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Evaluation of lipid peroxidation [TBARS] and antioxidant status for monitoring oxidative stress in students of Physical education and sport science.

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Abstract:
The main purpose of this study was to demonstrate the impact of different exercises on the oxidative stress in students of first class physical education and sport sciences in Kufa university/ Iraq. Plasma enzymetic [super oxidedismutase (SOD),catalase (CAT), glutathione reductase (GSH) and glutathione peroxidase (GPX) activities], non enzymetic [total antioxidant capacity (TAC), uric acid (UA) and some serum trace elements(Cu, Zn, Se)] as antioxidant ,and a marker of lipid peroxidation thiobarbituric acid –reactive substances[TBARS] were measured in(30) students in beginning year before they received any training or exercise programs (T0) and after three months (T1) then after 6 months (T2), when these students exposure for multi and variety training and games who take as lessons in first class of physical education and sport sciences faculty which is moderate physical activities and mixed aerobic and anaerobic exercise. Our results showed increased plasma values of (SOD) ,(Cu, Zn, Se) after 3 months (T1) and 6 months (T2) when compared at baseline (T0), while plasmatic (CAT), (GPX),(GSH) were similar in three groups. On the other hand a lower (TAC) levels after 3 months (T1)and 6 months (T2) when compared with levels at base line(T0). Nevertheless, (TBARS) levels were found to be significantly higher in the students after 6 months when compared with levels at 3 months and at base line levels.
Key words: lipid peroxidation [TBARS], antioxidant status, oxidative stress, moderate training.

Introduction

Exercise can be defined as any planned structured activity that leads to increase in energy expenditure and heart rate. There are different modes of exercise in relation to intensity (aerobic and anaerobic), to muscle contraction (isometric, concentric, and eccentric), and to frequency (acute and chronic).

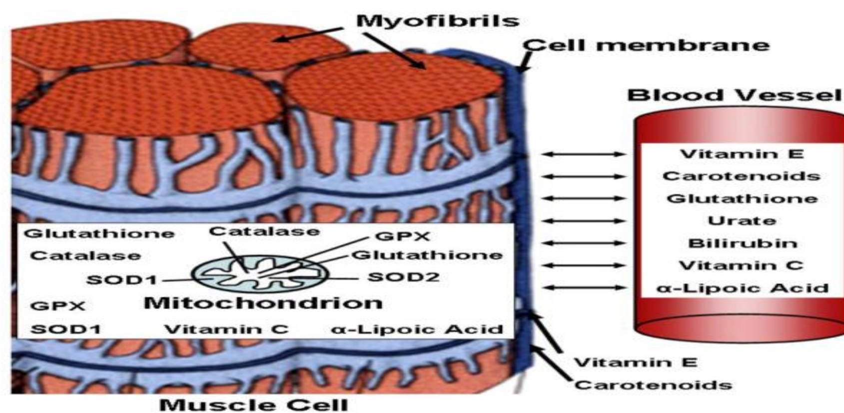
The beneficial effects of regular, no exhaustive physical exercise have been known for a long time. There is irrefutable evidence of the effectiveness of regular physical activity in the primary and secondary prevention of several chronic diseases (e.g., cardiovascular disease, diabetes, cancer, hypertension, obesity, depression, and osteoporosis) and premature death [1]. However, the beneficial effects of exercise are lost with exhaustion. It is well known that exhaustive exercise (especially when sporadic) causes structural damage to muscle cells or inflammatory reactions within the muscles, for instance, as evidenced by an increase in the plasma activity of cytosolic enzymes and sarcolemma and Z-line disruption [2]. Some of this damage is due to the production of free radicals and it may be prevented by optimizing nutrition, particularly by increasing the dietary content of nutritional antioxidants [3,4].

Moreover, free radicals are involved in the pathogenesis of many diseases, such as diabetes, cardiovascular diseases, inflammation, or pulmonary diseases. Free radicals are also involved in important physiological processes, such as aging.
Intense physical activity is known to increase oxidative reactions and possibly oxidative stress [5]. Although free radicals appear to play important regulatory roles in intracellular signal transduction and pathways [5], the unbalanced increase in oxygen reactive species during heavy exercise can seriously impair physical performance due to muscle injury and to damage of other critical cells such as erythrocytes [6]. However, the body is equipped with both non-enzymatic and enzymatic antioxidant systems to prevent the potentially harmful effects of the oxygen-reactive species [5]. The delicate physiological balance between oxidative reactions and antioxidant capacity may be affected by intense physical activity [7]. Moreover, the type of sport may be an important factor affecting antioxidant status. Aerobic modalities, such as classic triathlon and long-distance running, may generate more oxygen reactive species than those anaerobic, such as short-distance running and swimming, thus affecting the antioxidant capacity. The associated impact in some sport modalities may also affect the antioxidant status. The degree to which oxidative stress increases with an acute exercise bout is likely related to training status. Training down regulates the activity of antioxidant enzymes[8], suggesting that athletes have adapted to the demands of exercise and require less enzyme activity than those completing unaccustomed exercise. Antioxidant status in athletes can be assessed by one or more of 3 options[9]. First is measurement of individual plasma concentrations of antioxidants, which typically include vitamin C (ascorbic acid), uric acid, vitamin E, and selenium [8]. The second option is the measurement of total antioxidant capacity in plasma (TAC). TAC is a measure of how well a food product or biological sample can reduce an oxidant and thus takes into account the synergies among the various antioxidants found in a sample. The TAC measurement is defined as the moles (or millimoles) of radicals neutralized per gram or milliliter of tested sample [10]. Third, activity of the enzymes responsible for scavenging reactive species can be used to determine antioxidant status. Erythrocyte antioxidant enzyme activity (including superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]) has been used as an indicator of oxidative stress, with higher activity suggesting higher concentrations of reactive oxygen species present [11]. Erythrocytes are vulnerable to oxidative damage because of their continuous exposure to oxygen and their high concentrations of polyunsaturated fatty acids and heme iron. As oxidative stress may be proportional to oxygen uptake, it is not surprising that antioxidant enzymes in erythrocytes can display high activity during and after exercise [12]. Previous studies report GPx and SOD to be up-regulated in response to exercise, especially high intensity and/or prolonged-duration exercise, while CAT activity is less responsive [10]. It is important to measure a range of the possible options to fully assess antioxidant status. Overall, antioxidant status may influence how athletes cope with maximal exercise [13].

Antioxidants can be both synthesized in vivo and absorbed through diet. They can be divided into two groups: enzymatic and nonenzymatic. The main enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). Each of these enzymes is responsible for the reduction of a different ROS, and they are located in different cellular compartments. (1) SOD: there are 3 isoforms of this antioxidant, two of them are present within cells, whereas the other

one is located in the extracellular space. Specifically in skeletal muscle cells, the highest percentage of SOD (65–85%) is found in the cytosol, and the remaining (15–35%) is present in the mitochondria of the muscles. SOD catalyses the reaction of superoxide radicals into oxygen and hydrogen peroxides (H_2O_2). (2) GPX: located in both the cytosol and the mitochondria of cells, it is responsible for the removal of a wide range of hydroperoxides—from complex organic hydroperoxides to H_2O_2—thus, it may protect membrane lipids, proteins, and nucleic acids from oxidation. GPX is also present in muscle cells, but its activity varies depending on the muscle fiber type, with the greatest activity present in slow twitch muscle fibers (type I) which have higher oxidative capacity. (3) CAT: it is extensively distributed within the cells, and its main function is to degrade H_2O_2 into H_2O and O_2. Nevertheless, it has a lower affinity for H_2O_2 compared with GPX. Similarly to the latter, CAT can be found in higher concentration in type I muscle fibers (for further details on these enzymes, refer to Powers and Jackson [14].

The nonenzymatic antioxidant group includes glutathione, vitamin C, vitamin E, carotenoids, uric acid, and Zinc , copper, selenium which are mineral nutrients known to participate in several antioxidant systems, including metalloproteins, such as the copper-zinc enzyme superoxide dismutase and the low-molecular weight zinc- and copper-binding protein metallothionein [15, 16]. Similarly to the enzymatic antioxidants, these are present in different cellular compartments and elicit distinct antioxidant properties which maximize their effectiveness [10]. Below are more details on the enzymetic and nonenzymatic antioxidants—glutathione, vitamin C, and vitamin E in scheme (1) .



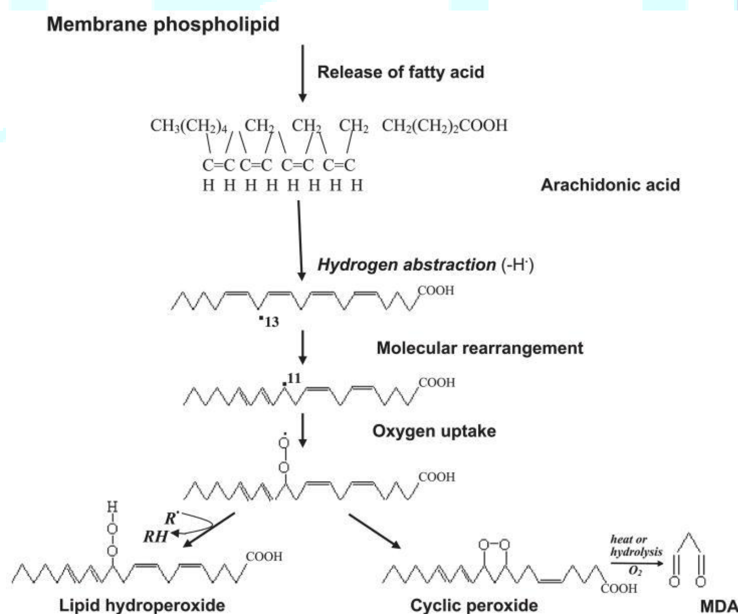
Scheme (1): Elucidate the enzymatic and non-enzymatic anti-oxidant in blood vessel and muscle cell. .

Interestingly, recent work has shown that the increase in reactive species during exercise leads to favorable exercise-induced adaptations. Pertaining literature documents well that both aerobic and anaerobic training causes an enhancement in the antioxidant enzyme activity in various tissues [17,18,19]. This is an adaptation process that happens because the free radicals, produced during muscle contraction, act as signaling molecules. This stimulates the gene expression and, hence, increases production of antioxidant enzymes and modulates other oxidative stress protection pathways, such as enhancing the activity of DNA repair enzymes in skeletal muscles

[17]. This stimuli associated with enhanced antioxidant protection occurs not only in the muscles but also systemically so vital organs, such as liver and brain, also go through this beneficial adjustment [20].

In this regard, the type and duration of training are key for a significant upregulation of the endogenous antioxidants with long-duration high-intensity endurance training being more effective [21]. Niess et al. [22] reported that trained individuals presented less DNA damage after an exhaustive bout of exercise compared to untrained men. Other evidence for this training adaptation was reported by Miyazaki et al. [23], which showed that free radical production was reduced after 12 weeks of endurance training. More specifically, they reported a decrease in the neutrophil superoxide anion production and attenuation in the lipid peroxidation process. The contrary is also true as sedentarism not only reduces various physiological functions but also decreases the body's oxidative stress protection mechanisms. Consequently, there may be an increase in the occurrence of oxidative stress associated with diseases such as cancer, atherosclerosis, cardiovascular, and neurodegenerative diseases [20].

On the other hand, regular training is known to increase the resistance against ROS induced lipid peroxidation, and to decrease the accumulation of oxidative protein and DNA damage [22]. The lipid peroxidation of polyunsaturated fatty acids in the sarcolemma can compromise its integrity, resulting in leakage of muscle-derived enzymes into the blood. In fact, lipid peroxidation appears to be an important mechanism underlying exercise-induced muscle damage and delayed-onset muscle soreness [24].



Scheme (2): Elucidate how the malonyldialdehyde (MDA) release from membrane phospholipid.

Materials and methods

Study design:

Thirty (30) students of first class of physical education and sport science faculty were enrolled in the study. The study was conducted in a week in November (2014) when the students were on the beginning year before they taking any training or exercise program (T0), then repeated this experiment after three months in a week in January (2015) (T1), and after six months in a week in March (T2), when the students take different training and games as lessons in first class. The controlled physical training consisted of five games per week involved (track and field, basketball, football, swimming and weightlifting), 2-4 hour per day. All participants had a body-mass $<20 kg/m^2$ and were healthy (self-reported) , non-smokers, were not taking non-steroidal anti- inflammatory medication or anabolic drugs, and had not used antioxidant supplements for at least 3 months.

Blood collection and handling:

After obtaining a written consent total of 10 ml blood was withdrawn aseptically from the anticubital vein from each participants at rest between 8 and 9 a.m. by a trained phlebotomist after the participants had fasted overnight (8-12 hour). Blood samples were drawn into EDTA-treated Vacutainer tubes and nonadditive serum Vacutainer tubes and immediately placed on ice in the dark until centrifugation. An aliquot of whole blood was separated to measure hematocrit and hemoglobin. An aliquot of the whole blood in serum tubes was immediately centrifuged for total antioxidant capacity (TAC) analysis and the remaining blood allowed to clot for 30 min, at room temperature, and then centrifuged at 2,000 rpm for 10 min for serum separation. To obtain the plasma fraction, the remaining whole blood in EDTA-containing tubes was immediately centrifuged. Erythrocytes were washed and centrifuged three times with a 0.9% sodium chloride solution and lysed with ice-cold distilled deionized water. Serum, plasma, and washed erythrocytes were separated into several aliquots and frozen at –80 °C for later biochemical analysis. Samples were analyzed in duplicate, and the mean value was used for statistical analysis. A major limitation of the current study is that, because of technical problems, ascorbic acid was not measured .

Biochemical analysis :

Hemoglobin and hematocrit were assessed from EDTA-treated blood using an automated analyzer (Horiba ABX Micros 60, ABX Diagnostic, Montpellier,France).

Uric acid was determined by an enzymatic method at 550 nm using a commercial kit (Horiba ABX A11A01670, ABX Diagnostic, Montpellier, France) according to the manufacturer's specifications.

Serum TAC was measured spectrophotometrically using a commercial kit (Randox NX2332, Randox, Crumlin, UK) [25].

Enzyme activities were analyzed according to the standard spectrophotometric–colorimetric procedures. Whole-blood GPx activity was determined using cumene hydroperoxide as the oxidant of glutathione (Ransel RS 505, Randox, Crumlin, UK).

The plasmatic activity of glutathione reductase was measured by monitoring the oxidation of NADPH to NADP+ during the reduction of oxidized glutathione (Ransel GR 2368; Randox, Crumlin, UK) and expressed in U/L. A value of 10 U/L was considered the detection limit, This method is based on Paglia DE and Valentine WN [26]. Superoxide dismutase (SOD) was measured by using RANSOD kit (Randox Laboratories Ltd . Crumlin, UK) using the Pyrogallol method [27] . Catalase activity in RBCs was determined as Goth method [28]. The determination of thiobarbituric-acid-reactive substances (TBARS) in serum was performed using a commercial kit (Oxi-tek TBARS assay kit, Zeptometrix Corp., Buffalo, NY) according to the manufacturer's instructions. Briefly, serum (100 µl) was mixed with an equal volume of 8.1% sodium dodecyl sulfate and 2.5 ml of 5% thiobarbituric acid/acetic acid reagent. The sample was incubated at 95 °C in capped tubes for 60 min and thereafter cooled to room temperature in an ice bath for 10 min before being centrifuged at 3,000 rpm for 15 min.The supernatant was removed, and its absorbance was read at 532 nm. The results are expressed as malondialdehyde equivalents by interpolation from a malondialdehyde standard curve (0–100 nM/ml) as per the method of Kei Satho[29].

For zinc and copper measurements, standard zinc and copper solutions of 0.1%, 0.2%, 0.3% and 0.4% were prepared and then defreeze the serum. One cc of defreeze serum sample was put into a Balloon Jujeh and 5% glycerol solution was added into it. In the standard solutions and the serum sample solution, zinc and copper levels was then measured using Flame Atomic Absorption Spectrometry (Perking Elmer Analyst 100). Using 213.9 nanometer wavelength for Zn and 324.8 nanometer wavelength for Cu, the standard solution curves and equation line Serum zinc and copper levels drawings were calculated. Selinuim was measured by the direct graphite furnace AAS (AA 220, GTA 110, Varian,Australia) equipped with pyrolytically coated graphite tubes and deuterium background correction after a further dilution of serum with Triton X-100 (0.1% v/v). Direct determination of Se in body fluids by graphite furnace AAS may suffer from problems like severe background, matrix effects, preatomization losses, and spectral interferences. So, the mixture of Pd+Mg (NO3)2 was used as matrix modifier in graphite furnace AAS for the direct determination of Se in the serum [30] .

Statistical analysis

The results are expressed as mean ± SD., Statistical significance of paired differences in means and standard deviations of the related hematological and biochemical changes among at base line (T0), after 3 months (T1),and after 6 months (T2) values were calculated using by one-way ANOVA analysis to compare the homogeneity of the variance, the levenne test was used. When variances were homogeneous, comparisons were made using the *post hoc* Tukey test. The level of significance was set at $P<0.05$ in all cases.

Results and discussion

Table (1) show the mean and standard deviations of physical characteristics of students who they participate in this study were not significantly different.

Few studies have extensively addressed the hematological and biological changes in athletics .this investigation elucidate the effects of moderate and regular exercise on athlete health and the findings can be used to help participants in future competition .

Hb and Hct , two indicators of anemia, were normal before the training or exercise , the two indicators remained same value between months three and six , so called . Under normal conditions , red cells with a mean life of 120 days are renewed at approximately 1 % daily . However , this turnover rate increases following moderate and regular training, as reflected in the participants in this study. The increased turnover rate is good for the athletes as the young red cells can carry oxygen more efficiently than the older cells [31,6].

Table (1): physical characteristics of students.

Variables	Mean ± SD
Age (y)	20 ± 2
Weight (kg)	66 ± 4
Height (cm)	176 ± 5
Body mass index (kg/m^2)	21.8 ± 2
Average training (h/wk)	12 ± 3
Hemoglobin (g/dl)	15.6 ± 0.7
Hematocrit (L/L)	46.1 ± 2.2

Table (2) presents antioxidant status, non-enzymatic (TAC),(UA) and enzymatic, SOD,GPX,GSH,CAT, in three cases before and after the intervention period (training). There were no statistically significant differences between status for post training values of enzymes CAT,GPX, and GSH. TAC decreased significantly in (T1) and (T2) respectively. For uric acid (UA) values significant increase was observed for T1 and T2 status when compared with (T0) status. (T1), (T2) status showed a statistically significant increase in levels of SOD enzyme.

The antioxidant biomarkers TAC was negatively associated with training dose, that is, students athletes who had trained for more months, completed more training hours a week, competed at a moderate intensity, and trained further had lower antioxidant activity in their plasma and red cells at rest.

Association of the exercise induced change in uric acid with regular training were mentioned in other studies [32 ,33]. Although chronic training may lead to raised serum uric acid levels [33,34].Our students athletes presented a statistically significant tendency to have higher concentration than their case when were untrained, as has been described by others [32,24].

The higher uric acid levels seen in student athletes could, at least partially, explain their lower TAC values in comparison with their case when were untrained, resembling the result obtained by other researches[34,35]. The lower TAC in the students athletes can also be explained by a lower plasma concentration of other antioxidant not measured in the current work (e.g; ascorbic acid) or increased ROS generation resulting from the intense physical- training program. Actually, it has been recognized that overtraining reduces TAC [36].

Exercise- induced changes in the redox status of tissues may initiate intracellular signal – transduction processes that trigger antioxidant protein expression [32]. It has been reported that regular exercise training causes an up- regulation of the activity of

antioxidant enzymes at rest and immediately after exercise [37]. Our data elucidated that erythrocyte SOD activity was significantly augmented in the students athletes after training when compared before training, as has been described by other authors [34,36].

Erythrocyte superoxide dismutase activity appears to increase as a result of increased superoxide radical production during exercise [3]. In human studies, the effect of type of exercise and duration of training on erythrocyte superoxide dismutase activity is controversial. Most studies measured activity after exhausting training in athletes and non-athletes [37, 38, 38]. Higher response was observed in trained than in sedentary individuals [37]. An increase in the enzyme activity was reported after training in swimmers and long-distance runners [37,38], whereas no change in activity was seen in decathlon after competition [40]. Marzatico et al. [37] reported that marathon runners had higher erythrocyte superoxide dismutase activity than sprinters when compared in basal conditions and also immediately after exercise. Discrepancies between studies may be explained by different superoxide dismutase assays used, different frequency and intensity of training tested in each study and different resting time allowed before blood draws [6].

Catalase (CAT), widely distributed in all cells, is present in high amounts in erythrocytes. It is an intracellular enzyme made up of four polypeptide chains with four porphyrin heme groups. Catalase is responsible for detoxification of hydrogen peroxide in the cells [28]. In present study, the enzymetic antioxidants CAT in the hemolysate were resemble in all situations (T0,T1,T2) . The consistency in the activity of CAT nearly could be due to increase in the lipid peroxidation product malonyldialdyhyde, which can form crosslinks, therapy inactivating several membrane bound enzymes [36].

Glutathion peroxidase (GPX) belongs to group of antioxidant selenoenzymes that protects the cell damage by catalyzing the reduction of lipid hydroperoxides. This action requires the presence of glutathione.

Glutathione peroxidase levels in the body are in close relation with the glutathione, which is the most important antioxidant present in the cytoplasm of the cells[41]. The present study demonstrated non-significant elevation in red cell GPX .Augmented levels of GPX is due to activation by the increased super oxide anion production leading to an increase in oxidative stress[41]. Our findings are in confirmation with the study of [42], in accordance with other study [43],although other findings have also been published, indicating either higher [8] or lower [42] activity levels.

Contrary to the findings of [44], we did not find difference in the glutathione rductase activity (GSH) in plasma of either group. The equivocal data reported in the literature on antioxidant enzyme activities could be linked to difference s in the athletes nutritional intakes and status; the type, intensity, and duration of their training program ;or the biological material analyzed [42].

Table (2): Blood antioxidant parameters in students in three cases (T0, T1,T2).

Parameters	T0 Mean ±SD	T1 Mean ±SD	T2 Mean ±SD
Total antioxidant capacity TAC (m mol)	1.25±0.22	1.07±0.19[a]	0.88±0.09[b]

Uric acid UA (mg/dl)	7.15±1.88	18.28±4.55[a]	26.48±5.92[bc]
Superoxide dismutase SOD (U/ml)	97.5±22.2	108.2±32.8[a]	125.8±42.3[bc]
Catalase CAT (U/L)	122.2 ±23.4	120.8±24.7	123.2±25.7
Glutathion peroxidase GPX (U/L)	18.45±6.82	20.54±5.88	23.36±6.21
Glutathion reductase GSH (U/L)	15.22±5.28	14.72±4.72	12.92±3.86

a $P<0.05$ *vs* statistically significant when T0 compared with T1. b $P<0.05$ *vs* statistically significant when T0 compared with T2. b $P<0.05$ *vs.* statistically significant when T1 compared with T2.

Table (3) shows changes in trace elements Se, Cu, Zn,levels in three cases. There were significant increase in all elements for T1 and T2 cases respectively.

The increased levels of serum antioxidant trace elements (Cu, Zn, Se) and SOD activity observed in the current study were insufficient to counterbalance the rise in lipid peroxidation observed in the student athletes.

Moderate exhausting exercise increases the oxygen demand 10 to15 times as compared with resting conditions, resulting in increased oxygen consumption, increased oxygen cell uptake, and increased flow in the electron transport chain [5]. According to the intensity and duration of the exercise, there is an increased release of oxygen reactive species, mainly in muscle but also in erythrocytes, resulting in stimulation of antioxidant mechanisms [5,6]. These mechanisms include copper/zinc-dependent metalloproteins, such as superoxide dismutase in erythrocytes. Superoxide dismutase and have been extensively studied in relation to physical activity [37,38,39].

In this study, we compared zinc- and copper-dependent indices of antioxidant status in 30 elite student athletes modalities of different aerobic and impact conditions. The antioxidant capacity was evaluated as a result of long-term adaptation rather than an acute response to exhaustive exercise. Exercise has a pronounced effect on zinc metabolism [6]. In the present study, plasma zinc concentrations were adequate in all student athletes. Additionally, aerobic exercise may increase zinc uptake by erythroid cells in the bone marrow in order to meet the increased zinc demand for protection of sulphahydryl groups in cell membrane proteins and for synthesis of metalloproteins such as carbonic anhydrase, superoxide dismutase, and metallothionein [8, 24]. Increased cell zinc uptake associated with erythropoiesis may result in a decrease in plasma zinc and an increase in erythrocyte zinc such as we observed in our study , particularly in (T2) status. Copper metabolism plays an important role in physical activity, since it is involved in many aspects of energy metabolism and synthesis of hemoglobin, myoglobin, cytochromes, and peptide hormones [31]. Moreover, copper is a component of ceruloplasmin and superoxide dismutase. However, the effect of physical activity on plasma copper is unclear. Plasma copper has been found

unchanged in studies comparing different sport modalities [31]. In another study, plasma copper was higher in anaerobic than aerobic modalities [36], while in present study plasma copper was higher in two modulates anaerobic and aerobic.

One of the essential trace elements in human plasma is selenium. Selenium was first discovered as a byproduct of sulfuric acid production. It is a well-known electrometalloid and is mostly famous due to its anti-cancerous properties. It is an essential constituent of the enzyme glutathione peroxidase and also incorporates in various important proteins such as hemoglobin and myoglobin. It helps in preventing free radical damage caused by ferrous chloride, and heme compounds. Its deficiency may affect the iron binding capacity of transferrin which leads to iron stores and subsequent tissue damage [45]. The study indicates a significant increase in plasma concentrations of the essential element selenium as well as increase in plasma activity of selenium dependent antioxidant enzyme glutathione peroxidase (GPx).

Table (3): Biochemical data for elements (Mean ±SD) in students with T0, T1 ,T2 cases.

Elements	T0 Mean ±SD	T1 Mean ±SD	T2 Mean ±SD
Selenium (µg/dl)	113.33±28.22	125.67±32.11[a]	137.87±35.23[bc]
Zinc (µg/dl)	111.72±23.84	126.72±27.8[a]	141.11±38.76[bc]
Copper (µg/dl)	89.48±24.80	96.5±32.22[a]	135.44±33.62[bc]

a $P<0.05$ *vs* statistically significant when T0 compared with T1. b $P<0.05$ *vs* statistically significant when T0 compared with T2. c $P<0.05$ *vs.* statistically significant when T1 compared with T2.

From the results of this study ,it appears that there are significant increase in values of TBARS in T1,T2 cases when compared at baseline T0 show figure (3).

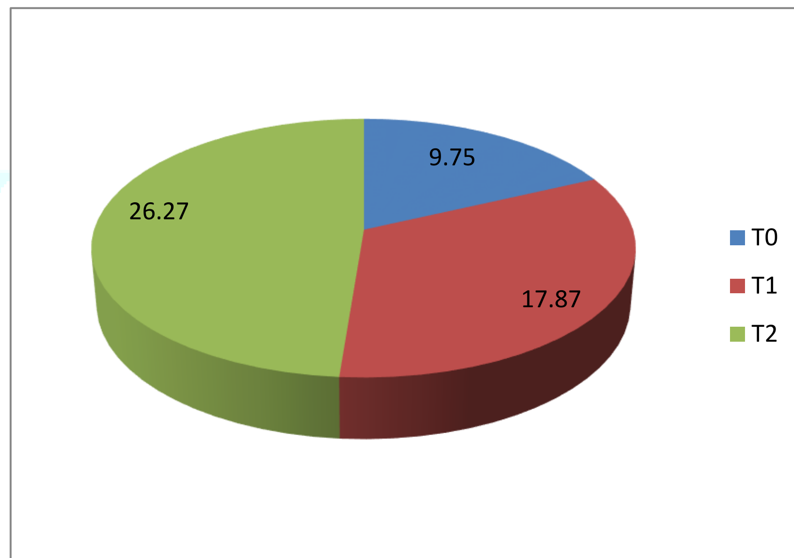
Thiobarbituric acid reaction substance (TBARS) levels were higher in the study groups (T1,.T2) than(T0) groups these results agree with previos studies,[42].Although this may suggest that the stress imposed by strenuous training exceeds the individuals increased capacity to detoxify ROS several published studies failed to find adifference in the peroxidation status between athletic and non athletic group[44], or even describe lower oxidant damage in the former [11].

The inconsistent results among studies that compared resting lipid peroxidation levels between trained and untrained individuals might be attributable to differences in the sports examined, the time athletes spent training, their training and nutritional status, and the biomarkers used to detect oxidative stress [8]. Despite TBARS being one of the most commonly studied markers to evaluate plasma pro-oxidant status, it has been criticized for a lack of specificity and sensitivity [8,42],and it would have been useful to determine another biomarker of lipid peroxidation, such as F2-isoprostanes [35]. Inconsistencies could also be related to the diverse circannual rhythms of this lipid peroxidation marker for untrained and trained participants [46].

In the current work, athletes' TBARS levels were significantly elevated in(T1,T2) comparison with (T0) , indicating a potential basal oxidative stress caused by daily training lessons.

Actually, although the adaptation to training involves, to some extent, a degree of strengthening of antioxidant defense (as indicated by the increase of SOD activity in this study).Other study revealed intensive training could cause muscle cells to lose membrane integrity, with loss of intramuscular enzymes, even in highly trained athletes [47].

Nevertheless, the lipid peroxidation increase and the antioxidant status did not increase consistently over the course of the lessons. This leads us to theorize that the training load was enough to induce beneficial adaptations in antioxidant defense , however, exceeding the threshold level of exercise above which antioxidant status is compromised, oxidative stress is chronic, and, therefore, antioxidant supplementation is requird.



Scheme (3): The means of thiobarbituric acid –reactive substances [TBARS] in three cases.

The present study is aimed at investigating CAT,GPX,GSH and SOD, antioxidant enzymes. In addition to (UA), (TAC), some trace elements and TBARS at baseline and after 3 months, 6 months for followed the oxidative stress in students and impact of training on antioxidant status.

Conclusion:

we still have insufficient knowledge about the interaction between exercise and antioxidants, which are important in assessing the adequacy of protection against oxidative damage and about the necessity of dietary manipulation and/or

supplementation. Thus physical exercise is a double-edged sword: when practiced strenuously it causes oxidative stress and cell damage; in this case antioxidants should be given. But when practiced in moderation, it increases the expression of antioxidant enzymes and thus should be considered an antioxidant. A schematic representation highlighting the role of ROS generated in moderate exercise in the upregulation of antioxidant enzymes and, thus, the fact that moderate exercise is an antioxidant.

References:

1. Warburton, D. E., Nicol C. W., Bredin S. S. Health benefits of physical activity: the evidence. Can. Med. Assoc. J.**2006** 174:801–809
2. Armstrong, R. B., Ogilvie, R. W.; Schwane, J. A. Eccentric exercise induced injury to rat skeletal muscle. J. Appl. Physiol. **1983** 54:80–93;.
3. Jackson, M. J. Muscle damage during exercise: possible role of free radicals and protective effect of vitamin E. Proc. Nutr. **1987** 46:77–80;.
4. Vina, J.; Gomez-Cabrera, M. C.; Lloret, A.; Marquez, R.; Minana, J. B.;Pallardo, F. V.; Sastre, J. Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. IUBMB Life **2000** 50:271–277; .
5. Radak,Z.S.,Zhongfu., Z.H., Erika K., Hideki O.S., and Mustafa A.T. Oxygen Consumption and Usage During Physical Exercise: The Balance Between Oxidative Stress and ROS-Dependent Adaptive Signaling. Antioxidants & Redox Signaling, Discoveries J **2013** 18,10, p1211**.**
6. Smith, J.A., M. Kolbuch-Braddon, I., Gillan, R.D., Weidmann, M.J. Changes in the susceptibility of red blood cell to oxidative and osmotic stress following submaximal exercise. Eur. J. Appl. Physiol.**1995** 70:427-436.
7. Clarkson, P.M., and Thompson,H.S. Antioxidants: what role do play in physical activity and health? Am. J. Clin. Nutr **2000**. **72**:637S-646S.
8. Knez ,W.L., Jenkins ,D.G., Coombes ,J.S. Oxidative stress in half and full Ironman triathletes. **2007***Med Sci Sports Exerc.***2007**39(2):283–288.
9. Aguilo, A., Fuentespina, E., and Tauler P. Antioxidant die supplementation influences blood iron status in endurance athletes. *Int J Sport Nutr Exerc Metabol*;**2004**14(2):147–160.
10. Dragland, S., Senoo ,H ., Wake, K ., Holte, K ., Blomhoff ,R .Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr. **2003**;133(5):1286.
11-.Cazzola, R., Russo-Volpe ,S., Cervato ,G., Cestaro, B. Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status in professional soccer players and sedentary controls. Eur J Clin Invest.**2003**; 33(10):924–930.
12. Smith, JA., Exercise, training and red blood cell turnover. *Sports* Med. **1995**; 19(1):9–31.
13. Lafay ,S., Jan, C., and Nardon , K. Grape extract improves antioxidant status and physical performance in elite male athletes. J Sports Sci Med. **2009** ;8(3):468–480.

14. Powers, S, K., and Jackson, M, J. "Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production," Physiological Reviews **2008** vol. 88, no. 4, pp. 1243–1276.
15.Hamer, D.H. Metallothionein. Ann. Rev. Biochem **1986** 55:913-951.
16. McCord, J.M., and Fridovich, I. Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). J. Biol. Chem **2009** 244:6049-6055.
17. Gomez-Cabrera, E., Domenech, M., and Viña, J "Moderate exercise is an antioxidant: upregulation of antioxidant genes by training," Free Radical Biology and Medicine **2008** vol. 44, no. 2, pp. 126–131.
18.Kanter, M "Free radicals, exercise and antioxidant supplementation," Proceedings of the Nutrition Society **2012** vol. 57, no. 1, pp. 9–13.
19. Wilson, D.O., and . Johnson, P "Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver," Journal of Applied Physiology **2000** vol. 88, no. 5, pp. 1791–1796.
20- Radak, Z., Chung, H., Goto, S "Systemic adaptation to oxidative challenge induced by regular exercise," Free Radical Biology and Medicine **2008** vol. 44, no. 2, pp. 153–159.
21. Powers, S.K., Ji , L.L ., and Leeuwenburgh, C ."Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review," Medicine and Science in Sports and Exercise 2013 vol. 31, no. 7, pp. 987–997.
22. Niess, A.M., Hartmann, A ., Grünert-Fuchs, M ., Poch, B., and Speit, G "DNA damage after exhaustive treadmill running in trained and untrained men," International Journal of Sports Medicine **2006** vol. 17, no. 6, pp. 397–403.
23.Miyazaki, H ., Oh-ishi, S ., and Ookawara T. "Strenuous endurance training in humans reduces oxidative stress following exhausting exercise," European Journal of Applied Physiology **2001** vol. 84, no. 1-2, pp. 1–6.
24. Robertson, J.D., Maughan, R.J., Duthie, G.G., & Morrice, P.C. Increased blood antioxidant systems of runners in response to training load. Clinical Science **1991**, 80(6), 611–618.
25. Kampa, M., Nistikaki, A ., Tsaousis, V . A New Automated Method for the Determination of the Total Antioxidant Capacity (TAC) of Human Plasma, Based on the Crocin Bleaching Assay, Bio. Med. Central Clin. Path **2002** 2(1), 3-6 .
26. Paglia ,D.E ., and Valentine,W.N. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase, J. Lab. Clin. Med **1967**,70, 158-169 .
27. Marklund, S., and Marklund, G. Involvement of the superoxide dismutase anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur.J. Biochem. **1974**, 47:469-471.
28. Goth,L. A Simple Method for Determination of Serum and Erythrocyte Catalase Activity and the Revision of the Reference Range, Clin. Chim. Acta, **1991** 196, 143-145 .
29. Satho, K. Serum Lipid Peroxide in Cerebrovascular Diseases, Determined by a New Colorimetric Method, Clin. Chim. Acta **1987** 90, 37-43 .
30. Joha*n*nessen, J.,Gammelgaard ,B., and Jons, O. Comparison of Chemical Modifiers for SimultaneousDetermination of Different Selenium Compounds in Serum and Urine by Zeeman-Effect Electrothermal Atomic Absorption Spectrometry, J. Anal. At Spectrom.,**1993** 8, 999-1004 .

ISSN: 2455-7676

31. Jordan, J., Kiernan, W., Merker H .J., Wenzel, M., Beneke, R. Red cell membrane skeletal changes in marathon runners. Int J Sports Med **1998**; 19: 16-19.
32. Finaud, J., Lac, G., & Filaire, E. Oxidative stress:Relationship with exercise and training. Sports Medicine (Auckland, N.Z.), **(2006)** 36(4), 327−358.
33. Lekhi, C., Gupta, P.H., & Singh, B. Influence of exercise on oxidant stress products in elite Indian cyclists. *British Journal of Sports Medicine,* **(2007)** 41(10), 691−693.
34. Vitor, T., Hugo, V., and Susana, C .Antioxidant Status, Oxidative Stress, and Damage in Elite Trained Kayakers and Canoeists and Sedentary Controls, Int J Sport Nutri and Exercise Metabolism, **2009**, 19, 443-456
35 . Gougoura, S., Nikolaidis, M.G., Kostaropoulos, I.A., Jamurtas, A.Z., Koukoulis, G., &Kouretas, D. Increased oxidative stress indices in the blood of child swimmers. European Journal of Applied Physiology and Occupational Physiology, 2007 100(2),235−239.
36. Andrea, J. , Will, G. , and Timothy, E. Effect of Dietary Antioxidants, Training, and Performance Correlates on Antioxidant Status inCompetitive Rowers, International Journal of Sports Physiology and Performance, **2013**, 8, 565-572.
37. Marzatico, F., Pansarasa, O., Bertorelli, L., Somenzini, L., & Della Valle, G. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. Journal of Sports, Medicine, and Physical Fitness, **1997** 37(4), 235–239.
38. Lukaski, H.C., Hoverson, B.S., Gallagher, S.K ., and Bolonchuk W.W. Physical training and copper, iron, and zinc status of swimmers. Am. J. Clin. Nutr, **1990** 51:1093-1099.
39. Mena, P., Maynar, M., Gutierrez J.M ., J. Maynar, J. Timon, and J.E. Campillo. Erythrocyte free radical scavenger enzymes in bicycle professional racers. Adaptation to training.Int. J. Sports Med,**1991**12:565-566,
40.Tauler, P. I ., Gimeno, A., Aguiló, M.P., Guix ., and A. Pons. Regulation of erythrocyte antioxidant enzymes during competition and short-term recovery. Pflügers Arch.–Eur. J. Physiol **1999** 438:782-787.
41. Elisa, C ., Silva, S ., and Marta , R . Oxidants, Antioxidants, and the Beneficial Roles of Exercise-Induced Production of Reactive Species Oxidative Medicine and Cellular Longevity, Int. J. Sports Med, **2012** 24:1022-1024.
42. Balakrishnan, S.D., & Anuradha, C.V.. Exercise, depletion of antioxidants and antioxidant manipulation. Cell Biochemistry and Function, **1998** 16(4), 269–275.
43. Chang, C.K., Tseng, H.F., Hsuuw, Y.D., Chan, W.H., & Shieh, L.C.**.** Higher LDL oxidation at rest and after a rugby game in weekend warriors. Annals of Nutrition & Metabolism, **2002** 46(3–4), 103–107.
44. Banfi, G., Malavazos, A., Iorio, E., Dolci, A., Doneda, L., Verna, R., . Plasma oxidative stress biomarkers, nitric oxide and heat shock protein 70 in trained elite soccer players. European Journal of Applied Physiology and Occupational Physiology, **2006** 96(5), 483–486
45. Elham, A. Relationship between oxidative stress and antioxidant status in beta thalassemia major patients, Acta Chim. Pharm. Indica , 2014 4(3), 137-145.
46. Balog, T., Sobocanec, S., Sverko, V., Krolo, I., Rocic, B., Marotti, M.The influence of season on oxidant-antioxidant status in trained and sedentary subjects.*Life Sciences,* **2006** 78(13), 1441−1447.

47.Palazzetti, S., Richard, M.J., Favier, A., & Margaritis, I.. Overloaded training increases exercise-induced oxidative stress and damage. Canadian Journal of Applied Physiology, **2003** 28(4), 588–604.