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# Comparison of the Effects of Total Body Resistance Exercise and Traditional Resistance Training on the Immune System Biomarker in Inactive Obese Women

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## Abstract

**Objective:** This study aimed to compare the effect of eight weeks of traditional resistance training and total body resistance exercise (TRX) on the immune system of obese sedentary women.

**Materials and Methods:** In this quasi-experimental study, 28 obese women with a mean body mass index (BMI) of 30.39± 4.65 (kg/m$^{2}$) were randomly divided into three groups of TRX training (N= 9), traditional resistance training (N= 9) and control (N= 10). The experimental groups performed exercise training in three sessions per week for 8 weeks. Blood samples were taken from all subjects 48 hours before and after the last training session and were used for neutrophil, lymphocyte, monocyte, basophil, and eosinophil analysis. Data were statistically analyzed using dependent T-test and one-way analysis of variance (ANOVA) by SPSS software, the significance level was $P< 0.05$.

**Results:** The results of the study did not show significant differences within and between groups in the variables of the immune system (neutrophils, lymphocytes, monocytes, eosinophils) ($P< 0.05$). Also, the results of ANOVA showed a significant difference in basophil count only between the three groups ($P= 0.006$).

**Conclusion:** According to the results of the study, no significant difference in immune system indices was observed between the groups. Therefore, performing traditional resistance exercises and moderate-intensity TRX has no significant effect on the immune system of sedentary obese women.

**Keywords**: Traditional resistance, TRX, Immune system, Women, Obese

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## Introduction

Obesity is associated with high blood pressure, hypercholesterolemia, heart disease and tumors, but in addition, obesity is associated with impaired immune function, leading to increased infections and increased risk of ulcers. A number of studies have reported a decrease in antibody levels in obese patients. Animal studies have also shown that obesity may impair the immune response. Thus, obesity leads to several complications such as inflammation and involvement of the immune system, which can be exacerbated after a period of exercise (1).

Different aspects of physical activity have different effects on the components of this system, and identifying these effects broadens our insight into understanding and interpreting the body's physiological mechanisms and biological reactions (1,2)**.**

Physical activity, depending on its type, duration and intensity, has different effects on the human immune system, for example; light, continuous and regular exercise increases the level of activity of the immune system, as well as increases the body's resistance to infections such as infections of the upper respiratory tract. Intense exercise, however, also affects the immune system. It significantly reduces the body's resistance to such infections (2) and also causes many changes in the body (3). Regarding the effect of exercise on the cells of the immune system, excessive exercise pressure causes dysfunction of the endocrine glands, so that the Thyroxine and Testosterone hormones in the blood decrease and the Cortisol increases. It also suppresses the function of the immune system, which suppresses a line of defense against the invasion of bacteria, viruses, and special cells (lymphocytes, granulocytes, macrophages, and antibodies), as a serious consequence of overtraining (4).

Much research has been done on the effects of strenuous exercise. Dastbarhagh and et al. studied the effects of intense intermittent exercise (hypoxia 14O2% and normoxia 21O2%) on leukocytes and blood cells in response to debilitating activity and they found that strenuous exercise increased the power of red blood cells and could reduce the risk of thrombosis in response to strenuous exercise (5).

Yadegari et al. also studied the effect of progressive and intense periodic aerobic activity on the number of leukocytes and blood platelets in non-athlete men. In this study, they counted the number of leukocytes, neutrophils, lymphocytes, monocytes and platelets before, immediately and two hours after the intense physical activity. They found that progressive and intense periodic aerobic activity can significantly increase the number of immune system cells and blood circulation, and there is no significant difference between these two types of activity in the amount of stimulation of immune system cells (6).

To perform different types of resistance exercises, various devices have been designed and invented, which are generally divided into three groups: resistance exercises with free weights, resistance exercises with machines, and resistance exercises with bodyweight (7,8)**.**

Traditional resistance training is one of the resistance training types which is common among professional bodybuilders and due to the movement pattern implemented in it, is less generalizable to functional sports activities (9). Unstable training is a relative innovation in resistance training that allows people to train with multiple upper and lower body training positions. These exercises simultaneously challenge joint strength, balance, and stability. The TRX suspension is a unique training method that allows you to use your body weight as resistance with two bands and handles (10). The most important feature of this device, in addition to focusing on the target muscle, is the imbalance, in which the athlete has to use the muscles of the central part of the body to maintain balance (9,11). Unlike traditional resistance training exercises where stress and strain are applied to one muscle group, TRX allows the use of a large number of muscle groups simultaneously as well as with a wider range of multi-stage movements (10)**.**

Among resistance activities and high-intensity exercise, TRX is a component of strength training. But strength training can affect the immune system, especially in obese people, and weaken their immune system, which can be exacerbated after a period of exercise. On the other hand, adaptation created by regular exercise can moderate this event. Therefore, selecting multiple training patterns is a serious challenge for coaches and sports science professionals.

Therefore, according to the researchers' views on reducing the ability of the immune system due to the performance of this group of heavy exercise and also due to different findings and opinions in this regard, more detailed research is needed to determine the exact mechanism and effect of various models.

This study aimed to compare the effect of eight weeks of traditional resistance training and TRX on immune system biomarkers (neutrophils, lymphocytes, monocytes, basophils, eosinophils) in obese inactive women.

## Materials and Methods

This quasi-experimental study consisted of three groups. Obese and non-athletic women who volunteered to participate in this study and then completed the informed consent form. They then completed a medical history and physical activity questionnaire that included questions about personal characteristics, health, smoking, and physical activity history. Then their height and weight were measured and their body mass index (BMI) was calculated (weight in kilograms in height in meters).

Out of 167 volunteers who were eligible to participate in this study, 45 were selected by random sampling with replacement. The subjects were not athletes and ranged in age from 18 to 25 years. Their BMI was between 30 and 33 kg* m². In addition, they had no history of smoking or allergies and had not taken any medication for at least 2 weeks before the start of the study period. Subjects were also not allowed to take any medication during the study period and followed their normal diet. Then, all subjects were divided into three groups: TRX, traditional resistance, and control group, with 15 subjects in each group, using simple random sampling. During the research protocol, 17 subjects were excluded from the test due to illness or personal reasons. Therefore, the research protocol ended with 9 people in the traditional resistance group, 9 people in the TRX group and 10 people in the control group.

## Exercise protocol

The subjects of the experimental groups first participated in 2 orientation sessions to get acquainted with the training program and how to perform it. The main purpose of these sessions was to acquaint the subjects with different resistance and TRX activities using the device. The exercises were based on TRX weights and straps, as well as their familiarity with performing a maximum repetition test (RM-1). The Brzycki formula was used to calculate a maximum repetition.

Weight÷ [1.0278- (0.0278× Number of repetitions)]

The training program was performed for the experimental group for 8 weeks, 3 sessions per week and 90 minutes per session. Each training session included 15 minutes of warm-up, 65 minutes of specific exercises, and 10 minutes of stretching to cool down. The specialized program of the TRX training group also consisted of 10 movements (Chest Press, Suspended Lunge (both legs), 2-Arm Row, Squat, YTW, Single Leg Stiff Leg Dead lift (both legs), Triceps Extension, Hamstring Curl, Plank, Isometric Side Hold with Pallof Press) (Table 1).

The movements in the traditional resistance training protocol were similar to each in the TRX training protocol (Table 2).

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**Table 1. List of TRX exercises accompanied by performance descriptions plus prime movers involved for each exercise.**

| TRX exercise | Description | Prime mover |
|---|---|---|
| **Chest Press** | Position body facing away from anchor point. Grip TRX handles and angle the body. Flex the elbows bringing body to chest level. Extend elbows, returning to starting position. | Pectoralis major Anterior deltoids Triceps |
| **Suspended lunge (both legs)** | Place one foot in both TRX stirrups so strap it against the dorsal side of the foot. Step away from anchor point. Flex weight-bearing knee while keeping it behind the toe. Extend leg back to starting position | Quadriceps Hamstrings Gluteals |
| **2-Arm row** | Body facing the anchor point; grasp both handles with a closed pronated grip. Create the appropriate angle keeping the body in a straight line. Arms start extended. Pull body to chest level and return to fully extended starting position. | Rhomboids Trapezius Latissimus Dorsi Deltoids |
| **Squat** | Grasp both handles facing the anchor point with hands in neutral grip. Flex the knees until you reach parallel to the floor keeping the knees behind the toes while using arms as balance support only. Extend back to starting position. | Quadriceps Hamstrings Gluteals |
| **YTW** | Facing anchor point, grasp both handles. Arms start and remain fully extended throughout all three movements. After each movement (letter) return to starting position. Body is slightly angled. Raise arms above head in a Y position. Move arms laterally to make a T. Pull arms down and through to hips to form a W. | Deltoids Trapezius Rhomboids |
| **Single leg stiff leg dead lift (both legs)** | Place the dorsal side of one foot into both stirrups. Step away from anchor point. Hinge at the hips keeping back flat and leg fixed with a slight bend. Lower torso towards ground. Slowly come back to standing position | Gluteals Hamstrings |
| **Triceps extension** | Facing away from anchor point, grasp both handles with arms fully extended above head. Body should be in a straight line with both elbows in aligned with the shoulders. Flex the elbows to 90 degrees and then extend back to the starting extended position. | Triceps |
| **Hamstring curl** | Lay on floor in supine position. Place each heel in a stirrup with bottom of foot on handles. Lift and keep the hips in a bridge position while simultaneously bringing the heels to the glutes and back with an in and out motion. | Hamstrings |
| **Plank** | Lay on floor in a prone position with elbows supported on ground with dorsal side of feet placed in TRX stirrups. Keep feet, hips and back in alignment elevated from floor. Hold for designated amount of time. | Rectus abdominis Transverse abdominis Obliques Erector Spinae Multifidus |
| **Isometric side hold with pall of press** | Stand lateral to anchor point with TRX bands extended out. Hold both handles in center of chest. Angle body away from anchor point. Pulse the arms up into complete extension and down holding the body in an aligned isometric position. | Obliques Quadratus Lumborum |

Considering that the exercises of the experimental groups were in 3 sets and the range of 8 to 12 repetitions and the rest between each set was 1 minute, the rest between movements was 3 minutes; these exercises were performed under the principle of overload. In this way, traditional resistance training was performed in the first two weeks of training with an intensity of 65% of a maximum repetition; then, every two weeks, 5% of maximum repetition intensity was increased, so that the training intensity reached 80% of a maximum repetition in the eighth week. The TRX group also had a magnitude of 65 to 80% of a maximum repetition on the Borg Scale of 20 ranks, i.e. in the range of pressure perception of 14 to 17, which was considered an increased unit for overloading every two weeks.

To determine the amount of exercise pressure, individuals were introduced to the Borg scale (standards 6 to 19) and its range before beginning the exercise program.

For example, the range of perception of exercise pressure from 13-17 on this scale is equal to the training pressure of 80-45% of the training load. In addition, the intensity of exercise was controlled using a heart rate monitor. The maximum heart rate was calculated using the method of Tanaka et al. Equation (HRmax= 208− (0.7× age)). Percentage of heart rate was calculated using

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reserve heart rate (HRR method: Target HR (THR)= [(HRmax− HRrest)× % Intensity]− HRrest). Subjects were asked to measure and report their resting heart rate each morning. The equations and instructions implemented in this method were controlled using ACSM instructions (12). Due to the development of neuromuscular adaptation and increased muscle strength, once every two weeks, a maximum repetition of the subjects was calculated and the intensity of the training program was rewritten based on a new maximum repetition (13).

## Method of Measuring Research Variables

To measure biochemical variables, subjects were first asked to abstain from any exercise 2 days before the experiment and to maintain a normal diet. Then blood sampling (5 ml of blood after 12 hours of fasting from the left antibiotic vein in the sitting position and at rest) was performed at 8 am in the experimental and control groups. After this stage, the experimental groups performed the traditional resistance training program and TRX for 8 weeks. Also, after 8 weeks of resistance training and TRX and 48 hours after the end of the last training session and observing 12 hours of fasting, the second stage of blood sampling was performed from the experimental and control groups as in the first stage. Immediately after blood sampling, the serums were stored in the refrigerator at 20-24 °C until the day of the experiment. CBC of serum was measured by cell counter method according to the instructions of Japanese manufacturer with K2 EDTA anticoagulants and with a sensitivity of CV< 3 to determine the number of lymphocytes, neutrophils, monocytes, eosinophils and basophils. Leukocyte subclasses were calculated by

**Table 2. List of RT exercises accompanied by performance descriptions plus prime movers involved for each exercise.**

| Traditional exercises | Description | Prime mover |
|---|---|---|
| **Bench press** | Lay supine on a bench, grasp bar with both hands shoulder width apart, lower to chest and extend back up. | Pectoralis major<br>Anterior deltoids<br>Triceps |
| **Lunge (both legs)** | Step forward with one leg. Lower body down. Thigh and knee make a 90 degree angle keeping front knee behind toe. Rise back up to starting position and switch legs and repeat process. | Quadriceps Hamstrings Gluteals |
| **Seated row** | Seated, hold handles in a closed pronated grip. Using arms only, pull bar back to chest, and extend back to original position | Rhomboids Trapezius Latissimus Dorsi Deltoids |
| **Squat** | Place a barbell across the upper back. While keeping the back straight and, flex hips and knees until thighs are parallel to ground and extend back to starting position. | Quadriceps Hamstrings Gluteals |
| **YTW** | Lay prone on bench. Arms start and remain fully extended throughout all three movements. After each movement (letter) return to starting position. Raise arms in front of head to body level in a Y position. Move arms laterally to make a T until level with body. Extend arms back until level with hips forming a W. | Deltoids Trapezius Rhomboids |
| **Single leg stiff leg dead lift (both legs)** | Stand on one foot and hinge at the hips keeping back flat and leg fixed with a slight bend. Lower torso towards ground. Slowly come back to standing completely upright. | Gluteals Hamstrings |
| **Triceps** | Extension Grab the head of a DB with both hands. Lift DB above the head and lower behind the head keeping elbows close to ears. | Triceps |
| **Hamstring curl** | Lay prone on machine with footpad across the ankles; bend the knees bringing heels to glutes. Return to starting position. | Hamstrings |
| **Plank** | Lay on floor in a prone position with elbows supported on ground. Keep feet, hips and back in alignment elevated from floor. Hold for designated amount of time. | Rectus abdominis Transverse abdominis Obliques Erector Spinae multifidus |
| **Isometric side hold** | In the Roman chair, position body at an angle in the chair with one hip lying against pad. Hold the body in an aligned isometric position with weight in hand over edge of chair. Hold for designated amount of time. | Obliques Quadratus Lumborum |

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multiplying the observed ratio of each of them in the flow cytometer by the total number of leukocytes per unit volume. In the flow cytometry method, non-stained blood samples were passed through a flow cytometer calibrated according to the standard protocol of the manufacturer and the ratio of each type of leukocyte subclass was determined.

## Statistical method

The natural distribution of data was assessed using the Kolmogorov-Smirnov test. Also, one-way analysis of variance (ANOVA) was used to examine the differences between the groups and the dependent T-test was used to examine the differences within the group. Statistical operations were performed using SPSS 22 software and a significant level was considered ($P$< 0.05).

## Ethical considerations

This quasi-experimental study consisted of three groups, which was approved by the Ethics and Research Committee of Qom University (No. IR.QOM.REC.1399.014) and was conducted according to the principles set out in the Helsinki Declaration.

## Results

The general characteristics of the subjects are presented in Table 3.

Based on the data in table 3, there was no significant difference in BMI, age, height, and weight of the subjects at the beginning ($P$< 0.05). In this study, the results of the Kolmogorov-Smirnov test showed that all variables have a normal distribution.

The results showed that according to the values of significance level in paired T-test to compare pre-test and post-test, there are significant differences in research variables in basophil rate in TRX and control groups**.** As in the TRX group, the basophil content increased by 2.72% and in the control group decreased by 0.91% but for the traditional resistance group, this was not significantly different from the level of ($P$= 0.40) more than 0.05% (Table 4).

There was also no significant difference between the mean of lymphocytes, neutrophils, monocytes and eosinophils of sedentary women before and after the control,

**Table 3. General characteristics of the subjects (mean± standard deviation)**

| Variables | Control (n=10)<br>Mean (±SD) | TRX exercise (n=9)<br>Mean (±SD) | Traditional resistance (n=9)<br>Mean (±SD) | *P* |
|---|---|---|---|---|
| **Age (year)** | 20.88 (±1.83) | 21.22 (±1.39) | 20.88 (±1.92) | 0.851 |
| **Height (cm)** | 160.54 (±4.82) | 160.72 (±3.74) | 158.2 (±2.53) | 0.79 |
| **Weight (Kg)** | 77.34 (±13.60) | 79.25 (±12.25) | 75.40 (±6.61) | 0.321 |
| **Body mass index (kg/ m$^2$)** | 30.21 (±4.89) | 30.85 (±5.48) | 30.12 (±2.31) | 0.142 |

**Table 4. Mean changes and standard deviation of research variables in experimental and control groups before and after eight weeks**

| Variables | Groups | Before test<br>mean (±SD) | After test<br>mean (±SD) | *P* |
|---|---|---|---|---|
| **Neutrophils** | TRX Resistance<br>Control | 58.25 (±8.75) | 58.25(± 8.75) | 0.68 |
| | | 55.04 (±0.832) | 56.27 (±7.81) | 0.62 |
| | | 60.11 (±6.96) | 59.53 (±7.52) | 0.51 |
| **Lymphocyte** | TRX Resistance<br>Control | 30.65 (±11.41) | 31.55 (±7.97) | 0.141 |
| | | 33.68 (±7.54) | 32.97 (±6.69) | 0.073 |
| | | 28.49 (±7.27) | 30.41 (±6.69) | 0.118 |
| **Monocyte** | TRX Resistance<br>Control | 7.60 (±1.90) | 7.56 (±0.99) | 0.94 |
| | | 8.11 (±1.62) | 7.71 (±2.70) | 0.48 |
| | | 8.97 (±2.73) | 7.27 (±2.58) | 0.18 |
| **Basophil** | TRX Resistance<br>Control | 0.22 (±0.10) | 0.82 (±0.32)† | 0.001 |
| | | 0.33 (±0.14) | 0.52 (±0.41) | 0.40 |
| | | 0.48 (±0.17) | 0.92 (±0.18) | 0.061 |
| **Eosinophil** | TRX Resistance<br>Control | 1.53 (±0.81) | 1.88 (±1.03) | 0.08 |
| | | 2.64 (±1.59) | 2.56 (±1.12) | 0.59 |
| | | 2.41 (±1.68) | 2.41 (±1.68) | 0.40 |

† Sign of significant difference with the pre-test stage ($P$<0.05)

traditional resistance and TRX groups. By using one-way analysis of variance test (ANOVA), no significant difference was observed between the groups in lymphocyte, neutrophil, monocyte and eosinophil variables and only the basophil count showed a significant difference between the three groups, so that basophil levels between TRX group with resistance Traditional (*P*= 0.006) showed a significant difference between the control and traditional resistance groups (*P*= 0.033).

## Discussion

The immune response to exercise is dual, meaning that although white blood cells increase after a session of intense exercise, they decrease 72 hours after it too so that if they decrease to baseline levels before exercise or the same levels at rest, this issue will be related to the "open window hypothesis". That is a situation in which the person is at greater risk for infections and disease (14).

The results of this study showed that safety indices including lymphocytes, monocytes, eosinophils due to traditional resistance training and TRX did not show a significant difference from pre-test to post-test. There was also no significant difference between groups in this index. But basophil level showed a significant difference between groups.

Aldemir et al. also showed that the number of neutrophils, monocytes, and lymphocytes increased during the first few hours after exercise and decreased several hours after exercise due to redistribution and replacement in the affected areas. Elevated white blood cell, neutrophil, and monocyte levels are probably in response to elevated glucocorticoids, growth hormone, and cytokines such as interleukin-6, and granulocyte-stimulating factors derived from bone marrow myeloid cells. On the other hand, there is a positive relationship between the innate immune system and stress hormones, especially cortisol. Cortisol has a suppressive effect on the immune system as well as anti-inflammatory properties. Hormones play an important role in regulating exercise-induced changes in white blood cell counts and their subtypes. Hormones such as cortisol and epinephrine have been shown to affect the distribution of white blood cells. Cortisol moves neutrophils out of the red bone marrow, but there is a delay in the increase in cortisol in the blood and neutrophils. Therefore, since cortisol release occurs 30 minutes after exercise, it does not affect the increase in white blood cell count immediately after strenuous exercise. Cortisol is responsible for increasing the second delayed phase of white blood cells, which works by increasing their release from the red bone marrow (15).

Research shows that the epinephrine hormone is responsible for increasing leukocytosis during strenuous and short-term exercise because the increase in cortisol in response to very slow activity and the increase in the number of white blood cells after exercise is related to the increase in cortisol (16). Catecholamines produced during exercise work to increase circulating white blood cells. Epinephrine also increases the number of white blood cells during exercise. The increase in white blood cell count during short-term exercise (less than an hour) is due to an increase in epinephrine. There is also a positive relationship between the amount of epinephrine and the number of white blood cells after exercise. Catecholamines and cortisol, which are target receptors in white blood cells and their subclasses, have been shown to increase white blood cell counts and their subclasses (called bone marrow) and increase their activity.

The decrease in white blood cells and their sub clinics at intervals of 48 and 72 hours after both maximal and submaximal exercise is because neutrophils, lymphocytes, and monocytes are excreted in large amounts during the recovery period under the influence of glucocorticoids. Findings of the present study on two-stage changes in increasing the number of white blood cells and their subclasses during the first day after both exercises and decreasing the number of white blood cells and its subclasses at intervals of two and three days after both exercisesare consistent with findings (14,15,17,18), while it is not consistent with findings (19,20). The researchers' findings showed an increase in leukocytes after a session of physical exercise and no return of white blood cells to resting levels. Whereas, the findings of the present study on the lack of significant differences between the maximum and minimum intensities of resistance training are consistent with the findings of Yadegari et al. (2017). They showed that one session of progressive aerobic activity and one session of intense intermittent activity in young non-athlete men increased the number of white blood cells and there was no significant difference between the two types of exercise in changing the number of white blood cells. Differences in various reports of white blood cell, neutrophil, lymphocyte, and monocyte counts in response to exercise appear to be due to factors such as the use of different exercise protocols, different training intensities, fitness levels, and mental health of subjects. Also differences in laboratory techniques used for measurements (21). Platelets and factor VIII increased significantly immediately, 2 hours, 24 hours after both exercises, and then returned to resting levels at 48 and 72 hours after exercise. Platelets and factor eight were significantly higher after maximal resistance training compared with sub-maximal training. Studies show that platelet counts increase during exercise. This increase may be related to physical activity and the release of fresh platelets from the vascular bed of the spleen, bone marrow, and other body reserves. Studies have also shown that epinephrine secretion causes strong contraction of the spleen (where one-third of the body's platelets are stored), and since epinephrine levels rise during activity, especially intense activity, this may be the reason for the increased platelet count in this study (22). This mechanism can justify platelet proliferation in exercise (34,35). Other factors such as troponin levels, ATP, blood, lactic acid pH, and blood catecholamines can be considered effective in increasing platelets after exercise (23). The set of risk factors for leukocytosis (increase in white blood cells), especially cardiovascular changes, local muscle damage, the release of catecholamines and cortisol, appear to significantly increase platelet counts after exercise. Therefore, it can be said that over time and by reducing the short-term and temporary effects of a session of maximal and sub-maximal resistance training in the present study and possibly by lowering the level of cortisol in the blood, platelet counts returned to baseline 48 and 72 hours after exercise. The increase in white blood cell counts in this study is probably due to the release of these substances from the spleen and lung arteries as well as the red bone marrow. Another possible reason for this increase is an increase in body temperature, perspiration rate, or plasma catecholamine concentrations (24). Although the results of the researches differ, researchers believe that long-term exercise suppresses the immune system and gentle, short-term exercise strengthens the immune system. According to the literature of the subject, it has been determined that physical activity is one of the effective factors in changing the work process of the defense system, which depends on the intensity, duration, exercise plan and physical condition, type of nutrition, mental and psychological factors and hormonal factors. According to the results of the study, no significant difference in immune system indices was observed between the groups. However, only in the basophil index, the difference between control and TRX groups was significant. Due to the lack of significant changes in safety indices in the two experimental groups during the 8 weeks of traditional resistance training and TRX, sedentary women can be advised that they can use both training protocols and these exercises lead to it does not weaken their immune system. In this study, the motivation and mental state of the subjects, the amount of sleep and rest, daily activity and nutrition of the subjects were not controlled, which is one of the limitations of the research, so it is recommended to control these cases in future research.

## Conclusions

In general, it can be stated that the immune system parameters, except for basophils, were not affected by eight weeks of traditional resistance training and TRX, and the change of these indicators may require a longer time than the training. In other words, it can be said that changes in the parameters of the immune system and the strengthening of these indicators may occur over a longer period of time.

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### Conflict of Interest

The authors of the article did not declare any conflict of interest.



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