Original Article

The effects of grape seed extract supplementation on exercise-induced oxidative stress in young untrained males

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Abstract

Background and Purpose: The aim of this study was to determine the effect of acute aerobic exercise and 14-days grape seed extract supplementation on total antioxidant capacity, lipid peroxidation and muscle cell damage biomarkers in untrained males.

Materials and Methods: In a randomized, double-blinded, and placebo-controlled study, twenty-two male students (age 19±1 years, weight 67.44±7 kg, BMI 22±2, $\dot{V}O_2max$ 39±2 ml/kg^{-1}/min^{-1}) were randomly assigned to two groups of grape seed extract and placebo (PLA) (GSE: 200 mg/day for two weeks). After subsequent 14-days of supplementation, subjects did a single session of aerobic exercise (running) on treadmill at 75% VO2max for 30 minutes. Blood samples were taken 3 times: prior to supplementation (baseline), 14-days after supplementation, and immediately after exercise (post-exercise). Total antioxidant capacity (TAC), malondialdehyde (MDA), creatine kinase (CK), lactate dehydrogenase (LDH) and lactate were measured. TAC was determined by ABTs method. The collected data were then analyzed by running analysis of variance (ANOVA) with repeated measure and Bonferroni post-hoc tests as appropriate using $SPSS_{17}$ at $p<0.05$.

***Results*:** Malondialdehyde, CK, LDH, Lactate were significantly increased after aerobic exercise ($p<0.05$). Short-term GSE supplementation significantly prevented MDA and CK cascade after exercise compared to PLA ($p<0.05$) but, it had no significant effect on basal parameters ($p>0.05$). The exercise had also no significant effect on total antioxidant capacity in any of the groups ($p>0.05$).

***Conclusion*:** Aerobic exercise could increase blood oxidative stress biomarkers and GSE supplementation, due to influential antioxidant effect; yet, it could attenuate exercise-induced oxidative stress in men.

Key words: Aerobic exercise; Grape seed extract; Oxidative stress; Antioxidant effect; Supplementation

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1. *Introduction*

In recent years, comprehensive research indicates that exercise exacerbates the generation of reactive oxygen (ROS) and other free radicals (1, 2). Free radicals have been implicated over a hundred disease conditions in humans, including arthritis, atherosclerosis, advancing age, Alzheimer and Parkinson's disease (3, 4). The generation of main types of free oxygen radicals, such as superoxide radical, hydroxyl radical, hydrogen peroxide radical, and single oxygen occurs regularly as part of normal cellular metabolism (5). A cascaded of free radicals has been demonstrated following both intense aerobic and anaerobic exercise (6). Under normal physiological conditions, various intensities and durations of aerobic exercise are major sources of free radical generation with an increase in oxygen consumption (VO_2), and is ultimately used for ATP production (7, 8) and water in the mitochondria. This increased oxygen flux through the mitochondrial respiratory chain contributes to an increased production of free radicals (9). During exercise, oxygen consumption may increase up to 10-20 fold in the whole body while it may reach 100 times in active muscles compared to the resting condition. Therefore, it has been confirmed that contracting muscles produce ROS (8-10).

Overall, exercise can cause an imbalance between ROS generation and antioxidant activity, a situation known as the oxidative stress state with cell damages in cellular lipids, proteins, or DNA, and it can inhibit their normal function (8, 11-13). It has been shown that moderate and high-intensity aerobic exercise, as a physiological stressor, could increase generation of free radicals and outbreak oxidative stress (7, 11, 14).

The assessment of total antioxidant capacity (TAC) is an established methodology of coincident measurement of various indices of antioxidant defense system (15). Several studies suggested that aerobic endurance training can raise the level of TAC in trained and untrained individuals. However, measurement of TAC itself can lay out limited information about the antioxidant status as TAC assays are not capable of measuring all antioxidant component (11). One of the mostly-used methods implemented to indicate exercise-induced oxidative damage regarding aerobic exercise is the assessment of lipid peroxidation, with malondialdehyde (MDA) (16). Goldfarb et al. (17) showed that MDA levels increased in both genders in response to exercise with 80% $\dot{V}O_2max$ for 30 min. Furthermore, MDA levels during exercise have been correlated with some muscular cellular damage markers, such as creatine kinase (CK) and lactate dehydrogenase (LDH) (18-20). Previous studies have indicated the rise of CK (18, 21, 22) and LDH as markers of muscle cell damage and MDA (17, 21, 23), as biomarker of oxidative stress and lipid peroxidation following aerobic exercise. Several studies have also demonstrated that aerobic exercise elevates lactate concentration as muscle fatigue index (21, 24, 25). Nevertheless, free radicals are neutralized by a specified antioxidant defense system which consists of enzymes, such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including glutathione, ubiquinone, flavonoids, vitamins C, A and E (2, 26).

Recently, numerous studies have revealed the antioxidant effects of plant extracts (27). One of these natural extracts is the

grape seed extract (GSE) which has various medicinal characteristics (28). Grape seed extract is a rich source of plant flavonoids and proanthocyanidin oligomers (29). The polyphenols in grape seeds are mainly flavonoids including gallic acid, epicatechin, the monomeric flavan-3-ols catechin, epicatechin 3-O-gallate, gallocatechin, epigallocatechin and procyanidin dimers, trimers, and more highly polymerized procyanidins. They also exhibit a strong antioxidant activity (30). Grape seed extract is highly bioavailable and provides significantly greater protection against free radicals and free radical induced lipid peroxidation and DNA damage than b-carotene, vitamin E and vitamin C (3, 31, 32).

GSE has been proved to have an antioxidant effect in numerous studies, but there are limited studies following the impact of concomitant aerobic exercise and supplementation with GSE. In this respect, for example, Taghizadeh et al. (1) and Belviranli et al. (33) demonstrated that GSE supplementation can alleviate exercise-induced oxidative stress by preventing lipid peroxidation and increasing antioxidant enzyme activities. In general, given what has been said, intense aerobic exercise can increase the production of reactive oxygen species (ROS) and other free radicals, leading to exercise-induced oxidative stress. In contrast, the use of natural antioxidant supplements of plant origin, such as grape seed extract, is one of the methods that has been proposed to counteract and reduce the adverse effects of oxidative stress. Thus, considering the studies with little information in this regard, the current study aimed at investigating the effect of two-week grape seed extract supplementation, as a powerful antioxidant, on the possible reduction of oxidative and cellular damage caused by acute aerobic exercise.

2. *Materials and methods*

The current study was a randomized, double-blinded, and placebo-controlled trial involving young males. Twenty-two untrained male collegiate students (age 19±1 years, weight 67.44±7 kg, body fat 12±3%, BMI 22±2, $\dot{V}O_2$max 39±2 ml/kg^{-1}/min^{-1}) were randomly recruited to participate in the present study (Table 1).

The sample size was estimated based on previous similar studies (14). Following the invitation to participate in the present research project, 22 subjects were selected from among the volunteers. All participants were advised on the purposes of the study and attendant risks, and they subsequently completed a health history questionnaire and informed consent. The participants were healthy, with no history of regular exercise for at least six months, and consumed any supplements, such as vitamins, minerals or medications that might have affected oxidative stress markers before the exercise session. The study protocol and the procedures conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee by the Tabriz University of Medical Sciences Ethical Committee (IRCT 2013081614371N1).

Briefly, at least 10-days prior to the supplementation period, the participants performed preliminary Bruce treadmill-based tests to determine maximal oxygen consumption ($\dot{V}O_2$max). In addition, the physiological characteristics of all participants were measured. Then, they were randomized in a double-blind manner to either a supplement (n=10; 100 mg/12 h;

GSE) and/or placebo groups (n=12; 100 mg/12 h; PLA). The standardized grape seed extract (GSE) and PLA capsules were provided by Drug Applied Research Center, Tabriz University of Medical Sciences (34). In short, grape seeds (Vitis vinifera) were washed with water and chopped, the raw extract was divided between H_2O and n-hexane for removing lipoid components. Next, GSE was provided by utilizing ethanol 95% and water (water/ethanol, 30/70) as solvents with mechanical agitation for 2 to 3 hours. This process was constantly repeated twice. After that, the organic solvent was evaporated and remaining dried extract was kept at 4 °C for experimentation (35).

The first blood samples (5 ml) were taken via venipuncture from the antecubital vein (baseline), and the participants received either 100 mg of PLA, and or 100 mg of GSE per 12 h. After 14-days supplementation period, the second blood samples were taken before exercise protocol (pre-exercise). Then, after 10-min warm-up (consisting of stretching and running on a treadmill), all subjects participated in aerobic exercise protocol with 75% $\dot{V}O_2$max on a treadmill for 30-min. The last blood samples were taken immediately after the exercise protocol (post-exercise). The blood samples centrifuged for 10-min at 3000 rpm. The serum samples were stored at −70°C until the analysis of total antioxidant capacity (TAC), malondialdehyde (MDA), creatine kinase (CK), lactate dehydrogenase (LDH) and lactate in laboratory.

Table 1. Participants characteristics in grape seed extract supplement (GSE) and placebo (PLA) groups, N=11 /group. ($p<0.05$)

Characteristics	GSE	PLA
Age (ears)	19.07±0.77	19.22±0.88
Height (Cm)	174±12	172±14
Weight (kg)	68.08±7.44	66.85±7.55
Body fat (%)	12.00±2.85	12.73±2.97
BMI (kg/m^2)	22.03±1.83	22.38±1.42
$\dot{V}O_2$max ($ml/kg^{-1}/min^{-1}$)	39.21±1.60	39.35±1.26

Total antioxidant capacity was measured using a colorimetric assay (Randox Laboratories Ltd, Crumlin, U.K.). In this method, ABST is incubated with methemoglobin (as peroxidase) and hydrogen peroxide to produce cation radicals ABST. This radical is in greenish blue color, relatively stable, and measurable at 600 nm wavelength. Antioxidants in samples were reduced, and color intensity was formed, which was proportional to its concentration in the sample (36). Serum malondialdehyde levels were measured based on the reaction with thiobarbituric acid (TBA). The extraction was performed with normal butanol, the measurement was done using a spectrophotometer, and the absorption was compared with the standard curves. Serum levels of CK and LDH were measured by commercial assay kits (Randox, U.K.) using an auto analyzer apparatus. Serum level of lactate was also determined using commercial kit (Giesse, Italy) and auto analyzer. All analyses were performed in accordance with the manufacturers' recommendations.

Values are expressed as the mean±SD. The distribution of all variables was examined

using the Shapiro-Wilk test for normality. Afterwards, Mauchly's test of sphericity was checked and the collected data were assessed by the analysis of variance (ANOVA) with repeated measures and Bonferroni Post-Hoc test as appropriate through SPSS15, and $p<0.05$ was considered significantly meaningful (Table 2).

3. *Results*

Descriptive characteristics of the subjects in GSE and PLA groups are presented in Table 1. The baseline resting serum TAC levels was not different between groups (0.95±0.17 vs. 0.98±0.15 mmol/ml, $p>0.05$). After two-week supplementation, serum TAC levels increased in GSE, but not significantly (0.97±0.12 vs. 0.96±0.18 mmol/ml, $p>0.05$). The total antioxidant capacity increased immediately after exercise in both groups was negligible, and not again significant (1.01±0.14 vs. 0.98±0.21 mmol/ml, $p>0.05$) (Table 2).

Table 2. Effect of time and intervention, and their interaction on measured variables (TAC, MDA, CK, LDH and Lactate) assessed by repeated measurements ANOVA

Variables	Source	Sum of Squares (SS)	df	Mean Square (MS)	F	Sig
CK (IU/l)	Between-Subjects					
	Group	100.91	1	100.91	0.059	.801
	Error	34129.28	20	1706.64		
	Within-Subjects					
	Times	14234.02	2	7117.01	3.98	0.027 *
	Times*Group	3609.84	2	1837.02	1.00	0.372
	Error	71520.36	40	1788.00		
LDH (IU/l)	Between-Subjects					
	Group	174.02	1	174.02	0.59	0.801
	Error	58528.33	20	2926.41		
	Within-Subjects					
	Times	17782.52	2	9267.24	11.770	0.000 *
	Times*Group	475.85	2	247.99	0.315	0.723
	Error	30216.77	40	787.41		
MDA (nmol/ml)	Between-Subjects					
	Group	1.34	1	1.34	1.26	0.275
	Error	21.24	20	1.06		
	Within-Subjects					
	Times	3.79	2	1.97	7.53	0.002 *
	Times*Group	1.66	2	0.86	3.30	0.049 *
	Error	10.06	40	0.262		
TAC (mmol/l)	Between-Subjects					
	Group	0.01	1	0.01	.007	0.935
	Error	1.50	20	0.07		
	Within-Subjects					
	Times	0.017	2	0.012	1.36	0.267
	Times*Group	0.009	2	0.006	0.698	0.460
	Error	0.245	40	0.006		
Lactate (mmol/l)	Between-Subjects					
	Group	0.001	1	0.001	0.003	0.955
	Error	3.14	20	0.157		
	Within-Subjects					
	Times	4.39	2	2.19	25.14	0.000 *
	Times*Group	0.026	2	0.018	0.150	0.799
	Error	3.49	40	0.117		

*** Significant (P < 0.05)**

Baseline serum MDA concentration was not different between groups. Malondialdehyde levels also elevated protocol in both groups that resulted in a significant increase in PLA (3.43 ± 0.35 vs. 4.16 ± 0.80 nmol/ml, p<0.05) (Figure 1).

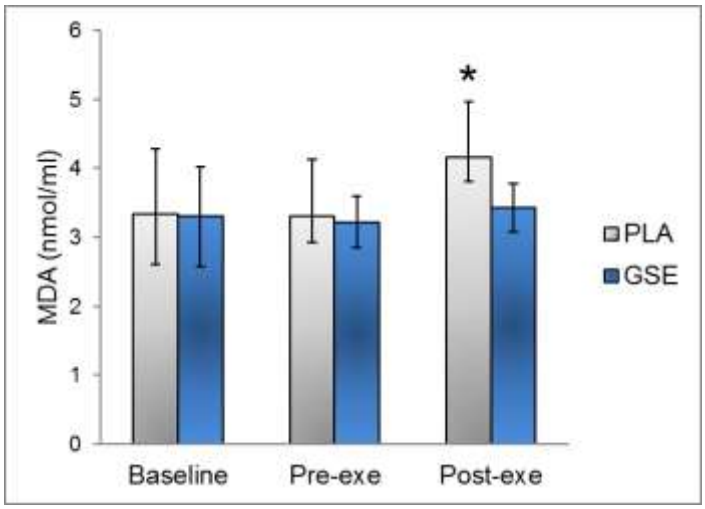

Figure 1. Serum MDA baseline, before and after exercise. Results are presented as means ± SD. ∗ Indicates significant differences between pre-test and post-test (p<0.05). PLA: Placebo, GSE: grape seed extract.

The blood CK concentration for the study groups is shown in Figure 2. As indicated there, creatine kinase increased immediately after aerobic exercise in both groups, whereas the amount of CK significantly increased after exercise, only in the PLA group (151±33 vs. 174±46 U/L, p<0.05).

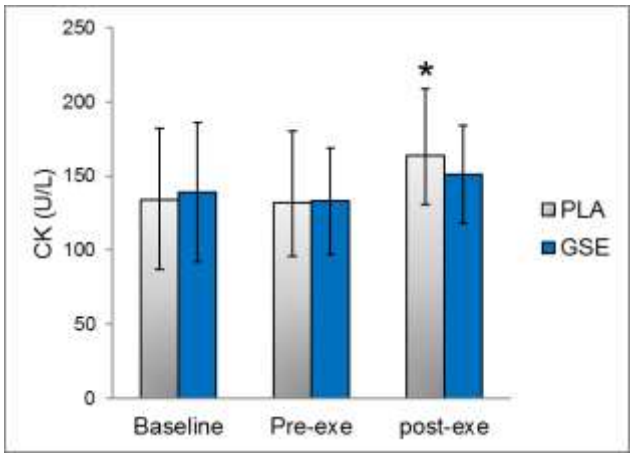

Figure 2. Serum CK baseline, before and after exercise in study groups. Results are presented as means ± SD. ∗ Indicates significant differences between pre-test and post-test (p<0.05). PLA: Placebo, GSE: grape seed extract.

Serum lactate dehydrogenase (LDH) concentration is shown in Figure 3. The findings showed that the baseline serum LDH was not different between groups, and lactate dehydrogenase concentrations significantly increased immediately after exercise in both groups (p<0.05). However, there were no significant differences between the LDH concentrations in both groups after the exercise protocol (295±46 vs. 292±43 U/L, p<0.05).

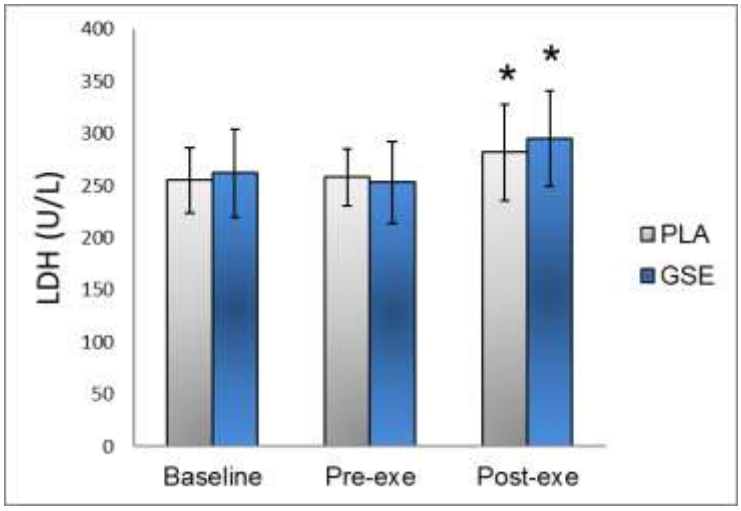

Figure 3. Serum LDH baseline, before and after exercise. Results are presented as means ± SD. ∗ indicates significant differences between pre-test and post-test in both groups ($p<0.05$). PLA: Placebo, GSE: Supplement.

Also, the blood Lactate concentration for study groups is shown in Figure 4. Serum Lactate concentrations elevated immediately after exercise in both groups ($p<0.05$), but, as illustrated in the figure, there were no detectable differences between PLA and GSE (1.46±0.54 vs. 1.41± 0.4 mmol/L, $p>0.05$).

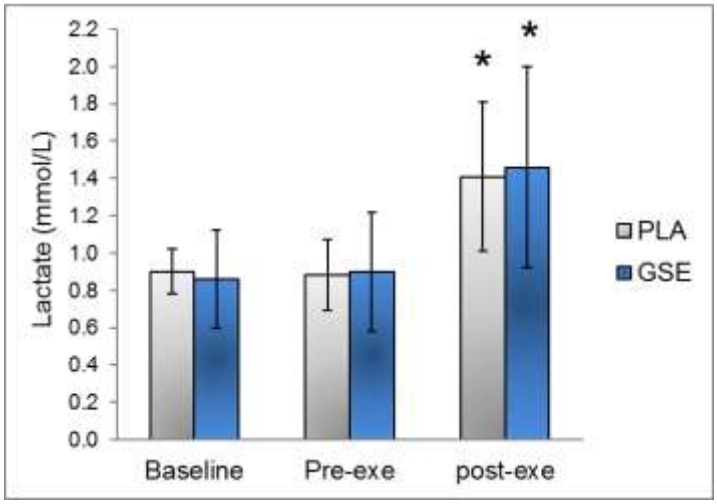

Figure 4. Serum Lactate baseline, before and after exercise. Results are presented as means ± SD. ∗ Indicates significant differences between pre-test and post-test in both groups ($p<0.05$). PLA: Placebo, GSE: Supplement.

4. Discussion

The main purpose of this study was to examine the effect of aerobic exercise protocol with 75% $\dot{V}O_2max$, and short-term grape seed extract supplementation on lipid peroxidation (MDA), muscle cell damage (LDH, CK) and muscle fatigue response in the serum of untrained young males.

Generally, measurement of the body antioxidant capacity is utilized as a marker of oxidative stress (16). Previous studies have indicated that aerobic exercise with various protocols could accompany with changes to cause increase (14, 37, 38) or decrease (22, 23, 39) in TAC levels. For example, Babaei et al. (23) reported that after 30-min aerobic exercise, TAC decreased significantly as compared to pre-exercise. In contrast, Taghizadeh et al. showed that the administration of GSE had

no significant effect on creatine phosphokinase (CPK) and total antioxidant capacity (1).

In the present study, TAC had no significant alterations after exercise as compared to pre-exercise in both groups. This suggests that GSE supplementation had no significant effect on TAC level. These findings were in agreement with Kar et al. (40) and Taghizadeh et al. (1) but not with Natella et al (41). The differences between dose and duration of GSE supplementation to ameliorate antioxidant capacity, type of the participants, and their characteristics may be among the possible reasons for the contradictions in studies.

Peroxidation measurement of membrane lipids or fatty acids is a basic approach to study oxidative stress (11). Production of free radicals during exercises has an important role in lipid peroxidation and muscle damage (42). In this study, a drastic increase was observed in lipid peroxidation marker (MDA) after 30-min of aerobic exercise in PLA. This finding was in line with the finding of Skishahr et al. (21) and Goldfarb et al., (17) and inconsistent with Bloomer et al. (43).

In comparison to PLA, supplementation with GSE significantly prevented MDA increase after exercise (Figure 1). The results of the present study were found to be in agreement with those of Sano et al. (44) and Natella et al. (41). Grape seed extract may decrease the content of MDA levels and cellular membrane damage by improving the activity of specific antioxidant enzymes, such as SOD, and GSPx (45) and also, free radical scavenging ability (3).

It was also reported that the marker of muscle damage (CK) increased immediately after aerobic exercise in both groups (21). Creatine kinase is an indicator of muscular cellular damage that can also be considered as indirect markers of oxidative stress because lipid peroxidation induces damage of cellular membranes (11). Moreover, elevation of serum CK may be due to disruption of Z-disk in muscle fiber structures, which can then result in the leakage of this protein into the circulation. This efflux relates to the raise in ROS-induced membrane permeability of the muscle cells (8, 23). Supplementation with GSE, as compared to PLA, significantly prevented CK elevation after exercise which is in agreement with the results reported by Saada et al. They indicated that pre-irradiation GSE administration significantly decreased radiation-induced oxidative stress in heart tissues, which was substantiated by a significant amelioration of serum lactate dehydrogenase (LDH), and creatine kinase (CK) activities. It seems that any reduction in CK was most likely related to blunting MDA by GSE pretreatment (46).

The extent of muscle cell damage can be estimated by following a variety of enzymes released by the disrupted cells into blood (47). Lactate dehydrogenase is an enzyme that increases in blood after aerobic and anaerobic exercise (48-50). The results of the present study showed that the LDH levels were significantly increased after aerobic exercise in GSE and PLA groups. It seems that short-term supplementation (14-days) with GSE had no effect on LDH level as a muscle damage marker.

Several studies have indicated that exercise at various intensities and duration increased lactate concentration as a muscle fatigue parameter (21, 24, 51, 52). Furthermore, muscle fatigue may be associated with increased free radicals in skeletal muscle fibers (39). In the present study, a drastic increase was observed in lactate

concentration after the exercise protocol in both groups (Figure 4).

These results were found to be in line with the findings of Sachek et al. (24) and Buttner et al. (51). Therefore, it was suggested that GSE supplementation had no significant impact on lactate concentration. However, there were inadequate studies regarding the effect of GSE supplementation on exercise-induced oxidative stress.

In conclusion, short-term supplementation with the grape seed extract (100 mg per 12 hrs), before exercise could significantly attenuate MDA level, CK activity, and exercise-induced oxidative stress in untrained young males, but has no effect on LDH level as a muscle damage marker. However, more studies are needed to draw a complete conclusion.

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Conflicts of interest

The authors declare that they have no conflict of interests.

References

1. Taghizadeh M, Malekian E, Memarzadeh MR, Mohammadi AA, Asemi Z. Grape seed extract supplementation and the effects on the biomarkers of oxidative stress and Mmetabolic profiles in female volleyball players: A randomized, double-blind, placebo-controlled clinical trial. Iranian Red Crescent Medical Journal. 2016;18(9):e31314.
2. Yimcharoen M, Kittikunnathum S, Suknikorn C, Wichuda N, Petcharee Y, G. T, et al. Effects of ascorbic acid supplementation on oxidative stress markers in healthy women following a single bout of exercise. Journal of the International Society of Sports Nutrition. 2019;16(2):1-9.
3. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, et al. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology. 2000;148(2-3):187-97.
4. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clinical interventions in aging. 2018;13:757-72.
5. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine. 2018;54(4):287-93.
6. Bloomer RJ, Falvo MJ, Fry AC, Schilling BK, Smith WA, Moore CA. Oxidative stress response in trained men following repeated squats or sprints. Medicine & Science in Sports & Exercise. 2006;38(8):1436-42.
7. Park S-Y, Kwak Y-S. Impact of aerobic and anaerobic exercise training on oxidative stress and antioxidant defense in athletes. Journal of Exercise Rehabilitation. 2016;12(2):113-7.
8. Powers SK, Deminice R, Ozdemir M, Yoshihara T, Bomkamp MP, Hyatt H. Exercise-induced oxidative stress: Friend or foe? Journal of sport and health science. 2020;9(5):415-25.
9. Jackson MJ, Pye D, Palomero J. The production of reactive oxygen and nitrogen species by skeletal muscle. Journal of Applied Physiology. 2007;102(4):1664-70.
10. Tucker WJ, Rosenberry R, Trojacek D, Chamseddine HH, Arena-Marshall CA, Zhu Y, et al. Studies into the determinants of skeletal muscle oxygen consumption: novel insight from near-infrared diffuse correlation spectroscopy. The Journal of Physiology. 2019;597(11):2887-901.
11. Finaud J, Lac G, Filaire E. Oxidative Stress. Sports Medicine. 2006;36(4):327-58.

12. Cooper C, Vollaard N, Choueiri T, Wilson M. Exercise, free radicals and oxidative stress. Biochemical Society Transactions. 2002;30(2):280-5.
13. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology. 2003;189(1-2):41-54.
14. Diaz KM, Feairheller DL, Sturgeon KM, Williamson ST, Brown MD. Oxidative stress response to short duration bout of submaximal aerobic exercise in healthy young adults. International Journal of Exercise Science. 2011;4(4):247–256.
15. Gawron-Skarbek A, Chrzczanowicz J, Kostka J, Nowak D, Drygas W, Jegier A, et al. Physical Activity, Aerobic Capacity, and Total Antioxidant Capacity in Healthy Men and in Men with Coronary Heart Disease. Oxidative Medicine and Cellular Longevity. 2015;2015:197307.
16. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. Dynamic Medicine. 2009;8:1-25.
17. Goldfarb AH, McKenzie MJ, Bloomer RJ. Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation. Applied Physiology Nutrition and Metabolism. 2007;32(6):1124-31.
18. Nazmi S, Fatma U, Zuhal H, Bekir Ç. Effects of acute twelve minute run test on oxidative stress and antioxidant enzyme activities. African Journal of Pharmacy and Pharmacology. 2011;5(9):1218-1222.
19. Ammar A, Chtourou H, Hammouda O, Turki M, Ayedi F, Kallel C, et al. Relationship between biomarkers of muscle damage and redox status in response to a weightlifting training session: effect of time-of-day. Physiology international. 2016;103(2):243-61.
20. Withee ED, Tippens KM, Dehen R, Tibbitts D, Hanes D, Zwickey H. Effects of Methylsulfonylmethane (MSM) on exercise-induced oxidative stress, muscle damage, and pain following a half-marathon: a double-blind, randomized, placebo-controlled trial. Journal of the International Society of Sports Nutrition. 2017;14(24):1-11.
21. Seifi-Skishahr F, Siahkohian M, Nakhostin-Roohi B. Influence of aerobic exercise at high and moderate intensities on lipid peroxidation in untrained men. The Journal of sports medicine and physical fitness. 2008;48(4):515-21.
22. Nakhostin-Roohi B, Babaei P, Rahmani-Nia F, Bohlooli S. Effect of vitamin C supplementation on lipid peroxidation, muscle damage and inflammation after 30-min exercise at 75% VO2max. The Journal of sports medicine and physical fitness. 2008;48(2):217-24.
23. Babaei P, Rahmani-Nia F, Nakhostin-Roohi B, Bohlooli S. The effect of VC on immunoendocrine and oxidative stress responses to exercise. Journal of Clinical and Diagnostic Research. 2009;3:1627-32.
24. Sacheck JM, Milbury PE, Cannon JG, Roubenoff R, Blumberg JB. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. Free radical biology & medicine. 2003;34(12):1575-88.
25. Gonzalez-Izal M, Lusa Cadore E, Izquierdo M. Muscle conduction velocity, surface electromyography variables, and echo intensity during concentric and eccentric fatigue. Muscle & nerve. 2014;49(3):389-97.
26. Thirumalai T, Therasa SV, Elumalai EK, David E. Intense and exhaustive exercise induce oxidative stress in skeletal muscle. Asian Pacific Journal of Tropical Disease. 2011;1(1):63-6.
27. Khani M, Motamedi P, Dehkhoda MR, Dabagh Nikukheslat S, Karimi P. Effect of thyme extract supplementation on lipid peroxidation, antioxidant capacity, PGC-1α content and endurance exercise performance in rats. Journal of the International Society of Sports Nutrition. 2017;14(11):1-8.
28. Pourghassem Gargari B, Abedini S, Babaei H, Aliasgarzadeh A, Pourabdollahi P. Effect of supplementation with grape seed (Vitis vinifera) extract on antioxidant status and

lipid peroxidation in patient with type II diabetes. Journal of Medicinal Plants Research. 2011;5(10): 2029-2034.
29. El-Ashmawy IM, Saleh A, Salama OM. Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats. Basic & clinical pharmacology & toxicology. 2007;101(5):320-327.
30. Shi J, Yu J, Pohorly JE, Kakuda Y. Polyphenolics in grape seeds-biochemistry and functionality. Journal of medicinal food. 2003;6(4):291-299.
31. Grases F, Prieto RM, Fernández-Cabot RA, Costa-Bauzá A, Sánchez AM, Prodanov M. Effect of consuming a grape seed supplement with abundant phenolic compounds on the oxidative status of healthy human volunteers. Nutrition Journal. 2015;14(1):94.
32. Sakr HF, Abbas AM, Bin-Jaliah I. Modulation of the neurological and vascular complications by grape seed extract in a rat model of spinal cord ischemia-Reperfusion injury by downregulation of both osteopontin and cyclooxygenase-2. Canadian Journal of Physiology and Pharmacology. 2016;94(7):719-27.
33. Belviranlı M, Gökbel H, Okudan N, Başaralı K. Effects of grape seed extract supplementation on exercise-induced oxidative stress in rats. The British journal of nutrition. 2012;108(2):249-56.
34. Najafi M, Vaez H, Zahednezhad F, Samadzadeh M, Babaei H. Study the effects of hydroalcoholic extract of grape seed (Vitis vinifera) on infarct size and cardiac arrhythmias in ischemic-reperfused isolated rat heart. Journal of Pharmaceutical Sciences. 2011;16(4):187-194.
35. Razmaraii N, Babaei H, Mohajjel Nayebi A, Assadnassab G, Ashrafi Helan J, Azarmi Y. Cardioprotective Effect of Grape Seed Extract on Chronic Doxorubicin-Induced Cardiac Toxicity in Wistar Rats. Advanced Pharmaceutical Bulletin. 2016;6(3):423-33.
36. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical science. 1993;84(4):407-12.
37. Carlsohn A, Rohn S, Mayer F, Schweigert FJ. Physical activity, antioxidant status, and protein modification in adolescent athletes. Medicine & Science in Sports & Exercise. 2010;42(6):1131-9.
38. Carvalho J, Marques E, Ascensão A, Magalhães J, Marques F, Mota J. Multicomponent exercise program improves blood lipid profile and antioxidant capacity in older women. Archives of gerontology and geriatrics. 2010;51(1):1-5.
39. Leelarungrayub D, Khansuwan R, Pothongsunun P, Klaphajone J. N-acetylcysteine supplementation controls total antioxidant capacity, creatine kinase, lactate, and tumor necrotic factor-alpha against oxidative stress induced by graded exercise in sedentary men. Oxidative Medicine and Cellular Longevity. 2011;329643.
40. Kar P, Laight D, Rooprai HK, Shaw KM, Cummings M. Effects of grape seed extract in Type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. Diabetic medicine : a journal of the British Diabetic Association. 2009;26(5):526-31.
41. Natella F, Belelli F, Gentili V, Ursini F, Scaccini C. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. Journal of agricultural and food chemistry. 2002;50(26):7720-5.
42. Moflehi D, Kok L-Y, Tengku-Kamalden T-F, Amri S. Effect of single-session aerobic exercise with varying intensities on lipid peroxidation and muscle-damage markers in

sedentary males. Global Journal of Health Science. 2012;4(4):48-54.
43. Bloomer RJ, Goldfarb AH, McKenzie MJ. Oxidative stress response to aerobic exercise: comparison of antioxidant supplements. Medicine & Science in Sports & Exercise. 2006;38(6):1098-105.
44. Sano A, Uchida R, Saito M, Shioya N, Komori Y, Tho Y, et al. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. Journal of nutritional science and vitaminology. 2007;53(2):174-82.
45. Shan Y, Ye X-H, Xin H. Effect of the grape seed proanthocyanidin extract on the free radical and energy metabolism indicators during the movement. Scientific Research and Essay. 2010; 5(2):148-153.
46. Saada HN, Said UZ, Meky NH, Abd El Azime AS. Grape seed extract Vitis vinifera protects against radiation-induced oxidative damage and metabolic disorders in rats. Phytotherapy research: PTR. 2009; 23(3):434-8.
47. Gleeson M. Biochemical and immunological markers of over-training. Journal of sports science & medicine. 2002;1(2):31-41.
48. Diaz E, Ruiz F, Hoyos I, Zubero J, Gravina L, Gil J, et al. Cell damage, antioxidant status, and cortisol levels related to nutrition in ski mountaineering during a two-day race. Journal of sports science & medicine. 2010;9(2):338-46.
49. Bulduk E, Ergene N, Baltacı AK, Gümüş H. Plasma antioxidant responses and oxidative stress following a 20 meter shuttle run test in female volleyball players. Journal of Human Sciences. 2011;8(2):510-27.
50. Marin DP, dos Santos Rde C, Bolin AP, Guerra BA, Hatanaka E, Otton R. Cytokines and oxidative stress status following a handball game in elite male players. Oxidative Medicine and Cellular Longevity. 2011;2011:804873.
51. Büttner P, Mosig S, Lechtermann A, Funke H, Mooren FC. Exercise affects the gene expression profiles of human white blood cells. Journal of applied physiology. 2007;102(1):26-36.
52. Coco M, Perciavalle V. Creatine ingestion effects on oxidative stress in a steady-state test at 75% VO(2max). The Journal of sports medicine and physical fitness. 2012;52(2):165-9.