Research Paper

The Effects of Eight Weeks of Plyoball and Resistance Band Training on Glutathione Peroxidase and Malondialdehyde in Handball Players

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ABSTRACT

Purpose: Acute aerobic and anaerobic exercise can cause a state of oxidative stress characterized by an increase in oxidized molecules in a variety of tissues and body fluids. The amount of oxidation depends on the mode of exercise, intensity, and duration, and is specifically related to the degree of oxidant production. This research investigates the effect of eight weeks of plyoball and resistance band training on glutathione peroxidase, malondialdehyde, and performance indicators of female handball players.

Methods: In this semi-experimental study, 30 female handball players aged between 10 and 16 were randomly divided into the following three groups: 1) Handball training (n=10), 2) Handball+resistance band (n=10), and 3) Handball+plyoball training group (n=10). The plyoball and resistance band training program consisted of eight weeks (two sessions per week) and each session lasted 45 to 60 min. The one-way analysis of variance repeated measures were used for within and between group changes, respectively.

Results: Eight weeks of plyoball and resistance band training caused a significant increase in glutathione peroxidase levels and decreased salivary malondialdehyde levels in handball players. The accuracy and strength of the handball shot increased significantly at the end of the eight-week intervention period.

Conclusion: According to the results, the greatest increase in the levels of glutathione peroxidase was in the group of handball exercises with a resistance band and the greatest decrease in the levels of malondialdehyde was in the group of handball exercises alone. The highest amount of improvement in the accuracy and strength of the handball shot was achieved in the group of handball training with the resistance band. Therefore, the performance of handball exercises with resistance bands can be recommended to improve the levels of oxidative stress, accuracy use, and shot strength in handball players compared to other training groups.

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Highlights

• The highest increase in glutathione peroxidase levels occurred in the group of handball exercises with traband.

• The greatest decrease in malondialdehyde levels occurred in the group of handball exercises alone.

• The accuracy and strength of the handball shot increased significantly at the end of the eight-week intervention period.

• The most improvement in the accuracy and strength of handball shots was achieved in the group of handball training with traband.

Plain Language Summary

Physical exercise is a complex biological activity that affects homeostasis at the level of cells, tissues, organs, and the whole body. Reports show that physical exercise increases reactive oxygen species production. There is little information about the level of oxidative stress when comparing the exercise modes of traband and plyoball. This research investigates the effects of eight weeks of plyoball and resistance band training on glutathione peroxidase, malondialdehyde, and performance indicators of female handball players. The results demonstrated that eight weeks of plyoball and resistance band training caused a significant increase in glutathione peroxidase levels and decreased salivary malondialdehyde levels in handball players. The accuracy and strength of the handball shot increased significantly at the end of the eight-week intervention period. Considering the considerable variation in the subjects, types, intensity, and duration of exercises, the duration of the training periods of most related studies has been controversial, and the effect of exercise on antioxidant enzymes is also at issue. Hence, the appearance of conflicting results about antioxidant enzyme activity levels between different studies is not unexpected. Overall, traband plyoball exercises can affect the activity level of antioxidant enzymes in female handball players.

Introduction

Oxidative stress is observed as an imbalance between the production of reactive oxygen species (ROS) and their elimination by protective mechanisms, which can lead to chronic inflammation [1]. Oxidative stress in the cellular environment leads to the formation of unstable lipid peroxides and reactive oxygen species. One of the most important products of lipid peroxidation is malondialdehyde (MDA), which has been widely studied and is considered a primary marker of oxidative stress [2, 3]. MDA is a carbonyl group produced during lipid peroxidation and plays a role in the diagnosis of oxidative stress [4]. Antioxidants are the body's defense mechanisms against oxidants, playing an important role in maintaining the redox status, eliminating active species, and establishing a balance between reduction and oxidation reactions in the body. The most important and abundant enzymatic antioxidants include catalase, glutathione peroxidase, and superoxide dismutase [5], and non-enzymatic antioxidants include flavonoids [6], albumin, glutathione [1], and thioredoxins [7]. Glutathione tripeptide is an intracellular thiol antioxidant. Lower levels of glutathione tripeptide lead to increased ROS production, which can result in an imbalance in the immune response, inflammation, and susceptibility to infection [8]. Participation in physical exercises can have a dual effect on the body's antioxidant defense system and susceptibility to inflammation. Engaging in moderate-intensity aerobic activities does not lead to the production of free radicals, and regular and moderate physical exercises are thought to improve the body's antioxidant status and reduce the production of free radicals in the body [9]. On the other hand, long-term intense anaerobic physical exercises confirm the production of free radicals and cellular damage following intense and heavy exercises, leading to the stimulation of the production of reactive oxygen species and the antioxidant defense system [10].

Regular exercise increases the activity of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. The basic approach to measuring oxidative stress is to measure the level of lipid peroxidation or fatty acids [11]. In this regard, various studies have been conducted to determine the relationships between different exercise conditions and their effects on oxidative stress markers [12-14]. Bagheri et al. (2012) showed that the MDA oxidative stress index in female handball athletes was significantly lower than in non-athletic girls, and the total antioxidant capacity of these athletes was higher than that of non-athletic girls. These results indicate that handball athletes had lower levels of oxidative stress and higher total antioxidant capacity compared to non-athletic individuals [15]. Tayebi et al. (2017) investigated the effects of eight weeks of resistance training at two different intensities on oxidative stress markers in 30 healthy men aged 20-25 years. The participants were randomly assigned to the three following groups: Moderate-intensity resistance training (hypertrophy), high-intensity resistance training (strength), and a control group. The results showed a significant decrease in MDA concentration in the exercise groups compared to the control group, with higher levels in the hypertrophy training group compared to the strength training group. Additionally, the concentration of reduced glutathione increased significantly in the exercise groups compared to the control group [16]. Based on the studies conducted, some have reported an increase in oxidative stress and lipid peroxidation markers after physical activity and exercise [15, 17], while the results of some studies indicate a decrease in these markers [18]. In this regard, the results obtained from current research in this area are contradictory and require further investigation.

In short, considering the nature of handball, where players engage in intense physical activity throughout the game, athletes in this sport may be susceptible to injuries resulting from lipid peroxidation; therefore, addressing and investigating the level of oxidative stress in female handball athletes is essential. Furthermore, there are currently various forms of resistance training that can be implemented, with the most common being the use of free weights and elastic bands. Resistance band exercises, due to their ease and safety, are widely used today. Among the advanced resistance exercises, the use of resistance bands can lead to increased muscle size and strength [19]. Exercise with resistance bands has been established as a safe tool and an effective strategy for improving neuromuscular function, enhancing muscle strength, and increasing the ability to perform functional tasks [20]. Using free weights requires facilities and equipment and can sometimes be hazardous and it requires significant supervision by coaches in schools and sports clubs. Moreover, the use of this training method in handball, especially for upper-body strength, has been less studied and researched. Accordingly, given the need for strength and sufficient power in the upper body, and the apparent weakness of students in this area, the use of these training methods can be beneficial. Hence, this research investigates the effects of an eight-week plyoball and resistance band training on changes in glutathione, salivary malondialdehyde, and functional indicators in female handball players.

Materials and Methods

This was a semi-experimental study with a pre-test and post-test design. Given that talent identification in the field of handball occurs between the ages of 10 and 16 years, this age range corresponds to a critical period of personal and social growth known as adolescence. During this time, individuals experience notable physical, cognitive, and behavioral changes. They are in the process of shaping their identity and facing new and exciting challenges. It is within this age group that the most talented handball players can be identified and recruited. The statistical population of this research included girls aged 10 to 16 years who were members of the handball teams in school sports clubs. The sample of the study consisted of 30 students from Sabzevar City, Iran, who were purposefully selected and then randomly assigned to three groups. The following groups were included in this research: 1) Regular handball exercises (n=10), 2) Handball exercises+resistance band (n=10), and 3) Handball exercises+plyoball exercises (n=10).

In the initial stage, individuals were familiarized with the nature and the ways of collaborating with the implementation of the research. The inclusion criteria for the study were as follows: Being in good health based on a health questionnaire, aged between 10 and 16 years, having no mobility restrictions for participating in sports activities, not using saliva-thinning medications, not taking any medication, not smoking, and not using drugs or dietary supplements that could affect the research results. The criteria for exiting the study were non-participation in two consecutive training sessions, suffering from cardiovascular, renal, or hepatic diseases during the eight-week protocol, neuromuscular incapacity to perform the exercise and a history of smoking. Based on the personal information questionnaire, medical history, examination, and the physician's opinion, all participants were deemed healthy. The level of physical activity of individuals was also determined using the Kaiser physical activity survey questionnaire, which had a reliability of 0.87 [21]. In this study, the physical activity readiness questionnaire was used to assess the fitness of the participants. The participants voluntarily participated in the study, following the study conditions, and their parents signed the consent form.

For the assessment of body composition, the following measurements were taken: The height of the participants was measured using a SECA stadiometer (manufactured in Germany) with an accuracy of 5 mm; the body weight was measured using a digital scale (manufactured in China) with an accuracy of 0.1 kg; the circumference of the hips and waist was measured using a measuring tape (manufactured by Mabis, Japan) with an accuracy of 5 mm; the waist-to-hip ratio in cm was calculated by dividing the waist circumference by the hip circumference; the body mass index was calculated by dividing the body weight by the square of the height in meters. To measure the waist-to-hip ratio, the researcher measured the waist circumference at the narrowest point (between the lower end of the chest and the navel) and the hip circumference at the widest point (over the buttocks) in cm using a measuring tape and then calculated the ratio for each participant.

In this study, performance indicators, including the power and accuracy of handball shots were collected. The Cornish handball test was used to determine the strength and power of the subjects in handball throwing and the 9-m throw test was used to obtain the shot accuracy of the subjects.

The participants in the exercise group were 8 weeks (2 sessions per week) and the duration of each session was 45 to 60 min. Evening training (16:00 to 17:00) was held which included a warm-up with stretching (six stretches were maintained for at least 30 s). The intensity of exercise was controlled by the Borg scale in addition to heart rate. The intensity was increased during the training period (increased repetition and decreased rest).

Normal handball training consisted of 10 min of general warm-up, 30-40 min of movement with a dedicated ball, training of skills and techniques of this field, reviewing tactical and playing tasks, and 10 min of group cooling, which were performed alternately and variably by the triathlete groups for eight weeks and repeated two sessions at 60 to 90 min per week. The cooling program consisted of 5 min of stretching [22].

According to the objectives of the study in which upper limbs were considered, the selected activities were performed by the experimental group 2 periodically and according to the instructions for 8 weeks and 2 sessions per week for 30 to 40 min. The time of the selected training with traband was immediately after the end of the normal handball training with experimental groups one and three. The training protocol group with the elastic band in pink has 4 upper body and 4 lower body movements (upper body: Pectoral muscle stretching, biceps stretch with bodybuilding elastic band, lateral stretching of scapula muscle with bodybuilding elastic band, triceps stretching with bodybuilding elasticity; lower-body movements: Squat, hip girder (outward), close the thigh, knee opening without opening the thigh (Table 1)). The perceived intensity of exercise pressure was considered between 10 and 15 [23].

The duration of these exercises was 30-40 min after the joint training. The start and end times of all three groups should be as close as possible. All the activities were repeated in 2 to 3 sets and the process frequency was 6 to 10 repetitions, and between each set was 30 to 45 s, and between the two activities was a rest intermission of 2 to 3 min. In designing the program, this study arranges the exercises from simple to difficult in each session and from one session to the next. Also, the weight of the ball was selected in such a way that the subjects could perform the activity correctly and with maximum speed. At the time of implementation of the program, the subjects were not allowed to participate in any resistance exercises, skill training, or other regular sports (Table 2).

Saliva sampling was done in two stages before and after eight weeks. First, one day before the start of the research protocol, 3 mL of unstimulated full saliva was collected from each person in a sitting position and resting state. The sampling conditions were that they did not consume any substance for 90 min before sampling and rinse their mouth with water. After 5 min in the test tube, every 2 to 10 min, they emptied their saliva in the test tube, which was frozen at -80°C. Then, at the end of the eight-week training program, 24 h after the last training session, resampling was performed. To determine the level of salivary MDA and glutathione peroxidase

Table 1. Program of selected exercises with traband

Weeks	1st	2nd	3rd	4th	5th	6th	7th	8th
Set	3	3	3	3	3	3	3	3
Repetition	8-10	8-10	10-12	10-12	10-12	10-12	12-14	12-14

PHYSICAL TREATMENTS

Table 2. Selected plyoball training program

	Movement	Set×Repetition	Rest
1	Circle move overhead with Madison ball	Three sets of 8 repetitions once with rotation on the right and the left	30-45 s
2	Moving the woodcutter with the Madison ball	Three sets of 10 repetitions on each side	30-45 s
3	Kicking Madison's ball to the ground	12 repetitions	30-45 s
4	Knee lift with Madison ball	For 30 s	30-45 s
5	Moves Swedish swimming with Madison ball	3×10-12	30-45 s
6	Climbing moves with Madison ball	Performed for 20 s quickly and without deformation to fatigue; 30 s of rest; repeated twice	30-45 s

PHYSICAL TREATMENTS

levels purchased from Padgin Teb Company and the enzyme-linked immunosorbent assay laboratory method was performed by ZellBio kit made in Germany with a sensitivity of 0.5 U/mL for glutathione peroxidase activity with serial number ZB-GPX-48A/ZB-GPX-96A and sensitivity of 0.1 µM for MDA activity with serial number ZB-MDA-48A/ZB-MDA-96A.

The collected data were analyzed by the SPSS software, version 20. After confirming the normality of the data using the Shapiro-Wilk test and the homogeneity of variances using the Levene test. The one-way analysis of variance with repeated measures was used to compare the mean of intra and intergroup means. Meanwhile, the Bonferroni post hoc test was used to evaluate the comparison of two-to-two groups. To determine the significance of the results, the level of $P>0.05$ was considered a decision-making instrument.

Results

The participants were randomly selected for this research. The characteristics of the samples, including mean age, height, weight, and body mass index, are presented in Table 3.

The results of body composition in Table 4 showed no significant difference between the three groups in any of the mean body weight, body mass index, and waist-to-pelvic ratio. The results of intragroup changes in body weight and body mass index significantly decreased in all three groups ($P=0.001$); however, no significant change in waist-hip ratio was observed at the end of the training period.

Table 5 showed that changes in lipid peroxidation levels and performance indices of handball girls showed that interactive time changes in the group were significant only in handball shot strength. However, there was no significant difference between the three groups in salivary MDA and glutathione peroxidase and handball shot accuracy. The results of intragroup changes in MDA levels in all three groups decreased significantly and the amounts of salivary glutathione peroxidase increased significantly ($P=0.001$). The strength and accuracy of handball shots increased significantly in all three groups.

Based on Bonferroni's post hoc test results, there was a significant difference between the handball training group and the handball+traband training group ($P=0.001$), handball training group+plyoball training group ($P=0.001$), while there was no significant difference between handball training+traband group of handball+plyoball training group ($P=0.329$) in the handball shooting strength variable (Table 6).

Table 3. The characteristics of the samples taken from the pre-test results

Variables	Mean±SD				
	Age (y)	Height (cm)	Weight (kg)	Body Mass Index (kg/m²)	Waist to Hip Ratio (cm)
Handball	13.40±1.77	150.90±5.46	40.94±3.39	18.04±2.03	0.88±0.04
Handball+traband	13.60±1.95	151.95±5.88	43.25±3.94	18.78±1.94	0.90±0.04
Handball+plyoball	13.90±1.85	153.50±5.29	40.63±3.74	17.29±1.93	0.91±0.04

PHYSICAL TREATMENTS

Table 4. Changes in body composition of handball players

Variables	Groups	Mean±SD		Changes (%)	P**	p		
		Stages				Between Group Mean Changes		
		Pre-test	8th Week			Time	Group	Time×Group
Weight (kg)	Handball	40.94±3.39	40.05±3.23	-2.22	0.001†	0.001	0.207	0.658
	Handball+traband	43.25±3.94	42.42±3.98	-1.95	0.002†			
	Handball+plyoball	40.63±3.74	39.55±3.47	-2.73	0.002†			
BMI (kg/m^2)	Handball	18.04±2.03	17.65±1.94	-2.20	0.001†	0.001	0.210	0.670
	Handball+traband	18.78±1.94	18.42±1.92	-1.95	0.002†			
	Handball+plyoball	17.29±1.93	16.81±1.64	-2.85	0.003†			
WHR (cm)	Handball	0.88±0.04	0.89±0.04	1.12	0.084	0.049	0.549	0.506
	Handball+traband	0.90±0.04	0.89±0.03	1.12	0.347			
	Handball+plyoball	0.91±0.04	0.90±0.02	1.12	0.606			

PHYSICAL TREATMENTS

Abbreviations: SD: Standard deviation; Kg: Kilogram; BMI: Body mass index; WHR: Waist-to-hip ratio.
**P within the group; †Significant level P<0.05.

Table 5. Comparison of intra- and inter-group mean changes in salivary malondialdehyde and glutathione peroxidase enzyme of handball players

Variables	Groups	Mean±SD		Changes (%)	P**	p		
		Stages				Between Group Mean Changes		
		Pre-test	8th Week			Time	Group	Time×Group
Malondialde-hyde (nmol/L)	Handball	6.81±0.67	5.88±0.69	-15.81	0.014†	0.001	0.204	0.256
	Handball+traband	7.13±0.63	6.17±0.45	-15.58	0.001†			
	Handball+plyoball	6.37±0.80	5.87±1.01	-8.51	0.001†			
Glutathione peroxidase (units per mL)	Handball	54.54±8.52	62.47±7.46	12.69	0.001†	0.001	0.622	0.759
	Handball+traband	52.80±5.65	63.03±7.58	16.23	0.008†			
	Handball+plyoball	51.28±2.90	60.91±6.78	10.59	0.001†			
Strength shoot	Handball	6.21±0.34	6.85±0.14	9.34	0.001†	0.001	0.001	0.001
	Handball+traband	6.20±0.41	7.72±0.37	19.68	0.001†			
	Handball+plyoball	6.41±0.19	7.76±0.46	17.48	0.001†			
Accuracy shot	Handball	12.44±0.34	13.47±0.38	7.64	0.001†	0.001	0.238	0.075
	Handball+traband	12.30±0.85	14.08±0.66	12.64	0.001†			
	Handball+plyoball	12.31±0.80	18.28±0.64	13.79	0.001†			

**P within the group, †Significant level P<0.05.

PHYSICAL TREATMENTS

Table 6. Results of the Bonferroni post hoc test to evaluate the shoot strength of handball players

Variable	Group 1	Group 2	Mean Difference	P
Strength shot	Handball	Handball+traband	-0.430	0.002
		Handball+plyoball	-0.555	0.001†
	Handball+traband	Handball+plyoball	-0.125	0.329

†Significant level of P<0.05.

PHYSICAL TREATMENTS

Discussion

This study investigated the effect of eight weeks of plyoball and traband training on changes in salivary glutathione peroxidase, MDA, and performance indices of female handball players. There was no significant difference between the three groups in salivary MDA and glutathione peroxidase. The results of intragroup changes in malondialdehyde levels in all three groups decreased significantly and the amounts of salivary glutathione peroxidase increased significantly.

The results are consistent with the findings of Mehregan et al. (2023) [24]. However, it is not consistent with the findings of Koubaa et al. (2023) and Sharifi et al. (2014) [25, 26]. Mehregan et al. (2023) investigated the effect of four weeks of high-intensity interval training compared with aerobic training on serum ceruloplasmin and MDA levels in elite female handball players. The results showed no significant change in serum levels of MDA after aerobic training [24]. In contrast, Koubaa et al. (2023) investigating the effect of three warm-up periods on post-exercise oxidative stress in 14 amateur handball players, concluded that the concentration of most oxidative stress indices, such as glutathione peroxidase, superoxide dismutase, glutathione reductase, and thiobarbituric acid reactive substances were significantly increased [25]. Sharifi et al. (2014) reported that six months of handball training, three sessions per week in 30 students divided into two groups of athletes and non-athletes, resulted in a decrease in the levels of MDA in athletes compared to non-athletes [26]. Antioxidants can act by different mechanisms, such as removal of oxygen or local oxygen concentration, extraction of catalytic metal ions such as Cu^{2+}, Fe^{2+}, ROS, such as superoxide and hydrogen peroxide, and interruption of chain reactions. The ability of an antioxidant to neutralize reactive oxygen and free radicals depends on various factors that can be related to the location of the ROS. Free radical production and its activity, interaction with other antioxidants, and adsorption, distribution, and metabolism of antioxidants were noted [27]. Exercise training can improve the regulation of the antioxidant system create protective adaptations and ultimately protect cells against the harmful effects of oxidative stress. Exercise training increases the amount of glutathione peroxidase by affecting the gene expression of antioxidant enzymes. Glutathione peroxidase has more stable changes among other oxidative enzymes and causes incremental changes compared to long training [28]. After exercise training, the cellular defense system tries to balance or increase antioxidant enzymes against oxidative stress. Probably regular and continuous exercise training increases cellular defense and antioxidant enzyme activity and prevents the activity of free radicals. By increasing the antioxidant activity of glutathione peroxidase, beneficial effects on lipid peroxidation of the membrane have been observed. As a result, regular and regular exercise training makes people more resistant to oxidative stress and provides a healthy life.

The results showed that interactive time changes in the group were significant only in handball shot strength. However, there was no significant difference between the three groups in handball shoot accuracy. The strength and accuracy of handball shots increased significantly in all three groups. there was a significant difference between the handball training group and handball+traband training group, handball training group+plyoball training group, while there was no significant difference between handball training+traband group of handball+plyoball training group in the handball shooting strength variable. The results of this study are consistent with the findings of Carter et al. (2007) [29] and Hoff et al. (1995) [30]. Hoff et al. (1995) investigated the effect of bench press exercises with maximum combined strength and normal team skill training on female handball team players. Standing throwing speed, shooting speed with 3-step firing in set, and one repetition maximum in bench press significantly improved in all variables [30]. Recently, it has been found that there is a positive relationship between physical activity and cognitive function in schoolchildren (7-18 years) which indicates the positive role of exercise on cognitive function [31]. In addition, exercise may be a strong protective factor against the neurological analysis that occurs with age. It can also increase cognitive function in older adults and decrease the progression of cognitive decline that is based on dementia [32]. In other studies, it has been observed that exercise leads to neurodegeneration and performance improvement in learning and memory behavioral tests and changes synaptic plasticity in rat dentate torture [33]. From one perspective, memory is concerned with attention in terms of processing resources allocated to a task. From another point of view, attention is involved in selecting these essential resources and preventing the processing of other unnecessary items [34]. On the other hand, attention and accuracy have a special importance in learning. Concentration abilities are a prerequisite for learning [35]. Considering the relationship between memory and learning with attention and attention and on the other hand the effect of exercise on memory and learning, the effect of exercise on attention is also justified.

Conclusion

The data show that eight weeks of plyoball and traband training significantly increased glutathione peroxidase levels and significantly decreased the levels of salivary MDA in handball trees. The accuracy and power of handball shooting increased significantly at the end of the eight-week intervention. The highest increase in salivary glutathione peroxidase levels was observed in the group and handball training with traband, and the highest decrease in MDA levels was achieved in the handball group alone. The results showed that the average of the three groups in throwing power in the handball training group and the traband group had the highest improvement and there was a statistically significant difference between the three groups. Therefore, the implementation of all three types of training programs for 8 weeks can be recommended to improve levels of oxidative stress and shot accuracy and power in handballers, so using these interventions is probably a good way to improve the performance of handball girls.

Ethical Considerations

Compliance with ethical guidelines

Informed consent was obtained from the participants following the Declaration of Helsinki. The right foot was the dominant limb for all participants. This study was approved by the local Ethics Committee of Sabzevar University of Medical Sciences (Code: IR.HSU.REC.1401.010).

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Authors' contributions

Conceptualization and methodology: Keyvan Hejazi and Azizeh Toghdori; Data collection, data analysis, and original draft preparation: Keyvan Hejazi; Review and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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