Jundishapur J Nat Pharm Prod. 2019 February; 14(1):e61419. doi: 10.5812/jjnpp.61419.
Published online 2019 January 9. **Research Article**

Green Tea Supplementation During Resistance Training Minimally Affects Systemic Inflammation and Oxidative Stress Indices in Obese Men

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Received 2016 November 11; **Revised** 2017 September 26; **Accepted** 2017 December 30.

Abstract

Background: It is well known that green tea has antioxidant properties. Accordingly, it is important to investigate the effects of green tea on systemic inflammation and oxidative stress indices in humans during high intensity resistance training, especially in obese men.

Objectives: The aim of the present study was to examine the effect of green tea extract supplementation during high intensity resistance exercise training on oxidative stress and systemic inflammation indices in obese men.

Methods: Twenty obese men (body mass index $\geq$ 30) voluntarily participated in the current study and were randomly assigned to groups of green tea and high intensity resistance training (RT) (GR; n = 10) and placebo and high intensity resistance training (PR; n = 10). RT was performed three times a week on non-consecutive days for eight weeks. The training started at 80% of one-repetition maximum (1RM), and training intensity reached to 90 - 95% of 1RM till the end of the eighth week. The GR group consumed a green tea capsule (500 mg) each day during the eight weeks. Blood samples were collected before and after the intervention and were tested for malondialdehyde (MDA) and total antioxidant capacity (TAC). Concentrations of ofinterleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and C-reactive protein (CRP) in plasma were also measured.

Results: The results indicated significant ($P < 0.05$) or near significant improvements in all the measured blood parameters with training, but no further effects (interactions) with ingestion of green tea. Also, we found that there was not any significant relationship between MDA and TAC changes and TNF-alpha and IL-6 in either group after RT intervention and supplementation ($P > 0.05$).

Conclusions: Thus, it can be stated that low-dose green tea supplementation does not influence inflammatory and oxidative stress indices when provided along with exercise training.

Keywords: Catechin, Weight Training, TAC, Inflammation, Lipid Peroxidation

1. Background

It has been reported that obesity is one the most important risk factors of ill health in many countries, especially in the developing ones (1). One of the popular recommendations for weight loss, weight control, and increasing strength is resistance training. Resistance training has been proven to have several benefits, including weight management, prevention of musculoskeletal diseases, improvement of the cardiovascular system, and prevention of sports injuries. However, compared to aerobic rhythmic exercises, resistance training has received much less consideration as a prescription for general health and disease prevention (2).

On the other hand, resistance training such as weight training may cause oxidative stress, cell necrosis, and cell damage (3), and it has been reported that resistance training exacerbates the formation of free radicals through ischemic contracture and reperfusion (4). Increased free radicals such as reactive oxygen species (ROS) causes lipid peroxidation of polyunsaturated fatty acids in biological membranes and blood, which are more sensitive to oxidative damage than proteins (5). These free radicals are released from various types of muscle cells. Endothelial and leukocyte cells stimulate further changes during intense physical activity including synthesis of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (6) also known as proinflammatory cytokines.

The inhibition of xanthine oxidase activity, which produces ROS, by allopurinol reduces lipid peroxidation in rodents' skeletal muscles subjected to repeated contractions (7). Further, tissue priming with antioxidants has been shown to reduce ischemia/reperfusion injury in an animal model (8), suggesting that at least some of the oxidative damage related to strenuous skeletal muscle contractile activity might be reduced by increasing animals' oxidative capacity.

One of the most commonly consumed beverages rich in polyphenols is green tea, and it is well approved that polyphenols found in green tea have high antioxidant properties (9). Some studies have reported the effects of green tea polyphenols on systemic indices of oxidative stress and inflammation, and most studies have examined the damage induced by oxidative stress in athletes. For example, in weight-trained men, Panza et al. observed protective effects of 7-day intake of green tea on blood markers of oxidative stress after a bout of resistance exercise (10). However, there is little published information concerning the effect of green tea supplementation during a high intensity resistance training program on inflammation and oxidative stress in obese people. It is worth noting that obese individuals are more sensitive to oxidative stress, free radicals, and inflammation than those with a healthy body weight (11).

2. Objectives

The purpose of the current research was to investigate whether daily consumption of green tea for two months along with intense resistance training benefits oxidative stress and inflammatory adaptation in obese men, and in particular, whether markers of oxidative stress and inflammation are attenuated.

3. Methods

3.1. Subjects

Twenty untrained male volunteers aged 18 - 25 years (body mass index [BMI] $\geq$ 30) were participated in the present study. They were randomly assigned to green tea and resistance training (GR; n = 10) and placebo and resistance training (PR; n = 10) groups (Table 1) (12). The subjects had not attended any strength and resistance training programs for at least 12 months before taking part in the study. All the subjects had daily physical activity such as walking, swimming, or soccer at least once a week. The subjects were asked to fill out a medical history form, provide a written approval from a specialist, and sign an informed consent form. The exclusion criteria included having any of the contraindications defined by the American College of Sports Medicine (ACSM) and taking any supplements assumed to have ergogenic properties within 12 weeks prior to the study. The inclusion criteria were not smoking, not having any surgical operations or illnesses, and not using any drugs or dietary supplements such as antioxidant supplements and anti-inflammatory drugs within two months prior to the study. Also, none of the subjects reported taking exogenous anabolic-androgenic steroids, drugs, medications, or dietary supplements with potential effects on redox and inflammatory responses prior to or during the study. The research design of the study was confirmed by the Ethics Committee of Medical Research at Kurdistan Medical University, and the Declaration of Helsinki principles were observed in this study.

Table 1. Physical Characteristics of the Subjects Before the Intervention[a]

Variables	Resistance Plus Green Tea	Resistance Plus Placebo
Age, y	23.9 ± 2.9	22.8 ± 2.2
Weight, kg	101.5 ± 7.3	98 ± 7.8
Height, cm	176.8 ± 4.5	178.5 ± 4.8
Body mass index, kg.m^{-2}	31.8 ± 2.1	30.8 ± 1
Body fat percentage	26.9 ± 1.9	25.9 ± 2.1
1RM chest press, kg	48.5 ± 7.5	45.5 ± 7.8
1RM squat, kg	63.2 ± 11	58.7 ± 6

Abbreviation: 1rm, one-repetition maximum.
[a]All data were expressed as Mean ± SD.

3.2. Functional and Descriptive Assessments

Prior to the intervention, all the participants visited the specified sports clinic on three consecutive days for baseline assessments. In the first session, about 10-cc blood samples were collected from the antecubital vein of the left arm in seated position after a short rest. In the second session, we measured the anthropometric and physiological characteristics of the subjects including height, BMI (Seca, Mod 704, Germany) and skin fold thickness at three sites using caliper (Lafayette, Mod 01127, USA). Afterwards, the participants were trained how to perform movements and exercises properly. The exercises were chest press (CP), lat pull-down machine (LM), leg extension (LEM), and flexion (LFM) with machine and biceps (BC) and triceps curl (TC) and squat (SQT) with barbell. On the next day, maximum strength in the lower and upper body was measured in bench press and squat by an expert technician. These evaluations were repeated every two weeks using the same procedure. For this purpose, the subjects were asked to perform 10 repetitions at 50% of one-repetition maximum (1RM) estimated according to each subjects' capacity. After 120 - 150 sec, the subjects performed 1RM of each exercise until the 1RM was determined within three attempts, with 180 - 300 sec of rest between attempts. After two days, a 1 RM test was performed to guarantee optimal test-retest reliability.

3.3. Controlled Diet and Capsule Content

All the participants were asked to avoid any changes in their daily diet during the study, except for avoiding any products containing green tea and caffeinated drinks. The subjects also filled out a dietary recall questionnaire (a weekday and a weekend day) before and after any intervention. The daily dietary intake of some common antioxidant vitamins was analyzed using software (Esha Research, Salem, OR, USA). To minimize the effect of other dietary products with a high polyphenol content, the subjects were asked to limit fruits, juice, tea, chocolate, and cocoa consumption for two days before and after blood sampling (pre-test, post-test). During the study, the subjects were taking either green tea capsules (500 mg capsules, Green teadin, Iran) or placebo capsules (500 mg sucrose, after breakfast) daily. Capsules were similar in all respects (shape, size, and color). The timing of intakes was standardized to avoid possible interference with the results, and the dosage was carefully monitored.

3.4. High Intensity Resistance Training Programs

High intensity resistance training was performed three times a week on non-consecutive days for eight weeks. The exercises were chest press (CP), lat pull-down machine (LM), leg extension (LEM), and flexion (LFM) with machine, biceps (BC) and triceps curl (TC), squat (SQT) with barbell, and sit-ups. The subjects carried out three sets of eight repetitions at an intensity corresponding to 80% of 1RM, with a 90 - 120 sec rest interval between sets. The load for each exercise increased by approximately 5% every two weeks, such that the intensity of training reached 90% - 95% of 1RM by the end of the eighth week. A 30-sec rest interval between sets was added to the rest period between sets when the intensity of training increased after each two weeks. One of the limitations of the current study was the intensity of training, because the intensity was too high for the subjects at the starting point. Therefore, the intensity increased once every two weeks so that participants adapt to their training load. The subjects were advised to do the repetitions as much as they can. If they were not able to complete the repetitions, after a short recovery, we asked them to try again to complete the repetitions. It should be mentioned that the volume of resistance training was calculated in the two groups at the end of each week by the following equation: Load $\times$ repetitions $\times$ sets. A 20-minute light warm-up and cool-down using aerobic exercise was included at the beginning and after the end. All the training sessions were held at the same time of day (16.00 - 18.00 PM) at a sports clinic under the supervision of an experienced coach and technicians.

3.5. Blood Sampling and Biochemical Analyses

After overnight fasting, between 10 - 12 am and prior to any intervention and assessment, a 10-mL blood sample was obtained from the antecubital region. For plasma collection, heparinized blood samples were centrifuged at 2500 (4°C) rpm for 20 min. The same procedure was repeated exactly three days after eight weeks of the intervention. Plasma was separated from cells and transferred to sterile micro-tubes and stored at -70° until analysis. Plasma samples were used for the measurement of malondialdehyde (MDA) and total antioxidant capacity (TAC). A commercially available kit (Randox, Cat No, NX 2332, and UK) was used to measure the plasma level of TAC (13). Plasma MDA was measured based on the method of Buege and Aust. This method is based on the reaction between MDA molecules and thiobarbituric acid, which generates red agents whose absorbance is measured at 532 nm by a spectrophotometer (14). TAC and MDA are expressed based on mmol/ and nmol/mL, respectively. Also, Tumor necrosis factor-alpha (TNF-α), IL-6, and C-reactive protein (CRP) were evaluated in plasma samples. TNF-alpha, IL-6, and CRP were evaluated in duplicate by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Bender Med System). TNF-alpha and IL-6 are presented in $pg.mL^{-1}$ and CRP is presented in $mg.L^{-1}$. The inter-assay and intra-assay coefficients of variation were less than 6% for the mentioned variables. To reduce inter-assay variation, samples from each participant in each group were analyzed using the same ELISA microplate on the same day by a single technician.

3.6. Statistical Analysis

All the values are expressed as mean $\pm$ SD. Firstly, the normal distribution of all the dependent variables was confirmed with the Kolmogorov–Smirnov test. Physiological characteristics of the subjects before the intervention were homogenized by independent *t*-test. Also, independent *t*-test was used to compare the results of the dietary survey. The data regarding biochemical parameters were compared using two-way ANOVA, and repeated measures ANOVA was run to investigate the influence time or training and supplementation intervention. A significant interaction between group and time indicates the significant effect of training or supplementation on the dependent variable of interest. Correlation of MDA, TAC and BF% with TNF-α, IL-6, and CRP was tested with Pearson's correlation coefficient. All the data were analyzed by SPSS version 21, and P value less than 0.05 was considered statistically significant.

4. Results

The 1RM intra-class correlation coefficients for exercise were as follows: CP = 0.90, LM = 0.92, LEM = 0.94, TC = 0.91, BS = 0.90, and SQT = 0.91. Although total antioxidant intake increased during training in both GR and PR groups, there was no significant difference between the GR and PR groups. Antioxidant analysis of the dietary records of the GR and PR groups before and after the high intensity resistance training period is presented in Table 2.

We found that BMI was not affected by resistance training and supplementation ($P > 0.05$). However, body fat percentage (BF%) decreased by 19.7% and 15% in the GR and PR groups, respectively, although there was no significant exercise type resistance training with supplement × time for BF% ($P > 0.05$).

There were no significant differences between the GR and PR group in upper (chest press) and lower body (squat) strength before the outset of the study ($P > 0.05$). However, chest press increased significantly by 22.4% and 25.9% in GR (pre: 48.5 ± 7.5 vs. post: 59.4 ± 5.7 kg; $P < 0.001$) and PR (pre: 45.5 ± 7.8 vs. post: 57.3 ± 4.9 kg; $P < 0.001$), respectively. In addition, squat exercise significantly improved by 22.6% and 24.7% in GR (pre: 63.2 ± 11 vs. post: 77.5 ± 4.6 kg; $P < 0.001$) and PR (pre: 58.7 ± 6 vs. post: 73.2 ± 9.9 kg; $P < 0.001$) groups, respectively. However, there were no significant differences between the GR and PR groups in all the mentioned exercises after resistance training and supplementation.

There were no significant main effects for the treatment (green tea) in any of the measured blood parameters. As expected, significant main effects ($P < 0.05$) for exercise were noted for TAC ($P = 0.003$), MDA ($P = 0.021$), TNF-alpha ($P = 0.001$), and CRP ($P = 0.007$). Near-significant main effects ($P < 0.06$) for exercise were noted for IL-6 ($P = 0.054$) and CPK ($P = 0.053$). Further, there were no significant treatment × time interactions for the mentioned variables ($P > 0.05$), that is, there was no significant difference between the GR and PR groups in MDA, IL-6, TNF-alpha, CRP, and TAC after resistance training and supplementation (Table 3).

Lastly, BF% and MDA did not correlate with TNF-alpha, IL-6, and TAC in either group after the resistance training intervention (Table 4), nor did any changes occur in BF%.

5. Discussion

In the present study, we studied the effect of green tea supplementation, a substance rich in phenolic antioxidants, on MDA, lipid peroxidation, inflammation, and blood biochemical markers before and after eight weeks of high intensity resistance training in obese healthy males. For this purpose, 10 subjects performed high intensity resistance training for two months while consuming 500 mg green tea capsules on a daily basis. These data provide evidence that taking green tea pills along with high intensity resistance training for eight weeks does not attenuate lipid peroxidation and some inflammatory indices in obese. In other words, in comparison with placebo, we found that taking green tea extract was not able to improve resting oxidative stress and inflammatory markers any further than exercise training alone, indicating that changes in these markers would not occur without high intensity resistance training.

We found that high intensity resistance training caused significant increase in maximal strength in chest press (25.9%) and squat (22.5%) in the subjects. This issue indicates that resistance exercise training has been effective, while BMI had not changed significantly in the GR and PR groups.

We found that in both GR and PR groups a significant reduction in BF% had occurred, although there was no significant difference between the groups, that is, 60 days of green tea supplementation alone could not influence BF%. Since body fat may separately influence oxidative stress and inflammatory indices (11), studying BF% changes seemed to be important. Indeed, it has been reported that 12-week administration of tea catechins (400 - 600 mg/d) reduces body fat parameters (15-17). Although we asked the subjects to refrain from consuming any products containing green tea and caffeinated drinks during the study, it seems that prior habitual caffeine use led to tolerance of the anticipated effects of caffeine and catechins on body fat; therefore, green tea supplementation was ineffective in decreasing BF% in our study. Also, subjects in the present study were relatively low consumers of catechins (according to the brochure, each capsule has 350 mg/d of catechins), and they had to avoid caffeine-containing foods and beverages during the experiment (only three cups of tea per day) (18). Differences in dosage used in these studies may explain why GT did not noticeably affect body composition during training.

Our results showed that green tea did not significantly influence MDA and TAC levels during training. In our previously published work, we have shown that progressive resistance training alone can improve antioxidant defense and decrease MDA in untrained men (19, 20). In the present

Table 2. Antioxidant Analysis of the Dietary Records of the GR and PR Groups Before and After the Training Period[a,b]

Variables	GR	PR	*t*	P Value
Vitamin C (mg/d)				
Pre-training	67 ± 9.8	58.8 ± 6.5	2.57	0.29
Post-training	58.5 ± 6.2	55.1 ± 4.5	0.609	0.61
α- tocopherol (mg/d)				
Pre-training	4.8 ± 1.1	4.5 ± 1.2	0.91	0.57
Post-training	4.3 ± 0.9	4.1 ± 0.8	0.45	0.37
Vitamin A (μg/d)				
Pre-training	419.1 ± 118	435.8 ± 88.7	0.17	0.18
Post-training	438.7 ± 172.4	457.3 ± 115	0.406	0.99

[a]Nutrient data comparison between GR and PR was done with independent *t*-test. $P < 0.05$.
[b]All data were expressed as Mean ± SD.

Table 3. Biochemical Variable for GR and PR Groups Before and After Training

Variables	Resistance Training Plus Green Tea	Resistance Plus Placebo
MDA (nmol/mL)		
Pre	3 ± 0.8	3.1 ± 1
Post	2 ± 0.9	2.2 ± 1.2
TAC (mmol/L)		
Pre	1.4 ± 0.2	1.2
Post	2 ± 0.8	1.8 ± 0.7
IL-6 (pg/mL)		
Pre	4 ± 1.25	3 ± 0.9
Post	3.3 ± 1.3	2.6 ± 1.5
TNF-alpha (pg/mL)		
Pre	6 ± 1.1	5.5 ± 2
Post	2.7 ± 2.1	3.6 ± 2.4
CRP (mg/L)		
Pre	2.4 ± 1	2.5 ± 0.6
Post	1.8 ± 0.6	1.9 ± 0.4

study, green tea could not influence MDA and TAC.

One of the limitations of this study was that we did not measure catechin concentrations pre- and post-supplementation, we only measured TAC as a marker of circulating antioxidant capacity. Thus, we cannot be certain whether GT increased catechin level in the body. Others have reported that despite an increase in total plasma catechins, no changes in TAC plasma level after acute ingestion of green tea have been observed (21). However, recently Jowko et al. showed that treatment with 980 mg of this polyphenol for 2 - 4 weeks could increase TAC. Discrepancies may be due to differences in doses used in these studies (22) and the duration of supplementation.

In the current study, MDA, a marker of oxidative damage, decreased in the PR group, but green tea consumed along with training could not influence MDA. Also, near-significant main effects ($P < 0.06$) for exercise were noted on IL-6 ($P = 0.054$). The lack of GT-induced change in MDA may be attributed lack of GT-induced change in TAC.

Although it has been reported that catechins existing in green tea are effective scavengers of free radicals in vitro (23), some authors stated that catechins existing in green tea may not be as effective in vivo since even with very high intake doses, plasma, intracellular flavonoids and polyphenol concentrations in humans are likely to be 100–1000 times lower than concentrations of other antioxidants such as vitamin C or glutathione. On the other hand, it has been stated that polyphenols such as catechins in plasma are metabolites of polyphenol metabolism, which have a lower antioxidant activity than the parent polyphenol and flavonoid. For these reasons, it seems that the relative contribution of dietary flavonoids to plasma and tissue antioxidant function in vivo is likely to be relatively minor (24).

High intensity resistance training alone significantly decreased TNF-alpha, CRP, and IL-6, although the latter did not quite reach significance ($P = 0.054$). Circulating markers of inflammation such as TNF-alpha, CRP, and IL-6 have been reported as risk factors for cardiovascular diseases (25). Recently, it has been reported that 10 weeks of moderate to high intensity resistance training can reduce the systemic inflammatory milieu in sedentary elderly women (26). However, unlike the present results, we previously showed that eight weeks of resistance training at 65 - 70% and 85% - 90% of 1RM was not able to change IL-6 and TNF-alpha basal concentrations (19). Others have also reported that 16 weeks of resistance training in middle-aged healthy

Table 4. Relationships (Correlation Coefficients) of MDA and BF% with TNF-α and IL-α as Inflammatory Factors and CK as the Index of Muscle Injury After Resistance Training and Supplementation[a]

Variables	BF%	TNF-Alpha	IL-6	CRP
Resistance training plus green tea				
MDA	0.089	0.41	0.92	0.51
BF%	-	0.92	0.31	0.76
TAC	0.35	0.21	0.93	0.52
Resistance training plus placebo				
MDA	0.52	0.58	0.22	0.20
BF%	-	0.13	0.15	0.55
TAC	0.52	0.405	0.44	0.65

Abbreviations: BF%, body fat percentage; MDA, malondialdehyde.
[a] $P < 0.05$.

men did not affect IL-6 and TNF-alpha levels (27). These contradictions may be justified by the difference in training period, subjects' adaptation abilities, factors affecting inflammatory response, gender differences, hormonal factors, age, and intensity and load of training.

In the present study, we applied progressive resistance training that started at 80% of 1RM and increased each two weeks by 5% of 1RM, which reached to 90% - 95% of 1RM at end of the study (circuit fashion in progressive mode). The exercise-related reduction of inflammatory cytokine levels was clearly demonstrated in the present study, but green tea could not influence CRP, IL-6 and TNF-alpha measures. This is probably related to the inability of the intervention to effect changes in oxidative capacity and damage and could be a function of the relatively low dose of supplementation.

In conclusion, 500 mg/d of GT supplementation along with a progressive resistance training program could not further attenuate oxidative stress and inflammation indices in obese men compared to a placebo.

Acknowledgments

We would like to thank our participants. Also, we would like to appreciate the Deputy of Research of Islamic Azad University, Sanandaj Branch, for their financial support.

Footnotes

Conflict of Interests: The authors declare that they have no conflict of interests concerning for this article.

Ethical Considerations: The research design of the study was confirmed by the Ethics Committee of Kurdistan Medical University, and the Declaration of Helsinki principles were observed in this study.

Funding/Support: This study was funded by the Deputy of Research of Islamic Azad University, Sanandaj Branch, Iran.

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