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Research Article

The effect of chocolate milk consumption on muscle damage enzymes of men professional football players

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Abstract

Background: Muscle damage caused by exercise occurs in all sports activities with eccentric contractions. The aim of the research was the effect of consuming chocolate milk on muscle damage enzymes of men professional football players.

Materials and Methods: 22 male soccer players (24.5 ± 1.2 years old) were voluntarily and randomly placed in two experimental and control groups. The training protocol for two weeks, 3 sessions per week and each session for 60-90 minutes included speed/plyometric training (ladder legs, agility training and coordination training) followed by resistance training. Subjects used 672 ml of chocolate milk supplement after training. Blood samples were taken before starting the research protocol and 48 hours after the last training session. Analysis of covariance test was used at a significance level of p<0. 05.

Results: Consuming chocolate milk caused a significant decrease in CK, LDH, AST, ALT, ALP and muscle pain in male soccer players (P = 0.001).

Conclusion: According to the results, chocolate milk supplement can be used as a factor in reducing muscle damage enzymes in male football players.

Keywords:
chocolate milk, muscle damage, football players

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1. Introduction

Football is considered one of the most attractive and popular sports in the world, and it is one of the most popular sports among men and women in Iran (1). This sport is performed as a relatively intense and short-term alternating activity, along with active rest (1). Football players must have a very high ability of maximum efficiency. During a 90-minute soccer match, most players usually run a distance of 10-12 km with a maximum heart rate of 80-90% (2). Muscle damage is a complication that leads to a disorder of muscle structure and is accompanied by symptoms such as delayed muscle contusion, reduction in maximum force production and increase in plasma proteins (3). Serum indices such as creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) are used to measure muscle damage. The increase in the concentration of these proteins in the blood is a sign of changes in the cell structure, including the rupture of the cell membrane and disruption of the internal integrity of the cell (4,5). CK is an enzyme that is found in the cytosol and mitochondria of tissues that have a high energy demand (4). Creatinine is one of the important plasma metabolites and is obtained as the final product of creatine breakdown. Creatine is a non-structural protein that is used to form phosphocreatine from the family of compounds with high-energy phosphate bonds (6). LDH is an enzyme that is abundantly present in the cytoplasm of all body tissues with different concentrations and plays a role in converting pyruvate to lactate or vice versa in the glycolysis pathway. This enzyme is found in many tissues of the body, especially the heart, liver, red blood cells, kidneys, muscles, brain and lungs (7).

AST is an aminotransferase that catalyzes the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. This reaction happens between mitochondria and cytosol and produces energy in the cell. This enzyme is present in skeletal muscles, heart, liver and red blood cells. The amount of AST increases immediately after performing the muscular activity and in some cases its effect remains up to 24 hours after the activity (8). Gudarzi et al. investigated the effect of futsal competition on creatinine and AST of soccer players. The results indicated an increase in the serum concentration of Cr and AST (9). Nutritionists and athletes are trying to find a post-workout nutrition strategy to increase muscle glycogen, accelerate recovery, and improve the quality of future workouts. The time of consumption and composition of nutrients can have a significant effect on recovery after heavy sports (10). Carbohydrate and protein supplement reduce the symptoms of sarcolemma disorders such as increased creatine and muscle pain and improves muscle function (11) compared to only carbohydrate drink. The results of Forrati et al.'s research showed that the creatine kinase enzyme in the muscles of runners decreased after consuming carbohydrates and proteins compared to carbohydrates (12). Chocolate milk is a potentially effective drink that contains carbohydrate and protein similar to carbohydrate and protein drinks associated with recovery, and due to its taste, availability, and low cost, it can be a suitable alternative to commercial sports drinks (13).

Considering the few studies on the effect of chocolate milk on muscle damage enzymes and the presence of conflicting results in previous researches and the necessity of recommending healthier and more effective supplements to athletes, the present study investigate the effect of chocolate milk consumption on muscle damage enzymes of professional football players.

2. Materials and Methods

The statistical population of this semi-experimental research included all football players in the age group of 19-30 years old in the Tehran province league in 2017-2018. 22 football players voluntarily participated in the research as a statistical sample and were randomly placed in two groups of chocolate milk consumption and control. The criteria for entering the research included being in the age range of 19-30 years old, being a member of one of Tehran's first division football clubs, not using supplements, drugs, tobacco, alcohol, and not having any disease or physical disability. The criteria for leaving the research included not participating in exercises for more than 1 session, unwillingness to cooperate with the researcher, and injury during the exercise protocol. After explaining the method and purpose of the research, the examinees completed the questionnaire of personal information and physical condition, such as history of lung disease, heart disease, vascular disease, and history of surgery, and were examined and approved by the relevant doctor.

The participants were advised to participate in training sessions and competitions. Others do not participate and maintain their usual diet during the implementation of the research. The examinees also filled out a written consent form. A calibrated wall-mounted height meter with an accuracy of 1 mm (Seka Company, Japan) was used to measure height, and a digital scale with an accuracy of 100 grams (Seka Company, Japan) was used to measure weight. Body mass index was calculated using people's weight and height.

Exercise protocol

The training protocol is presented in Table No. 1. Football training for 6 sessions, during two weeks, each session was held for 60 to 90 minutes under the supervision of the researcher in the Keshvari sports complex. At the beginning and end of the training, 10 minutes of warming up and 10 minutes of cooling down were performed. The main training consisted of speed/plyometric exercises (ladder legs, agility and coordination) followed by resistance training. The length of the main exercises varied from 55 to 70 minutes. Basic exercises were considered for two sessions and increased training duration for 4 days. The rest interval between skill tests was 3 to 5 minutes and between repetitions of tests was 2 to 3 minutes (14).

Table 1: training protocol

Week	Days of training	Type of training	period of time
The first week	**Monday**	coordination and skill exercises *(basic)*	70 minutes
The first week	**Wednesday**	coordination and skill exercises *(basic)*	70 minutes
		Intense training	
second week	**Saturday**	Cardiovascular training	55 minutes
second week	**Monday**	Plyometric training	55 minutes
second week	**Wednesday**	Strength and speed training	55 minutes
The third week	**Saturday**	Plyometric and recovery	45 minutes

Coordination and skill exercises included dribbling, passing and shooting skill tests by Moore-Christian (١5). Speed exercises including ٣٥meters speed test and agility exercises including Ili Noise agility test.(١٤) . In order to perform strength training, the test of one maximum repetition for the squat movement was used, using the Berzyski method, in two shifts with moderate intensity weights (15). Plyometric exercises included high jump, pair jump, sideways jump over hurdles and pair jump over hurdles, which included an average of 210 movements. These exercises were performed in 4 stations and 3 sets per station (15).

Cardiovascular training included Hoff's training method. The training intensity was equal to 90-95% of the maximum heart rate of each player. The formula (220 - age = maximum heart rate) was used (15). The training method was that the players dribbled the first 10 cones in a spiral shape and jumped over 30 cm high obstacles with the ball. After that, they spiraled through the next cones and from point A to B while controlling the ball; they would move backwards and then turn around and move towards the starting point. The working periods of activity included four four-minute periods separated by three minutes of active rest with 70% of the maximum heart rate (15).

In order to cause fatigue and increase the muscle damage of the athletes, the field test of periodic anaerobic power recovery yo-yo 2 was used. This test consists of running 2 consecutive distances of 20 meters back and forth to the starting point. The heart rate at the end of the test was measured with a heart rate monitor (Polar model F7 made in Finland) (15).

Prepare chocolate milk

Based on Gilson's article, equivalence was made and Manizan Company's product was prepared with the conditions of the following food table (Table 2). Immediately after each training session, the supplement group was given 672 mL of chocolate milk (16).

Table 2: Nutritional value of chocolate milk

Nutrient	Volume (mL)	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Sodium (mg)	suger (g)	VIT C	VIT E	CA
Chocolate milk	672	504	80	22.8	10	403	47	7	0	873

24 hours before the start of the training protocol and 48 hours after the last training session, blood was drawn to evaluate muscle damage indicators, in the amount of 5 cc in the sitting position from the vein of the right arm by a laboratory technician in Farabi laboratory. The samples were centrifuged for 10 minutes at 3000 rpm (made by Hetish, Germany). Then the resulting serum was separated and kept frozen at -80°C until the experiments. The amount of creatine kinase concentration was measured by chemical colorimetric method using the kit of Iran's Pars Azmoun Company with the sensitivity of 1 unit per liter with the auto analyzer of Kobas Mira Plus Company, made in Switzerland. Lactate dehydrogenase was measured by enzyme colorimetric method and using a color kit of Pars Azmoun Iran with a sensitivity of 5 units/liter. The level of liver enzymes AST, ALP and ALT was measured using a greener kit made in Germany. To measure pain perception, the subjects filled in the Magill Pain Questionnaire 24 hours after the exercise session (17).

The participant chose his perception on a 5-point continuum that was graded from mild pain (12) to unbearable pain. Shapiro-Wilk test was used to determine the normality of data distribution and Levine's test was used to check the homogeneity of variances. Analysis of covariance method was used to check the significant changes of each research variable. The level of significance was considered $p < 0.05$ for all calculations.

3. Results

The characteristics of age, height, weight, body mass index and sports history of the participants of the two groups are shown in Table 3.

Table 3: Demographic characteristics of the participants

group . variable	history (years)	BMI	weight (kg)	height (cm)	age (years)	Number
supplement	6.2±7	24.1±1	71.2±9	173.6±4	25.5±1	11
Control	7.3±7	24.1±6	71.4±7	174.4±1	23.6±2	11

The mean and standard deviation of creatine kinase, lactate dehydrogenase, AST, ALT, ALP and perceived muscle pain of the participants of the two groups in the pre-test and post-test phases are shown in Table 4.

Table 4: Mean and standard deviation of the variables measured by the participants in the pre-test and post-test phases

Group. variable	stage	chocolate milk	control	P value
creatine kinase (units per liter)	Pre-test	160.4±7،8	3،158±1،5	
	Post-test	2،149±9	6،164±7،10	*003،0
(units per liter) LDH	Pre-test	5،310±4،8	2،311±8،8	
	Post-test	9،301±7،7	8،313±5،10	*007،0
(units per liter) AST	Pre-test	7،18±1،3	7،15±5،3	
	Post-test	6،14±5،2	2،17±3،2	*010،0
(units per liter) ALT	Pre-test	1،24±7،4	5،27±7،7	
	Post-test	1،18±5،5	1،26±5،2	*001،0
(units per liter) ALP	Pre-test	88±7،11	1،87±4،8	
	Post-test	9،67±7،7	88±3،7	*001،0
Muscular pain	Pre-test	2،35±5،4	3،36±3،3	
	Post-test	7،22±7،3	9،32±3،3	*001،0

The results showed that the consumption of chocolate milk caused a significant decrease in the enzyme creatine kinase (sig = 0.003), lactate dehydrogenase (sig = 0.007), AST (sig = 0.010), ALT (sig = 0.001), ALP (sig = 0.001) and muscle pain (sig = 0.001) in professional football players.

4. Discussion

The results of the present study showed that the consumption of chocolate milk led to a decrease in creatine kinase in male professional football players compared to the control group. The results of the present study are consistent with the findings of Atashak et al. (2016) and potter et al (2015) (18, 19). It seems that the consumption of chocolate milk immediately after training can increase the speed of recovery and reduce muscle damage, and as a result, players are better prepared for the next training session. In Gilson et al.'s (2010) study, 13 subjects performed normal exercise for one week followed by four days of intense exercise (ITD) and then received high carbohydrate or chocolate milk. The results showed that serum creatine kinase significantly decreased after four days of ITD with the consumption of chocolate milk (16). Although the mechanism of the effect of chocolate milk is not completely clear, the combination of carbohydrates, milk protein and electrolytes may promote muscle glycogen production and regeneration. Fast body between training sessions. During light and moderate exercise, a higher percentage of milk fat compared to an electrolyte carbohydrate drink may increase blood free fatty acid and delay muscle glycogen depletion (3).

The results of the present study showed that the consumption of chocolate milk led to a decrease in lactate dehydrogenase in male professional football players compared to the control group. These results are in line with the findings of Gilson et al., Shirreffs et al. (16,20) and The results of Zardoost and colleagues are not consistent (21). One of the possible reasons for the inconsistency of the results may be attributed to the age range of the participants or to the type of drink. In Zardoost's research, the carbohydrate drink included saffron drink, which is different from the ingredients of chocolate milk.

Consuming chocolate milk immediately after exercise and again 2 hours after exercise appears to be beneficial for post-exercise recovery and may reduce indices of muscle damage. Compared to many carbohydrate-rich electrolyte drinks, chocolate milk has more carbohydrates per milliliter and is highly rehydrating, as well as providing sodium and fluids that must be replaced due to sweating during exercise (22). Chocolate milk is high in calcium, which is one of the main components of muscle contraction and building and maintaining strong bones. Recent studies have investigated the effectiveness of chocolate milk on protein synthesis following endurance training (23). The results of these studies show that chocolate milk is more effective than carbohydrate drinks in creating an environment of intracellular anabolism after exercise (24).

The results of the present study showed that after consuming chocolate milk, AST, ALT and ALP enzymes decreased significantly in the experimental group compared to the control group. The activity of liver enzymes is affected sports activities are intensified, which is affected by the duration, intensity, type and method of training. In a research, it was reported that serum aldolase and AST increased in people who walked on a treadmill for only five minutes (25). As complex metabolic cells, liver cells contain high amounts of enzymes. When muscle damage occurs, enzymes such as AST, lactate dehydrogenase, creatine kinase and ALT, which are all located in muscle fibers, increase in the blood. AST and ALT enzymes are abundant in the liver, AST is abundant in other tissues such as the heart, kidneys, skeletal muscles, and red blood cells.

In fact, the increase of serum AST and ALT indicates the entry of muscle and liver enzymes into the blood circulation (26). Therefore, changing the concentration of these enzymes can cause muscle damage. One of the most important indicators for the regulation of liver indicators is body fitness and the level of physical fitness, and changes in the mentioned parameters can affect the levels of these enzymes (25). In the present study, considering that the participants were young and professional athletes, and performing the desired exercises in this study was effective on the fitness indicators of these athletes, it could be one of the reasons for the decrease in liver enzymes.

Also, due to having a large amount of indirect carbohydrates and protein, the consumption of chocolate milk has beneficial effects on reducing the amount of liver enzymes after intense activities and has a positive effect on muscle recovery after strenuous exercises. The results of the present study showed that the consumption of chocolate milk led to a decrease in the pain perception of male professional football players compared to the control group. The time of consumption and the combination of nutrients can have a significant effect on recovery after heavy sports. The increase in carbohydrate consumption immediately after training, it increases the speed of muscle glycogen recovery and reduces the side effects of heavy endurance training (nervous states, excessive pressure and poor performance) (11). Carbohydrate and protein supplement, compared to only carbohydrate drink, interrupts the symptoms of sarcolemma disorders such as serum myoglobin and creatine kinase and increased muscle pain and improves muscle function. In addition, protein and carbohydrate consumption during recovery improves the performance of the whole body it shows in all sports (27). potter et al reported that chocolate milk improves people's performance while climbing. In this study, which involved ten male climbers, the participants received chocolate milk 20 minutes after climbing. The results showed that the muscle pain was reduced three days after climbing with the consumption of chocolate milk (19). However, in the study of Gilson et al. (2010), it was shown that the consumption of chocolate milk after a period of intense training does not lead to a reduction in pain (16) that these results are not consistent with the findings of the present study. Among the possible causes of inconsistency of information can be mentioned the level of preparation of the participants, gender, motor skill and age range and type of endurance sports.

۵. Conclusion

Considering the significant reduction in the levels of cell damage indicators and also the reduction of pain following the consumption of chocolate milk in professional football players, it is suggested that football players and football coaches use chocolate milk in all stages of training in order to recover faster, improve performance, and reduce the levels of liver enzymes and increase the efficiency of the body.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: A.R.J., R.B.; Methodology: A.R.J., R.B.; Software: A.R.J., R.B.; Validation: A.R.J., R.B.; Formal analysis: A.R.J., R.B.; Investigation: A.R.J., R.B.; Resources: A.R.J., R.B.; Data curation: A.R.J., R.B.; Writing - original draft: A.R.J., R.B.; Writing - review & editing: A.R.J., R.B.; Visualization: A.R.J., R.B.; Supervision: A.R.J., R.B.; Project administration: A.R.J., R.B.; Funding acquisition: A.R.J., R.B.

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