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The effects of garlic supplements and exercise on the levels of Lipocalin-2 and insulin resistance among middle-aged obese women

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ABSTRACT

The aim of this study was to investigate the effect concurrent exercise training at 70-80%HR with consuming garlic supplement on Lipocalin2, insulin level and insulin resistance in obese woman. For this purpose , 38 non diabetic woman that have no exercise training before last 6 month ago ,randomly put in 4 groups of control, exercise, supplement, exercise + supplement. Subjects in exercise groups have aerobic exercise (70-80%HR) and resistance exercise program for 10 weeks. Two garlic tablet daily for 10 weeks has been considered for supplement groups .Anthropometric values and blood samples has been taken before and after 10 weeks. Data were analyzed using the repeated measures of ANOVA and Paired-sample T test. Data analyses revealed that exercise has not significant effect on weight and body fat percentage. After exercise training and consuming garlic supplements, lipocalin2, insulin and insulin resistance have significant difference by t test analyze in groups. Based on the findings of the present study, exercise training and consuming garlic supplement have beneficial effect on metabolic risk factors in obese woman.

KEY WORD: Lipocalin2, insulin resistance, garlic supplement, metabolic risk factors, concurrent exercise.

INTRODUCTION

Over the last decade, obesity has increasingly developed and at nowadays it is one of the most serious issues of public health. Metabolic syndrome or syndrome of insulin resistance is a metabolic disorder that is specified through the presence of several risk factors of cardiovascular diseases such as abdominal obesity, hyperlipidemia, high blood pressure, and insulin resistance [1,8,28.30]. Metabolic syndrome and type-2 diabetes are the most important risk factors that are pertinent to cardiovascular disease in the obese. However, the mechanism through which metabolic syndrome develops in obese people is not clear [24]. Aerobic exercises improve metabolic syndrome and its risk factors.[5, 6]. In different studies, useful effects of regular physical activities have been referred to as a medical strategy in abdominal obesity and cardiovascular complications; however, the special effect of combine aerobic and power exercises in adults with metabolic syndrome is not clear yet [5]. The role of garlic in decreasing risk factors of cardiovascular disease has long been distinguished. Garlic has a variety of biological activities such as anti-diabetic activity [21,26,17].

It is obvious that diet factors play an important role in prevention of diabetes and other metabolic factors [21]. Special Committee on Diabetes of World Health Organization recommended that an extensive research should be conducted on traditional methods of diabetes treatment. Since garlic is a popular spice in cooking and the oldest medicinal plant with a long history of use in healing infections, some cancers, heart diseases, and diabetes, its effects are being examined in a large number of studies [17].

Evidence indicates that extra intake of lipids results in insulin resistance in muscles [15]. Most type-2 diabetic patients are resistant to insulin, i.e. insulin sensitivity in the target cells such as muscles, liver, and its lipid has decreased [25]. Adipose tissues release multiple proteins called adipokine which plays a role in biological application like insulin function [22].

Adipokines are a group of secreted cytokines from adipose tissues like IL-6, RBP4, Lcn2, and TNFα that play an important role in developing insulin resistance and atherosclerosis. Lipocalin-2 is made of a large amount of adipose tissue and its secretion is activated during infections and inflammation. Its serum concentration is positively related to waist circumference, percentage of body fat, systolic blood pressure, fasting glucose, insulin, triglycerides, and inflammatory markers of coronary, and the development of its gene expression increases with factors that improve insulin resistance. Lipocalin-2 is an adipokine that affects the metabolism of glucose and insulin sensitivity [4, 9, 6, 27]. Compared to slim individuals, obese ones have

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higher levels of lipocalin-2. Moreover, there is a significant relationship between lipocalin-2 and several variables related to metabolic syndrome, including adverse lipid profile, hyper-insulinoma, hyper-glycaemia, and insulin resistance [4, 14]. In addition, its increase results in improvement of insulin sensitivity [4]. Meanwhile, local response to inflammation caused by obesity and tissue damage motivates the released adipokine, which in turn results in an increase in inflammatory markers like lipocalin-2 [6].

In addition to diet criteria, participation in regular physical activities is one of the most significant strategies to prevent obesity and related inflammatory. In spite of changes in levels of adipokine that cause useful effects of sports, there is no any access to information on changes of lipocalin [4, 6]. Exercise increases insulin sensitivity in people with and without diabetes [27]. Evidence indicates that only a slight decrease in weight as a result of change in diet and exercise is very effective in reduction of type-2 diabetes [5].

Several studies have indicated the effects of regular physical activities as medicinal strategy on abdominal obesity and risk factors of cardiovascular diseases. However, the role of aerobic exercises in combination of resistance in metabolic syndrome is unclear [4, 6]. The levels of cytokines are related with the type of exercise and the scope of the changes in them is highly dependent on the intensity and duration of the exercise [6].

One of the important physiological changes in health and heart metabolic effects caused by exercise is related to the change in adipokine secretion involved with inflammation. Eduardo, et al (2009) conducted a review study on adipokines and insulin resistance. In a variety of studies that were included in his study, it was concluded that lipocalin-2 has a positive relationship with waist circumference, percentage of body fat, systolic blood pressure, fasting glucose, insulin, triglycerides, and inflammatory markers of coronary [9].

Limiting the intake calorie and average-intense to intense aerobic activity is necessary to control the factors causing type-2 diabetes. However, one should not forget the point that the amount of needed exercise to remarkably reduce weight is more than the one needed to control blood glucose [37, 38].

Change in lifestyle and ability to exercise in improvement of heart metabolic markers may be related to modification of adipokine secretions [6, 11]. Some studies have studied the effects of different exercises on adipokines and reported contradictory results [6].

Choi et al (2009) ,determine the effects of three months of aerobic and power exercise on lipocalin and factors of metabolic syndrome, they investigated 30 obese women and 15 non-obese ones (control group). The obese group had aerobic activity with intensity of 60-75%, maximum heart rate, 300 calories of consumption, and 20 minutes of power activity with 100 calories of consumption. After three months of combined exercise, a significant decrease in weight, BMI, waist-to-hip ratio, fasting glucose, and cholesterol. However, there was no significant difference in the level of lipocalin and insulin resistance among the subjects [4].

Koiou et al (2012) conducted a study on 47 obese or over-weight female patients in order to figure out the effect of weight reduction on lipocalin level in obese women. In their study, they used low-calorie diets, exercise, and consumption of orlistat and sibutramine for 6 months. In another group, 25 women with normal weight were treated using metformin over 6 months. In the beginning, the level of lipocalin-2 in the obese group compared to the normal one was higher. Weight drop among obese individuals caused a reduction in lipocalin-2 level while consumption of metformin had no effect on it [14].

Padiya, et al (2011) studied the effect of garlic on improvement of insulin sensitivity and metabolic syndrome factors in mice. They concluded that garlic consumption results in a significant drop in weight, blood glucose, insulin, and improvement of insulin sensitivity in diabetic mice (21).

Recent medicine studies focus on modifying insulin resistance which is the fundamental disorder in obesity and type-2 diabetes. There are natural compounds with medical value like garlic that over years have gained a unique place as a potential to control diabetes and its metabolic complications. There are many natural materials with anti-diabetic effects [12]. Garlic is one of the most popular spices in cooking and is the most selling plant supplementary all over the world. Although garlic has been reported to be used as a medicine for diabetes in Europe, India, and the Middle East, there are many studies with contradictory results [17, 23].

Since exercise alone has a positive effect on risk factors of metabolic syndrome, and studies of the effects of garlic supplementary on metabolic syndrome indicate the positive effects of garlic on insulin resistance; therefore, the present study was an attempt to respond to the question whethersimultaneous use of garlic supplementary and selective combined exercise has an effect on metabolic syndrome risk factors among middle-aged obese women or not.

Study sample

The study was consisted of 38 middle-aged healthy women of 30-50 years of age, with overweight, without history of exercise and BMI≥25 who participated in the study voluntarily and were randomly divided into three experimental groups.

The method for measuring weight and the percentage of body fat

To measure the subjects' weight, the device of analyzing body composition (Model biospace.co, inbody3.0 made in Korea) was utilized.

The method for measuring waist circumferences

The method proposed by the National Committee of Health was used to measure waist circumferences. In so doing, after the highest and the most outermost of iliac crest margin was touched, a horizontal line along this point was drawn up to the middle line of the body. Afterwards, by putting a tape meter around the subjects' abdomen in a way that the low margin of the meter placed on this line, the waist circumferences were recorded [16].

Initial measures included:

- Measuring anthropometric indices such as: height, weight, BMI, body circumference, and percentage of body fat
- Specifying the heart rate at rest (HRrest) (immediately after night sleep) and maximum heart rate (HRmax): (220-age), specifying the intensity of exercise
- 1RM of each subject through free scales and according to the following formula:

A maximum repeat = Weight (Kg) x [1 + (0.033 x Number of repetitions)]

Based on the calculated numerical values for a maximum repeat (RM1) and the maximum heart rate (HRmax) and also library-based studies, the progressive protocol for 10 weeks including combined exercises for the experimental groups was designed.

Resistance training with weights (bench press, latpulldown, rowing, leg press, leg extentionandleg curl) wastwo sets with 10-12 repetitions per every session and with an intensity of 40-60% of maximum repetition and rest time of 60-90 seconds. Aerobic exercise was walking and running on treadmill with an intensity of 60-75% of maximal heart rate. The exercise schedule was 3 times a week and continued for 10 weeks. Once every 4 weeks a new maximum repetition of the individuals was re-measured and weights changed with new 1RM. In addition, aerobic exercises increased for 5%.

Every exercise session included 7-10 minutes of warm-up and 7-10 minutes of cooling down including stretching exercises.

The subjects were recommended not participate in any other sports activities for 10 weeks.

Anthropometric indices like height, weight, BMI, body circumferences, and fat percentage were measured and recorded again after the 10-week period.

PROTOCOL	GROUPS
7-10 min warm up	
bench press, latpulldown, rowing, leg press, leg extention and leg curl 40-60% 1RM,60-90s rest Endurance training, running 60-70% maximum heart rate	**concurrent training (22 min endurance training and 22 min resistance training)**
7-10 min cooldown	
10 weeks training **-every 4 weeks new 1RM has been measured** **- every 4 weeks endurance training intensity 5% increased**	

In the group of daily consumption of garlic supplementary, 2 bulbs of garlic were considered to be eaten with meals.

Blood sampling and laboratory analysis

The first phase of blood sampling was carried out right before the first exercise session using the subjects left vein. The blood factors that were considered in the present study included insulin, and Lcn2. Forty-eight hours after the 10-week period, blood sampling was again carried out in order to evaluate the mentioned parameters. Blood samples ware centrifuged for 15 minutes with a speed of 3000 rpm in order to separate the plasma and they were stored at -70˚C.

Insulin and Lcn2 were measured by ELISA-Enzyme method, and, insulin resistance was measured using the following formula:

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

Data analysis

Normality of the collected data was determined using Kolmogorov-Smirnov test. To check the effect of different exercises on dependent variables, paired-samples t-test was employed. One-way ANOVA was employed to determine before- and after-exercise changes, and whenever it was significant, Bonferroni post hoc test was utilized. In all of the tests, the error level was set at $P<0.05$. SPSS 16.0 was used for data analysis. Excel was used to draw the graphs.

RESULTS

Groups	control	supplement	Supplement + exercise	exercise
age	41.3±5.91	38.8±4.47	41.4±6.11	37.8±9.12
height	160.9±5.28	162.2±5.92	164.9±5.25	162.2±6.90
Weight(kg)	77.94±6.09	79.83±5.61	79.18±7.12	75.72 ± 4.78 75.72±4.78
Body fat percent	44.33±3.26	43.94±4.53	42.02±3.75	42.62±6.10
Lcn-2	1371.56±474.47	1412.62±314.93	1112.84±128.72	1403.80±162.58
insulin	6.7±2.16	7.18±3.38	6.53±3.39	5.39±1.71
Insulin resistance	1.40±0.45	1.46±0.70	1.45±0.76	1.12±0.33

calculated P-value for body fat percent (P=0.706) and body weight (P=0.068) was more than the significant level, there for 10-week concurrent exercise and consumption of garlic supplementary had no significant effect on body fat percent and weight among middle-aged inactive women.

The results of the present study indicated that changes in serum levels of lipocalin-2 among middle-aged obese women in response to 10-week combined exercise and consumption of garlic supplementary was not significantly different from the control group (P=0.459). However, according to the results of paired-samples t-test, it was concluded that 10-week combined exercise and consumption of garlic supplementary had a significant effect on the increase of lipocalin-2 among middle-aged obese women (P=0.000). However, the level of lipocalin-2 in the group of combined exercises and garlic supplementary consumption did not alone a significant effect (P>0.05).

According to the results of one-way ANOVA, there was no significant difference between the two groups in regard with the serum of before and after the study as a result of 10-week combined exercise and consumption of garlic supplementary (P=0.479). However, according to the results of the t-test, conduction of 10-week combined exercise and consumption of garlic supplementary had a significant effect on reduction in the level of insulin serum among middle-aged inactive women (P=0.028). Moreover, conduction of 10-week combined exercise and consumption of garlic supplementary had a significant effect on decreased insulin resistance (P=0.018).

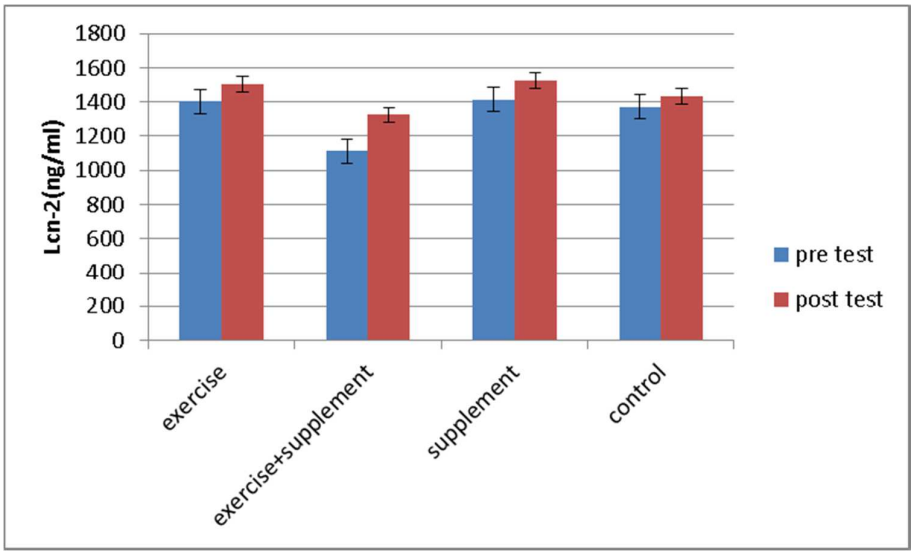

Figure 1. Lcn2 before and after 10 weeks training in groups

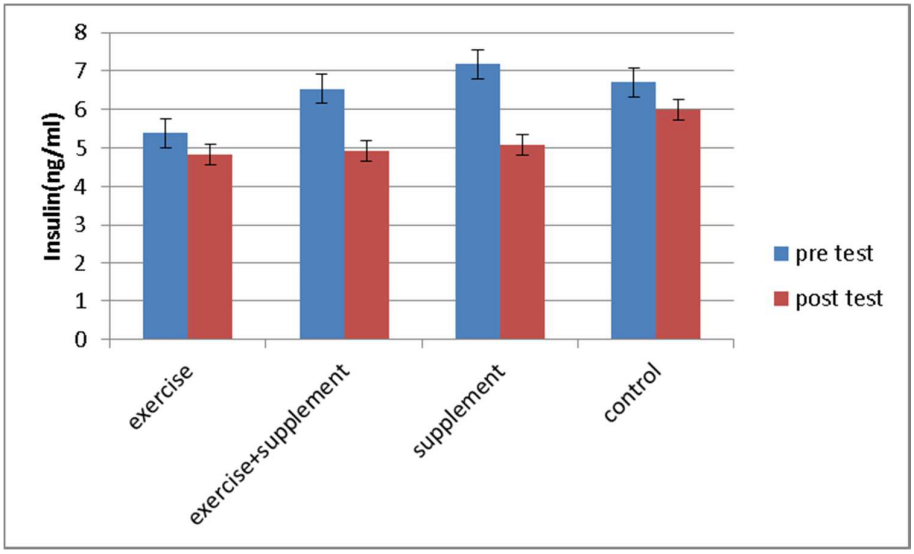

Figure 2. Insulin before and after 10 weeks training in groups

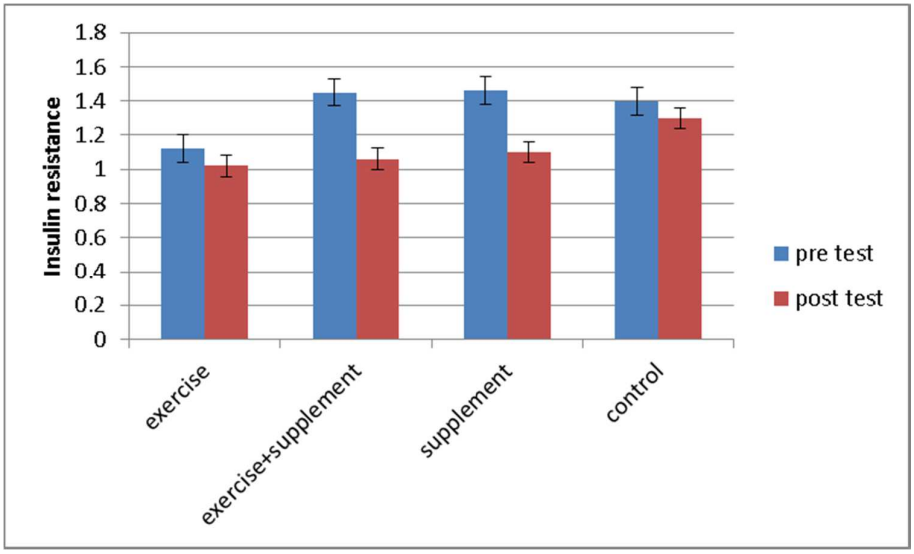

Figure 3. Insulin resistance before and after 10 weeks training in groups

DISCUSSION

Obesity is an epidemic inflammatory disorder in public health [29]. Presence of general inflammation in obesity, especially central obesity, accompanies increased occurrence and development of cardiovascular diseases such as atherosclerosis[13]. Inactivity and obesity are two major mechanisms in justifying and interpreting the relationship between development of diabetes and cardiovascular diseases. With an increase of 1 unit in BMI, the risk of cardiovascular diseases rises 8%. On the contrary, an increase of 1 day in physical activities, the probability of cardiovascular diseases drops 8% [2]. Conducted studies over the last decade focused on the role of adipose as an active endocrine organ and secretory paracrine and effective in controlling the physiological and pathological trends of the body indicated that the probability of secretion of cytokines, adipokines, and other secretory factors from adipose tissue is responsible for occurrence of chronic inflammatory conditions accompanying visceral obesity among obese or overweight subjects [4,22, 9].

Obese individuals develop resistance to the cellular actions of insulin, characterized by an impaired ability of insulin to inhibit glucose output from the liver and to promote glucose uptake in fat and muscle. Insulin resistance can be defined as a condition in which normal concentrations of insulin produce an inadequate metabolic response by insulin sensitive tissues (skeletal muscle, liver, and adipose tissue).

Lipocalin-2 has been recognized as an adipocyte-derived acute phase protein that is positively correlated with obesity, insulin resistance, type 2 diabetes, and cardiovascular disease[19].

Our study have demonstrated that after 10 weeks combined training program serum Lcn2 increased in exercise group with garlic supplement not other groups .

Moghadasi (2014) carry out 8 weeks endurance training program and resistance training program in sedentary young men. The results showed that Lcn 2 decreased in both training group compared with the control group[19].Choi (2009) indicated that there was no significant change in the Lcn2 in obese women after 12 weeks moderate exercise training[4].This discrepant result may be attributed to variation in the exercise protocols and differences in subject.

The results showed that body weight, body fat percent did not change after training. Previous studies mentioned that exercise-induced changes in body fat, especially visceral adipose tissue, may attribute to plasma Lipocalin2 decrease after the training.

The results are in agreement with previous reports showing that there was a significant positive relationship between plasma lcn2 levels with body mass, body fat percentage and WHR, suggesting that the increased fat mass might account for the elevated blood levels of this adipokine in obese individuals.

Wang (2007), showed a higher concentration of Lcn2 in obesity and this adipokine is positively related to the BMI, Waist circumference and body fat percentage[30]. Choi et al (2008) demonstrated that a positive relationship between Lcn2 and body mass [3]. Damirchi et al (2011) showed a positive relationship between Lcn2 level with waist circumference, fat mass ,BMI .Body fat percent decreased 8.8% after 8 weeks aerobic training, thus it seems that the aerobic training could offer a sufficient stimulus for plasma Lcn2 decreases[8].

Mohamadi (2014) found a significant related between Lcn2 and insulin resistance determined by HOMA-IR. They reported that chronic aerobic exercise training resulted in improved glucose tolerance during glucose clamp conditions and ultimately improved insulin sensitivity[20].

We observed no significant change in body fat percent after exercise. The lack of exercise effect on lipid profile in the present study might have been attributable to the lack of weight lose during the experimental period. Furthermore, a limitation for the present study was the experimental period. A longer study period may yield different results.it is indicated that garlic has a favorable effect on lipid profile.

The results of the studies on the effects of exercise on plasma concentrations of cytokine molecules are contradicting. Some studies have referred to an increase in the serum level of lipocalin-2 [6, 7]; however, some of them have not reported a significant change [4]. In a study, a reduction in the level of lipocalin-2 serum was observed after weight loss [14]. In the present study, there was a significant increase in the amounts of lipocalin-2 serum after conduction of 10-week combined exercise and consumption of garlic supplementary compared to the control group (P=0.000). However, there is no study conducted in order to examine the effect of garlic on this factor. The results of some studies indicate that garlic might apply the effects of hypoglycemic from two mechanisms. Garlic contains a compound called s-allyl cysteine sulfoxide (also called allicin) that possesses the same hypoglycemic effects as standard anti-diabetic medicines like glibenclamide and insulin in diabetic animals. The most probability of action mechanism for the effects of allicin is that its action compared to inactivating insulin sulfhydryl (cysteine) keeps insulin inactive and stored. Other evidence indicates the effects of garlic on increased insulin sensitivity in regard with hypoglycemic effects. Since presence of insulin signal increases liver glycogen, treatment with allicinenhances the amount of liver glycogen in diabetic animals, and the increased secretion of insulin results in improvement of insulin sensitivity. In another study, acillin supply had a clear effect on the activity of alpha glucanphosphorylase and a preventive effect on glucose-6-phosphatase in normal mice; therefore, it seems that allicin enhances insulin function [17].

Higashikawaet al (2011) studied garlic fermented extract and concluded that consuming this product can cause hyperlipidemia after 12 weeks [10]. Seo et al. investigated the effect of regular exercise and aged garlic extract (AGE) in combination among postmenopausal women. They suggested that AGE supplementation reduced cardiovascular risk factors independently of exercise in postmenopausal women (26). Therefore, the effect of combined intervention is largely unknown. Considering the literature, combining intervention may be more effective than either intervention alone (1).

In a study conducted in order to examine the effects of garlic on improvement of insulin sensitivity and its relationship with metabolic syndrome risk factors, Padiya et al (2011) concluded that the group of mice that consumed garlic experienced a significant decrease in their weights after 8 weeks. In addition to a reduction in glucose and insulin serum, triglyceride also dropped [21]. Most studies are in agreement with the present study and have reported positive effects for garlic in preventing and treating risk factors of metabolic syndrome.

Based on the findings of the present study, it is concluded that garlic supplementation along with exercise training might have additive effect on insulin resistance. Future research should investigate the efficacy of this combined intervention over a longer duration. Moreover, further studies are needed to determine the effect of this combined intervention in other populations such as diabetic patients.

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