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The Effect of Aerobic and Anaerobic Exercises with Drinking Green Tea on Malondialdehyde Enzyme

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Abstract: Considering the lack of adequate information about the effect of aerobic and anaerobic exercises with drinking green tea on Malondialdehyde, the current study is aimed to investigate the effect ofan aerobic and anaerobic selected exercises (resistance) course with drinking green tea on Malondialdehyde enzyme in male athletes 20-35 years old. 60 male athletes (with average age of 26.30 and standard deviation 10.73 and average weight 71.68 and standard deviation 12.47) were selected and divided into 6 groups randomly: group aerobic exercise with green tea, group aerobic exercise, group anaerobic exercise (resistance) with green tea, group anaerobic exercise (resistance), group only green tea and group they only did their own exercises (control). All six groups implemented 8-week protocol that included exercises in two types of cardio exercises (aerobic exercise) with intensity of 65-75% of maximum heart rate and anaerobic exercises (resistance) that worked with weights by 60-70% of one repetition maximum. The experimental group received green tea three days a week at a rate of 200 mL. Before doing the exercises and 8 weeks after the last session of exercise program, blood samples were taken from the participants while they were fasting. Inferential statistics were used for unilateral variance analysis in addition to that Tukey post hoc test was used in all hypotheses in significant level of (p = 0/05). The rate of rest MDA changes didn't have significant difference in all six research groups. Changes in all 6 groups were increasing MDA but they didn't have significant difference statistically. Despite in two groups of aerobic exercise with green tea and resistance exercise with more tea MDA increased so results show that aerobic exercise, anaerobic exercise, aerobic and anaerobic exercise with green tea and green tea don't have significant effect on MDA and their effect isn't different. Therefore, the results showed that doing aerobic exercises, anaerobic ones, aerobic and anaerobic with green tea may increase MDA.

Key words: Anaerobic exercise (resistance), aerobic exercise, oxidative stress, inflammation, inferential statistics

INTRODUCTION

Oxidative stress causes cell and physical function decline, fatigue and muscle damage through damaging different types of cell infrastructures (Alok and Mandal, 2003). Some of researchers believe that through taking different strategies in order to inhibit oxidative stress and lipid peroxidation and its reduction, a decline in sport performance can be prevented and even go forward due to improving that and increasing tolerance to exercise (Urso and Clarkson, 2003). Oxidative stress happens when creation of reactional types which are known as free radicals go further than system ability for neutralization and elimination of these molecules in a system. Extra free radicals can damage lipids and proteins or DNA of a cell. The creation of these oxygen and nitrogen species happens regularly as a part of normal cell metabolism and increases under stress (Bloomer *et al*., 2004). Since, oxidative stress during and after sport only happens when producing Reactive Oxygen Species (ROS) caused by sport go beyond potential capacity of antioxidant defenses of the body (Konig *et al*., 2000).

The plant of green tea is cultivated and grown in east south Asia including China, India, Japan as well as many African countries such as South Africa (Ramma *et al*., 2005). One of the most important antioxidants can be flavonoids. Green tea is an important source of flavonoids. Tea contains a group of flavonoids polyphenolic compounds called catechins and among these catechins, EpiGallo Catchin Gallat (EGCG) is a strong antioxidant and the most common and affluent polyphenols in green tea (Murase *et al*., 2002).

Some studies have been done about the relationship with the effect of short-term supplementation of green tea on total antioxidant capacity and lipid peroxidation of young women after a strenuous resistance training

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session. Obtained findings included that consuming green tea for 14 day causes significant increase of total antioxidant capacity ($p<0.001$) and significant reduction of MDA ($p<0.01$) in exercise group with supplementary while one session of intense resistance exercise caused significant reduction of Total Antioxidant Capacity (TAC) ($p<0.04$) and significant increase of MDA ($p<0.01$) in alone exercise group. Based on current findings, it can be concluded that green tea supplementary through increasing total antioxidant capacity of plasma, can increase undesired changes of lipid peroxidation caused by resistance activities severely. In another study namely the effect of supplementary and anaerobic exercise on the indexes of oxidative stress in water polo athletes, they understood that the rate of MDA and the activity of anti-oxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase or (GPX) and catalase or (CAT) significantly decreased ($p<0.05$) and total antioxidant capacity increased significantly ($p<0.05$). According the results of this research, it can be said that consuming vitamins C and E may increase TAC and because of that destructive effects of oxidizing on lipid membrane of cells and anti-oxidative enzyme activity decreased while in a research, the effects of using vitamin supplementary (Beta-carotene 24 and 100 mg ascorbate and 800 IU of alpha-Tocopherol) were investigated. Findings showed that using vitamins decreases fat oxidation (MDA) significantly. The amount of this reduction was more in people with diabetes who were more prone to oxidation of lipoproteins (Anderson *et al*., 1999).

Therefore, considering the obtained contradictory results and the paucity of information in the field of green tea supplementation on aerobic and anaerobic activity (resistance) and its role on some of inflammation factors and oxidative stress index in athletes following these exercises and since there aren't accurate and controlled studies around this subject,the current study was designed to cover this subject.

MATERIALS AND METHODS

The research method is semi-experimental test. Statistical population includes all Tehran male athletes that 100 people were volunteers to participate with recall that among them (N = 100) 60 ones purposeful (Inventory) were selected randomly as sample and divided into 6 groups (5 experimental groups (N = 50) and 1 control group (N = 10) that included:

- Group A: Aerobic exercise with green tea
- Group B: Anaerobic exercise (resistance) with green tea
- Group C: Only drank green tea
- Group D: Only aerobic exercise
- Group E: Only anaerobic exercise (resistance)
- Group F: Only performed their ordinary exercises (control)

One week before implementing the test, participants were measured in height, weight, age and familiarity with the test procedure and getting written consent. All participants were also in perfect physical and mental health and didn't have any history of cardiovascular disease, respiratory diseases and certain diseases. Participant was first taken a pretest before starting the procedure and after the end of the exercises that is after 8 weeks, they were taken post test.

About 30 participants were given 200 mL of green tea (2 g of dry leaf green tea in 200 ml of water at a temperature 80-100°C) as a drink (Panza *et al*., 2008) and 30 other participants were without green tea. For pretest and posttest, blood test (fasting) was used. the exercises were three days a week including 24 sessions that is 8 weeks and each exercise session was 60 min.

Aerobic exercise: Cardio exercises included aerobic ones including 15 min as warmup (walking, movements and stretching exercise, running and doing exercises with bulk, weight (light), ball, working on the steppe and doing a series of rhythmic movements which is 35 min and 10 min was for cool-down that total was 60 min aerobic which was as 3 sessions a weeks (Every other day). The intensity of exercises was among 65-75% of maximum heart rate (Modern aerobic exercises, Kasten and Jordan, edition 1), (aerobic-teaching and its benefits, Veisi and Keshtidar, edition 1).

Anaerobic exercises (resistance): Anaerobic exercise (resistance) included 10 min warmup and then starting doing exercises with weights that included 2 programs as the program of first to fourth weeks (The front foot with the back foot device with sitting device, chest press with the device, publication of the shoulder with the device, biceps with the device, triceps with the device, pull back with device and crunch with the device) which included 1 set and 8-12 repetition and 1-2 min rest.

The exercise plan of fifth to eight weeks (Leg press, instep with the device sitting, chest with the device, publication of the back of shoulder with the device, publication of behalf with the device, biceps with the device, triceps standing with cable, pull back with device and abdominal crunch with the device) that 1 set and 8-12 repetition and 1-2 min rest and doing the intensity of exercises of 60-70% (1RM) (Reference book of strength

Table 1: Tests and used solutions in research

Blank	Test	Reagent
-	1 mL	Serumpatient
1mL	-	D.waverage
1mL	1mL	Reagent 1
1mL	1mL	Reagent 2
1mL	1mL	Reagent 3

training plan: Physiology of power and weight training systems/[National Association of strength and fitness United States] Lee in 2012, edition 1).

Supplementation with green tea: In groups that supplementation with green tea was considered, participants were wanted during 8 weeks by keeping their diet, 3 days a week to the rate of 200 milliliter (2 g of dry leaf green tea in 200 ml of water at a temperature 80-100°C) to brow green tea and after doing exercises the participants were given green tea.

Blood sampling and measurement of research indexes: Blood sampling was done after 12-14 h of fasting in two steps (before the beginning of exercises that is one day before beginning exercises and after 8 weeks of exercise). In first step of blood sampling which was done in Al-Nabi mosque's gym from participants at 8 a.m. right hand vein of each participants sitting down and in rest mode 5cc ml of blood was taken. In second step, also after finishing 8 weeks their blood were taken at the same way.

How to measure malondialdehyde: For measuring serum Malondialdehyde, Pars Azmoun company kit, made in America, Nanomol/mL in wavelength of 534° nm nanometers by UV-spectrophotometry device, spectrophotometric method (HPLC) was used. For measuring MDA, two below methods are used which include:

- Spectrophotometry method (TBARS)
- (HPLC) method/532 nanometer

MDA in serum will be separated by connecting to TBA and serum proteins are depositedby TCA centrifuge then TBA complex will be measured in wavelength of 534 nanometers. Necessary reagent include in Table 1:

- 17.5% TCA reagent
- 70% TCA reagent
- 0.6% TBA reagent

The method of test is as follows: All pipes will be shook and placed in Ben Murray 100°C for 15 min. Then after cooling dawn in room temperature, they will be centrifuged for 20 min at around 2000 rpm (rotations per minute) for 15 min. The surface reagent which contains MDA will be called in length of 534 nanometers with spectrophotometer.

Calculating the results: MDA concentration based on nanomol/milliliter will be calculated through below formula:

$$\text{Abs (test)-Absblank} = 1/56\times10^{5}, 10^{5}\times56.1$$

In HPLC method, after preparation sample for measuring MDA, prepared reagent will be injected to HPLC system by a special syringe and in wavelength of 532 nanometers against determined concentration standard of MDA, MDA amount of samples will be obtained using standard curve (Al-Anee *et al*., 2009).

Statistical methods: For recognizing and naturalization of data, Kolmogorov and Smirnov test has been used and it was identified that groups are not different from each other. Descriptive statistics was used in order to calculating central indexes and dispersion was used. Inferential statistics was used for unilateral variance analysis statistical test and tukey post hoc test. Significance level was considered as alpha 0.05. All statistical operations were done through SPSS software version 20.

RESULTS

Investigating the effect of aerobic exercise and green tea on MDA variable

First null hypothesis: Aerobic exercise with green tea doesn't have significant effect on male athletes of 20-35 years old. The results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes didn't show significant difference between changes in four groups of aerobic exercise with green tea, aerobic exercise, green tea and control ($F_{3.36}$ = 1.124, Sig. = 0.352). Table 2 shows the results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes in four groups of aerobic exercise with green tea, aerobic exercise, green tea and control.

Considering the results, it can be perceived that although the values of rest MDA in all four groups of aerobic research with tea, aerobic exercise, green tea and control have increased (positive changes) but these changes weren't significant statistically. It can also be said that the most increase of MDA has been in aerobic exercise with green tea group and after that the most changes of MDA were in green tea group.

Table 2: Results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes in four groups of research

Groups	Sum of squares	d	Mean square	F	Sig.
Intergroup	154/0	3	051/0	124/1	352/0
Intragroup	648/1	36	046/0		
Total	803/1	39			

Table 3: Results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes in four groups of research

Variance	Statistic	df1	df2	Sig.
Welch Amendment	3.276	3	86/18	044/0

Table 4: Results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes in six groups of research

Variance	Statistic	df1	df2	Sig.
Welch Amendment	2.256	5	28/24	081/0

Therefore, it can be concluded that green tea with aerobic exercise, aerobic exercise and consuming green tea don't have significant effect on the values of rest MDA of 20-30 year old men and the hypothesis of research will be rejected.

Investigating the effect of anaerobic exercise (resistance) and green tea on variable MDA

Second null hypothesis: Resistance exercise with green tea won't have significant effect on the rate of MDA of 20-35 year pol male athletes. The results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes didn't show significant difference between changes in four groups of anaerobic exercise with green tea, anaerobic exercise, green tea and control ($F_{3.18.86}$ = 3.276, Sig. = 0.044) (there wasn't the homogeneity of variance among four groups so Welch Amendment has been used). Table 3 shows the results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes in four groups of anaerobic exercise with green tea, anaerobic exercise, green tea and control.

Considering the results, it can be perceived that the values of rest MDA changes in all four groups of anaerobic research with tea, anaerobic exercise, green tea and control have had significant difference. The results of Games-Howell post hoc tests showed that this difference is between the group of resistance exercise with green tea with green tea group and control. But three groups of resistance exercise. Green tea and control weren't significantly different from each other. Resistance exercise and green tea have also increased MDA alone but their effect has not been significant but when resistance exercise has been with green tea the increasing effects of both two factors means resistance exercise and green tea have led to significant increase of MDA. Therefore, it can be concluded that green tea with anaerobic exercise have significant effect on the values of rest MDA of 20-30 year old men and the hypothesis of research will be confirmed.

Comparing the effect of aerobic and anaerobic exercise (resistance) and green tea on MDA variable

Third null hypothesis: There isn't significant difference between aerobic exercise with green tea and resistance one with green tea and green tea on the rate of MDA in 20-35 year old men.

The results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes didn't show significant difference between changes in six groups of research ($F_{5.24.28}$ = 2.256, Sig. = 0.081) (there wasn't the homogeneity of variance among four groups so Welch Amendment has been used). Table 4 shows the results of unilateral variance analysis statistical test for comparing the rate of plasma rest MDA changes in six groups of research.

Considering the results, it can be concluded that the rate of rest MDA changes in all six research groups didn't have significant difference. The changes in all groups were increasing MDA but that wasn't significant statistically. Despite in two groups of aerobic exercise with consuming tea and resistance exercise with tea the most range of increase MDA was observed, so these results show that aerobic, anaerobic exercise, aerobic and anaerobic exercise with green tea don't have significant effect on MDA of 20-30 year old men and their effect isn't different therefore, the hypothesis of research will be rejected (notice that in this hypothesis, the concentration is on investigating the difference of two exercise methods with green tea not their effect. In hypotheses 1 and 2, the effects were investigated but in third one the comparison of effects was investigated).

DISCUSSION

The current research showed that doing aerobics and anaerobic exercise, aerobic and anaerobic exercise with green tea may increase MDA and be effective on some factors of oxidative stress and inflammation. Researchers believe that although different cells and textures of body produce free radicals as a part of metabolismbut sometimes such as during work and physical activity, producing these radical species will go beyond body's antioxidant capacity and will lead to oxidative stress (Bloomer *et al*., 2005).

Green tea as a strong antioxidant supplementary paly and effective role in neutralization free radicals and increasing the capacity of body's antioxidant system and reducing lipid peroxidation (Kuriyama, 2008) and (Yuan *et al*., 2011). In extreme resistance exercises, the process of ischemia and reperfusionand mechanical loads exerted on the involved soft tissues have effective role in the creation of lipid peroxidation and the production of

free radicals (Dixon *et al*., 2006). During exercise diversion of blood to the skin and active muscles cause transient tissue hypoxia and lack of coordination of active oxygen consumption and oxygen requirements in the active textures during high intensity exercise; although following re-oxygenating of these textures and cutting off or reducing the intensity of activity, producing reactive oxygen species (ROS) will be provided with increasing lipid peroxidation and cell function decline (Ogonovszky *et al*., 2005; Watson *et al*., 2005).

Catechins found in green tea can increase the capacity of antioxidant through increasing intracellular antioxidant such as glutathione, uric acid and bilirubin and increasing the capacity of intracellular antioxidant enzymes such as glutathione reductase and glutathione peroxidase and catalase in protecting cell against depletion of reduced glutathione and by this mechanism help increasing TAC (Raihan *et al*., 2009). Generally, these compounds because of having hydroxyl groups can neutralize free radicals and can act as the electron or hydrogen donor (Katiyar *et al*., 2007). Catechins found in green tea especially (EGCG) may inhibit the process of lipid peroxidation through reducing the production of free radicals (mainly because of having dihydroxy phenol structure) as well as reconstruction of tocopherol (converting tocopherol to tocopherol radical). Through connecting copper element and preventing connecting this element to lipoproteins, Catechins can significantly prevent reducing tocopherol concentrations in plasma and cause a delay in beginning the process of plasma lipid peroxidation. (Ostrowska *et al*., 2005).

Ghasemi *et al*. (2013) reported the consumption of green tea for 14 days as the reason for increasing TAC significantly and significant decrease of MDA by intense resistance activity with the strength of 85% (RM1) in health non-athlete women and also in a research that (Alkhamees, 2013) did, they found out that through using Moran consumption (Portugal Osage), (15 and 30 milligram/kilogram/day) for five weeks that with Moran treatment (Portugal Osage: The most potent flavonoid inhibitor is fatty acid synthesis) in diabetic mice respectively after reducing MDA and increasing the levels of DNA,T-GSH and increasing the activity SOD in liver cells decreased oxidative stress induced by STZ significantly. These biochemical findings were similar to the confirmations of Histopathologic.

But, Swamy *et al*. (2011) noticed in their obtained results that rats fed by polyphenols showed important increase during swimming and increased the activities of lactate Dehydrogenase (LDH) and Creatine and also decreased the value of MDA in liver, muscles and blood but the concentration of DNA and RNA will be increased by this in muscles.

The reasons of these disagreements may be because of supplements, exercise, exercise intensity, samples (healthy or patient human or animal model) and gender (male or female) because on the contrary of (Alkihamees, 2013), in current study the type of supplementary has been green tea and considering misalignment in current findings the type of done exercise has been aerobic exercise and intensity aerobic exercise 65-75% of maximum heart rate and on the contrary of Swamy *et al*. (2011) in current research, it can be said that all participants were healthy athlete men.

The researches have also showed that Malondialdehyde is a secondary product of lipid peroxidation that is measured as the index of oxidative stress and final product of oxidation of lipid peroxidase is in lipid membrane of cells in the body that inflammation processes have been increased there and in case of neutralization by body's respiratory system can damage structure and function of cell membranes of the body (Devasagayam *et al*., 2004; Nielsen *et al*., 1997).

We have some evidences out of recent researches that intensive physical activity not only long-term aerobically but also in short-term anaerobic may stimulate oxidative stress (Alessio *et al*., 2000). It was assumed that oxidative stress more likely will help the fatigue and damaging muscle cells and as result it may affect sport performance (Watson *et al*., 2005).

One of the effects of oxidative stress is intensification of lipid peroxidation which is reflected by increasing blood concentration with its products that is Hydroxy fatty peroxidase (LOOH), Mda as well as Thiobarbituric substances with acid reactive (TBARS) (Davis *et al*., 1982). In done researches by MCBride *et al*. (1998), intense resistance training stimulates increasing in MDA concentration in blood which in higher level before exercise was maintained even till 24 h.

Most of researches have also showed that one level of boring exercise or intensive sport activity or in long period cause increasing the index of oxidative stress (MDA) and decreasing the capacity of total serum antioxidant (Tauler *et al*., 2006; Jafari *et al*., 2011).

In spite of this, one hand available oxygen increase production of free radicals and along intensive physical activities which we face increasing consuming oxygen their producing will reach to several times of the resting mode (Taular *et al*., 2006), Malondialdehyde or MDA as a free radical is a deformation of hydrogen peroxide (H_2O_2) that is effectiveon creation of conditions of oxidative stress and blood, MDA of tissue damage (Taular *et al*., 2006). Increasing the concentration of MDA in blood is also dependent on the intensity of sport and whatever the

intensity of activity is more, production and releasing MDA will increase as well (Valado *et al*., 2007). Considering that one of the very likely mechanisms involved in cell damage is increasing leakage of free radicals and oxidative stress caused by them, Malondialdehyde index which represents the value of lipid peroxidation (Cell membrane lipid oxidative damage), it was investigated as possible mechanisms involved in causing harm (Chiaradia *et al*., 1998; Frankiewicz-Jozko *et al*., 1996). Also considering that glutamine is the precursor of glutathione so glutathione can decrease lipid peroxidation and free radicals through increasing the capacity of plasma antioxidant (Cotgreave and Gerdes, 1998). On the other handthe reaction of free radicals with cells skin will lead to produce one of the stress indexes that provide the possibility of measuring oxidative stress indirectly by an oxidative called Malondialdehyde (MCbride and Kraemer, 1999).

CONCLUSION

Considering the implemented researches, it can be said that other factors related to type of exercise, sports field, exercise intensity, exercise duration, type of training, supplements, supplementation dose, gender, samples (human or animal model) and other factors can also be effective on decreasing or increasing MDA.

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