

## DETERMINATION OF OXIDATIVE STRESS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN HANDBALL PLAYERS

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### ABSTRACT

**Purpose:** Acute exercise, lipid peroxidation and free radicals, formed in consequence of oxidative stress, show a change in terms of type and time and intenseness of exercise. In this study, determination of degrees of oxidative stress and some antioxidant enzyme activities in handball players has been aimed.

**Method:** In this study, 14 male sportsmen who play handball and 14 sedantery individuals have been taken into the study. The degrees of malondialdehyde (MDA) and catalase(CAT) activities and reduced glutathione have been measured as spectrophotometric.

**Conclusions:** Statistically significant difference between degrees of MDA, CAT and GSH of control group and handball players has been found( $p<0.05$ ). A significant decrease in values of MDA and catalase, which is an important antioxidant enzyme activity, has taken place in handball players compared to control group, value of GSH in handball players has been found higher than that of control group( $p<0.05$ ).

Consequently, in sportsmen who take part in handball team, an increase in GSH has been seen whereas a decrease in Catalase and MDA has occurred. It can be said that reduced glutathione which has the characteristic of antioxidant enzyme is a good protector against oxidative damage in individuals who do intensive exercise.

**Keywords:** Handball, Catalase, Glutathione.

### INTRODUCTION

Oxidative stress is defined as an increase of reactive oxygen species as a result of the product caused by deterioration between oxidant and anti-oxidant balance in body<sup>24</sup>. Reactive oxygen species(ROS) can emerge as stress observed during aerobic endurance. ROS is thought to be in mitochondria of the muscles working actively<sup>16</sup>. Free radicals are physiologic products of aerobic metabolism and are formed continually. Free radicals have important role in pathologies of such diseases as atherosclerosis, cancer, inflammation, neurodegenerative. The increase in muscle contractions during exercise enhances metabolic activity. Consequently, there is an increase in the formation of such free radicals as superoxide, hydrogen peroxide and hydroxide radical. Consisted free radicals corrode lipids, proteins and DNA<sup>22</sup>. It has been determined that free radicals increase during physical activity and exercise<sup>6</sup>. From a different viewpoint, the balance between antioxidant activity and formation of free radical can have an important role in pathogen heart disease. Antioxidant defense system becomes active against free radicals consisting with exercise and tries to protect body against possible damages<sup>28</sup>.

Catalase removes hydrogen peroxide by turning it into water and oxygen, which plays an important role in abatement of oxidative stress<sup>18,25</sup>. Malondialdehyde(MDA) is often used as indicator of oxidative stress in determination of lipid peroxidation. MDA leads to damage cell membrane by reacting with vicinal fatty acids<sup>23</sup>. Found abundantly in cytosol and

mitochondria, glutathione is a non-enzymatic, thiol antioxidant in tripeptide structure. Glutathione protects tissues from oxidative damage during exercise. Also, Glutathione protects erythrocytes, leucocytes and eye-lenses from oxidative stress<sup>2, 10, 7, 26, 27, 23</sup>. So far, determination of oxidative stress and antioxidant enzyme levels in handball players has not been reported. In this study, determination of oxidative stress degree and some antioxidant enzyme activities in handball players has been aimed.

### **Material and Method**

In this study, 14 sedantary students and 14 healthy volunteer students, who plays in handball team of Van Yuzuncu Yil University, were used as subjects. Blood samples were taken from sportsmen and sedanteries, and then, their serums were separated in Biochemistry laboratory of Faculty of Science at our university. Then, levels of malondialdehyde(MDA), catalase(CAT) enzyme activity and reduced glutathione(GSH) were determined spectrophotometricly in Biochemistry laboratory of Department of Chemistry, Faculty of Science at Yuzuncu Yil University.

### **Determination of Malondialdehyde(MDA) level**

Being one of peroxidation products which are formed as a result of reaction of fatty acids with free radicals, malondialdehyde has been measured spectrophotometricly in consequence of its getting into colorful form with thiobarbutiric acid<sup>13</sup>.

### **Determination of Reduced Glutathione(GSH)**

Reduced glutathione(GSH) has been measured through formation of yellow colour as resut of the reaction of sulfhydryl groups, found in erythrocyte, with DTNB(5',5'-(2-dithiobis nitro benzoic acid). Measurement of reduced glutathione level in the blood with EDTA has been made in 412 nm on spectrophotometer in 24 hours<sup>8</sup>.

### **Determination of Catalase(CAT) Activity**

In this study, in which hydrogen peroxide was used as substrate, catalase activity was determined according to Aeibi method. Firstly, two tubes were taken. 30 mM-H<sub>2</sub>O<sub>2</sub> was added to the blind tube in the amount of 1.4 ml and then, over it phosphate buffer was added in the amount of 0.1 ml. To the sample tube, 30 mM-H<sub>2</sub>O<sub>2</sub> was added in the amount of 1.4 ml. Enzyme was added over it in the amount of 0.1 ml and it was mixed with vortex. Absorbencies were studied twice in 240 nm in every 30 seconds. So, the activity was determined<sup>1</sup>.

### **Statistical Analysis**

In this study, descriptive statistics were expressed as Average, Standard Deviation, and Minimum and Maximum value. Student t test was used in comparison of the groups. Level of statistically significance was taken as 5% in calculations and SPSS statistical package program was used for calculations.

### **FINDINGS**

In the study, results of comparison of individuals playing handball with control group have been given in table 1 and graphic 1,2 and 3. Accordingly, for each three feature, differences of individuals playing handball from control groups have been found statistically significant (p<0.05). An important decrease has occurred in individuals playing handball in terms of values of Catalase and MDA, value of GSH has increased in individuals that play handball

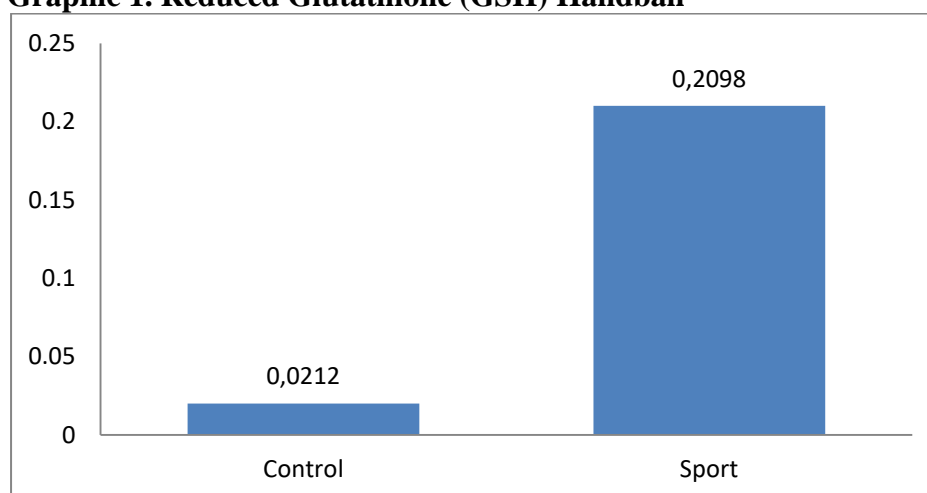
compared to control group. In other words, in handball players, while Catalase and MDA has decreased, GSH has shown an increase.

Table 1. Descriptive statistics according to groups and results of comparisons.

		N	Mean	Std. Dev	Std. Error	Min	Max	p
CAT	Control	14	,0062	,0009	,0002	,0048	,0073	0,010
	Sport	14	,0000	,0000	,0000	,0000	,0002	
	General	28	,0031	,0032	,0006	,0000	,0073	
MDA	Control	14	16,9722	2,5311	,6764	12,3764	21,0139	0,019
	Sport	14	14,2462	2,5449	,6801	11,4418	22,1978	
	General	28	15,6092	2,8512	,5388	11,4418	22,1978	
GSH	Control	14	,0212	,0081	,0021	,0110	,0390	0,010
	Sport	14	,2098	,0472	,0126	,1415	,2955	
	General	28	,11555	,1016	,0192	,0110	,2955	

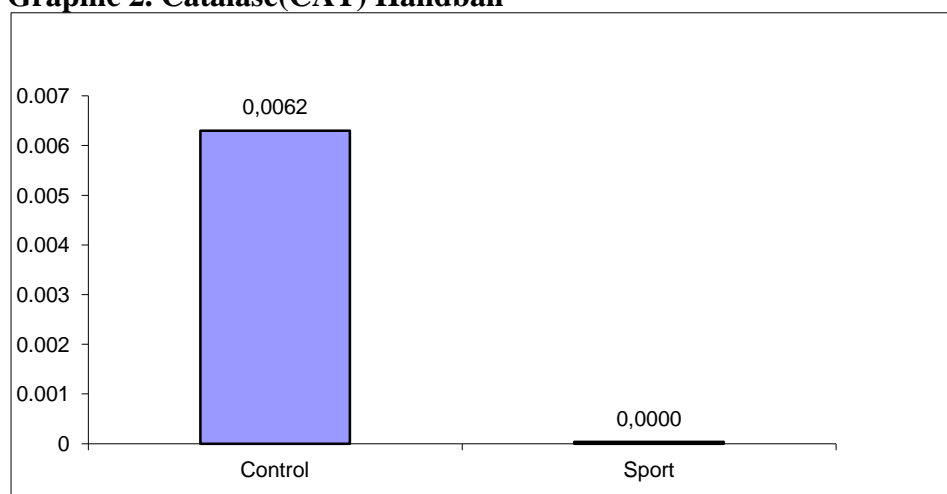
$p < 0.05$ ; shows significant difference between groups

**Graphic 1. Reduced Glutathione (GSH) Handball**

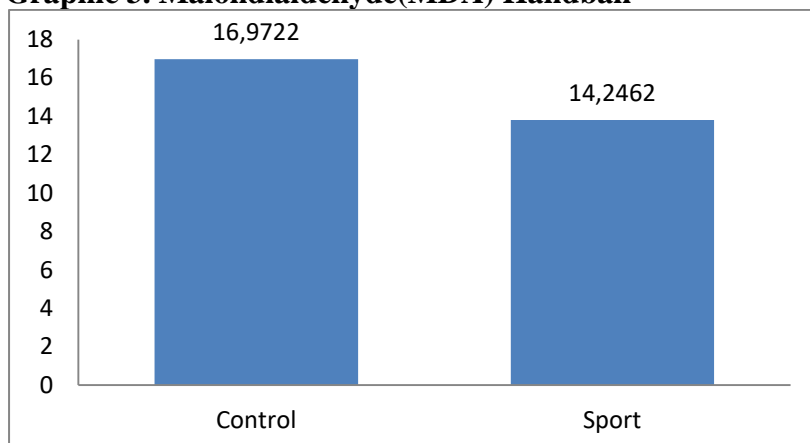


Value of GSH has been found high and significant in handball players compared to control group ( $p < 0.05$ )

**Graphic 2. Catalase (CAT) Handball**



Value of CAT has been found low in handball players compared to control group ( $p < 0.05$ ).

**Graphic 3. Malondialdehyde(MDA) Handball**

The value of MDA has been found low and significant in handball players compared to control group ( $p < 0.05$ ).

## DISCUSSION

Free radicals are shown as cause of many diseases including aging and cancer<sup>17</sup>. Aging, and ischemia-reperfusion causing excessive production of free radicals, and balance between antioxidant and oxidant system that living being has for various situations, which may lead to numerical increase and accumulation of neutrophils in circulation, may change on behalf of oxidant substances<sup>19</sup>. Exercise is a term that is used to describe every kind of muscular movements, however, emergent results depend on number and type of muscle fibre, severity and time of movement, and the way that consumed energy has been obtained. It has been reported by many researchers that exercise causes an increase in oxygen and formation of other free radicals. Antioxidant capacity changes depending on type of muscle fibre. In literature, there is no agreement about effects of exercise on muscle fibres and antioxidant enzyme activities. While some have recorded increase in enzyme activities, others state that there is not any change<sup>21</sup>. Regular physical activity is shown as an important factor in treatment and prevention of cardiovascular disease<sup>14</sup>. Malondialdehyde(MDA) is the last product of lipid peroxidation and is usually used as oxidative stress reagent in determination of lipid peroxidation. MDA leads to various damages on cell membrane by combining with fatty acids<sup>23,9</sup>. In some literature studies, it has been found that MDA level rises in some diseases; on the contrary, it decreases in healthy individuals<sup>5,11,12</sup>. In our study, a significant decline in MDA levels has been seen in handball players compared to control group( $p < 0.05$ ). It is important that MDA level is low in healthy individuals, especially those who do exercise; because oxidative stress has decreased in blood serums of those who do exercise. This situation affects metabolism positively and may prevent or stop any possible disease that can develop in body.

Catalase is an enzyme which detracts  $H_2O_2$  from inside of an oxygen molecule and a water molecule and which is important for antioxidant protecting reactive oxygen radical against this process by means of elimination. Antioxidant systems protects all cells against free radical damage in livings<sup>4,15</sup>. Catalase enzyme has been found solvable in erythrocyte<sup>20</sup>. Catalase is an enzyme that can be used for removing hydrogen peroxide, used as oxidizing or bleaching or for the purpose of sterilization, and that can be widely and analytical purposefully used as compound of hydrogen peroxide or glucose biosensors<sup>3</sup>. In literature studies, Bulduk(2010) have found CAT level low in both sportsmen and control group in the studies that they did. A significant decline has been seen in catalase enzyme activity in

individuals who play handball compared to control group( $p<0.05$ ). Available findings can be said to be in concordance with literature data.

In the studies, it has been determined that reduced glutathione is a good protective in those who do intensive exercise against oxidative damage. Moreover, reduced glutathione protects erythrocytes, leucocytes and eye-lenses against oxidative stress<sup>2, 10, 7, 26, 27, 23</sup>. In some other studies, it has been found that reduced glutathione level decreases in some diseases and increases in healthy individuals<sup>5, 11, 12</sup>. As Bulduk(2010) expressed, there are different results contradicting with each other according to their features in the studies on effects of antioxidant enzymes based on exercise. In this study, GSH level has risen in the individuals who play handball compared to control( $p<0.05$ ).

Consequently, glutathione which has antioxidant enzyme feature can be said to be a good protective in individuals who do intensive exercise against oxidative damage.

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