International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(11) November -2015

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THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 11, pp. 7494-7499, November, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

EFFECTS OF HIGH-ALTITUDE ON THE STATUS ANTIOXIDANT ENZYME IN LONG DISTANCE RUNNERS

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ARTICLE INFO

Article History:

Received 06th August, 2015
Received in revised form
14th September, 2015
Accepted 23rd October, 2015
Published online 28st November, 2015

Key words

Athletics, Catalase, carbonic anhydrase, high altitude, sea-level.

ABSTRACT

Aim: The aim of the study was to investigate the high-altitude and sea-level blood parametersof elite athletes living in high altitude permanently and to contribute to the literature by discussing the findings in the light of present knowledge.

Material and Method: The sample included 16 people who participated voluntarily, 8 of them were elite athletes in the province of Agri, and rest of them was randomly selected sedentary from the same age group.

Results: Results of the study can be summarized as in terms of the catalase values of athletes no significant difference has been observed. In terms of the catalase values of athletes and sedentaries, there was a statistically significant difference between the valuesof athletes before exercise at high altitude and the values of sedentaries at high altitude ($p<0.05$). In terms of carbonic anhydrase values of athletes, there was no statistically significant difference between the values before exercise at high altitude and the values after exercise at sea-level. On the other hand there were statistically significant differences for all other comparisons. In terms of peroxidase values of the athletes, a statistically differencewas observed between the values of before and after exercise at high altitude.

Conclusion: As a result of the study it can be said that exercise raises the values of catalase levels of athletes were lower than sedentaries, and finally exercise raises the catalase level in blood or tissuesboth at high altitude and at sea-level.

INTRODUCTION

There is a limited body of evidence suggesting that oxidative injury mediated by free radicalsis increased at altitude (Biselli, 1992; Simon, 1994). Simon-Schnass (Simon, 1994) identified significant increases in indirect indices of free radical mediated lipid peroxidation at altitude, which included increased pentane excretion and thiobarbituric acid reactive substances, decreased erythrocyte filterability, and increased leucocyte and granulocyte counts. Daily supplementation with an antioxidant such as tocopherol (vitamin E) equivalent to 300-400 mg has been shown to improve endurance performance, by theoretically limiting tissue damage (Biselli, 1992; Bailey , 1997). An accelerated production of the highly toxic hydroxyl radical may occur as a consequence of an increased production of free iron derived from altitude induced and training induced destruction of red blood cells (Simon, 1994). Thus it would appear that hypobaric hypoxia significantly increases oxidative stress, which has been shown to negatively influence energy metabolism and membrane integrity (Bailey,1997). During the resting state the human body produces Reactive Oxygen Species (ROS), but at levels well within the capacity of the body's antioxidant defense system. Comparatively, during endurance exercise there is a 10- to 20-fold increase in whole body oxygen consumption (Astrand *et al*,1986) and oxygen uptake in the active skeletal muscleincreases100-to200-fol8 (Keul, 1972; Mastaloudis, 2001). Despite the many known health benefits of exercise, there is a body of evidence suggesting that endurance exercise causes oxidative stress (Astrand *et al*, 1986). Astrand, 1982conducted one of the earliest studies addressing the issue of oxidative stress associated with exercise. The most frequently used tests included determination of the whole body maximal and submaximal oxygen consumption and the lactate turnpoint, (Costill *et al*, 1973) oxidative enzyme activities and the percentage of slow twitch fibers (Booth *et al*, 1974; Bylund *et al*,1977).

There are an increased number of investigations on the effects of high altitude on the antioxidant system. Unfortunately, the findings seem to vary considerably. It is meaningful that some native highlander tribes in India often eat the seeds of Trichopus zeylanicus, which have been shown to scavenge free

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radicals andreduce the levels of lipid peroxidation and DNA damage by their antioxidant capacity, depending upon the polyphenol and sulfhydyl content (Tharakn, 2005). In a subsequent study, we could not detect a significant effect of a 4-week exposure to 4000m on the activities of antioxidant enzymes (Radak, 1998). However, the exposure to altitude was longer and less severe than in the former study, which could account for the discrepancy (Nakanishi, 1995). Imai *et al*. compared the activities of GSH-Px in serum of native highlanders (4000 m) and subjects from sea level. They found that people residing at high altitude had lower levels of GPX activity. (Imai H,1995) High altitude exposure decreases the level of reduced glutathione (GSH) and increases oxidized glutathione concentration. (Ilavazhagan G, 2001, Joanny P, 2001). Thus, it appears that the capacity of enzymatic and non-enzymatic antioxidant systems is somewhat decreased at high altitude (Dosek *et al*, 2007)It appears that exposure to high altitude decreases the activity and content of some antioxidant enzymes.

Thus, both human and animal studies are relatively consistent in reporting that high altitude-associated hypoxia causes oxidative damage to lipids, proteins, and DNA. This damage can be due to the increased levels of ROS production and/or the decreased levels of the antioxidant capacity. (Gulcin I, 2012)The oxidative stress seems to be linearly related to the altitude: higher altitude leads to greater oxidative challenge to the body. It also appears that long term acclimatization and/or genetic adaptation attenuate or eliminate the high altitude-induced oxidative stress. On the other hand, physical exercise at high altitude could further increase the altitude-induced oxidative stress and the associated oxidative damage. (Dosek *et al*, 2007)

There is growing evidence that exercise increases oxidative stress within muscle tissue. (Biselli R,1992, Booth FW,1974,- Packer L,1997) Muscle damage results from the attack of reactive oxygen species (ROS) on polyunsaturated fatty acids in cellular membranes, resulting in lipid peroxidation, decreased membrane fluidity, altered ionic gradients, ellular (Biselli R,1992) swelling, tissue inflammation, DNA damage, and protein changes. (BolcalC, 2007)Such effects are linked to muscle soreness, delayed-onset muscle soreness, fatigue, increased recovery time, (Maughan RJ, 1989) and reduced oxygen delivery to tissue due to peroxided red blood cells. (Simon-SchnassI,1994) At higher altitudes, oxidative stress may be greater due to a combination of causative factors: decreased partial pressure of oxygen in inspired air, high rates of energy expenditure, decreased anaerobic threshold, increased UV light intensity, cold temperatures, and large, swift temperature changes (Simon, 1994; Askew, 1995; Saltin, 1967; Squires *et al*, 1982). The purpose of the study was to investigate a comparison in high altitude and sea level of various blood parameters, of the elite-level athletes who constantly living in high altitude conditions (Agri/Turkey).

MATERIAL AND METHODS

Selection of Subjects

In this study joined 8national athletes male volunteer and 8 volunteer individual as a sedater grouping the elite level makes sport athletics within the Turkey Agri Provincial Directorate Youth and Sport as the experimental group. The numbers of total subjects participating were 16 people in the study and these individuals live constantly in the 1640 meters altitude in the city Agri of Turkey. Test subjects in the experimental were selected group national elite athletes trained an average of 2 hours each day of the week. People in the sedentary group don't do the sport and who are residing in the city Agri of Turkey. Within this research to the athletes in the experimental group was implementeda training program immediately after from first blood sample. According to this programthe athlete was performed maximal loading with 3000 meters distance after 15-20 minute from warm-up. After this running were taken the second blood samples. This application was performed both at high altitude and sea level

Taking of the Blood Samples from the test subjects

Implementing various training programs during 12 months from athletes and sedentary individuals at 15.00 was receipt the first blood sample at height 2200 meters in the mountain foothills Bubi Mountain in the near city of Agri. Later to the athletes was performed maximal loading with 3000 meters distance after 15-20 minute from warm-up. After only from athletes were taken the second blood samples. The same people, was downloaded to the sea level on 7 days later and was taken to Trabzon Akçaabat Söğütlü athletics runway in the 10 meters altitude. Blood samples were obtained in the same manner and athletes was per for med the same training program and from just athletes were taken second blood samples. Blood sample was taken to the tubes with ETDA and normal biochemistry. Sample was centrifuged 10 min at and serum was stored in the -70^{o}Cuntil to the day analysis. Biochemmistry parameters measurement was performed in Atatürk University Faculty of Science Biochemmistry laboratory. In this study was evaluated EU/mL (catalaseactivity), EUmL (Peroxidase activity), EUmL (carbonic anhydrase activity) levels

Catalase Activity Assay

Catalase activity was assayed by monitoring the decrease in absorbance at 240 nm due to H_2O_2 consumption according to Aebi (1984). The reaction mixture contained 25 µL haemolysate, 1.5 mL 40 mM H_2O_2 and1.475mL0.1M phosphate buffer (pH 7.0). The decrease in absorbance was recorded at 240 nm for intervals 15 sec for three minutes. Enzyme activity was expressed as µmoles H_2O_2 decomposedby H,1974)

Peroxidase Activity Assay

Peroxidase activity was measured by the method of (Shannon *et al*. (1996). For this purpose, 25 mL haemolysate was taken up into 1 mL 0.1 M potassium phosphate buffer (pH 6.0). Then 1 mL ABTS was added. The reaction was started by addition of 1 mL 3.2 M H_2O_2. Change in absor bance at 470 nm was recorded for 2 min at intervals of 15 s. The enzyme activity was expressed as enzyme unit/min. gHb. (Shannon LM, 1966)

Detection of carbonic anhydrase

Different methods are applied according to the source and isolation of carbonic anhydrase isoenzyme in a mammalian species. More work is done on the cytoplasmic carbonic anhydrase isoenzymes of carbonic anhydrase I and II d. These two are the most easily obtainable source of isoenzyme erythrocytes. In excess of hemoglobin in erythrocytes in the studies, chloroform - ethanol precipitation, ammonium sulfate could be removed by precipitation or denaturation by heat. Besides these three-phase separations method has an alternative. In this method, a salt such as ammonium sulfate tert-butanol - water - and thus could be added to the protein mixture separated by denaturing hemoglobin and myoglobin. Then, upon receipt of the remaining 90% ammonium sulfate saturation it is collected in the middle phase of gel-like proteins. It is found in addition to carbonic anhydrase and superoxide dismutase, catalase phase. Isolation of carbonic anhydrase I and II is the most widely used method of affinity chromatography. For the first time 1970 after which this method being implemented in s carbonic anhydrase I and II isoenzymes could be successfully separated. In these methods, the support material is a strong inhibitors bind to the enzyme gel is provided which is inserted and kept in the enzyme column through these molecules. In this sense, it is also the most widely used inhibitor sulfanilamide. $HCO3- + CO2 + H2O \rightarrow H +$

When now the red blood cells by diffusion, through this enzyme found in red blood cell is converted to carbonate very quickly. The carbonate and bicarbonate ions released into the blood again separated into protons and blood pH of 7.4't like to be compensated by providing a natural buffer to works.

Statistical Analysis

The findings of the study were statistically evaluated. Calculations were performed using SPSS15.0 package program compatible with Windows. The results obtained were presented as Mean ±SD. The data that the non-parametric Mann-Whitney-U test was applied to test. Statistically different in the parameters, pair wise comparisons with Wilcox on test was performed. $P < 0.05$ was considered significant.

RESULTS

Results of oxidative stress markers of the in each group were Shown in tables 1.catalase activity in serum decreased markedly beaha (60.75±22.27) andaeaha (75.72±33.17) compared with the sgha(157.625±81.69) group. A significant decrease in the activity of catalase was observed in beaha and aeahagroup compared with sgha group. The difference between the beaha, aeaha and sgha groups was also significant ($p<0.05$, fig.1). The peroxidase levels of the serum were as follows: beaha group, 9.50±6.02; and aeaha group, 23.50±19.05. The serum peroxidase levels in the beaha group were significantly lower than the aeaha group ($p < 0.05$). The difference between the beaha group and aeaha group and i/r group was significant ($p < 0.05$; see table 1, fig.2).the carbonic anhydrase levels of the serum were as follows: beaha group, 0.78± 0.043; asana group 1.21±0.27; and aeasl group, 1.14±0.28. The serumcarbonic anhydraselevels in the beaha group were significantly lower than the aeaha and aeasl groups. The difference between the beaha and the aeaha and aeasl groups was also statistically significant ($p <0.05$, fig. 3). The carbonic anhydrase levels of the serum were as follows: beasl group, 1.07±0.27; and aeasl group, 1.14±0.28. The difference between the beasl and the aeasl groups was also statistically significant ($p <0.05$, fig. 3).

Table 1 Comparison Of Beaha, Aeaha, Beasl,Aeasl,Sgha And Sgsl Groups On The Serum Peroxidase, Catalase And Carbonic Anhydrase Levels

	N	Mean±SD Catalase (Eu/ml)	Peroxidase (Eu/ml)	Carbonic anhydrase (Eu/ml)
Before Exercise of Athletes at High Altitude (BEAHA)	8	60.75±22.27	9.5±6.02	0.78± 0.043
After Exercise of Athletes at High Altitude (AEAHA)	8	75.72±33.17	23.5±19.05	1.21±0.27
Before Exercise of Athletes at the Sea Level (BEASL)	8	81.88±39.09	13±13.84	1.07±0.27
After Exercise of Athletes at the Sea Level (AEASL)	8	108.44±70.44	15.5±17.55	1.14±0.28
Sedentary Group High Altitude (SGHA)	8	157.625±81.69	25.25±11.5	1±1.01
Sedentary Group Sea Level (SGSL)	8	132.125±104.37	11.5±6.56	1.01±0.13

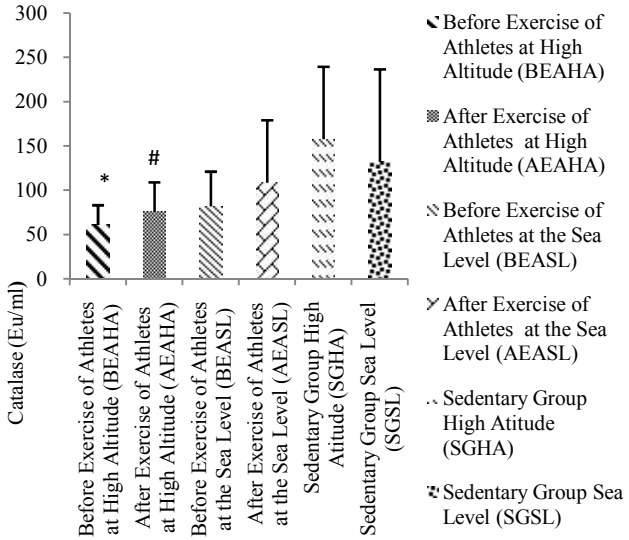

Figure 1 The effects of before exercise and after exercise of athletes at high altitude, before exercise and after athletes'exercise at sea level, sedentary group at high altitude and sedentary group at sea level on the CAT levels. *: Between the BEAHA and SGHA statistically difference (P=0.009, p<0.05), #: Between the AEAHA and SGHA statistically difference (p=0.046, p<0.05)

DISCUSSION

This study was made in order to compare of values blood parameters in the high altitude and sea level of elite level athletes permanently living in conditions high altitude (Turkey/Ağrı). ROS at physiological concentrations may be required for normal cell function. They are also capable of damaging crucial biomolecules such as lipids, polyunsaturated fatty acids, nucleic acids, proteins, and carbohydrates. Also, they may cause DNA damage that can lead to mutations.

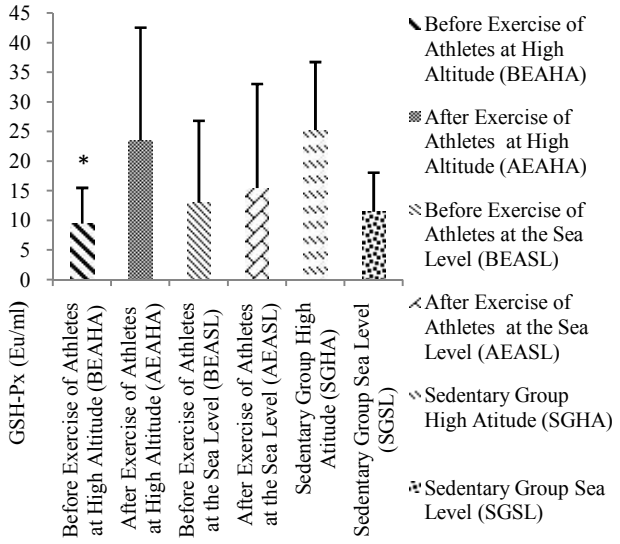

Figure 2 The effects of before exercise and after exercise of athletes at high altitude, before exercise and after exercise of athletes at sea level, sedentary group at high altitude and sedentary group at sea level on the CAT levels. *: Between the BEAHA and AEAHA statistically difference ($p<0.05$).

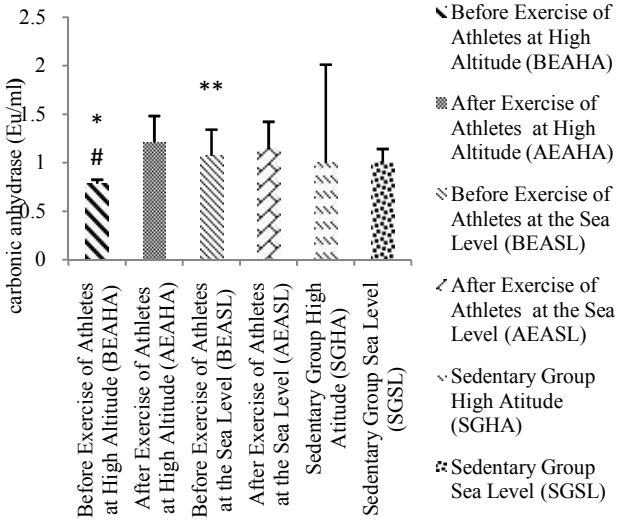

Figure 3: The effects of before exercise and after exercise of athletes at high altitude, before exercise and after athletes'exercise at sea level, sedentary group at high altitude and sedentary group at sea level on the CAT levels. *: Between the BEAHA and AEAHA, AEASL statistically difference ($p=0.012$, $p<0.05$), #: Between the BEAHA and BEASL statistically difference ($p=0.025$, $p<0.05$), **: Between the BEASL and AEASL statistically difference ($p=0.012$, $p<0.05$).

If ROS are not effectively scavenged by cellular constituents, they can stimulate free-radical chain reactions subsequently damaging the cellular biomolecules such as proteins, lipids and nucleic acids and finally, they lead to disease conditions.H_2O_2, which is a product of the oxidative metabolism, that can spread easily is non-radical a reactive species between the same timelive cells and produced in the organism Catalase enzyme removes harmful effects converting to water H_2O_2. (Gulcin I, 2012) did not find a significant difference in the süperoxide dismutase, peroxidase and catalase values in his study in order to determination of the effect to free radicals and antioxidant levels of the environmental stresses such as cold-hot and melatonin in the different room temperature in rats exercised for a long time. (Şıktar E,2008)has not detected significant differences CAT levels in the exercise group (18M, 28M, 38M) compared according to the temperature difference both in the exercise groups l-carnitine treated (18WM, 28WM and 38WM) according to temperature differences in the study research the effects of antioxidant levels and produce free radical of l-carnitine and thermal stress, different water temperatures exhausting swimming exercise in rats (Aguilo A,2000) stated that the fall of the CAT enzyme activity in the result submaximal (%80 maksVO_2) exercise test 9 of marathon runner age means25,6. (Salminen and *et al*, 1984) endurance study; Ohno, H, 1988) our study acute-exercise was found in training endurance does not change CAT of value. Effect of training to CAT activity skeletal muscle on the studies seems contradictory. Some studies, when talking increase in the CAT activity as a response to steady training in the skeletal muscle, if some studies are talking decrease of the enzyme activity under the same conditions. (Powers SK, 1999) In our study was observed, when evaluating CAT activity, decrease on the ratio important according to SGHA group in the BEAHA group CAT activity ($p<0.05$) However, was observed decrease on the significant level according to SGHA in the AEAHA group CAT activity ($p<0.05$). Even if different CAT activity between the other groups, was considered to be not statistically a different in the between groups. Peroxidase enzyme is an oxidoreductase which catalyzed reaction in the between H_2O_2 compound that may receive these atoms with compounds that tends to give hydrogen atoms.

Studies is show generally increase result steady exercise in the active skeletal muscle of peroxidase activity. (LeeuwenburghC, 1994) did not find a difference in the peroxidase values in his study in order to determination of the effect to free radicals and antioxidant levels of the environmental stresses such as cold-hot and melatonin in the different room temperature in rats exercised for a long time (Şıktar E, 2008) Peroxidase enzyme levels was found highly difference before and after exercise under the high altitude, before and after exercise under the sea level and under sea level and high altitude inthe sedentary groups. But peroxides levels were evaluated showed a statistically significant difference between the BEAHA and AEAHA groups. ($p<0.05$) Hyperventilation, provide more excretion. Formed of CO_2 although more lead to organism receives of O_2. Therefore, decreases CO2 in the arterial blood and increases the amount of alkaline substances. (Astrand PO,1988) It was determined regulate amount of the CO_2 the in tissues and blood of the CA enzyme.CA catalyzes the reversible hydration of CO_2 to bicarbonate (HCO_3^-). (Silverman DN,1988)

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How to cite this article:
Metin Bayram, Elif Şiktar., Effects of High-Altitude On The Status Antioxidant Enzyme In Long Distance Runners. *International Journal of Recent Scientific Research Vol. 6, Issue, 11, pp. 7494-7499, November, 2015*

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ISSN 0976-3031