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The effect of a moderate intensity resistance training course with garlic supplementation on the lipid profile in overweight women

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Abstract

Background: Obesity carries great risks related to health, such as the development and progression of chronic inflammation and obvious metabolic disorders. This study aimed to examine the effect of eight weeks of moderate-intensity resistance training with garlic supplementation on the lipid profile in overweight women.

Methods: The present research was a semi-experimental study and the participants were 60 overweight women from Gachsaran City with (body mass index: 28.45±6.72) who were randomly divided into four groups (n = 15): supplemental exercise, placebo exercise, garlic supplemental, and placebo. Two training groups performed moderate-intensity resistance training for eight weeks and three sessions per week. One day before the start of training and 48 hours after the last training session, blood samples were collected to measure total cholesterol (TC), triglyceride (TG), LDL-C, and HDL-C variables. Data were analyzed by one-way ANOVA and LSD post-hoc test.

Results: Findings showed that there is a significant decrease in the serum levels of TC, TG, and LDL-C and a significant increase in HDL-C (P-Value < 0.001) serum levels in three groups of supplemental exercise, placebo exercise, and garlic supplemental compared to the placebo group.

Conclusion: Moderate-intensity resistance training with and without consuming garlic improves overweight women's lipid profile, and when exercise is accompanied by consuming garlic, the amount of these changes and improving the lipid profile is greater.

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Highlights

What is current knowledge?

- Obesity is a direct and indirect cause of serious diseases such as hypertension, heart disease, cancer, and diabetes.

What is new here?

- Although performing a course of moderate-intensity resistance training without garlic consumption can improve lipid profiles, if the training is accompanied by garlic supplementation, the changes in the levels of TC, TG, LDL-C, and HDL-C can be more significant.

Introduction

Today's lifestyle has been mixed with increased consumption of high-fat foods and reduced physical activity, which is associated with metabolic complications such as obesity and metabolic syndrome (1). Despite global warnings and increasing awareness of the complications and harms of obesity, we are witnessing a significant increase in the prevalence of obesity and overweight in the world (2). The process of increasing fat accumulation increases the infiltration of macrophages and subsequently chronic inflammation, which plays an essential role in the onset of insulin resistance, one of the important components of metabolic syndrome (3). Thus, excessive fat accumulation is associated with a decrease in the sensitivity of glucose absorption due to insulin stimulation, a decrease in the re-esterification of free fatty acids, and an increase in lipolysis resistance to the inhibitory effect of insulin in abdominal and peripheral adipose tissue (4). The incidence of obesity and increase in body fat levels is one of the major health problems because it leads to an increase in obesity-related diseases, such as hypertension, cardiovascular diseases, and diabetes. Therefore, dealing with obesity and controlling body fat levels is one of the measures to control or prevent cardiovascular and metabolic diseases (5).

Today, cardiovascular diseases are known as one of the main causes of death in the world (6). Various factors such as a sedentary and inappropriate lifestyle, high-fat diet, and lack of sufficient exercise play a part in the occurrence of these diseases (7). One of the most important risk factors for cardiovascular diseases is an increase in low-density lipoprotein (LDL-C), total cholesterol (TC), triglyceride (TG), and a decrease in high-density lipoprotein (HDL-C) (8). However, research shows that some people with normal levels of LDL-C and HDL-C may also suffer from cardiovascular diseases. Therefore, it is very important to pay attention to the markers that can predict the risk of cardiovascular diseases with greater accuracy and sensitivity (9). To deal with obesity, it is recommended to reduce calorie intake and increase calorie consumption. To reduce energy intake, appetite suppressants are mainly prescribed, however, these drugs may not be very effective and their use may be associated with some side effects. Therefore, the approach of increasing energy consumption has received more attention (10). Despite the advancement of technology in producing new chemicals to control obesity, the prevalence and complications associated with obesity are still increasing, which indicates the urgent need for new effective treatment options (11).

The use of complementary and alternative medical methods in healthcare is growing at a fast pace. Examples of complementary and alternative treatments for weight loss include nutritional supplementation, including medicinal plants and their effective ingredients, acupuncture, and homeopathy (12). Research shows that garlic has multiple biological effects including cardiovascular protection (13), anti-inflammatory properties (14), nerve cell protection, and protection against mitochondrial damage (15). In fact, by inhibiting nuclear factor kappa (NF-KB), garlic can inhibit the transcription of IL-6, TNF-α, IL-12, and IL-8 cytokines, which are the most important factors of pro-inflammatory reactions (16). The results of some research have shown that in rats fed with high-calorie food along with various garlic products consumption (garlic decoction, fresh garlic, and aqueous garlic extract), the levels of TC, LDL-C, and TG significantly decrease following the consumption of garlic products (17,18). Many studies have examined the impact of exercising on fat levels and related factors (19,20). As a body stressor, sports exercises can create a negative energy balance and consequently activate the mechanisms involved in energy regulation and balance. Nevertheless, most of the research conducted has been related to endurance training, while little research has been conducted on the effect of resistance training on the fat profile. Alternatively, it has been suggested that resistance training can be related to the individuals' reduced cardiovascular problems (21) so that this type of training has become increasingly more popular, especially among the youth.

However, the effect of resistance training with different intensities on the factors related to cardiovascular diseases is not clearly known and the results of studies in this regard are inconsistent. For example, in Benz et al.'s study (2003), it was shown that resistance training caused a significant reduction in the waist-to-hip ratio, but there was no change in LDL-C and TG levels after 10 weeks of training (22). On the other hand, in Najafi et al.'s study (2020), eight weeks of resistance training alone and with vitamin D supplementation caused a significant change in the lipid profile of overweight women (20). In their study, Cunha et al. (2019) investigated the difference between high-intensity and low-intensity resistance training protocols on lipid profiles; the results showed that high-intensity resistance training has a greater effect on improving the lipid profile of elderly women (23). Also, in another study, Saydi et al. (2018) examined the effects of resistance training with high and moderate intensities on lipid profile, glycemic index, and FGF21 in type 2 diabetic patients. The results showed that glycosylated hemoglobin, HDL-C, LDL-C, fat percentage, and muscle strength in the experimental groups improved significantly compared to the control group (24). Considering cardiovascular diseases and their relation to training variables, especially the intensity of training and the condition of individuals, the difference

in the results of the studies may be due to the intensity of training, gender, age, and type of the subjects. A decrease in growth rate and increase in the population of middle-aged people in the coming years, on the one hand, and an increase in the sedentary lifestyle, followed by an increase in the risk of cardiovascular and metabolic diseases indicates the need for research on the benefits of an active lifestyle in the overweight population. Since most research has focused on endurance training, this study is one of the few researches that investigated the simultaneous effect of garlic supplementation and resistance training on lipid profile. Considering the importance of designing training protocols to achieve better results and avoid wasting time as well as the lack of research on the effect of resistance training with moderate intensity and the simultaneous use of herbal medicines on the serum levels of TC, TG, LDL-C, and HDL-C in overweight women, as well as the contradictions in this regard, the present study aimed to investigate the effect of eight weeks of moderate intensity resistance training with garlic supplementation on the lipid profile in overweight women in Gachsaran, Iran.

Methods

The research method of this applied study was semi-experimental with four groups, for which the medical ethics permit for the project implementation was obtained from the Islamic Azad University of Gachsaran with the code IR.GACHSARAN.1402.278. The statistical population comprised overweight women in Gachsaran, Iran. The samples of the study were selected through simple randomization of this statistical population with average weight 73.0±67.59 kg and height 161.0±83.05 cm in a purposeful manner according to the inclusion criteria. At first, a call notice was posted to identify and invite overweight women in Gachsaran who wished to perform sports activities to adjust their weight and improve their physiological condition. Then, the individuals were invited for preliminary evaluations, and based on the results of the general health questionnaire and the physician's examination of clinical symptoms, 60 participants with physical and mental health were selected and randomly assigned into four groups, including training + garlic supplement, placebo training, garlic supplement, and placebo.

The main criteria for the inclusion of participants in the current research were not suffering from cardiovascular, skeletal-muscular, and metabolic diseases, not participating in regular sports activities more than once a week in the last six months, and not smoking. In the course of the research, the subjects in the experimental groups carried out a training protocol, and the subjects in the placebo and garlic supplement groups carried out their daily activities without intervention. The briefing session initially included introducing the conditions of the research, including potential benefits and risks; then, the necessary recommendations were presented to the subjects, and consent was obtained from them to participate in the research. In the course of the research, all the subjects were asked not to participate in any activity outside of the protocol, and to inform the researcher immediately in case of changes in their lifestyle. Also, they were asked to follow their ordinary diet, according to the pre-research period. On the other hand, all subjects were prohibited from taking any supplements or drugs or taking a specific diet. Before starting the training protocol, the participant's height and weight were evaluated in the experimental conditions. In this vein, 24 hours before the beginning of the research period, the initial blood sampling was performed; also, 48 hours after the completion of the 8-week training period, following 12 hours of fasting, the second stage of blood sampling was performed to evaluate the levels of serum TC, TG, LDL-C, and HDL variables. In the case of observing the disease symptoms such as fever, dizziness, and nausea as well as subjects' non-presence over two sessions, they were excluded from the rest of the research.

Implementation of garlic supplement and the placebo

Garlic capsules with a dose of 500 mg, manufactured by the U.S. Naturemid Company, were prepared for supplementation, being available by Tehran *Pura teb* Pharmaceutical Company under the supervision of the Food and Drug Organization in Iran (25). To administer, the subjects consumed two garlic capsules 12 hours apart. Also, the subjects in the placebo group consumed lactose in capsules per the garlic supplement group.

The first training day was the same as to start taking capsules and the last training day was the ending day of taking them. It is noted that both taking the placebo and garlic supplement was a double-blind procedure. Thus, neither the subjects nor the researcher knew about the contents of the capsules.

Training protocol

In a preliminary session, one week before the start of the protocol, one repetition maximum (1RM) was performed using the Brzeski formula for all resistance movements, so that the intensity of the training was based on the determined percentage of one repetition maximum for each person. For twelve weeks and three sessions per week, the subjects performed resistance training that included chest press, shoulder press, forearm, back arm, front leg, back leg and armpit (lat) stretch. In each session, the movements for the experimental groups were performed in 3 sets with 10-12 repetitions with 60% of one repetition maximum. Rest intervals between sets were 1 minute and between movements were 2 minutes. Each training session included 3 stages warming up, specific movements, and cooling down. To control the training intensity and to observe the principle of overload and gradual progress, 1RM of the mentioned movements was recorded once every two weeks (24).

Blood sampling and laboratory evaluation

After fasting for 8-12 hours, 10 cc of venous blood sample was taken from the radial artery of the subjects' left hand in the pre-test and post-test stages (24 hours before the start of the training protocol and 48 hours after the last training session) by a laboratory specialist following 10-minute rest. Next, biochemical variables were investigated. To measure serum levels of TC, TG, HDL-C, and LDL-C, *Pars Azmun* Co. kit (Tehran, Iran) was used.

Statistical analysis

Descriptive statistics were used to obtain the mean and standard deviation. The normality of data distribution in the pre-test stage was checked using the Shapiro-Wilk test. A dependent samples *t*-test was used to examine the inter-group findings, and a one-way analysis of variance with LSD *post hoc* test was used to compare between-group findings. The statistical operations were performed using SPSS version 22 and the significance level was considered 0.05.

Results

Levene's test showed that all groups have the same or similar variance. Therefore, one-way ANOVA test was used. The results of the one-way ANOVA statistical test showed that there was no significant difference between the measurement variables in the pretest for TC (P-Value=0.598), TG (P-Value=0.080), LDL-C (P-Value=0.202), and HDL-C (P-Value=0.062). Demographic characteristics of the study participants are presented in Table 1; the normality of data distribution for TC, TG, LDL-C and HDL-C variables in the pre-test stage based on Shapiro–Wilk test was tested. Mean and SD of these variables are shown in Table 2; also, comparison of the average inter-group changes in different research groups for the aforementioned variables are tabulated in Table 3.

As shown in Table 3, the results of the one-way analysis of variance indicated a significant difference between the four groups of training + supplement, training + placebo, garlic supplement and placebo for TC, TG, LDL-C and HDL-C variables, with significant levels of (P-Value=0.001), (P-Value=0.001), (P-Value=0.001), and (P-Value=0.001), respectively. The results of the LSD *post hoc* test for the variables measured in the research indicated that TC serum values decreased significantly in the training supplement, training + placebo, and garlic supplement groups compared to the placebo group, with significant levels of (P-Value=0.001), (P-Value=0.006), and (P-Value=0.006), respectively. Also, there was a significant difference between the training + supplement group and the training ++ placebo group (P-Value=0.028).

The values of TG in the training + supplement, training + placebo, and garlic supplement groups compared to the placebo group decreased significantly with significant levels of (P-Value=0.001), (P-Value=0.001), and (P-Value=0.001) respectively, while there was no significant difference between the training + supplement group and the training + placebo group (P-Value=0.542). The values of LDL-C in the training + supplement, training + placebo, and garlic supplement groups decreased significantly compared to the placebo group with significance levels of (P-Value=0.001), (P-Value=0.001), and (P-Value=0.002) respectively, while there was no significant difference between the training + supplement group and the training + placebo group (P-Value=0.262).

Table 1. Demographic characteristics of the study participants

Group	Pre-test Weight (Kg)	P-Value	Post-test Weight (Kg)	P-Value	Height (cm)
Training+Garlic	74.88±4.73	0.859	70.45±4.62	0.901	161.13±1.26
Training+Placebo	74.02±5.56		71.92±5.08		165.13±1.26
Garlic	73.37±3.95		72.72±4.11		163.93±0.56
Placebo	72.42±4.17		72.36±3.68		162.13±1.26

P-Value is based on the one-way ANOVA test

Table 2. Mean ± SD for TC, TG, LDL-C, and HDL-C variables in the pre-test stage

Variable	Training+Garlic supplement	Training+Placebo	Garlic supplement	Placebo
TC (mg/dl)	193.00±6.02	183.20±6.24	185.13±5.41	**188.52±4.09**
TG (mg/dl)	178.46±4.36	172.73±4.91	166.66±3.70	**164.00±3.70**
LDL-C (mg/dl)	144.13±2.78	140.13±2.46	135.06±4.00	**136.60±3.13**
HDL-C (mg/dl)	42.33±4.10	41.80±4.99	44.46±4.86	**42.13±5.59**

Table 3. Comparison of the average intergroup changes of TC, TG, LDL-C, and HDL-C variables in different research groups

Variable	Group	Pre-test	Post-test	P-Between groups	P-Between 1&2	P-Between 1& 3	P-Between 1&4	P-Between 2&3	P-Between 2&4	P-Between 3&4
TC (mg/dl)	Training + Garlic supplement	193.00±6.02	154.06±6.82	0.001*	0.028*	0.001*	0.001*	0.025*	0.001*	0.006*
	Training + Placebo	183.20±6.24	152.46±5.96							
	Garlic supplement	185.13±5.41	162.46±5.55							
	Placebo	188.53±4.09	190.80±3.47							
TG (mg/dl)	Training + Garlic supplement	178.46±4.36	138.4±6.46	0.001*	0.542	0.035*	0.001*	0.473	0.001*	0.001*
	Training + Placebo	172.73±4.91	139.4±46.09							
	Garlic supplement	166.66±3.70	141.3±13.60							
	Placebo	164.00±3.70	168.4±80.47							
LDL-C (mg/dl)	Training + Garlic supplement	144.13±2.78	108.2±66.17	0.001*	0.2620	0.005*	0.001*	0.365	0.001*	0.002*
	Training + Placebo	140.13±2.46	116.2±33.50							
	Garlic supplement	135.06±4.00	123.3±13.82							
	Placebo	136.60±3.13	138.2±86.96							
HDL-C (mg/dl)	Training + Garlic supplement	42.33±4.10	41.6±40.07	0.001*	0.045*	0.019*	0.001*	0.978	0.001*	0.001*
	Training + Placebo	43.33±5.13	44.5±86.15							
	Garlic supplement	40.46±4.86	16.1±71.54							
	Placebo	42.13±5.59	14.2±6.28							

* Indicates a significant difference between the study groups based on the one-way ANOVA, and LSD post-hoc test. Group 1: Training + Garlic supplement; Group 2: Training + Placebo; Group 3: Garlic supplement; Group 4: Placebo

The values of HDL-C in the training + supplement, training + placebo, and garlic supplement groups compared to the placebo group increased significantly with significant levels of (P-Value=0.001), (P-Value=0.001), and (P-Value=0.001), respectively, and there was a significant difference between the training + supplement group and the training + placebo group (P-Value=0.045).

Discussion

Today, cardiovascular diseases are listed at the top of the mortality causes all over the world, and millions of people are hospitalized every year due to the complications resulting from diseases such as heart attacks, heart arrests, and angina pains. Obesity is one of the main reasons for cardiovascular disease, which is mostly due to a lack of sufficient physical activity, an inappropriate diet, and a sedentary lifestyle (26). Obesity in society has harmful effects on individuals' spiritual, mental, and physical health, and it also triggers metabolic risk. Therefore, it seems necessary to implement effective measures to control or reduce it. In this study, the effect of eight weeks of moderate-intensity resistance training with garlic supplementation on serum levels of some cardiovascular risk factors (TC, TG, LDL-C, and HDL-C) in overweight women was investigated.

The results showed that the implementation of eight weeks of moderate-intensity resistance training with and without garlic consumption improved the lipid profile in overweight subjects, and when the training was accompanied by garlic supplementation, changes and improvements in serum levels were significant. Fortunately, clinical and epidemiological evidence shows a significant reduction in mortality from cardiovascular diseases among consumers of fruits, vegetables, and substances extracted from plants. In this vein, in the present study, consumption of garlic supplements alone for eight weeks improved TC, TG, HDL-C, and LDL-C indicators in overweight women.

Based on studies, it can be suggested that garlic supplementation can affect the lipid profile through mechanisms such as reducing inflammatory factors, reducing oxidative stress, and increasing antioxidant capacity (27). In addition, it has been suggested that garlic supplementation can be effective in the treatment of cardiovascular diseases through phytochemicals, as it inhibits key enzymes in the synthesis of cholesterol and fatty acids such as acetyl coenzyme A, carboxylase, and hydroxymethylglutaryl coenzyme, and finally affects the blood triglyceride levels (28,29).

Animal studies also show that the activities of lipogenic and cholesterogenic enzymes such as malic enzyme, glucose-6 phosphate dehydrogenase, fatty acid synthetase, 3-hydroxymethylglutaryl coenzyme A reductase are altered under the influence of garlic supplementation and controlled diet (30), affecting the lipid profile. In this regard, the results of a study showed that the consumption of garlic extract along with the control of a high-fat diet led to a decrease in leptin receptor, modification of AgRP and NPY expression in vasculitis-induced rats by increasing the number of beta-adrenergic receptors 1-3 in adipose tissue and improving the fat profile (31). Garlic extract inhibits TLR signaling, decreases phosphorylation of NF-κB, and activates AMP-activated protein kinase, and hence can improve inflammation and lipid profile (32). Thus, based on the mechanisms mentioned above, the fact that eight weeks of garlic supplementation improved the serum levels of TC, TG, LDL-C and HDL-C variables in overweight women can be justified

Another result of this research was that eight weeks of resistance training with moderate intensity alone reduced the serum levels of TC, TG, LDL-C and increased HDL-C, which is consistent with the results of the studies by Cunha et al. (2019) (33), Stojanović et al. (2021) (34) and Perhampour et al. (2021) (35), and is inconsistent with the results of some other studies (36,37). In the study of Allah verdi et al. (2017), eight weeks of interval aerobic training with high intensity and moderate intensity did not significantly change the levels of TG, LDL-C, and HDL-C, and only the amount of cholesterol decreased significantly (36).

In general, the reason for these differences can be related to the type of subjects and training protocol (especially the amount of training). Based on the evidence, the affectability of the four cardiovascular indicators measured in the current research is not in line with the exercise training. In their study, Stojanović et al. (2021) generally stated that resistance training reduced the levels of TC, TG, and LDL-C in the postmenopausal period, while an increase in HDL-C was less common and the highest amount of improvement occurred in overweight women (34).

Likely, the low energy consumption caused by resistance training compared to aerobic training as well as the short training period compared to previous studies are the main reasons for lack of significant changes in HDL-C. In their study. Oh et al. (2023) investigated the effect of combined resistance training (TRX) and aerobic training (with high and moderate intensity) on body fat percentage, body weight, and serum levels of TC, TG, LDL-C, and HDL-C in 40-year-old overweight women. The results showed that in both TG training groups, the percentage of fat and body weight decreased and the serum levels of HDL-C increased, but the serum levels of cholesterol and TC decreased only in the combined resistance and low-intensity aerobic training group (38).

These results suggest that moderate-intensity aerobic training may activate fat metabolism more effectively and may lead to additional physiological effects, such as increased muscle strength and lean body mass, due to TRX, which is included in a combined exercise regimen. In the current research, there was no significant difference between the weight of the subjects in the four groups, which cannot be definitively commented on due to the lack of measurement of fat percentage and fat-free mass. This is because it is likely that eight weeks of resistance training along with garlic supplementation reduced fat mass and increased muscle mass in overweight women. In this regard, it has been shown that by doing resistance training, the lean body mass increases and the fat mass decreases, thus improving the body composition; the reason for this can be the high caloric expenditure of resistance training due to the high oxidation of body fat mass (39). In general, it has been proposed that resistance training increases the rate of resting metabolism by causing muscle fatigue, so recent studies have shown that resistance training increases muscle mass, and strength, improves insulin sensitivity, blood sugar, fasting insulin, glucose tolerance levels, and decreases visceral fat (40).

Nevertheless, it should be noted that in performing effective exercises to prevent and treat obesity, one should consider important factors such as intensity, volume, frequency, and type of exercise (41). It is worth noting that today, to change the lipid profile, training volume, and the combination of resistance and aerobic training further attention should be paid. There are very limited studies that have investigated and analyzed the effect of the volume of resistance training. One of the studies to explore the volume of resistance and aerobic training on lipid parameters was conducted by Fett et al. (2009). They generally stated that the amount of movement may be as important as or even more important than the amount of the weight lifted in resistance training (42). In their study, the effect of resistance training and aerobic training with low to moderate intensity on the risk factors of cardiovascular disease in overweight women was investigated. The study was conducted in a period of two months, and during the study, the volume of training increased from three sessions (180 minutes per week) in the first month to four sessions per week (240 minutes per week) in the second month. The results showed that in the resistance training group, levels of TC and TG significantly reduced, while in the aerobic training group, only LDL-C and total cholesterol to HDL-C ratio significantly decreased (42).

Regarding the positive effect of eight weeks of moderate-intensity resistance training on related cardiovascular indicators, what can be noticed in the following

research is the amount of training performed during these eight weeks, which improved the lipid profile. Regarding the possible mechanism of the change in lipid profile levels in the present study, it can be suggested that human growth hormone is secreted more under the influence of resistance training (43) and thus it can be responsible for the increase in fatty acid called. Therefore, with the increase in the duration of training, the amount of growth hormone increases and is maintained at an increased level for hours after the activity in the period of returning to the initial state. In addition, in the course of exercise, fat tissue becomes more sensitive to the sympathetic nervous system or to the increase in the levels of circulating catecholamines, both of which will increase the fat call; this call is a response to a special fat call substance that is highly sensitive to an increased level of activity (44). Regarding the effect of eight weeks of garlic supplementation along with resistance training, it was observed that the effect on lipid profile was more significant, which shows the positive effect of resistance training and garlic supplementation. The present research had some limitations, including the lack of measurement of the subjects' cardiovascular risk factors as well as the lack of control of their nutrition. Accordingly, it is suggested that, in analogous research, other levels of these indicators should be possibly investigated along with garlic supplementation or other supplements following resistance training and controlling the subjects' nutrition. It should also be noted that the subjects of the present study were healthy and did not suffer from glucose homeostasis and blood lipid profile disorders, hence it is recommended to conduct a research study in overweight women with glucose homeostasis and blood lipid profile disorders. Limitations of the present study were the lack of measurement of other indicators involved in obesity, and participants' sleep as well as nutrition.

Conclusion

A striking finding of the current research is the exploration of the effectiveness of garlic supplementation alone on the lipid profile in overweight women, which can be an effective factor to be attended on the indicators related to cardiovascular diseases in the diet of the relevant group of society. Although performing a course of moderate-intensity resistance training without garlic consumption could improve the subjects' lipid profile, if the training is accompanied by garlic supplementation, the intensity of changes in the levels of TC, TG, LDL-C, and HDL-C would be higher. In general, the results of this research can expand our information on the salience of the volume and intensity of resistance training as well as garlic consumption.

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Ethical statement

The Islamic Azad University of Gachsaran Ethics Committee approved this study's protocol (IR. GACHSARAN.1402.278).

Conflicts of interest

The research was self-funded, and the equipment necessary for conducting the study was provided by the Islamic Azad University of Gachsaran. All authors were involved in data interpretation and presentation. All authors approved the final manuscript.

Author contributions

Yalda Sadeghi played pivotal roles in data collection, Statistical population collection, and laboratory coordination. Their expertise and insights were crucial to the success of this research. Ali Khajehlandi, Mohabat Salehi, and Amin Mohammadi played pivotal roles in setting up the background, and statistical analysis of the research.

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