The effect of high-intensity functional training on performance and oxidative stress in adolescent soccer players

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ABSTRACT

While regular exercise training is known to decrease oxidative stress (OS) and enhance antioxidant functions in adults, limited data exist on OS responses in the pediatric population. This study aimed to investigate the effects of high-intensity functional training (HIFT) on oxidative stress and physical performance in adolescent soccer players. In this randomized controlled trial, 20 well-trained adolescent soccer players were divided into HIFT (n=10) and moderate-intensity soccer technical training (MITT, n=10) groups. Both groups trained for 8 weeks (3 days/week). Oxidative stress biomarkers (MDA, 8-OHDG, H2O2, GPx, CAT) and physical performance (VO2peak, vertical jump height [VJH], maximal explosive power [MEP]) were assessed pre- and post-intervention. Repeated measure mixed ANOVA (2×2) with a significance level of P<0.05 was used to determine differences between and within subjects. The main effect of interaction was significant for VO2peak, VJH and MEP (P<0.05). The main effect of interaction was not significant for CAT, 8-OHDG, GPx, H2O2 and MDA (P>0.05). Following intervention, VO2peak and VJH performance in the HIFT were significantly higher than MITT (P<0.05). MDA and H2O2 in the HIFT decreased significantly (P<0.05), but no significant changes were observed in the MITT (P>0.05). CAT activity decreased significantly (P<0.05), while GPx activity increased significantly (P<0.05) in both groups. 8-OHDG did not show significant changes in both groups (P>0.05). Eight weeks of HIFT did not cause greater OS compared to MITT, while could improve physical performance parameters. These findings suggest that HIFT is a viable and time-efficient training strategy for enhancing athletic performance in youth sports.

Keywords: Antioxidants, Exercise training, Functional training, Oxidative stress, Performance, Power

1. *INTRODUCTION*

Ball and team sports such as soccer are very popular among teenagers. According to statistics, nearly 22 million players bellow the age of 18 are active in the soccer [1]. Due to the aerobic and intense nature of soccer [2, 3], one of the main challenges for coaches and experts in this field is to use efficient, safe and special training methods to improve the performance of adolescent athletes ultimately. On the other hand, exercise training during puberty has a significant effect on the development of bio-motor abilities and performance, which is also known as the open window of trainability [3, 4]. Adolescence, affects on adaptive responses to training due to physical (height, muscle mass), physiological (hormones, aerobic capacity, strength and body composition) and cognition changes [4]. On the other hand, due to special situations of adolescents regarding less free time and financial dependence in most societies, training protocols which require shorter duration and fewer facilities are more applicable for them.

Nowadays, a method of exercise training called high intensity functional training (HIFT), which contain high-intensity functional and resistance movements in a circular and interval pattern, has been attracted attentions [5-8]. Not requiring expensive equipment and short duration of HIFT have made it a good option for fitness promotion [9]. In young people, HIFT with body weight bearing has been shown to provide the same cardiorespiratory adaptations as traditional high intensity interval training (HIIT) on an ergometer cycle [10]. Also, increased aerobic endurance, anaerobic peak power and flexibility after HIFT have been shown [5, 11]. In contrast, some studies have found that HIFT has no significant effect on maximal oxygen consumption (VO2max) and fatigue index [11]. However, an increase in muscle strength after body weight functional training has been reported [12]. In addition, a meta-analysis study (2020) reported that chronic HIFT improved endurance capacity and strength compared to non-exercised individuals [7]. However, the authors

suggested that moderating factors such as age and gender should be investigated in future studies. In this regard, another meta-analysis (2023) reported that HIFT had a small to large effect on improving strength, power, flexibility, and sport-specific performance in young athletes (10-24.5 years), while not affecting endurance [8]. However, the authors noted that due to the limited number of studies and heterogeneity in HFT training protocols, more research is needed to explore the potential factors that influence physical fitness and specific performance of athletes [8].

Despite the positive effects of HIFT on bio-motor abilities and its growing popularity, there are also concerns about stress related to intensive training which involve immunological, hormonal, oxidative, metabolic, and mechanical during growth and development periods. However, there is limited research on adolescent athlete's stress responses, especially oxidative stress (OS), to HIFT. OS is a state where the generation of free radicals surpasses the body's antioxidant defenses. This imbalance leads to potential cellular damage as reactive oxygen species (ROS) and other free radicals accumulate faster than they can be neutralized by antioxidants [13]. This change in redox status can stimulate a number of physiological and biochemical responses [14, 15]. On other hand, the relationship between OS and sports performance is complex. For example, in situations that OS is excessive such as acute strenuous exercise or excessive training, reactive oxygen and nitrogen species (RONS) damage the function and structure of biological molecules such as DNA and proteins [14-17]. This can disrupt the regeneration of cellular damage. Therefore, it can also reduce training adaptations and exercise performance [18]. 8-Hydroxy-2-deoxyguanosine (8-OHDG) and malondialdehyde (MDA) are respectively the biomarkers of oxidative damage to DNA and membrane lipids [14, 19]. These markers are associated with a wide range of pathological conditions, decreased training adaptation, and overtraining [14, 19]. however, low to moderate production of ROS according to Hormesis theory increases cellular resistance to cytotoxic stress by strengthening repair systems and antioxidant defenses (such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD)) [14]. In turn, these changes lead to increased longevity, cell survival, and exercise adaptation [14]. According to emerging evidence, Hormesis is one of the underlying frameworks for the body's adaptive responses to exercise. Despite this growing body of evidence, there remains a significant gap in the literature regarding the specific impact of hormesis on athletic performance among adolescents. Further investigation is essential to elucidate the nuances of this relationship and to better understand how Hormetic responses can influence training outcomes in younger athletes.

According to our knowledge, there is no evidence about HIFT effects on OS markers in adolescent soccer players. However, it has been shown that the OS response to a one bout strenuous running and CrossFit (a common type of HIFT) are similar [13]. Some studies have not found changes in 8-OHDG [20, 21], but others reported a decrease in 8-OHDG after regular exercise training [22]. Zainuddin et al [23] showed that intense training periods can increase 8-OHDG [23], while Yushino et al [24] showed that regular and voluntary exercise couldn't alter serum 8-OHDG levels [24]. In non-trained adolescent subjects (mean age 17.4 years) after 12 weeks of soccer training (10 to 15 hours of high-intensity training per week, which was a combination of soccer technical training and conditioning), no change in MDA levels was observed [25]. Also, the activity of SOD and CAT antioxidant enzymes increased after 6 months of soccer training [26]. In 11 to 13 years old adolescents, after 16 weeks of swimming training, SOD and CAT activity increased and GPx activity decreased [17]. In contrast, there was no difference in the activity of SOD and GPx of red blood cells in young soccer players during the soccer season [27].

In light of these inconsistent findings and the recognized importance of exploring new, effective training approaches for adolescents, this study aims to directly evaluate the effects of an 8-week HIFT program on both oxidative stress (OS) markers and physical fitness in adolescent soccer players. By focusing on these two aspects, this research seeks to clarify the physiological impacts of HIFT in young athletes, shedding light on both the potential benefits for physical performance and the associated stress responses during a critical developmental period.

2. Materials and methods

The present study is a Parallel arm design randomized controlled trial (registration number IRCT20190530043762N1, 25/09/2019) which has been approved by the National Ethics Committee in Biomedical Research of the Ministry of Health, Treatment and Medical Education with the ethics ID IR.UT.SPORT.REC.1398.014 (Iran). All experimental procedures followed the principles of the Helsinki Declaration. No important methodological changes were done after trial commencement. All methods were performed following the relevant CONSORT 2010 guidelines and regulations. We used a simple method for randomization (shuffled deck of cards). First, each participant was assigned a unique ID code. Then each ID

code was written on a card and all cards were placed in a ball. Next, the cards inside the sphere were shuffled and one card was taken out from inside the ball. ID codes that were taken out of the ball in odd turns were placed in the HIFT group and ID codes that were taken out of the ball in even turns were placed in the MITT group. It should be noted that after each time the cards were taken out, the contents of the ball were shuffled again. All steps were carried out by two members of the research team. It is noteworthy that all functional and experimental evaluations took place in the Sports Physiology Laboratory located in the Faculty of Sport Sciences at the University of Tehran (Iran).

Table1. Anthropometric characteristics of participants

Variable/Group	HIFT	MITT	Total
	Mean (SD)	Mean (SD)	Mean (SD)
Age (year)	15.2 (0.41)	15.04 (0.40)	15.1 (0.4)
Weight (kg)	56.6 (3.6)	54.7 (3.1)	55.7 (3.4)
Height (cm)	174.2 (3.42)	173.8 (2.68)	174 (2.99)
BMI (kg/m2)	18.7 (1.3)	18.1 (1.2)	18.4 (1.2)
APHV (year)	14.5 (0.35)	14.4 (0.4)	14.5 (0.4)
Maturity offsets (year)	0.7 (0.1)	0.6 (0.3)	0.6 (0.2)

2-1. Participants

Forty U-16 male soccer players volunteered to participate in this research after a public announcement that was delivered 20 days after the end of the Tehran League. The inclusion criteria for the candidates were: male sex, age 14– 16 years, at least 2 years of experience in soccer league matches, being in the same developmental stage based on maturity offset, not participating in HIFT previously, not consuming any kind of supplements during the last 3 months and physical health certification from clinical centers. Sample size estimation

*The number of participants in this study was determined based on the study by Varamenti et al. [28], according to which chronic exercise training led to a significant improvement in antioxidant capacity (glutathione) compared to control (effect size = 1.13). Using G*Power 3.1, considering the confidence interval of 95%, and the analysis power of 0.78, it was found that at least 20 participants (10 participants for each group) are needed for this study.*

2-3. Trial design

According to the inclusion criteria, 20 teenage soccer players were selected as participants of the study (Figure1). In a familiarization session, all research programs were explained to the volunteers and their parents, then all of them (volunteers and their parents) also signed a consent form to participate in the intervention. The anthropometric characteristics of the participants is listed in Table 1. Functional tests and blood sampling were performed before and after the intervention. To control the effect of maturity, non-invasive method was used to estimate the maturity offset (Equation 1) and age at peak height velocity (APHV) (Equation 2) [29, 30]. Indeed, all participants were in PHV1 (maturity offset = +0.5 to +1.49 years) [30]. Accordingly, participants were divided into high-intensity functional training (HIFT, n=10) and moderate-intensity technical soccer training (MITT, n=10) groups based on maturity offset [30]. Two participants were excluded from the study, one participant due to a cold (MITT) and another one (HIFT) due to not cooperating in intervention programs.

Equation 1:

$$\text{Maturity offset (year)} = -9.236 + ((0.0002708 \times (\text{leg length (cm)} \times \text{sitting height(cm)})) + (-0.001663 \times (\text{age (year)} \times \text{leg length(cm)})) + (0.007216 \times (\text{age(year)} \times \text{sitting height(cm)})) + (0.02292 \times ((\text{weight (kg)} / \text{height (cm)}) \times 100)$$

Equation 2:

$$\text{Age at Peak Height Velocity (APHV) (year)} = \text{chronological age at measurement(year)} - \text{maturity offset(year)}$$

Before the intervention, the participants were banned from any exercises for 48 hours. On the morning of the third day (7-7.30 am and 12 hours fasting), blood samples were taken from all participants. First, the site of needle entry (brachial vein) was disinfected and 5 ml of blood was taken. Then, the collected samples were placed in closed tubes at normal room temperature (22-25 C) for 35-45 minutes to perform normal blood coagulation. The samples were then centrifuged (KUBOTA, JAPAN) for 10 to 15 minutes and centrifuged at 3000 RPM. After serum separation, the samples were frozen and stored at -80 to -85 °C by recording the code

and sampling date. Immediately after blood sampling, the subjects ate the same meal (350-400 kcal, 64% carbohydrates, 20% protein and 16% fat). Two hour later, the vertical jump test was performed, then peak oxygen consumption (VO2peak) was measured indirectly by the 20-meter shuttle test [31]. To control the effect of diet during pre-test and post-test evaluations, participants' diets were adjusted based on 3000-3100 kcal (65-60% carbohydrates, 20-25% protein and 20-25% fat). Participants were asked to avoid dietary and ergogenic supplements during the intervention. We asked the participants not to inform the evaluators in which group they were placed in the final assessment.

2-4. Performance assessment

Vertical jumping was used to determine the explosive power of the lower body. Each participant performed three vertical jumps and the maximum vertical jump height (VJH) was recorded as their score [3]. An acceptable validity and reliability for the vertical jump test in adolescent athletes was reported by Rodríguez-Rosell et al [32]. Maximal explosive power (MEP) was also calculated by Johnson & Bahamonde equation [33] (Equation 3). VO2peak was measured indirectly using a 20-meter shuttle-run test. Previous studies have shown that the 20-meter shuttle run test is a valid and reliable test for teenagers (Test-retest reliability coefficients were 0.89) [34, 35]. Finally, VO2peak was calculated according to Mahar and colleagues (2011) equation [31] (Equation 4). It should be noted that the standard error of estimate (SEE) of this test is 6.17 ml/kg. minute [31].

Equation 3:

$$MEP\ (Watt) = 78.6 \times VJ\ (cm) + 60.3 \times mass\ (kg) - 15.3 \times height\ (cm) - 1.308$$

Equation 4:

$$VO2peak = 41.76799 + (0.49261 \times laps) - (0.00290 \times laps2) - (0.61613 \times BMI) + (0.34787 \times 1 \times age\ (year))$$

$$Standard\ error\ of\ estimate\ (SEE) = 6.17\ (ml \cdot kg^{-1} \cdot min^{-1})$$

2-5. Measurement of OS markers

Serum levels of 8-OHDG were measured by ELISA method (sensitivity 0.25 ng/ml, made in China, BT LAB). H2O2 (sensitivity 5 µmol), MDA (sensitivity 0.1 µmol), CAT activity (sensitivity 0.5 units/ml) and serum GPx activity (sensitivity 5 units/ml), were measured by simple colorimetric method (Assay kits made by the Germany, ZellBio). All analysis processes were performed according to the instructions in the kit manual. It should be noted that all laboratory tests were performed at Noor Laboratory (Tehran, Iran).

Training protocols and monitoring

*HIFT protocol consisted of 8 stations with body weight resistance exercises (1- Dribbling with high speed with soccer ball for 20 meters, 2- Push-Up, 3- Squat jump, 4- Plank jack, 5- Nordic curl, 6- Mountain Climber, 7- Sprint on the spot, 8- Burpee). Each station was performed for 30 seconds with maximum effort (all out) and 30 seconds of active rest was considered between each station. Also, at the end of each cycle (8 stations), 2 minutes of inactive rest was considered (Table 2). HIFT was performed progressively for 8 weeks (3 days/week). The MITT group also performed moderate-intensity soccer technical training (rondo soccer drill, 10 x 10-yard area, 1 soccer ball, 4 players possessing, 2 defenders) for 8 weeks (3 day/week * 60-65% maximum heart rate (MHR)). MHR was calculated based on the 220-age equation. In order to match the two groups, the duration of MITT was similar to the HIFT group (table 2). Also, in addition to HIFT and MITT, the subjects performed habitual soccer training (3 days/week) and weight resistance training (1 day/week) for other days under the supervision of coaches. These training mode, intensity and duration in both groups were similar in two groups. It should be noted that all the participants were members of the same soccer training camp and their training regime (except for HIFT and MITT) was the same. Soccer training in each session included general warm-up (15 minutes), specific warm-up with the ball (10 minutes), technical drills (20 minutes), tactical drills (40 minutes), and cooling (15 minutes). Intensity was monitored using the Beurer heart rate monitor watch (PM 80, made in Germany). Weight training also included eight types of exercises (3 set,10-12 repetitions, 75-80% one repetition maximum) in each session.*

2-6. Statistics

SPSS software (version 26, IBM-SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Due to the assumption of normal distribution of data and homogeneity of variances, repeated measures mixed ANOVA (2×2) and Bonferroni's post hoc test were used. The significance level was considered as $P<0.05$.

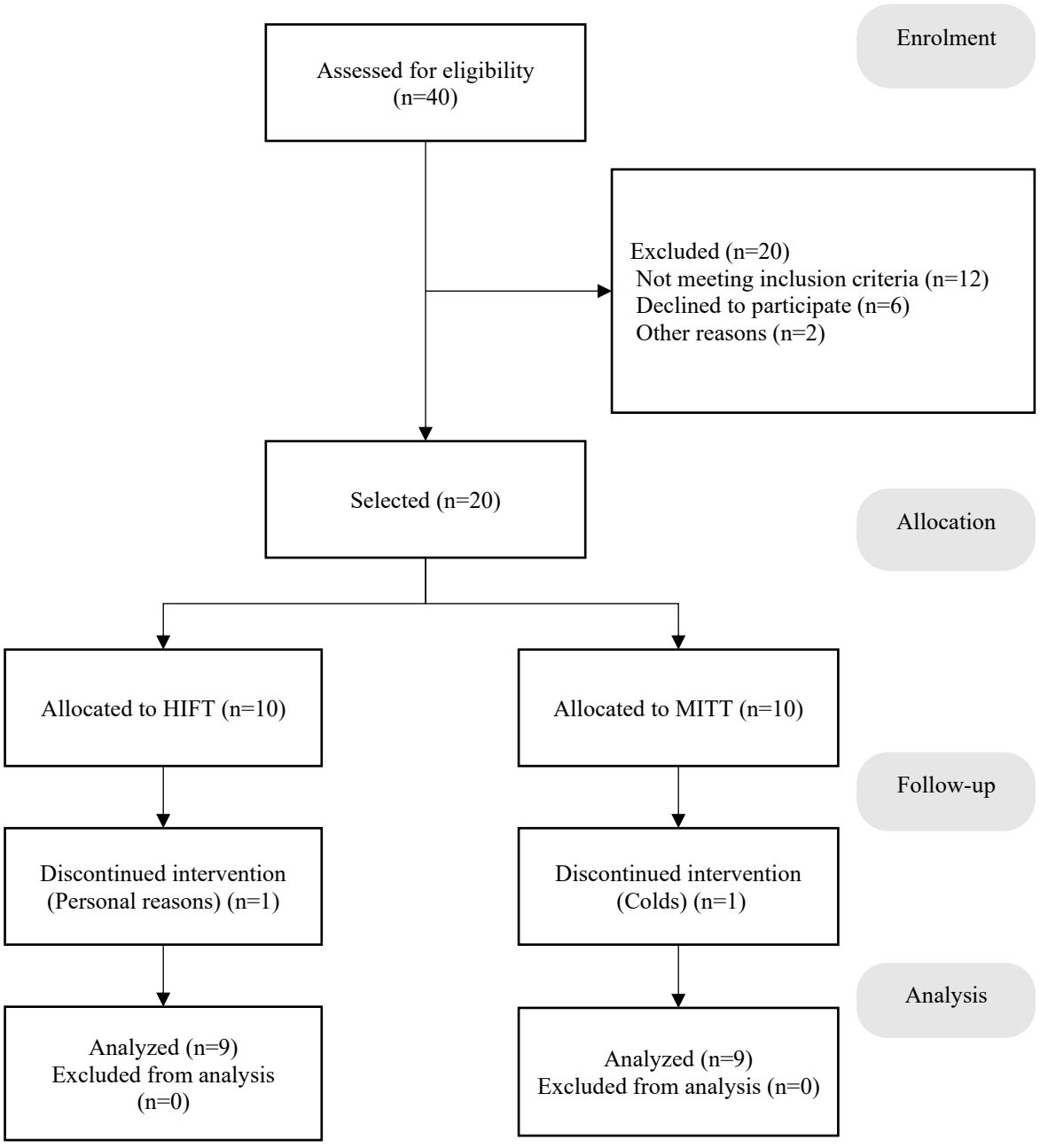

Figure 1. Participant flow diagram. HIFT: High intensity functional training, MITT: Moderate intensity technical training

Table 2. Training protocols

Group	Week	Frequency (day/week)	Round per session	Duration	Intensity
High intensity functional training (HIFT)	1-2	3	1 × (8 station)	Work interval: 30 s Recovery interval: 30s Rest between round: - Total time: 7:30 min:s	All-out Session means HR: 85-90% MHR
	3-4	3	2 × (8 station)	Work interval: 30 s Recovery interval: 30s Rest between round:2min Total time: 17 min	All-out Session means HR: 85-90% MHR
	5-6	3	3 × (8 station)	Work interval: 30 s Recovery interval: 30s Rest between round:2min Total time: 26:30 min:s	All-out Session means HR: 85-90% MHR
	7-8	3	4 × (8 station)	Work interval: 30 s Recovery interval: 30s Rest between round:2min Total time: 36 min	All-out Session means HR: 85-90% MHR
	HIFT Stations: 1- Carrying the soccer ball (20 m/shuttle), 2- Push-Up, 3- Squat jump, 4-Plank jack, 5-Nordic hamstring, 6-Mountain Climber, 7-Sprint on the spot, 8-burpee				
Moderate intensity soccer technical training (MITT)	Week	frequency	Round per session	Duration (min:s)	Intensity
	1-2	3	1	Work interval: 7:30 Rest between round: - Total time: 7:30	Session means HR = 60-65% MHR
	3-4	3	2	Work interval: 7:30 Rest between round:2min Total time: 17 min	Session means HR = 60-65% MHR
	5-6	3	3	Work interval: 7:30 Rest between round:2min Total time: 26:30	Session means HR = 60-65% MHR
	7-8	3	4	Work interval: 7:30 Rest between round:2min Total time: 36 min	Session means HR = 60-65% MHR
	MITT content: rondo soccer drill, 10 x 10-yard area, 1 soccer ball, 4 players possessing, 2 defenders. If the defending players reached the target heart rate, they would be replaced by other players. The possessing players had to keep their heart rate within the target range with short movements				
All subjects		Week	frequency	Duration	Intensity
	HST	1-8	3	90 min general warm-up (15 minutes), specific warm-up with the ball (10 minutes), technical drills (20 minutes), tactical drills (40 minutes), and cooling (15 minutes)	Session means HR = 55-65% MHR Session mean RPE = 4-6
	RT	1-8	1	60-70 min Squat with smith machine (3set×10rep), Leg press (3set×10 rep), Bench Chest press (3set×10 rep), Seated cable row (3set×10 rep), Knee extension (3set ×10 rep), Lying leg curl (3 set×12rep), Stand calf raises (3set×12rep), Shoulder press (3set×10 rep).	75-80% 1RM

HR: heart rate, MHR: maximal heart rate, HST: habitual soccer training, RT: resistance training, RM: repetition maximum

3. *Results*

According to repeated measure mixed ANOVA, the main effect of the group was significant only in VO2peak (P<0.05) and no significant effect was observed for other variables (P>0.05) (Table 3). The main effect of time was not significant for 8-OHDG (P>0.05), but for VO2peak, VJH, MEP, CAT, GPx and H2O2 the main effect of time was significant (P<0.05) (Table 3). Also, the effect of time×group interaction was significant only for VO2peak, VJH and MEP (P<0.05) (Table 3). The effect of interaction was not significant for other variables such as CAT, 8-OHDG, GPx, H2O2 and MDA (P>0.05) (Table 3).

The post hoc test showed that VO2peak decreased significantly in the MITT group (P<0.05), while there was no significant change in the HIFT group (P>0.05) (Figure 2, a). In the post-test, VO2peak was lower in the MITT compared to the HIFT (P<0.05) (Figure 2, a). VJH also increased significantly in the HIFT (P<0.05),

but did not change in the MITT (P>0.05) (Figure 2, b). In the post-test, VJH was higher in the HIFT compared to the MITT (P<0.05) (Figure 2, b). The MEP also increased in both groups compared to the pre-test (P<0.05), but no significant changes were observed between the two groups in the post-test (P>0.05) (Figure 2, c). MDA (Figure 3, e) and H2O2 (Figure 3, b) levels in the HIFT showed a significant decrease (P<0.05), but no significant changes were observed in the MITT (P>0.05). Compared to the pre-test, CAT activity (Figure 3, d) significantly decreased in both groups (P<0.05) while GPx activity (Figure 3, c) significantly increased (P<0.05). Also, the changes of 8-OHDG are shown in Figure 3, a.

Variables (unit)	MITT (n=9)		HIFT (n=9)		ANOVA (η^2) df = (1,16)		
	Pre Mean (SD)	Post Mean (SD)	Pre Mean (SD)	Post Mean (SD)	Time	Group	interaction
VO_{2peak} (ml/kg.min)	60.50 (2.69)	58.10 (1.27) *	62.50 (2.28)	62.66 (3.35) #	0.041 (0.23)	0.008 (0.36)	0.022 (0.28)
VJH (cm)	39.66 (6.50)	41.22 (5.14)	41.33 (5.5)	47.11 (4.7) *#	0.000 (0.67)	0.15 (0.12)	0.005 (0.40)
MEP (watt)	2481.02 (880.10)	2658.20 (789.80) *	2724.59 (604.46)	3192.52 (658.87) *	0.000 (0.69)	0.27 (0.073)	0.016 (0.31)
8-OHDG (ng/ml)	2.55 (1.16)	2.43 (0.93)	2.52 (0.68)	2.49 (0.91)	0.60 (0.17)	0.97 (0.000)	0.75 (0.006)
CAT (U/mol)	9.52 (2.09)	7.13 (1.88) *	8.68 (2.89)	6.36 (1.57) *	0.007 (0.37)	0.24 (0.08)	0.96 (0.000)
GPx (U/mol)	73.07 (34.84)	141.23 (35.90) *	60.17 (37.89)	153.70 (63.91) *	0.000 (0.66)	0.98 (0.000)	0.39 (0.046)
H_2O_2 (µmol)	142.74 (32.15)	122.15 (49.69)	155.24 (48.77)	122.15 (49.69) *	0.043 (0.23)	0.96 (0.000)	0.40 (0.044)
MDA (µmol)	3.12 (0.70)	3.05 (0.57)	3.41 (0.89)	2.71 (0.62) *	0.048 (0.22)	0.93 (0.000)	0.1 (0.16)

* *Significant within group (P<0.05).* # *Significant between groups (P<0.05).*

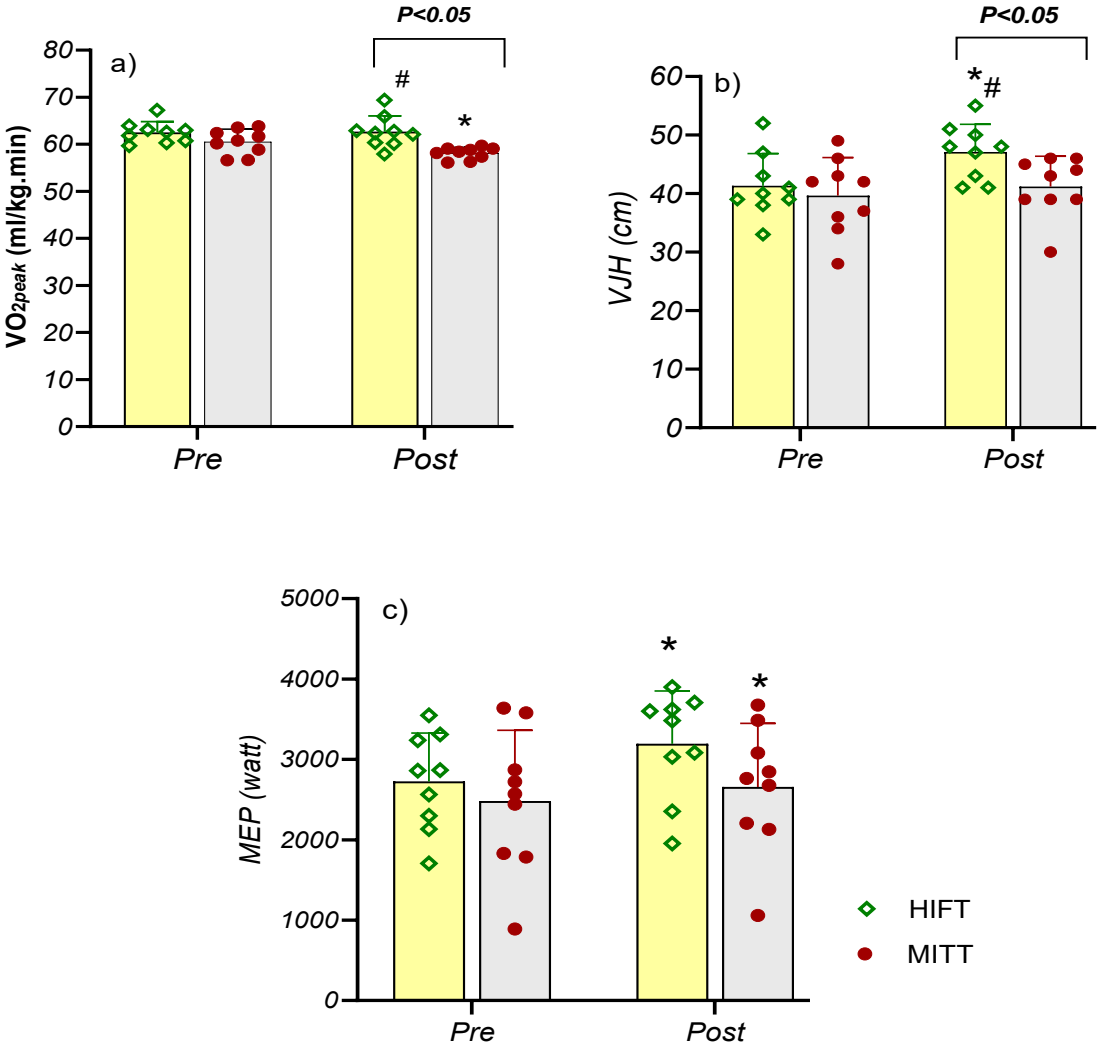

*Figure 2. Changes in performance parameters in pre-test and post-test with individual value. VO_{2peak}: Peak oxygen uptake, MEP: maximal explosive power, VJH: vertical jump height. *Significant difference within group (P<0.05). # Significant difference between groups (P<0.05).*

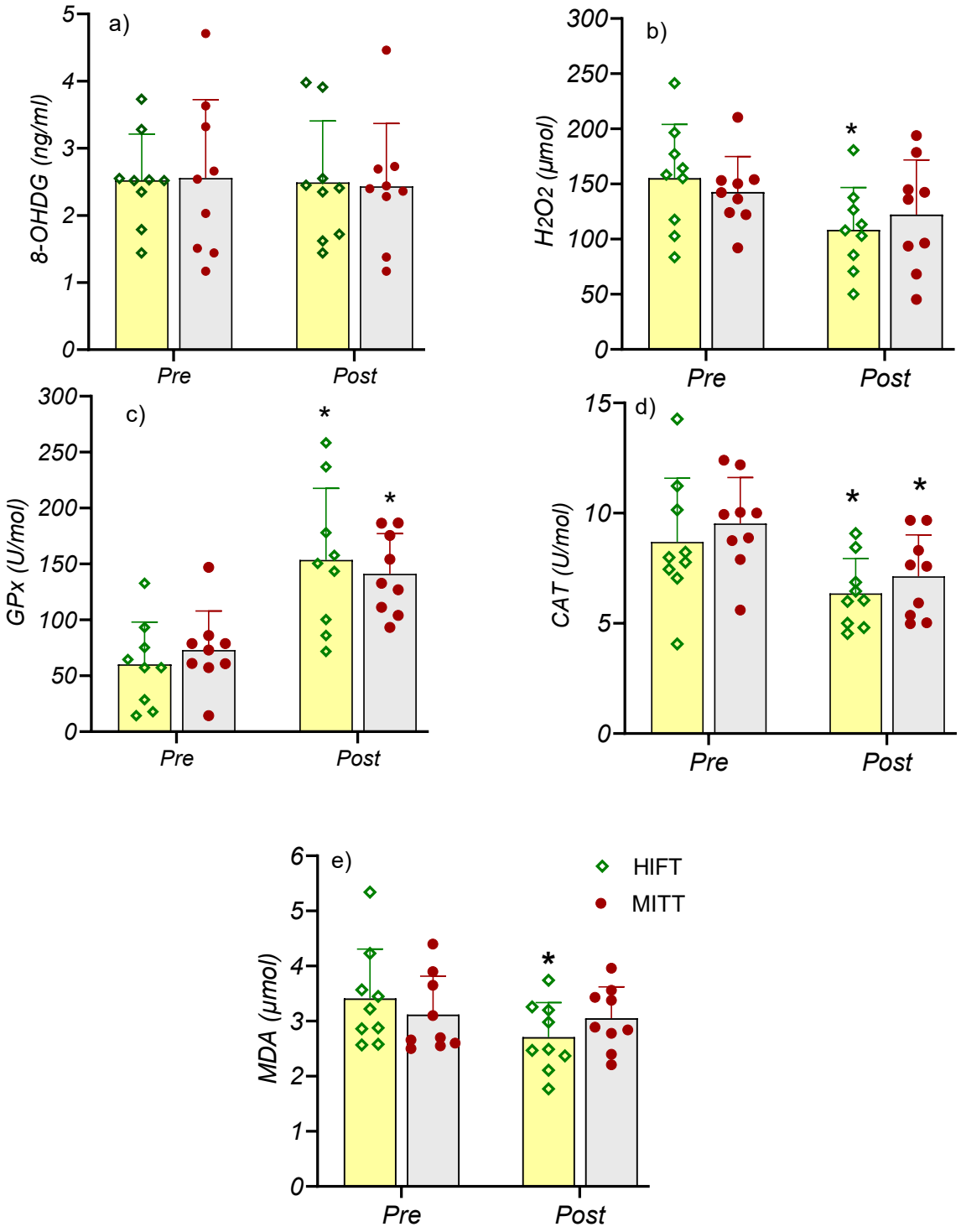

*Figure 3. Changes in oxidative stress markers in pre-test and post-test. The hatched bars belong to the HIFT and the white bars belong to the MITT. 8-OHDG: 8-hydroxy-2'-deoxyguanosine, MDA: Malondialdehyde, GPx: Glutathione peroxidase, CAT: Catalase, H_2O_2: Hydrogen peroxide. * Within group Significant difference.*

4. Discussion

Overall, according to the findings of this study, 8 weeks of HIFT did not cause greater OS compared to MITT, but could improve performance parameters. Notably, the reduction in MDA and H_2O_2 levels in the HIFT group suggests that integrating HIFT into adolescent soccer players' training routines can be an effective strategy for reducing basal OS.

One of the most notable findings of this study was that HIFT significantly improved power performance, including vertical jump height (VJH), compared to MITT. The increase in maximal explosive power and VJH of the HIFT compared to the MITT group can be explained by the neuromuscular and metabolic mechanisms (phosphocreatine stores and glycolytic enzyme activity) induced by HIIT/HIFT [36, 37]. In confirmation of this finding, Buckley et al. (2015) showed that 6 weeks of HIFT increased anaerobic power, strength and muscle power [38]. A previous study also reported an increase in explosive power in untrained adolescents following 6 weeks of HIFT [39]. In this context, earlier research has demonstrated that HIFT enhances motor unit recruitment and activation, as well as improves neuromuscular conduction, motor unit firing, and synchronization, all of which contribute to increased strength, efficiency, and muscle coordination [36, 37]. In addition, Bermiju et al. (2018) showed that intense cycling training improves performance on the Wingate anaerobic test compared to HIFT [36]. They believed that the better results of the cycling group compared to HIFT were due to the biomechanical similarities of the tests and exercise characteristics [36]. Therefore, in this study, the improvement in vertical jump height (VJH) may be attributed to the resemblance between the biomechanical patterns of the movements in HIFT and the vertical jump test. For instance, one of the HIFT stations involved squat jumps, which closely mimic the motion of the vertical jump.

Furthermore, the results of this study revealed a decrease in VO_{2peak} in the MITT group, whereas it maintained in HIFT. The decrease in VO_{2peak} of the MITT may be mediated by the decrease in exercise intensity during the intervention period. Considering that the present study was conducted after the competition season (high-intensity and volume cycle of competition and training), the high VO_{2peak} in the pre-test seems reasonable. However, during the intervention period, average heart rate in MITT was usually less than 70% of the MHR. Therefore, reducing the overall intensity of training can be one of the most important reasons for reducing VO_{2peak} in the MITT group. In contrast, the mean heart rate during HIFT sessions was more than 80% of the MHR. Therefore, the workload performed in the HIFT was more than the MITT group, which can explain the maintenance of higher amounts of Vo_{2peak} in the HIFT group. Previous studies have also shown that high intensity training can improve or maintain high VO_{2peak} by inducing central (cardiac output, stroke volume, ventricle contractility) and peripheral (increased skeletal muscle oxidative enzymes, mitochondrial biogenesis and Angiogenesis) adaptations; While low-intensity training can even reduce VO_{2peak} [40]. Also, not increasing the VO_{2peak} in the HIFT group can be due to the high level of VO_{2peak} and the training experience of the participants before the intervention [12, 41]. Previous studies have shown that improvements in VO_{2peak} are greater in untrained individuals than in trained individuals [42]. Also, the lower VO_{2peak} in the MITT group compared to the HIFT group can be due to lower running economy (Work output/ O2 consumption) and lactate threshold in the MITT group and maintaining a proper running economy, lactate threshold and more lactate tolerance in the HIFT group. For example, in trained subjects, high intensity interval training has been shown to maintain muscle oxidative capacity and endurance performance compared to lower intensity training [43]. In addition, according to reports by Gonzalaz et al.[44], the running economy in the HIIT group was better than the moderate-intensity training group [43, 44]. In contrast, a new study has shown that HIFT is an effective training method for multifaceted improvement of physical fitness parameters (strength, power and endurance) of untrained adolescents [39]. Overall, based on the findings of the present study, it seems that HIFT can be a good strategy to maintain the aerobic fitness of adolescent soccer players. However, the impact of HIFT on the running economy and its contribution to the modulation of VO_{2peak} requires further study.

Additionally, the results of this study revealed a significant decrease in CAT activity in both groups, whereas GPx activity showed a notable increase. Although a decrease in CAT activity was observed in both groups, it should be noted that CAT activity increases only at high concentrations of H_2O_2 and severe OS [45]. On the other hand, GPx has a more important role in the elimination of H_2O_2 at low concentrations due to its greater affinity [18]. Therefore, the decrease in CAT activity in both groups is consistent with the increase in GPx activity and the decrease in H_2O_2, especially in the HIFT group. These results are consistent with the

findings of some previous studies [41, 46]. However, contrary to the findings of the present study, Costa et al. (2018) in blood erythrocytes showed that four weeks of HIIT significantly increased catalase activity and decreased thiobarbituric acid reactive substances (TBARS) [47]. Differences in subjects, duration of intervention (4 weeks vs. 8 weeks), intensity of exercise, and location of catalase activity (serum versus blood erythrocytes) may partly explain the differences in results. Another study, with participants similar to the present study, showed that 6 months of soccer training increased blood CAT and SOD activity and lipid peroxidation, while decreased glutathione levels [26]. These findings suggest that increased catalase activity is associated with a marked increase in oxidative damage (increased lipid peroxidation index). While in the present study, MDA levels significantly decreased in the HIFT group. In addition, it should be noted that CAT is considered as an intracellular enzyme and its increase in serum levels is partly due to cellular and tissue damage [48-50]. Some studies have reported an increase in serum CAT associated with erythrocyte and hepatocyte damage in haemolytic and liver diseases [49]. Also, the increase in serum CAT after acute eccentric exercise has been shown to be mainly due to CAT leakage out of damaged muscle fibers [48]. Also, another study showed that catalase and MDA increased in overtraining situations where OS and cellular damage was greatest [12]. Therefore, it seems that the decrease in serum CAT activity in both groups could be due to the decrease in damaged cells. Nevertheless, there are still many questions about the sources of serum CAT and its biological role in the serum. In fact, it has been suggested that CAT may be involved in the removal of serum H_2O_2 [51]. Therefore, it is suggested that more attention must be paid to the biological role of serum CAT and its interaction with HIFT in future studies.

In the present study, after 8 weeks of training, H_2O_2 and MDA were significantly reduced, while in the MITT group, only a slight and non-significant decrease was observed. It has been shown that H_2O_2 is continuously produced and removed in plasma. In particular, during acute intensive exercise, cellular damage and inflammation status, circulating xanthine oxidase and endothelium-bound xanthine oxidase increase [52], which can increase plasma H_2O_2 production. However, the H_2O_2 can diffuse into other cells [53] or react with proteins and other plasma compounds and cleared from the environment. Therefore, the decrease in hydrogen peroxide after HIFT in this study could be due to reduced cellular and tissue damage, decreased xanthine oxidase activity, increased plasma antioxidants (GSH, GPx) and also increased H_2O_2 diffusion capacity. Due to the lack of sufficient information about the effect of HIFT on each of the mechanisms, it is suggested to study more of these possible mechanisms in future studies. However, a decrease in MDA after HIFT reflects a reduction in cellular damage. Consistent with the findings of the present study, previous studies have shown that regular aerobic or resistance training can reduce MDA [54, 55]. This decrease in MDA also appears to be independent of the intensity of exercise [54]. MDA is mainly formed in cells due to peroxidation of membrane lipids and then released into the extracellular space [56]. Therefore, the decrease in MDA in the exercise group may be due to the reduced formation of MDA in the cells. Because MDA is mainly formed due to mitochondrial ROS [57, 58], improving mitochondrial function and antioxidant defense (increase GPx activity and decrease H2O2 levels) after HIFT may have led to a reduction in MDA formation. Also, removal of MDA from plasma [54], possibly due to increased catabolism, excretion or redistribution in the body, may play a role in reducing MDA in the HIFT group.

The findings of this study showed that 8-OHDG did not induce significant changes in the two groups. Unlike any other form of oxidized guanine, 8-OHdG can cross cell membranes, so it is usually detected in the urine or serum as a marker of OS. Inconsistent with these findings, a study using 14 adolescent athletes (7 girls and 7 boys) showed that long-term of intensive endurance training (a 10-month period including training and competition) can increase 8-OHDG [23]. However, the subjects in this study had about 9 to 15 hours per week of various endurance activities such as swimming, cycling, and running, and covered a total distance of about 144 km per week. Therefore, different volume of training can be considered as one of the main reasons for the contradiction of the findings, which needs to be paid more attention in future research. However, another study by Hamurcu et al. (2010) found that 8-OHDG levels in adolescent wrestlers were significantly lower than in the sedentary group [59]. It seems that higher sample size in the study of Hamurcu et al. (2010) (18 adolescent male wrestlers and 18 adolescent male control) than the present study are effective in different findings. Also in the present study, the MITT group performed football technical training, while mainly, previous studies have shown the effects of training compared to inactivity on 8-OHDG. No study has examined the chronic effect of

exercise intensity on 8-OHDG changes. However, the findings of the present study showed that, at least in basic conditions, the intensity of training does not seem to have a significant effect on 8-OHDG. Given the limited theoretical literature on the effect of chronic HIFT/HIIT on 8-OHDG, further studies in this area, especially in adolescents, seem necessary. It has been reported that there is a positive dose-response relationship between the intensity of acute endurance exercise and 8-OHDG [16]. However, the HIFT training protocol is different from the endurance aerobic training protocols. In addition, the interaction of the volume and intensity of the acute exercise changes the overall stress and 8-OHDG levels [60]. Also, it has been reported that oxidative DNA damage due to acute exercise is recovered within about 72 hours [16]. Therefore, definitive conclusions about the effect of training variables on 8-OHDG in adolescents need further studies. Also, although many studies have shown an increase in 8-OHDG under OS conditions, the exact biological role of 8-OHDG has not been investigated [61]. It may have mutagenic or at least harmful effects on cells, and it is the reason that mammalian physiology is working hard to eliminate this oxidized guanosine [61]. Surprisingly, the production of this molecule could be one of the cells' defense mechanisms against inflammation caused by OS [61]. For example, inhibition of Rac1 by exogenous 8-OHDG has been shown to significantly inhibit ROS-induced inflammation [61]. Compared to other nucleoside products, 8-OHDG has a strong anti-inflammatory effect. In fact, compared to aspirin, 8-OHDG has been shown to be more effective in reducing tumor necrosis factor (TNF-a), interleukins, myeloperoxidase activity, and neutrophil uptake in severe pneumonia due to injection of lipopolysaccharide into mice [62]. This finding suggests that 8-OHDG levels increase under severe inflammatory conditions and high levels of OS. Therefore, the lack of significant changes in 8-OHDG in the present study can be related to the anti-inflammatory and antioxidant effects of chronic HIFT. However, a meta-analysis (2020) showed that acute and chronic exercise are stressors on the redox status of adolescent athletes. This study showed that the changes in biomarkers of OS are more pronounced after acute exercise, while the changes after chronic exercise training are low to moderate (except for overtraining conditions and illness) [15]. However, the effects of chronic exercise on 8-OHDG levels were not reported in this meta-analysis. Therefore, due to the limitations of this meta-analysis, it is suggested that more research be done to compare different types of chronic training methods on 8-OHDG levels.

It should be noted that the current study had some limitations including not being able to exclude some moderators. A key limitation of this study is the small sample size, which may limit the generalizability of the findings. Additionally, the lack of a non-exercising control group makes it difficult to attribute changes solely to the intervention. In addition, the amount of received antioxidants and the details of the participant's diet during the intervention were not measured and not assimilated in two groups which may affect the antioxidant adaptations caused by HIFT. Therefore, it is suggested that future studies investigate the interaction of nutritional and exercise patterns in adolescent athletes. Also, in this study, OS biomarkers were measured only in serum and resting conditions (48 hours after exercise), it is possible that in other time intervals, such as immediately after acute exercise, the responses are different compared to resting conditions.

5. Conclusion

In conclusion, this study demonstrated that High-Intensity Functional Training (HIFT) was not associated with greater oxidative stress compared to Moderate-Intensity Technical Soccer Training (MITT) in adolescent soccer players. Instead, HIFT led to improvements in power performance and the maintenance of high aerobic capacity. The enhanced performance observed in the HIFT group may be attributed to antioxidant and neuromuscular adaptations. These findings suggest that HIFT is a time-efficient and effective training strategy for improving physical performance while reducing oxidative stress in adolescent athletes. Future research should investigate the long-term effects of HIFT and include larger, more diverse populations to confirm these results.

Competing interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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