



ORIGINAL ARTICLE

Comparison of the Effect of Two Different Types of HIIT on Serum Homocysteine and 25-hydroxyvitamin D Levels in Overweight Women with Low Vitamin D Status

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ABSTRACT: Vitamin D deficiency along with obesity, which is a significant health problem, can increase the risk of many diseases. Regular exercise has a favorable effect on this problem, but some people spend little or no time to benefit from these favorable effects. In this study, the effect of two different types of high intensity interval training on serum homocysteine and 25-hydroxyvitamin D levels in overweight women with low vitamin D levels was compared. This semi-experimental study was conducted on 39 overweight women with vitamin D deficiency. Subjects were randomly divided into control groups, high intensity interval resistance training, and high intensity interval running training after being selected using the available sampling method. Interval training were performed during 8 weeks and 3 sessions per week. High intensity interval resistance training was performed in three sets with an intensity of 80% of a maximum repetition and a 2.5minute rest between sets. high intensity interval running training was performed with the 12 x 1-min running bouts at 80-90% HRmax interspersed with 1-min active recovery at 50% HRmax. Data were analyzed using one-way ANOVA tests (Kruskal-Wallis for non-normal data) and paired t at a significance level of less than $P < 0.05$. After 8 weeks, the level of homocysteine in the experimental groups decreased and the level of 25-hydroxyvitamin D increased significantly ($P < 0.05$). In addition, there was no significant difference between the effect of two types of intermittent exercise on reducing homocysteine or increasing 25-hydroxyvitamin D ($P < 0.05$). It seems that both types of intense interval training (repetitive running and resistance) can have similar protective effects in obese women with vitamin D deficiency, by reducing homocysteine, improving vitamin D status, and reducing body weight.

INTRODUCTION

Obesity is recognized as a major health problem worldwide, especially in youth. Vitamin D deficiency is one of the complications of obesity [1]. Obesity is associated with atherosclerotic risk factors, including decreased blood flow, endothelial dysfunction, lipid disorders, and hyperinsulinemia. Also, the increase of

homocysteine is known as a risk factor for atherosclerosis [2].

Recently, it has been shown that hyperhomocysteinemia may be caused by smoking or alcohol consumption, improper nutrition, kidney dysfunction, or enzyme deficiency. It has been the focus of researchers in terms

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of its relationship with skeletal muscles, physical performance, and obesity [3].

Vitamin D deficiency is closely related to the disease of metabolic disorders, fat profile and unfavorable serum glycemia and homocysteine [4]. Also, an inverse relationship between the concentration of 25-hydroxyvitamin D and homocysteine has been observed in people with low vitamin D status [5,6]. Nutritional interventions, drug treatments, as well as performing various sports activities, are among the methods that each of them in turn can be very important in preventing and dealing with obesity. Sports activities are considered as one of the least dangerous methods of dealing with obesity, and various researches conducted in this field have also resulted in different results. In many studies, the effects of different types of sports activities, such as aerobic, resistance and combined exercises, have been investigated [7].

Most of the research in the field of high-intensity interval training (HIIT), of its endurance type, has been done, while high-intensity interval resistance training protocols have rarely been evaluated [8].

In observational studies from a recent meta-analysis, the inverse relationship between 25-hydroxyvitamin D and body mass index has been confirmed in diabetic and non-diabetic subjects [9]. The probability of homocysteine increase in participants with 25-hydroxyvitamin D concentrations less than 25, 25-50, 50-75 and more than 75 nmol liter⁻¹ was 0.92, 0.52, 0.34 and 0.32, respectively. and temporary improvement in vitamin D status reduces serum homocysteine concentrations and thus may potentially contribute to the primary prevention of cardiovascular disease.

High plasma homocysteine levels are a risk factor for cardiovascular diseases [10]. Cardiometabolic diseases are one of the main causes of death in the world [11]. Some studies have shown an inverse correlation between homocysteine blood levels and regular physical exercise. However, the effect of different training methods on plasma homocysteine levels is not clear [10]. Physical activity can reduce blood homocysteine levels [12]. In addition, various exercise protocols can increase the serum concentration of 25-hydroxyvitamin [13]. In addition, researchers noted that serum 25-hydroxyvitamin D concentrations increased significantly

after a high-intensity exercise program in a group of young adults, and this increase in 25-hydroxyvitamin D levels attributed to the increase in the concentration of albumin in the blood circulation [14]. serum 25-hydroxyvitamin D concentrations increased significantly after 12 weeks of a high-intensity resistance training program compared to baseline values [15].

Some researchers believe that the increase in serum 25-hydroxyvitamin D levels may be related to the significant increase in intramuscular CYP27B1 after high-intensity exercise [16]. New findings suggest that high-intensity interval training (HIIT) may be associated with increased inflammation after a short period of exercise [17].

The results of a study showed that 8 weeks of Pilates training, vitamin D intake and combined intervention were associated with increased levels of 25-hydroxyvitamin D. In addition, the combined intervention was associated with more development in this variable compared to the other two therapeutic interventions [18].

The results of the researchers' study showed that the levels of 25-hydroxy vitamin D increased significantly (10.75%) after 12 weeks [19].

Also researchers showed that the homocysteine concentration was significantly higher in the control group than in obese patients, regardless of nutritional status, eating habits, resistance to Insulin, history of certain diseases, history of drug use, genetic background, etc., were lower [20].

The results of a study showed that there was no significant non-linear relationship between waist circumference and body mass index with hyperhomocysteinemia (Hcy level >15 μmol L⁻¹) in these people. Also, in those who did not have diseases related to cardiovascular disease, abdominal obesity had a positive relationship with the risk of hyperhomocysteinemia. Therefore, people can reduce the risk of hyperhomocysteinemia by maintaining a relatively low body mass index and a normal waist circumference [21].

To date, most research has focused on endurance-type HIIT, while high-intensity interval resistance training (HIIRT) protocols have rarely been studied. Therefore, this research seeks to determine whether two different types of intense interval training have similar effects on

homocysteine and 25-hydroxyvitamin D levels in overweight women with low vitamin D status.

MATERIALS AND METHODS

This semi-experimental study was conducted using a pre-test-post-test design with a control group. The statistical population of the present study included inactive young overweight women (body mass index equal to or higher than 25 to less than 29 kg m²), with an age range of 23 to 29 years, referring to sports clubs in Babol city in 1400. Among them, 39 volunteers were selected in an accessible and purposeful manner and after meeting the necessary conditions for entry and obtaining written consent, they were randomly divided into three groups: control, intermittent running and intermittent resistance training (13 people in each group).

Entry criteria for subjects with a body mass index between 25 and 29 kg m², not having regular sports activity in the last six months, not suffering from cardiovascular diseases, high blood pressure and inflammatory diseases, having 25-hydroxyvitamin D levels less than 20 ng ml and the use of any special medicine or supplement, pregnancy was considered. Exclusion criteria were taking vitamin D or not participating in more than three sessions in sports activities. Subjects were examined by using the nutrition tracking form, the type of food they consumed, and during the entire research process, the subjects had their normal diet. The amount and duration of exposure to sunlight was not controlled, which of course is one of the limitations of the present research. (But on the other hand, the subjects were uniformed in terms of clothing). Vitamin D with a dose of 50,000 units (made by Zahrawi company, made in Iran) was taken every week with a meal by the subjects of the intervention group. While the control group had their normal activity and consumed gel capsules containing paraffin (made by Zahrawi company, made in Iran) on a weekly basis. In addition, the sample size was determined by taking into account the confidence factor of 95% and the power of 80%, taking into account the strong effect of the independent variable of exercise on the studied variables using Gpower software.

Training protocols

The schedule of each training session for the exercise and exercise + vitamin D groups included three parts: warm-up (5-10 minutes warm-up), intense interval resistance training, and 5 minutes cool-down (return to the initial state). The training load of each subject was determined based on the initial test of 5 maximum repetitions (5RM) before and during the protocol every two weeks. One week before the start of the protocol, in order to get familiar, the subjects did two sessions of resistance training similar to the main training program, with an intensity of 60% of one repetition maximum (equivalent to 15RM).

Intense intermittent resistance training was performed in three sets. The first set consisted of 6 repetitions with an intensity of 80% of one maximum repetition (equivalent to 6RM) with a 20-second rest, which was continued with the same weight lift and another 20-second rest, until reaching exhaustion (usually 2 or 3 repetitions). (This sequence was considered as a set). Then, after resting for 2.5 minutes, the subjects followed the next sets (6 + 2 or 3 repetitions).

Exercise movements included leg press for the lower body, chest press for the chest muscles, underarm cable puller for the back muscles, military press for the shoulder muscles, reverse forearm press for the arm muscles, and plank for the abdominal muscles. The intense interval running training protocol included 8 weeks of intense interval training, 3 sessions per week. Intermittent running training for each training session includes three minutes of warm-up and cooling down with an intensity of 50% of the maximum heart rate and the main exercise with 12 repetitions of one minute of running with an intensity of 80 to 90% of the maximum heart rate and one minute of active rest with an intensity of 50% of the heart rate. was the maximum [22].

In the first week, the exercises were performed with an intensity of 80% of the maximum heart rate and 6 repetitions, and in the fifth week, it reached an intensity of 90% of the maximum heart rate with 12 repetitions (with a gradual increase of 5% to the intensity of the exercise, 3 repetitions every two weeks) and until the last week continued. In addition, the reserve heart rate was controlled using a polar heart rate monitor, and the

maximum heart rate of the participants was calculated using the relationship $(220 - \text{age})$.

Blood sampling

The subjects' blood samples were collected from the brachial vein of the left arm in a sitting position after 12 hours of overnight fasting (light diet the night before blood sampling), in two phases, pre-test and post-test (48 hours after the last training session). After 15 minutes of rest, it was collected in the morning (5 cc). Then the resulting serum was frozen at -80°C and used to measure the research variables. Blood sampling was done in the luteal phase of menstruation.

Biochemical analysis

The amount of 25-hydroxyvitamin D was determined by ELISA method and commercial 25-hydroxyvitamin D kit (25-OH-VitaminD-ELIZA KIT) manufactured by Gostar Isar Antibody of Iran with a sensitivity of 2.6 ng ml^{-1} . Serum concentration of homocysteine was measured using a commercial special measurement kit by ELISA method.

Statistical analysis

The research data were described in terms of central and dispersion indicators. Also, Shapiro-Wilk and Levin tests were used to check the normality of data distribution and homogeneity of variances, respectively. In addition, paired t-tests were used to examine intra-group changes, and one-way analysis of variance (Kruskal-Wallis and U-Man-Whitney tests for non-normal data) were used to examine inter-group changes, and if there is a significant difference between groups, Tukey's post hoc test was used to determine the location of differences at the significance level of $P < 0.05$. All statistical analysis was done using SPSS version 22 software.

RESULTS

The results of the paired t-test to investigate the difference in homocysteine levels before and after 8 weeks of two types of interval training are presented in Table 1. The calculated t value and its significance at the level of $P < 0.05$ indicated a decrease in homocysteine serum levels in the intense intermittent resistance training and intense intermittent running training groups after 8 weeks, but its levels did not change significantly in the control group (Figure 1).

Table 1. The results of the t-test to investigate intra-group changes in homocysteine serum levels in the research groups

Source of changes	Intense interval resistance training	Intense interval running training	Control group
Mean differences + standard deviation	0.742 ± 0.138	0.900 ± 0.231	-0.042 ± 0.076
The value of t	19.365	14.051	-2.008
p value	*0.000	*0.000	0.068

*: sign of significant difference compared to before training

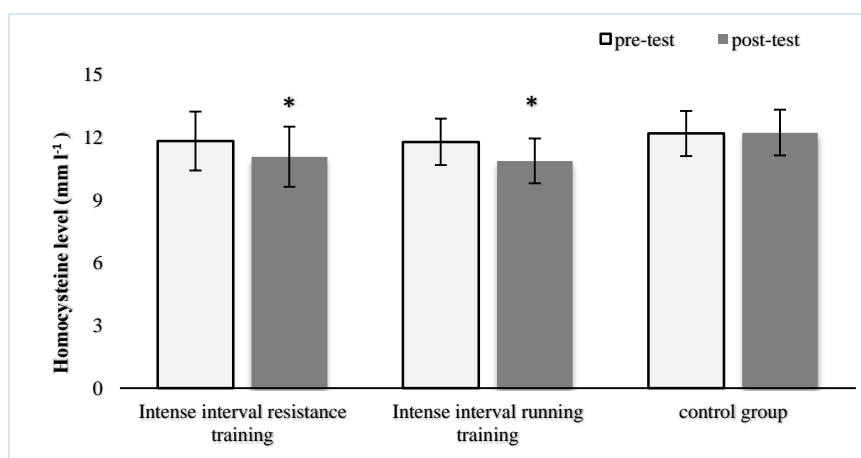


Figure 1. Comparison of homocysteine serum levels of the research groups in the post and pre-test
*: sign of significant difference compared to the pre-test

The results of Tukey's post hoc test and two-by-two comparison of homocysteine serum levels in the research groups are presented in Table 2. Based on this, after 8 weeks, the mean percentage of homocysteine changes in both intense intermittent exercise groups was

significantly higher compared to the control group. While there was no significant difference between the mean percentage of homocysteine changes between the interval training groups ($P=0.904$).

Table 2. Tukey's post hoc test results of serum homocysteine levels of different groups after the test

	control group	Intense interval resistance training	Intense interval running training
	-----	M =7.736 * $p=0.000$	M =8.003 * $p=0.000$
Average percentage of homocysteine changes	control group		M =-1.268
	Intense interval resistance training	-----	$p=0.102$
	Intense interval running training		-----

The results of the paired t-test to investigate the difference in the levels of 25-hydroxy vitamin D before and after 8 weeks of two types of periodic training are presented in Table 3. The calculated t value and its significance at the $P<0.05$ level indicated an increase in

the serum levels of 25-hydroxyvitamin D in the groups of intense intermittent resistance training and intense intermittent running training after 8 weeks, but its levels in the control group had a significant change. Not found (Figure 2).

Table 3. The results of the t-test to investigate intragroup changes in serum 25-hydroxyvitamin D levels in the research groups

Source of changes	Intense interval resistance training	Intense interval running training	Control group
Mean differences + standard deviation	-3.325±0.742	-3.416±0.813	-0.179±0.202
The value of t	-16.152	-15.148	3.194
p value	*0.000	*0.000	0.08

*: sign of significant difference compared to before training

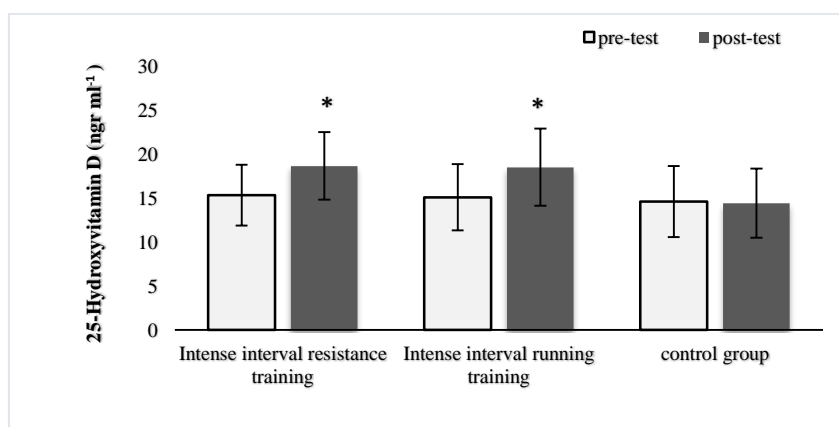


Figure 2. Comparison of serum levels of 25-hydroxy vitamin D of the research groups in the post and pre-test

*: sign of significant difference compared to the pre-test

The results of the two-by-two comparison in Table 4 indicated the existence of a significant difference between the percentage of changes in the levels of 25-

hydroxyvitamin D in the experimental groups and the control group. However, no significant difference was observed between the two training groups.

Table 4. The results of the Mann-Whitney test, the percentage of changes in 25-hydroxyvitamin D levels in different groups

		control group	Intense interval resistance training	Intense interval running training
Average percentage of homocysteine changes	Control group	-----	Z =-4.359 * p=0.000	Z =-4.359 * p=0.000
	Intense interval resistance training		-----	Z =0.026 p=0.980
	Intense interval running training			-----

DISCUSSION

Based on the findings of this research, both types of exercise were associated with a decrease in homocysteine levels and an increase in 25-hydroxyvitamin D. Previous studies showed that the increase in fat tissue is related to the increase in homocysteine concentration [23]. Therefore, it seems that the interventions of the present research may improve the increase in homocysteine levels associated with overweight and vitamin D deficiency, by reducing weight and increasing serum 25-hydroxyvitamin D levels. Although in the comparison of the means in the post-test, only a significant difference was observed between the homocysteine levels of the subjects of the intense intermittent running group and the control group, but both the interventions of running and intermittent resistance training were associated with the same percentage of changes in the reduction of homocysteine levels (respectively 7.66% and 6.39%).

The results of this study are in line with some other research results. For example, in a new research, it was observed that 8 weeks of interval training with an intensity equivalent to 90-95% heart rate led to a decrease in plasma levels of homocysteine and BMI in young obese women with a body mass index of 30-35 kg m⁻² [24]. It was also reported that 12 weeks of high-intensity interval training (including 40 meters back and forth at maximum speed, 30 seconds of work and 30 seconds of rest with 4 repetitions in the first week and 10 repetitions in the twelfth week) With the reduction of homocysteine in overweight men, it was associated with a body mass index between 25 and 30 kg m² [25]. In addition, consistent with the results of the current research, In a study it was shown that plasma levels of homocysteine in inactive overweight men with a body

mass index of 31.6 kg m², following eight weeks of low-volume high-intensity interval training including 10 intervals of 1 minute with an intensity of 85 to 90% of the maximum heart rate and 1 minute of recovery with an intensity of 50 to 55% of the maximum heart rate) decreased significantly [26]. The researchers showed that the effect of both types of aerobic and anaerobic exercise in reducing homocysteine in sedentary young men with normal body mass index was the same [10]. It has been suggested before that homocysteine levels are affected by the two factors of exercise intensity and duration, and increasing the duration or intensity of exercise can be associated with stronger effects in reducing homocysteine [26]. Although the results of the discussed studies indicate the effect of various sports interventions on the negative regulation of homocysteine, but there were no related studies, especially in overweight conditions combined with abnormal vitamin D status, which is one of the limitations of the present study. In conditions of overweight or obesity, various reasons such as low exposure of obese people to ultraviolet B (UVB) radiation and dilution of vitamin D can be considered as mechanisms of low levels of 25-hydroxyvitamin D in these people [23]. In the results of the present research, an increase in the levels of 25-hydroxy vitamin D was observed following both intensive intermittent running and resistance training interventions. Although the levels of 25-hydroxy vitamin D did not increase to the normal level following the research interventions, both types of resistance training and intense intermittent running were associated with an increase in the levels of 25-hydroxy vitamin D by 22.34% and 23.19%, respectively. These results point to the same effect of intense interval training interventions with different methods of repeated

running and resistance training on the vitamin D status of the subjects of the present study. These results are similar to the findings of other researchers. In this regard, it was reported that 12 weeks of aerobic exercise, including cycling on an ergometer, for 40 minutes and three times a week with an intensity of 70% of the maximum heart rate for 5 minutes and active recovery with an intensity of 50% of the maximum Heart rate for 5 consecutive minutes led to a significant increase (10.75%) of 25-hydroxy vitamin D levels in 30 to 40-year-old obese women with myalgia [19]. Also, in the findings of other researchers, the increase in the levels of 25-hydroxyvitamin D after eight weeks of intermittent aerobic exercise including 30-45 minutes of intermittent aerobic exercise with an intensity of 50-70% and 40-45% of the maximum heart rate, in the form of 2 minutes of exercise and 2 minutes of active rest, three sessions per week in overweight women with an age range of 30-50 years and an average body mass index of $31.71 \pm 2.59 \text{ kg m}^2$ [27] or after 8 weeks of Pilates training , with an intensity of 50 to 70% of the reserve heart rate, was observed in men with lower than normal levels of vitamin D [18]. But researchers showed the effect of 12 weeks of resistance training (3 sessions per week) on the levels of 25-hydroxyvitamin D in elderly men and women (50 to 80 years old) with a body mass index of 26.3 kg m^2 , that resistance training was associated with a decrease in fat mass, an increase in net body mass and muscle strength of the whole body, but it did not have a significant effect on the levels of 25-hydroxyvitamin D of these subjects, the reason for this difference may be related to the age of the subjects [28]. Physical activity can increase the lipolytic process and the release of vitamin D metabolites through the release of some hormones such as insulin and beta-adrenergic hormones. In addition, all exercise protocols can increase energy consumption and expenditure through additional post-exercise oxygen consumption. For this reason, the increase in serum 25-hydroxy vitamin D level after endurance training can be related to lipolytic processes during exercise [29]. Some researchers believe that the increase in serum 25-hydroxyvitamin D levels may be related to the significant increase in intramuscular CYP27B1 after high-intensity exercise and may enhance the local metabolism of vitamin D in skeletal muscles

[16]. In previous studies, it was observed that both interventions of high-intensity intermittent aerobic training (90 seconds to 12-minute intermittent training) and high-intensity intermittent resistance training (single-set resistance training for fatigued muscles) lead to an increase in the index in parallel. Ventricular stroke volume and myocardial (cardiac) mass increased in middle-aged inactive obese or overweight men aged 30 to 50, but with the greater effect of intermittent aerobic exercise. Although high-intensity intermittent aerobic endurance exercise had a positive effect on cardiometabolic risk, especially cardiac performance, both exercise methods had a positive effect on cardiac metabolism [8].

CONCLUSIONS

Based on the findings, both types of intense interval training (repetitive and resistance running) can exert similar protective effects in obese people with vitamin D deficiency, by reducing homocysteine, which may partially improve vitamin D status and also related to weight loss.

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ETHICAL CONSIDERATION

This study has been approved by the ethics committee in the research of Islamic Azad University of Sari branch with the code IR.IAU.SARI.REC.1402.245.

Conflict of interest

There is no conflict of interest between the authors.

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