Original Article

Effect of 12 weeks of Aqua training and green tea supplement on serum TNFα, CRP and cardiovascular risk factors in inactive obese women

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Received 03 Oct 2019, **Accepted** for publication 26 Dec 2019

Abstract

Background & Aims: The pre-inflammatory cytokines responsible for TNF-α, CRP, and lipid profiles have an important role in the development and prevention of systemic inflammation and also the development of obesity-related diseases, including cardiovascular diseases. The purpose of this study was to investigate the effect of 12 weeks of Aqua training and green tea supplement on serum TNF-α, CRP, and cardiovascular risk factors in inactive obese women.

***Materials & Methods*:** In this quasi-experimental study, 60 women with body mass index of over 30 kg / m ^{2} were randomly assigned into 4 groups: control group, Aqua training group, green tea group, and Aqua training + Green tea group. The 12-week training program consisted of three 60-minute sessions per week with an intensity of 65-75% heart rate. Green tea supplements were also taken at 500 mg per day and in three equal portions. Blood samples were used for pre-test and post-test for TNF-α, CRP, and lipid profiles. Data were analyzed using SPSS software and the level of significance was set at 5%.

***Results*:** The data showed that in the combination group (Aqua training + green tea) ($p< 0.05$), green tea group ($p<0.05$), and Aqua training ($p <0.05$) there was a significant decrease in TNF and CRP in comparison with the control group. And the results of ANOVA test showed that there is a significant difference between the effects of different interventions on alpha TNF-α ($p< 0.05$). The results of Tukey's post hoc test for TNF-α and CRP showed that there was a significant difference between the control group and the combined group (Aqua training + green tea) ($p <0.05$), between the green tea group and the exercise group ($p <0.05$), and between the exercise group and the combination (Aqua training + green tea) ($p <0.05$). In addition, in the case of lipid profile changes following different interventions, there were significant differences between the three Aqua training, the green tea group and the combined group (Aqua training + green tea) compared to the control group ($p <0.05$).

***Conclusion*:** Based on the findings of this study, it seems that supplementation of green tea supplement and Aqua training can have better results on the level of inflammatory factors in obese women.

***Keywords*:** Aqua training, TNF-α, CRP, lipid profile, obese women

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Introduction

Many studies have shown that obesity is associated with inflammation, but this inflammation is different from inflammation observed in autoimmune infections or diseases and it is a chronic inflammation (1-3)

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Inflammation in obesity is a systematic process that affects a large number of organs. When calorie and fat intake increases, the activation of inflammatory pathways in the cells begins through the perception of the brain materials and cytokines, and the process of perceiving the brains materials is done through molecular pattern recognition, including the receptors of innate immunity which is known as TLR 2,4.(4, 5) The adipose tissue secretes a large number of active peptides called Adipokines(6), and excessive increase in the normal cytokines of the tumor necrosis factor (TNF-α) and C-reactive protein (CRP) leads to mild chronic inflammation. And the presence of these cytokines indicates chronic diseases, including chronic arthritis, hypertension, cardiovascular disease, and cancer(7). CRP has been widely studied as a sensitive and non-specific index. CRP is a member of the Pentraxins family, which plays an important role in the immune response. Although this substance is produced in the liver, new research has shown that it is also produced in the atherosclerotic vascular endothelium layer(8). CRP, in addition to the inflammatory marker, can cause vessel destruction by various mechanisms such as decreasing nitric oxide production (NO), increasing adhesion of the molecules, and altering low-density lipoprotein (LDL) absorption by macrophages, increasing the amount of this protein (As the most sensitive inflammatory and predictor of cardiovascular risk) and consequently increasing the risk of cardiovascular events to 5 times(8). TNF-α is also an important pro-inflammatory cytokine that secretes from single-nucleotide phagocytes, T lymphocytes, Kupffer cells, and endothelial cells.

And the first cytokine is produced in the inflammatory cascade, and it increases the risk of heart disease by increasing the expression of the Adhesion molecules(9). On the other hand, these cytokines play a important role in regulating inflammation and tissue damage, and also lead to increased stress Oxidative, and in general, excessive increase of TNF-α cytokines lead to chronic and mild inflammation(9). There is a significant relationship between levels of these cytokines and cardiovascular diseases(10). Therefore, given the strong association between these inflammatory factors and the occurrence of cardiovascular disease, any intervention that reduces these factors can help prevent or improve the process of cardiovascular disease treatment. Inappropriate diet and inactivity are the most important causes of overweight and obesity, which are the underlying factors of no communicable diseases(11). Previous studies have shown that both the absolute fat of the whole body and the central distribution of fat, which includes abdominal visceral fat, are closely related to diabetes, hypertension, increased blood fats, and cardiovascular disease. The mortality rate of cardiovascular disease among obese men and women is three times higher than other people(12). However, the genetic background can also contribute to the development of obesity in different individuals(12). According to the findings, the best way to treat obesity is to reduce blood lipoproteins using a diet that is compatible with exercise(12). On the other hand, studies have recently discovered active bio-phytochemicals in foods contribute to the prevention of chronic diseases such as cardiovascular disease, inflammation, and metabolic diseases, including obesity (13). One of the phytochemicals is polyphenol. Green tea has a high percentage of polyphenols called catechins. Green tea has five major catechins, the most important of which is Epigallocatechin gallate (EGCG) (14). And the green tea's protective effects are essentially attributed to its polyphenolic content(15). Also, the polyphenol green tea has anti-mutagenic, anti-diabetic, anti-bacterial, and anti-inflammatory properties(16). Likewise, green tea fluids, in addition to the effect on fat metabolism improve lipid

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liposuction(16). The results of epidemiological studies have shown that long-term consumption of green tea is significantly associated with a reduction in the risk of cardiovascular disease(17). Although there is recent evidence that EGCG has a metabolic role and can be effective in reducing body fat(16). EGCG also reduces food intake, triglyceride, and blood cholesterol levels and, on the other hand, stimulate energy recovery and increase HDL levels(16). The use of green tea as a treatment for obesity may be due to its role in reducing body fat mass, apoptosis, adipocyte, and inhibition of adipogenicity, as well as other biological and pharmacologic benefits of green tea including its anti-allergic, antioxidant activity and its anti-inflammatory effects (18, 19). This results in the reduction of inflammatory factors such as lipoxygenase, cyclooxygenase, nitric oxide synthase, and tumor necrosis factor-alpha (TNF-α) (19). Contradictory results have been reported concerning the effect of exercise on TNF-α and CRP. Also, controversial results have been reported regarding the effects of green tea on TNF-α and CRP at resting levels. In a study, Crouzier et al. studied human leukocytes exposed to epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) and observed that the catechins did not affect TNF-α while the TNF-α inhibitor was reported with EGCG (20).To reduce the deleterious effects of TNF-α and CRP performance, antioxidant and anti-inflammatory supplements can be used(18) and exercise activities suppress low-grade inflammation (21). Sport exercises are effective in reducing the TNF-α and CRP receptors and adhesion molecules in patients, such as those with obesity or chronic heart failure, and reductions are associated with improved aerobic fitness. Long-term exercises decrease the severity and frequency of body weight, body fat, and TNF-α (22). Kenah et al. (2013) evaluated the effect of eight weeks of green tea extract on reducing obesity and lipolysis pathways in high-fat diets. Results showed that a daily intake of 400 mg/kg body weight of green tea extract improves lipolysis and weight loss(23). In the study of green tea consumption and exercise training, body fat was decreased(22). Recently, in a study by Haggini et al. (2013), the use of green tea extract and sport exercises resulted in a significant decrease in fat percentage and body mass index and increased cardiac and respiratory capacity in obese men(24). Considering that most surveys independently have studied the effect of green tea and no study directly investigated the effects of simultaneous consumption of green tea supplementation and Aqua training on cardiovascular factors, therefore, the present study evaluated the effect of 12 weeks of Aqua training and green tea supplement on serum TNFα, CRP, and cardiovascular risk factors in inactive obese women.

Materials and Methods

The present study was a semi-experimental pre-test-post-test study with a control group. The subjects of this study were 60 inactive woman within the age range of 35-35 years who had no regular exercise during the last two years. Initially, by installing announcements, overweight or obese people who wanted to exercise to adjust their weight and improve their physiological status were identified by the researcher and 60 of them randomly and according to the criteria for entering the research were chosen. The criteria for entering the study included: Women aged 30 to 35 years old, BMI greater than or equal to 30 kg / m^2 not having a regular physical activity (meaning regular activity in average intensity of more than 30 minutes on most days of the week), not smoking in the last six months, not having the underlying disease that prevents physical activity or dieting, not consume a drug that has a heart rate effect associated with body weight. And the exclusion criteria were pregnancy during the study, the reluctance to continue to participate in the study. Subjects were randomly assigned into four groups of 15, including

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control group, green tea group, Aqua training group, and combined group (Aqua training with green tea). 500 milligrams of Green Tea Leaf Powder with a standard stamp from Dineh Pharmaceuticals Company was consumed and three meals were taken daily for 12 weeks by the subjects(13). The reason for choosing a green tea pill was due to the ease of use by the subjects. The control group continued to change its daily lifestyles without changes during the 12 weeks. There was no sample loss in this study. The height and weight of the subjects were measured with an accuracy of 5 mm and 0.2 kg, respectively. Body mass index (BMI) was also calculated by dividing the weight by squared height (kg / m^2). Blood samples were taken from the Brachiocephalic vein (5 ml) after 12 hours of fasting and 24 hours before the start of the protocol and 24 hours after the activity and then were poured into ethylene diamine tetracyclic glasses and, after transfer to the laboratory, they were centrifuged at 3000 rpm for 10 minutes, and the plasma obtained from the isolation was kept until the day of the experiment in two-ml micro-tubes at -70 ° C to measure TNF-α, CRP and lipid profiles. TNF-α of plasma was measured by ELISA method using an ELISA Buster Immunoassay Kit, using the Eliseo BioTech ELX800 Model, manufactured by the United States. The sensitivity threshold for TNF-α was one picogram per ml, and the serum CRP level was measured by nephelometric method using the CRP kit (England's Building Site Company Kit) with a sensitivity of 0.14 mg/dl and lipid profile (TG, TC, HDL, LDL) was measured using the Pars kit made in Iran.

Aqua training Program:

After explaining the stages of the research process, a satisfactory knowledge form was obtained from the subjects. Control group and green tea group did not attend the training program. However, each group was trained for 12 weeks and 3 one hour sessions per week in Aqua training. Initially, they participated in a 4-session program on how to practice water activities. During these sessions, the learner was first trained to determine the maximum heart rate following various exercises in the water by touching the cervical artery (carotid) with two fingers in 15 seconds and multiplying it in number 4 within two weeks, and on the basis of this information, feedback was given on the intensity of training. The Aqua training briefly included increased movements with an intensity of 50-75% of the maximum heart rate that were performed in the following three steps:

1- *Heating phase*: The duration was 10 to 15 minutes. During this stage, the subjects prepared their own body to practice the exercise program by walking in the pool and doing tensile and relaxation exercises.

2- *The main program phase*: The duration of this phase was 35-40 minutes, during this time, the main training was given for each session. Generally, the exercises performed at this stage included a series of tensile gestures, walking in different directions, water jogging and tightening movements, and performing 10 simple moves on the upper and lower extremities of big muscles that were exercised by a sports instructor in water and regarding the physiological status and ability of the subjects. One minute was considered for each move and the subjects were asked to perform the required maximum power. The principle of overload was applied with tightening movements, using combined movements, and shortening the rest time between the sets.

3- Cooling phase: the subjects did gentle walking with simple movements in low-intensity with sleeping on water, they did cool down for 5-10 minutes(25).

Statistical Analysis:

In order to analyze the data, initially after the test, the assumption of the natural distribution of variables using Shapirovailk and the equality of the variances test, in the

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general linear model, dual-mode analysis of variance was used to determine the interaction between the two factors of training and complement on the variable research was used. In the case of the ANOVA significance, Tukey's post hoc test was used to determine the difference between groups. To determine the difference between the pre-test and post-test values in each group, t-test was used. Significant level was considered at the alpha error level of 5% (p <5%).

Results

The results of the statistical test showed that, in comparison to the pre-test, the mean of pre-test variables of weight, BMI, waist to hip ratio in two groups (exercise in green tea and Aqua training condition) and Aqua training after 12 weeks of interventions showed a significant decrease (p <0.05) (Table 1). On the other hand, the results of ANOVA test showed the effect of interaction between Aqua training and green tea in reducing the concentration of TNF-α and CRP in obese women was significant (p <0.05). Therefore, changes in serum TNF-α, CRP, and TG were not only influenced by the effect of the exercise in green tea and Aqua training, but the combined Aqua training + green tea had a synergistic effect on reducing the concentrations of these factors. The contribution of reducing these factors in the combined group (Aqua training + green tea) was higher than other groups (Table 2). Tukey's post hoc test showed that there was a significant difference in the mean distribution of these variables between the combined groups (Aqua training + green tea) and Aqua training (p <0.05). The highest range of changes and reduction of these indicators were related to the Aqua training + green tea (Table 2). Also, the results of the ANOVA test showed that the effect of the training, regardless of complementary effect, was significant in decreasing LDL-C and cholesterol variables (p <0.05). However, the interaction effect of Aqua training and green tea on LDL-C reduction was significant (p <0.05). On the other hand, HDL-C increased in all groups (except for the control group) and intervention of two exercise factors in Aqua training and green tea increased this factor.

Table 1: The values of the mean change in pre- and post-test anthropometric variables studied in groups

value	Stage of test	Aqua training + green tea	Aqua training	green tea	control
Age		32/58±5/93	33/94±1/37	32/93±27/26	32/8±1/24
Height		161/37±23/1	163/2±3/27	160/26±1/88	161/66±6/46
Weight	Pre - test	83/28±1/1	83/21±54/2	83/39±1/7	82/85±7/9
	Post - test	87/18±3/2*	80/43±3/76*	80/28±5/36*	83/74±4/1
BMI (kg/m2)	Pre - test	32/48±4/85	32/39±6/23	32/61±1/49	32/21±1/24
	Post - test	29/38±2/29*	30/01±1/31*	30/18±5/14*	32/92±6/37*
Waist to hip	Pre - test	1/8±0/5	1/9±0/3	1/8±0/7	1/7±0/4
ratio	Post - test	1/1±0/7*	1/3±0/9*	1/5±0/4*	1/8±0/8

Values are expressed as Mean ± standard deviation.

* Significance related to the pre-test values in each group

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Table 2: The results of ANOVA test and Tukey's post hoc test were used to determine the difference between the studied groups

value	Stage of test	Aqua training + green tea	Aqua training	green tea	control	Supplement	Aqua training	Interaction of Supplement * Aqua training
TNF-α	Pre - test	18/5±5/6	18/1±5/4	17/87±5/9	18/3±4/3			
	Post - test	13/2±4/5*	15/1±3/6*	15/2±4/1*	18/5±7/5*	P<0/05	P<0/05	P<0/001
CRP	Pre - test	1/6±2/7	1/6±3/1	1/61±2/7	1/6±2/34			
	Post - test	0/52±2/6*	0/75±1/7	0/86±3/6	1/7±1/21	P<0/05	P<0/05	P<0/001
LDL-C	Pre - test	107/9±15/3	107/4±18/3	107/3±14/4	106/3±16/2			
	Post - test	100/4±2/7*	103/9±17/3*	104/9±23/5*	107/1±12/1	P<0/05	P<0/05	P<0/001
HDL-C	Pre - test	42/9±12/3	42/2±5/1	42/4±11/8	42/2±13/1			
TG	Post - test	48/7±4/9*	46/3±13/6*	46/8±2/6*	43/1±1/2	P<0/05	P<0/05	P<0/001
	Post - test	167/1±84/1*	171/2±27/3*	172/9±28/1*	173/3±18/2	P<0/05	P<0/05	P<0/001
TC	Pre - test	176/8±38/9	178/5±15/3	176/8±23/2	177/9±23/8			
	Post - test	168/4±36/3	170/1±12/5	172/3±11/8	178/1±25/1	P<0/05	P<0/05	P<0/001

Values are expressed as Mean ± standard deviation

*Significant amount of pre-test values

Discussion and Conclusion

The purpose of this study was to investigate the effect of 12 weeks of Aqua training and green tea supplement on serum TNF-α, CRP and cardiovascular risk factors in inactive obese women. In the present study, after 12 weeks of Aqua training, there was a significant decrease in weight, BMI, and waist to hip ratio in groups (green tea, Aqua training, combination in Aqua training + green tea) in comparison to control group and also, lipid profiles showed a significant improvement. TNF-α, and CRP also showed a significant decrease in all three groups compared to the control group The decrease was observed in Aqua training + green tea group more than in Aqua training treatment group and green tea group. Traditional bio-indicators such as lipid profiles combined with new inflammatory markers (TNF, CRP) of the immune system are used to predict the risk of chronic diseases(26). TNF-α is the first cytokine produced by the inflammatory cascade and is associated with a reduction

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in blood glucose eradication, in fact, an increase in the level of TNF-α in the circulation with the rise and development of atherosclerosis(26).

One of the most important human advances in understanding obesity is the emergence of the concept of chronic low-grade inflammation, that basis of this view is related to the increase in serum levels of several inflammatory markers, including pro-inflammatory cytokines and acute-phase proteins. In obesity, their levels increase, and the most important of them are TNF-α and CRP (27). Decreased levels of TNF-α due to green tea and exercise compared to the control group in obese women may lead to a reduction in inflammatory responses in these subjects(28). In the present study, there was a significant reduction in TNF-α and CRP levels in all three groups (green tea, Aqua training, and Aqua training + green tea). Molina et al. showed that the eight weeks of exercise, along with the use of green tea, reduced the pro-inflammatory cytokines and decreased TLR-4 expression in obese mice(28). Therefore, one of the mechanisms responsible for the reduction of TNF-α in the present study is due to the inhibition of the TLR-4 signal by green tea. Previous studies showed that EGCG inflammatory responses were directly regulated with suppression of TLR-4 expression and protein levels(29). Activation of TLR-4 leads to the stimulation of the signal pathway of MAPK and NF-Kb, which leads to an increase in the transcription of pro-inflammatory agents(28). Since the NF-Kb signal pathway results in the transcription of pro-inflammatory factors, it seems that one of the mechanisms of TNF-α reduction in the present study is to inhibit the NF-Kb signal, as reported in previous studies(30). Also, Bayan et al.(29) and Joe et al.(31) showed that the reduction of pathogenic TLR-4 pathway caused by catechine EGCG green tea is responsible for reducing the expression of protein cytokines such as TNF-α by inhibiting or reducing the binding of NF-Kb to the DNA. It was also observed in human intestinal epithelial cells and brain microvascular endothelium that EGCG can inhibit NF-Kb(32). Dehghan pisheh et al. (2015) concluded that 8 weeks of aerobic training led to a significant decrease in TNF-α in women (33). Also, Farazandeh Nia et al. (2017) found that 8 weeks of exercise plus supplementation significantly reduced the risk of obesity in male mice(11). The obesity induces m2 macrophages to m1 and via reducing the production of arginase and increasing the production of TNF-α that cause inflammation(34). This inhibits the tyrosine IRS-1 phosphorylation in fat cells and skeletal muscles and causing disease (34). The concentration of TNF-α and CRP in obese individuals and green tea exercises in all three groups was lower than the control group, which was consistent with findings of Bogdansky et al. (35). Overall, green tea consumption in obese women over 12 weeks led to a decrease in TNF-α and CRP after Aqua training, indicating a positive effect of green tea on decreasing the pro-inflammatory factor. Regular physical activities can also be effective in maintaining low levels of inflammatory factors, as immobility, and along with age they lead to an increase in systemic inflammation and increase the risk of disease(27). The results of this study showed improvement of lipid profile after 12 weeks of Aqua training and green tea consumption in all three groups (green tea Aqua training and Aqua training + green tea) compared to control group. This study showed that consumption of catechin-containing beverages for 12 weeks leads to a significant reduction in body fat and lipid profiles in obese women. According to this study, the mechanism of reducing fatty content and lipid profiles in this study could be due to the following reasons:

1. Catechin inhibits lipoprotein absorption with an inhibitory effect on phospholipase A2 and also inhibits lipoproteinase by inhibiting the transcription of synthetic fatty acids and acetyl carbonate carboxylase inhibitors.

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2- Catechins present in Green tea increases fat oxidation even when resting, and when combined with moderate physical activity, this increase even is more. Catechin also stimulates the prolonged sympathetic nervous system by inhibiting the catechol-methyl transferase enzyme (the enzyme responsible for the breakdown of noradrenaline in the synapse), thereby causes an increase in energy consumption.

3. Caffeine in green tea inhibits phosphodiesterase activity and decreases the breakdown of adenylate cyclase and, on the other hand, increases the activity of sympathetic nerves. The sympathetic nervous system stimulates fat and carbohydrate oxidation(36). Regarding the findings of this study, the levels of cholesterol and triglyceride and LDL in all three groups were significantly lower than the control group. Also, HDL levels in the intervention groups showed a significant increase compared to the control group. In the same study, the findings showed a decrease in LDL and cholesterol and an increase in HDL due to the consumption of green tea for 8 weeks in obese women(37) which is inline with the findings of present research. One of the mechanisms is due to the catechin in green tea, which inhibits the oxidation of LDL by CuSO4 (13), and also affects the cholesterol biosynthesis restriction inhibitor, and inhibits the synthesis of cholesterol(13). The other mechanism of action in reducing the harmful lipoproteins of the body is due to the interference with the cholesterol micelles in the digestive system, which, by the formation of insoluble cholesterol, results in cholesterol excretion through the stool and reduces the absorption of cholesterol(38). On the other hand, according to results, Phenol in green tea reduces LDL-oxidation by inhibiting serum antioxidant activity and increasing HDL levels(38).

Conclusion

In general, the findings of this study indicate that exercise Aqua training with green tea consumption with TNF-α, CRP level modification, and lipid profile improvement have a positive effect on inflammation.In comparison with different types of interventions, and the combination of two methods of exercise intervention, green tea could have a better effect on this category. It is expected that this adjustment will reduce chronic inflammation and its related complications, such as cardiovascular disease. Regarding the beneficial effects of Aqua training, this type of exercise can be achieved with the necessary precautions as an effective factor in the prevention and control of obesity and its related complications in obese people.

Acknowledgments

In this way, the researchers announce their gratitude and thanks to all the participants in the project and to the loved ones who helped in this study.

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