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Research article

How Does Altering the Volume-Load of Plyometric Exercises Affect the Inflammatory Response, Oxidative Stress, and Muscle Damage in Male Soccer Players?

Jian Tian and Miao Miao ✉
School of Physical Education, Henan University, Kaifeng, China

Abstract

Incorporating plyometric exercises (PE) into soccer players' conditioning routines is vital for boosting their performance. Nevertheless, the effects of PE sessions with diverse volume loads on inflammation, oxidative stress, and muscle damage are not yet clearly understood. This study aimed to examine the effects of altering the volume-loads of PE on indicators of oxidative muscle damage and inflammation. The study involved forty young male soccer players who were randomly assigned to three different volume-loads of PE (Low volume-load [100 jumps]: LVL, n = 10; Moderate volume-load [150 jumps]: MVL, n = 10; and High volume-load [200 jumps]: HVL, n = 10) and a control group (CON = 10). The levels of various biomarkers including delayed onset muscle soreness (DOMS), serum lactate dehydrogenase (LDH), creatine kinase (CK), 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), protein carbonyl (PC), leukocytes, neutrophils, interleukin-6 (IL-6), and C-reactive protein (CRP) were measured at different time points. These measurements were taken at rest, immediately after completion of PE, and 24-, 48-, and 72-hours post-PE. The CK, LDH, DOMS, 8-OHdG, MDA, and PC levels were significantly increased ($p < 0.05$) after the PE protocol, reaching their peak values between 24 to 48 hours post-PE for all the volume-loaded groups. The levels of leukocytes, neutrophils, and IL-6 also increased after the PE session but returned to resting values within 24 hours post-PE. On the other hand, CRP levels increased at 24 hours post-PE for all the treatment groups ($p < 0.05$). The changes observed in the indicators of muscle damage and inflammation in response to different volume-loads of PE was not significant. However, the HVL and MVL indicated significant differences compared to LVL in the 8-OHdG (at 48-hour) and MDA (at 72-hour). Athletes engaging in higher volume-loads demonstrated more pronounced responses in terms of biochemical variables (specifically, LVL < MVL < HVL); however, these changes were not statistically significant (except 8-OHdG and MDA).

Key words: EIMD, eccentric exercise, inflammation, WBC.

Introduction

Soccer is a team sport characterized by intermittent activity, often involving explosive movements such as jumping, accelerating, and short-distance sprints (Helgerud et al., 2001; Douchet et al., 2023; Mitrousis et al., 2023). Plyometric training (PT) is commonly used to improve the performance of soccer players by enhancing their vertical jump, strength, agility, and sprint performance (Ramirez-Campillo et al., 2018). The PT method, commonly referred to as the stretch-shortening cycle, entails expeditious eccentric movement succeeded by swift concentric movement within the identical muscle-tendon unit, which is highly beneficial for training soccer players (Ramirez-Campillo et al., 2018). However, it is important to note that eccentric actions during PT can lead to skeletal muscle trauma, increased muscle soreness, elevated levels of creatine kinase activity and C-reactive protein, as well as an increase in circulating neutrophils, and myeloperoxidase (Arazi et al., 2016; 2019). These factors contribute to producing free radicals, oxidative stress, and inflammation.

The magnitude of inflammation, oxidative stress, and muscle damage is contingent upon a multitude of training variables, including type of exercise, training load, repetition, intensity of training, and allocation of rest periods (Nikolaidis et al., 2008; Margonis et al., 2007; Mirzaei et al., 2014; Rasouli mojez et al., 2021, Sayevand et al., 2022). Regarding exercise training volume-load, established guidelines suggest that a suitable volume-load for producing positive effects in performance adaptations is 100 jumps per training session (Ramirez-Campillo et al., 2020; Asadi et al., 2016). In contrast, strength and conditioning coaches design various PT regimens during training sessions in soccer players conditioning schedule, wherein athletes may experience varying biochemical reactions based on the different volume-loads assigned in their training program. It is critical to closely monitor indicators of muscle damage and oxidative stress to prevent the symptoms associated with overreaching or overtraining (Margonis et al., 2007; Nikolaidis et al., 2008). While the optimal design of PT sessions has the potential to enhance the performance of soccer players (Ramirez-Campillo et al., 2018), a substantial augmentation in volume-load without sufficient recovery can induce mild trauma, thereby increasing inflammation and the onset of overtraining syndrome (Arazi et al., 2016; Nikolaidis et al., 2008). In a comprehensive experimental investigation conducted by Margonis et al. (2007), the authors revealed a significant correlation between an augmented resistance training volume-load and a decline in performance, accompanied by an elevation in oxidative stress biomarkers. Furthermore, this increase in biomarkers was found to be directly associated with the rise in training volume-loads. Consequently, the monitoring of biochemical variables at varying volume-loads of training is essential, and enables coaches to modify the training program, ultimately leading to the optimization of athletic performance (Sheykhlouvand et al., 2022).

To date, little is known about the effects of plyometric exercise (PE) volume-loads on the symptoms of muscle damage, especially in male soccer players, wherein PT plays an essential role in improving their performance ad-

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aptations. As far as our knowledge, numerous studies have investigated the implications of various elements of PE (i.e., intensity, rest interval, and PT surface) on inflammation and muscle damage (Nikolaidis et al., 2008; Twist and Eston, 2005; 2007; 2009; Chatzinikolaou et al., 2010). However, there have been no recent studies that have explored the impact of various PE volume-loads (i.e., sets × repetitions × intensity) on indicators of muscle damage, oxidative stress, inflammation, and leukocytes in soccer players. Therefore, the primary aim of the present study was to evaluate the impact of low-, moderate, and high volume-loads of PE on markers of muscle damage, oxidative stress, and inflammation in young male soccer players. This investigation sought to clarify the influence of PE volume-loads on the monitoring of inflammation and muscle damage and clarify time-course changes during the following days in this specific population.

Methods

Participants

Using the G*Power software, the estimated sample size for each group was determined to be 10 participants, based on an alpha level of 0.05 and a beta of 0.8 (Gonzalez et al., 2014). The study involved the participation of forty young male soccer players who had similar soccer training habits (Table 1). The players were matched based on their playing position and were subsequently assigned to one of four groups: Low volume-load (LVL, n = 10), Moderate volume-load (MVL, n = 10), High volume-load (HVL, n = 10), or a control condition (CON, n = 10). All participants were familiar with PT but had not engaged in this training for at least six months before the study. Participants were excluded if they had experienced upper or lower body injuries within three months prior to their inclusion or any medical or orthopedic issues that could impact their participation or performance. The participants were instructed to abstain from engaging in any physical exercise for a minimum of seven days prior to the commencement of the experiments. The participants were instructed on the study protocol and provided with comprehensive information concerning the nature of the study and its objectives. All participants provided informed consent and volunteered to participate. The study was conducted by the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Chuzhou University.

Experimental design

This study employed a randomized-control design. Participants attended the laboratory on five separate occasions to assess the impact of different volume-loads of PE on symptoms related to muscle damage, oxidative stress, and inflammation. During the initial visit, the subjects' height was measured using a wall-mounted stadiometer (Bodymeter, Seca, Germany), and their body mass was measured using a digital scale (Ironman Body Composition Monitor, USA). Additionally, the participants were instructed on the proper technique for performing plyometric vertical jumps (i.e., to achieve 90° of knee flexion), and the maximal vertical jump was assessed (Arazi et al., 2019; Twist and Eston, 2007). One week following the initial visit, the players were recruited to the laboratory (Sheykhlouvand et al., 2016) and performed PE (see more details in the Plyometric exercise section). The CON group remained in the laboratory without engaging in any physical exercise (Fereshtian et al., 2017). Blood samples were collected before and after the PE session. Subsequent visits to the laboratory occurred at 24 hours (24 h-post), 48 hours (48 h-post), and 72 hours (72 h-post) post-PE to evaluate delayed onset muscle soreness (DOMS), levels of lactate dehydrogenase (LDH), creatine kinase (CK), 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), protein carbonyl (PC), leukocytes, neutrophils, interleukin-6 (IL-6), and C-reactive protein (CRP) (Figure 1). Participants were asked to avoid rigorous physical activity (Sheykhlouvand et al., 2018a), adhere to their regular diet 24 hours before the test (Sheykhlouvand and Forbes 2017), and abstain from caffeine and alcohol (Gharaat et al., 2020; Barzegar et al., 2021).

Table 1. Subject characteristics (mean ± SD).

Groups	N	Age (y)	Weight (kg)	Height (cm)	Training age (y)	Maximal vertical jump (cm)
LVL	10	20.1 ± 0.9	79.1 ± 3.5	178.9 ± 3.6	5.3 ± 1.1	48.3 ± 3.9
MVL	10	20.9 ± 0.8	78.9 ± 2.7	179.1 ± 3.2	5.6 ± 1.6	45.6 ± 4.7
HVL	10	20.7 ± 0.6	78.1 ± 4.8	179.6 ± 3.4	5.4 ± 1.3	47.4 ± 4.8
CON	10	20.5 ± 0.8	79.4 ± 2.2	180.4 ± 5.1	5.9 ± 0.9	47.9 ± 4.2

LVL: low volume-load plyometric exercise, MVL: moderate volume- load plyometric exercise, HVL: high volume-load plyometric exercise, CON: control condition.

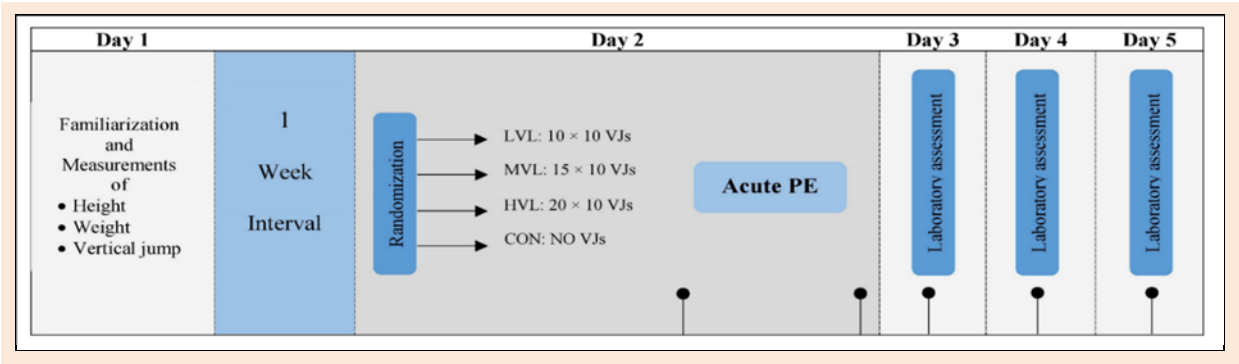

Figure 1. Experimental design. Blood samples and muscle soreness measurements at pre, 30-min post, 24-h post, 48-h post and 72-h post plyometric exercise (PE).

Table 2. Evaluation of dietary consumption in the three days prior to the exercise session (mean ± SD).

Groups	Energy intake (Kcal)	Carbohydrate (g)	Fat (g)	Protein (g)	Vitamin E (mg)	Vitamin C (mg)
LVL	2660 ± 341	353 ± 35	83 ± 17	125 ± 12	8.4 ± 1.8	68 ± 27
MVL	2495 ± 348	328 ± 27	79 ± 20	118 ± 15	8.5 ± 1.7	65 ± 24
HVL	2580 ± 366	349 ± 38	78 ± 18	120 ± 13	7.7 ± 1.4	67 ± 28
CON	2730 ± 317	378 ± 22	85 ± 21	117 ± 10	7.9 ± 1.6	67 ± 21

LVL: low volume-load plyometric exercise, MVL: moderate volume- load plyometric exercise, HVL: high volume-load plyometric exercise, CON: control condition.

Diet control

To control any potential interference from diet, the participants were required to provide a detailed account of their dietary intake for three days before their inclusion in the study. Using the Nutritionist IV diet analysis software, the participants' total calorie, protein, carbohydrate, fat, vitamin C, and vitamin E intake were analyzed using SAS 9.4 software (SAS Institute, Cary, NC, USA), as presented in Table 2.

DOMS measurement

Each participant in the study assessed the soreness of their leg muscles by performing 1 active squat exercise until their knees reached an approximate angle of 90°. Subsequently, participants were requested to indicate the degree of muscular soreness experienced in their knee extensors using a visual analog scale. This scale ranged from 1 to 10, with 1 representing no muscle soreness and 10 indicating extreme soreness. A picture of the muscle was inserted to enhance the scale's accuracy. This method has proven effective in previous research (Naughton et al., 2018). The reliability coefficient for repeated measurements of muscle soreness was 0.98.

Blood sampling and analysis

The participants were instructed to arrive at the laboratory after a 12-hour fast, and 8 hours of sleep, specifically between the hours of 9 and 11 A.M. This was confirmed through a personal interview prior to the commencement of measurements. Blood samples were collected from the antecubital vein using plain evacuated test tubes with a volume of 10 cc (Sheykhlouvand et al., 2018b). The blood was allowed to clot at room temperature for 30 minutes and then centrifuged at 1500 × g for 10 minutes. The serum layer was extracted and stored in multiple aliquots at a temperature of -20 ◦C for further analysis. The serum CK (Milton Roy, NY, USA) and LDH (MAK, Sigma Diagnostics, USA) were determined using a commercially available kit through spectrophotometry in duplicate. The CRP (DBC, Austria) was determined using the enzyme-linked immunosorbent assay (ELISA) method. The serum 8-OHdG, MDA, and PC levels were measured using commercially available ELISA kits (ZellBio, Germany). Using an automated hematology analyzer (PocH100i, Kobe, Japan), complete blood cell analyses were conducted to count leukocytes and neutrophils. The IL-6 (R&D Systems Inc. UK) was determined using a commercially available ELISA kit with a spectrophotometric plate reader (Dynex Technologies 268 Inc. USA). Pre-exercise (Pre) blood samples were collected following a 15-minute equilibration period, while post-exercise (Post) blood samples were taken 30 minutes after exercise cessation in the supine position. Blood samples were also collected at 24, 48, and 72-h post-PE, following a 1-minute equilibration period (Townsend et al., 2013). The coefficient of variation for all blood measurements was less than 7%.

Plyometric exercise

Following a 10-minute warm-up (i.e., 5 minutes of cycling and 5 minutes of stretching movements), players in each group performed their exercise programs. The LVL group performed a total of 100 jumps, divided into 10 sets of 10 repetitions, during their maximal-effort vertical countermovement jumps. On the other hand, the MVL group completed 150 jumps, spread across 15 sets of 10 repetitions. Lastly, the HVL group accomplished 200 jumps, split into 20 sets of 10 repetitions. Meanwhile, the CON group stayed in the laboratory and did not participate in any physical exercise. The participants assigned to the experimental groups were provided with explicit instructions to exert their utmost effort during each jump, aiming to attain the highest possible height. Following the landing phase, participants were directed to flex their knee joint to an angle of 90° (Twist and Eston, 2007). A one-minute rest interval was implemented between each set to allow for recovery. Throughout the exercise session, participants received verbal encouragement. An experienced strength and conditioning coach monitored all exercise protocols. The laboratory maintained a temperature and humidity level of 27-28° and between 40 to 50% during all visits, respectively.

Statistical analysis

The data are presented as mean ± standard deviation (SD). Before statistical comparisons, all data were checked by the Shapiro-Wilk test to assess normal distribution. To determine the existence of significant differences between the four groups, a 4 × 5 (group × time) repeated-measures analysis of variance (ANOVA) was performed for each variable tested. In cases where a significant F value was obtained, the Bonferroni post-hoc test was utilized to identify differences between the groups. Additionally, a one-way ANOVA was conducted to compare the area under the curve (AUC) for all assessments (Gonzalez et al., 2014). The significance level was set at 0.05.

Results

There were significant increases in serum CK and LDH levels and DOMS following the PE protocol in all treatment groups, with the highest values observed at 24-h post-PE ($p < 0.05$). A significant group × time interaction was observed for CK ($F = 44.52$, $p = 0.001$), LDH ($F = 6.26$, $p = 0.001$), and DOMS ($F = 46.55$, $p = 0.001$), indicating a greater increase in CK, LDH, and DOMS for the treatment groups compared to the CON group after the PE. Although the HVL indicated a higher level of changes in muscle damage indicators in comparison to the MVL and LVL, these differences did not reach statistical significance

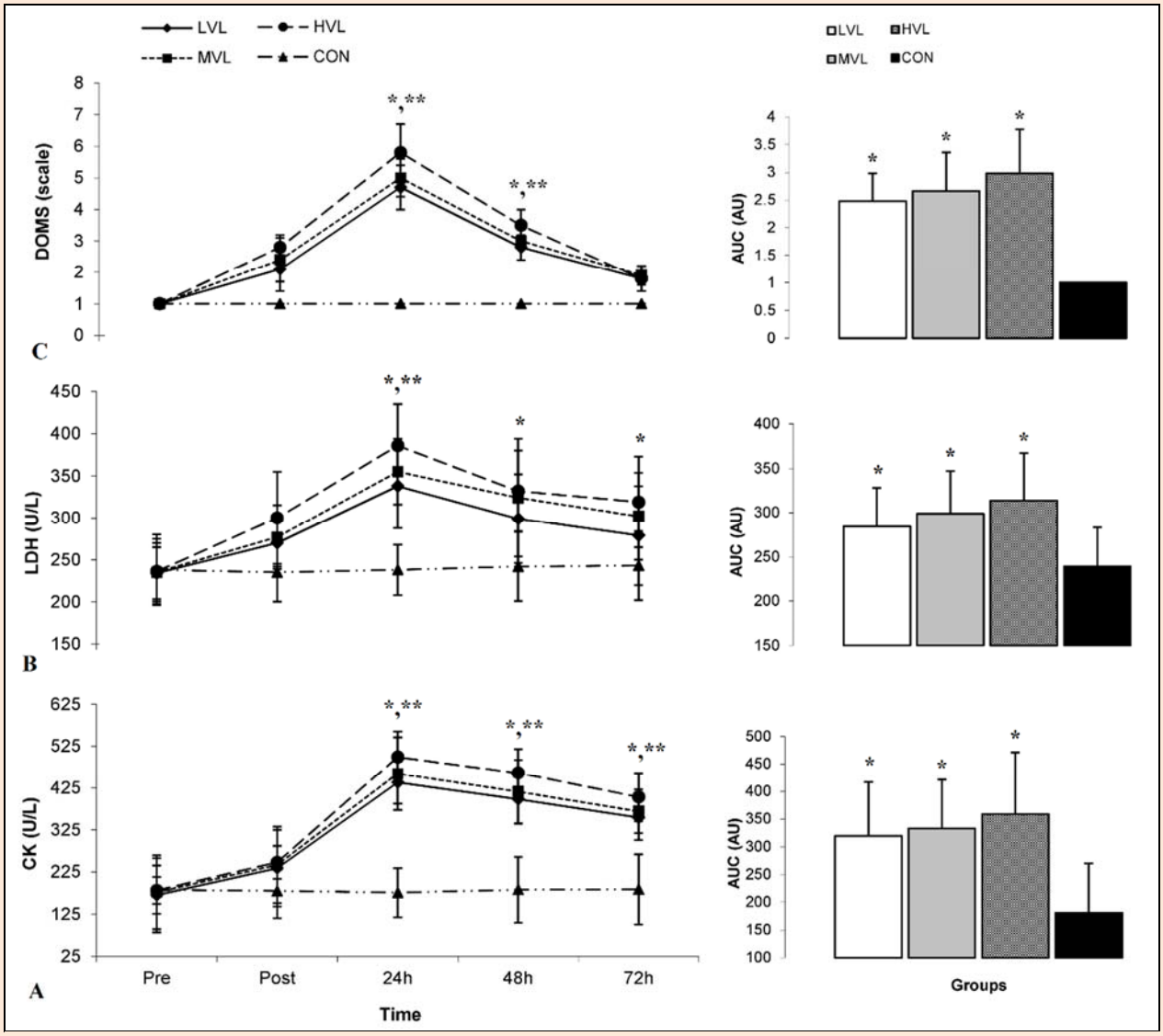

Figure 2. Time course changes in muscle damage (mean ± SD). LVL: low volume-load plyometric exercise, MVL: moderate volume-load plyometric exercise, HVL: high volume-load plyometric exercise, CON: control condition. * Significant differences compared to pre and CON for all experimental groups ($p \leq 0.05$), ** Significant differences compared to post for all experimental groups ($p \leq 0.05$). In AUC, * denotes significant differences compared to CON for all experimental groups ($p \leq 0.05$).

($p > 0.05$). The analysis of the AUC for CK, LDH, and DOMS demonstrated greater increases in the treatment groups compared to the CON group ($p = 0.001$) (Figure 2; A, B, and C). There was a significant increase in serum 8-OHdG levels following the PE protocol in all treatment groups, with the highest values observed at 24-h post-PE ($p < 0.05$). However, at 48-h post-PE, the MVL and HVL groups still exhibited higher 8-OHdG levels compared to the pre-treatment levels ($p < 0.05$). A significant group × time interaction was observed for 8-OHdG ($F = 22.06$, $p = 0.001$), indicating a greater increase in 8-OHdG for the treatment groups compared to the CON group at post and 24-h post-PE. In addition, the MVL and HVL indicated significant differences compared with LVL and CON groups at 48-h post-PE. The analysis of the AUC for 8-OHdG demonstrated only greater increases for the MVL and HVL groups compared to the CON group ($p = 0.02$) (Figure 3, A).

There was a significant increase in serum MDA levels following the PE protocol in all treatment groups, with the highest values observed for the LVL group at 24-h post-PE and for the MVL and HVL groups at 48-h post-PE ($p < 0.05$). However, at 72-h post-PE, the LVL indicated declines until pre-value; the MVL and HVL groups still exhibited higher MDA levels compared to the pre-test levels ($p < 0.05$). A significant group × time interaction was observed for MDA ($F = 8.44$, $p = 0.001$), indicating a greater increase in MDA for the treatment groups compared to the CON group at 24-h and 48-h post-PE. In addition, the MVL and HVL groups indicated significant differences compared to LVL and CON groups at 72-h post-PE. The analysis of the AUC for MDA demonstrated only greater increases for the MVL and HVL groups compared to the CON group ($p = 0.001$) (Figure 3, B).

There was a significant increase in serum PC levels following the PE protocol in all treatment groups, with the highest values observed at 48-h post-PE and remained elevated until 72-h post-PE ($p < 0.05$). A significant group × time interaction was observed for PC ($F = 13.16$, $p = 0.001$), indicating a greater increase in PC for the treatment

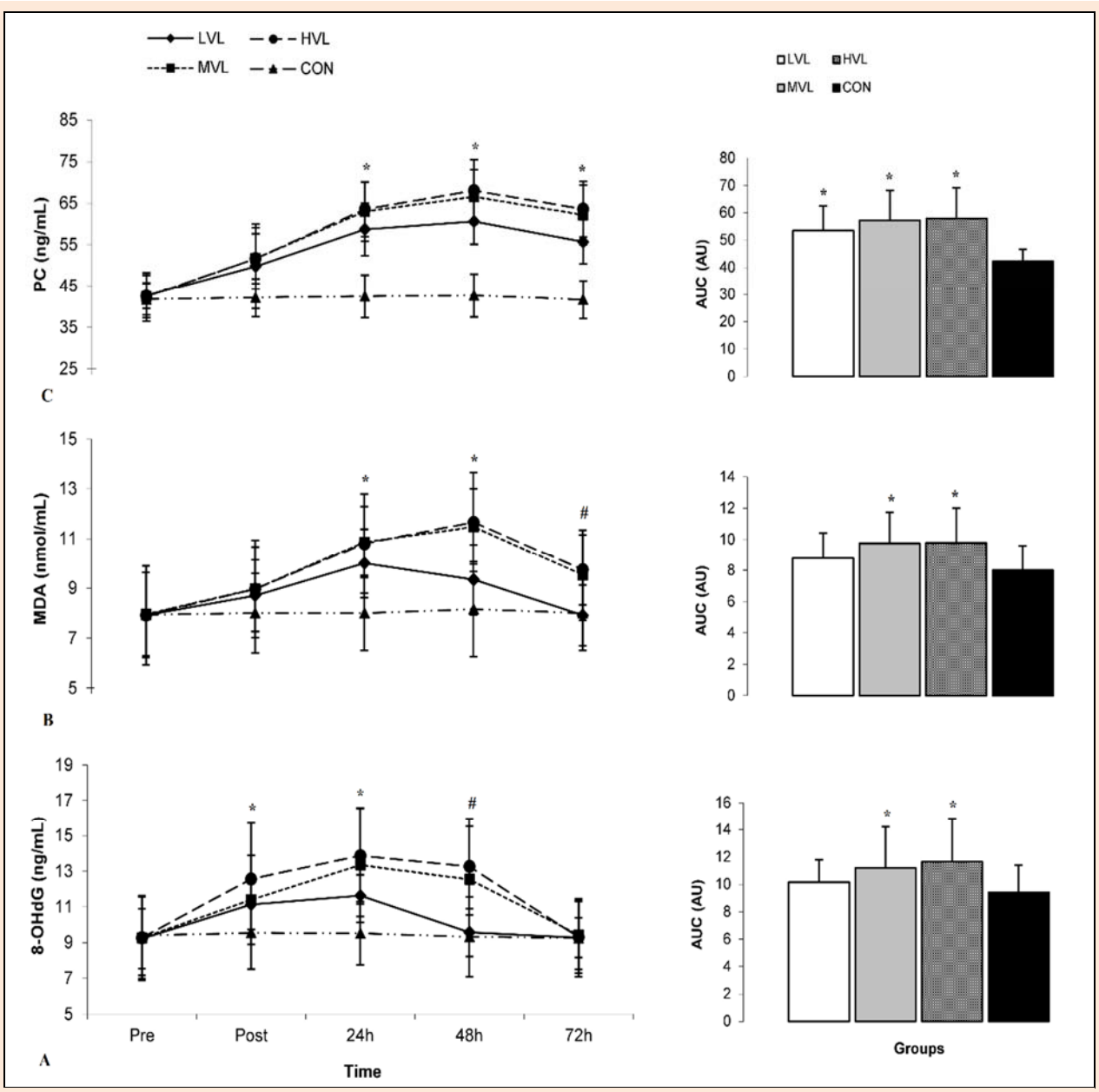

Figure 3. Time course changes in oxidative stress (mean ± SD). LVL: low volume-load plyometric exercise, MVL: moderate volume-load plyometric exercise, HVL: high volume-load plyometric exercise, CON: control condition. * Significant differences compared to pre and CON for all experimental groups ($p \leq 0.05$), # Significant differences between MVL and HVL with LVL ($p \leq 0.05$). In AUC, * denotes significant differences compared to CON for all experimental groups ($p \leq 0.05$).

groups compared to the CON, whereas no significant differences were observed between the treatment groups at any time point ($p = 0.32$). The analysis of the AUC for PC demonstrated greater increases in the treatment groups compared to the CON group ($p = 0.001$) (Figure 3, C).

There were significant increases in leukocytes and neutrophils following the PE protocol in all treatment groups, with the highest values observed post-PE ($p < 0.05$). A significant group × time interaction was observed for leukocytes ($F = 11.68$, $p = 0.001$) and neutrophils ($F = 13.51$, $p = 0.001$), indicating greater increases in leukocytes and neutrophils for the treatment groups compared to the CON group at post-PE. There were no significant differences between the treatment groups in the leukocytes and neutrophils at any time point post-PE. The analysis of the AUC for leukocytes ($p = 0.125$) and neutrophils ($p = 0.238$) demonstrated no significant differences between the groups (Figure 4; A and B).

There were significant increases in IL-6 and CRP levels following the PE protocol in all treatment groups, with the highest values observed at post-PE and 48-h post-PE for each group ($p < 0.05$), respectively. Significant group × time interactions were observed for IL-6 ($F = 25.62$, $p = 0.001$) and CRP ($F = 34.96$, $p = 0.001$), indicating greater increases in IL-6 and CRP for the treatment groups compared to the CON group at post-PE and 48-h post-PE. The analysis of the AUC for IL-6 ($p = 0.001$) and CRP ($p = 0.004$) demonstrated greater increases in the treatment groups compared to the CON group (Figure 5; A and B).

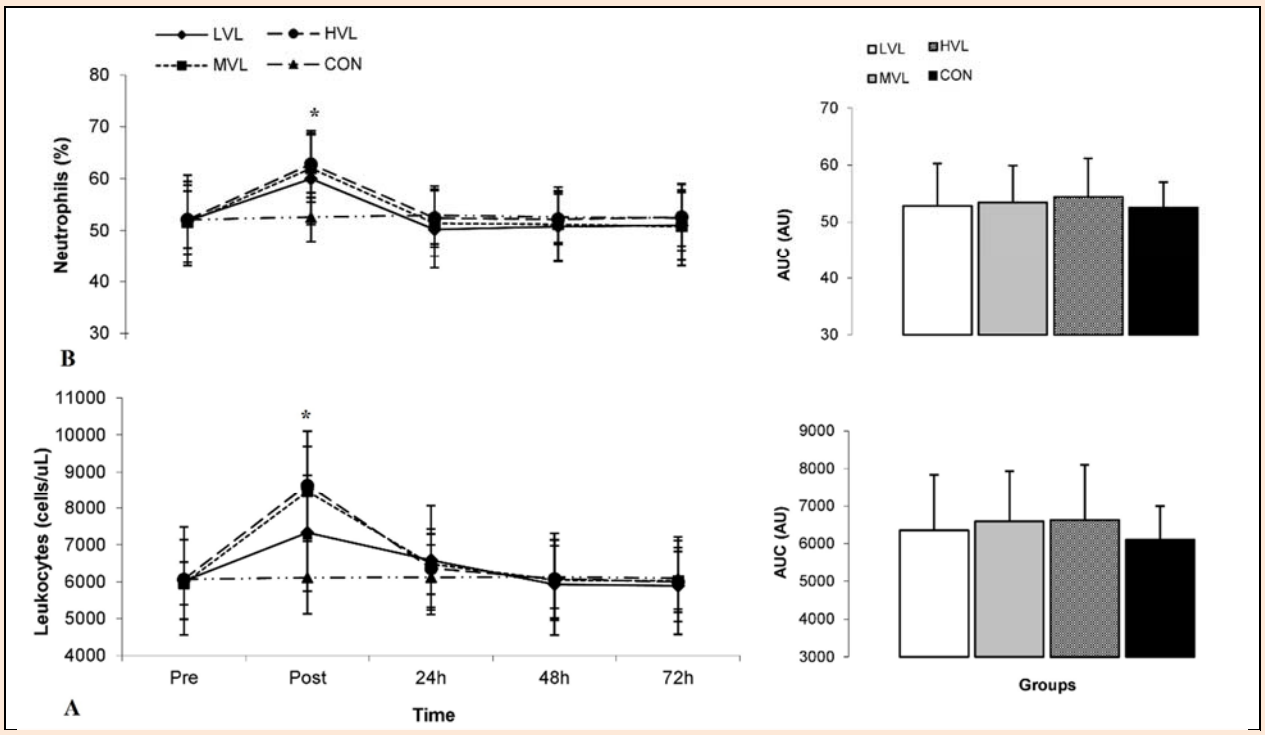

Figure 4. Time course changes in leukocytes and neutrophils (mean ± SD). LVL: low volume-load plyometric exercise, MVL: moderate volume-load plyometric exercise, HVL: high volume-load plyometric exercise, CON: control condition. * Significant differences compared to pre and CON for all experimental groups ($p \leq 0.05$).

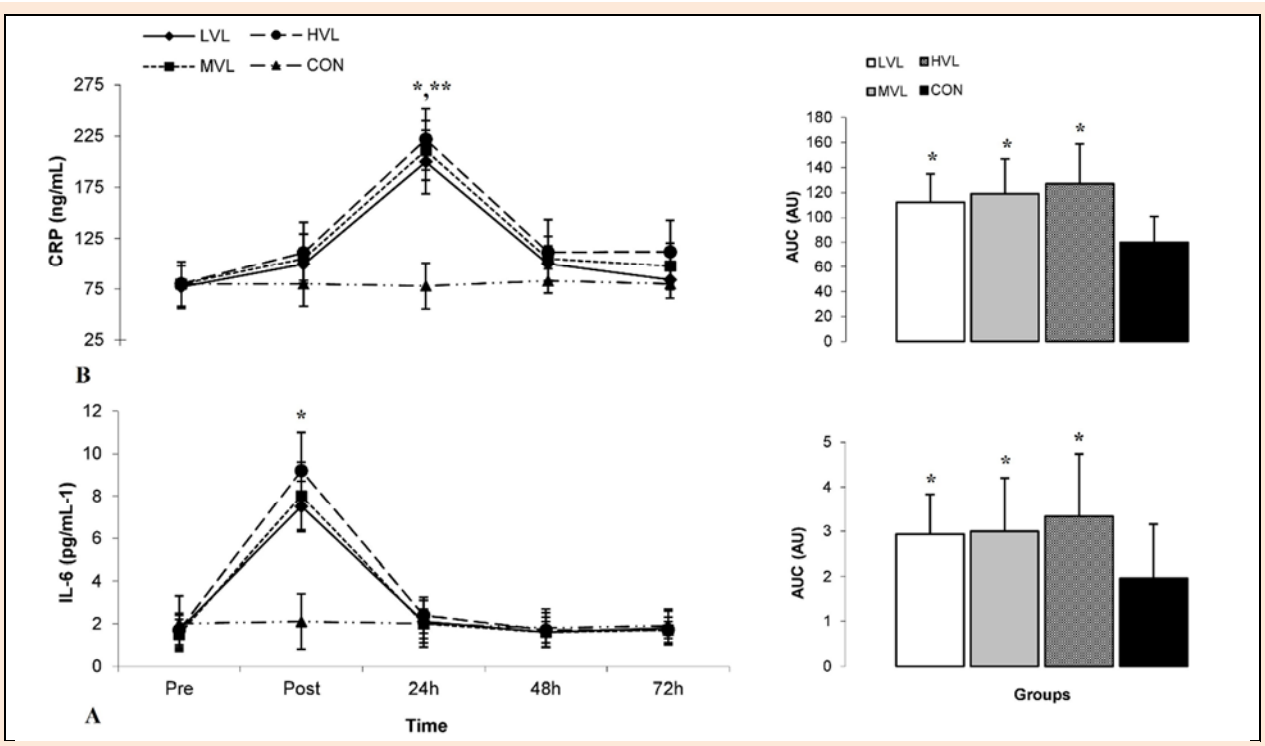

Figure 5. Time course changes in inflammation (mean ± SD). LVL: low volume-load plyometric exercise, MVL: moderate volume-load plyometric exercise, HVL: high volume-load plyometric exercise, CON: control condition. * Significant differences compared to pre and CON for all experimental groups ($p \leq 0.05$), ** Significant differences compared to post for all experimental groups ($p \leq 0.05$). In AUC, * denotes significant differences compared to CON for all experimental groups ($p \leq 0.05$).

Discussion

The results of the present study indicated that a session of PE induced increases in the CK, LDH, DOMS, 8-OHdG, MDA, and PC levels with peaking values between 24 to 48 hours post-PE for all the volume-loaded groups. The levels of leukocytes, neutrophils, and IL-6 also increased after the PE session but returned to resting values within 24-h post-PE. In addition, CRP levels increased at 24-h post-PE for all the treatment groups. In relation to differences between

the treatment groups, the HVL and MVL indicated significant differences compared to LVL in the 8-OHdG (at 48-hour) and MDA (at 72-hour). To the best of our knowledge, this study is the first to investigate the effects of incorporating plyometric exercise (PE) with diverse volume loads into soccer players' conditioning routines on inflammation, oxidative stress, and muscle damage.

In the present study, symptoms of muscle damage such as CK, LDH, and DOMS increased significantly after PE, peaking values at 24-h post-PE in soccer players. The alterations in CK levels persisted at an elevated state until 72-h post-PE across all groups. In addition, the LDH remained only elevated for the MVL and HVL groups at 72-h post-PE. In DOMS, all treatment groups exhibited similar responses, with increases observed at 24-h and 48-h post-PE. The observed symptoms of muscle damage at 24 to 72-h post-PE are consistent with previous studies that measured CK, LDH, and DOMS (Twist and Eston, 2005; Tofas et al., 2008; Byrne and Eston 2002; Marginson et al., 2005). Furthermore, it has been observed that a higher volume-load of training (i.e., resistance training) results in a greater degree of muscle damage compared to a lower volume-load (Margonis et al., 2007; Chatzinikolaou et al., 2010). The underlying cause of this phenomenon may be attributed to the presence of muscle microtrauma (Twist and Eston, 2009). Previous studies have indicated that the forces generated during ground impact and the associated eccentric contractions during PE may lead to muscle damage, particularly in type II muscle fibers and the Z-line (Marginson et al., 2005; Cho et al., 2022). To resolve landing force, the quadriceps muscles execute an eccentric action that entails a counter-extension movement to dissipate kinetic energy (Byrne et al., 2004). In the negative phase of PE, eccentric activation generates greater tension per unit of active muscle mass than concentric actions, leading to notable structural muscle injury as well as elevations in markers of DOMS, CK, and LDH (Byrne and Eston 2002; Byrne et al., 2004). There were no statistically significant differences observed when comparing the treatment groups. Nevertheless, it was observed that soccer players who underwent higher PE volume-loads experienced more changes compared to those who underwent lower or moderate PE volume-loads (i.e., LVL < MVL < HVL). It is crucial to take this into account for better recovery after an intense and high volume of training sessions. These findings provide additional information that supports the effects of manipulating volume-loads during PE, which have minimal impact on symptoms of muscle damage in soccer players. However, these slight increases during the periodization of training could affect the ability of soccer players and should be considered for optimal recovery post-PE.

Another important finding of this study was that oxidative damages significantly increased following PE in soccer players, with varying peak values (e.g., 8-OHdG at 24-h post-PE, MDA and PC at 48-h post-PE). The findings support that a session of PE has a significant impact on enhancing oxidative damage, which is consistent with previous studies that have reported an increase in 8-OHdG, MDA, and PC in the human population during PE involving stretch-shortening cycle actions (Arazi et al., 2019; Chatzinikolaou et al., 2010). The possible explanations for the increase in 8-OHdG following eccentric exercise could be attributed to the anoxic state experienced by the exercising muscle after eccentric (i.e., plyometric) exercise, the activation of neutrophils, or the involvement of xanthine oxidase, which is a source of superoxide anions during eccentric exercise (Chatzinikolaou et al., 2010; Suzuki, 2017; Rahimi et al., 2011). Furthermore, the increase in MDA and PC following PE can be attributed to the disruption of cell membranes and proteasome pathways, respectively (Margonis et al., 2007; Chatzinikolaou et al., 2010; Rahimi et al., 2011; Ramel et al., 2004; Street et al., 2011). In fact, cellular membranes contain fatty acids, and the act of engaging in eccentric exercise has the potential to disrupt these cell membranes (Nikolaidis et al., 2008). This disruption can subsequently lead to an increase in the concentration of polyunsaturated fatty acids in the bloodstream, ultimately resulting in lipid peroxidation and subsequent enhancements in MDA levels (Nikolaidis et al., 2008; Ramel et al., 2004). Similarly, the elevation in muscle damage caused by eccentric exercise is consistent with the activation of proteolytic events and muscle disruption, including intracellular protein degradation and ubiquitin-proteasome proteolysis pathways leading to increased PC (Atashak et al., 2014).

After comparing the volume-loads of PE, it was found that players who performed more volume-loads showed greater responses in 8-OHdG and MDA. Notably, the HVL and MVL showed significant differences compared to LVL in the 8-OHdG at 48-h post-PE and MDA at 72-h post-PE. However, no differences were observed between the MVL and HVL. It appears that the response of 8-OHdG and MDA to volume-load of PE is dependent on dose responses. These results suggest that the extent of stimulation of the oxidative system following plyometric activity has a specific range, and an additional load cannot cause a greater response. Hence, the oxidative stress response to different PE volumes exhibits a stimulation threshold, which, in this particular study, was observed to be moderate volume. This could be attributed to various factors, such as the heightened generation of ROS through the augmentation of mitochondrial metabolic rate during the moderate and high volume-loads, increased oxidative DNA damage, and greater tissue damage and cell membrane disruption (Peak et al., 2017). Consequently, this discovery implies that the activity of oxidative stress in soccer players can be influenced by varying volume-loads of PE. However, further research is necessary to elucidate the specific biochemical changes that occurred following moderate and high volume-loads, which contributed to the exacerbation of oxidative damage symptoms.

Another observation of this study was that leukocytes and neutrophils increased significantly post-PE for all the treatment groups. In line with these findings, Arazi et al. (2019) reported that a session of moderate-load of PE could induce increases in leukocytes and neutrophils in male subjects. The initial stage of immune function is typically marked by the response of the white blood cell count (i.e., neutrophils and leukocytes) (Suzuki, 2018). Upon infection, these cells swiftly migrate to the site of inflammation and engulf microbes and cellular debris within phagosomes (Suzuki, 2017; 2018). Notably, the production of

ROS occurs, which plays a role in both microbe elimination and tissue damage (Suzuki, 2017). The present study suggests that the observed increase in leukocytes and neutrophils following PE may be attributed to the up-regulation of oxidative mechanisms and inflammatory cytokines (Arazi et al., 2019; Margonis et al., 2007), which aligns with our findings.

In the present study, IL-6 indicated peaking value at post-PE, and the CRP increased with peaking value at 24-h post-PE, which aligns with previous studies that reported PE is a training method for enhancing inflammation. During the process of inflammation, CRP, which is an acute-phase protein, is synthesized and subsequently released by the liver following stimulation by IL-6 (Steensberg et al., 2003). Furthermore, IL-6, functioning as a pro-inflammatory cytokine present in the plasma, serves as an indicator of chronic low-grade inflammation and exhibits significant predictive capabilities for future damage (Coffey and Hawely, 2007). The physiological stimulus of exercise has been observed to elicit an inflammatory response that aligns with intra-cellular substrate replenishment (Donges et al., 2010). Previous studies have suggested that elevations in circulatory IL-6 and CRP are correlated with exercise-induced muscle damage (Chatzinikolaou et al., 2010; Marginson et al., 2005). There is evidence that connects the stress load of training and the subsequent response of these markers in the bloodstream, indicating a relationship between the volume-load of training and the magnitude of their increase (Tofas et al., 2008; Neubauer et al., 2008). Additionally, CRP rise has been associated with the activation of recruit leukocytes (Suzuki, 2017). These findings suggest that exercise-induced activation of muscle trauma increases cytokine and inflammation (Suzuki, 2017). However, further research is necessary to examine the impact of exercise training volume-load on possible acute mechanisms involving CRP and other cytokine and inflammation markers to identify muscle damage pathways following exercise.

Conclusion

The present study revealed that a single session of PE results in an increase in symptoms related to muscle damage, oxidative stress, and inflammation among soccer players. Although engaging in plyometric activity during a session led to higher levels of inflammation and muscle damage, there were no statistically significant differences observed between the groups that were studied. However, when comparing the HVL and MVL groups to the LVL group, significant differences were found in the levels of 8-OHdG (at 48 hours) and MDA (at 72 hours). Therefore, it can be concluded that athletes who participate in higher volume-loads exhibit more noticeable responses in terms of biochemical variables (specifically, LVL < MVL < HVL). In addition, these changes were not statistically significant, except for 8-OHdG and MDA, in soccer players, which indicated greater oxidative stress for the MVL and HVL groups.

Acknowledgements

The experiments comply with the current laws of the country in which they were performed. The authors have no conflict of interest to declare.

The datasets generated and analyzed during the current study are not publicly available, but are available from the corresponding author who was an organizer of the study.

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Key points

- The extent of muscle damage and inflammation varies minimally between different volume-loads of plyometric exercises. Understanding such divergent outcomes has practical implications for coaches, trainers, and sports practitioners involved in designing and implementing training programs for soccer players.
- Athletes who engage in higher volume-loads tend to exhibit more noticeable changes in biochemical variables, with the order of response being LVL < MVL < HVL. This finding highlights the importance of individualized training plans, strategic periodization, monitoring and adjusting intensity, and balancing intensity for desired adaptations.
- In terms of oxidative stress, both the HVL and MVL interventions impose greater changes compared to the LVL group in the levels of 8-OHdG (at 48 hours) and MDA (at 72 hours). By implementing strategies mentioned above, sports professionals can enhance the effectiveness of training programs while safeguarding athletes against the potential negative effects of oxidative stress.

AUTHOR BIOGRAPHY

Jian TIAN
Employment
School of Physical Education, Henan University
Degree
MSc
Research interests
football training and physical education
E-mail:
10180019@vip.henu.edu.cn

Miao MIAO
Employment
School of Physical Education, Henan University
Degree
PhD
Research interests
Physical Education and Training
E-mail: kugua-00000@163.com

✉ **Miao Miao**
School of Physical Education, Henan University, Kaifeng, Henan 475000, China.