL. 24 hours after the match, the average lactate dehydrogenase result increased to 273.3 mg/dL, and 48 hours after the match, the average lactate dehydrogenase result increased to 228.8 mg/dL. The difference in average lactate dehydrogenase results can be seen in graph 4.

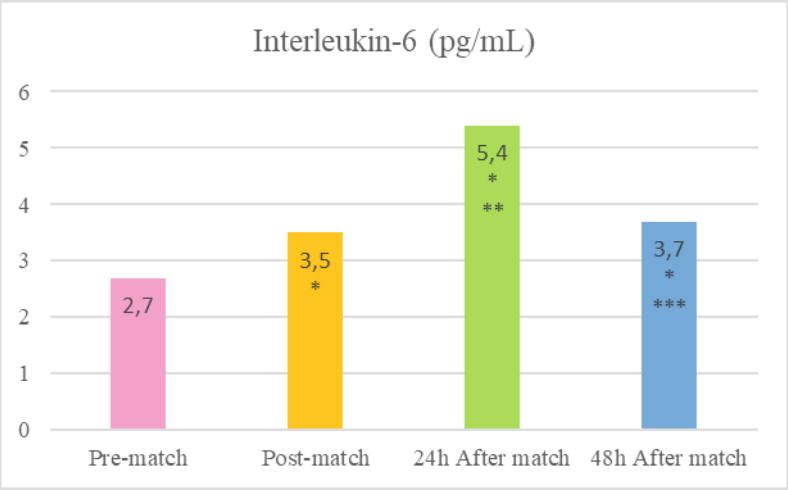


Graph 4. *The difference in average lactate dehydrogenase results*

*ANOVA followed by Post Hoc Analysis by SPSS 26. *P < 0.05 has significant diferent LDH levels with pre-match. **P < 0.05 has significant diferent LDH with post-match. ***p<0.05 has significant diferent LDH with 24h after match*

Interleukin-6 (IL-6)

The results of the normality test using the Shapiro-Wilk test for measuring interleukin-6 before the match, after the match, 24 hours after the match, and 48 hours after the match proved that all groups had a normal distribution. This is indicated by the results of the Shapiro-Wilk test, with a significance value of <0.05. Then the data was tested for influence using the one-way ANOVA test. The results of the one-way ANOVA test obtained a significance value of 0.00. This proves that there is a significant difference in influence between before the match, post-match, 24 hours after the match, and 48 hours after the match. In this study, soccer matches were held in the afternoon with a playing time of 45x2. Soccer matches will certainly increase the results of interleukin-6, which is caused by damage to tired muscles, significantly (p<0.05). Before the match, the average interleukin-6 result was 2.7 pg/mL. Then, after the match, the average interleukin-6 result increased to 3.5 pg/mL. 24 hours after the match, the average interleukin-6 result increased to 5.4 pg/mL, and 48 hours after the match, the average interleukin-6 result increased to 3.7 pg/mL. The difference in average interleukin-6 results can be seen in graph 5.



Graph 5. *The difference in average interleukin-6 results*

*ANOVA followed by Post Hoc Analysis by SPSS 26. *P < 0.05 has significant diferent Il-6 levels with pre-match. **P < 0.05 has significant diferent Il-6 with post-match. ***p<0.05 has significant diferent Il-6 with 24h after match*

Discussion

This study aimed to examine the time interval for performance recovery in female amateur soccer athletes as measured by checking physical performance and through biomarkers after a soccer match. More specifically, physical performance parameters were measured by a vertical jump, which was checked before the match, immediately after the match, 24 hours after the match, and 48 hours after the match. The muscle damage profile also shows an increased response, measured by checking CK, LDH, and IL-6 parameters. The parameters CK, LDH, and IL-6 increased significantly after the game after 24 hours. Then, during an examination 48 hours after the match, the results showed a significant decrease in CK, LDH, and IL-6 levels.

Our research shows that this recovery time is at least 48 hours. It should be noted that we intentionally limited the definition of training recovery to the ability to replicate or exceed baseline performance on our measures. To be clear, we are not saying that our subjects have fully recovered because we recognize that other disturbances to homeostasis, such as changes in muscle tissue, nerves, and hormonal status, may have occurred; however, we chose not to investigate this. Instead, we looked into what we consider a useful, performance-based recovery assessment that coaches and athletes can use. Additionally, because our performance measures are general and straightforward, coaches can easily evaluate performance recovery, and they also apply to other sports.

The average HR recorded before the match averaged around 81 bpm. After the match, the average HR increased to 154 bpm. 24 hours after the match, the average HR of the athletes returned to normal at 88 bpm, and 48 hours after the match, the average HR was still at a normal number of around 84 bpm. This is similar to Tessitore's study, which found that women's soccer post-match HR ranged between 120 and 140 bpm. Additionally, Capranica's research indicates that among teenage athletes, the HR of female soccer players will rise to about 170 bpm (Capranica et al., 2001). Women's soccer involves explosive actions, including sprinting, acceleration, or peak jump height. For example, at the elite level, competition demands include a total distance of between 9 and 11 km, with 1.5 km covered in high-speed running (>13 km/h) and around 4.7 km in sprinting (>22 km/hour) per player (Goulart et al., 2022). Competition-related acute fatigue will cause HR to rise to over 70% of HR max. This can also illustrate that soccer activities are carried out at low to moderate intensity; this sport is described as an "intermittent aerobic sport," with approximately 90% of total energy expenditure provided by aerobic energy sources or pathways. However, current data lack evidence to completely explain the recovery time of all physical performance actions on female soccer players. Different fatigue levels resulting from various skill sets, muscle mass recruitment, and intermuscular coordination between tests may cause the various recovery profiles for performance parameters (Alexandre et al., 2012).

Soccer matches that take place at high intensity will result in muscle fatigue. Muscle fatigue usually occurs after high-intensity exercise or prolonged physical activity (Dambroz et al., 2022). Fatigue can be defined as a decrease in tension capacity or force output after repeated muscle contractions. This can result in a negative impact on an individual's overall performance. One way to check for leg fatigue is to check the power output during a jump height test (vertical jump) (Cooper et al., 2020). The vertical jump results in the pre-match showed it was 44.7cm. After the match, the vertical jump test results decreased to 38.2cm. 24 hours after the match, the vertical jump result increased to 43.4cm. The vertical jump test results 48 hours after the match were still in the same condition at 44.3cm. These findings corroborate Jesper Sjokvist's research, which demonstrated a decrease in vertical jump height in women following soccer activities (Sjökvist et al., 2011). Apart from that, there are also other studies, such as those conducted by Kreamer et al., who reported finding the effect of physical recovery after fatigue in the recovery period after 24 hours of a tennis match in female players (Girard et al., 2014).

According to Rodrigues research, moderate to high-intensity physical activity can cause significant muscle damage to muscle fibers. These findings explain the increase in CK levels, which lasts up to 24 hours after activity. Several studies report that soccer activities cause severe microdamage to muscle tissue at the cellular level, resulting in higher CK activity (Rodrigues et al., 2010). Another thing that deserves attention is that lower speeds, especially in the eccentric phase, increase muscle damage. CK is a good indicator to use in monitoring athlete recovery, and an increase in CK indicates a muscle damage response after a soccer game. Some studies show that CK concentrations can remain high for hours or even days after intense competition or prolonged training sessions (YAPALI & KÜRKLÜ, 2022). In this study, CK levels were checked several times. The first check is at pre-match, the second check is immediately after the soccer match, the third check is 24 hours later, and the fourth check is 48 hours later. When pre-matched, the average was 153.4 U/mL. The average CK level increased to 165.5 U/mL in the second examination after the match. On the third examination, 24 hours after the match, the average CK level had increased to 233.7 U/mL. On the fourth examination, 48 hours after the match, the average CK level had decreased to 228.8 U/mL.

Another important finding of this study concerns the LDH response. A significant increase in LDH can be experienced after exercise. Previous research by Kobayashi, demonstrated that primarily aerobic exercise can increase LDH activity for 12 to 24 hours (Kobayashi et al., 2005). Since both aerobic and resistance exercise are linked to stress-strain, it is thought that these changes in the immune system and hormones may be caused by increasing the amount and intensity of exercise (Walsh et al., 2011). This is, of course, in line with the research we conducted. The peak of LDH production occurs 24 hours after a soccer match. After that, LDH will decrease until it returns to normal. In this study, the LDH level was 201.7 mg/dL before the match. After the match, it increased to 207 mg/dL. 24 hours after the match, this is the peak of LDH production; the LDH levels examined had an average of 273.3 mg/dL. After 48 hours, LDH levels tended to decrease; the results showed that the average LDH level after 48 hours was 228.8 mg/dL.

This study showed a statistically significant difference between basal levels and levels 24 hours after the match in soccer players. This means a rapid homeostatic reorganization of the immune system, which lasts at least 24 hours in soccer players. However, contracting muscles are not the only source of IL-6 after exercise. For example, small discharges from the internal jugular vein suggest central nervous system (CNS) attribution in IL-6 secretion during exercise (Nybo et al., 2002). Under normal conditions, IL-6 levels are almost undetectable in the CNS. However, in pathological situations, such as trauma, hypoxia, or ischemia, IL-6 can be reduced by activated astrocytes (Van Wagoner et al., 1999). However, it can also be expressed in the form of the hypothalamus after acute and prolonged stress, such as intense and long-lasting exercise. Exercise-induced increases in athletes' serum IL-6 depend on the type, intensity, and duration of exercise. The initial view that IL-6 is a result of muscle damage that often accompanies some types of exercise has yet to be confirmed by current bibliographic data (Athanasios, 2014). Some research showed that IL-6 production was the same in athletes who performed isokinetic exercise, regardless of fitness level. In addition, IL-6 and creatine kinase, which are sensitive markers of muscle damage (Bouzigon et al., 2021).

Conclusion

Based on our research, soccer matches are light- to moderate-intensity sports played for 2 x 45 minutes. This makes the body feel tired, and performance decreases acutely, heart rate increases, and muscle tissue damage occurs, as indicated by the biomarkers CPK, LDH, and IL-6. Peak fatigue, characterized by increased biomarkers, will occur

in the first 24 hours. Then, 24 hours later (48 hours after the match), physical performance and muscle tissue damage return to normal. The slow recovery time experienced by an athlete is certainly not influenced by just one or two factors but by various complementary factors.

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Conflict of Interest
The authors declare that there are no conflicts of interest.

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