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Comparing of Cu/Zn SOD Gene Expression of Lymphocyte Cell and Malondialdehyde Level in Active Men and Women after Physical Training

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Abstract

***Background*:** The purpose of this study is to compare Cu/Zn SOD mRNA and MDA level as a result of a session incremental exercise in active women and men.
***Materials and Methods*:** This research is a quasi-experimental study with repeated measurements in which 14 active female and 13 male subjects with age range 22-24 participated voluntarily. Then, blood was taken from brachial vein of the subjects in three stages before and after GXT (Graded exercise test) and 3 hours after that and SYBER Green PCR Master mix reagent Kit and Real time-PCR were used to measure Cu/Zn SOD mRNA and spectrophotometer was used to measure MDA level.
***Results*:** MDA levels increased significantly in men during the recovery stage and after the exercise (p_1=0.012 and p_2 =0.014), but it did not increase significantly in active women. Also, MDA difference between the two genders was not reported significant in any of the exercise stages. Cu/Zn SOD gene expression did not increase significantly in either sex.
***Conclusion*:** The risk of injury from free radicals is more probable in active men than active women and vigorous physical activity does not significantly increase the Cu/Zn SOD gene expression.

Introduction

Available oxygen increases free radical production which reaches to a few times as much as rest state during vigorous physical activity of the body when we are faced with increased oxygen consumption [1]. As a free radical, MDA (Malondialdehyde) is the modified form of hydrogen peroxide (H_2O_2) which is effective in creation of oxidative stress conditions and tissue damages [1]. In addition, the increase of MDA concentration in the blood is dependent on exercise intensity and the higher the intensity of activity is, the more the production and release of MDA will also increase [2].

Superoxidase dismutase is an enzyme cu/zn SOD type of which is present in the cellular cytoplasm and is the first line of defense against free radicals. It is reported that physical fitness and activity level are of factors affecting the enzyme Cu/Zn SOD [3]. Active people are those individuals who perform their exercise regularly at least three sessions a week and at least 30 minutes per session. Valado et al. reported that active people in the base state have higher antioxidant levels and lower MDA level than sedentary individuals [2].

On the other hand, given the physiological differences between men and women, the studies on human and animal models have reported that free radical production in females is less than males and females produce less free radical due to estrogen hormone [4-7]. However, no research was found by the researchers of the present study regarding the expression of antioxidant enzymes in active people and their gender differences. Thus in this study, researchers aim to examine the effect of intense physical activity on Cu/Zn SOD gene expression in active women and men.

Materials and Methods

This research is a quasi-experimental study the statistical population of which is composed of active individuals. As a result of the calling, 60 young men and women in Urmia volunteered to participate in the research and completed health questionnaire and consent to participate in the study. Then, their physiological characteristics, including height (cm), weight (kg), fat, and heart rate (beats per minute) and etc. were studied. People with history of chronic diseases, women who menstruate, and those with lack of desired criteria of physiological parameters for being active, were excluded from the study and from them, 14 active young female subjects and 13 active young male subjects with age range 22-24 were included in the study.

At base and fasting state, the amount of 4 ml venous blood was collected and kept at the temperature 4^{o}C in falcon tubes containing blood. Exercise protocol: incremental exercise test was performed based on GXT

(Graded exercise test) (speed: 12Km/h, slope: 5 degree, time: 20 minutes) [8]. Five seconds after the completion of the activity and 3 hours after that, the amount of 4 ml blood samples were taken again. Blood samples were transferred to the laboratory of immunology, Tabriz University of Medical Sciences.

MDA measurement laboratory approach: MDA was measured according to the method of Pars Azmoon Company. MDA measurement started by dissolving 500µl of serum in 3ml of phosphoric acid 1%. After vortex, 1ml thiobarbituric acid solution 0.67% was added to the tube and after complete vortex; it was placed in a boiling water bath for 45 minutes.

After the required period, the test tubes were cooled under cold water, 2ml of normal butanol was added and vortexed for 1 to 2 minutes and then it was centrifuged for 10 minutes on 3000rpm. After isolation of the organic phase (supernatant), optical absorption was measured at wavelength of 532 nm as blanch against butanol and the results after transfer to the standard curve, the concentration of serum MDA of samples was determined.

Laboratory method of Cu/Zn SOD gene expression

RNA Isolation: 5ml peripheral blood was taken in anticoagulant EDTA and its red blood cells got lysis using ammonium chloride and were centrifuged for 15 minutes at 4^{o}C and 600g. Then, supernatant was speared, the cells were washed with 1ml cold PBS. Then, they were transferred to 1/5ml tubes of DNase Free and RNase Free. In the next stage, one ml of RNXTM-PLUS solution was added to microtube for every 6×10^{6} cells.

Carefully and without shaking the tube, the supernatant phase containing RNA was isolated and was transferred to the other microtube. 20µl DEPC-treated eater was added to every microtube and was kept in the freezer -70 ^{o} C for further stages.cDNA production: RevertAID TM Firs Standard cDNA synthesis Kit (Fermentas) Was used to produce cDNA according to manufacturer's instructions (primer used for Cu/zn SOD expression was showed in table 1).

Table 1. Primer used for Cu/zn SOD expression

H Cu/Zn-SOD Forward	5'-AAGGCCGTGTGCGTGCTGAA-3
H Cu/Zn-SOD Reverse	5'-CAAGTCTCCAACATGCCTCT-3'
H β-actin Forward	5'-CAGGTCATCACCATTGGCAAT-3'
H β-actin Reverse	5'-TCTTTGCGGATGTCCACGT-3'

Real-time PCR: the respective device Corbett-Rotor (gene-6000) was used to measure Cu/Zn SOD gene expression. For data analysis, first ΔCt of genes in each sample was calculated from differentiation of Ct of the relevant gene and Ct of β-actin gene as the reference. Statistical analysis of data: the normal distribution of all data in this study was tested and *t-test* was also used to compare the differences between the two genders. The effect of exercise on various parameters of the present study was also determined using mixed model statistical method and Bonfreoni post hoc test was performed to compare various stages of activity at base state at significance level of $p<0.05$ and using the software SPSS-17.

Results

Physiological characteristics of active men and women were showed in table 2. Mixed Model statistical analysis showed that MDA level has increased significantly in men after the exercise ($p=0.012$), while this change was not reported significant in women. Three hours after the exercise, MDA level in active men decreased compared to after the exercise but it was still higher than basal level, and this difference was statistically significant ($p=0.014$) and MDA level in active women was significantly increased in this interval ($p=0.029$) (Table 3). In addition, no difference was reported statistically significant between active men and women in basic mode, after the exercise and recovery.

The results of mixed model statistical analysis showed that increasingly intense exercise in overall state (overall and regardless of gender) has no significant effect on MDA level ($p>0.993$). MRNA level of Cu/Zn SOD enzyme in active men and women partly increased after exercise and 3 hours after that, but mixed model statistical analysis showed that these changes were not significant in either genders ($p>0.05$) (Table 3).

However, the difference between men and women was not different at base state in terms of parameter Cu/Zn SOD ($p>0.079$), but this difference was reported significant after the activity ($p=0.017$). Nevertheless, it was not reported significant again at recovery mode ($p>0.222$). However, in the overall state, increasingly intense activity had a significant effect on the Cu/Zn SOD gene expression in active individuals ($p=0.044$) (Table 3).

In addition, regression statistical analysis showed that there is no significant correlation between the changes in Cu/Zn SOD mRNA and MDA during the stages of increasingly intense activity in active men ($p>0.51$) and Cu/Zn SOD gene expression decreased by -2.62% for each unit of increase of MDA (Fig. 1). There was a significant relationship and effect between the changes in enzyme Cu/Zn SOD and MDA in active women ($p=0.014$) and Cu/Zn SOD gene expression increased by 2.9 unit per unit increase of MDA (Fig. 1).

Table 2. Physiological characteristics of active men and women

Physiological characteristics	Men	Women
Age (years)	23±1	21±1
Height (cm)	177.59±7.02	160.8±4.35
Weight (Kg)	70.54±6.1	53.1±5.78
Fat tissue (%)	11.16±4.73	35.15±2.58
Vo_2max (ml/kg/min)	55.97±1.35	52.6±1.83
Body Mass Index (BMI) (kg/m^2)	22.5±1.5	25.6±1.83
Heart Rate (beat/min)	78±7.52	67.3±8.67

Table 3. Effect of incremental exercise on Cu/zn SOD mRNA (fold induction) and MDA activity (µm)

Variable		Male		Female		*p*-Value
		Mean ± SD	Median (range)	Mean ± SD	Median (range)	
MDA activity	Overall					0.993
	Baseline	2.95 ± 0.84	3.2 (1.2 to 3.8)	2.5 ± 1.24	2.15 (1.2 to 4.2)	0.512
	After	3.37 ± 0.99	3.6 (1.7 to 4.6)	2.84 ± 1.38	2.75 (1.3 to 4.9)	0.349
	P1	0.012		0.255		
	Recovery	3.36 ± 0.85	3.6 (1.8 to 4.4)	3.04 ± 1.16	3.35 (1.4 to 4.3)	0.605
	P1	0.014		0.029		
	P2	>0.99		0.893		
Cu mRNA	Overall					0.044
	Baseline	3.77 ± 1.88	4.54 (0.5 to 5.62)	5.38 ± 2.09	5.55 (0.33 to 7.72)	0.079
	After	4.04 ± 0.86	3.87 (3.17 to 6.27)	5.89 ± 2.17	6.73 (2.45 to 9.08)	0.017
	P1	>0.99		>0.99		
	Recovery	5.03 ± 2.37	4.8 (0.4 to 8.79)	6.35 ± 2.42	6.08 (3.55 to 10.47)	0.222
	P1	0.346		>0.99		
	P2	0.64		>0.99		

P1 Comparison with Baseline, P2 = in comparison with after time

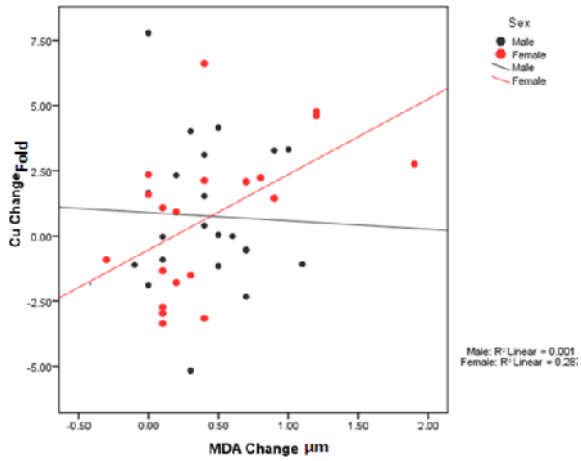

Figure 1. Correlation between the changes in Cu/Zn SOD mRNA and MDA activity

Discussion

In this study, MDA level in men significantly increased at all stages, but it significantly increased in women only in recovery state. The researchers believe that estrogen in women is effective on levels of plasma free radicals and reduces its level [7]. In the present study, insignificance of plasma concentration of MDA in women can be attributed to this hormone in them. There is a report regarding the increased MDA suggesting that increased MDA concentration in the blood is dependent on exercise intensity [2].

Given that the exercise protocol used in this study is an intense aerobic activity, this increase in MDA concentration can be due to the high consumption of oxygen by the tissues or muscle and cell damages during intense exercise.

However, Increase of plasma levels of MDA may also be influenced by gender of subjects. In this study, although MDA level in active men, was higher than in active women, but its difference between the two genders was not significant due to the intense exercise. This shows that regular exercises activities they do every week, have had similar effect on their physical fitness level. Michalis et al. who investigated the effect of swimming activity on free radicals in both genders reported that swimming activity decreases free radicals in both young men and women and this effectiveness and reduction have been reported to be similar in both genders [9].

In the present study, Cu/Zn SOD gene expression increased in both active genders after intensive exercise and 3 hours after that, but it was not reported to be statistically significant. Morikawa et al. also reported the non-significant increase in Cu/Zn SOD gene expression in professional football players to be relatively intensive after the exercise, [10] but Lambertucci et al. reported that the concentration of mRNA of Cu/Zn SOD has increased subsequent with intensive aerobic exercises in studied exercised rats in response to free radicals [11]. Although free radicals are of the factors affecting Cu/Zn SOD gene expression; their increase does not necessarily mean a significant increase in Cu/Zn SOD gene expression.

Studies of Fisher et al. have also shown that the immune system in exercised people has increased Cu/Zn SOD activity as a result of increased MDA level, while at the same time with this process; gene expression of this enzyme did not change significantly [3].

One of the factors affecting the expression of this enzyme is gender. In the present study, although the difference between the genders in terms of Cu/Zn SOD gene expression was reported significant only after the exercise. However, in all exercise stages, its level of activity in women was reported more than men on which estrogen hormone in women and stress and androgen hormones in men are effective [12, 13].

The important findings of this study was to determine the relationship between MDA and Cu/Zn SOD gene expression quantitatively (numerical) and this relationship was not mentioned like this in previous studies. Our findings showed that for every unit increase in MDA Cu/Zn SOD gene expression decreased by -2.62% in men, but in active women mRNA level of cu increased by 2.9 unit per unit increase in MDA that indicates antioxidant and peroxiding disequilibrium and the damage caused by free radicals in men and is very applied in presenting detailed strategies to improve immune system of both

groups of active men and women.The results of this study revealed that the likelihood of damages caused by free radicals in active men is more than active women and considering insignificance of Cu/Zn SOD gene expression in both genders, both men and women are likely to respond to the increase of free radicals through enhanced activity of Cu/Zn SOD and gene expression of this enzyme does not significantly increase and accordingly, immune system of women and men somewhat responds to the increase in free radicals.

In the limited studies conducted on this issue in European and American races, significant gender differences have been also reported in terms of gene expression of this enzyme [4, 14] but in the Iranian population and active people, especially the impact of gender is not clearly determined and studied in which regard the results of this study can be used.

In this study, other free radicals and other affective hormones that may affect the results of this study have not been measured, which is suggested to be reviewed in other studies.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

No conflict.

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