

Original Article**The Effect of Six-Week Aerobic Exercise Combined with Green Tea Consumption on PON1 and VO₂max Increase and Apelin, Blood Pressure, and Blood Lipids Reduction in Young Obese Men****Hematinezhad Touli, M¹*, Elmieh, A¹, Hosseinpour, A¹***1. Rasht Branch, Islamic Azad University, Rasht, Iran*Received 27 February 2022; Accepted 20 April 2022
Corresponding Author: hematinezhad.mehdi@gmail.com**Abstract**

Obesity is among the major health problems; therefore, the present study aimed to investigate six-week aerobic training with green tea supplementation on some cardiovascular markers in young obese men. After measuring physiological markers, 57 overweight men in the age range of 28-35 years old were randomly divided into four groups: aerobic exercise + green tea consumption (AE+GE; n=10), aerobic exercise (AE; n=10), green tea consumption (GE; n=10), control group (C; n=10) in which members of this group neither participated in aerobic exercise nor consumed green tea. Eighteen sessions of aerobic exercises were held over the six-week study period (three 45-minute training sessions per week). The variables were examined before the intervention and 48 hours after the last training session and tea consumption. Body Mass Index and fat percentage significantly decreased in AE+GE, AE, and GE groups ($P=0.001$ for all). Aerobic power significantly increased in AE+GE ($P=0.001$) and AE ($P=0.001$) groups. Systolic blood pressure significantly decreased in AE+GE ($P=0.006$) and AE ($P=0.002$) groups. Diastolic blood pressure significantly decreased in AE+GE ($P=0.001$) and AE ($P=0.015$) groups. Total cholesterol and low-density lipoprotein decreased in AE+GE ($P=0.001$), AE ($P=0.004$, $P=0.002$) and GE groups ($P=0.02$; $P=0.012$). High-density lipoprotein significantly increased in AE+GE ($P=0.001$) and AE groups ($P=0.04$). Apelin levels decreased in AE+GE ($P=0.001$) and AE groups ($P=0.001$). Paraoxonase-1 significantly increased in AE+GE ($P=0.001$), AE ($P=0.001$), and GE ($P=0.30$) groups. Aerobic exercise and green tea consumption effectively controlled cardiovascular risk factors in obese or overweight people. However, combining aerobic exercise and green tea consumption led to better results.

Keywords: Green tea, Aerobic exercise, Obesity, PON1**1. Introduction**

Obesity is among the major health problems in societies, and its occurrence is significantly increasing in both developed and developing countries (1). According to recent reports, the rate of obesity has tripled in developing countries over the past two decades (1), and Iran is no exception. According to the latest World Health Organization (WHO) report, more than half of Iranian adults are overweight or obese (1).

The most common and practical indicator for assessing obesity in the adult population is the Body Mass Index (BMI) (1). Abdominal obesity is a significant risk factor for cardiovascular disease, diabetes, hypertension, and dyslipidemia (1). Studies showed that, regardless of BMI, the maximal oxygen uptake in people with sedentary or inactive lifestyles is less than that of active people (2). Other studies have shown that weight gain increases the heart's workload resulting in

higher blood pressure. Jiang, Lu (3) found a relationship between obesity and hypertension.

On the other hand, apelin is an endogenous peptide identified as a ligand for the G-protein-coupled receptor APJ. Apelin belongs to the family of adipokines, which are bioactive mediators released by adipose tissue. Extensive tissue distribution of apelin and its receptors suggests that it may be involved in many physiological processes, including blood pressure regulation, body fluid homeostasis, endocrine stress response, cardiac contractility, angiogenesis, and energy metabolism (4). As one of the adipose tissue-derived peptides, apelin may be involved in obesity-related disorders; moreover, it may be related to BMI and serum apelin level. However, its role is not clear, and the empirical findings are contradictory. Some studies have shown that serum apelin-12 level is lower in young obese patients than in healthy individuals, suggesting that it may be associated with the severity of insulin resistance and obesity (4). In contrast, one study showed that serum apelin level was significantly higher in obese female children than in non-obese ones (5). According to studies, apelin and APJmRNA expression levels decreased in hypertensive mice (6), significantly associated with lower serum apelin levels in the newly diagnosed hypertensive patients (6). Numerous findings suggest that the antihypertensive effect of apelin and APJ receptors may be mediated through Nitric Oxide Synthase (4).

Paraoxonase-1 (PON1) is another protein that affects metabolism and blood pressure. Some studies have shown that PON1 decreases in central obesity, exacerbating obesity-related inflammation and oxidative stress (7). Other studies have shown that decreased obesity-associated PON1 activity may simply reflect a reduction in the concentration of its plasmatic carrier (8). A relationship has also been reported between PON1 activity and blood pressure (9).

An active lifestyle and proper eating habits can effectively control cardiovascular metabolic indicators. Green tea contains phenolic and antioxidant compounds that may effectively control cardiovascular risk factors. Other studies indicated that green tea

intake effectively lowers blood pressure (10). Green tea consumption probably leads to better results if combined with physical activity. Bagheri, Rashidlamir (11) found that green tea consumption combined with endurance training improves the body composition of obese women.

Nevertheless, Maki, Reeves (12) reported that green tea consumption combined with aerobic exercise did not significantly impact total body fat mass. Ichinose, Nomura (13) reported that moderate-intensity aerobic exercise combined with green tea intake increased peak aerobic power. However, green tea consumption alone had no significant impact on aerobic power (13). Sheibani, Hanachi (14) reported increased apelin levels in obese participants due to aerobic exercise.

In contrast, Kadoglou, Vrabas (15) reported decreased apelin levels in diabetic patients due to aerobic exercise. In general, no clear results have been reported concerning the effect of aerobic exercise and green tea consumption on the variable mentioned. Therefore, the present study examined the impact of a six-week aerobic exercise program combined with green tea consumption on blood pressure, aerobic power, fat percentage, BMI, serum apelin, and PON1 levels.

2. Materials and Methods

2.1. Participants

Fifty-seven overweight men in the age range of 28-35 years old from Talesh, Iran, voluntarily announced their willingness to participate in this semi-experimental study. Therefore, a questionnaire covering personal information, medical records, and sports experiences was designed and distributed among the participants. This study required the participation of physically healthy young men with BMIs between 25 and 30. After collecting and analyzing the volunteers' information, 40 participants were included in the study. They announced their willingness to participate in the study after the authors explained the study process. In doing so, each of them completed a letter of commitment designed to ensure their continued cooperation during research data production.

2.2. Procedure and Data Collection

After selecting the study's participants, an introductory session was held before the onset of the exercise program to help them get acquainted with the training environment and different phases of the study. After measuring the participants' height, weight, and BMI, they were randomly divided into four groups:

Group 1 (aerobic exercise + green tea consumption) (AE+GE; n=10)

Group 2 (aerobic exercise) (AE; n=10)

Group 3 (green tea consumption) (GE; n=10)

Group 4 (control group; members of this group neither participated in aerobic exercise program nor consumed green tea) (C; n=10)

Each participant's systolic and diastolic blood pressure, height, weight, fat percentage, and aerobic power were measured 48 hours before the onset of the training protocol. Then, a participant information sheet (i.e., a form containing the explanation of the conditions each participant was required to comply with during the study) was distributed among the participants of the study. At the end of the session, the participants received their green tea.

Moreover, 48 hours after the end of the exercise program (six weeks), the participants' systolic and diastolic blood pressure, height, weight, fat percentage, and aerobic power were premeasured in similar conditions to those of the pretest.

2.3. Green Tea Supplementation and Consumption

Members of the groups AE+GE and GE were asked to consume green tea three times per day for six weeks. In doing so, 200 ml of boiling water were poured into a cup containing three grams of dry green tea leaves; the combination was brewed for 6 to 8 minutes and used 2 hours after each meal (11).

2.4. Aerobic Exercise Program

Eighteen aerobic exercise sessions were held over the six-week study period (three 45-minute training sessions per week). Each session included a 10-minute warm-up exercise, 30-minute aerobic exercise, and 5-minute cool-down exercise; the intensity of the exercise

protocol began by 50% of the participant's maximum heart rate and increased 5% each week until it reached 70% in the last two weeks (Table 1) (11).

Table 1. Aerobic Exercise Program

Week	Heart Rate	Warm-up Duration	Exercise Duration	Cool-down Duration
1	50%	10 min	30 min	5 min
2	55%	10 min	30 min	5 min
3	60%	10 min	30 min	5 min
4	65%	10 min	30 min	5 min
5	70%	10 min	30 min	5 min
6	70%	10 min	30 min	5 min

To determine the participants' target heart rates, they were asked to record their resting heart rates for three consecutive days. Then, after determining the participants' resting heart rates (the average of the three resting heart rates for each participant), their target heart rates were calculated by the Karvonen formula (3):

Karvonen formula= ((220-age) - resting heart rate) * (resting heart rate + exercise intensity)

2.5. Height, Weight, and Fat Percentage Measurement

The participants' heights were measured in centimeters using a tape measure with an accuracy of 0.1 cm. The participants' weights were measured in kilograms (minimal clothing and no shoes) via a HARDSTONE digital scale, model HS-HS018 (made in Korea). By pinching their chest, abdomen, and thigh skins, participants' subcutaneous fat was measured using a SAEHAK caliper model SH5010 (made in England). The measurement was repeated three times and at 20-second time intervals on the right side of each participant's body, and the average of the three measurements was calculated and recorded. The fat percentage was calculated using the Jackson-Pollock Formula:

Jackson-Pollock Formula=1.1093800-0.0008267SSF +0.0000016 SSF² -0.0002574 age

2.6. Blood Pressure Measurement

Systolic and diastolic blood pressure were measured at the beginning and the end of the experiment; for this

purpose, the participants were asked to rest for 15 minutes. Then, using a GALA digital wrist blood pressure monitor, model TD-3022 (made in Thailand), each patient's blood pressure was measured three times with at least one-minute time intervals while the patient was in a sitting position; the average of the three measurements was considered his final blood pressure (3).

2.7. Aerobic Power Measurement

The participants' weights were measured while they were putting on running clothes. They walked one mile (1,609 meters) on flat ground at maximum speed. Then, their 15-second heart beats were multiplied by four and placed in the formula. The time was recorded by a timer in seconds and placed in the formula in hundredths of a minute (3).

Maximal oxygen uptake (ml/kg/min) - $132.85 - (0.1692 * \text{weight (kg)}) - (0.3877 * \text{age}) + (6.315 * \text{gender}) - (3.2649 * \text{time (milliseconds)}) - (0.1565 * \text{heart beats per minutes})$.

In this study, the normality of the data was determined using the Kolmogorov-Smirnov test. The collected data were analyzed via analysis of variance (ANOVA), Bonferroni post hoc test, and paired t-test. Tables, graphs, means, and standard deviations were also used to represent the collected data. All the statistical calculations were conducted using SPSS software (version 28) at the error level of 0.05.

3. Results

3.1. Body Mass Index and Fat Percentage

The BMI and fat percentage significantly decreased in AE+GE ($P=0.001$), AE ($P=0.001$), and GE groups ($P=0.001$); no significant difference in BMI and fat percentage was observed in the control group ($P=0.267$, $P=0.99$, respectively). The results of one-way ANOVA showed significant differences in BMI ($P=0.04$) and fat percentage ($P=0.03$) between the study's groups. Bonferroni post hoc test results showed that members of the AE+GE group had lower BMIs

($P=0.025$) and fat percentages ($P=0.031$) compared to members of the control group, though no significant difference was found between other groups ($0.05 \leq P$). (Table 2 and Figure 1).

3.2. Aerobic Power

Although aerobic power significantly increased in AE+GE ($P=0.001$) and AE ($P=0.001$) groups, it showed no significant change in GE and control groups ($P=0.385$). The results of one-way ANOVA showed significant differences between the study's groups concerning aerobic power ($P=0.001$). Furthermore, significant differences in aerobic power were observed between AE+GE and control groups ($P=0.005$), AE+GE and GE groups ($P=0.002$), AE and GE ($P=0.005$), and AE and control groups ($P=0.010$).

3.3. Systolic and Diastolic Blood Pressure

Systolic blood pressure significantly decreased in AE+GE ($P=0.006$) and AE ($P=0.002$) groups. Diastolic blood pressure significantly decreased in AE+GE ($P=0.001$) and AE ($P=0.015$) groups. The Bonferroni post hoc test results indicated significant differences between AE+GE and control groups ($P=0.001$) and between AE and control groups ($P=0.001$) concerning systolic and diastolic blood pressure measures.

3.4. Fat Profile

Total cholesterol and low-density lipoprotein (LDL) significantly decreased in AE+GE ($P=0.001$), AE ($P=0.004$, $P=0.002$) and GE groups ($P=0.02$; $P=0.012$). Although high-density lipoprotein (HDL) significantly increased in AE+GE ($P=0.001$) and AE groups ($P=0.04$), no significant difference was observed between other groups. The results of the Bonferroni post hoc test indicated significant differences in total cholesterol and LDL between AE+GE and control groups ($P=0.001$), AE and control groups ($P=0.007$; $P=0.004$), and GE and control groups ($P=0.030$; $P=0.021$). Furthermore, significant differences were observed in serum HDL levels between AE+GE and control groups ($P=0.001$), AE and control groups ($P=0.003$), and AE+GE and GE groups ($P=0.03$) (Figure 2).

Table 2. Markers changes in four groups

Variables	Exercise Green Tea		Exercise		Green Tea		Control	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
BMI (kg/m ²)	29.3±2.3	25.11±1.0	28.9±3.1	26.5±3.12	29.73±1.16	27.11±1.15	28.32±3.3	28.81±3.8
Fat Percentage (%)	22.46±3.7	19.01±3.2	22.5±2.22	19.81±1.5	22.9±3.7	20.46±3.7	22.9±3.6	22.1±1.8
VO ₂ max	38.72±2.6	42.45±3.7	38.55±3.31	41.7±2.16	39.22±1.43	39.7±3.31	38.9±2.3	38.21±2.3
Systolic Blood Pressure	140±12.88	120±10.88	140±13.17	125±10.12	140±19.12	138±17.12	140±19.5	140±18.5
Diastolic Blood Pressure	90±5.2	78±3.2	89±4.5	79±4.9	90±6.12	89±9.8	90±7.21	90±4.2
Cholesterol (mg/dL)	187.43±27.88	140.23±12.32	192.43±29.2	157.43±9.1	190.43±24.78	165.43±27.14	196.55±27.13	200.21±13
LDL (mg/dL)	100.34±20.12	68.15±12.31	105.43±30.14	70.17±14.25	104.16±22.31	80.17±14.25	106.21±20.15	109.3±9.2
HDL (mg/dL)	41.3±5.12	49.17±6.2	40.2±4.3	48.17±3.4	41.5±7.2	42.4±4.8	41.18±4.2	41.2±2.7
Apelin (pg/ml)	350.4±3.22	200.6±20.18	360.4±27.5	250.5±22.5	360.55±27.16	340.2±15.1	370.4±20.3	372.16±12
PON1 (nmol/min/ml)	130.4±19.4	154.3±22.12	132.4±20.15	148±12.3	130±22.13	138.3±18.23	132.3±18.2	131±16.4

LDL: low-density lipoprotein; HDL: high-density lipoprotein; PON1: Paraoxonase-1

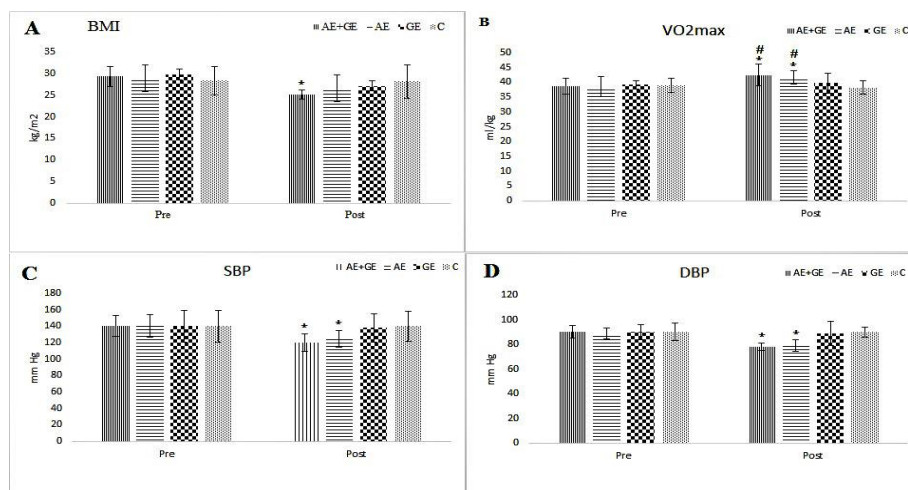


Figure 1. BMI, VO₂max, SBP, and DBP values in four groups of AE+GE, AE, GE, and Control A (value), BMI (value), SBP (C), VO₂max and DBP (D)

*Significant in comparison to the control group in the post-test phase.
#Significant in comparison to the GE group in the post-test phase.

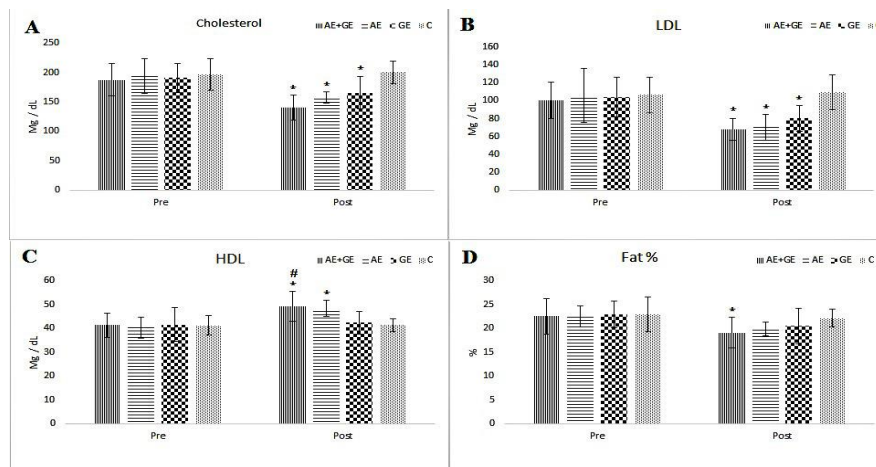


Figure 2. Cholesterol, HDL, LDL, and fat percentage in four groups of AE+GE, AE, GE, and Control A (cholesterol level), B (HDL level), C (LDL level), and D (fat percentage)

*Significant compared to the control group in the post-test phase.
#Significant compared to the GE group in the post-test phase.

3.5. Apelin

Apelin levels significantly decreased in AE+GE ($P=0.001$) and AE groups ($P=0.001$). However, no significant difference was observed between other groups. The Bonferroni post hoc test results indicated significant differences in apelin level between AE+GE and control groups ($P=0.001$) and between AE and control groups ($P=0.001$).

3.6. Paraoxonase-1 (PON1)

Although PON1 significantly increased in AE+GE ($P=0.001$), AE ($P=0.001$), and GE ($P=0.30$) groups, it did not change substantially in the control group. The results of the Bonferroni post hoc test indicated significant differences between AE+GE and control groups ($P=0.001$), AE and control groups ($P=0.03$), and GE and control groups ($P=0.03$) (Figure 3).

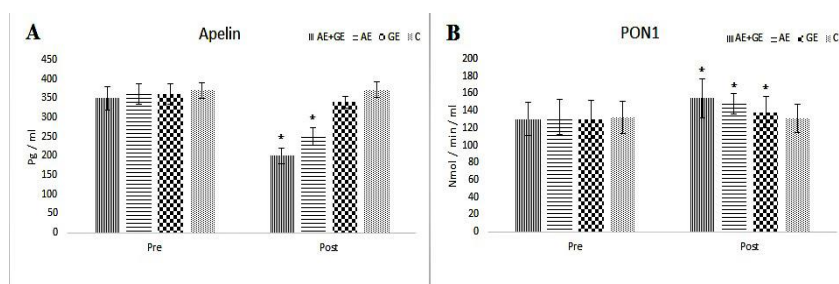


Figure 3. Apelin and PON1 levels in four groups of AE+GE, AE, GE, and Control
A (apelin level), B (PON1 level)

*Significant in comparison to the control group in the post-test phase.

4. Discussion

The present study examined the effect of a six-week aerobic exercise program combined with green tea consumption on blood pressure, aerobic power, fat percentage, BMI, and serum apelin and PON1 levels in overweight young men. The results indicated a significant decrease in BMI and fat percentage among members of the AE+GE, AE, and GE groups. Although BMI was not statistically different between the study's groups, the fat percentage was significantly lower in the AE+GE group than in the control group. As Bagheri, Rashidlamir (11) reported similar results. However, Maki, Reeves (12) reported that green tea consumption combined with aerobic exercise showed no significant impact on total body fat mass.

Energy expenditure is several times higher during exercise than at rest; during exercise, the absolute rate of lipolysis and fat oxidation also increase (16). According to the results of previous studies, anthropometric measurements showed a 39% increase in body fat percentage reduction when green tea consumption is combined with aerobic exercise,

indicating that green tea increases fat metabolism during aerobic exercise (16). Ichinose, Nomura (13) observed that green tea consumption increased the total body fat expenditure ratio during exercise. Feeding animals with green tea leaves increases the expression of fatty acid translocase gene CD36 and medium-chain acyl-CoA dehydrogenase (MCAD), proteins involved in fat transfer and oxidation. In addition, GTE reduces malonyl-CoA to increase the activity of carnitine palmitoyltransferase I (17).

The increase of aerobic power is effective in reducing body fat percentage. Dhara and Chatterjee (18) found a relationship between body composition and VO_2 max and reported that overweight people with higher fat percentages have lower maximum aerobic power. Therefore, one of the causes of reduced-fat percentage is probably increased aerobic power and visceral fat consumption as a source of energy.

According to the results of the present study, a six-week aerobic exercise program combined with green tea consumption and aerobic exercise alone significantly increases aerobic power. Reducing

oxidants production by using antioxidants is one of the most important factors in increasing VO_2 max. Moreover, EGCG probably increases the arteriovenous oxygen difference in the skeleton (19). Furthermore, green tea contains other components such as caffeine and theanine in addition to catechins. It has been shown that combining such components increases fat metabolism (12). Therefore, the increase in aerobic power due to drinking catechins may be due to the synergistic effects of catechins, caffeine, and theanine.

Results of this study also showed that aerobic exercise paired with green tea intake, aerobic exercise alone, and green tea consumption alone significantly decreased systolic and diastolic blood pressure. In vitro studies have shown that green tea catechins improve blood pressure through various mechanisms, including inhibiting oxidation, vascular inflammation, and thrombogenesis, and improving endothelial dysfunction (10). Reducing the percentage of body fat as well as lowering blood lipids (cholesterol and LDL) can also lower blood pressure (20).

In the present study, cholesterol and LDL levels decreased in AE+GE, AE, and GE groups, and HDL levels increased in AE+GE and AE groups. The exact mechanisms by which green tea exerts its fat-reducing effects are still unknown. The hypothetical mechanisms are through suppressing cholesterol biosynthesis, interfering with fat absorption, and increasing cholesterol excretion. On the other hand, green tea's polyphenols increase HDL-C level by inhibiting LDL-C oxidation and increasing serum antioxidant activity (21). In addition, the effectiveness of exercise in improving fat profile is related to the enzymatic processes involved in fat metabolism, where an increase in the enzymatic activity of lipoprotein lipase (LPL) has been reported (22). Variations in fat profile may also be related to other mechanisms, such as changes in plasma hormones and LPL concentrations.

On the other hand, changes in blood fats in response to exercise depend on the initial levels of blood fats. Sugiura, Sugiura (23) reported that regular exercise

significantly decreases TC and LDL-C levels by increasing LPL and lecithin-cholesterol acyltransferase (LCAT) activities. Increasing aerobic power can lower blood lipid profiles. In other words, increasing aerobic power causes the body to use subcutaneous and visceral fats as well as blood fats as a source of energy (23).

The PON1 level also affects the fat profile. The PON1 is a Ca^{2+} -HDL-dependent enzyme related to ester hydrolase that protects LDL and cell membranes from oxidation by hydrolysis of biologically active lipid peroxides. The PON1 inhibits macrophage cholesterol biosynthesis (24). Peritoneal macrophages of PON1-knockout mice increased cellular cholesterol biosynthesis and the administration of PON1 directly inhibited macrophage cholesterol biosynthesis. This mechanism was probably related to PON1-A2 phospholipase-like activity, which led to the formation of lysophosphatidylcholine and the inhibition of cellular cholesterol biosynthesis (24).

Moreover, PON1 stimulates the flow of HDL-mediated cholesterol from macrophages and reduces the oxidized LDL absorption by macrophages (9). In this regard, the PON1 levels significantly increased in AE+GE, AE, and GE groups. This increase was higher in the AE+GE group. It seems that aerobic exercise and green tea consumption increase PON1 by reducing oxidative stress and increasing antioxidants.

In the present study, apelin levels significantly decreased in AE+GE and AE groups. Moreover, there were significant differences in apelin level between AE+GE and control groups and between AE and control groups. It seems that apelin level increases in obese people, and it can be reduced by weight loss; therefore, one of the reasons for the decrease of apelin in the present study could be weight loss and decreased fat percentage. Insulin and TNF- α reduction have also been reported as one of the mechanisms involved in apelin reduction (14).

4. Conclusion

The results of this study indicated that aerobic exercise and green tea consumption effectively control

cardiovascular risk factors in obese or overweight people. However, a combination of aerobic exercise and green tea consumption leads to better results.

Authors' Contribution

Performing exercise training, acquisition of data, drafting the manuscript: M. H. T.

Drafting the manuscript, drafting the manuscript and critical revision of the manuscript: A. A.

Statistical analysis, acquisition of data, drafting the manuscript: A. H.

Ethics

This paper was extracted from the Ph.D. thesis in sports physiology in the Islamic Azad University, Rasht Branch, with the Ethics Code of IR.SSRC.RES.1400.063.

Conflict of Interest

The authors declare that they have no conflict of interest.

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