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Research Article

Randomized Clinical Trial Effect of Sesame and Training on Heat Shock Protein 70 Gene Expression and Light Chain 3-I Proteins of Inactive Middle-Aged Men

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Abstract

Background: Clinical studies have indicated that exercise activity and supplementation may have different effects on the immune system and health. The present study aimed to determine the effect of sesame and interval training on heat shock protein 70 (HSP70) gene expression and light chain 3-I (LC3-I) proteins among inactive, middle-aged men.

Methods: In this randomized clinical trial and quasi-experimental study which was conducted in fall, 2019, 60 middle-aged inactive men who had the habit of going to the parks in Karaj, Iran were selected as the study's statistical subject and, then, were randomly divided into four groups (i.e., sesame supplement, interval training, sesame supplement + interval training, control). Sesame, and sesame supplement + interval training groups received sesame. The training protocol lasted for 12 weeks. HSP70 gene expression and LC3-I were measured before and after study using the enzymatic method. As for the homogeneity of variances, the Levene's test and the Shapiro–Wilk test were used to investigate the natural distribution. Analysis of variance was used to investigate differences in four groups.

Results: Significant differences were found regarding LC3-I ($P<0.001$) and HSP70 ($P<0.001$). According to the results of the Tukey post hoc test, there was a significant difference between the training + sesame group and controls ($P \leq 0.05$).

Conclusion: It was concluded that high interval intensity training with sesame reduced HSP70 gene expression and LC3-I. The novelty of this study lay in the fact that it found consuming supplementation and training capable of synergistically reducing chaperone protein and autophagy indicator.

Keywords: Dietary supplements, Exercise training, Autophagy, Heat shock proteins

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Background

The compounds of heat shock protein (HSP) and light chain3-I (LC3-I) have been found effective in improving the immune system of cells. The HSP family participates in the proteostasis stages and the regulation of protein synthesis and degradation by autophagic pathways (1).

One of pharmaceutical plants is sesame, an important traditional health food that has been used to improve nutritional status and prevent various diseases. Sesame is rich in protein lignin, vitamin, and antioxidants. Employing nutritional intervention and antioxidants has been recognized as an effective strategy to reduce the side effects of training and exercise. According to one study, one hour after receipt, sesame oil exerts its effect by changing the fluidity of cell membranes or altering the function of membrane ion channels. This finding may have been attributed to the increase in acetylcholine and sesame list in the hippocampus (2).

Some studies have investigated the effect of acute and chronic sesame oil consumption on the strychnine induced seizure in adult rats. Seemingly, the chronic consumption of sesame oil has the potential to delay the onset of seizure and reduce the kindled seizure acquisition. This effect may be associated with a possible mechanism whereby antioxidants prevent damage caused by free radicals. The presence of sesame, sesamol, and sesamolin lignans in sesame oil reduces lipid peroxidation and increases the activity of antioxidant enzymes. Vitamin E in sesame oil also increases glutathione. It goes without saying that these possibilities have a significant impact on improving the immune system and improving athletic performance (3).

Some other studies have explored the effect of white sesame supplement protocol on muscle damage, markers, systemic inflammation, oxidative stress, and aerobic capacity among soccer players. The results have shown that sesame consumption may reduce muscle damage and oxidative stress while improving the aerobic capacity in soccer players. A previous study has also reported an improved aerobic capacity concomitant with reduction of oxidative stress markers in runners supplemented with grape juice. However, the mechanisms responsible for reducing oxidative stress or the systemic inflammation contributing to better aerobic capacity have remained relatively unknown (4).

Interval training with sesame supplementation has been found to improve metabolism and strengthen the immune system and antioxidants, and serum malondialdehyde has been regulated in untrained middle-aged men (5).

Yuan et al investigated the altered expression levels of autophagy-associated proteins during exercise preconditioning in order to indicate the involvement of autophagy in cardio protection against exercise-induced myocardial injury (6).The results of Pesce et al showed that considering the beneficial effects that regular physical exercise has on promoting health and reducing age-related injuries, it seems necessary to understand the relationship between exercise and the human phenotype that characterizes aging and pathology. Chronic low-grade inflammation was found associated with aging and obesity. Inflammation was also discovered to be related to resistance exercise stress triggering (7).

The mechanism behind the functions of molecular chaperone complexes as well as macrophage complexes contributing to degradation has also been investigated (8). Some studies have examined the role of nutrient and exercise-mediated autophagy in making metabolic and immune adjustments in response to exercise. Sesame consists of tetrahydro-1H, 3H-furo furan and has been categorized as a unique healthy food with a high level of mono- and polyunsaturated fatty acids, fiber, nutraceutical components, and vitamin E (9). Several research studies have focused on the modulation of antihypertensive effects, lipid peroxidation, lipid metabolism, and anti-oxidative activity. Their effects on immune regulation have also been the subjects of studies (9). Resistance exercise training has been shown to improve muscle strength in chloroquine-treated rats and to prevent chloroquine-induced increases in Beclin-1 and p62 (10).

However, the LC3-I and HSPs response in human muscle to exercise seems to be more complex. Interval training is a type of training that involves a series of intensity workouts interspersed with rest (11). Varying in the intensity of exercise affects the cardiac muscle and improves aerobic capacity. HIIT (High-intensity interval training) has the capacity to increase autophagy. A recent study on rats has found no significant changes in these markers (12).

A previous study in young rats involved running on a treadmill for four weeks. The exercise program increased the change in LC3II / I ratio and decreased the p62 protein (13-14). One of the primary types of autophagy is chaperone-mediated autophagy. Metabolic adaptations are primarily mediated by adenosine monophosphate-activated protein kinase (6). The majority of the studies have only focused on the field of nutrition or exercise; however, sesame and exercise have not received a due research attention. Therefore, this study aimed to investigate the effects of interval training with sesame on LC3-I and HSP70 gene expression among inactive middle-aged men.

Material and Methods

Subjects

This study was a randomized clinical trial and semi-experimental study, adopting a pre and post-test design. The statistical population included 60 inactive middle-aged men from Karaj, Iran. The sample size was determined based on a comparison of several means by using one-way analysis of variance and alpha error tables of 1%-5%. Exclusion criteria were having cardiovascular disease, smoking, consuming alcohol, and not taking antioxidant supplements during the previous three months. To determine the sample of the study, first all middle-aged men who had the habit of going to big parks in five different areas of the city were enrolled in the study as volunteers. Taking into account the subjects' availability and willingness as well as the inclusion and exclusion criteria of the study, then, 60 of them were randomly selecte. (Figure 1). The characteristics of the subjects and their related groups were recorded on blood collection tubes.

Grouping

All subjects were informed of the content of the study, and a written informed consent was obtained from them. Then, they were randomly divided into four groups, namely control, interval training + sesame, sesame, interval training groups.

Exercise Protocol

The high-intensity interval training program consisted of 12 weeks, 4 sessions per week, wherein a gradual increase in duration and intensity was included. The content of each training session included 10 minutes of warm-up. The intensity of training sequences in the final sessions reached 85% of the maximum heart rate. Heart rate was calculated using Equation (Age -220), measured by a polar heart rate monitor, and tracked during the program by measuring the radial pulse (Table 1).

Laboratory Measurements of LC3-I and HSP70

A skilled technician from the reference laboratory attended the Iran gym two times, before and immediately after the end of the last session of the protocol and supplementation, and collected the blood samples at the site before and after centrifugation. The subjects' blood samples were taken from antecubital vein and, then, the collected samples were immediately transferred to the laboratory. Proteins were extracted using the buffer and quantified with the Bio-Rad Laboratories. The part of total protein extract of each sample was separated and

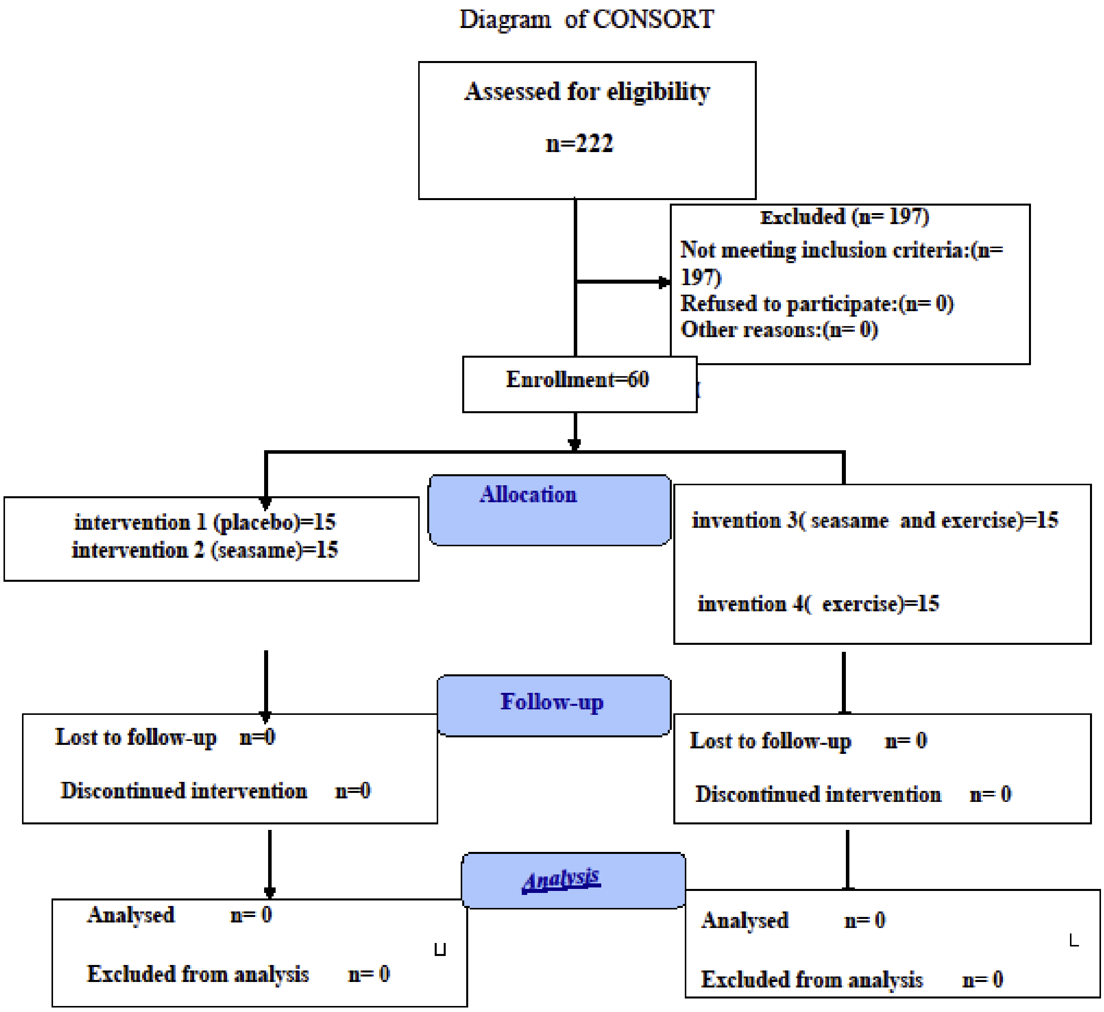

Figure 1. CONCORT Flowchart of the Study.

electroblotted on a polyvinylidene fluoride membrane. The transferred membranes were probed. The polyclonal antibody was used as a loading control. Furthermore, the peripheral venous blood of the study subjects was obtained before and after the study course in the seated position and collected in the ethylene diamine tetraacetate vial. Five mL of blood was collected, from which one mL was used for RNA isolation, and the plasma was separated by centrifugation of the remaining volume of blood at 3000 rpm for 15 minutes. Whole blood samples were examined using RNA blood according to the manufacturer's protocol. The examination of LC3 gene expression was performed by experts in the laboratory using partial real-time PCR and SYBR Green Master Mix (2X) (Ampliqon Company). In the present study, GAPDH gene was used as the reference gene. The data on primers are presented in Table 1. The primer designing was performed by Gene Runner software.

HSP70 gene expression was assessed using the real-time PCR method. First, the primer was designed and the extracted solution was purified with enzyme X. The absorbance ratio found by spectrophotometry was 260-280 nm for all absorption samples between 2.8-2. Then electrophoresis and 1% agarose gel were used. As for the cDNA synthesis, micrograms of each mRNA sample X-primers and reverse transcription enzymes were used in the kit synthesis instructions.

Supplementation

The supplement group and the supplement + exercise group took 4 200 mg sesame tablets during the study period. Sesame supplement in the form of capsules (Kholaor Brand , Thailand) was given to the study subjects. The taken dose was determined based on previous studies and references (15). Placebo group received the same amount of starch, with no specific smell, color, or taste.

Statistical Analysis

The normality of the data was assessed using the Shapiro-Wilk test, and the homogeneity of variances was evaluated using the Levene's test. One-way analysis of variance and the Tukey post hoc were adopted to examine the differences between groups using SPSS software version 21 ($P \leq 0.05$ was considered significant).

Table 1. High Intensity Interval Training Protocol With Course, Repetition and Rest Between Sets

Week	Warm-up	Running (min)	Heart Rate (% max)	Proportion	Exercise content
1-4	10	30	%60	1:1	1. Warm-up:10 min. 2. Stretching exercises 5 min 3. Dynamic exercises 5 min 4. jogging and running 30-60 min
5-8	10	45	%75	2:1	
9-12	10	60	%85	3:1	

Results

After determining the number of study samples (60 people), participants were randomly divided into four groups of 15 individuals. Their demographic characteristics, as well as their anthropometric and physiological indices (age, weight, height, body mass index) were measured and recorded (Table 2). Mean and standard deviation of LC3-1 changes in four groups were assessed before and after the implementation of study protocol. After completing training period and taking sesame, according to the results, the highest mean of LC3-1 in terms of GAPDH changes was observed in the control group whereas the lowest mean was detected in the training + sesame. The comparison of the changes in LC3-1 gene expression in different research groups was performed using analysis of variance and Tukey post hoc test (Figure 2). A significant decrease was observed in LC3-1 gene expression compared to the control group ($P < 0.001$).

Mean and standard deviation of HSP70 gene expression changes were measured and recorded before and after training protocol and sesame. According to the results, moreover, the highest mean was found in the control group ($P < 0.07$) while the lowest mean was discovered in the training + sesame. The results from one-way analysis of variance on HSP70 gene expression were evaluated in different groups ($F = 7.37$, $P < 0.001$), and a significant difference was observed. Tukey post hoc test showed that in the exercise + sesame group compared to the control group, the expression level of HSP70 gene decreased significantly ($P < 0.001$)(Figure 3).

Table 2. Mean ± SD of Descriptive Characteristics of Subjects (n = 15)

Group	BMI (%)	Height (cm)	Weight (kg)	Age (y)
Sesame + interval	28.86 ± 1.91	1.72 ± 0.06	85.67 ± 8.08	46.93 ± 4.99
Sesame	28.50 ± 3.48	1.75 ± 0.08	88.13 ± 8.98	48.07 ± 5.27
Interval	28.46 ± 1.35	1.73 ± 0.07	86.93 ± 9.5	47.93 ± 4.4
Control	28.44 ± 1.67	1.76 ± 0.2	87.00 ± 8.6	47.80 ± 5.88

BMI, body mass index.

Discussion

Intensity interval training caused a decrease in the level of peripheral leukocytes HSP70 gene expression and LC3-I. The results from this study were similar to those from a study by Cho et al (11). Contrary to the findings of the present study, the findings from some other studies had reported increased levels of HSP70. If there had been an evaluation exercise in the first sessions, however, this increase might have been observed. The contradictory results may also have been attributable to the reduction of sesame consumption and the synergistic effects of training and supplement compatibility (16).

Furthermore, endurance training had been adopted in other studies; while intense interval training was used in the present study. In other studies, heart and muscle tissue had been the subjects of study; whereas gene expression was explored in this study. The increase in body temperature and the cycle of ROS formation as well as the decrease in glycogen when adopting endurance training have been also found to cause an increase in HSP70 levels (17). However, physical activity increase and accumulate HSP70 and then is affected by the protected molecules. (18). Exercise has been recognized as a stress factor, which increases the HSP; however, this study found a contradictory result in this regard since it determined the adaptability of exercise. Differences in the type, intensity, and duration of exercise, muscles involved, nutrition, age, and gender are also factors that contradict the results of this study with others(19). Moreover, tissue hypoxia, and pressure in the cell, might trigger the HSP expression (19).

The heat stress response modulates autophagy and inhibits light chain protein 1. Chronic and prolonged exercise has been reported to lead to autophagic responses in skeletal muscle (20). In a study by Tarawan et al, moderate intensity of exercise was found to increase autophagy gene expression since, in their study compared to this study, different protocol training and subjects were employed and no supplementation was used (20).

The response of LC3-I to exercise is variable. The data from the current study suggested that different exercises for autophagy activation may have been affected by population and environmental stresses. Intense interval training reduced autophagic LC3-I, which may have been indicative of adaptive responses to HIIT. Escobar et al determined autophagy responsible for loss of muscle mass, fiber destruction, decreased muscle strength, and metabolic disorders. But it is effective on adaptive responses to exercise. Exactly this part is seen in this research (21). Brandt et al showed that inhibition of autophagy in rat led to reduced body growth, myofibril size, degenerative changes in muscle tissue, and decreased strength (22), none of which was observed in this study. According to the results from a study by Smiles et al, however, autophagy played a role in developing endurance capacity, skeletal tissue homeostasis, physical function, and antioxidant defense (23), which were consistent with the results of this study. On the other hand, some

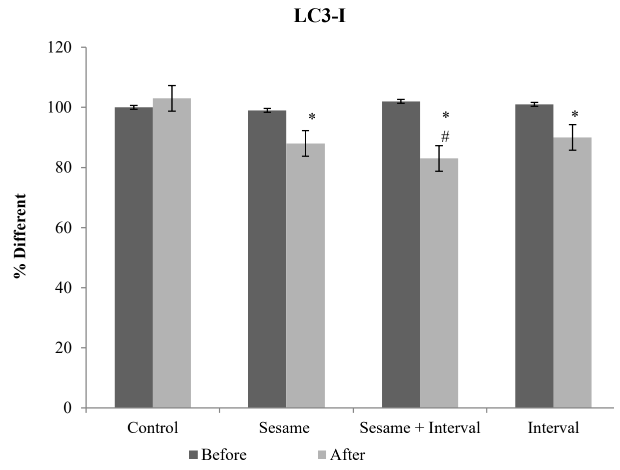

Figure 2. Comparison of Mean, % Different and Significant Changes LC3-1 Between Four Groups of Inactive Middle-aged Men. * $P\leq 0.05$, significant difference compared to control group. # $P\leq 0.05$, significant difference sesame+interval training compared to the control group.

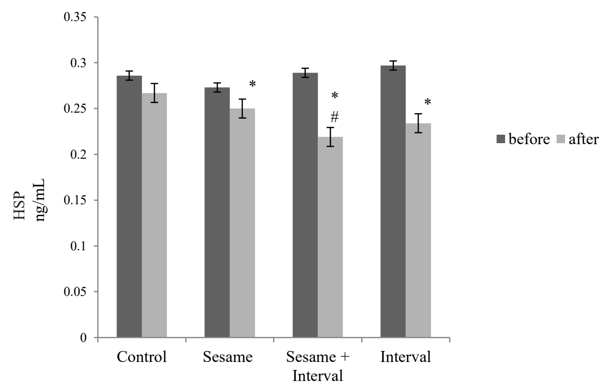

Figure 3. Comparison of Mean and Significant Changes HSP70 Gene Expression Between Four Groups of Inactive Middle-aged Men. * $P\leq 0.05$, significant difference compared to control group. # $P\leq 0.05$, significant difference sesame+interval training compared to the control group.

researchers have reported that shorter-term exercise reduces the LC3-I content (24).

Sesame helps prevent various diseases and may have antioxidant effects (25). The results of the present study were supported by several studies regarding the emphasis on the antioxidant effects of sesame (26). Visavadiya and Narasimhacharya, for instance, had reported similar results for rat. Sesame has lignin, sesamolin and vitamin E content. Antioxidant effects of lignin have been documented by many studies. Sesame, containing polyphenols and flavonoids, increases catalase and superoxide dismutase activity, decreases malondialdehyde, improves lipid profile (27), suppresses oxygen species production, and possesses an ability to increase vitamin E and gamma-tocopherol levels in tissues, which in turn may facilitate the suppression of different free radicals (25). Dalibalta et al demonstrated that sesame induced its effects through multiple pathways with the antioxidant and anti-inflammatory properties of sesame (15). The duration of the intervention in studies might also vary. No exact mechanism has ever been identified to explain how sesame products are able to decrease oxidative stress (25).

The strengths of this study lay in the facts that healthy human sample, combination of exercise and supplementation, and plant compounds were used in the study. However, the study suffered from some weaknesses including the reduction in strict control of the subjects in terms of nutrition and activity, the high cost of the experiments, and the use of small experimental groups.

Conclusion

It was concluded that high interval intensity training with sesame reduced HSP70 gene expression and LC3-I. The novelty of this study lay in the fact that it found consuming supplementation and training capable of synergistically reducing chaperone protein and autophagy indicator. It should be noted that these indicators usually cause damage, immune weakness, and lack of cellular recovery of the tissue among middle age. Therefore, the inclusion of sesame in the diet of people and the use of training can contribute to better health and quality of body function, without side effects.

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Conflict of Interests

None to declare.

Ethical Approval

All stages of the study were carried out in accordance with the principles of the Helsinki Declaration, and all the ethical principles were followed during the research. Therefore, the study was approved by Ethics Committee (ID no.: IR.IAU.VARAMIN.REC.1398.006), and was also registered on the Iranian Registry of Clinical Trials website (identifier: IRCT20171210037809N6).

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