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## **Effect of eight weeks of electrical muscle stimulation on the interleukin-6 and interleukin-10 levels in overweight women**

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**Abstract**--Introduction: Nowadays more people are overweight more than before. Overweight can be considered as having more body fat than desired. Overweight or obesity increases the relative risk of diabetes and coronary artery disease in women. Therefore, this research investigates the effect of eight weeks of electrical muscle stimulation on the interleukin-6 and interleukin-10 levels in overweight women. Research method: In this research, we selected 34 overweight women and then randomly divided into three groups: control (10 people), resistance training (12 people) and resistance training + EMS (12 people). Then, two experimental groups performed resistance exercises for 8 weeks and 2 sessions per week, each session 20 minutes. In order to check the research variables, we collected 5 cc of blood from the research samples 48 hours before and after the test. We used Shapiro-Wilk test and analysis of covariance with SPSS version 24 software for statistical analysis of the research. Results: The analysis of IL-6 serum levels of the participants before and after eight weeks of intervention through covariance analysis showed that the difference between groups (control groups, resistance training and resistance training + EMS) was not statistically significant ( $p = 0.307$ ). The null hypothesis of the research is confirmed, that is, eight weeks of resistance training alone and combined with EMS does not have a significant effect on CRP levels in overweight women. Analysis of the FFM values of the participants before and after eight weeks of intervention through the analysis of covariance test showed that the difference between the groups was not statistically significant ( $p=0.431$ ).

**Keywords**---obesity, interleukin-6 and interleukin-10, EMS, Tehran women.

## Introduction

A person's overweight is generally describable by body mass index (BMI). Overweight is definable as BMI $\geq$ 25 or more, thus including pre-obesity as a BMI between 25 and 29.9 and obesity as a BMI of 30 or more. However, pre-obesity and overweight are often used interchangeably, so overweight is definable as a BMI between 25 and 29.9 plus overweight. However, there are several other common methods for measuring the fat in a person's body (1).

Obesity is one of the major health problems in the world and its prevalence is increasing significantly in developed countries as well as third world and developing countries. (2) (26-28)

A sedentary life is associated with the risk of many chronic diseases, so that about two million deaths in the world occur annually due to a sedentary life. Physical activity is effective not only in reducing the prevalence of cardiovascular diseases, but also in the incidence of other physical diseases and psychological disorders such as depression, anxiety and stress. Meanwhile, 60% of the world's population does not do the minimum recommended activity with moderate intensity. In Iran, the prevalence of inactivity among women aged 15 to 24 years has been about 68% and for men aged 15 to 24 years about 14.5%. The function of various body systems, including the immune system, undergoes changes through different training methods. We assume that training with moderate intensity and duration will improve immune function and intense and long training will suppress it (3).

Cytokines are polypeptides that interact with the immune system. The different types of immune and non-immune cells produced them (4). Cytokines have fallen under two categories: pro-inflammatory and anti-inflammatory. Pro-inflammatory cytokines are IL-1, TNF- $\alpha$ , IL-6, IL-17, IL-18, IL-22, IL-23 and anti-inflammatory cytokines are IL-4, IL-10, IL-1R, IL-13 (19).

An exercise movement of local cytokines including IL-6, TNF- $\alpha$ , IL-1 $\beta$  affects skeletal muscle. This local inflammatory response takes place through an orderly reaction by increasing the production of IL-6 from the liver and the initiation of the acute phase response by stimulating the production of CRP. (5)

There are various methods to lose weight, including diet, pharmaceutical and hormonal methods, surgical methods, use of massage, and use of slimming belts along with exercise, all of which have side effects. Using acupuncture is another method used to lose weight and applied on acupuncture points in the body or ears. It is an invasive and painful method due to the use of needles.

Exercising while managing a proper diet can be very effective. Obesity is caused by excess fat in the body. This body fat can be burned with exercise. The only problem is that exercising is harder and more tiring. On the other hand, sports

are fun. A person continues playing even after exhaustion because he enjoys it (6).

Electrical stimulation is a method often used to rehabilitate and strengthen muscles. As a treatment, it applies electrodes to the surface of the skin and treats muscle spasms and pain. Electrical stimulation helps prevent atrophy and build or maintain muscle strength, reduce inflammation, and improve range of motion. Electrical muscle stimulation (EMS), also known as neuromuscular electrical stimulation (NMES) or electromyostimulation, is a protocol that induces muscle contraction using electrical impulses that directly stimulate a person's motor neurons (23-27). This stimulation causes muscle contractions that can be rapid and frequent, rapid with long pauses or contractions held for several seconds or minutes at a time.

EMS has many amazing benefits - one of which is its ability to help the individual lose weight. EMS helps with weight loss by creating a calorie deficit. One of the main reasons EMS is beneficial for weight loss is that exercise helps create a calorie deficit, which is the main way to lose weight (7).

EMS technology is especially effective for those who find it difficult to go into a caloric deficit due to its low impact nature. For example, if the person has difficulty exercising due to old age or excess weight, it becomes more difficult to engage in calorie reduction because the main emphasis is on dietary changes. EMS can help those people exercise safely and effectively and burn much calorie. EMS also promotes weight loss by building muscle, which in turn helps increase metabolism and burn fat. Weight loss with EMS training is most effective when combined with a healthy, balanced diet that increases the calorie deficit. The aim of the current research was to study the effects of electrical muscle stimulation on some inflammatory/anti-inflammatory indicators in overweight women.

Bahrami, Elnaz, 2019, reviewed sports exercises with the help of EMS electric currents. This method has different effects, from strengthening the muscles of the lower back, abdomen and pelvic floor, which are difficult to change, to physical strength, shaping the body and the growth of muscles. In the meantime, the improvement of endurance has also been provable. Especially, in people who have done a little exercise before (8). Farzin Far, Mahbobeh, Vahid Saatchian and Amin Azim Khani (2018) stated in their research that, based on the results of the studies conducted on the effects of WB-EMS, this tool has significant positive effect on parameters such as muscle strength, muscle size, body composition, obesity, sarcopenia, obesity caused by Sarcopenia and pain reduction in people with non-specific chronic back pain. Conclusion: Considering the lack of studies on the effects of WB-EMS, the contradiction between the studies and the lack of consensus, there is a need to conduct more research in this field. (9) Nakhazri et al. concluded that combined exercise with a dominant aerobic supplement and coenzyme Q10 did not change significantly the serum levels of IL-10 and TNF $\alpha$  in patients with multiple sclerosis in none of experimental groups of the levels of IL-10 and TNF $\alpha$  (10). Tahir Kilich (2018) in a study stated that, investigating the effect of six-week electro-muscle stimulation training on physical changes in sedentary women and men, EMS training has increased the maximum strength associated with exercise. (11) according to Nejc Šarabon et al., Electrical muscle

stimulation and whole-body vibration in older adults/RT or a combination of RT and WBV or EMS is probably the most efficient method for improving muscle strength and performance, while the best method for increasing muscle mass in older adults needs further study. (12) The results of Priska Dyana Kristi's research (2020) showed that the average weight difference was 6.49%. The average difference in fat percentage is 15.54% and the difference in waist circumference is 6.74%. Therefore, body weight training with EMS can significantly reduce body fat percentage and waist circumference. (13) The research of Seung-Ah Yoo et al. showed a significant increase in muscle activity in all measurements made after the intervention. Abdominal subcutaneous fat thickness also showed a significant decrease between each measurement. The results of the experiment showed that the values of abdominal subcutaneous fat thickness taken eight weeks after the intervention were significantly lower than the values before the intervention and four weeks of the intervention. (14) D. RELJIC1 et al. (2020) stated that the effects of whole body stimulation electromyosystem exercises and caloric restriction on cardiac risk profile and muscle strength in obese women with metabolic syndrome decreased body weight by an average of 3 kg; fat percentage decreased only in the EMS group. This study shows that WB-EMS can be a suitable option for improving body composition, muscle strength and heart health in obese women. (15) In their research, Evangelista et al. (2019) answered the question whether electrical stimulation of the whole body muscles along with strength training causes morpho-functional changes. Our data shows that the combination of ST+EMS may cause changes in muscle strength and MT muscle thickness in active people (16). Investigating the effect of isometric exercise with electrical muscle stimulation on inflammatory cytokine levels, muscle strength, and knee joint function in elderly women with early knee osteoarthritis, Park et al. (2021) showed that eight weeks of isometric exercise (3 sessions per week/30 minutes each session) with EMS led to a significant decrease in IL-6-CRP-TNF $\alpha$  serum levels in elderly women. It was associated with weight loss and body fat percentage. (86)

According to the articles and results, exercise can have both positive and negative effects on the immune system. Regular mild exercise seems to reduce the spread of infection, while intense long-term exercise causes an immediate suppression of a large number of immune parameters according to the intensity and duration of exercise. (17)

The findings of the existing researches show the dual nature of the immune response to exercise. The cause of this issue can concern the wide variety of sports activities in terms of intensity, duration, the involvement of other physiological factors such as the role of hormones, as well as psychological factors and the effect of environmental factors.

The researches have not studied the response of inflammatory/anti-inflammatory factors in the training method of electrical muscle stimulation. Considering the current situation and the spread of the corona disease, exercising individually with an EMS device in a completely isolated environment can help people to use its benefits. Therefore, interventions that lead to the prevention or reduction of problems related to overweight and inactivity are important. This research

determines the effect of eight weeks of electrical muscle stimulation on the levels of some indices of interleukin-6 and interleukin-10 in women with overweight.

This research can be useful and practical for the following people:

- Coaches, athletes, students and researchers of sports fields.
- People who have not much time and are interested in sports.
- Those who are unable to do club/traditional exercises due to injuries.
- People who are looking for a sport with new technology and want to make a radical change in their fitness regimen.
- People with local obesity.

### ***Materials and methods***

This research was applied and semi-experimental, with a pre-test and post-test design. We randomly assigned to two test and control groups 34 subjects, including all 30-40-year-old healthy non-athlete women in Tehran with a body mass index (0.25-9.29). Before the start of the test, we explained the entire process of the research along with its objectives to the subjects in a session and obtained their demographic information from them along with the written consent of the candidates.

In the current research, we determined the exclusion of subjects from the study according to the following criteria:

- Not participating in more than 2 sessions during the training period.
- Inability to tolerate electrical stimulation to the extent that it causes contraction.
- Illness in such a way that it affects the continuation of exercises without risk and in a positive way.

After a brief warm-up, basic instructions and familiarization with the testing devices and correct training techniques, the participants performed strength tests on the following five sports equipment:

(Chest press, pull down machine, lower back machine, abdominal crunch and leg press).

We calculated based a maximum repetition on Barziki's equation as follows:

(1)

$(0.0278 * \text{number of repetitions until fatigue}) - 1.0278 / \text{weight moved (kg)} = \text{one maximum repetition}$

To measure the interleukin-6 and interleukin-10 levels, the laboratory team took blood samples after 10 hours of overnight fasting, 48 hours before the start of the training program and again 48 hours after the end of the training. Then, during the specified sessions (16 sessions), two days a week, the test group participated in the electrical test. In the control group, in the first and last session, without any intervention, we measured and recorded weight, height, body mass index, blood sampling.

### ***Exercise protocol***

At first, we randomly divided the statistical population of the research into a ST + EMS training group (12 participants, group 1), a ST training group (12 participants, group 2) and a control group (10 participants, group 3). An individual, who did not participate in this study, carried out the randomization process. Then, two intervention groups performed exercise twice a week (20 minutes each session) for 8 weeks and totally 16 sessions at the sports center. Group 1 (ST+EMS) performed exercises with electrical muscle stimulation and group 2 (ST) performed the same exercises without electrical muscle stimulation, and the control group did not perform any exercise during the period (16, 19).

During each session, participants wore a WB-EMS vest (AQ2, Spain) and performed a resistance training protocol guided by an instructor.

The WB-EMS vest and pants are equipped with 20 conductive electrodes to stimulate the muscles of the arm (2 electrodes), lower back (2 electrodes), chest (2 electrodes), middle back (2 electrodes), gluteal (2 electrodes), trapezius muscles (2 electrodes), quadriceps (2 electrodes), abdominal (2 electrodes), and free electrode (2 electrodes).

The controller and specific program (table) set the device for 20 minutes with a frequency of 80 Hz, duration of contraction 4 seconds, duration of rest 4 seconds, depth of penetration 350. (70-71)

Subjects warmed up with dynamic stretching movements 5 minutes before the main exercise and cooled down about 5 minutes after the main exercise. (13)

For each training session, respondents performed bodyweight exercises including squats, lunges, crunches, and planks (images). Subjects performed 2 sets of movements bearing body weight with a 20-second rest period between each movement. (13)

Squats and lunges are exercises that can strengthen the quadriceps, hamstrings and glutes in the lower body. Crunches are an abdominal exercise that strengthens the right part of the abdomen. Plank is the most basic exercise for central muscles that strengthens the muscles around the spine. (13)

Both training groups performed Squat, lunge, crunch 20 times each and plank exercise for 1 minute (2 sets). The number of sets and repetitions was equal between the groups. (13)

Exercises are carried out following the prepared training program. Before being used for research, expert professors approved the curriculum. Below are pictures of the exercise protocol:





Figure 1: Pictures of the exercise protocol

After collecting the data, we used SPSS version 24 statistical software to test and analyze the data. We used Shapiro-Wilk test to check the normality of data distribution and Anocava analysis of covariance test to compare changes between groups.

### **Results**

#### Description of research findings

Table 1 reports the serum levels of IL-6, CRP and IL-10 as well as body weight, BMI, body fat percentage, WHR, SMM, FFM and BFM of subjects in three groups of control, resistance training and resistance training + EMS before and after eight weeks of training Resistance alone and in combination with EMS as mean  $\pm$  standard deviation.

Table 1: Levels of the variables under study (mean  $\pm$  standard deviation)

Variable	Measurement stage	Control	Resistance training	Resistance training + EMS	Intra-group p
IL-6	Before				

)pg/ml(	training				
	After training				
IL-10 )pg/ml(	Before training				
	After training				
Significance (Intra-group p)					
BMI (kg.m <sup>2</sup> )	Before training				
	After training				
Significance (Intra-group p)					
body weight (kg)	Before training				
	After training				
Significance (Intra-group p)					

### **Data distribution**

We used Shapiro-Wilk test to check the data distribution, and the results of this test showed the normal distribution of the research data ( $p < 0.05$ ). Table 2 reports the results of the Shapiro-Wilk test for the variables under study.

Table 2: Shapiro-Wilk test results

Variable	Statistic	Sig.
IL-6	955.0	151.0
CRP	951.0	110.0
IL-10	944.0	100.0
BMI	981.0	732.0
Boy weight	967.0	340.0

### **Testing research hypotheses**

1- Eight weeks of resistance training alone and with EMS has no significant effect on IL-6 levels in overweight women.

The analysis of IL-6 serum levels of the participants before and after eight weeks of the intervention through the analysis of covariance test showed that the difference between the groups (control groups, resistance training and resistance training + EMS) was not statistically significant (307/30  $p = 0$ ). Based on these findings, the null hypothesis of the research is confirmable, that is, eight weeks of resistance training alone and with EMS does not have a significant effect on IL-6 levels in overweight women (Table 3).

Table 3: Covariance analysis test for IL-6 serum levels

	Degree of freedom	Mean squares	F	Sig. (p)
Corrected model	3	575.104	784.427	001.<0
	1	540.302	601.1237	001.<0
Initial values	2	301.0	232.1	307.0

Error group	28	244.0		
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Table 4 has given the effect size of changes in serum levels of IL-6 and the amount of intra-group changes (in control groups, resistance training and resistance training + EMS) of this cytokine.

Table 4: Effect size and intra-group changes of IL-6

Group	Intra-group significance	Effect size
Control	624.0	036.0
Resistance training	100.0	227.0
Resistance training + EMS	156.0	174.0

Figure 1 shows IL-6 serum levels in the control, resistance training and resistance training + EMS groups before and after the eight-week intervention.

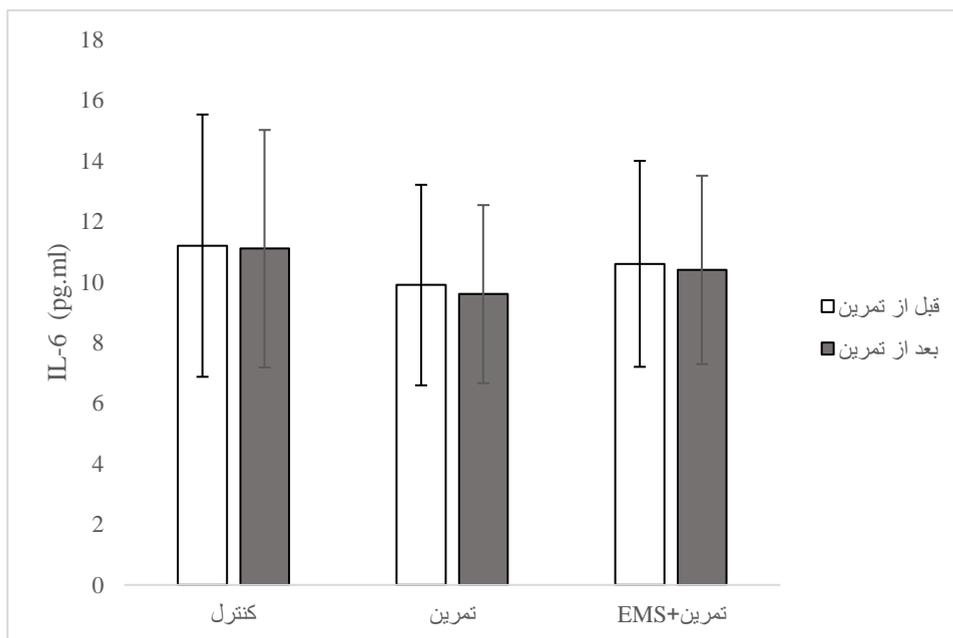


Diagram 1: IL-6 serum levels.

Figure 2 shows The percentage of changes in IL-6 serum levels after eight weeks of intervention compared to the pre-test values in the control, resistance training and resistance training + EMS groups.

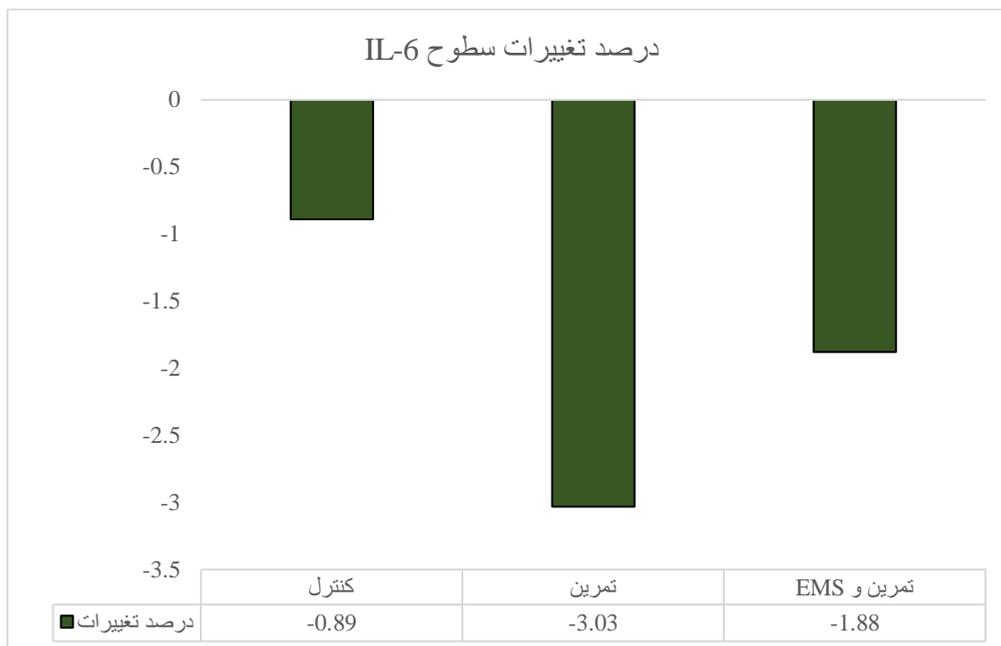


Diagram 2: percentage changes of IL-6

2- Eight weeks of resistance training alone and with EMS has no significant effect on IL-10 levels in overweight women.

The analysis of IL-10 serum levels of the participants before and after eight weeks of intervention through analysis of covariance test showed that the difference between groups (control groups, resistance training and resistance training + EMS) was not statistically significant ( $p = 0.361$ ). Based on these findings, the null hypothesis of the research is confirmable, that is, eight weeks of resistance training alone and with EMS does not have a significant effect on IL-10 levels in overweight women (Table 5).

Table 5: Covariance analysis test for IL-10 serum levels

	Degree of freedom	Mean squares	F	Sig. (p)
Corrected model	3	844.12912	072.133	001.<0
	1	011.37444	783.382	001.<0
Initial values	2	443.102	056.1	361.0
Error group	28	037.97		

Table 6 reports the effect size of changes in serum levels of IL-10 and the amount of intra-group changes (in control groups, resistance training and resistance training + EMS) of this cytokine.

Table 6: Effect size and intra-group changes of IL-10

Group	Intra-group significance	Effect size
Control	421.0	095.0
Resistance training	233.0	127.0
Resistance training + EMS	169.0	165.0

Figure 3 shows the serum levels of IL-10 in the control, resistance training and resistance training + EMS groups before and after the eight-week intervention.

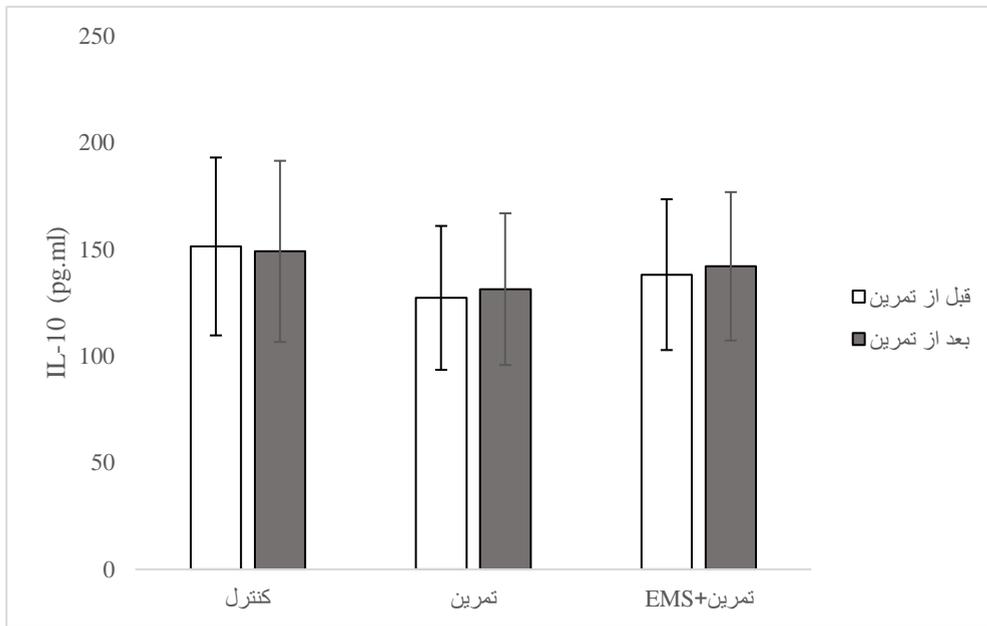


Diagram 3: IL-10 serum levels.

Figure 4 shows the percentage of changes in IL-10 serum levels after eight weeks of intervention compared to the pre-test values in the control, resistance training and resistance training + EMS groups.

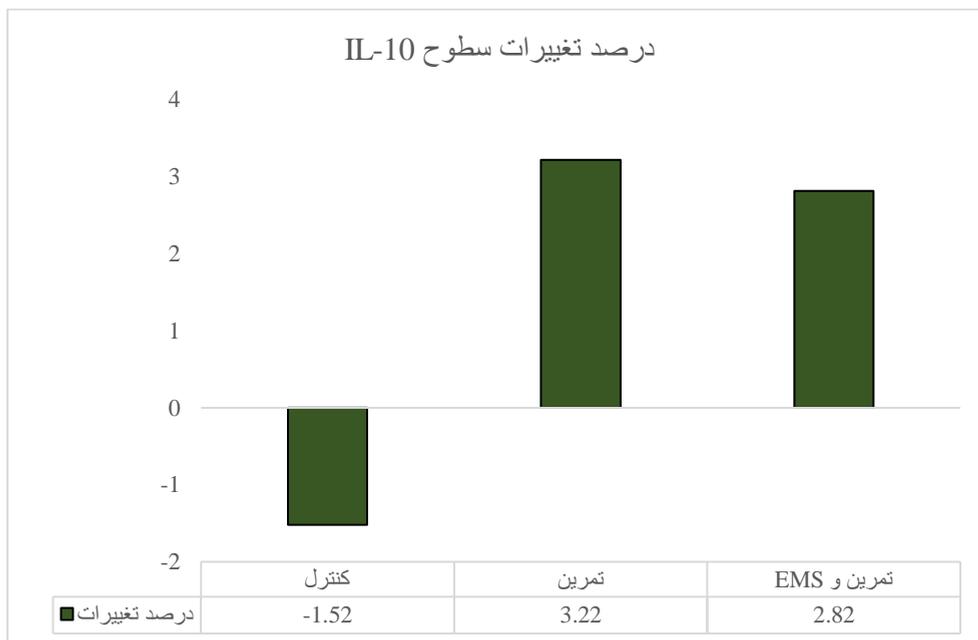


Diagram 4: percentage changes of IL-10

### Discussion

The present study investigates the effect of eight weeks of resistance training alone and with EMS on the levels of IL-6, CRP, and IL-10 on overweight women. The main finding of the present study was that eight weeks of resistance training and resistance training + EMS did not have a significant effect on the variables under study (IL-6, CRP, IL-10 levels).

In confirming the anti-inflammatory effects of exercise, researchers have shown that aerobic and resistance exercise leads to a reduction in chronic inflammation, especially in obese people with high levels of inflammatory markers (You et al., 2013). (20) According to studies, the anti-inflammatory effects of exercise are applied through various mechanisms such as reducing visceral fat mass, reducing the expression of Toll-like receptors (TLRs) on monocytes and macrophages, inhibiting the infiltration of monocytes and macrophages into fat tissue and changing the phenotype. Macrophages in fat tissue from inflammatory to anti-inflammatory (Gleason et al., 2011). (21) Some researchers also attribute the anti-inflammatory effects of exercise to the effect of exercise on muscle tissue to produce anti-inflammatory myokines secreted from skeletal muscles, Fat tissue hypoxia, reducing local inflammation of fat tissue. Its effect on endothelial cells is to reduce leukocyte adhesion and systemic production of cytokines, and to reduce the immune system the number of pro-inflammatory cells and reduce production of Pro-inflammatory cytokines per cell (You et al., 2013). (20)

Despite the anti-inflammatory effects reported for sports training, the present study showed that eight weeks of resistance training alone and with EMS did not have a significant effect on IL-6 and IL-10 levels. In line with the results of the

present study, the researchers showed in a research that eight weeks of intense intermittent exercise (three sessions per week) in the form of pedaling did not have a significant effect on IL-6 and CRP levels in type 2 diabetic patients. They considered the lack of significant change in the levels of inflammatory factors (including IL-6 and CRP) associated with the short period of the exercise program (Madsen et al., 2015). (22) In the present study, the exercise program was implemented for eight weeks.

In another research, Salamat et al. (2016) confirmed the current findings and stated that despite the reduction of IL-1 $\beta$  and IL-6 levels through aerobic and combined (resistance-endurance) training, resistance training could not have a significant effect on IL-1 $\beta$  and IL-6 levels. The researchers concluded that aerobic training is more effective in modulating and reducing inflammation compared to other sports training (Salam et al., 2016). (23)

In a study, Nakhazri et al. (2018) investigated the effect of eight weeks of combined exercise with aerobic dominance and coenzyme Q10 supplementation on serum levels of IL-10 and TNF- $\alpha$  in the patients with multiple sclerosis. In line with the study, they showed that IL-10 and TNF- $\alpha$  levels did not change significantly in all three experimental groups compared to the control group (Nakhzari et al., 2018). (24) Despite the mentioned cases, Farinha et al. (2015) showed, contrary to the present findings, that 12 weeks of aerobic exercise in women with metabolic syndrome cause a significant decrease in the IL-6 and TNF- $\alpha$  levels and an increase in the levels of IL-10 as an anti-inflammatory cytokine. These changes in the levels of cytokines are associated with a decrease in body fat percentage and insulin resistance (Farinaha et al., 2015). (25) Accordingly, the contradiction in the present findings with the above study is attributable to the lack of change in body composition (reduction in body fat percentage or increase in muscle mass) of the subjects of the present study following an eight-week intervention. In confirmation of the importance of changes in body fat mass on changes in the levels of inflammatory factors, it has been reported that IL-6 gene expression is associated with the amount of fat mass. Several studies have shown a positive correlation between the amount of body fat mass and body mass index (BMI) with IL-6 plasma levels (Chodak and Viccek, 2006). (26) Thus, in order to change significantly the levels of cytokines examined in the present study, the duration and intensity of the exercise program should be such that can lead to a significant reduction in body fat mass of subjects.

It seems that the duration of the exercise period also affects the observed changes in the levels of inflammatory mediators. In confirmation of these statements, Segurian et al. (2011) in a research on postmenopausal women showed that 12 weeks of aerobic exercise and aerobic exercise + omega-3 supplementation had no effect on IL-6 levels. However, they showed a significant decrease in IL-6 levels in the groups of omega supplementation, aerobic exercise and aerobic exercise + omega 3 supplementation after 24 weeks of intervention (Tartibian et al., 2011). (27) These results indicate that that by increasing the duration of the exercise period, significant changes can be made in the inflammatory conditions. Of course, the lack of effect of sports training and EMS in the present study is attributable to the low frequency of training (two sessions per week) in addition to the short duration of the intervention (eight weeks).

## Conclusion

Our results showed that eight weeks of resistance training alone or in combination with EMS (two sessions per week) had no significant effect on the serum levels of inflammatory (IL-6) and anti-inflammatory (IL-10) markers in healthy overweight women. The lack of change in the levels of inflammatory and anti-inflammatory markers in the present study is partially attributable to the lack of effect of EMS on the subjects' body composition (percentage of fat and muscle mass) as well as the low frequency of EMS. According to the present findings, in order to increase the effectiveness of sports training and EMS, we can recommend to increase the duration of intervention and their frequency for overweight and obese people. The future studies should deal with the effect of the number of different training sessions and EMS on the present variables.

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