Measurement of GSH, SOD and MDA some Antioxidant level after Excises Athletes

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Abstract

Antioxidants are a line of defense that protects the cells of the body. Attacking the stray oxygen molecules. The function of these antioxidant is to add a group of electrons to the blood vessels to give them to electrolytes or monovalent free radicals so that they stabilize and calm down and do not destroy cells. Thus, antioxidants work to achieve a balance between free radicals and antioxidants In the cell and keeps the cell from being damaged. Increased electrolytes or free radicals work To weaken the ability of antioxidants' and enzymes secreted by cells despite the fact that the cell has a self-protection and a defensive line to secrete self-antioxidants, and here the importance of antioxidants arises. These metabolic changes increase the oxidative effort and affect the ability to achieve and the efficiency of athletes, as well as the body contains a lot of antioxidant systems Oxidative stress that includes enzymes such as GSH) Glutathione Dos) Super oxide D ismutses. Exercise can produce an imbalance between ROS and antioxidants, which is referred to as oxidative stress. Dietary antioxidant supplements are marketed to and used by athletes as a means to counteract the oxidative stress of exercise. Whether strenuous exercise does, in fact, increase the need for additional antioxidants in the diet is not clear. This research showed the role of free radicals in causing oxidative stress during exercise. The results showed significant increased in malondialdehyde (MDA) level (as an index of lipid peroxidation) glutathione (GSH) superoxide dismuatse activity (SOD) in subjects after efforts of exercise in comparison with the control group.

Key Word : *SOD, antioxidant , MAD, Athletes, Sport*

Introduction

The cells in our body continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. Free radicals are molecules or part of molecules which have one or more unpaired electrons in external electronic shell. Main characteristics of these molecules are very short life span and extremely high reactivity. Injurious effects of free radicals are induced by necessity to establish electronic stability and therefore they react with next stable molecule, taking its electron and creating new free radical. The at way this molecules also becomes unstable and further interferes with other molecules from its surrounding which leads to impairments of cellular components. Free radicals are created during the process of oxidative phosphorylation in mitochondria [14]. Oxidative stress occurs as a result of ROS activity and reduced protective mechanisms that lead to impairements in cells and tissues functions. It causes secondary damage through late cell death and inflammation [15]. Various studies have shown that oxidative stress represents pathogenetic foundation of many diseases [16]. ROS are normally neutralized by complex system of antioxidant defence [17]. The system of antioxidant defence can be divided into two groups: enzymes including superoxid dismutase (SOD), catalase (CAT), glutathione preoxidase (GPX); and non-enzymes including vitamins C and E, retinol, bilirubin, uric acid, redox glutathione, thiols, coenzyme Q□□, stress proteins, albumins, as well as transport proteins and storage proteins for Fe□+ i Cu□+ which disable potentially harmful metal ions and their involvement in

production of free radicals [18]. Nevertheless, low levels of ROS appear to be necessary for important physiological functions such as cell signaling, immune response, and apoptosis [19]. Many studies have shown that exercise induces oxidative stress and causes adaptations in antioxidant defenses [20]. Training can have positive or negative effects on oxidative stress depending on training load, training specificity and the basal level of training. Data suggest that regular long term training can induce antioxidant response to the oxidative stress. The results of a study which investigated the relationship between oxidative stress and exercise overtraining/ overreaching support the possibility that the beneficial effect of physical exercise on oxidative stress might be associated with increased antioxidant defences [21]. It is also well known that active and non active skeletal muscles produce reactive oxygen and nitrogen species although it is not quite clear where oxidants originate during physical activity [22]. The degree of oxidative damage, as well as the time course for elevation in oxidative stress markers has varied across studies, and appears to be dependent, among all, on the type, intensity, volume and duration of exercise [23]. This leads to differences in oxidative status between athletes in different sport disciplines, but the results of the previous studies are inconsistent.

Subjects and Exercise Programmed:

12 male student volunteers as Control from education college– from Almustaqbal university college , were recruited to participate in the study. And 45 male volunteers athletes from different gym sport in Babylon province , ages between (20-40) years ,. All subjects were The questionnaire taken from the case and control sheets involved: types of food, occupation, age, type exercise, gender, Supplements, smoking habit, alcohol intake type Sport . All tests took place in human performance laboratory of Almustaqbal university college. The temperature ranged from 14°C to 20°C. Athletes performed all testing in the same equipment conditions and drank the same energy beverage.

Blood Sampling Procedures

About (5 ml.) Blood samples were collected by puncture from an antecubital vein in resting after Exercise conditions The blood samples were centrifuged (at 3000 rpm for 300 sec), and serum was divided into aliquots and frozen in dry ice prior to storage at –20°C until assayed for glutathione, MDA, and superoxide dismutase.

Antioxidant Analysis

Determination of Reduced Glutathione(GSH) in Blood Serum

GSH level was determined in blood serum using a modified method dependant by Sedlack and Lindsay, (1968). The method depends on Ellman's reagent which contains [5,5-dithiobis(2- nitrobenzoic acid)] or known as DTNB which reacts with the thiol group of reduced glutathione to form a coloured solution absorbed at (412) nm.

Determination of Lipid Peroxidation in Blood Serum (Malondialdehyde, MDA)

MDA level was determined in blood serum through the measurement of MDA concentration. The method depends on the reaction between MDA and thiobarbituric acid or (Beruge and Aust, 1978) to form a coloured solution absorbed at (532)nm.

Determination of Superoxide Dismutase (SOD) Activity in Blood Serum

(SOD) activity level in blood serum was determined using photochemical method. The method included using sodium cyanide as peroxidase inhibitor. This methods depends on an indirect approach to determine the SOD activity through the change in formazene absorbance formed from the reduction of O**2 •** (which is produced by radiating the sample of serum with light) for nitroblue tetrazolum (NBT) dye (Brown and Goldstein, 1983). Decreased difference in formazene absorbance means increased SOD activity.

Data Analysis

All values are presented as means ± SD. Between-group, differences for selected variables were determined by SPSS Statistic Analysis .

Results and Discussion

Table 1 show the relationship in Parameters of Antioxidant level enzyme between the control and case according the Job.

JOB		Parameters of Antioxidant level enzyme		
		GSH µmol/L	SOD µmol/L	MAD µmol/L
Student	Mean	4.5380	165.3750	3.2176
	Std. Deviation	.88014	158.5280	1.01551
	Std. Error of Mean	.31118	5.58366	.35904
Control	Mean	1.9425	212.0833	3.8078
	Std. Deviation	.48931	21.16368	.19139
	Std. Error of Mean	.14125	6.10943	.05525
Non Employ	Mean	4.0472	186.3000	3.1068
	Std. Deviation	1.24248	171.4722	.97982
	Std. Error of Mean	.27783	38.34237	.21909
Employ	Mean	4.9095	180.0000	2.8985
	Std. Deviation	1.25936	84.85281	1.39088
	Std. Error of Mean	.89050	60.00000	.98350

Table 2 show the relationship in Parameters of Antioxidant level enzyme GSH SOD and MAD between the control and case according the Type Sport .

		Parameters of Antioxidant level enzyme		
TYPE		GSH µmol/L	SOD µmol/L	MAD µmol/L
Athletes	Mean	4.3744	228.1000	2.4047
	Std. Deviation	.62194	143.49019	.85927
	Std. Error of Mean	.19668	45.37558	.27172
Building body	Mean	4.1661	156.4000	3.4813
	Std. Deviation	1.35567	169.76591	.83348
	Std. Error of Mean	.30314	37.96081	.18637
control	Mean	1.9425	212.0833	3.8078
	Std. Deviation	.48931	21.16368	.19139
	Std. Error of Mean	.14125	6.10943	.05525

Table 3 show the relationship in Parameters of Antioxidant level enzyme GSH SOD and MAD between the control and case according the taken Protein .

		Parameters of Antioxidant level enzyme		
PROTIEN		**GSH µmol/L**	**SOD µmol/L**	**MAD µmol/L**
No protein	Mean	3.8762	193.6667	2.9368
	Std. Deviation	1.19026	179.68666	1.13605
	Std. Error of Mean	.28055	42.35255	.26777
Have protein	Mean	4.7745	160.2500	3.4009
	Std. Deviation	.89422	138.24953	.60910
	Std. Error of Mean	.25814	39.90920	.17583

Table 4 show the relationship in Parameters of Antioxidant level enzyme GSH SOD and MAD between the control and case according the Smoker .

		Parameters of Antioxidant level enzyme		
SMOKER		GSH µmol/L	SOD µmol/L	MAD µmol/L
Non Smoker	Mean	4.2071	200.2083	2.9079
	Std. Deviation	1.24584	152.6012	.97181
	Std. Error of Mean	.25431	31.14959	.19837
Smoker	Mean	4.3492	100.6667	3.9808
	Std. Deviation	.76080	191.8339	.25474
	Std. Error of Mean	.31060	78.31588	.10400
Control non Smoker	Mean	1.9425	212.0833	3.8078
	Std. Deviation	.48931	21.16368	.19139
	Std. Error of Mean	.14125	6.10943	.05525

Table 5 show the relationship in Parameters of Antioxidant level enzyme GSH ,SOD and MAD between the control and case according the Nergla Smoker .

		Parameters of Antioxidant level enzyme		
Nergela SMOK		GSH µmol/L	SOD µmol/L	M µmol/LAD
No smoker	Mean	4.3333	154.6190	2.9071
	Std. Deviation	.65906	142.3304	1.04360
	Std. Error of Mean	.14382	31.05904	.22773
smoker	Mean	3.7716	215.5000	3.6767
	Std. Deviation	1.95318	167.5405	.54845
	Std. Error of Mean	.61765	52.98097	.17344
Control no smoker	Mean	1.9657	246.1429	3.7736
	Std. Deviation	.46054	104.4229	.17367
	Std. Error of Mean	.17407	39.46815	.06564
Control smoker	Mean	1.9750	207.2500	3.7838
	Std. Deviation	.65000	22.44066	.24365
	Std. Error of Mean	.32500	11.22033	.12183

Table 6 show the Correlation in Parameters of Antioxidant level enzyme GSH ,SOD and MAD in the case according the Type Sport .

Correlations type of sport (football, body sport, control)						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
SOD µmol/L	Between Groups	42931.288	2	21465.64	1.135	.332
	Within Groups	737820.61	39	18918.47		
MAD µmol/L	Between Groups	11.753	2	5.877	11.31	.000
	Within Groups	20.247	39	.519		
GSH µmol/L	Between Groups	45.358	2	22.679	21.55	.000
	Within Groups	41.034	39	1.052		

The increase in energy consumed during exercise increases the oxygen demands of the active tissues, increasing up to 20 times in comparison with basal state [1]. The oxygen flow in the peripheral skeletal muscle tissue can increase up to 200 times, increasing 30 times the blood flow, and the oxygen difference in the arteriovenous blow increases 3 times. As a result, the oxidative metabolism is increased, maximizing the energy produced by unit of substrate and avoiding lactate accumulation [2]. Dillard et al. (1978) [3] first described that extenuant exercise induced lipid damage in tissues. After that, many other investigations focused on the effects of exercise and training in oxygen toxicity and the body defense response. It is accepted that oxygen toxicity can be implicated in some pathologic situations. The understanding of the mechanisms associated with physiological responses that explain how exercise increases the oxygen toxicity and the design of appropiate measures to minimize toxicity are indispensable to: **1.** Increase exercise efficacy as a preventive and therapeutic instrument in clinical practice **2.** Control the damaged tissue induced by exercise Oxidative stress induced by extenuant exercise is a situation by which cells are exposed to a prooxidant environment and defense mechanisms are not enough, affecting the redox estate of the cells. Due to this, nutritional supplements of antioxidants such as vitamin C, vitamin E, carotenids, and polyphenols in the diet are important [4].

Further research is required to support these hypotheses Where the hypotheses said the Malondialdehyde (MDA) is one of the most frequently usedindicators or biomarkers of lipid peroxidation. It was significantly increased as a result of exercise in the present study. Two theories support the concept that resistance exercise could lead to an increase in the production of oxygen free radicals in active muscle sites. A widely held hypothesis involves the ischemia-reperfusion injury . Intense muscle contractions can result in a temporary decrease in blood flow and oxygen availability and subsequent ischemia. The following reperfusion period (muscle relaxation) produces an abundant reintroduction of O2 and results in the formation of the O2- radical. Mechanical stress is another hypothesis used to explain an increase in free radicals. In particular, eccentric exercise causes high levels of force that has been shown to initiate muscle tissue damage. This initiates the inflammation process that eventually produces oxygen free radicals [5] and lipid peroxidation. The significant increase in MDA agrees with other investigators [6] They reported that moderate intensive treadmill running exercise was sufficient to result in muscle damage and increases in the susceptibility of erythrocytes to in vitro peroxidation represented by MDA. Similar result was reported by others [7] where they suggested an increase in plasma MDA immediately after exercise. It was also recorded a significant increase in MDA at 6 hours post exercise [8]. Glutathione (GSH) oxidation in different tissues is a valid parameter to appreciate oxidative stress. In this situation, intracellular GSH rapidily oxidizes to GSSG. Intracellular GSSG can be reduced to GSH in the presence of a reductase glutathione and NADPH as cofactor. When the oxidative stress is high, the relation between GSSG/GSH can be higher than the reduction ability of the cells. In this situation, the heart and skeletal muscle cells pour GSSG out of the cells [24]. In extenuant exercise, an increase of GSSG and a decrease of total glutation (GSSG + GSH) in the skeletal muscle tissues such as the liver and heart has been observed [9,10]. This increasing production of GSSG exceeds the reductase glutathione's ability to reduce disulfide group, thus explaining that the GSSG spill from the tissue to the plasma [11]. The increasing oxidized glutathione plasma concentration as a result of the exercise has been demonstrated in many studies [10,12]. The glutathione synthesis ability in the liver is high and exercise induces a decrease of glutathione, promoting a protective response of the liver [10]. Up to now, the work has been focused in the damaging effect of exhaustive exercise. However, moderate exercise results in a healthy and beneficial practice that prevents diseases, due to its ability to prevent oxidative stress [13]. Oxidative stress induced by exercise depends on the type, intensity, and the length of the exercise. However, interindividual variability is attributed to the level of training, sex, nutrition, and genetic factors .

In biological tissues, the superoxide anion can be converted into the nonradical species hydrogen peroxide and singlet oxygen by the aid of SOD enzyme. Thus, SOD provide the primary defense against ROS generated during exercise. The activity of this enzyme is known to increase in response to exercise in both animal and human studies [10] . like to the present study in which SOD activity was increase significantly ($p<0.001$) in training athletes.

Therefore, athletic performance increases the production of free radicals That lead to cell damage, and then it has been observed that physical performance leads to an increase in the blood level as well as an increase in the expiratory air content of the lungs, both of which are indirect signs of metabolic oxidation, even if they differ in different people. Take an anti-oxidant Oxidation during food or through preparations before training reduces muscle fibers resulting from athletic training. A decrease in the level of fat in the blood was observed as a result of an increase Training time due to increased adaptation, Also, an increase in the blood content of the reduced GSH image was observed. In another hand, it was found that running training for athletes improves the anti-oxidant amount of blood compared to non-athletes in terms of the red blood cell content of Vitamin E for Glutatione and the activity of the catalase enzyme. So this research show the Regular exercise increases the efficiency of the antioxidant defense system Reduced amount of oxidative stress in the absence of regular exercise.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Almustaqbal University College and all experiments were carried out in accordance with approved guidelines.

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