THE PROTECTIVE EFFECTS OF PISTACHIO NUT (*PISTACIA VERA L.*) ON THIOL/DISULFIDE HOMEOSTASIS IN YOUNG SOCCER PLAYERS UNDERGOING A STRENUOUS EXERCISE TRAINING PROGRAM

Hakim Celik[1], Mehmet Kucuk[1], Yakup Aktas[2], Mustafa Zerin[1], Ozcan Erel[3], Salim Neselioglu[3], Davut Sinan Kaplan[4]

[1]Harran University, Medical Faculty, Department of Physiology, Sanliurfa, Turkey - [2]Harran University, School of Physical Education and Sports, Sanliurfa, Turkey - [3]Yildirim Beyazit University, Medical Faculty, Department of Clinical Biochemistry, Ankara, Turkey - [4]Gaziantep University, Medical Faculty, Department of Physiology, Gaziantep, Turkey

ABSTRACT

Introduction*: The exercised skeletal muscle may induce oxidative stress (OS) leading to contractile dysfunction, fatigue and weakness during a strenuous exercise training. Pistachio nut, a rich source of potent antioxidants, can prevent OS by reducing reactive oxygen species (ROS) production and by strengthening the antioxidant defense system. Therefore, we aimed to investigate the thiol/disulfide homeostasis (TDH) parameters, a potent indicator of OS, to evaluate both the harmful effects of the strenuous soccer training program (STP) and the protective effects of pistachio nut against OS caused by a strenuous STP.*

Materials and methods*: Forty male soccer players from two professional soccer teams and twenty-one healthy control male subjects of a similar age and body mass index who were not interested in any sports were enrolled in this study. Immediately after a three month resting period, the forty soccer players were randomly divided into a soccer player group (SPG) and an experimental group (EG). The players in the SPG underwent a twenty-one day strenuous STP only, and those in the EG underwent the same twenty-one day STP and received raw pistachio nut kernels (25 g/day) as a dietary supplement. A baseline venous blood sample was obtained from all participants, and a subsequent venous blood sample was obtained after the STP in only the STG and EG. Serum TDH parameters were measured using an automated technique.*

Results*: No significant difference was observed in TDH parameters among SPG, EG, and control group before STP. Although TDH impaired significantly over time in the SPG (at baseline and after the twenty-one day STP), no significant alterations in the TDH parameters were observed over time in the EG.*

Conclusions*: The results indicate that a strenuous STP may induce OS and that dietary supplementation with pistachio nuts may prevent this OS-associated damage in young soccer players.*

Keywords*: Oxidative stress,soccer players, exercise training, nutrition, thiol/disulfide homeostasis, pistachio nut.*

DOI: 10.19193/0393-6384_2019_2_135

Received *November 30, 2018;* ***Accepted*** *January 20, 2019*

Introduction

During a strenuous physical exercise, active oxygen consumption in the whole body may increase by up to 20 times of that during resting period and by up to 100 times in the active skeletal muscles[(1)]. Majority of oxygen (≈95%–98%) in the skeletal muscles is used to form ATP and water, and the remaining ≈2%–5% oxygen is reduced to produce reactive oxygen species (ROS)[(1,2)]. ROS overproduction during a strenuous exercise training induce oxidative stress (OS), leading to muscle atrophy, contractile dysfunction, fatigue and muscle weakness[(2-4)].

Pistachio nut (*Pistacia vera L.*), which belongs to the botanical family Anacardiaceae, has long been consumed alone or as an ingredient of traditional recipes in many countries in Central Asia, Western Asia, and Mediterranean basin (Figure 1)[(5)]. Among tree nuts, pistachio nuts are one of the richest source of water- and fat-soluble antioxidants[(6-8)]. Moreover, pistachio nuts are among the top 50 food products with the highest antioxidant content[(9)] and are an abundant source of phenolic compounds, which possess high antioxidant activity (Table 1)[(10,11)].

Thiols, which contain a sulfhydryl functional group, are potent antioxidants that protect organisms

from the destructive effects of OS[12,13]. During OS when ROS production has increased and/or antioxidant status has decreased, thiol groups in albumin, cysteine, homocysteine, glutathione, and other proteins form disulfide bonds[12,14]. However, when antioxidants levels exceed ROS production, the disulfide bonds reversibly reduce to thiol groups, thus maintaining a dynamic thiol/disulfide homeostasis (TDH)[14].

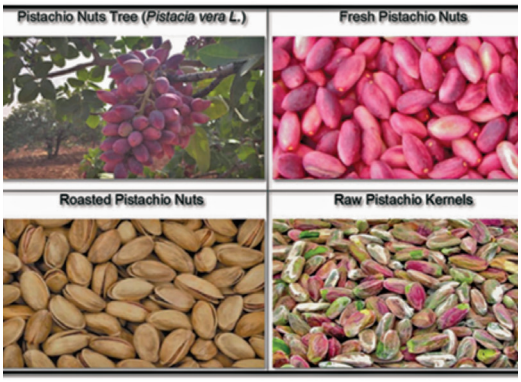

Figure 1: Tree, and fresh, roasted and kernels of Pistachio nuts (*Pistacia vera L.*).

Pistachio nuts, per 100 g	
Energy, kcal	572
SFA, g	5.60
PUFA, g	13.3
MUFA, g	24.5
Protein, g	21.0
Carbohydrates, g	28.3
Fiber, g	10.3
Water, g	1.9
Ashes, g	3.0
Vitamin C, mg	3.0
Vitamin A, IU	266
α-Tocopherol, mg	2.17
β-Tocopherol, mg	0.13
γ-Tocopherol, mg	23.42
δ-Tocopherol, mg	0.53
Beta carotene, µg	159

Table 1: Nutritional composition of pistachio nuts per 100 g (dry roasted).
Data obtained from US Department of Agriculture (USDA), Nutrient Database for Standard Reference, Release 28[11].
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; IU, international units.

Therefore, the dynamic TDH is suggested to be a marker of OS and to play a crucial role in the antioxidant defense system[15].

Strenuous soccer training can induce OS, which may cause diseases such as atherosclerosis, and cancer[16] and other complications such as fatigue and muscle weakness[2,4,17]. To the best of our knowledge, no study has reported the protective effects of pistachio nuts, which are one of the richest source of water- and oil-soluble potent antioxidants and phenolic compounds, on OS induced by a strenuous soccer training. Therefore, the present study investigated the protective effects of pistachio nuts on OS induced by a newly initiated strenuous soccer training program (STP) after a 3-month resting period by assessing the dynamic TDH, a new indicator of OS, in young soccer players.

Materials and methods

Subjects

This study included 40 young male soccer players (age: 19.55 ± 1.08 years; body mass index [BMI]: 21.30 ± 0.84 kg/m^2) from 2 professional soccer teams and 23 healthy control male subjects (age: 20.04 ± 1.15 years; BMI: 21.72 ± 3.12 kg/m^2) who were not interested in any sports. All the participants provided a written consent for participation in the study and received a detailed description of study procedures, which were approved by the ethics committee of the university and were in accordance with the Declaration of Helsinki. The soccer players were divided randomly into two groups, namely, soccer player group (SPG) and experimental group (EG), with each group containing 20 players. After a 3-month resting period, the players in the SPG underwent a 21-day STP and those in the EG underwent the same 21-day STP and received pistachio nut kernels (25 g/day) as a dietary supplement. At the beginning of the study, all the participants were asked to complete a questionnaire for obtaining information on their general health status, medical history, and nutritional patterns. Participants with suspicious pathological findings, recent illnesses, or injuries and those using drugs, cigarettes, alcohol, vitamins, and nutrients, which could affect OS markers, were excluded from the study. Consumption of eggs, meat, vegetables, dairy products, fruits, and soft drinks was similar among the participants.

The soccer training program

All the soccer player underwent the STP for 3 weeks. During each week, the players performed technical, tactical, and basic training for 5 days;

played a 90-min game for 1 day; and rested for 1 day. During the 5-day training sessions, the players underwent regeneration, endurance, speed and agility, coordination, and technical–tactical training, respectively. The daily training session lasted for 85-100 min in total and was divided into three parts, namely, warm up (15-20 min); technical, tactical, and basic soccer skills (50-60 min); and cool down (15-20 min). Finally, the players played a 90-min game on the sixth day of the week and were rested on the seventh day of the week.

Blood Collection

Just before the initiation of the STP, venous blood samples were collected from each participant. Venous blood samples were collected after the 3-week STP in the morning after a minimum of 8 hours of overnight fasting from only the SPG and the EG. Next, the collected samples were centrifuged at 1500 × g for 10 min, and the separated serum samples were stored at -86°C for further analysis.

Thiol/disulfide homeostasis parameters

Serum TDH parameters were examined using a new, automated measurement technique improved by Erel and Neselioglu[18]. Briefly, serum native thiol and total thiol levels were determined spectrophotometrically by using Cobas c501 (Roche Diagnostics, Indianapolis, Indiana, USA). First, serum native thiol levels were measured by reacting the samples with 5, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) without any previous processing. Second, total thiol levels were measured by reducing dynamic disulfide bonds in the serum samples by using sodium borohydride (NaBH4) to produce free functional thiol groups. Next, formaldehyde was used to completely remove unused NaBH4, and total thiol groups, including reduced and native thiol groups, were measured by reacting the samples with DTNB. Because the reduction of a disulfide bond generates two distinct thiol groups, the amount of the dynamic disulfide bonds was calculated by determining 50% difference between the total thiol and native thiol groups. In addition, percentage disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios were calculated.

Statistical analysis

Statistical analyses were conducted using SPSS for Windows version 23.0 (IBM SPSS Inc, Chicago, IL, USA). Shapiro-Wilk test was used to assess the normal distribution of data. Numerical variables showing normal distribution are presented as mean ± standard deviation and those not showing normal distribution are presented as median (interquartile range). The One-Way ANOVA test was used to conduct multiple comparisons. To compare intragroup alterations over time (from baseline to the end of the STP), all dependent variables were examined using paired Student's t-test and Wilcoxon t-test. Confidence interval was accepted as 95% throughout the analyses, and a two-tailed p value of <0.05 was considered statistically significant.

Results

Before the initiation of the 21-day STP, no significant differences were observed among the SPG, the EG, and the control group with respect to the TDH parameters, BMI, age, and albumin level ($p > 0.05$ for each) (Table 2).

	Experimental Group (n = 20)	Soccer Player Group (n = 20)	Control Group (n = 23)	ANOVA P
Age (years)	19.35 ± 1.04	19.75 ± 1.12	20.04 ± 1.15	0.130
BMI (kg/m^2)	21.39 ± 0.78	21.21 ± 0.92	21.72 ± 3.12	0.703
Native thiol (μmol/L)	530.8 ± 32.2	535.3 ± 27.8	529.2 ± 23.3	0.762
Total thiol (μmol/L)	563.5 ± 37.7	570.3 ± 33.4	562.9 ± 21.5	0.702
Disulfide (μmol/L)	16.31 ± 8.05	17.47 ± 6.09	17.28 ± 4.98	0.827
%Disulfide/native thiol	3.07 ± 1.51	3.25 ± 1.12	3.28 ± 0.99	0.831
%Disulfide/total thiol	2.86 ± 1.33	3.04 ± 0.98	3.07 ± 0.88	0.789
%Native thiol/total thiol	94.28 ± 2.65	93.92 ± 1.97	94.00 ± 1.63	0.853

Table 2: The comparison of demographic and thiol/disulfide homeostasis parameters among experimental, soccer player and control groups, before soccer training program.
BMI, body mass index. The data are presented as mean ± standard deviation.

Throughout the 21-day STP (at baseline and at the end of the training program), no significant alterations were observed in the albumin level and TDH parameters of the players in the EG ($p > 0.05$ for each) and in the albumin and native thiol levels of the players in the SPG ($p > 0.05$ for each). However, the total thiol and disulfide levels and the disulfide/native thiol and disulfide/total thiol ratios were significantly increased ($p = 0.015$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) and the native thiol/total thiol ratio was significantly decreased ($p < 0.001$) throughout the 21-day STP in the players in SPG (Table 3 and Figure 2).

	Variable	BISTP	AESTP	p Value
Experimental Group	Albumin (g/L)	4.47 ± 0.14	4.55 ± 0.13	0.071
	Native thiol (μmol/L)	530.84 ± 32.17	542.82 ± 19.80	0.071
	Total thiol (μmol/L)	563.45 ± 37.74	576.62 ± 23.43	0.055
	Disulfide (μmol/L)	16.00 [12.62]	14.89 [14.97]	0.940
	%Disulfide/native thiol	2.99 [2.36]	2.77 [2.63]	0.911
	%Disulfide/total thiol	2.83 [2.10]	2.62 [2.32]	0.940
	%Native thiol/total thiol	94.35 [4.19]	94.76 [4.65]	0.940
Soccer Player Group	Albumin (g/L)	4.54 ± 0.13	4.50 ± 0.15	0.140
	Native thiol (μmol/L)	535.31 ± 27.79	536.60 ± 24.52	0.830
	Total thiol (μmol/L)	570.26 ± 33.36	588.29 ± 32.99	0.015
	Disulfide (μmol/L)	17.47 ± 6.09	25.85 ± 10.21	<0.001
	%Disulfide/native thiol	3.26 ± 1.12	4.82 ± 1.92	<0.001
	%Disulfide/total thiol	3.04 ± 0.98	4.34 ± 1.60	<0.001
	%Native thiol/total thiol	93.92 ± 1.97	91.32 ± 3.20	<0.001

Table 3: The comparison of thiol/disulfide homeostasis parameters at baseline and at the end of the soccer training program, in both experimental and soccer player groups.
BMI, body mass index; BISTP, before the initiation of the soccer training program; AESTP, after the end of the soccer training program. The data are presented as mean ± standard deviation and median [interquartile range].

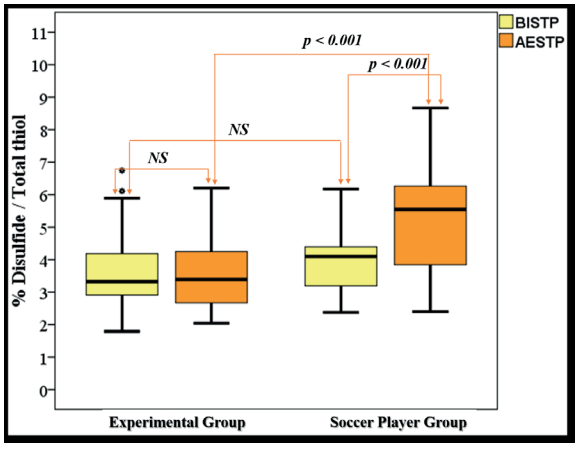

Figure 2: The comparison of disulfide / total thiol ratio at baseline and at the end of the soccer training program, in both experimental and soccer player groups.
BISTP, before the initiation of the soccer training program; AESTP, after the end of the soccer training program; NS, non significant

Discussion

Although a confirmed association exists between OS and strenuous exercise training[1-4] and between the antioxidant system and pistachio nuts[6-10], no study has addressed the protective effects of pistachio nuts on OS induced by a strenuous exercise training to the best of our knowledge. The present study showed that TDH, a new and important indicator of OS, was impaired after the 21-day strenuous STP in the young soccer players who did not consume antioxidant compounds as a dietary supplement. However, TDH was not impaired in the young soccer players who consumed pistachio nut kernels (25 g/day) as a dietary supplement throughout the strenuous STP.

STP involving a long period of strenuous physical activity can induce OS, which is characterized by an imbalance between ROS production and the antioxidant defense system[19]. Strenuous physical activity induces OS by increasing ROS production in the mitochondria of skeletal muscle cells through nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase enzymes[20,21]. OS induced by a strenuous exercise training may trigger muscle atrophy and contractile dysfunction, leading to fatigue and decreased physical performance[2,3,22]. Similar to previous studies, the present study showed that TDH, a new and important indicator of OS, changed in favor of disulfide after the 21-day strenuous STP among the players in the SPG who

did not consume additional antioxidant nutrients in their diet. Moreover, the disulfide level and disulfide/native thiol and disulfide/total thiol ratios increased significantly over time (at baseline and at the end of the STP) in the players in the SPG. Therefore, TDH, which is an indirect measure of possible ROS production, may help coaches and sports practitioners in regulating exercise load and dietary antioxidant content in soccer players to minimize cellular damage and injury.

Antioxidant nutrient deficiency weakens the antioxidant defense system and increases strenuous exercise-induced OS and tissue damage[23]. Pistachio nuts, which are among the top 50 food products with the highest potent antioxidant content[9] and a rich source of phenolic compounds[10], may prevent OS-induced tissue damage and strengthen the antioxidant defense system during a strenuous physical activity. Several studies have shown that pistachio nuts prevent OS by reducing ROS production and by strengthening the antioxidant defense system[5,24,25]. An experimental human study by Kocyigit et al. showed that a 3-week diet of pistachio nuts ameliorated OS by decreasing malondialdehyde level, an important indicator of lipid peroxidation, and by increasing antioxidant potential[5]. An experimental animal study conducted by our research team showed that pistachio nut consumption of up to 20% of the daily calorie intake increased paraoxonase 1 and arylesterase activities, which prevented LDL cholesterol oxidation, which causes coronary artery disease[25]. Sharika et al. showed that a methanolic extract of pistachio nuts exerted hepatoprotective effects against ROS production and lipid peroxidation[26]. Consistent with the findings of these studies, the present study indicated that although TDH changed significantly toward disulfide, which is an ROS, among the players in the SPG after the 21-day strenuous STP, TDH was not impaired among the players in the EG who consumed pistachio kernels (25 g/day) as a dietary supplement. These findings indicate that pistachio nuts prevent the impairment of TDH, an important indicator of OS, induced by the 21-day newly initiated strenuous exercise training after a 3-month resting period.

Limitations

This study has two limitations. First, the extraction and characterization of pistachio nuts could not be performed in the present study. Second, the participants included in the present study were not given a fixed and specific diet.

Conclusions

The present study provided three important conclusions and/or suggestions. First, a newly initiated strenuous STP after a 3-month resting period may impair TDH, which is a potent indicator of OS, leading to fatigue and muscle weakness. Second, pistachio nut, which is a rich source of potent antioxidants, can prevent OS induced by strenuous STP in soccer players. Third, these data may help coaches and sports practitioners in regulating exercise load and diet of soccer players to reduce OS-induced cellular damage and injury.

References

1) Andersson H, Karlsen A, Blomhoff R, Raastad T, Kadi F. Plasma antioxidant responses and oxidative stress following a soccer game in elite female players. Scand J Med Sci Sports 2010; 20(4): 600-8.
2) Brites FD, Evelson PA, Christiansen MG, Nicol MF, Basilico MJ, et al. Soccer players under regular training show oxidative stress but an improved plasma antioxidant status. Clin Sci (Lond) 1999; 96(4): 381-5.
3) Steinbacher P, Eckl P. Impact of oxidative stress on exercising skeletal muscle. Biomolecules 2015; 5(2): 356-77.
4) Pal S, Chaki B, Chattopadhyay S, Bandyopadhyay A. High-intensity exercise induced oxidative stress and skeletal muscle damage in postpubertal boys and girls. J Strength Cond Res 2018; 32(4): 1045-52.
5) Kocyigit A, Koylu AA, Keles H. Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers. Nutr Metab Cardiovasc Dis 2006; 16(3): 202-9.
6) Bolling BW, McKay DL, Blumberg JB. The phytochemical composition and antioxidant actions of tree nuts. Asia Pac J Clin Nutr 2010; 19(1): 117-23.
7) Dreher ML. Pistachio nuts: Composition and potential health benefits. Nutrition Reviews 2012; 70(4): 234-40.
8) Paterniti I, Impellizzeri D, Cordaro M, Siracusa R, Bisignano C, et al. The anti-inflammatory and antioxidant potential of pistachios (Pistacia vera L.) in vitro and in vivo. Nutrients 2017; 915(9): 1-15.
9) Halvorsen BL, Carlsen MH, Phillips KM, Bohn SK, Holte K, et al. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am J Clin Nutr 2006; 84(1): 95-135.
10) Martorana M, Arcoraci T, Rizza L, Cristani M, Bonina FP, et al. In vitro antioxidant and in vivo photoprotective effect of pistachio (Pistacia vera L., variety Bronte) seed and skin extracts. Fitoterapia 2013; 85: 41-8.

11) Hernandez-Alonso P, Bullo M, Salas-Salvado J. Pistachios for health: What do we know about this multifaceted nut? Nutr Today 2016;51(3):133.
12) Baba SP, Bhatnagar A. Role of thiols in oxidative stress. Curr Opin Toxicol 2018;7:133–9.
13) Hanikoglu F, Hanikoglu A, Kucuksayan E, Alisik M, Gocener AA, et al. Dynamic thiol/disulphide homeostasis before and after radical prostatectomy in patients with prostate cancer. Free Radic Res 2016;50(1):79–84.
14) Cakici EK, Eroglu FK, Yazilitas F, Bulbul M, Gur G, et al. Evaluation of the level of dynamic thiol/disulphide homeostasis in adolescent patients with newly diagnosed primary hypertension. Pediatr Nephrol 2018; 33(5): 847-53.
15) Zubarioglu T, Kiykim E, Cansever MS, Neselioglu S, Aktuglu-Zeybek C, et al. Evaluation of dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in maple syrup urine disease patients under treatment. Metab Brain Dis 2017; 32(1): 179-84.
16) Tapia-Vieyra JV, Delgado-Coello B, Mas-Oliva J. Atherosclerosis and cancer; A resemblance with far-reaching implications. Arch Med Res 2017; 48(1): 12-26.
17) Qaisar R, Bhaskaran S, Premkumar P, Ranjit R, Natarajan KS, et al. Oxidative stress-induced dysregulation of excitation-contraction coupling contributes to muscle weakness. J Cachexia Sarcopenia Muscle 2018; 9(5): 1003-17.
18) Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014; 47(18): 326-32.
19) Lafay S, Jan C, Nardon K, Lemaire B, Ibarra A, et al. Grape extract improves antioxidant status and physical performance in elite male athletes. J Sports Sci Med 2009; 8(3): 468-80.
20) Powers SK, Duarte J, Kavazis AN, Talbert EE. Reactive oxygen species are signalling molecules for skeletal muscle adaptation. Exp Physiol 2010; 95(1): 1-9.
21) Perrea A, Vlachos IS, Korou L, Doulamis IP, Exarhopoulou K, et al. Comparison of the short-term oxidative stress response in national league basketball and soccer adolescent athletes. Angiology 2013; 65(7): 624-9.
22) Powers SK, Nelson WB, Hudson MB. Exercise-induced oxidative stress in humans: Cause and consequences. Free Radic Biol Med 2011; 51(5): 942-50.
23) Sureda A, Ferrer MD, Tauler P, Romaguera D, Drobnic F, et al. Effects of exercise intensity on lymphocyte H2O2 production and antioxidant defences in soccer players. Br J Sports Med 2009; 43(2): 186-90.
24) Kay CD, Gebauer SK, West SG, Kris-Etherton PM. Pistachios increase serum antioxidants and lower serum oxidized-ldl in hypercholesterolemic adults. J Nutr 2010; 140(6): 1093-8.
25) Aksoy N, Aksoy M, Bagci C, Gergerlioglu HS, Celik H, et al. Pistachio intake increases high density lipoprotein levels and inhibits low-density lipoprotein oxidation in rats. Tohoku J Exp Med 2007; 212(1): 43-8.
26) Shahraki J, Kamalinejad M, Shahraki J, Zareh M. Cytoprotective effects of hydrophilic and lipophilic extracts of Pistacia vera against oxidative versus carbonyl stress in rat hepatocytes. Iran J Pharm Res 2014; 13(4): 1263-77.

Corresponding Author:
Dr. Hakim Celik
Harran University, Medical Faculty,
Department of Physiology
63000, Sanliurfa
E-mail: hakimcell@gmail.com
(Turkey)