

Comparison of Post–Yo–Yo Intermittent Recovery Test Creatine Kinase Responses
Across Professional Soccer Leagues

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ORIGINAL ARTICLE

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Abstract

This study aimed to compare the acute muscle damage responses of professional soccer players from different league tiers following a standardized high-intensity loading protocol. It was hypothesized that there would be no statistically significant differences in Creatine Kinase (CK) responses among leagues despite varying seasonal match and training intensities. The study population consisted of 75 professional soccer players recruited from the Turkish Football Federation (TFF) 1st (n=26), 2nd (n=25), and 3rd (n=24) Leagues. All participants performed the Yo-Yo Intermittent Recovery Test (Level 1). Venous blood samples were collected 24 hours post-test, a timing chosen based on literature indicating peak CK levels at this interval. Data were analyzed using SPSS (v.24) via One-Way ANOVA, and effect sizes (partial eta-squared, η^2) were calculated to determine the magnitude of differences. The mean CK levels were 462.69±384.44 U/L for the 1st League, 433.00±335.97 U/L for the 2nd League, and 466.25±350.56 U/L for the 3rd League. The ANOVA results revealed no statistically significant differences in CK responses across the leagues (F=0.102, p=0.903, $\eta^2=0.003$). The findings suggest that professional league standing does not significantly influence the extent of acute muscle damage when a standardized exercise protocol is applied. The lack of significant variance and the small effect size indicate that professional soccer players across different tiers exhibit comparable physiological adaptations and sarcolemmal stability. Variations in individual responses are likely attributable to intrinsic biochemical signatures and training status rather than league hierarchy.

Keywords: Soccer, Muscle Damage, Creatine Kinase, Yo-Yo Test, Professional Leagues.

**Profesyonel Futbol Liglerinde Yo-Yo Sonrası Aralıklı Dinlenme
Testi Sonucunda Ortaya Çıkan Kreatin Kinaz Tepkilerinin
Karşılaştırılması**

Öz

Bu çalışma, farklı lig seviyelerindeki profesyonel futbolcuların standartlaştırılmış yüksek şiddetli bir yüklem protokolü sonrası sergiledikleri akut kas hasarı yanıtlarını karşılaştırmayı amaçlamıştır. Sezonluk maç ve antrenman yoğunluklarındaki farklılıklara rağmen, ligler arasında Yo-Yo testi sonrası Kreatin Kinaz (CK) yanıtları açısından istatistiksel olarak anlamlı bir fark olmayacağı hipotez edilmiştir. Araştırma grubu, Türkiye Futbol Federasyonu (TFF) 1. Lig (n=26), 2. Lig (n=25) ve 3. Lig'den (n=24) seçilen toplam 75 profesyonel futbolcudan oluşmuştur. Tüm katılımcılar Yo-Yo Aralıklı Recovery Testi (Seviye 1) protokolünü uygulamıştır. Venöz kan örnekleri, literatürde CK seviyelerinin bu aralıkta zirve (pik) yaptığına dair bulgulara dayanarak, testten 24 saat sonra alınmıştır. Veriler SPSS (v.24) programında Tek Yönlü ANOVA kullanılarak analiz edilmiş ve farkların büyüklüğünü belirlemek için etki değeri (kısmi eta-kare, η^2) hesaplanmıştır. Ortalama CK seviyeleri; 1. Lig için 462,69±384,44 U/L, 2. Lig için 433,00±335,97 U/L ve 3. Lig için 466,25±350,56 U/L olarak belirlenmiştir. ANOVA sonuçları, ligler arasında CK yanıtları açısından istatistiksel olarak anlamlı bir fark olmadığını ortaya koymuştur (F=0,102; p=0,903; $\eta^2=0,003$). Elde edilen bulgular, standart bir egzersiz protokolü uygulandığında profesyonel lig düzeyinin akut kas hasarı derecesi üzerinde anlamlı bir etkisinin olmadığını göstermektedir. Anlamlı bir varyasyonun bulunmaması ve düşük etki değeri, farklı kademelerdeki profesyonel futbolcuların benzer fizyolojik adaptasyonlar ve sarkolemma stabilitesi sergilediğine işaret etmektedir. Bireysel yanıtlardaki farklılıkların, lig hiyerarşisinden ziyade sporcuların özgün biyokimyasal imzaları ve antrenman durumlarıyla ilişkili olduğu düşünülmektedir.

Anahtar Kelimeler: Futbol, Kas Hasarı, Kreatin Kinaz, Yo-Yo Testi, Profesyonel Ligler.

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Introduction

Football is a high-intensity sport that emphasizes various physical fitness components, such as strength, endurance, agility, and speed (Dugdale et al., 2019; Mathivanan, 2018). Furthermore, the sudden and continuous repetition of activities including changes of direction, rapid decelerations, and accelerations inherent to the system of play places significant mechanical stress on the muscles. This repetitive stress can lead to muscle damage, thereby increasing the risk of injury among football players (Young et al., 2022; Bate, 2014). In addition to many factors such as the type, intensity and frequency of training, lack of adequate recovery and environmental factors, the level of difficulty of the league in which the soccer player plays is also an important factor influencing the extent of muscle damage in soccer players (Markus et al., 2021; Gabbett et al., 2014). The seasonal schedule of soccer players playing in the top leagues is intense, so the training programme is also more intense and demanding (Hostrup & Bangsbo, 2023; Scott et al., 2014). Muscles and tissues are exposed to microtears and stress during these intense training sessions, so muscle damage may be higher in players in the top leagues (Ivancevic et al., 2015).

Muscle damage occurs in two ways: traumatic muscle damage and microtraumatic muscle damage (McHugh & Tyler, 2019). As soccer is a system of play in which collisions, falls and impacts are experienced intensely due to sudden movements and changes in direction, traumatic muscle injuries are a common occurrence (Young et al., 2022; Bate, 2014). However, the likelihood of microtraumatic muscle injuries is increasing due to the increase in loads caused by intense training characteristics, particularly in high-level leagues, and the inability of muscles to recover sufficiently afterwards (Chou et al., 2021).

The effects of high-intensity intermittent and continuous training on the activation of some enzymes in the organism have become the subject of numerous studies in recent years (Taş et al., 2019). As soccer is a sport that requires intense athletic performance and efficiency (Ivancevic et al., 2015). It is of great importance to minimise the risk of injury and maintain muscle health for athletic success by optimising the overall health shape of soccer players (McBurnie et al., 2021). Muscle damage that negatively impacts the injury risk and athletic efficiency of soccer players can be one of the serious health issues that can change the direction of their careers (Hunkin et al., 2014).

In light of this information, the effects of muscle damage on athletic performance in soccer are of great importance. It is necessary to prevent muscle damage and eliminate potential injury risks in order to easily integrate into the intense game programme of soccer players competing in top leagues and thus meet the expectations of success (Chou et al., 2021). However, it is inevitable that lower league athletes, who have less intense playing schedules compared to the top leagues, will also

suffer muscle damage if regular training and adequate rest periods are not provided and nutrition and hydration are not maintained (Burke et al., 2019). Therefore, it is important to further investigate the extent of muscle damage in soccer players in different leagues, especially depending on the intensity of play and training, and to develop optimal training programmes and alternative strategies to prevent muscle damage (Nedelec et al., 2012).

The Yo-Yo Intermittent Recovery Test, developed by Jens Bangsbo and inspired by the 20-meter shuttle run test, is a field test designed to measure the aerobic and anaerobic endurance of athletes (Biçer, 2021; Can & Cihan, 2013). Although structurally similar to the standard 20-meter shuttle test, its primary distinction lies in the inclusion of a 10-second active recovery period following each shuttle bout (Bangsbo et al., 2008). The test is administered in two different intensity versions: Level 1 (YYIR1) and Level 2 (YYIR2). While YYIR1 focuses on general aerobic capacity, YYIR2 emphasizes the ability to perform repeated high-intensity exercise with a significant anaerobic energy contribution (Schmitz et al., 2018).

The primary objective of implementing the Yo-Yo test in football is its high alignment with the intermittent nature of the sport. Football is characterized by constantly changing intensities, involving actions such as sprinting, walking, running, or standing (Can & Cihan, 2013). By incorporating 10-second recovery intervals, the Yo-Yo test effectively simulates this intermittent structure to measure football-specific endurance (Bangsbo et al., 2008).

Furthermore, the test is crucial for evaluating high-intensity effort and recovery capacity. Since a key performance indicator in football is the ability to recover rapidly following strenuous bouts during a match, the Yo-Yo test provides a clear profile of players' capacities through heart rate monitoring, lactate profiles, and recovery durations (Bayrakdaroğlu et al., 2021). Consequently, it serves as a fundamental benchmark for training programming and monitoring, allowing for the assessment of training effectiveness and the individual development of athletes (Biçer, 2021).

Additionally, the Yo-Yo test offers a practical alternative to laboratory assessments. While laboratory tests measuring maximal oxygen uptake (VO_2 max) are often costly and time-consuming, the Yo-Yo test is a highly valid and reliable tool that can be administered to large groups on natural pitch surfaces within a short timeframe (Schmitz et al., 2018; Bangsbo et al., 2008). Finally, the test serves as a strong predictor of match performance. Research has demonstrated that the distances covered during the Yo-Yo test correlate highly with the total high-intensity running distance and sprint volume of footballers during actual match play (Bangsbo et al., 2008; Can & Cihan, 2013).

The rationale for utilizing the Yo-Yo Intermittent Recovery Test (YYIR1) as a stimulus for muscle damage lies in its high mechanical and metabolic demands. Unlike continuous running protocols, the Yo-Yo test requires frequent and intense decelerations, changes of direction, and rapid accelerations. These actions impose significant eccentric loading on the lower extremity musculature, particularly during the braking phase of the 180-degree turns. Scientific evidence suggests that such eccentric-dominant movements are the primary drivers of mechanical disruption in sarcomeres, leading to the leakage of muscle-specific enzymes like Creatine Kinase (CK) into the bloodstream (Bangsbo et al., 2008; Young et al., 2022). Therefore, the Yo-Yo test serves as a valid field-based model to evaluate the acute muscle damage profile in professional soccer players.

The aim of this study was to investigate and compare the acute muscle damage responses in soccer players competing in different professional leagues. We hypothesized that players in higher leagues would demonstrate greater CK elevation due to their higher chronic training and match loads. While the existing literature extensively examines muscle damage in relation to post-match processes, specific training programs, varying playing surfaces, and fatigue or recovery (Karabulak & Aslan, 2023; Kızıltoprak, 2020; Young et al., 2012; Baird et al., 2012; Freire et al., 2020), there is a notable lack of research directly comparing biochemical markers across different professional league tiers under a standardized stimulus. This study, therefore, seeks to fill this gap by providing objective data on the extent of muscle damage caused by standardized high-intensity stress in athletes across leagues with different competitive levels and training intensities.

Therefore, the insights gained from examining muscle damage profiles across different league levels are expected to provide critical guidance for designing training programs that incorporate adequate recovery periods. Such data will facilitate the optimization of training loads and the implementation of alternative strategies to mitigate exercise-induced muscle damage, thereby reducing the risk of injury. Furthermore, these findings are anticipated to contribute significantly to characterizing the specific physiological and training requirements of soccer players at various professional tiers.

Material and Method

This study employed a Cross-sectional comparative design to compare biochemical muscle damage responses to standardized exercise across professional footballers from the TFF 1st, 2nd, and 3rd Leagues. During the competitive season, 75 professional athletes underwent the Yo-Yo Intermittent Recovery Test (Level 1) on standardized natural grass pitches. Venous blood samples were collected 24 hours post-test to analyze Creatine Kinase (CK) levels as the primary marker of exercise-induced muscle damage.

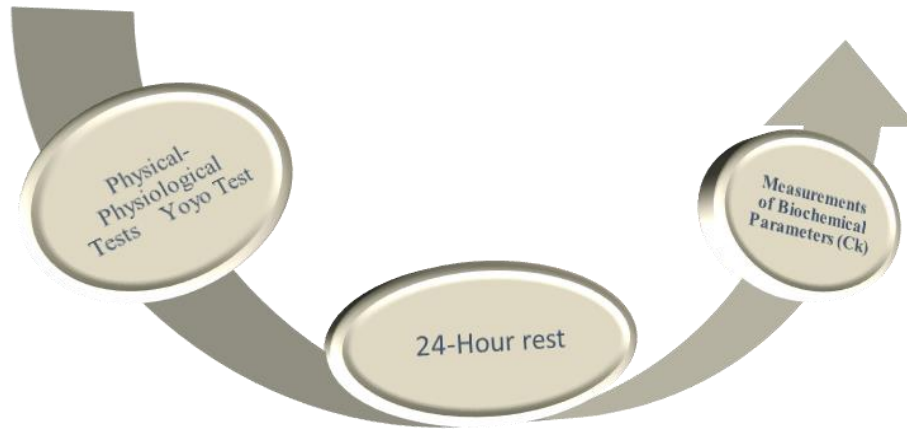


Figure 1. Work design

Model of the Research

The study was conducted using an cross-sectional comparative design involving three independent groups. This model allows for a robust inter-group comparison of measured variables, providing significant advantages in the evidence-based design and optimization of training programs for professional soccer players.

Sample Size, Groups and Power Analysis

The sample size of the study was determined using G*Power software (v3.1.9.7). Based on a power analysis conducted with an alpha significance level of $\alpha=0.05$ and a medium effect size ($f=0.25$), it was targeted to achieve a statistical power ($1-\beta$) of over 80%. Consequently, the minimum required total sample size was calculated to ensure the robustness of the statistical findings.

The study population comprised a total of 75 professional soccer players from three different divisions of the Turkish Football Federation. The sample included 26 players from Kocaelispor (1st Division; mean age: 28.50 ± 4.25 years), 25 players from Çorum Football Club (2nd Division; mean age: 26.56 ± 5.70 years), and 24 players from Karşıyaka Sports Club (3rd Division; mean age: 27.12 ± 3.45 years). It was confirmed that all athletes had been active at a professional level for at least five years and had not sustained any lower extremity injuries affecting their performance within the last three months. Prior to the data collection process from the high-level clubs, coordination was established with the technical staffs. The athletes were provided with detailed information regarding the study protocol, and a 'Written Informed Consent Form' was obtained from each participant before their inclusion in the study.

To ensure a homogeneous study group, the following criteria were applied for participant selection

Inclusion Criteria: Participants were required to be professional football players active in the TFF 1st, 2nd, or 3rd Leagues; have participated in at least 80% of the team's seasonal training sessions; and be aged between 18 and 35.

Exclusion Criteria: Athletes with any musculoskeletal injuries within the last six months, those using performance-enhancing supplements or medications that might affect muscle damage markers (e.g., NSAIDs, antioxidants), and those who did not complete the entire Yo-Yo Intermittent Recovery Test protocol were excluded from the analysis.

Training Routine and Seasonal Period

The study was conducted during the competitive phase (in-season) of the football calendar. The participants' weekly training schedule typically comprised an average of six days, encompassing daily sessions of 90–120 minutes that included variations in endurance, speed, and technical-tactical workloads. Data collection took place during the final weeks of the first half of the league season—a period characterized by high levels of physical adaptation and optimized conditioning among the players.

Yo-Yo Intermittent Recovery Test Protocol

The aerobic performance capacities of the participants were determined using the Yo-Yo Intermittent Recovery Test Level 1 (YYIR1) protocol, administered according to the standardized procedures established by Bangsbo et al. (2008). The test consisted of repeated 2×20-meter shuttle runs at progressively increasing speeds, regulated by calibrated audible cues. Following each 40-meter bout (out and back), participants were allotted a 10-second active recovery period within a designated 5-meter (2.5m×2) zone. The test commenced at an initial speed of 10 km/h. Following the initial stages, speed increments were strictly regulated according to the YYIR1 protocol (e.g., 10.5 km/h, 11 km/h, 11.5 km/h, etc.), with stages increasing as defined in the literature. Participants were instructed to reach the finish line in synchronization with the acoustic signals, and the test was terminated if a participant failed to reach the line twice. The total distance covered (meters) was recorded as the final performance measure. All testing procedures were conducted on a natural grass pitch to ensure ecological validity (Totsuka et al., 2002). Estimated maximal oxygen uptake (VO₂max) was calculated from the total distance covered using the following standardized regression formula:

$$\text{VO}_2 \text{ max(ml/kg/min)} = [\text{distance(m)} \times 0.0084] + 36.4$$

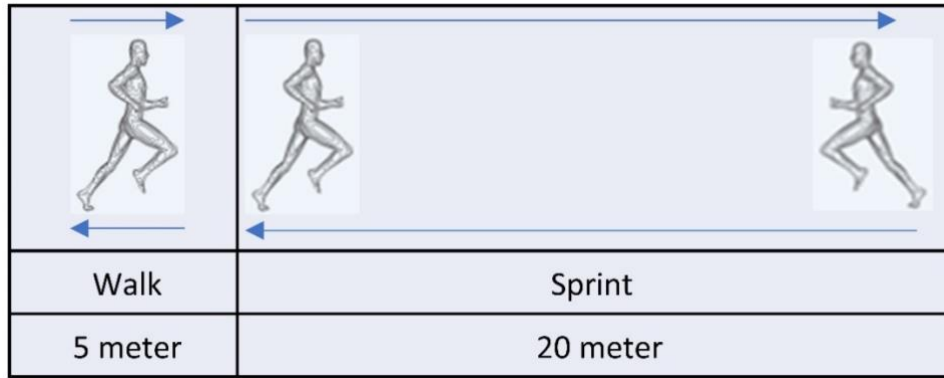


Figure 2. Yo-yo Intermittent Recovery Test Soccer

Data Collection and Biochemical Analysis

All physical and physiological parameters were measured in the performance laboratories of the respective football clubs. To evaluate exercise-induced muscle damage, 5-ml venous blood samples were collected from each participant exactly 24 hours after the Yo-Yo test protocol. The 24-hour post-exercise period for blood collection was specifically chosen based on the physiological kinetics of Creatine Kinase (CK). According to existing literature, CK levels do not peak immediately after strenuous activity; instead, they show a delayed increase as the enzyme gradually leaks from the damaged muscle fibers into the bloodstream. Peak serum CK activity typically occurs between 24 and 48 hours following eccentric-heavy or high-intensity intermittent exercise (Totsuka et al., 2002; Baird et al., 2012). Therefore, collecting samples at the 24-hour mark allowed for the objective assessment of the acute delayed-onset muscle damage response across different league levels.

Blood sampling was conducted during the morning hours while athletes were in a fasted and resting state. Samples were drawn from the antecubital vein by certified healthcare professionals and transferred into vacuum gel tubes. Biochemical markers were analyzed in the clinical laboratories of the following affiliated institutions: Private Konak Hospital (Kocaeli), Private Elit Park Hospital (Çorum), and Karşıyaka Medical Point Hospital (İzmir). Creatine Kinase (CK) levels were determined and reported in units per liter (U/L) using standardized enzymatic methods to ensure data reliability (Brancaccio et al., 2007).

Physical Parameters / Anthropometric Measurements

The anthropometric measurements of the participants were conducted under standardized conditions. Stature (height) was measured using a manual stadiometer with a precision of 0.1 cm while the soccer players were barefoot and dressed in lightweight sports attire, such as shorts and T-shirts (Şenel et al., 2009). Following the stature assessment, body mass was determined using a Tanita BC-418 Segmental Body Composition Analyzer. To ensure maximum measurement accuracy and

reliability, all body mass assessments were performed with participants barefoot and in minimal clothing. All anthropometric evaluations were carried out by the same researcher to eliminate inter-observer variability and ensure data consistency across all study groups (Şenel et al., 2009).

Statistical Analysis

Statistical analyses were performed using SPSS software (Version 24.0). The normality of the data distribution was assessed and confirmed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Additionally, the homogeneity of variances was evaluated using Levene's test to meet the required assumptions for parametric analysis ($p > 0.05$). Descriptive statistics were expressed as means and standard deviations (\pm SD). A One-Way Analysis of Variance (ANOVA) was employed to determine statistically significant differences in continuous variables—including age, height, body weight, and Creatine Kinase (CK) levels—among the soccer players across the three different league levels. In addition to null hypothesis significance testing, partial eta-squared (η^2) was calculated to determine the effect size. Values of 0.01, 0.06, and 0.14 were used to define small, medium, and large effects, respectively. The level of statistical significance was set at $p < 0.05$ for all analyses.

Ethical Considerations

The study protocol was reviewed and approved by the Manisa Celal Bayar University Non-Interventional Clinical Research Ethics Committee (Decision Date: 20.12.2023; Decision No: 20.478.486/2146). The research was conducted in strict accordance with the Helsinki Declaration and the Directive on Scientific Research and Publication Ethics of Higher Education Institutions. All participants were comprehensively informed about the study's objectives, experimental procedures, and potential risks regarding muscle injury profiling. Participation was entirely voluntary, with the explicit right to withdraw from the study at any stage without any penalty. To ensure data privacy, all personal information was anonymized and kept strictly confidential. Written informed consent was obtained from each athlete prior to the commencement of the data collection process.

Results

The anthropometric and physiological characteristics of the professional soccer players, categorized by their respective league levels, are presented in Table 1. The mean height of the entire study population was 179.06 ± 5.96 cm, while the mean body weight was determined to be 74.64 ± 5.01 kg.

Anthropometric and Performance Parameters

One-way ANOVA results revealed no statistically significant differences between the 1st, 2nd, and 3rd League players in terms of height ($F=0.336, p=0.715$) or body weight ($F=0.585, p=0.560$). Similarly, performance-related parameters including distance covered ($F=0.420, p=0.637$) and estimated Max VO₂ levels ($F=0.510, p=0.590$) did not show significant variation across the three competitive levels.

Table 1

Comparison of Anthropometric and Performance Parameters Across League Levels Mean Body Weight and Height of the Athletes Who Participated in the Study (n=75)

Parameters	League Level	n	Mean±sd	F	p
Height (cm)	1st League	26	179.80±6.01cm	0.336	0.715
	2nd League	25	178.88±6.13cm		
	3rd League	24	178.44±5.90cm		
	Total	75	179.06±5.96cm		
Body Weight (kg)	1st League	26	74.99±3.87kg	0.585	0.560
	2nd League	25	75.16±6.38kg		
	3rd League	24	73.73±4.54kg		
	Total	75	74.64±5.01kg		
Distance covered	1st League	26	3240±6.72m	0.420	0.637
	2nd League	25	3360±5.73m		
	3rd League	24	3080±4.84m		
	Total	75			
Max VO₂	1st League	26	65.4±6.44ml/kg/min	0.510	0.590
	2nd League	25	66.2±5.42ml/kg/min		
	3rd League	24	62.4±4.32ml/kg/min		
	Total	75			

Table 1 presents the comparative analysis of anthropometric and physiological performance parameters among professional soccer players across different league levels. The statistical evaluation via One-Way ANOVA indicated that there were no significant differences between players in the 1st, 2nd, and 3rd Leagues regarding height ($F=0.336, p=0.715$) and body weight ($F=0.585, p=0.560$). Furthermore, performance metrics obtained from the Yo-Yo Intermittent Recovery Test, specifically total distance covered ($F=0.420, p=0.637$) and estimated Max VO₂ ($F=0.510, p=0.590$), showed no statistical variance between groups. These results suggest that the physical profiles and aerobic capacities of the participants were homogeneous across the professional tiers studied * $p<0.05$

Biochemical Analysis of Muscle Damage

The comparison of Creatine Kinase (CK) levels as a marker of exercise-induced muscle damage is illustrated in Table 2. The average CK level for the total sample 24 hours post-exercise was 453.93 ± 281.61 U/L. Statistical analysis indicated that there was no significant difference in CK responses between the league levels ($F=0.102, p=0.903$). The mean values for the 1st, 2nd, and 3rd Leagues were 462.69 ± 288.78 U/L, 433.00 ± 335.97 U/L, and 466.25 ± 214.46 U/L, respectively. These findings suggest that the acute biochemical response to the standardized Yo-Yo Intermittent Recovery Test is consistent among professional soccer players, regardless of their competitive tier.

Table 2
Comparison of Creatine Kinase (CK) Levels Across League Levels

Parameters	League Level	n	Mean±sd	F	p	η ²
CK (U/L)	1st League	26	462.69 ± 288.78 U/L	0.102	0.903	0.003
	2nd League	25	433.00 ± 335.97 U/L			
	3rd League	24	466.25 ± 214.46 U/L			
	Total	75	453.93 ± 281.61 U/L			

The comparison of serum Creatine Kinase (CK) levels, measured 24 hours post-exercise as a marker of acute muscle damage, is summarized in Table 2. The analysis showed that 1st League players had a mean CK level of 462.69 ± 288.78 U/L, 2nd League players 433.00 ± 335.97 U/L, and 3rd League players 466.25 ± 214.46 U/L. The overall mean CK concentration for all participants was 453.93 ± 281.61 U/L. Statistical analysis revealed no significant difference in muscle damage responses between the league levels ($F=0.102, p=0.903, \eta^2 = 0.003$), indicating that the biochemical response to the standardized Yo-Yo test protocol was similar across all competitive professional divisions and that the practical significance of the difference was negligible.

Discussion and Conclusion, Recommendations

In this study, acute muscle damage profiles following the Yo-Yo Intermittent Recovery Test were compared among professional soccer players competing in the TFF 1st, 2nd, and 3rd Leagues. The prevention and management of muscle damage are of paramount importance for protecting athlete health and ensuring career longevity in soccer. The primary finding of our study is that there were no statistically significant differences in Creatine Kinase (CK) responses among soccer players from different leagues following a high-intensity protocol such as the Yo-Yo test.

According to the research findings, while the Creatine Kinase (CK) values of players in the 1st League (462.69 U/L) and 3rd League (466.25 U/L) followed a remarkably similar course, it was observed that players in the 2nd League (433.00 U/L) exhibited lower values. Although not statistically significant, it is suggested that this relative decrease in the 2nd League stems from training loads, match surface types, recovery periods, and individual differences. This observation is further supported by the Max VO₂ levels and total distance covered during the Yo-Yo test. Existing literature indicates that training level, along with exercise intensity, duration, and contraction type, are decisive factors in the extent of muscle damage. Indeed, Baird et al. (2012) demonstrated that low-intensity training induces less muscle damage compared to maximal eccentric training when performed with identical sets and repetitions. Creatine Kinase (CK) is an enzyme located in the cytosol of muscle cells that plays a critical role in energy metabolism, specifically within the ATP-CP system. During exercise, mechanical stress and the accumulation of metabolic waste within muscle fibers increase the permeability of the sarcolemma (the muscle cell membrane), leading to the leakage of this enzyme into the bloodstream. The absence of a statistically significant difference between leagues in our study ($p=0.903$) can be explained by several key mechanisms at the cellular level (Kurapati et al. 2026). In terms of Sarcolemmal Integrity and Mechanical Stress: High-intensity activities such as soccer, particularly eccentric contractions involving sudden stops and changes of direction, cause micro-trauma to the Z-discs within the sarcomere structure. However, in professional soccer players, the 'repeated bout effect'—which develops through regular training—strengthens the muscle cell cytoskeleton (specifically desmin and titin proteins) and preserves sarcolemmal integrity. The fact that participants in this study were professional athletes with similar physical profiles (VO₂ max) indicates that cell membrane durability has reached a comparable level of adaptation, regardless of league division (Marcus et al. 2021). In terms of Metabolic Adaptation and CK Release: Creatine Kinase (CK) leakage is dependent not only on mechanical damage but also on the disruption of intracellular calcium (Ca²⁺) homeostasis. During high-intensity exercises such as the Yo-Yo test, the accumulation of intracellular Ca²⁺ activates proteolytic enzymes, thereby triggering membrane damage. The homogeneous results obtained in our study suggest that athletes in all three leagues possess a similar metabolic recovery capacity and have developed comparable physiological adaptations in maintaining intracellular ionic balance (Bair et al. 2012).

The wide standard deviations observed in the Table 2 data of our study (e.g., 433.00±335.97 U/L for the 2nd League group) reveal that the muscle damage response following the Yo-Yo test in professional soccer players exhibits an extremely individual character. This situation is directly associated with the phenomenon defined in the literature as 'High Responders' and 'Low Responders'. According to Brancaccio et al. (2007), serum CK levels are influenced by various genetic and physiological factors that can be considered an individual's 'biochemical signature,' extending beyond

age, gender, and training status. As emphasized by Brancaccio et al. (2007), serum CK levels are influenced by numerous genetic and physiological factors that can be considered an individual's 'biochemical signature,' extending beyond age, gender, and training status. Serum CK levels correlate with total muscle mass and the predominant muscle fiber type. Type II (fast-twitch) fibers, due to their high glycolytic capacity and greater sensitivity to eccentric contractions, tend to release more CK into the bloodstream in the event of damage. In this context, the lack of a statistically significant difference between the leagues ($p=0.903$) confirms that the players in all three leagues exhibit a professional profile with similar muscle mass and fiber distribution adaptations. One of the most fundamental causes of individual variation is genetic differences. In particular, polymorphisms in the alpha-actinin-3 (ACTN3) gene directly affect Z-disc stability and, consequently, the CK response to mechanical stress. In 'high responder' athletes, this genetic structure may lead to a more rapid increase in the permeability of the cell membrane (sarcolemma) (Clarkson et al., 2005; Massidda et al., 2019). Since Creatine Kinase (CK) is a large molecule (approximately 86 kDa), it cannot pass directly into the capillaries; instead, it is primarily transported via the lymphatic system. Variations in the clearance rate among athletes constitute a significant biochemical mechanism that explains the wide standard deviations observed in serum levels at the 24-hour mark. As highlighted by Brancaccio et al. (2007), trained individuals exhibit significantly lower peak CK levels compared to untrained individuals due to the 'repeated bout effect'. The fact that all athletes in our study are of professional status suggests that this protective adaptation (blunted response) developed similarly across all leagues, indicating that cellular adaptation capacity is the primary determinant rather than the league level. Consequently, the observation that CK values in the 2nd League were slightly lower than in other leagues—without reaching statistical significance—stems from individual biochemical response differences and homogeneous physical performance levels among participants rather than a league hierarchy.

In the present study, the collection of blood samples at the 24-hour mark was critical for determining the acute phase of muscle damage. Şenel and Akyüz (2010) reported that Creatine Kinase (CK) levels begin to rise at the end of a competition and reach their peak at 24 hours post-exercise. However, it is also documented in the literature that CK peak values can vary between 1 and 5 days depending on the specific type of training performed. The lack of significant differences between the leagues in our study may be attributed to the substantial influence of individual variability during periods of intense training, as emphasized by Lazarim et al. (2009). In high-tempo games like soccer—which involve sudden changes of direction, stopping, and sprinting the impact of explosive movements on muscle damage can exhibit significant person-to-person variation.

The similarity in the mean ages of the participants (1st Division: 28.50 ± 4.25 ; 2nd Division: 26.56 ± 5.70 ; 3rd Division: 27.12 ± 3.45 years) may have played a role in the emergence of comparable biochemical responses. Age is one of the most critical factors upon which personalized training programs aimed at preventing muscle damage are based. Plews et al. (2014) found that younger athletes exhibited higher CK levels when compared across the same training frequency and intensity, likely due to the greater muscle mass density typically found in younger individuals. The fact that the athletes in our study belong to a mature age group and operate at a professional level suggests that they may have developed a specific 'repeated bout effect' against muscle damage.

One of the underlying assumptions of this study was that players in higher leagues are exposed to greater chronic physical stress due to more intensive training schedules and match demands. While our study did not utilize objective workload monitoring tools such as Global Positioning Systems (GPS) or heart rate telemetry to quantify these differences—which remains a limitation—the literature supports the notion of a performance hierarchy across professional tiers. Research by Mohr et al. (2003) and Di Salvo et al. (2013) has consistently demonstrated that elite-level soccer players cover significantly more high-intensity running and sprinting distances during matches compared to their lower-league counterparts. Therefore, it was anticipated that the cumulative effect of these higher chronic loads in the 1st League might lead to a more pronounced acute muscle damage response (CK elevation) following a standardized stressor like the Yo-Yo test. However, our findings ($p=0.903$) suggest that despite these presumed differences in competitive intensity, the cellular adaptation and muscle recovery mechanisms across the three professional leagues appear to have reached a similar physiological threshold.

Balnave and Thompson (1993), examined 10 soccer players with an average age of 16 years in their study. The athletes trained for 8 weeks and 4-6 times per week for an average of 60-90 minutes. As a result of the study, it was reported that the athletes' CK levels decreased.

Khaitin et al. (2021), investigated the effect of exercise-related muscle damage in a group of 30 professional soccer players during the competition period, found that muscle damage caused by intense exercise significantly affects the strength of the adductor muscle and drew attention to the importance of the strength of the adductor muscle, as this muscle group has a critical function in stabilizing explosive movements such as changes in direction, sudden stops and sprinting, which are considered basic movements in soccer, a high-tempo game.

Limitations of the Study

Despite the significant insights provided regarding the muscle damage profiles of professional soccer players, this study has several limitations that should be considered when interpreting the results. First, the sample size consisted of 75 participants across three leagues; a larger cohort might

be necessary to identify more subtle statistical trends between competitive levels. Second, blood samples were collected only at the 24-hour mark to determine acute responses, which may not fully capture the complete kinetic profile of Creatine Kinase (CK) as peak values can occur between 1 and 5 days depending on individual and exercise-specific factors. Furthermore, the absence of baseline (pre-exercise) CK measurements constitutes a significant methodological limitation. Due to the intensive competitive schedules of the professional teams, the study focused solely on the 24-hour post-exercise response rather than the relative change from baseline. Consequently, the lack of direct control over pre-test factors such as sleep quality, precise nutritional intake, and caffeine consumption should be noted, although all participants were professional athletes following standardized club routines. Third, while efforts were made to ensure a homogeneous group through strict inclusion and exclusion criteria, inherent individual variables such as genetic predisposition, muscle fiber type distribution, and recovery quality were not fully controlled and may have contributed to the high variability in CK responses. Additionally, environmental factors and training surfaces, which can influence mechanical stress, were not standardized across the different clubs. Finally, as the study focused exclusively on professional male soccer players, the findings may not be generalizable to female athletes or amateur populations

Conclusion and Future Directions

This research demonstrates that professional athletes competing at different professional levels exhibit similar acute muscle damage responses to a standardized high-intensity intermittent loading protocol. The obtained data indicate that differences in league levels do not have a decisive influence on the degree of muscle damage when the same test protocol is applied. It is concluded that variations in Creatine Kinase (CK) levels are shaped by factors such as individual training status, exercise intensity, and the type of muscle contraction rather than a league-based hierarchy. Furthermore, the fact that athletes possess similar physical profiles and aerobic capacities proves that conditioning standards converge across all levels of professional soccer. Looking forward, future studies should incorporate longitudinal monitoring of muscle damage markers throughout an entire competitive season to better understand the cumulative effects of match and training loads. Additionally, investigating the role of genetic polymorphisms and specific recovery interventions across different league tiers could provide deeper insights into personalizing athlete care and optimizing performance longevity.

Abbreviations

CK: Creatine Kinase

Ethics Committee Approval Information

Ethics review board: Manisa Celal Bayar University, Faculty of Medicine, Health Sciences Ethics Committee

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Author Contributions Statement

All authors contributed to all stages of the study, including the methodology, results, introduction, discussion, and conclusion sections.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding this study.

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