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The Effects of Regular Moderate Intensity Exercise on Oxidative Stress and Serum Prolidase Levels: A Comparative Study

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Abstract

Background:The effects of exercise on oxidation state is still a controversial topic. Additionally, the relation between exercise and serum levels of prolidase enzyme has not been reported so far. We aim to compare sedentary and physically active individuals regarding the levels of oxidative stress biomarkers and prolidase enzyme. Materials and Methods: Healthy individuals, 19-22 years old, were enrolled in this study, encompassing the exercise group (n=79) and the sedentary group (n=48). The serum levels of glutathione peroxidase (GPX), catalase, malondialdehyde, total antioxidant status (TAS), total oxidant status (TOS), and prolidase were assayed. Statistical analyses were applied to the findings. Results: The groups demonstrate insignificant difference regarding the serum GPX ($p=0.558$) and catalase ($p=0.628$) levels. The serum levels of malondialdehyde ($p<0.001$) and prolidase ($p<0.001$) are significantly higher in the exercise group and sedentary group than the other group, respectively. The TOS and TAS levels are considerably higher in the exercise group ($p=0.025$) and sedentary group ($p<0.001$) than the other group, respectively. Statistical analysis demonstrates significant relationship between the prolidase and TAS levels in the exercise group ($r=0.243$, $p=0.031$). Conclusion: The remarkably lower prolidase levels in the exercise group suggest decreased collagen turnover in physically active individuals. Oxidative stress appears to occur without compensation by enzymatic antioxidant mechanisms in young adults, involved in moderate intensity exercises. This study also indicates a correlation between the serum levels of prolidase and TAS in this population.

Keywords: Exercise, exopeptidase, free radical scavengers, malondialdehyde, oxidative stress

Düzenli Orta Düzey Egzersizin Oksidatif Stres ve Serum Prolidaz Düzeyleri Üzerindeki Etkileri: Karşılaştırmalı Bir Çalışma

Özet

Amaç: Egzersizin oksidasyon durumu üzerine etkileri halen tartışmalı bir konudur. Ayrıca egzersiz ile sreum prolidaz enzim düzeyleri arasındaki ilişki henüz bildirilmemiştir. Çalışmamızda fiziksel olarak aktif bireyler ile hareketsiz yaşam süren bireylerin oksidatif stres belirteçlerinin ve prolidaz enziminin düzeylerine göre karşılaştırılması amaçlanmıştır. Gereç ve Yöntem: On dokuz ile yirmi iki yaş aralığında ve sağlıklı olan bireyler çalışmaya dahil edildi. İki çalışma grubu oluşturuldu: Egzersiz yapan grup (n=79) ve egzersiz yapmayan grup (n=48). Katılımcılarda glutatyon peroksidaz (GPX), katalaz, malondialdehit, total antioksidan kapasite (TAK), total oksidan kapasite (TOK) ve prolidazın serum düzeyleri çalışıldı. Bulgular istatistiksel analize tabi tutuldu. Sonuç: Çalışma grupları arasında GPX ($p=0.558$) ve katalaz ($p=0.628$) düzeylerine göre istatistiksel anlamlı fark bulunmadı. Malondialdehit düzeyi egzersiz yapan grupta, prolidaz düzeyi egzersiz yapmayan grupta anlamlı düzeyde diğer gruba göre yüksekti ($p<0.001$). Egzersiz yapan gruptaki TOK düzeyinin yüksekliği ($p=0.025$), egzersiz yapmayan gruptaki TAK düzeyinin yüksekliği ($p<0.001$) istatistiksel olarak anlamlı idi. İkili korelasyon analizi sadece egzersiz yapan gruptaki prolidaz ve TAK düzeyleri arasında anlamlı ilişki gösterdi ($r=0.243$, $p=0.031$). Tartışma: Egzersiz yapan gruptaki belirgin prolidaz düzey düşüklüğü fiziksel olarak aktif bireylerde kollajen döngüsünün daha az olduğunu öne sürmektedir. Orta düzey egzersiz yapan genç yetişkinlerde oksidatif stresin, enzimatik antioksidan mekanizmanın telafisi olmaksızın meydana geldiği görünmektedir. Bu sonuçlar egzersiz yapan genç yetişkinlerde serum prolidaz ile TAK düzeyleri arasında anlamlı ilişki olduğunu işaret etmektedir.

Anahtar kelimeler: Egzersiz, ekzopeptidaz, serbest radikal temizleyicileri, malondialdehit, oksidatif stres

INTRODUCTION

Prolidase (EC. 3.4. 13.9) is a manganese dependent cytosolic exopeptidase, found in some strains of bacteria and in the various tissues of mammalians – particularly erythrocyte, kidney and intestinal mucosa (23). It cleaves iminodipeptides which contain proline or hydroxyproline at the C-terminal end (23,26). The enzyme has an important role in the collagen turnover and thus in the maintenance of connective tissues (5,23,26). Many studies have suggested the prolidase activity to be related to the oxidation state of the patients with various disorders (13,17-19,23). The changes in the serum prolidase levels of healthcare staff during the hospital shifts have even been reported (5). To the best of our knowledge, the effects of exercise on the serum levels of prolidase enzyme have not been reported in the literature so far.

Reactive oxygen species (ROS), chemically reactive molecules containing oxygen, are generated by the mitochondrial respiration of human cells (31). ROS deplete the plasma antioxidants, damage DNA and cause inflammatory response by modification of the biomolecules (5,13,17,18). ROS elimination is supposed to counterbalance the formation in healthy humans through the activity of antioxidant scavenger enzymes such as glutathione peroxidase (GPX) (EC 1.11.1.9) and catalase (EC 1.11.1.6) and endogenous non-enzymatic antioxidants such as albumin, bilirubin, and uric acid (15,33). Besides, exogenous antioxidant molecules including beta-carotene, vitamin C, vitamin E, and thiol compounds (e.g. glutathione) reinforce the elimination of ROS (15,33).

ROS become more abundant because of oxidative stress (OS). OS is defined as the loss of balance between the formation and the elimination of ROS, resulting in increased concentrations of these molecules, despite the counterbalance mechanisms of human metabolism (5,13,15,17,18,29). OS can occur due to endogenous causes (inflammation, infection, malignancy, excessive physical activity, and mental stress) as well as exogenous causes (smoking, alcohol, cooking, medication, and exposure to environmental pollutants and radiation) (29). The response of antioxidant defense mechanism (ADM) to exercise in terms of the levels of OS biomarkers can be variable (15). Although investigated by many researchers, the effects of regularly performed, moderate intensity exercises on OS with regard to enzymatic and non-enzymatic biomarkers are still among the controversial and relatively less studied topics (11,15,29,33). Moreover, the correlation between the biomarkers of OS and prolidase enzyme in young adults, who are involved in regular exercises, has not been reported yet. The present study was designed to compare sedentary individuals with individuals who regularly perform moderate intensity exercises, considering the serum levels of OS biomarkers as well as the serum levels of prolidase, in order to describe the effects of aforementioned levels of exercises on these biochemical parameters. The correlations between the serum levels of OS biomarkers and prolidase were analyzed as well.

MATERIALS AND METHODS

This prospective, cross-sectional, comparative study was conducted with the approval of the institutional review board (approval date: November 8, 2017; document number: 18) and in accordance with the ethical principles for medical research involving human subjects, as outlined in the Declaration of Helsinki. Before enrollment, all subjects, who volunteered to participate in this study, were informed and written consents were obtained from them. The students of Medical Faculty (MF) and Physical Training and Sports Faculty (PTSF) at the age of 19 to 22 were included in the study. The latter (PTSF) group comprised subjects who were regularly involved in various sportive activities (such as football, volleyball, tennis, swimming, etc.) in accordance with the curriculum of the faculty. The exclusion criteria were the presence of acute or chronic disease, history of previous or current tobacco use, alcohol consumption habit, any kind of medication or supplementation use. The students of MF performing regular exercise (i.e. participation in sports activity or any kind of exercise at least three hours per week) were excluded from the study as well. The samples were obtained in the period of March 2018 to June 2018.

All blood samples were drawn from the antecubital veins of the participants using sterile vacutainer needles following skin antiseptic preparation. After taking 5 mL of venous blood into the polypropylene tubes containing ethylenediaminetetraacetic acid and gentle mixing, the tubes were centrifuged at 4000 rpm for 15 minutes at 4 degrees Celsius. The supernatant plasma samples, obtained after the centrifugation

process, were stored in plastic tubes at -80 degrees Celsius until the samples were assayed for prolidase enzyme activity and OS biomarkers. Initially thawed at room temperature, the plasma samples were diluted 40-fold using 2.5 mmol/L Mn^{2+} and 40 mmol/L Trizma HCl buffer (pH 8.0) and incubated at 37 degrees Celsius for two hours. Thereafter, the reaction mixtures, consisting of 30 mmol/L gly-pro, 40 mmol/L Trizma HCl buffer (pH 8.0), and 100 µL of incubation serum in 1 mL, were incubated at 37 degrees Celsius for 30 minutes. The incubation reactions were ended with the addition of 0.5 mL 20% trichloroacetic acid solution. The supernatants were assayed for proline using the method described by Myara et al. (25), a modification of Chinard's method (8). All reagents used were of analytical grade and acquired from Sigma-Aldrich (St.Louis, Missouri, USA) and Merck (Darmstadt, Germany). The standard curve was used to calculate the concentrations of the samples. Intra-assay coefficient of variation was 4 for the samples.

Total oxidant status (TOS) and total antioxidant status (TAS) were measured utilizing the commercially available kits (REL Assay Diagnostics, Mega Tip, Gaziantep, Turkey) in a microplate reader (Thermo Scientific Multiskan FC, USA). The standard curve was used to calculate the concentrations of all unknown samples. The serum TOS and TAS levels are expressed in mmol/L.

The measurements of malondialdehyde (MDA) levels were in accordance with the method described by Ohkawa et al. (27) and were based on the measurement of thiobarbituric acid-malondialdehyde absorbance. The catalase enzyme activity was measured using the method described by Beutler (1). The serum MDA and catalase activity levels are expressed in nmol/ml and U/g hb, respectively.

In the presence of t-butyl hydroperoxide, glutathione oxidation to glutathione disulfide takes place with the activity of GPX enzyme. The procedure is based on the principle of quantification of the change of absorbance value at 340 nm wavelength reflecting oxidization of NADPH to NADP in the reaction which reduces glutathione disulfide to glutathione by the glutathione reductase enzyme (EC 1.6.4.2) catalysis (2). The serum GPX activity levels are expressed in U/g hb.

The statistical analyses were performed using IBM SPSS version 22.0 for Windows (IBM Corp., Armonk, NY, USA) to investigate the differences between the study groups. Shapiro-Wilk test demonstrated normal distribution of the data. For the multiple group and two independent group comparisons, one-way analysis of variance (ANOVA) and independent samples t test were performed, respectively. Scheffe test and Tamhane T2 test were utilized for the pairwise post-hoc testing. The relation between the quantitative variables was investigated using Pearson's correlation analysis. The distribution of the categorical variables was evaluated using Chi-square test and exact test. The statistical parameters are expressed in mean ± standard error of the mean (SEM), percentage (%) and frequency (n). Statistical significance was considered for p values less than 0.05.

RESULTS

The demographic features of the study groups (MF students in the sedentary group and PTSF students in the exercise group) are depicted in Table 1. The mean serum GPX level of the sedentary group is higher than that of the exercise group and the mean serum catalase level of the exercise group is higher than that of the sedentary group (Table 2). However, the differences are trivial ($p=0.628$ for the mean catalase levels and $p=0.558$ for the mean GPX levels). The differences between the study groups are considerable in terms of the mean serum MDA and prolidase levels ($p<0.001$ for both comparisons). The former and the latter parameters are greater in the exercise group and the sedentary group, respectively. The mean serum TOS level of the exercise group is considerably high compared to that of the sedentary group ($p=0.025$). Conversely, the mean serum TAS level of the sedentary group is remarkably high compared to that of the exercise group ($p<0.001$), as shown in Table 2.

The analyses of the biochemical parameters by the pairwise comparison of the study groups within the genders are depicted in Table 3. The mean serum GPX levels of female ($p=0.737$) and male ($p=0.708$) subgroups do not demonstrate any remarkable difference when the sedentary and the exercise groups are compared. The differences between the study groups are trivial regarding the mean serum catalase levels of female ($p=0.306$) and male ($p=0.472$) subgroups. The mean serum MDA levels of the exercise group are significantly high compared to the sedentary group, both for female ($p=0.004$) and male ($p<0.001$) subgroups. On the other hand, the mean serum prolidase levels of the

sedentary group are significantly high compared to the exercise group, both for female (p=0.001) and male (p<0.001) subgroups. The mean serum TAS levels of the sedentary group are higher than that of the exercise group with considerable difference regarding female (p=0.005) and male (p=0.001) subgroups. The significance levels of differences between the study groups regarding the mean serum TOS levels within the genders are quite dissimilar. The male subgroup of the exercise group have considerably greater mean serum TOS level than the male subgroup of the sedentary group (p=0.018) while the mean serum level of female subgroup of the exercise group is higher than that of the sedentary group with minor difference (p=0.359). The mean levels of the OS biomarkers and prolidase were also compared between the genders within the study groups and no considerable differences could be identified between the genders, as shown in Table 3.

The multiple comparisons of the gender-based subgroups depict insignificant difference regarding the mean serum levels of GPX (p=0.943), catalase (p=0.591), and TOS (p=0.086) (Table 4). On the other hand, the mean serum levels of prolidase and TAS in the female and male subgroups of the sedentary group are considerably high compared to the female and male subgroups of the exercise group (p<0.001 for the comparisons of the mean prolidase and TAS levels). The mean serum levels of MDA in the female and male subgroups of the exercise group are considerably greater than that of the female and male subgroups of the sedentary group (p=0.001).

The bivariate correlation analyses between the mean levels of prolidase enzyme and OS parameters (prolidase – GPX: r=0.191, p=0.194; prolidase – catalase: r=-0.244, p=0.095; prolidase – MDA: r=-0.073, p=0.623; prolidase – TOS: r=-0.019, p=0.898; and prolidase – TAS: r=-0.251, p=0.085) do not reveal any significant correlation in the sedentary group. The analyses do not demonstrate any significant correlation between the mean levels of prolidase and GPX (r=0.141, p=0.215), catalase (r=0.174, p=0.124), MDA (r=-0.024, p=0.832), and TOS (r=0.103, p=0.366) in the exercise group as well. On the other hand, the analysis demonstrates positive correlation with statistical significance between the mean levels of prolidase and TAS in the exercise group (r=0.243, p=0.031).

DISCUSSION

ADM protects human body against the harmful effects of the natural byproducts – ROS – which are generated by the metabolic processes (11,15,29,33). The antioxidant scavenger enzymes, essential component of the defense mechanism, continually buffer ROS (10). A decline in the antioxidant enzyme levels is expected after exercise bouts. However, previous researches on the relation between exercise and the OS biomarkers report equivocal results for the enzyme level changes in terms of magnitude and direction of the change, either increase or decrease (11,15,33). The mean serum levels of GPX and catalase levels are higher in the sedentary group and the exercise group with no significant differences in the present study, respectively. While the findings of this study are contradictory to the concept of adaptation of human metabolism to chronic exercise, which depends on the hypothetical upregulation of enzymatic ADM (24), these findings are in line with those of Kanaley and Ji (21), Chang et al. (7), and Tauler et al. (32).

MDA is a product and a biomarker of the lipid peroxidation (5,7,11,15,21,24,29,32,33). The measurement of MDA levels is one of the most used assays to evaluate the oxidative damage, induced by physical activity (9). Discordant results have been reported in the studies investigating the relation between exercise and the MDA levels (11,15). Besides, numerous studies mention no significant difference in the MDA levels, even after high intensity exercises (15). The present study contrarily notifies remarkably high mean serum MDA level in the exercise group in comparison to the sedentary group, supporting the hypothesis of increased MDA formation with exercise (20). Like the findings of the present study, a study on the oxidative stress levels in sedentary individuals and judokas mentions considerably high mean plasma MDA levels in the latter compared to the former with regard to measurements at rest (11). Another study, comparing the mean MDA levels between handball players and sedentary participants, also demonstrates significantly greater values in the former than those found in the latter (30).

The measurement of TAS indicates the sum of antioxidant capacity of the body, thus eliminating the need for demanding measurement of various antioxidant enzymes and molecules (6). The measurement of TOS, on the other hand, indicates the sum of oxidation state of the body (14).

Numerous studies in the literature compare TAS levels between physically active and inactive individuals. Those studies mention significant difference in favor of physically active groups regarding the higher TAS levels of those groups (3,11,15,16). Improved antioxidant capacity in physically active individuals despite exposure to exercise induced ROS has been explained by the hypothetical compensatory increase in the ADM activity (11,15,24).

The results of the present study are unlike those of aforementioned studies with regard to significantly high mean TAS level in the sedentary group compared to the exercise group. Conversely, the mean TOS level of the sedentary group was significantly lower than that of the exercise group, consistent with the mean levels of TAS. In addition to no significant difference between the two study groups in terms of the antioxidant enzyme levels, the remarkably lower mean level of TAS in the latter group compared to the former group is also contradictory to the hypothesis of adaptive increase in ADM activity against exercise induced OS (11,15,24).

The discordance between the present study and some of those studies mentioned above in regard to the measurements of OS biomarkers may be explained by the differences in the study designs and the exercise programs of the participants (i.e. the duration of aerobic and anaerobic exercises) as well as the relatively low intensity of exercises of physically active group involved in this study compared to the studies including elite athletes (9,11,15).

Many studies investigate the gender-specific differences in oxidation state secondary to pathological conditions (4). Moreover, those studies notify equivocal results for the disease states (34). Although estrogen is reported to have antioxidant feature (22), some studies conducted on sedentary participants (4) as well as physically active participants (28,34) do not support the knowledge of antioxidant feature of estrogen. The present study, which includes healthy participants with sedentary and physically active lifestyle, also indicates no significant gender-specific difference regarding the levels of non-enzymatic and enzymatic OS biomarkers.

Prolidase, an essential enzyme for collagen recycling, has been investigated in numerous researches (5,13,17-19,23,26). Most of them studied the prolidase activity levels in various disorders (12,13,17-19,23,26). On the other hand, Buyukhatipoglu et al. investigated the relation between oxidation state and prolidase enzyme levels in healthy, non-smoking healthcare staff and non-healthcare staff (5). They report notable increase in serum prolidase levels in the former group, encountering stressful situations during the working hours, in contrast to trivial difference in the latter group, presumably free of stressful situations at the same period (5). However, the authors do not compare the prolidase levels between the two groups (5). Based on the search of the literature, the present study, which reports significantly high prolidase levels in sedentary group compared to exercise group, is the first investigation comparing sedentary as well as physically active young adults in terms of serum prolidase levels. The rationale behind the lower prolidase enzyme activity in the exercise group can be the need for the preservation of structural integrity of collagen containing tissues to achieve better physical performance.

Prolidase activity level has been shown to associate with oxidative stress in patients diagnosed with various disorders (13,18,19) as well as malignancies (17). The reported changes in prolidase activity levels due to the diseases are ambigious (23). Buyukhatipoglu et al. mention negative correlation between TAS and serum prolidase levels with no significant difference, both in healthcare staff and non-healthcare staff (5). The results of the present study indicate negative correlation with no significant difference between the mean prolidase level and TAS level in the sedentary group as well. On the other hand, the positive correlation with significance, found between the mean prolidase level and TAS level in the exercise group, is contradictory to the findings of the research conducted by Buyukhatipoglu et al. (5). The latter finding appears to suggest a relationship between the two parameters, particularly in physically active young individuals.

The strengths of the present study are the gender-specific comparisons of OS biomarker and prolidase activity levels as well as the analysis of the correlation between the former and the latter. The weaknesses of the present study are the relatively limited number of the participants in the study groups (particularly the small number of the male participants in the sedentary group), the diversity of the sports in which the subjects of the physically

active group involved, and the lack of measurements of non-enzymatic antioxidant levels.

To conclude, the results of the present study do not support the hypothesis of gender-specific difference in young, healthy adults with physically active and sedentary lifestyles considering OS biomarkers. The remarkably low levels of prolidase activity in the exercise group compared to those in the sedentary group suggest decreased collagen turnover in the former. OS appears to occur with no associated compensatory increase in the antioxidant enzyme activities in young adults who regularly perform moderate intensity exercises. This study also indicates a correlation between the serum levels of prolidase and TAS in this population, but further studies are seemingly required for deeper insight into the topic.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Table 1. The demographic features and pairwise comparison of the study groups.

		Sedentary group (n=48)	Exercise group (n=79)	p
Age (mean ± SEM) [year]		21.04 ± 0.2	20.61 ± 0.18	0.119
Height (mean ± SEM) [cm]		167.15 ± 1.23	170.76 ± 0.9	0.017*
Weight (mean ± SEM) [kg]		60.33 ± 1.35	63.05 ± 1.13	0.131
BMI (mean ± SEM) [kg/m²]		21.53 ± 0.36	21.49 ± 0.26	0.929
Genders	**Female [n (%)]**	34 (70.8)	42 (53.2)	0.049*
	Male [n (%)]	14 (29.2)	37 (46.8)	

Independent samples t test; Chi-square test; * Statistically significant difference ($p<0.05$) BMI: body-mass index, SEM: standard error of the mean

Table 2. The biochemical parameters and pairwise comparison of the study groups.

	Sedentary group (n=48)	Exercise group (n=79)	p
GPX (mean ± SEM) [U/g hb]	893.59 ± 82.96	851.68 ± 32.98	0.558
Catalase (mean ± SEM) [U/g hb]	32.46 ± 2.75	34.01 ± 1.84	0.628
MDA (mean ± SEM) [nmol/mL]	6.04 ± 0.19	7.83 ± 0.31	<0.001*
Prolidase (mean ± SEM) [U/mg prot.]	26.15 ± 1.97	10.7 ± 0.8	<0.001*
TOS (mean ± SEM) [mmol/L]	21.14 ± 3.43	31.31 ± 2.87	0.025*
TAS (mean ± SEM) [mmol/L]	0.79 ± 0.08	0.41 ± 0.04	<0.001*

Independent samples t test; * Statistically significant difference ($p<0.05$)
GPX: glutathione peroxidase, MDA: malondialdehyde, SEM: standard error of the mean, TAS: total antioxidant status, TOS: total oxidant status

Table 3. Gender and study group based pairwise comparisons of the study groups and genders regarding biochemical parameters, respectively.

		Genders		
		Female (n=76)	**Male (n=51)**	**p**
GPX (mean ± SEM) [U/g hb]	**SG**	900.31 ± 108.12	877.27 ± 114.96	0.901
	EG	863.05 ± 47.48	838.77 ± 45.91	0.716
	p	0.737	0.708	
Catalase (mean ± SEM) [U/g hb]	**SG**	30.42 ± 2.91	37.43 ± 6.19	0.25
	EG	34.53 ± 2.71	33.42 ± 2.49	0.766
	p	0.306	0.472	
MDA (mean ± SEM) [nmol/mL]	**SG**	6.02 ± 0.24	6.09 ± 0.28	0.873
	EG	7.96 ± 0.54	7.69 ± 0.25	0.667
	p	0.004*	<0.001*	
Prolidase (mean ± SEM) [U/mg prot.]	**SG**	24.17 ± 1.66	30.98 ± 5.32	0.116
	EG	11.88 ± 1.19	9.37 ± 1.02	0.119
	p	0.001*	<0.001*	
TOS (mean ± SEM) [mmol/L]	**SG**	22.74 ± 4.29	17.25 ± 5.56	0.474
	EG	28.33 ± 4.2	34.7 ± 3.85	0.271
	p	0.359	0.018*	
TAS (mean ± SEM) [mmol/L]	**SG**	0.78 ± 0.1	0.81 ± 0.16	0.883
	EG	0.43 ± 0.08	0.38 ± 0.04	0.503
	p	0.005*	0.001*	

Independent samples t test; * Statistically significant difference (p<0.05) EG: exercise group, GPX: glutathione peroxidase, MDA: malondialdehyde, SEM: standard error of the mean, SG: sedentary group, TAS: total antioxidant status, TOS: total oxidant status

Table 4. Multiple comparisons of gender based subgroups regarding biochemical parameters.

Biochemical Parameters	**Sedentary - female group (n=34)**	**Sedentary - male group (n=14)**	**Exercise - female group (n=42)**	**Exercise - male group (n=37)**	**p**
GPX (mean ± SEM) [U/g hb]	900.31 ± 108.12	877.27 ± 114.96	863.05 ± 47.48	838.77 ± 45.91	0.943
Catalase (mean ± SEM) [U/g hb]	30.42 ± 2.91	37.43 ± 6.19	34.53 ± 2.71	33.42 ± 2.49	0.591
MDA (mean ± SEM)[nmol/mL]	6.02 ± 0.24[c,d]	6.09 ± 0.28[c,d]	7.96 ± 0.54[a,b]	7.69 ± 0.25[a,b]	0.001*
Prolidase (mean ± SEM) [U/mg prot.]	24.17 ± 1.66[c,d]	30.98 ± 5.32[c,d]	11.88 ± 1.19[a,b]	9.37 ± 1.02[a,b]	<0.001*
TOS (mean ± SEM) [mmol/L]	22.74 ± 4.29	17.25 ± 5.56	28.33 ± 4.2	34.70 ± 3.85	0.086
TAS (mean ± SEM) [mmol/L]	0.78 ± 0.1[c,d]	0.81 ± 0.16[c,d]	0.43 ± 0.08[a,b]	0.38 ± 0.04[a,b]	<0.001*

One way anova, Post-hoc, Scheffe test, Tamhane T2 Test; * Statistically significant difference (p<0.05).
[a] Statistically significant difference with sedentary - female group; [b] Statistically significant difference with sedentary - male group; [c] Statistically significant difference with exercise - female group; [d] Statistically significant difference with exercise - male group
GPX: glutathione peroxidase, MDA: malondialdehyde, SEM: standard error of the mean, TAS: total antioxidant status, TOS: total oxidant status

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