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Research Article

Evaluating the performance of non-reactive and reactive agility tests in elite and average soccer players under the age of 14

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Abstract

Background: Agility is one of the most critical factors in the sport performance of soccer players. Although various tests have been designed to measure agility, there is no agility test, based on soccer-specific movement patterns. Therefore, this study aimed to develop and examine of a soccer Specific Reactive Agility Test (SSRAGT)for Players U14 Years.

Materials and Methods: 48 soccer players under the age of 14 years divided in two groups composed of 24 players. The group A were elite soccer players and active at the level of Asia vision, while group B were soccer players were active at the level of neighborhood and local competitions One hundred and seventy competitive soccer players under 14 years volunteered to participation in this study as subjects. The standard 505 Agility Test (505AT), Zig-zag Curl (ZZC)، Zig-zag straight (ZZS) the test was performed for all participants on two separate days within a two-day interval at the same time and place. The SSRAGT was performed after two days.

Results: In order to evaluate the intensity of agility test the number of steps and heartbeat after activity was applied. The 505-agility test with a heartbeat of 159.4±11.245b was lower than other tests. The number of steps in the 505-agility test indicated lower intensity and fewer steps 26.30±4.794[b.] The heartbeat and number of steps in the other test had a significant correlation with each other. The results of logistic regression between 48 player SSRAGT test can significantly predict the level of performance of young football players (OR = 1.437, P <0.01). As the ZZC test was able to significantly predict the performance level of the subjects in this study (OR = 1.05, P <0.01).

Conclusion: Based on the result the reactive agility test for the soccer player in comparison with non-reactive agility test had the potential to distinguish between average and elite soccer players and due to its reactive nature, it is similar to movement patter in soccer, so it can be used as an efficient field tool to evaluate players' agility levels.

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Journal of Sports Physiology and Athletic Conditioning

1. Introduction

Sport in general can be distinguished and known as team or individual sports in which the performance is exclusively determined by the individual compared to the group or team(1, 2). Another more complex evaluation is based on division by their movement and technical characteristics and physiological needs. Comparing individual sports with team sports we found that athletes need to perform high and low activities(3, 4). In this confrontation, the athlete in the field and field of completion need to run at maximum speed, hard activities for each specific sport, jumps etc. all of which rely on speed and strength(5, 6). A unified factor that all individual and team sports need is to make a change in their direction in order to have speed and appropriate position which is known as agility(7, 8). In team sports like soccer agility is a skill and important quality to escape from opponents when you attack or defend(9). Agility is a basic and fundamental element in performing sport activities(10, 11). Research has shown that a soccer player changes direction every 2-4 seconds, so as a whole he /she will have 1200-1400 change of direction during a game(12, 13). Another study in English premier league showed that each player on the average will have 727 turns during a 90-minute match, it shows the importance of changing direction and agility even more(14). Agility can be defined as the ability to change the direction in response to an external stimulus along with maintaining balance at maximum speed while performing as well as ability to perceive and make a decision, accordingly agility field tests are pre-planned and unplanned(15, 16).

In pre-planned agility tests the subject is fully aware of the movement direction and knows in which direction should move such as agility tests: Illinois, straight zigzag and spiral, 505 and other similar tests(17, 18). There is an evolved type of agility test which is called reactive agility tests, where subject's movement path is not predetermined and subject does not know in which direction should move(19, 20). So, this movement pattern is more similar to movement pattern in soccer(21, 22). Due to lack of prediction of movement path of soccer player on the field, this movement pattern has been taken as a model and different reaction agility tests haven been designed and validated to identify the agility and the level of readiness of the players(23, 24). In order to evaluated players and increase the level of their progress there is a need for several evaluations(25, 26). In this regard, there are various laboratory tests. Despite the high accuracy and value of the existing laboratory tests, they are not always accessible to the coach and applying them require spending a lot of money, so field test that do not need a lot of money and can be implement with the least possible facilities become important and as an efficient tool, a cheap and applicable tool will be accessible to the coach to distinguish more agile and elite players from each other(27, 28). Whereas the majority of the existing agility tests are based on pre-determined conditions that are not in accordance with movement pattern in soccer, therefore various test have been designed and validated based on reactive pattern but there a is a need for special test that despite being reactive be in accordance with movement pattern of the player in the football field and the purpose of the current research is to do initial designing agility test especially for the players under 14 years old and comparing it with other non-reactive tests to distinguish between average and elite players.

2. Materials and Methods

48 soccer players under the age of 14 years divided in two groups composed of 24 players. The group A were elite soccer players and active at the level of Asia vision, while group B were soccer players were active at the level of neighborhood and local competitions. Also, both groups were without any muscle damage and voluntarily announced their readiness in this study as subjects. The method of selecting subjects was based on their level of activity. Group A had four training sessions per week in Tehran province. Group B had three training session per week and 2 competitions per month. Puberty time for all participated performed according method of Moor et al(15). written consent was obtained from the subjects and their parents in order to participate in this test. Test execution protocol was explained to the subjects during a briefing session by the experts of physiology department of Islamic Azad University of central Tehran branch. Results and interpretation of data were done by a third party who was in connection with the subjects. General characteristics of the participants can be seen in Table 1.

Table 1: General characteristics of included participants (n=٤٨)

Characteristics	Mean ± SD	Min	Max
Age(year)	13.18±0.781	12	14
Height(cm)	136.66±11.326	112	165
Weight(kg)	34.23±7.277	23	74
BMI (kg/m^2)	18.080±3.797	13.03	32.08

General characteristics and familiarity with the agility test

The subjects were asked to visit the exercise physiology laboratory for familiarization with the test method and determining their general characteristics. Considering the number of subjects, it was impossible to measure all of them in one day. Therefore, the subjects were divided into three groups. First, their height and weight were measured, using a wall-mounted stadiometer and a digital scale. They were then instructed on how to complete the 505Agility Test (505AT), Zig-zag Curl (ZZC)، Zig-zag straight (ZZS), Specific Reactive Agility Test (SSRAGT).

SSRAGT

This reactive agility test for soccer players is modeled based on actual soccer games and resembles a virtual soccer game that is visual and less audible. The movement path of the players in the soccer game is constantly changing in reaction, and there is no predetermined path (player with the ball may change direction at any time) (1, 13) (1, 13) (1, 11) (1, 9) (1, 9) (1, 9) (1, 9) (1, 9). Based on this principle, the SSRAGT was designed. The SSRAGT includes four round trips of four meters, with a reactive ball in four main way the entire area of route change is 32 meters. The graphic representation of this test is shown in Figure 1. After dividing the field into four main directions, the performance protocol is determined by the cones. With the participant standing at point 0 (starting point) with the ball, the examiner indicates a direction to lead. Then, the subject starts from point 0, which is marked on the sides (to determine if the distance is 4 m), moves in a direction that is not predetermined (at least 20-30 cm away from the cones), and changes direction to point 0.

When the subjects reach the starting point, the examiner makes a short sound and indicates a new path so that the subject changes direction. This process is repeated four times in four main directions. The distance that each subject ran in this test was 32 meters. The Less time for each subject is considered as his best recorded. The SSRAGT includes four main directions and four change of direction. Agility has been shown to play a critical role in athletic performance. It requires the power of perception and quick decision-making skills to prepare for a new direction and path as quickly as possible. The principles of perception and decision-making have been considered in the SSRAGT. A subject must be ready to change direction quickly based on visual indication. In other non-reactive agility tests, such as 505AT, ZZC, ZZS, MICOD, ICOD the path includes obstacles that may affect an individual's ability to perform the test. Other reactive agility tests, such as Y test, are performed after the path is changed, indicate that individual skills may be influential. This important principle was considered in the design of SSRAGT. We tried to identify the weaknesses of previous tests, where individual skills were effective. One of advantage of SSRAGT this is it there are no obstacles or shots on the routes, and there are only swift changes of route in reactive and linear ways. Also, the ball movement is exactly designed, based on the pattern of soccer players' movements.

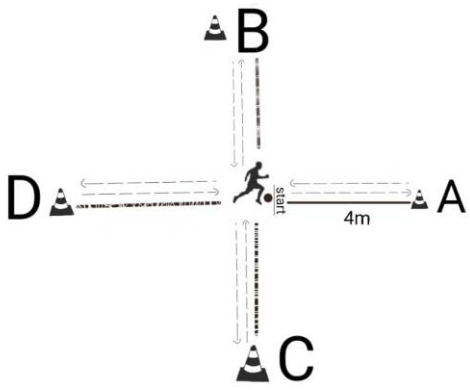

Figure 1: layout SSRAGT

Exercise protocol

A trial session was held at 3-5 p.m. to prevent daily diurnal variations. Each subject was tested three times, and their best record was documented. All players wore soccer uniforms, shorts, shoes, and socks to ensure that the test conditions were identical for everyone. Also, all of the tests were performed on artificial turf, and the conditions were the same for all soccer players.

Agility test

Forty-eight hours before the first test, the subjects were asked to avoid training hard. Fifteen minutes were allowed for warm-up before the test. The warm-up included a general warm-up, dynamic stretching, and a dedicated warm-up. The general warm-up included 800 meters of walk (200 m in 90 seconds, 200 m in 70 seconds, 200 m in 60 seconds, and 200 m in 45 seconds, respectively). Dynamic stretching included front and back lounges, squats to improve muscle contraction, and leg stretching movements. The dedicated warm-up included ten rotation movements at 90 degrees, with knee flexion and sprints in short distances at 70%, 80%, 90%, and 100% acceleration. Finally, after 3-4 minutes of activation rest, the subjects were be for the test.

Statistical analysis

The assumption of normality was examined using the Kolmogorov-Smirnov test at a significance level of $P<0.05$. Also we use the repeated measure ANOVA test to compare participation heart rate and steps.To assess the difference between the number of steps and heart rate were used Mean ± SD. The logistic model is used to model the probability of elite or not elite soccer players. The division of players into high and low soccer performance was based on their competitive level. high performance group competed in the national premier league and the low performance group competed in the provincial league. Logistic regression dependent variable Two variables

Surface (High performance = code 0 and low performance = code one) is considered. GraphPad Prism version 8.3 for Windows 10 was used to evaluate the data.

3. Results

It should be noted that the heart rate and the number of steps of the participants in the agility tests 505AT, ZZC, SSRAGT, ZZS were compared with each other. Significant differences in heart rate and number of steps were observed only between 505AT test whit SSRAGT. There was no significant difference in heart rate and number of steps between SSRAGT and ZZC, ZZS tests (Table 2).

Table 2: Comparison of heart rate and number of steps of the agility tests 505AT, ZZC, SSRAGT, ZZS

	Heart Rate Comparison		Step Comparison	
SSRAGT	166.4±11.149[a]		34.95±5.114[a]	
505AT	159.4±11.245[b]	P<0.001	26.30±4.794[b]	P<0.001
ZZC	167.63±10.448[a]		35.85±6.546[a]	
ZZS	168.33±8.480[a]		34.17±5.551[a]	

Diagnostic analysis (logistic regression)

The results of logistic regression between 48 players in 2 groups of 24 people with high and low soccer performance showed that SSRAGT test can significantly predict the level of performance of young football players (OR = 1.437, P <0.01). As the ZZC test was able to significantly predict the performance level of the subjects in this study (OR = 1.05, P <0.01) Table3.

Also, there is a positive correlation between agility test for the soccer players and 505 test as well as zigzag Slalom based on beta regression coefficient in the logistic model.

Table 3: Evaluation of diagnostic validity of tests based on logistic regression.

Agility test	B	P-value	OR	CI 95%	
				Lower	Upper
SSRAGT	.362	**<0.01**	**1.43**	1.35	1.52
505 AT	.017	0.50	1.01	0.96	1.06
ZZC	.049	0.27	**1.05**	0.96	1.14
ZZS	-.002	0.94	0.99	0.94	1.05

4. Discussion

The finding of this study showed that reactive agility test for the soccer players is not as a preliminary and reactive test where the movement path of the subject is not predetermined has a positive and significant agreement with other non-reactive agility test. One of the criteria for field evaluation to determine the severity of agility tests is the use of maximum heart rate. Accordingly, the heart rate of the subjects was compared in the 505AT, ZZC, ZZS and SSRAGT agility tests, and the results showed that the heart rate and the number of steps in the 505AT agility test were smaller than the other tests, which was related to the movement pattern is linear in this test and in SSRAGT agility test in comparison with other tests, there is no significant difference between heart rate and number of steps between tests, which shows that SSRAGT test is similar to other tests in terms of activity intensity. In all agility tests, the movement path of the players is predetermined and the player follows the movement path with full awareness, and finally the person's stagnation is recorded, which is different from the pattern and features of the soccer game, because in the soccer match a path is predetermined. There is no set and players are constantly changing direction depending on the conditions of the match.

During a 90-minute soccer match, there are about 1200-1400 diversions, and players are forced to change sudden and unpredictable routes during the match, based on the opponent's tactics(29, 30). Therefore, this very important pattern is used in the design of the SSRAGT test as far as the movement logo may not be predetermined and soccer's change direction to a new path in the shortest possible time based on perception, decision, cognitive factors and conditions(31, 32). Give soccer is a visual game and players must change their direction in the shortest possible time based on the movement of the ball and the opponent(33, 34). Therefore, the SSRAGT agility test is designed exactly accordingly, and the experimenter, after the test taker has determined a new path for him, should change the path to the determined path in the shortest possible time based on visual perception and timely decision. The results of previous studies in soccer's show that they have a lateral advantage in the right half of their body (ears, eyes, right foot) compared to the left half, and if they know the direction of movement before the test, lateral superiority may affect the record(35). The experimenter is incorrectly considered to have the desired agility.

However, in the SSRAGT agility test, this issue has been taken into consideration, and since the subject has to change direction in four main directions, the share of lateral superiority in the test result has been reduced to a minimum and its distorting effect has been curbed as much as possible. Regarding initial preliminary results of agility test for the soccer players as a reactive field test and in accordance with movement pattern of soccer players the results obtained based on logistic model indicates positive significance with other non-reactive agility tests which has a potential to distinguish between average and elite players.

Conclusion

The result showed that the preliminary reactive agility test for the soccer player enjoys an acceptable level of significance and in comparison as a reactive agility test compared with non-reactive agility test empowered to distinguish the average players from elite ones. So, it has recorded this important principle just like non-reactive agility tests. Therefore, as an efficient, field and accessible tool for the coach can be applied to distinguish between ordinary and elite players. Since, the change of direction in reactive agility test for soccer players is done with a ball in four main direction, therefore, it reduces the share of lateral advantage which is inclined toward right side of the players' body and presents him/her a more agile person.

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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: M.T., M.A.A., M.P.; Methodology: M.T., M.A.A., M.P.; Software: M.T., M.A.A., M.P.; Validation: M.T., M.P.; Formal analysis: M.T., M.P.; Investigation: M.T., M.A.A., M.P.; Resources: M.T., M.A.A., M.P.; Data curation: M.T., M.A.A., M.P.; Writing - original draft: M.A.A., M.P.; Writing - review & editing: M.T., M.A.A.; Visualization: M.T., M.A.A., M.P.; Supervision: M.T., M.A.A., M.P.; Project administration: M.T., M.A.A., M.P.; Funding acquisition: M.T., M.A.A.

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Research Article

Neuromuscular Electrical Stimulation in Muscular Adaptations in Exercise: A Narrative Review

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Abstract

Nowadays, sports science uses scientific methods and medical devices to assist people with any improvements in sports. Muscle adaptations have significantly benefited as a result of the use of these advanced devices. It has been shown that neuromuscular electrical stimulation (NMES) devices effectively improve muscle function. The use of NMES devices in exercise physiology shows that neuromuscular adaptation is a current research area in both athletes and non-athletes. This narrative review aims to address neuromuscular adaptations and describe neuromuscular changes based on research using NMES. Many researchers and sports trainers will benefit from the results of this article by better understanding neuromuscular adaptations. NMES training has been shown to be an effective way to improve muscle growth, maximum voluntary strength, neuronal drive, oxidative metabolism, and antioxidant defense systems. In addition, NMES is capable of regulating the homeostasis of muscle proteins and increasing oxidative enzyme activity. In animal models, it has also been shown to increase axonal outgrowth, fiber reinnervation, and motor axon regeneration. Various NMES methods may decrease age-related muscle atrophy and functional deterioration. The use of NMES, which is one of the most successful strategies for increasing athletic performance through neuromuscular adaptations, is one of the most promising areas of research.

Keywords:
Neuromuscular Electrical Stimulation, Muscle Adaptations, Exercise, Performance, NMES

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1. Introduction

The use of an electrical current to stimulate muscle contractions has been around since the 18th century when it was first used in neuromuscular tissue (1, 2). Electrical impulses can induce muscle contraction through electrical muscle stimulation. Alternatively, it is referred to as neuromuscular electrical stimulation (NMES) or electromyostimulation. In recent years, NMES has increasingly received more attention for several reasons. Both athletes and healthy individuals can use it for strength training (3-5). Rehabilitation and prevention can be achieved through their use by people who are partially or unable to move. This test can evaluate neurological and/or muscular functions and assist athletes in recovering after exercise (6).

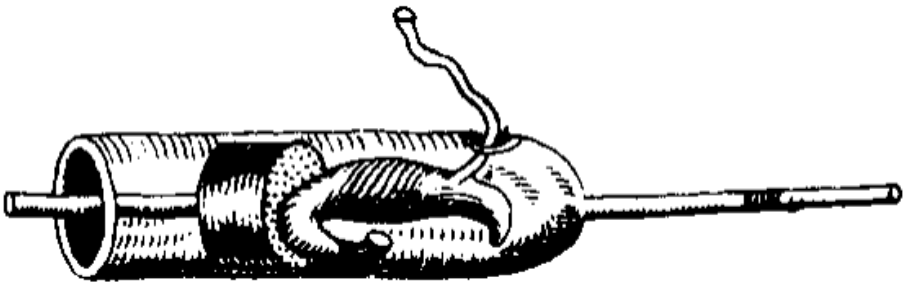

Figure 1: Swammerdam's illustration of a nerve-muscle preparation

About 350 years ago, it was seen that electrical current could be used to cause muscles to contract. Jan Swammerdam (1637–1680) demonstrated in the 1670s that a frog nerve-muscle preparation could be stimulated externally "irritated" via the nerve using scissors. However, he could not describe the specific process leading to muscle activation at that time (Figure 1) (7).

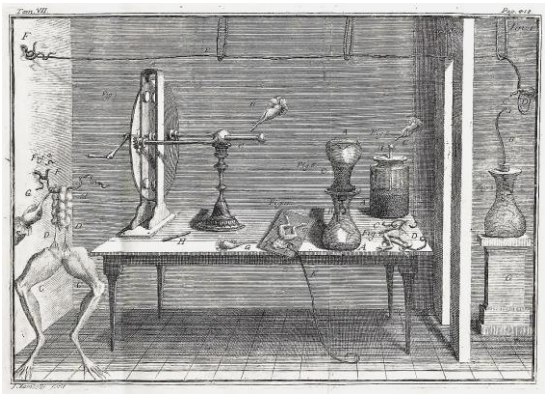

Figure 2: The illustration of Galvani's experiments

After a 3-month therapy session, Jean Jallabert (1712–1768) was able to persistently activate a patient's paralyzed right upper limb using electrical stimulation from a Leyden jar (in other words, a battery) (8). While conducting experiments with static electricity in 1791, Luigi Galvani (1737–1798) dissected a frog on a bench (figure 2) (8, 9). By chance, his assistant touched an exposed sciatic nerve with a metal scalpel that had picked up a charge, and they immediately saw sparks and a strong muscle contraction of the frog's leg as a result. Although Alessandro Volta first misunderstood and hotly contested the physiological principles underpinning muscle activation, Galvani's fundamental discovery was that electrical current might elicit muscular contraction.

Modern electrical generators were made possible by Michael Faraday's (1791–1867) discovery of electromagnetic induction, which was of utmost significance (10). Guillaume Duchenne de Boulogne (1806–1875), regarded as the pioneer of electrotherapy (11), was among the first to employ faradic currents to activate facial muscles using wet surface electrodes (figure 3). He clearly defined the connection between facial muscle contraction and the emotion conveyed by combining photography and electrical stimulation (12).

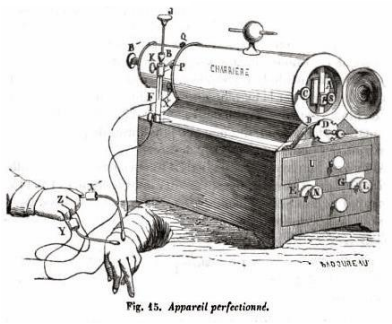

Figure 3: The Woodcut illustration of Duchenne's volta-electric device

Electrical stimulation became an effective therapy for countering muscular atrophy caused by denervation due to the high number of injuries sustained during wars in the first half of the twentieth century. The Russian scientist Yakov Kots hypothesized in 1971 that electrical stimulation of muscles might be more effective than voluntary contractions in boosting maximum strength (8). The groundbreaking Kots findings established the use of electrical stimulation as a technique of muscular performance enhancement, keeping with the well-known Olympic slogan "Faster, Higher, Stronger." This artificial training approach has been seen initially more as a technical gadget than a suitable tool for developing muscular strength, which is not surprising given the poorly managed use. Electrical stimulation has recently been shown to be an effective and legal supplement to voluntary resistance training programs for increasing muscular strength.

A scientific framework for improving the performance of athletes will be developed based on previous research on muscle adaptation. Afterward, according to neuromuscular stimulation, an explanation of possible changes in the mechanism of improving muscle contraction will be provided. For many coaches and trainers of any sports team, this article can help them understand how muscles adapt scientifically. NMES involves intermittent high-frequency trains of electrical stimulation [40–50 Hz] delivered via surface electrodes placed over the motor point to induce (vigorous) contractions of the skeletal muscles as a result of the activation of intramuscular nerve branches. By presenting an overview of the existing evidence about the alterations in muscle performance caused by many bouts of NMES in both healthy individuals and athletes, the advantages and limits of NMES training will be discussed in more detail.

The aim of this narrative review was to provide a general physiological overview of the NMES in muscle adaptations and performance improvement in athletes. Even though much progress has been made in this field, more research is still needed because medical knowledge is constantly changing.

Muscular Adaptations in Exercise

Human Muscle

Muscle fibers are equipped with many metabolic processes to facilitate this adaptation (2). These systems need sensing mechanisms (so the muscle fiber knows it has been worked out), amplification mechanisms (through different and often redundant metabolic pathways that lead to things like the phosphorylation of other proteins), and effector mechanisms (a change in net protein synthesis as a result). The nuclei present throughout the muscle fiber length are involved in the effector processes, which include alterations in transcription of some of the 30,000 genes that make up the genome and posttranscriptional mechanisms leading to changed protein synthesis.

The adductor pollicis muscle is an exciting model for investigating adaptations of muscle contractile properties following exercise. In a study for a 3-month period, one group of subjects performed maximal isometric contractions (10 contractions of 5 s duration) against a load of 30–40% of the maximum (13). This technique was used by Duchateau and Hainaut (1984) to compare the effects of two types of training on human volunteers (14, 15). The maximal muscle force increased significantly (20%) after maximal isometric contractions than after dynamic contractions.

The duration of the training program is also an essential factor in inducing changes in the muscle's force-generating capacity (16). After five weeks of isometric maximal voluntary contractions (MVC) of elbow flexors, McDonagh and colleagues observed no significant changes in the tetanic force of the biceps brachii (BB) (figure 3) (14).

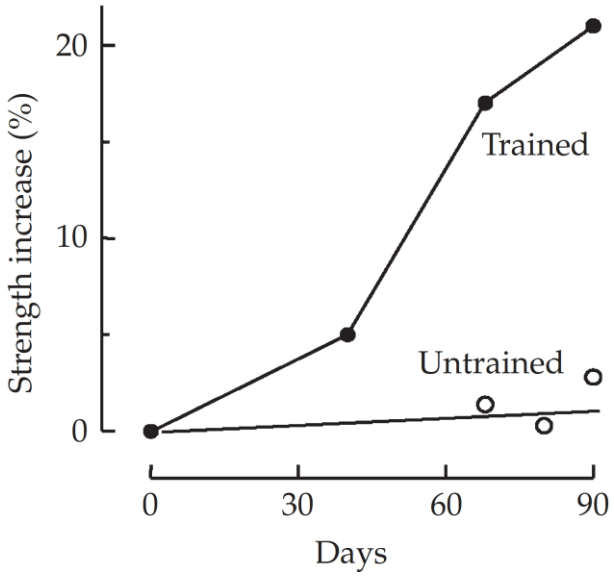

Figure 4: Changes in the adductor pollicis muscle's maximum tetanic force (100 Hz) over the course of a three-month strength-training regimen. The load ranged between 60% and 70% of full capacity. During the first 40 days of training, the trained muscle's tetanic force only went up by 5%. After that, it went up much more, reaching 21% at the end of the training program. The muscle of the contralateral hand remained unchanged.

Motor Unit

There was one research that measured the tetanic force of the motor unit earlier in a human before/after training (17). This technique employs percutaneous electrical stimulation to stimulate single motor axons at many places along the median nerve and to monitor their contractile properties in the thenar muscles. Because the form and magnitude of motor unit action potentials are highly consistent when electrodes are changed in the same position between sessions, longitudinal monitoring of the same motor units is feasible. Using this experimental method, Chan et al. (1999) found that motor unit adaptation to a program of high-frequency electrical stimulation varied in terms of their physiological properties (17). While the twitch and peak tetanic forces of the slower and fatigue-resistant unit grew, the force of the quicker and fatigue-prone unit unexpectedly decreased. The number of motor units per subject is relatively low, which is a significant limitation of the approach for analyzing the effects of a training intervention. For these reasons, other researchers decided to utilize a different technique known as spike-triggered averaging (18). Due to its unique architecture, this is the sole approach that can be utilized to evaluate the contractile properties of a deliberately triggered individual motor unit. In brief, action potentials from a single motor unit discharging at low frequency are employed as "spike triggers" when detected by an intramuscular needle electrode. Since the force produced by this motor unit is time-locked to the action potential (spike), it may be retrieved by averaging the force signal. When comparing the effects of isometric and dynamic training (as mentioned before), unique motor unit changes were detected (19).

Following isometric training, all motor units demonstrated a nearly proportionate increase in peak force without changing the twitch time course. As anticipated, motor units exhibited a minor increase in force after dynamic training, but the time-to-peak for the whole population of motor units was decreased. There was no indication of a change in the "size principle" after either dynamic or isometric training since a linear relationship between motor unit force and recruitment threshold was repeatedly seen. Similar modifications were seen in the tibialis anterior after dynamic exercise (20). These results demonstrate, once again, that muscle changes its contractile characteristics to the type of exercise.

Relation Between Muscle Size and its Strength

The number of parallel muscle fibers and sarcomeres in each fiber, as well as the angle between the fibers and the muscle's longitudinal axis (angle of pennation), are the main factors determining how much force a muscle can produce (2). Consequently, it is possible to measure the strength of a muscle anatomically by measuring its cross-sectional area (CSA)(21, 22). For technical reasons, measuring the anatomical CSA is more accessible, which involves taking measurements perpendicular to the muscle's long axis. This measurement should be done perpendicular to the direction of the muscle fibers. This measurement is often carried out using imaging methods (23).

Even though there is a chance of bias when measuring anatomical CSA, a strong correlation was found between the peak force of the calf muscles during electrically induced contraction and the CSA of a muscle group (23). On the other hand, there is more fluctuation in the connection when force is assessed during an MVC. For instance, the variance in CSA accounts for around half of the difference in strength across patients in specific research (22). This indicates that the capacity of a muscle to generate force is determined by parameters other than its size.

Specific Fiber Tension

Specific tension refers to the maximum force a muscle or muscle fiber exerts per unit CSA ($N.cm^{-2}$). This metric indicates the intrinsic capacity of the muscle or muscle fibers to generate force (2). At the whole muscle level, it has been discovered that depending on the muscle group under investigation, the tension in trained women is either higher or comparable to that in untrained women. In addition, it was found that there was no difference in strength per unit CSA between professional bodybuilders with severe hypertrophy and physical education student (24, 25). However, since the amount of connective tissue differs across individuals, measuring specific stress from the entire muscle may be misleading. The use of anatomical rather than physiological cross-sectional area as an indication of muscle size and the volitional drive's submaximal muscle group activation may contribute to this heterogeneity. Recording particular stress at the muscle-fiber level helps bypass these confounding variables. It has been shown that under these circumstances, type II but not type I vastus lateralis (VL) fiber-specific tension is higher in young, active adults than in older, sedentary adults, and type II but not type I fiber-specific tension is higher in bodybuilders than in sedentary individuals (26).

Additionally, it was shown that particular stress rose after a strength-training regimen. At least two processes may account for variations in specific tension at the muscle fiber level (27, 28). The number of myofilaments in each muscle fiber and how well force is transferred from the sarcomere to the skeleton are examples of these mechanisms.

Alteration in Total Muscle Mass and Construction

The maximum force of a muscle is highly correlated with its CSA, an increase in the latter metric results in an increase in maximal force (figure 4). There are two potential methods for increasing muscle mass: a development in the CSA of individual fibers of muscles (hypertrophy) and an increasing number of muscle fibers (hyperplasia). Most experimental data indicates that hypertrophy is the primary mechanism for increased muscle force (29). However, hyperplasia may occur in humans under certain situations (30).

Muscle Mass

It is now widely recognized that the size of the CSA increase with training relies on some variables, including the individual's starting strength, the load level, the length of the training program, and the training technique (23). For instance, six weeks of isometric training (80% MVC) in novices raised the CSA of the elbow flexor muscles by around 5%, but eight weeks of identical training increased the CSA of the quadriceps femoris by 15% (31). In contrast, 24 weeks of intense strength training by highly competitive bodybuilders had no significant effect on the CSA of BB muscle fibers (32).

However, the reasons are unclear 60–80% of 1-RM with 6–12 repetitions and 6–10 sets, as utilized by bodybuilders, seem to be more effective for muscular growth than larger weights (>80% of 1-RM) with fewer repetitions (33). Moreover, eccentric contractions have been shown to have a more significant effect on muscle hypertrophy, as indicated by the tremendous increase in CSA following a training program for knee extensor muscles that included both concentric and eccentric muscle actions compared to a training program with only concentric muscle actions. Strength training has also reportedly been linked to variations in the hypertrophy of a muscle group's various parts. For instance, following six months of strength training with a load of 80% maximum, the quadriceps CSA rose by roughly 19% in the distal and proximal areas but only by 13% in the central region (22).

Muscle Construction

Muscle architecture may alter with strength training in addition to CSA. Aagaard et al. (2001) evaluated muscle CSA and volume with MRI and VL pennation angle with ultrasound (34). After 14 weeks of training, knee extensor MVC force (16%), quadriceps volume (10%), and VL muscle fascicle pennation angle (36%) increased. The more significant increase in MVC force compared with muscle volume was ascribed to the pennation angle, which increases muscle force per unit volume. As mentioned, these findings affect muscle tension estimation. The physiological cross-section is more accurate than the anatomical cross-section for examining peripheral strength training responses. In specific muscles (e.g., calf muscles), excessive hypertrophy may elevate the pennation angle to a detrimental level for power or speed output.

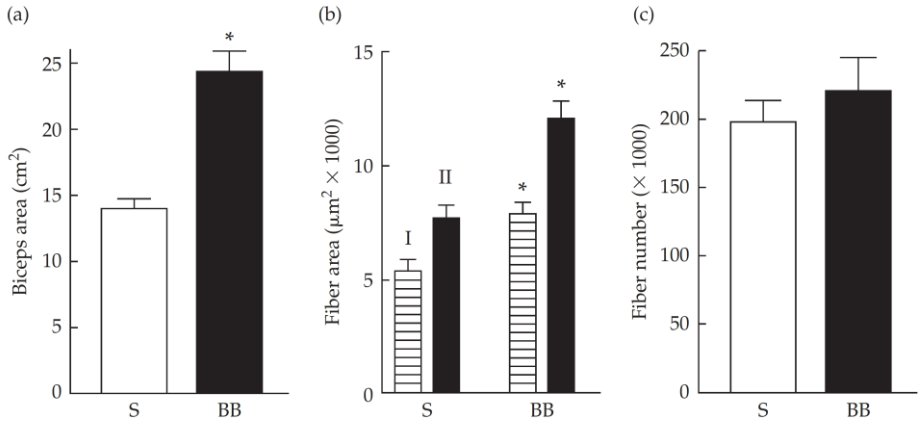

Figure 5: Compared muscle mass, muscle fiber size, and number of fibers in the biceps brachii of sedentary (S) and physically active (A) participants (BB). The mean CSA for the whole muscle, as determined by computed tomographic scans of the upper arm (a), and for the two major kinds of muscle fibers (type I and type II), as determined by needle muscle biopsies (b), differs between S and BB. In contrast, there is no significant difference in the projected mean fiber number derived by dividing the CSA of the whole muscle by the CSA of the average fiber (c).

P0.01, differences between the two groups.

Fiber Adaptations in Response to Exercise

The particular adaptations that result from the various strength-training techniques show that muscles are subject to general adaptations of their various muscle fiber types and changes in the makeup of their fiber types (2). Nevertheless, despite the variation in muscle biopsies, most research found that strength training did not change the percentage of type I and II fibers in muscles, even when MVC force is increased by 45% (32). Nevertheless, after a strength-training program, it is common to see an increase in type IIa fibers and a decrease in type IIb (IIx) fibers in VL (35).

There are more differences in the amount of hypertrophy amongst muscle fiber types. For instance, a 16-week isometric exercise raised the CSA of type I and type II fibers by 20% and 27%, respectively, in the SOL, but the lateral GAST showed a 50% increase in type II fibers but no change in type I fibers. Similar to this, after 14 weeks of exercising the knee extensors, the CSA of type II rose by 18%, while the type I fibers in the VL remained unchanged. In contrast to concentric contractions, eccentric contraction regimens seemed to encourage the hypertrophy of type II fibers (23).

Numerous studies show that the majority of the hypertrophy brought on by intense strength training occurred in type II fibers, even though the quantity of hypertrophy between type I and type II fibers did not consistently vary significantly (23). In fact, after six months of training, the area of type II fibers had risen by 29% and 13%, respectively, under severe load (70-100% of 1-RM) and dynamic contractions (10-60% of 1-RM done at full speed) (33).

Approximately a 4% increase in the CSA of type I and type II fibers was reported (36). Unexpectedly, it has recently been shown that stretch-shortening cycle training may cause a significant increase in muscle fiber CSA of the VL (22–30%). The pretraining level is a determining factor in the relative adaptability between the two primary fiber types, since trained athletes do not demonstrate such a large impact as beginners (32).

The Mechanism of Hypertrophy

Muscle hypertrophy involves changing net protein synthesis and increasing myonuclei to control contractile protein volume. Chronic muscular stretch overload increases protein synthesis over breakdown, resulting in a net contractile protein increase and muscle growth. "Satellite cells" become new myonuclei. Under the basal lamina are quiescent mononucleated satellite cells (23, 30). In response to increased activity, they proliferate, and some merge with a muscle fiber, adding additional nuclei to the current fibers, while others may form quiescent satellite cells (30). New myonuclei create mRNA and proteins like old ones. Protein synthesis causes fiber hypertrophy. In type I and II VL fibers, their quantity looks comparable. Few know the specific signal that causes satellite cells to contribute nuclei to muscle fibers. A single high-intensity exercise session may activate satellite cells, but it is not enough for final differentiation. Satellite cells must grow significantly to become myonuclei. When training increases fiber area by 17%, the number of myonuclei does not change, suggesting that the rise is due to protein synthesis, but more considerable hypertrophy (>30%) is accompanied by an increase in myonuclei (30). The quantity of myonuclei added following weight training correlates strongly with fiber hypertrophy.

Hypertrophy stimuli cause a net protein increase owing to a shift in protein synthesis and breakdown, followed by satellite cell activation and proliferation (23). The fusing of satellite cells to muscle fibers occurs later in the hypertrophy phase when protein synthesis alone cannot produce additional hypertrophy.

Intracellular signaling mechanisms enhance protein production and breakdown (23). The protein kinase B (Akt) triggers the mTOR transduction pathway. The growth factor-1 (IGF-1) isoforms generated by skeletal muscle in response to exercise stress stimulate Akt activation. This IGF-1 isoform, mechano growth factor, boosts protein synthesis. After interacting with a transmembrane receptor, it activates Akt, which promotes protein synthesis via mTOR pathways and inhibits protein breakdown by phosphorylating a transcription factor and reducing protein production. Other exercise cues than mechano growth factor may activate the Akt pathway, and many of the underlying processes of muscle hypertrophy are unclear. Myostatin inhibits muscle development, while IGF-1 stimulates it. Strength training reduces myostatin mRNA, whereas endurance exercise reduces it without muscle fiber growth. Although mRNA isoform expression was increased following a single strength training session, it takes 3–6 weeks of training before protein synthesis produces noticeable improvements in muscle growth (37) and primary muscular strength (23).

Hyperplasia

Despite the fact that the majority of experimental data points to hypertrophy as the primary mechanism behind the rise in muscle mass, hyperplasia may also be involved in the rise in muscle size (2).

However, the degree to which fiber hyperplasia may develop in the muscles of persons who engage in strength training remains debatable. Indirect research implies that athletes have more muscle fibers than untrained persons (33). This contradicts the findings of Sale et al. (1987), who compared the size of the BB of untrained participants to elite and intermediate bodybuilders (38). The number of muscle fibers was determined based on the ratio of total muscle area computed by CT scanning to the average fiber area from needle biopsies. According to the results, there were no significant differences in the average number of fibers across groups. McCall et al. (1996) found no change in BB fiber counts in young males after 12 weeks of rigorous strength training (39). Kadi et al. (2000) postulated that satellite cells might combine to produce new muscle fibers or repair broken muscle fibers (30). These scientists found that, unlike untrained persons, top power lifters had tiny diameter fibers that exhibited embryonic and neonatal myosin heavy chain compositions. Considering these essential breakthroughs, hyperplasia seems moderate in humans. Therefore fiber hypertrophy remains the predominant mechanism of mass muscle growth after strength exercise.

Force Transmission Adaptations to Exercise

Adaptation of muscle contractile characteristics following exercise may also affect MTC SEC. An increase in the SEC's stiffness may be required for more efficient force transmission. This shift in elastic properties improves performance by promoting the release of potential energy during stretch-shortening cycle workouts by decreasing the duration between the stretching and shortening phases. In DJs, plyometric training enhanced muscular pre-activity, supporting this idea.

This modification may have increased muscle-tendon stiffness at the moment of the impact, resulting in increased muscle-tendon stress. In addition to increases in muscle activation, Pousson et al. (1990) found increased stiffness of the SEC following six weeks of eccentric contractions of the elbow flexor muscles, as measured by a quick-release method (40). This shift occurred independent of elbow flexor force but was larger at low force. Cornu et al. (1997) found that plyometric training decreased the slope of the stiffness–force connection in plantar flexor muscles (41). The authors proposed that training can cause opposite changes in the passive structures of the SEC—tendon—and the active structures—muscles around the joints—so that the change in stiffness of the entire musculo-articular system will depend on their respective adaptations. This would explain the apparent discrepancy between the two studies.

Tendon–Aponeurosis Features

As a result of the quick-release technique's inability to discern between changes in either the contractile or tendon structures, ultrasonography is now often utilized to measure and quantify changes in tendon-aponeurosis complex stiffness (23). It has been documented that young and old persons had an increase in tendon stiffness after a strength training program with large loads (42). Similar results have been seen in young adults following training with eccentric movements (43). It indicates that the contraction mode is connected to the degree of tendon adaptation to training. In fact, data from the same lab show that isometric training tends to have a more significant effect than heavy-load training that moves.

In contrast, training with plyometric movements and ballistic isometric contractions did not affect tendon stiffness. Since plyometric exercise increases joint stiffness, the most significant changes may be in the contractile structures. It was further hypothesized that training-induced alterations in the internal structure of the tendon since none of these studies found that the training regimen altered tendon size. Contrary to this finding, long-distance runners had a higher CSA (22%) of the AT than nonrunners (44). This apparent difference may be described by endurance vs. strength training or by the delayed hypertrophic response that arises after a more extended exercise.

Neuromuscular Electrical Stimulation

To induce powerful muscular contractions, NMES involves transmitting preprogrammed trains of stimuli to muscles using surface electrodes placed on the skin. There is currently strong proof that NMES is an appropriate and legal addition to voluntary resistance training routines for increasing muscular strength and hypertrophy in healthy individuals (2).

In medicine, NMES is used for rehabilitation via medical devices in a laboratory (6). For example, it is utilized in physical therapy to prevent muscle atrophy due to inactivity or neuromuscular imbalance, which can occur for various reasons, for instance, after musculoskeletal injuries such as damage to bones, joints, muscles, ligaments, and tendons (21). This is not the same as transcutaneous electrical nerve stimulation, a pain treatment that uses an electric current. In the case of such stimulation, the current is often subthreshold. Therefore, muscle contraction is not detected. Progressive disorders such as cancer or COPD employ NMES to improve muscular weakness in individuals unable or unable to exercise whole-body.

NMES may enhance quadriceps strength, but further study is required since the data is weak (3). The same research suggests that NMES may result in more muscle hypertrophy (45). Insufficient data suggests that adding NMES to an existing fitness program may help ill individuals spend fewer days confined to their beds. During NMES training, complementary muscle groups are frequently targeted alternatingly for specific training goals, such as enhancing the ability to reach an object.

Resistance training has been demonstrated to be an effective treatment for neuromuscular deterioration (45). NMES has been used as a substitute to attenuate or treat muscular mass and strength reductions in aged individuals when resistance exercise intervention is infeasible due to circumstances such as injury and/or prolonged bed rest (46). Electrical stimulator devices cause the motor neuron axons and their branches, or the muscle fiber, to depolarize, which causes the muscle to contract (46). This can be done in several methods, including by stimulating a motor neuron directly or the superficial muscular bellies with self-adhesive surface electrodes. NMES, comprising stimulation-rest cycles, is administered over weeks or months to develop muscular tetany and muscle contraction (47). According to the Henneman size principle, the recruitment of MUs during voluntary contractions follows a pattern from slow twitch muscle fibers to fast twitch muscle fibers and from small MUs to larger ones (48).

On the other hand, the recruitment pattern by NMES is temporally synchronized, spatially fixed, and not selective, as shown by the early recruitment of a large number of fast twitch muscle fibers that get tired quickly (45).

In contrast to motor nerve stimulation, which engages all muscle fibers inside a MU, direct muscle stimulation stimulates all fibers close to the stimulating electrodes, which may or may not include whole MUs (45). Muscle or nerve excitation mainly relies on the proximity of stimulating electrodes, whereas axon depolarization is based on membrane resistance.

The primary effects of neuromuscular abnormalities are tissue atrophy and the inability to generate force effectively. The muscular structure is evaluated based on CSA, muscle fiber length, and pennation angle (49). It is possible that NMES positively increases total muscle size in humans, despite the contradictory evidence resulting from the diverse populations tested, the various aspects of NMES protocols, and the addition of resistance training and/or nutrition (50). In healthy elderly, eight weeks of high-frequency NMES (75Hz) administered to both the vastus lateralis and vastus medialis muscles increased CSA significantly (51). The combination of NMES and voluntary exercise resulted in an even higher rise in knee extensor CSA. Following nine weeks of NMES, histochemical and morphological studies showed that the diameter and proportion of fast-type muscle fibers increased while slow-type fiber diameter decreased (49). In addition, a significant rise in the number of satellite cells in the fast-type muscles was detected in the elderly following NMES, showing that NMES can stimulate muscle regeneration and hypertrophy (50). Evidence suggests that capillary growth and muscle fiber growth happen at the same rate in the skeletal muscles of humans (9, 15, 21, 45). This reveals that there is a positive relationship between capillarization and muscle fiber hypertrophy.

Although these findings on capillary supply adaptations in healthy elderly are limited, it has been observed that high-frequency NMES improved muscle capillarization and preceded the conversion of muscle fiber phenotype, illustrating the significance of angiogenesis and muscle fiber capillarization, specifically in older muscle.

Individual fiber and overall muscle atrophy are caused directly by an imbalance between muscle protein production and muscle protein breakdown. Muscle atrophy can be reduced or prevented by a protein diet plan and exercise. Regardless of dietary consumption, even moderate-intensity physical activity can preserve skeletal muscle mass, underscoring the potential of NMES as an interventional treatment (2). Five days of bed rest with NMES and protein supplementation in healthy older adults did not reduce muscle mass (52). Similarly, Dirks et al. (2016) examined the efficacy of NMES in conjunction with pre-sleep protein consumption on muscle protein synthesis (MPS) in older persons (53). Before 20g protein feeding, a 70-min single bout of NMES was performed unilaterally on the lower limb, and muscle biopsies after 4 hours revealed no change in myofibrillar MPS between the stimulated and control legs (53). However, the similar NMES procedure with 40g of protein rather than 20g increased muscle protein synthesis 8 hours after feeding, indicating that metabolic responses to NMES are sensitive to dietary intervention and time-dependent. Additionally, a single NMES session in elder type 2 diabetics, who are more sensitive to muscle atrophy and functional deterioration, increased MPS by 27% (54). In patients with knee osteoarthritis, four weeks of daily NMES performed at home increased muscle fiber size, which was associated with a rise in MPS.

Depending on the knowledge currently available on NMES and MPS, NMES can improve MPS whether used alone or as an addition to dietary (protein-based) therapies (45). As a result, it can help lessen the anabolic resistance frequently seen in aging muscles. Even though the pathophysiology of muscle aging and inactivity differ, they are frequently closely related. The reduction in their functional ability increases the elderly's tendency for falls and fractures, frequently resulting in immobilization (45). The use of NMES to facilitate the rehabilitation of older females following hip fracture surgery resulted in a quicker return to indoor mobility. In addition, a six-week follow-up evaluation revealed that NMES induced a longer-lasting effect on functional recovery, as evidenced by effectiveness in walking speed, postural stability, and muscle strength (3, 9, 21, 45).

Conclusion

NMES has been used in sports science to manage edema, maintain strength and muscle mass after prolonged immobility, and strengthen muscles. These effects have been achieved using a variety of stimulators, such as twin-spiked monophasic pulsed current, biphasic pulsed current, burst-modulated alternating current, or "Russian stimulator" stimulators.

Several studies have shown enhanced isometric muscular strength in NMES-stimulated and exercise-trained healthy, young individuals compared to unexercised controls, with no significant differences between the NMES and voluntary exercise groups. When NMES and voluntary activity are combined after training compared to either NMES or voluntary exercise alone, there does not appear to be a noticeable difference in muscle strength. NMES also improves functional performance in several strength activities.

Two mechanisms have been proposed to explain the NMES training effects. The first mechanism claims that the improvement of muscular strength by NMES happens similarly to increased muscle strength by voluntary exercise. This mechanism requires NMES strengthening programs to follow standard strengthening methods with few repetitions, high external loads, and high muscle contraction. The second mechanism argues that the muscular strengthening observed after NMES training is due to a reversal of the voluntary recruitment order and a selective increase in type II muscle fibers. Since type II fibers have a greater force than type I fibers, targeted augmentation of type II muscular fibers will boost the muscle's total strength. NMES has been widely explored to prevent muscle atrophy after knee ligament repair surgery or injury. NMES appears to protect against thigh muscle weakening, hypertrophy, and loss of oxidative capacity following knee immobilization.

In specific trials, NMES was found to be more effective at avoiding the atrophic alterations caused by knee immobilization than no exercise, isometric exercise of the quadriceps femoris muscle group, isometric co-contraction of the hamstrings and quadriceps muscle groups, and combination NMES-isometric exercise. Additionally, it has been found that NMES is supplied to the thigh muscles when the knee is immobilized during functional activities. NMES appears to strengthen muscles within muscle groups or sections of muscles selectively. Evidence has been shown for the selective strengthening of the abdominal muscles, back muscles, triceps brachii, and vastus medialis obliquus.

It is unknown whether this selective strengthening results from local changes in the stimulated muscle or muscular region or a change in the relative amplitude of recruitment of the different muscles within a muscle group or the different muscle sections. NMES has been recommended as a potential supplementary therapy for edema. Numerous studies have shown that monophasic pulsed stimulation pumps muscles to reduce acute edema. The last point is that it has been demonstrated that monophasic pulsed stimulation only affects acute edema when it is applied at amplitude values lower than those necessary to cause muscle contraction.

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Research Article

The effect of exercise and vitamin B6 on the expression of COX2 and IL-1B genes in endometrial tissue of endometriosis rats

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Abstract

Background: Endometriosis is a chronic disease, afflicting about 5-10% of women. Many possible environmental and molecular factors have been proposed for the pathogenesis of endometriosis; nevertheless, its real mechanism is still under investigation. This research aimed to examine the effect of physical exercise and B6 vitamin on the expression of COX2 and IL-1B genes on endometriotic tissue in endometriosis model rats.

Materials and Methods: In this experimental research, 25 Wistar rats were randomly assigned into five groups: control-healthy, endometriosis, endometriosis + exercise, endometriosis + B6, endometriosis + exercise + B6. Vitamin B6 were administered as 60 mg/kg per body weight of each rat. The swimming exercise program involved 8 weeks of exercise, each week 5 days, and each day for 30 min. For data analysis, one-way analysis of variance (ANOVA) and post hoc Tukey test were used.

Results: The extent of expression of COX and IL-1β genes increased significantly in the endometriosis group compared to the control-healthy group. All three groups of endometriosis + exercise, endometriosis + B6, and endometriosis + exercise + B6 showed a significant reduction of COX2 expression compared to the endometriosis group. Two groups of endometriosis + exercise and endometriosis + exercise + B6 indicated a significant decline in the extent of expression of 1L-1β gene, as compared to the endometriosis group. The endometriosis + exercise + B6 group revealed a significant reduction in the extent of expression of COX2 and IL-1β genes compared to endometriosis + B6. Finally, the endometriosis + exercise group revealed a significant decrease in the extent of expression of IL-1β gene, when compared with the endometriosis + B6.

Conclusion: Overall, the results suggest that changes in the key molecules or signaling pathways as well as gene expression in the endometriosis process can contribute to improving this condition. Doing physical activity and concurrent consumption of B6 vitamin will be helpful in curbing this disease and improving the level of this condition.

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1. Introduction

Endometriosis is a chronic disease afflicting around 5-10% of women (1). Endometriotic tissues emerge and grow in extrauterine region especially the pelvis (2). Endometriosis is often found in organs including cervix, ovaries, vagina, intestine, posterior cul-de-sac, uterine ligament, pelvic peritoneum and rectovaginal septum, urinary system, abdominal wall, chest cavity, lungs, and central nervous system (3). Although a large number of patients with endometriosis are asymptomatic, it may be associated with menstruation pain, painful intercourse, and chronic pelvic pain (4). Many possible environmental and molecular factors have been proposed for the pathogenies of endometriosis, but is real mechanism is still under investigation (5). Cyclooxygenase enzyme-2 (COX-2) converts arachidonic acid to prostaglandin H2, which is a precursor of different molecules including thromboxanes, prostacyclins, and prostaglandins. COX-2 enzyme is usually expressed only in inflammatory cells. COX-2 can be expressed in response to various stimuli such as hormones, mitogens, cytokines, inflammatory mediators, and growth factors. It is believed that COX-2 is involved in carcinogenesis through promotion of angiogenesis, increased cell attack, inhibition of apoptosis, and stimulating cell proliferation (6). Overexpression of COX-2 has been proven as a major regulator in the progression of endometriosis (7). IL-1β cytokines are produced out of macrophages and monocytes. IL-1β is another important cytokine, involved actively in inflammatory responses in humans. In endometriosis, peritoneal macrophages of women with this disease produce higher levels of IL-1β. IL-1β stimulates endometrial cells for secretion and production of cytokines plus growth factors, and plays a key role in binding, growth, and angiogenesis of endometriotic tissue (8).

Proinflammatory cytokine, IL-1β, is an established factor which regulates expression of COX-2 in patients with endometriosis. COX-2 gene is more sensitive to eutopic cell and to stimulation of IL-1β in extrauterine endometriotic stroma cells. These results indicate that inflammation plays a role in some pathogenic aspects of endometriosis (9). Various treatments have been considered for endometriosis; it has been shown that these treatments affect the quality of life and aerobic capacity of patients completely. Researchers believe that physical exercise may mitigate the pain resulting from the disease through some mechanisms (10). Nevertheless, there are correctible factors such as diet and physical activity which support the preventive and therapeutic methods against this disease. Physical activity can have different effects on this disease depending on energy reserves, exercise intensity and periodicity, along with oxidative stress resulting from exercise (11). Studies have shown that use of exercise interventions can be effective for patients with endometriosis (2). Nevertheless, what type of physical activity and through which molecular and cellular mechanisms it can have the best impact are still poorly understood. Research has shown that low-intensity aerobic exercise can lead to decreased expression of inflammatory cytokines, oxidative stress, and systemic inflammation in the uterus through establishing protective mechanisms, thereby inducing immune responses (12). The effect of physical activity on rest levels of IL-1β and COX-2 has not been specified completely. Research indicates that exercise can considerably inhibit COX-2 activity, thus leading to suppression of proinflammatory cytokines and alteration in the oxidation status (13).

Although no change has been observed in the COX-2 protein content according to some studies (14), the results suggest that exercise may, as least to some extent, mitigate the inflammation by reducing its formation. Further, IL-1β variations along exercise can occur in a non-inflammation dependent way. It was also observed that exercise may modulate inflammation formation or activation (15). Nevertheless, it should be noted that no study has explored the effect of physical activity on these two proteins in endometriosis, and the mechanism of their changes is poorly understood. From among aerobic exercises, swimming aerobic exercise with low intensity is among the exercises that can be used under various physiological conditions safely, and due to weight tolerance in water in relation to nonwatery sports, it is used in most physiological, biochemical, and molecular reaction studies. Mild to moderate physical activity can improve metabolic activity through increasing blood circulation, but severe activity, due to blood circulation displacement towards the muscles causes its reduction (10). Meanwhile, research has shown that a diet ricj in fruits and vegetables as well as cereal grains can be effective in prevention from growth of endometriosis metastasis (2). For example, Vitamin B6 is a central molecule in living creature cells. It is an important factor for a wide range of biochemical reactions, which regulates the essential cell metabolism (16). Vitamin B6 has been known as a potent anti-inflammatory, anti-mutagenic, and neuro-enhancer agent; through activating suppressor genes and deactivating angiogenesis as well as activating anti-inflammatory gene in induction of apoptosis causes inhibition of progression of cancerous tumors as well as many pathological changes that occur in response to penetration of inflammatory cells (17).

The group B family leads to pain alleviation as well as improvement in the endometriotic lesion and reduction of this disease symptoms (18). Nevertheless, no research has been performed so far with a focus on the influence of vitamin B6 on endometriosis. Endometriosis is a multifactorial disease with a complex pathophysiology, and most of its details have still remained unclear. Thus, considering the limited studies as well as the discrepancies and ambiguities regarding the effect of dietary and exercise interventions, regarding the preference of each of the mentioned methods and lack of similar studies, the present study was performed to examine the effect of physical activity and vitamin B on the expression of COX2 and IL-1B genes on the endometriotic tissue of endometriosis model rats.

2. Materials and Methods

In this experimental research, 25 adult 6-8-weak-old Wistar rats with the mean weight of 202.85±15.65 g were purchased from Pasteur Institute and transferred to the research center. The animals were kept according to the guidelines of Health International Institute, and the protocols of this study were done observing the principles of Declaration of Helsinki as well as medical ethics considerations. Pellet food and water were provided to the animals under treatment ad libitum. The food consumed by the animals was 10 g per each 100 g of the body weight based on weekly weighing. The protocol of this research was performed according to the international laws of handling laboratory animals. The rats were randomly assigned into five groups (5 rats in each group), including control-healthy group, endometriosis, endometriosis + exercise, endometriosis + B6, endometriosis + B6 + exercise. In order to induce the endometriosis model, first adult rats were anesthetized using ketamine and xylazine. Next, the abdominal region on the right side was cleansed using Betadine. Thereafter, an incision was made in the skin of the flank region in the pelvic part using a Bistoury blade. Once the abdominal muscle and peritoneum were opened, first the ovarian tissue alongside part of the uterine tube tissue were withdrawn. They were then placed inside a sterilized container with 1 cc PBS. Thereafter, each tissue was cut into a 1*1 mm piece. The tissue pieces, which were four for each rat, were grafted to the right pelvic muscular wall region, abdominal peritoneum, abdominal wall frontal region, and the visceral tissue around the ovaries. Next, the operated region was sutured, and the rats were transferred to the relevant cage (19).

Vitamin B6 was administered two weeks after induction of endometriosis on a daily basis and as gavage by 60 mg/kg of body weight in the rats of the endometriosis + B6 and endometriosis + B6 + exercise groups (11). The rats of endometriosis + exercise ad endometriosis + exercise + B6 groups, before initiating the main protocol, were placed inside water pool for 1 week (5 days) each time for 20 min, to get familiar with water and lower their swimming stress and get adapted to the exercise conditions. Then, they began swimming five days a week until the end of the research period inside a water tank with 50 * 50 * 100 cm dimensions over 8 weeks. The duration of exercise in water was 30 min daily until end of the exercise period (20). Once the period finished, in order to eliminate the acute exercise effect, samples were taken from the3 animals 48 h after the last swimming exercise program and consumption of Vit B6. For this purpose, first the animals were anesthetized through intraperitoneal injection of ketamine (20-30 mg/kg) and xylazine (20-30 mg/kg); after dissecting the abdominal cavity, the ovarian tissue was carefully withdrawn and frozen at -80˚C for investigating the expression of COX-2 and IL-1β genes and transferred to the laboratory. In order to examine the expression of COX-2 and IL-1B genes, in each group, the tissues were inspected using Real-time PCR. Next, cDNA was replicated via PCR technique, whereby qRT PCR was employed to confirm the expression of the studied genes quantitatively. After laboratory analysis of the tissue samples, descriptive statistical indices including mean and standard deviation for quantitative description of data along with inferential statistics. First, to determine the normality of data distribution, Shapiro-Wilk test was used, while for determining the variance homogeneity, Leven's test was applied.

Next, since the data distribution was normal, parametric tests including one-way analysis of variance and post hoc Tukey test were used at significance level of p-value≤0.05 to explore the changes in the expression of COX2 and IL-1B genes. For all statistical analyses, SPSS 23 was used, while for drawing the diagrams, Excel software was employed.

3. Results

The results of ANOVA test showed that there was a significant difference between the groups regarding levels of expression of IL-1β and COX genes (p-value≤0.0001, Table 2). The results of post hoc test also showed that the extent of expression of COX and IL-1β genes in the endometriosis group had a significant elevation compared to the healthy-control group (p-value≤0.0001).

All three groups of endometriosis + exercise, endometriosis + B6, and endometriosis + B6 + exercise showed a significant reduction in the extent of expression of COX gene, compared to the endometriosis group (p-value≤0.0001). Two groups of endometriosis + exercise and endometriosis + exercise + B6 revealed a significant decline in the extent of expression of IL-1β gene, as compared to the endometriosis group (p-value≤0.0001). The endometriosis + exercise + B6 group indicated a significant reduction in the extent of expression of COX (p-value=0.034) and IL-1β (p-value=0.035) genes, compared to endometriosis + B6 group. Further, the endometriosis + exercise group showed a significant reduction in the extent of expression of IL-1β gene compared to the endometriosis + B6 group (p-value=0.004) (Table 1).

Table 1: Central indices and distribution of Cox and IL-1β gene expression levels in different research groups

Statistic/Group	Control-healthy	endometriosis	endometriosis+B6	endometriosis+exercise	endometriosis+exercise+B6
Mean ± SD (Cox)	±0.028 0.062	0.749±0.045 ❋	#$0.363±0.091	$0.208±0.065	$0.186±0.033
Mean ± SD (IL-1β)	±0.045 0.219	0.871±0.076 ❋	#0.601±0.356	&$0.237±0.087	$0.293±0.066

*. Significant changes in relation to the control-healthy group, $: significant changes in relation to the endometriosis group, &: significant changes in relation to the endometriosis + B6 group, #: significant changes in relation to the endometriosis + exercise + B6 group.

Table 2: ANOVA test results for expression of Cox and IL-1β genes between different research groups

variables	Source of changes	Sum of Squares	df	Mean Square	F	p-value
(Cox)	Between Groups	2.552	4	.365	54.029	≤0.0001
	Within Groups	.216	20	.007		
	Total	2.768	24			
(IL-1β)	Between Groups	3.007	4	.430	20.438	≤0.0001
	Within Groups	.672	20	.021		
	Total	3.679	24			

P<0.05 significant difference

4. Discussion

The present research explored the effect of a period of regular swimming activity along with Vit B6 consumption on the expression of COX2 and IL-1B genes on the endometriotic tissue of endometriosis model rats. The results revealed that with induction of the endometriosis model, the expression of COX-2 and IL-1B genes in rats increased significantly compared to the control group. On the other hand, with implementation of the exercise and B6 treatment, the expression of the mentioned genes in rats showed a significant reduction compared to the endometriosis group, with this decline being greater in the exercise + B6 combined group. It has been reported that the endometrium completes a three-stage method (binding – aggression – angiogenesis), and eventually converts to endometriosis (21).

Recent studies have shown that immunological, inflammatory, angiogenetic, and environmental factors all can be heavily implicated in the pathogenesis of endometriosis. In addition, the peritoneum in patients with endometriosis is immunologically dynamic, linking the reproductive and immune system with each other. There is evidence suggesting that the peritoneal fluid around the endometriosis implant has pro-inflammatory tissue features, associated with altered immune response and cytokine production. It has been suggested that these cytokines with autocrine and paracrine effects may impair the intercellular interactions in immune cells, and include proliferative factors for implantation and development of extrauterine tissue (22). IL-1B is the most important anti-inflammatory cytokine, which reinforces the inflammatory state in endometriosis, causing secretion of other cytokines and growth factors, eventually supporting implantation and development of extrauterine lesion (21).

Endometriosis is a gynecological inflammation-dependent disorder. Overexpression of COX2 plays a key role in the development and progression of endometriosis. Nevertheless, the mechanism of endometriosis is unclear (21). In this study, similar to some studies, overexpression of COX-2 in extrauterine endometrium of patients with endometriosis was observed against the normal endometrium of the control group (22). Studies show that COX-2 plays a key role in the pathology of endometrium, especially in endometriosis. Expression of COX-2 is higher in local lesions of endometriosis than in endometrium, indicating increased synthesis of COX-2 enzyme and hence production of PGE2 in the peritoneal fluid. It has been reported that COX-2 is specifically regulated by IL-1b in endometrioses compared with normal endometrium stroma. Thus, these data support overexpression of COX-2 in extrauterine lesion and hypersensitivity of COX-2 expression resulting from IL-1b in endometrioses, which may aid the pathophysiology of endometriosis (21). Nevertheless, factors such as nutrition and physical activity help in prevention and treatment through regulating and modulating this disease (2). Research has shown that physical activity can reduce the risk of developing endometriosis in women. Some possible mechanisms introduced in this regard include decrease of oxidative stress, strengthening the immune system, and modification of hormonal factors (23). In addition, regular physical activity is associated with cumulative effect of menstruation, ovary stimulation, and estrogen functioning (11). Research indicates that exercise considerably inhibits COX-2 activity, and leads to suppression of proinflammatory cytokines and changes in oxidation status.

These results suggest that there is a molecular relationship between the central nervous system and the body's immune system (13). IL-1β is one of the most potent and important inflammatory mediators in acute phse response and in pathophysiology of chronic diseases (24). Data show that reduction of inflammatory cytokines following exercise intervention may occur through reducing the IL-1β activation (15). Recently, a review study on the relationship between vitamins and endometriosis disease has shown that there is a direct relationship between vitamin groups and improvement of endometriosis, whereby group B vitamin results in pain alleviation and improvement of endometriotic lesion as well as reduction of symptoms of this disease. Vitamin B6 has been known as a potent anti-inflammatory, anti-mutagenic, and neuro-enhancing factor (18). Group B vitamin has a key role in its prevention and treatment through modulating cellular signaling pathways. Since B6 vitamin has antioxidant properties, following exercise, it encourages rapid regeneration of exercise-induced damaged cells; it causes the body to be able to absorb fat-soluble nutrients, and lead to vasodilation, thereby facilitating blood flow (2). Vitamin B6 is considered essential for normal metabolism and immune response especially anti-inflammatory immune response. A study reported that Vitamin B6 inhibits expression of lipopolysaccharide (LPS) resulting from synthesis of nitric oxide synthase (iNOS) and COX-2 in mRNA as well as protein level through suppressing NF-kB activation in macrophages (25). Recent studies have shown that in groups undergoing treatment with B6 vitamin in comparison to the control group, expression of IL-1β, TNF-α, and IL-6 decreased. The results suggest the protective role of Vit B6 against hyperinflammation (26).

Conclusion

Overall, the results suggest that alteration of key molecules or signaling pathways as well as gene expression involved in endometriosis process can improve the level of this disease. Doing regular aerobic exercise as well as concurrent consumption of Vit B6 can be effective in mitigating this disease as well as its improvement.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval The Ethics Committee of Islamic Azad University-Sari Branch approved the study (IR.IAU.SARI.REC.1399.120).

Informed consent Informed consent was obtained from all participants.

Author contributions

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

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Research Article

The effect of L-arginine supplementation along with 6 weeks of Aquatic training on changes in blood pressure and fasting blood sugar in older peoples with Hypertension

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Abstract

Background: The factors that control blood pressure, the most important of which are inactivity and improper nutrition. The present study was conducted with the aim of investigating the effect of arginine supplementation on blood pressure and glucose metabolism in elderly people with mild Hypertension.

Materials and Methods: 43 elderly men and women in the age range of 55 to 70 years with mild hypertension were selected as participants and were randomly divided into 4 groups, including the aquatic training group (AT), L-arginine supplementation (ARG), aquatic training + L-arginine supplementation (AT+ARG) and control (CON). AT group did sports training in water for 6 weeks. ARG group received 100 mg of L-arginine per body weight and AT+ ARG group received L-arginine supplement during 6 weeks of water sports training. The control group also received only placebo. The values measured in the present study included aerobic power, systolic blood pressure, diastolic blood pressure and fasting blood glucose. The findings were compared using one-way analysis of variance and Tukey's post hoc test at a significance level of ≥0.05.

Results: The findings of the present study showed that the diastolic blood pressure of the subjects did not differ significantly (P=0.239), but the difference between the systolic blood pressure of the group was significant (P=0.031). Also, the change of fasting blood glucose of the samples in the 4 groups shows a significant difference (P=0.011). L-arginine consumption group and sports training + L-arginine consumption group showed a significant difference with the control group (P=0.0001 and P=0.001, respectively).

Conclusion: The results of the present study showed that consuming L-arginine for 6 weeks in the elderly with hypertension can lead to improvement of their systolic blood pressure and fasting blood sugar, but adding training cannot double its effect. slow All sports training have proven effects in modulating blood pressure and lowering blood sugar in people who have used it for some time, but it seems that observing the double effect of activities on these indicators requires long training programs, which future studies show. It will clarify more.

Keywords:
L-arginine, insulin resistance, hypertension, aquatic training.

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1. Introduction

The results of the world's largest study on blood pressure, which was conducted in collaboration with the World Health Organization and hundreds of researchers, showed that the number of people suffering from hypertension in the world has reached one billion and 130 million people, and the number of sufferers has more than doubled in the past 40 years; while, the number of Iranians suffering from hypertension has reached 10 million (Khamseh, 2021). Various factors affect blood pressure, the most important of which are inactivity and improper nutrition. With exercise, the incidence and intensity of cardiovascular and hypertension risk factors are reduced and it becomes possible to reduce the use of antihypertensive drugs and reduce premature death. Consuming special foods that can be effective in dilating blood vessels and regulating blood pressure is one of the other solutions that can be provided to control blood pressure. In the meantime, it has been determined that the consumption of some amino acids can be effective in controlling blood pressure (Vasdev, 2010). Human and animal studies have shown that certain amino acids combined with protein can have antihypertensive effects. Branched-chain amino acids (Jennings et al., 2016), cysteine (Vasdev, 2008), glutathione, glutamate (Stamler, 2009), and arginine (Lucotti, 2006) have a reducing and sometimes preventive effect on hypertension and factors related to insulin resistance and diabetes (Kass, 2012). On the other hand, type 2 diabetes as a metabolic disease has strong underlying and environmental factors among Iranians (Khamseh, 2021). This disease has a higher prevalence in people over 45 years old and studies on its possible mechanisms have been strongly focused.

By improving glucose metabolism in diabetics, the consumption of some amino acids leads to a reduction in the glycation of substances in the blood, and hence reduces the negative effects of high blood sugar on the vessel wall and hypertension in these people (Hu, 2017). It has been shown that the amino acid leucine has positive effects on diabetics (Zhang, 2020). Therefore, positive effects can be obtained by using amino acid supplements in people with hypertension and diabetes (Vasdev, 2010). On the other hand, it is known that sports activities, especially aerobic activities that are performed for long periods of time, lead to the adjustment of systolic and diastolic blood pressure. In a review study, researchers analyzed the results of 13 studies conducted on the effect of physical activities on blood pressure, and it was determined that the duration of the activity is the most important exercise variable in the effectiveness of exercise on blood pressure changes (Carpio-Rivera, 2016). The main mechanisms of cardiovascular diseases are dysfunction of vascular endothelium, so its dysfunction can increase its permeability to plasma components, especially low-density lipoproteins and its deposition in the sub endothelial space, and (LDL) can be used as it is one of the most basic events that occurs in the process of developing atherosclerosis. The effect of nutrition and physical activity on hypertension has been almost proven, however, the interaction of these two factors and the simultaneous actions of these two factors have been less discussed. It is not clear whether these two factors can have a synergistic effect.

Also, there is less information about the effectiveness of each of these factors alone in a simultaneous study in elderly people with hypertension, so in the present study, the researcher sought to answer the question that the effect of arginine supplementation on the indicators What is related to hypertension and glucose metabolism in the body? Also, if you combine the use of this supplement with aquatic training, will their effect increase or not?

2. Materials and Methods

Research method and samples: The statistical population of this research was all people with mild blood pressure in the age range of 55 to 70 years who did not take any special medicine to control blood pressure or other metabolic disorders. Among this population, 43 subjects who had the conditions to participate in the research were selected as subjects. In order to conduct the present study, first by referring to the centers that implement the Dignity Plan of Tehran Municipality, located in the 1st, 2nd and 3rd districts of Tehran, the necessary correspondence regarding the use of the elderly in these places was conducted with the officials of these centers, and the number of 43 women and Elderly men in the age range of 55 to 70 years with mild hypertension were selected and randomly divided into 4 groups including three experimental groups and one control group.

The criteria for entering the subjects in this study were systolic blood pressure above 140 and diastolic blood pressure above 90 mmHg, no consumption of alcohol, smoking, and no history of regular exercise, and none of the subjects took any special medication to control blood pressure or other disorders. They did not use metabolic drugs. There were some metabolic disorders such as diabetes, insulin resistance, blood fat and obesity in some subjects. Other demographic characteristics of the subjects are shown in Table 1:

Table 1: General characteristics of subjects (mean ± standard deviation)

Groups / Variables	control group	L-arginine group	Aquatic training group	L-arginine group along with aquatic training
Number	11	12	10	10
	6 men and 5 women	6 men and 6 women	6 men and 4 women	5 men and 5 women
age (years)	66.4±4.72	62.33±6.52	59.73±3.83	60.46±4.41
height (meters)	164.42±4.64	167.61±5.21	165.11±6.89	161.37±4.48
weight (kg)	71.56±6.90	74.27±8.01	69.12±6.23	68.73±7.37
body mass index (kilograms per square meter)	26.39±2.54	26.53±1.98	25.34±2.11	26.23±1.79
fat percentage (%)	28.46±3.17	26.11±5.09	23.55±5.54	25.11±4.09
Waist to hip ratio	1.01±0.17	0.97±0.09	0.89±0.11	0.91±0.19

After selecting the samples and explaining them about how to implement the work, written consent was taken from them to participate in the research. Then, the samples were randomly divided into 4 groups, including water sports training (AT) and L-arginine supplement consumption (ARG), aquatic training and L-arginine supplement consumption (AT+ARG) and control (CON). . Next, after measuring the dependent variables in the pre-test, the independent variables were applied in different groups for 6 weeks. For this purpose, the AT group performed 45 to 60 minutes of water sports three times a week for 6 weeks. The ARG group received 100 mg of L-arginine per kilogram of body weight twice a day in the form of oral capsules, and the AT+ARG group received L-arginine supplement during 6 weeks of aquatic training.

The control group also received only the placebo (dextrose) in the same capsules. 24 hours after the last activity session, the variables measured in the first week were measured and recorded in the fasting state under the same conditions as the pre-test. Evaluation of variables: The variables measured in this study included aerobic capacity, body composition, ratio of waist circumference to hip circumference, systolic blood pressure, diastolic blood pressure and fasting blood glucose. For this purpose, after coming to the laboratory, the subjects rested for 10 minutes on a comfortable chair, and then their systolic and diastolic blood pressure was measured using Citizen brand CH-456 digital sphygmomanometer made by CITIZEN company in Japan. It reports the pressure type along with the heart rate.

To measure fasting blood glucose, 10 cc of blood was taken from the subject's brachial vein and after clotting, it was kept in the refrigerator for FBS measurement. Before starting the training program and under completely identical conditions, the subjects' body composition and aerobic capacity were measured using the Quine staircase test. Their body composition was also done using the Inbody-270 body composition evaluation device made in South Korea, and after a full body scan, the ratio of waist circumference to hip circumference was provided to the researchers in printed form.

Exercise protocol

The exercise protocol implemented in the present study included a six-week aquatic training program, which was performed three sessions a week (18 sessions in total) and each session lasted 45 to 60 minutes. Each aquatic training session had three phases: warm-up, the main part of the aquatic training, and cool-down. The exercises of the experimental group were done in the indoor pool with water temperature between 26-28 degrees Celsius according to the protocol in Table 2.

Table 2: Steps of the aquatic training protocol

The first stage (warming up for 15 minutes)	the second stage (exercising for 30 minutes)	the third stage (cooling down for 15 minutes)
Stretching movements in water	transferring weight from front to back	doing stretching movements
Walking forward	turning around a square	buoyancy exercises, stretching exercises and deep breathing
Walking backwards	standing on one leg	
Walking sideways	transferring weight from one side to another	
Walking on heels and toes	stepping sideways	
Jacking in the water	Scott	
	Hamstring pull back	
	Open the thigh	
	bike leg	
	Pendulum movements of hands and feet	
	The movement of stepping forward	
	Step to the side	
	Military step forward movement	
	Coordinated walking movement	

Ethical Considerations

Before explaining the research, people were asked to participate in the research by completing the consent form if they were willing and gave informed consent. The exercise implementation process was fully and clearly explained and it was explained to the subjects that this research does not have any risk or harm in terms of intervention or assessment methods. Subjects were able to leave the research at any stage of the research for any reason.

Statistical analysis

After ensuring the normality of the data using the Shapiro-Wilks test, the data were analyzed using one-way analysis of variance and Tukey's post hoc test was used to compare the two groups. All calculations were done using SPSS version 26 statistical software at a significance level of 0.05.

3. Results

The findings of the present study are summarized in Table 3. The result of data analysis using one-way analysis of variance shows that the subjects' diastolic blood pressure did not differ significantly after 6 weeks of applying independent variables (P=0.239), but the difference between the systolic blood pressure of the samples in The post-test was statistically different (P=0.031). Also, the fasting blood glucose changes of the samples in the 4 groups showed a significant difference (P=0.011).

Table 3: Summary of research findings in groups of research groups

Variables / Groups	Fasting blood glucose mg/dl		diastolic blood pressure mmHg		systolic blood pressure mmHg		maximum oxygen consumption ml/kg.min	
	Post Test	Pre Test	Post Test	Pre Test	Post Test	Pre Test	Post Test	Pre Test
CON	122	126	86	85.8	133.8	134.3	22.7	22.1
ARG	113	135	84.2	88.9	131.2	135.7	25.2	24.3
AT	131	143	89.3	91.1	132.9	134.8	24.7	19.8
AT+ARG	100	126	86.5	90.3	132.1	135.1	26.3	23.2

Fasting blood glucose mg/dl diastolic blood pressure mmHg systolic blood pressure mmHg maximum oxygen consumption variable ml/kg.min

The systolic blood pressure values of the samples are shown in chart 1. Based on this, the results of Tukey's post hoc test show a significant difference with the control group (P=0.0001 and P=0.001 respectively). Other groups are not significantly different.

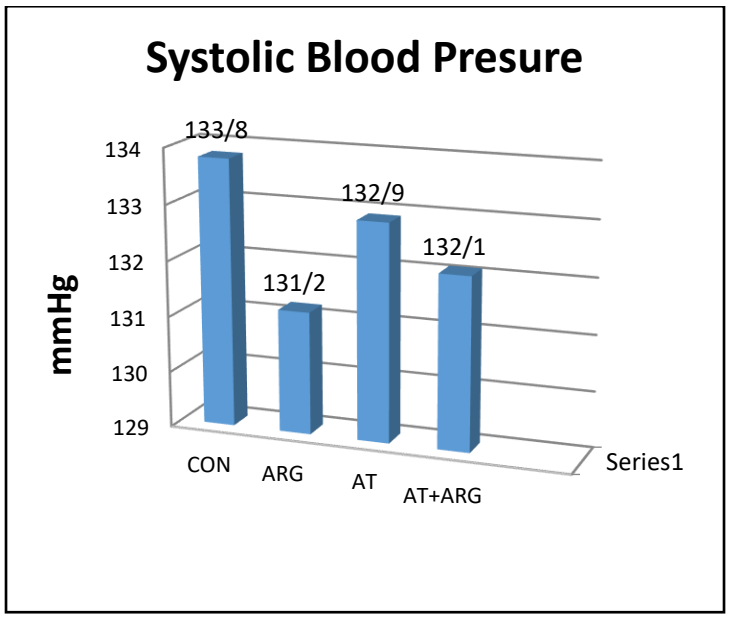

Chart 1: Comparison of systolic blood pressure after a 6-week intervention in research groups

Chart 2 also shows the changes in fasting blood sugar before and after the intervention program in different groups. These results indicate that there is a significant difference between the aquatic training group + L-arginine consumption and the control group (P=0.0001). Also, the aquatic training group is also different from the control group (P=0.001), but there is no significant difference between the other groups.

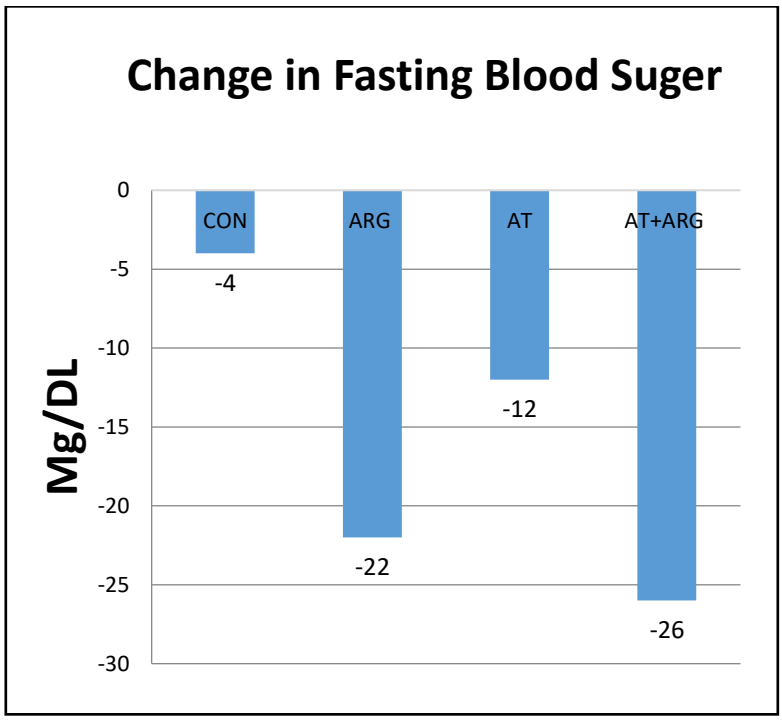

Chart 2: Comparison of changes in fasting blood sugar after 6-week intervention in research groups

4. Discussion

The results of the current research showed that consuming about 6 grams of L-arginine per day and doing sports exercises for 6 weeks lead to a decrease in systolic and blood pressure in the elderly with hypertension, but aquatic exercise alone do not have such an effect. This issue is consistent with the results reported by Mir Fatahi et al. (2018) and Bahrami et al. It is clear that the dosage of L-arginine and the subjects participating in the mentioned studies are the most important reasons for the difference in the results. In the study by Pahlavani et al. (2014), 2 grams of L-arginine per day were used for 45 days in the intervention group, while in the present study, the amount of about 6 grams of L-arginine was used. This was while the studies of Mirftahi (2018) and Bahrami (2018) used 3 and 5 grams of L-arginine, respectively.

More important than that was the study conducted by Asadi and his colleagues (2013) in order to investigate the supplemental effect of L-arginine in a double-blind randomized clinical trial of 57 patients with type 2 diabetes. The results showed that consuming 6 grams of L-arginine per day led to a decrease in total cholesterol, but there was no change in HDL and TG. This issue shows the role of dosage in creating effects of L-arginine on lipid profile. Other differences in different studies are related to the type of subjects. Considering the proven effects of exercise and L-arginine supplementation on blood pressure regulation, it seems that the greatest effect of this factor is in people who have hypertension.

On the other hand, in some researches that used healthy subjects with normal blood pressure, the use of L-arginine supplement and performing sports activities could not cause significant changes in blood pressure. Regarding the effect of the aquatic training program, the results showed that 6 weeks of aquatic training did not change much in adjusting and reducing the blood pressure of the subjects. 6 weeks of aquatic training does not seem to be sufficient for significant changes in blood pressure regulation in elderly subjects, while the American College of Sports Medicine in its latest opinion recommends 3-month exercise programs with an exercise volume of 150 to 250 minutes per week. has proposed for this purpose (). However, in the current study, the researcher was looking for the synergistic effect of two factors, sports activity and L-arginine consumption, and the results showed that this synergistic effect does not occur within 6 weeks. Regarding the reasons for the effect of l-arginine on blood pressure regulation, various mechanisms have been stated, and it seems that a set of these factors are effective in creating such results. Among them, we can mention the effect of L-arginine in stimulating the release of nitric oxide (NO) from vascular endothelium. Nitric oxide is the cause of vasodilation in many arterioles of the body. Nitric oxide synthase (NOs) catalyzes its production by endothelial cells. Since L-arginine is one of the factors that stimulate and activate the above enzyme, its increase in the blood flow can lead to an increase in the production of NO and by increasing the diameter of the vessels, it can reduce vascular resistance.

Arginase enzyme as L-arginine decomposer competes with nitric oxide synthase enzyme in getting L-arginine (Vasdev, 2010). Studies have shown that probably during old age, L-arginine goes more towards catabolic pathways, and for this reason, blood vessels lose their ability to produce nitric oxide, and one of the mechanisms of hypertension in old age is the lack of this amino acid. It is essential in the body of elderly people. For this reason, taking L-arginine can lead to the improvement of this condition in elderly people. Another mechanism by which L-arginine affects vasodilation is via insulin. L-arginine causes the release of insulin from the beta cells of the pancreas, and in turn, insulin causes a decrease in the concentration of asymmetric dimethyl arginine (ADMA) in the plasma. Asymmetric dimethyl arginine is an L-arginine analog that competes with L-arginine for uptake into vascular endothelial cells. Its increase leads to a decrease in cellular uptake of L-arginine (Ide 2007, Mann 2003). Therefore, by reducing the concentration of insulin in the blood, you help increase the absorption of L-arginine by the vascular endothelial cells. Also, the binding of insulin to insulin receptors causes the production of NO through the activation of an insulin-related signaling pathway, resulting in insulin-mediated vasodilation. L-arginine also modulates the function of the renin-angiotensin system (RAS). It inhibits angiotensin-converting enzyme (ACE) activity, thus reducing angiotensin II and its downstream effects (Campese, 1997). The effect of L-arginine on the RAS may be mediated by insulin. Low and moderate concentrations of insulin have been shown to decrease angiotensinogen expression in endothelial cells, while high concentrations upregulate the ACE receptor. L-arginine has been shown to have antioxidant activities that may help regulate redox-sensitive proteins and lower blood pressure.

Effect of L-arginine supplementation on blood pressure Arginine is involved in several important physiological processes and affects many vascular functions. L-arginine deficiency or lack of availability and changes in L-arginine metabolism can contribute to hypertension and endothelial cell dysfunction (Mann, 2003).

The results of the present study showed that consumption of about 6 grams of L-arginine reduces fasting blood glucose concentration. These results are consistent with what was reported by Lucotti et al. (2006), Pahlavani et al. (2014) and Boon et al. (2018). In the research of Bon et al. (2018), the effect of consuming 9 grams of L-arginine per day was investigated in 10 middle-aged Asian and European prediabetes men, and the results of this research showed that 6 weeks of consuming L-arginine had an effect on the basal metabolism and the activity of coffee fat cells. It does not exist in both races. However, glucose tolerance test results, circulating insulin, and peak insulin concentration were improved in European men, but not in South Asian men.

Conclusion

The researcher has concluded that L-arginine supplementation does not change the basic metabolism and activity of fat cells, but it improves some indicators of glucose metabolism in the blood of the European race. This research clearly shows that L-arginine can improve the blood sugar profile of pre-diabetic and diabetic people. However, some animal studies have reported that long-term L-arginine intake may increase insulin resistance. Since L-arginine acts as a stimulator of pancreatic beta-cells to produce insulin (Vassef 2008), it is possible that long-term stimulation of L-arginine leads to fatigue and laziness of pancreatic beta-cells.

Also, studies have shown that sports activities and exercises can reduce the effects of long-term abuse of L-arginine. In the same context, Barrera et al showed in 2017 that adding exercise to the L-arginine supplement use program in the long term can reduce the effects of abuse of this supplement in reducing insulin sensitivity in mice. Considering the role of exercise and L-arginine consumption in increasing the secretion of growth hormone from the pituitary gland, the results of this study confirmed the role of L-arginine in attenuated insulin resistance.

In general, the results of the present study showed that consuming L-arginine for 6 weeks in the elderly with hypertension can lead to improvement of their systolic blood pressure and blood sugar profile, but adding aquatic exercise could not double its effect. Although exercise training has proven effects in modulating blood pressure and reducing blood sugar in diabetic and pre-diabetic people, it seems that observing the double effect of activity on these indicators requires longer training programs, which future studies will investigate. It will clarify more.

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Conflict of interest None declared.

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Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: Y.K., P.H., Y.S.; Methodology: Y.K., P.H., Y.S.; Software: Y.K., P.H., Y.S.; Validation: Y.K., P.H., Y.S.; Formal analysis: P.H., Y.S.; Investigation: Y.K., P.H.; Resources: Y.K., P.H., Y.S.; Data curation: P.H., Y.S.; Writing - original draft: Y.K., P.H., Y.S.; Writing - review & editing: Y.K., P.H.; Visualization: Y.K., P.H., Y.S.; Supervision: P.H., Y.S.; Project administration: Y.K., P.H., Y.S.; Funding acquisition: Y.K., P.H.

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Research Article

The effect of caffeine consumption on some factors of physical fitness and skills of young footballers

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Abstract

Background: Caffeine is a supplement that is not yet fully effective in specific football training styles. The purpose of this study was to determine the effect of caffeine consumption on some of the physical fitness and skill factors of young footballers.

Materials and Methods: The research method is quasi-experimental and the participants of this study were 30 football players aged 16-18 years who were randomly selected and randomly assigned to one of the three groups of caffeine consumption, placebo use and control (30 = n). The variables of lower limb strength, agility, speed, maximum oxygen consumption and pass, shoot and dribble football skills were considered as dependent variables in pre-test and post-test of all participants. Data were classified by descriptive indices of mean and standard deviation and data analysis was performed using repeated measures analysis of variance test using SPSS-23 software at a significance level of $P < 0.05$.

Results: The results of statistical test showed that between the average lower limb strength (sig = 0.001), maximum oxygen consumption (sig = 0.001), speed (sig = 0.001) and agility (sig = 0.002) There is a statistically significant difference in the caffeine group with the other two groups from the pre-test to the post-test, but there is no statistically significant difference between the control and placebo groups. The results also showed that there was a statistically significant difference between the mean performance of shot, pass and dribble football in the caffeine group with the other two groups from the pre-test to post-test (sig = 0.001), but between the control and placebo groups. There is no statistically significant difference.

Conclusion: Based on the results of the present study, caffeine consumption can have a positive effect on the physical fitness and technical skills of young footballers. Therefore, football coaches are advised to use caffeinated beverages before training to improve their footballers' physical and skill factors.

Keywords:
caffeine, footballer, skill performance, physical fitness

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1. Introduction

Today, according to the available evidence, football is the most popular and most watched sport in the international arena, a phenomenon that has affected many social, political and economic issues of many countries and many countries in the world consider it their national sport. According to the statistics of the International Football Federation (FIFA), one out of every six people in the world has played football and one out of every three people is interested in football (1). Proper nutrition is important for success in a football game and can affect a player's ability to practice, play and recover after an activity. Also, dietary components such as vitamins, minerals, and supplements can be included in an athlete's diet through a varied diet and affect the body's neuromuscular and metabolic function (2). Foods and food ingredients that can improve a person's capacity to perform an exercise task have been defined as energizing aids (3). Most athletes use athletic assistance to improve the quality and quantity of their training and, in fact, to help them perform in competitive situations. Under certain circumstances, strength training aids may have positive effects on athletic performance, body composition, and strength (4,5). On the other hand, physical fitness is one of the most important parts of training in sports, which is necessary and a pioneer in achieving optimal sports performance. The main goal of physical fitness is to increase the practical capabilities of athletes and develop their capabilities to the highest level (6). In the present study, the factors of physical fitness were the score that was recorded for lower limb strength, cardiovascular endurance, speed and agility for young footballers. Technical skills in football, along with psychological skills and physical dimensions, are key factors influencing performance and performance in order to achieve appropriate results (7). Caffeine is one of the most widely used nutritional and ergogenic supplements used by athletes. Caffeine is a behavior-dependent active drug and is an example of the most widely used nutritional energy supplements in the world (8, 9). Fatty acid excretion, increased calcium release from the sarcoplasmic reticulum and improved skeletal muscle contractility, increased catecholamine secretion, increased energy production through increased neuromuscular transmission, and improved maximal muscle activity and decreased performance perception; Apply(10, 11).Caffeine produces a variety of physiological effects, including effects on the cerebral vascular system, blood pressure, respiratory function, gastrointestinal activity, urine volume, and exercise performance (12). Regarding the effect of caffeine on athletic performance, three main and possible mechanisms have been suggested. Caffeine increases cyclic adenosine monophosphate and consequently increases lipolysis, mobilization of intracellular calcium from the sarcoplasmic reticulum and competitive antagonist of adenosine receptors in the central nervous system (13, 14). Caffeine increases the oxidation of fatty acids and stores muscle glycogen. This practice can improve performance, especially during endurance activity (13). Because of these characteristics, athletes in various disciplines consume caffeine to increase performance. Although the effects of caffeine consumption on endurance performance have been well studied (15, 16), less research has been done on caffeine energy and its effect on other factors of physical fitness and skill of athletes.

In this study, football technical skills were the score that young futsal players received from the Moore Christian test series (dribble, pass and shoot). Vergayon et al. Examined the effect of carbohydrate supplementation alone and in combination with caffeine on kicking performance in 13 male tennis players. Caffeine consumption was given as 5 mg per kilogram of body weight one hour before training and 0.75 mg of caffeine was consumed for the additional two hours after that. The results showed that adding caffeine to carbohydrates had no effect beyond carbohydrates only on skill performance and the 70-meter shuttle test (17). Horneri et al. Examined the effect of caffeine on 12 top tennis players and found that caffeine consumption speeds up service compared to placebo (18). On the other hand, Lorino et al. Examined the effect of caffeine consumption on agility and showed that caffeine consumption did not have a significant effect on agility and anaerobic power in active men (19). Spradley et al. Also showed that caffeine supplementation (300 mg) before exercise significantly improved the selective reaction time in male athletes and had a lower effect on muscular endurance (20). Frati et al. In a study entitled the effect of acute caffeine consumption on muscle strength and endurance of amateur male football players showed that muscle strength and endurance in the experimental group increased significantly but did not see a significant change in the placebo group (21).

In their study, Lara et al. Showed the effects of caffeinated beverages on the performance of female soccer players and showed that consuming caffeinated beverages compared to placebo resulted in increased in-situ jumps and the average maximum sprint and, in general Participants in the energy supplement group ran faster than the placebo group (22). Haghighi et al. Also studied the effect of caffeine consumption on some indicators of skill and physical fitness of the best table tennis players and concluded that caffeine consumption has no effect on skill and physical indicators in table tennis (23). Due to the discrepancies in the results of the above studies and the lack of appropriate conclusions regarding the use and non-use of caffeine-containing supplements on young footballers, this study aims to examine the effects of caffeine supplementation on physical and skill indicators.

2. Materials and Methods

Methods of research and subjects: The method of this research is semi-experimental due to the use of human samples and lack of control of all disturbing factors and is applied research in terms of purpose. The statistical population of the present study was all football players in the age group of 16-18 years old in Tehran who were active in the provincial league in the 2017-17 season. Statistical samples included 30 footballers who participated in the study in an accessible and voluntary manner. Participants were randomly assigned to one of three groups: caffeine consumption, placebo use, and control.

Inclusion criteria include 16-16 years old age, membership in a sports club and issuance of playing cards for the current season of football history for at least 3 years under the supervision of a coach and exclusion criteria include not participating in training for more than 3 sessions, reluctance He continued to work with the researcher and was injured during the training protocol.

Exercise protocol

The study participants each participated in the study three times, one day apart, in three groups of caffeine users, placebo users and control.

The required information was collected by field method. After explaining the method and purpose of the research, participants completed a written consent form to participate in the research and a medical history questionnaire. Using a physical activity readiness questionnaire (which included 7 key questions) and a general practitioner examination, it was found that all participants were in good health and had no particular problem performing the activity (24). Participants were advised to avoid foods high in caffeine for 24 hours before the tests and to maintain a normal diet throughout the study. A few days before the tests, two candidates performed the tests on a trial basis, according to which the approximate time of the tests was determined. Participants in all test sessions first warmed up and stretched for 5 minutes, then participated in shoot, dribble, and pass football tests, respectively, and finally performed cardiovascular speed, agility, strength, and endurance tests. brought. At the end of the sessions, cooling was done for 5 minutes.

However, in sessions that included caffeine consumption (5 mg per kilogram of body weight) and placebo, participants came to the training site 45 minutes earlier and after consuming caffeine or placebo (starch powder) which was prepared as a capsule, along with Half a cup of water, 45 minutes in order to maximize the concentration of caffeine in the blood, sit in a chair without activity. It should be noted that the rest interval between skill tests was 3 to 5 minutes and between repetitions of tests was 2 to 3 minutes It took 48 hours for the participants to return to the gym, during which time the athletes stopped doing strenuous physical activity and caffeine consumption. It should be noted that all measurements and tests were performed under the same conditions in terms of temperature, humidity and time.

measurements:

Test the front foot of the device

In order to evaluate the maximum strength of the lower limb, a front wire device was used and the maximum lower limb strength was calculated based on the formula of one repetition (25). Maximum strength is 1RM (maximum weight that a person can have one repetition) (maximum repetition = shifted weight / (025 / × number of repetitions) -1).

YUT 2 Recovery Test (YIRT2)

To measure aerobic power, the Yuio 2 periodic recovery field test was used. This test involves running 2 consecutive distances of 20 meters in the form of going back and forth to the starting point, where the start and end time of the round trip is determined by the sound of the horn. Behind the starting line, the cone is 5 meters away, where the participant has 10 seconds to run 2 5-meter distances with a soft run after each round for recovery.

The test start speed will be 13 km / h, which will be increasingly increased. The test ends when the participant is unable to reach the line twice when he hears the beep. In order to ensure the maximum effort of the participants in performing the Yoyo 2 test, the heart rate at the end of the test was measured with a heart rate monitor (Polar model F7 made in Finland) (6)

35m Speed Test (40 Yards Speed)

This test measures the linear speed of athletes and is suitable for athletes who perform various sports activities at a very high speed. Test method: Using two cones at a distance of 35 meters, the test site was determined. The athlete is standing in the starting position, the starting position is maintained for 2-3 seconds, the timer starts working from the moment the sole of the foot begins to move. When the timer was held the athlete's foot was on the finish line.

Illinois Agility Test

This test is used to measure agility in running and different routes. The length of the ground is 10 meters and its width is 5 meters, the participant runs the route as fast as possible. Then touch the marked line and quickly move backwards on the new path. Here, in a zigzag motion, it moves side by side with the cones and returns to its original position. After going around the last cone, move back and forth again in the specified direction, and after touching the marked line, walk the straight path of about 10 meters again with a speed to the end. How to score in this test is how long it takes the participant to walk this route (6).

Moore-Christine dribble skill test

Equipment needed: 12 45 cm cones, chronometer, several balls, a circle with a diameter of 18 meters, one person who records the results, one person to return the balls, a scrapbook, a pencil or a pen. Draw a circle with a diameter of 18.5 meters. 12 cones with a distance of 4.5 meters are arranged on the circle. Then draw a 90 cm long starting line outside the circle. By announcing the start, the participant starts moving the ball from the starting point and passes through the cones with maximum speed and then returns to the starting point. The participant has performed this test twice, but each performance must be different from the previous one, so that in the first turn it moves clockwise and in the second turn counterclockwise. The final score of the test is calculated from the average time of 2 runs (26)

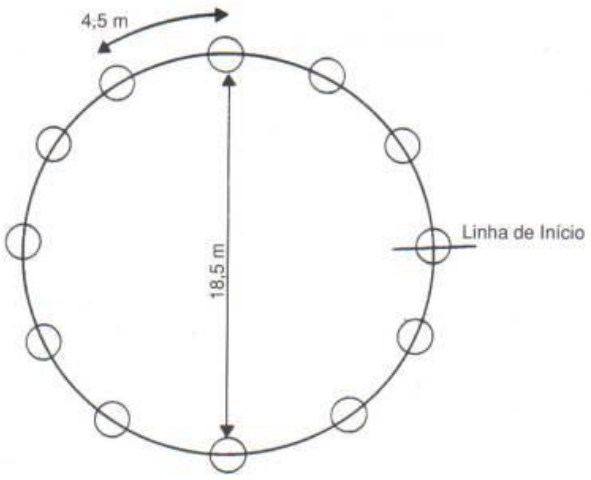

Figure 1: How to organize the Moore-Christine dribble test

Moore-Christine Pass Skills Test

Materials needed: cone, gate approximately one meter wide and half a meter high. In the skill of guarding two cones at a distance of approximately 1 meter using a rope approximately 135 cm will be created as a horizontal beam and two cones with an angle of 45 degrees to the gate line and another cone with a 90 degree angle at a distance of 15 meters They will be at the gate. People send 4 passes from each cone and a total of 12 passes to the goal. This test is repeated twice from each area and one point is awarded for each correct pass that hits the cone. The final score will be the total score of 12 assists (26).

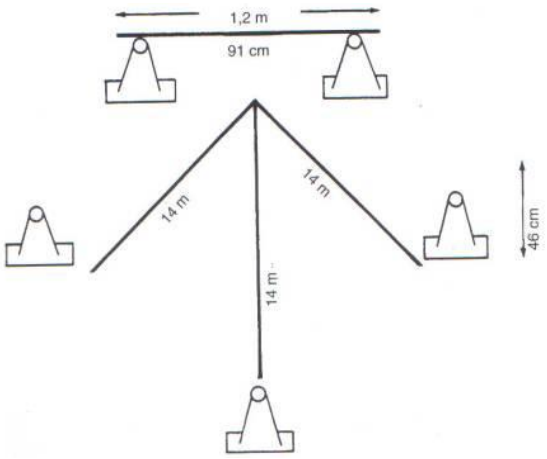

Figure 2: How to organize the Moore-Christine Pass test

Moore-Christine Shot Skills Test

Equipment needed: a few balls, enough space, two ropes, a football gate. In shooting skill, the execution method is as follows: with two strings, we separate the door rope from each vertical pole to the middle of the goal by 120 cm. From a distance of 14.5 meters to the gate, a line is drawn on the ground, which is the firing point. The subject is placed behind the starting line to shoot the ball into the goal. To prepare, each person is given the opportunity for 4 test shots. He then has the opportunity to repeat the test in 4 stages and 4 shots in each stage (16 shots in total). The scoring method is that 10 points are awarded to shots that hit the target and 4 points are awarded to shots that hit the target. For example, if a participant wants to shoot to the right and above the goal and the shot hits the same place as the arrow, he gets 10 points. If the ball is hit to the right but below the goal, 4 points will be awarded. Balls thrown on the ground are not awarded points and the final score is 16 shots (26).

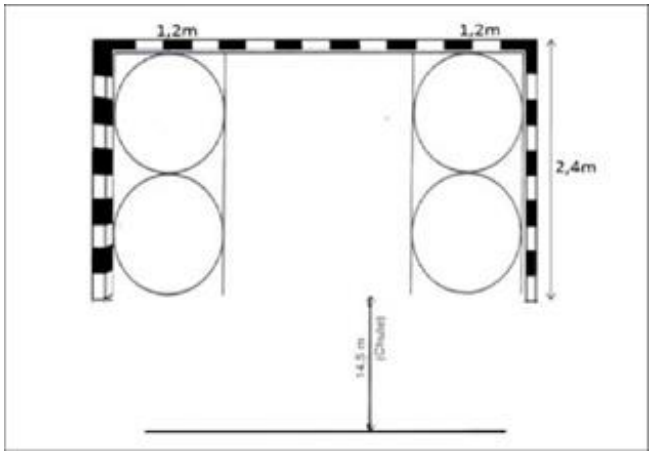

Figure 3: How to organize the Moore-Christine shot test

In inferential statistics, Kolmogorov-Smirnov test was used to evaluate

the normality of information distribution, and Levin test was used to check the homogeneity of variances. In order to test the research hypotheses, repeated measures analysis of variance test was used to evaluate the differences between groups and after the differences were significant, Bonferroni post hoc test was used to compare the two groups. In all tests, a significance level of p = 0.05 was considered. All statistical operations were performed using SPSS software version number 23.

3. Results

Frequency, mean and standard deviation of participants' body measurements are presented in Table 1. According to the information presented in the table, the participants have a similar average age. Also, the experimental group is 1 cm taller and weighs 2 kg more than the other two groups.

Findings related to the mean and standard deviation of participants' performance in all stages of the research are presented in Table 2. As shown in the table, the participants of all three groups in the pre-test stage are not significantly different from each other. In the post-test phase, the experimental group showed significant progress in all four subscales, but no difference was observed in the other two groups.

Findings related to the mean and standard deviation of participants' performance in all stages of the research are presented in Table 3. As shown in the table, the participants in the control group had poorer but minor performance in the pre-test stage in shooting and passing skills, and in the post-test stage the experimental group improved more than the other two groups. The Kolmogorov-Smirnov test was used to determine the normality of data distribution. According to this test, the distribution is normal when the value of P is greater than the critical number at the level of 0.05. The results of this test showed that the distribution of all measured data was normal. The results of this test are presented in Table 4.

Table 1: describes the descriptive demographic information of testers in three groups

Variable / Group	Experimental	placebo	Control
Abundance	10	10	10
Age (years)	17 ± 1	17.7±1.1	17.5±1.5
Height (cm)	173.8±2	172±3	172.2±2
Weight (kg)	68 ± 2.7	66±4.1	65±3.4

Table 2: Distribution of performance of physical fitness variables in different groups

Variable group		**power**	**VO2max**	**Speed**	**Agility**
		Mean ± standard deviation	Mean ± standard deviation	Mean ± standard deviation	Mean ± standard deviation
Experimental	pre-test	44.3±3	46.4±2.4	5.5±0.1	17.5±2
	Post-test	53±2.7	51.4±2.6	4.8±0.2	16±0.9
placebo	pre-test	42.8±4.5	45±1.9	5.5±0.1	19±1.7
	Post-test	45.1±2.8	46.2±2.2	5.4±0.1	18.8±1.7
Control	pre-test	42.6±4.9	45.9±1.3	5.3±0.2	18.2±1.7
	Post-test	45.1±2.8	46.8±2.8	5.3±0.1	18±2.2

Table 3: Information on the football skill performance of participants in different groups

Variable group		shoot	Pass	Dribble
		Mean ± standard deviation	Mean ± standard deviation	Mean ± standard deviation
Experimental	pre-test	152±12	4.2±1.3	18±2.2
	Post-test	176±5	7.3±1.7	14.8±1
placebo	pre-test	152±10	4.2±1.3	19±1.8
	Post-test	148±10	4.5±1.2	18.6±1.9
Control	pre-test	152±8	4.7±1.1	18.2±2
	Post-test			

Table 4: Summary of Kolmograph Smirnov test results for research variables

Indicator	the level	the power	VO2max	Speed	Agility	Pass	Dribble	shoot
Number	2	30	30	30	30	30	30	30
The value of Z	pre-test	0.164	0.187	0.201	0.162	0.192	0.189	0.203
	Post-test	0.179	0.145	0.138	0.148	0.174	0.195	0.172
Significance	pre-test	0.200	0.125	0.101	0.209	0.141	0.154	0.097
	Post-test	0.168	0.213	0.225	0.210	0.194	0.156	0.191

4. Discussion

The results showed that caffeine consumption has a significant effect on the lower limb strength of young footballers. The results of repeated measures analysis of variance test showed that among the three experimental groups, placebo and control, only the caffeine group had a change in lower limb strength from pre-test to post-test. The results also showed that there was a significant difference between the experimental group and the placebo and control groups, but this difference was not observed between the placebo and control groups. These results show that young athletes can increase and benefit from the strength of their lower limbs by consuming a certain amount of caffeine supplement. These results are consistent with the real findings of Azizi Masouleh et al., Strino et al. On the positive effect of caffeine consumption on fitness factors, and inconsistent with the results of Crow et al. (23, 27-29). Crow et al. Concluded in their study that caffeine had no energizing effect on repetitive cycling times with maximum intensity and could be detrimental to anaerobic performance (29). On the other hand, in a review study, Strino et al.

Examined the effect of caffeine on motor performance in high-intensity, low-duration activities. Studies have shown that 29 studies specifically addressed the effect of caffeine consumption on the performance of short-term high-intensity activities. The results showed that 11 out of 17 studies indicate that in team sports and strength-based sports, a significant improvement was observed after caffeine consumption. Six studies also showed the beneficial effects of caffeine consumption in resistance exercise (27).

Caffeine reduces muscle tissue fatigue. Fighting fatigue is stimulated by stimulation of the central nervous system as well as direct function on the muscle (30). Consumption of 300 mg of caffeine increases the capacity of individuals to do muscle work (31).

The results of the present study showed that caffeine consumption has a significant effect on the maximum oxygen consumption of young footballers. The results of repeated measures analysis of variance test showed that among the three experimental groups, placebo and control, only the caffeine group changed from the pre-test to the post-test in the maximum oxygen consumption. The results also showed that there was a significant difference between the experimental group and the placebo and control groups, but this difference was not observed between the placebo and control groups. These results show that young athletes can consume significantly more oxygen by consuming caffeine supplements. These results are consistent with the findings of Christensen et al., Lara et al., Azizi Masouleh et al., And inconsistent with the findings of Greer et al. (22, 28, 32, 33). One of the most likely factors influencing the response to exercise and sports supplements is age range. Research has reported that with age, the effects of aerobic exercise and the effects of supplementation decrease (34). The participants of the study were Greer and his colleagues were elderly people and based on theoretical principles, one of the possible causes of inconsistency of the results can be attributed to this issue (32).

The results showed that caffeine consumption has a significant effect on the speed and agility of young footballers. The results of the analysis of variance test showed repeated measures that among the three experimental groups, placebo and control, only the caffeine consumption group changed in speed and agility from pre-test to post-test. The results also showed that there was a significant difference between the experimental group and the placebo and control groups, but this difference was not observed between the placebo and control groups. These results show that young athletes can benefit from a considerable amount of speed and agility by consuming a certain amount of caffeine supplementation. These results are consistent with the findings of Ranjbar et al., Schnicker et al. Is inconsistent with the findings of Patton et al. (27, 35-37).

Regarding the effect of caffeine on athletic performance, three major and possible mechanisms have been suggested. Caffeine increases cyclic adenosine monophosphate (cAMP) and thereby increases lipolysis, mobilization of intracellular calcium from the sarcoplasmic reticulum, and competing antagonist agonist antigen system. . Caffeine increases the oxidation of fatty acids and stores muscle glycogen. This practice can improve performance, especially during endurance activity. However, it has been said that short-term and vigorous activities, such as speed and agility tests, are not limited by the amount of carbohydrates available. Therefore, it seems that the effect of caffeine through this mechanism does not play a major role in such activities (15). The results of the present study showed that caffeine consumption has a significant effect on the skill performance of young footballers.

The results of repeated measures analysis of variance test showed that among the three experimental groups, placebo and control, only the caffeine group had changes in pass, shoot and dribble football skills from pre-test to post-test. The results also showed that there was a significant difference between the experimental group and the placebo and control groups, but this difference was not observed between the placebo and control groups. These results suggest that young athletes can achieve significant success in dribble, shoot and pass skills by consuming caffeine supplements. These results are consistent with the findings of Lara et al., Schneiker et al., And inconsistent with the findings of Lara et al. (22, 23, 37). Although the exact mechanism that describes the ergogenic effects of caffeine in intense short-term activity, especially at physiological concentrations of caffeine, is not known; But these effects are probably multifactorial. However, it has been suggested that the main possible mechanism of action of caffeine in short-term and intense activities is the action of caffeine as a competitive antagonist of adenosine receptors (27). Caffeine binds to adenosine receptors in the central nervous system, causing more motor units to be used and increasing nerve drainage; Two factors that increase voluntary contraction and productive force (10). Caffeine can also increase performance by altering perceptions of exercise pressure, reaction time, or mental state (increased alertness and well-being) (13).

Conclusion

At the end of consumption of 5 mg of caffeine per kilogram of body weight by adolescent and young footballers, has a significant effect on physical indicators (lower limb strength, maximum oxygen consumption, speed and agility) and skill indicators (passes, shots and dribbles) and Leads to the improvement of each of these factors against placebo and non-use. Therefore, the use of these energizers for young athletes requires more research and encouragement from coaches.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest in publishing this article.

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

Informed consent Informed consent was obtained from all participants

Author contributions

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

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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.

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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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