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Influence of Intermittent Hypobaric Exposure on SOD and TBARS Levels in Trained Rats

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Abstract

Live high train low (LHTL) is a well-known training model for preparation of competitions. In this study, the thiobarbituric acid reacting substances (TBARS) levels and superoxide dismutase (SOD) activity were determined in heart, lung and muscle tissues of rats. They were intermittently exposed to hypobaric pressure of 523 mmHg, corresponding to an altitude of 3,000 m, and they performed swim training at sea level. Two groups of male rats were trained to swim for thirty minutes a day and 4 days a week, lasting 9 weeks. Two groups were exposed to hypobaria for 120 min a day and 4 days a week for 9 weeks in pressure cabin.

In heart tissue, TBARS levels of normobaric trained (NbT) group was higher ($P < 0,05$) than those of the normobaric sedentary (control) group. TBARS levels of hypobaric trained (HbT) group was higher than those of the control and hypobaric sedentary (Hb) groups ($P < 0.001$; $P < 0.01$, respectively). TBARS levels of lung tissue of HbT group was also higher than those of the same groups (control; $P < 0.01$, Hb; $P < 0.05$, respectively). In muscle tissue, TBARS levels of HbT group was higher than those of the sedentary groups (control; $P < 0.001$, Hb; $P < 0.05$, respectively). SOD activity of heart tissue of HbT group was higher ($P < 0.001$) than that of the other groups. In lung tissue, SOD activity of control group was lower than that of the other groups (HbT; $P < 0.001$, NbT; $P < 0.01$, Hb; $P < 0.01$, respectively). In muscle tissue, SOD activity of HbT group was higher ($P < 0.01$) than that of the control group.

The results of this study suggest that intermittent hypobaric exposure may augment exercise-induced oxidative stress in heart, lung and muscle of trained rats.

Key Words: swim training, intermittent hypobaric exposure, SOD, TBARS, rats

Introduction

Oxidative stress is defined as increased free oxygen (O_2) radicals, reactive oxygen species (ROS) and lack of antioxidant substances which are able to neutralize them. The electron transport associated with the mitochondrial respiratory chain is considered as the major process leading to ROS production at rest, and also during the exercise (9). ROS generated by a number of processes *in vivo* are highly reactive and toxic. ROS exert physiological conditions. They are known to be implicated in signaling pathways regulating cell growth (35, 36) and cell status redox control. However, when produced in excess, ROS exert detrimental effects and are able to damage cell macromolecules such as DNA, lipids or proteins

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Table 1. The characteristics of the experimental groups

Groups	n	Final Weight (g) Means ± SEM	Normobaric Swim Training	Intermittent Hypobaric Exposure
Group 1 (HbT)	12	273,08 ± 9,22*	Trained	Exposed
Group 2 (Hb)	10	268,00 ± 8,34	Sedentary	Exposed
Group 3 (NbT)	8	263,57 ± 10,65	Trained	Not
Group 4 (control)	9	237,56 ± 9,22	Sedentary	Not

*The means of the HbT group were significantly higher than those of the control group (Tukey HSD; $P < 0.05$).

(7). Among these targets the lipid peroxidation is particularly more damaging because it leads to a facile propagation of free radical reactions. However, biological systems have evolved an array of enzymatic and non-enzymatic antioxidant defence mechanisms to combat the deleterious effects of ROS. Superoxide dismutase (SOD) plays a key role in detoxification of superoxide anion, thereby protecting against ROS-induced damage. In addition, reduced glutathione (GSH) in conjunction with glutathione reductase (GR), glutathione peroxsidase (GPx), and catalase (CAT) enzyme also plays a central role in the defense against ROS (38).

Besides, hypoxanthine/xanthine oxidase, the inflammatory reaction of neutrophils, liver microsomal enzymatic functions, peroxisomal fatty acids and D-amino acid oxidation, stimulation of increased catecholamine on the beta-adrenergic receptors in myocard and skeletal muscles are potential factors increasing ROS production (19). Mitochondrial ROS production increases during exercise because the O_2 consumption in the organism rises dramatically during that period.

Additionally, results obtained from many experiments performed on rats show that oxidative stress indicators in the blood and various tissues increase in relation to the hypoxic state and under the laboratory conditions which simulate them (18, 30).

Oxidative stress in high altitude is not related to the increase of the O_2 consumption in tissues. Ultraviolet (UV) radiation, lack of dietary antioxidants, increased catecholamines, anoxi/reoxigenation conditions and hypoxanthine-xanthine oxidase contribute to the production of that process (2). Additionally, it is also shown that the exposure to high altitude decreases the activity and the effectiveness of the antioxidant enzyme system (25). Therefore, oxidative stress appears as a result of ROS production in high altitude.

Living at moderate altitudes and training at lower altitudes (Living High, Training Low-LHTL) is a well-known training model for preparation of competitions. Training models such as LHTL may augment physical performance by improving the exercise tolerance and maximum work capacity (14, 23). These methods may also attenuate some harmful conditions such as overtraining (4).

In this study, as a modification of the above-mentioned model LHTL, Wistar strain male rats that performed swimming exercise under normobaric sea level conditions were exposed to intermittent hypobaria. The aim was to examine SOD activity and thiobarbituric acid reacting substance (TBARS) levels and to determine adaptational changes in oxidant-antioxidant balance in heart, lungs and muscles where there is a heightened oxygen metabolism during exercise.

Materials and Methods

Animals

Forty-eight inbred, male Wistar strain Albino rats (5-6 months of age) were provided by the Centre for Reproduction and Research of Experimental Animals, Cerrahpasa Faculty of Medicine, University of Istanbul (The properties of the groups are given in Table 1). The rats were equally randomized and divided into four groups. Group 1 consisted of hypobaric trained rats (HbT); group 2: hypobaric sedentary rats (Hb); group 3: normobaric trained rats (NbT); group 4: normobaric sedentary rats (control). Nine rats were excluded from our experimental procedure due to some considerations (*i.e.*, death, infections, extremity injuries, wounded tissues, *etc.*). The rest of them (n = 39) were evaluated.

These animals were housed in conventional wire-mesh cages, four rats per cage, in a room with the temperature regulated at 21 ± 1°C, humidity 45-50% and in daily light-dark cycle (12 h). All rats were given *ad libitum* access to food, and tap water by drinking bottle throughout the experiment. They were fed a standard laboratory diet and received human care according to the criteria outlined in the "Guidelines for the Care and Use of Laboratory Animals" provided by the National Academy of Science and published by the National Institutes of Health (10), for the exercise to take place during their

most active period.

A hypobaric chamber or cabin, is an environment used during aerospace or high terrestrial altitude research or training to simulate the effects of high altitude on organisms, especially hypoxia and hypobaria. In our study, rats were exposed to hypobaric pressure of 523 mmHg, corresponding to an altitude of 3,000 m, for 120 min a day and four days a week for 9 weeks in a pressure cabin. To create a hypobaric environment, the air in the cabin was emitted by means of an air pump. The O_2 pressure in the cabin was kept constant. The pressure was followed automatically by means of a manometer connected to a Hg sensor.

Exercise

Trained rats were habituated to swimming for 5 min a day. The time of the exercise was gradually increased 5 min per day up to the level of 30 min a day. Trained rats performed swimming exercise four days a week. The procedure lasted for 9 weeks. Rats swam in groups of 4-5 animals simultaneously. Rats of HbT group immediately swam consecutively the hypobaric protocol on the same day. Swimming exercises were performed in a cylindrical glass beaker filled with water, approximately 35 cm deep and 50 cm wide. The beakers were submerged in a thermostatic water bath set between 30°C and 32°C. The fur of the rats was washed with liquid soap prior to the swimming. Air bubbles trapped in the fur were removed periodically to reduce buoyancy and ensure the imposed work load. Sedentary rats remained in their cages throughout the study.

Tissue Preparation

Nine weeks later, all rats were sacrificed by decapitation, 4 days after the last exercise. At the time of sacrifice, the rats were lightly anesthetized with ether. The tissues were removed from all rats in the same order: heart, lung and tibialis anterior muscles from both hind legs. The tissues were immediately covered with ice-cold buffer and kept on ice and then immediately transported to a cold room where they were processed to small preparations. All the tissue preparations were frozen on dry ice and then transferred to a –80°C freezer where they were kept until the measurement.

Biochemical Measurements

The TBARS levels and SOD activity in the tissues of rats were determined as a marker of lipid peroxidation (LPO), and the first line of defense against oxygen-derived free radicals, respectively.

Tissue Homogenization

The tissue samples were weighed (wet weight) and homogenized in ice-cold NaCl 0.9% to produce a 1/20 (w/v) homogenate. Tissue homogenization was performed with a tissue grinder fitting a Teflon pestle. The homogenate was sonicated with MSE sonicator two times at 30-second-intervals on ice, with a power output of 38 watts. The sonicated homogenates were centrifuged at 2,000 rpm for 10 min for the measurement of LPO and 15,000 rpm for 15 min for the measurement of the SOD activity, at 4°C. The biochemical assays were performed in the supernatants.

Measurement of LPO Levels

Tissue TBARS levels, as the marker of LPO was determined with the spectrophotometric method described by Buege and Aust (5). Stock solution: 15% trichloroacetic acid (TCA), 0.375% thiobarbituric acid (TBA), 0.25 N hydrochloric acid (HCl). The samples were heated in a water bath for 20 min and after cooling, centrifuged at 2,000 g for 15 min and the formation of a pink color as a result of the reaction in between one molecule of TBARS and two molecules of TBA, and was then measured at 560 nm spectrophotometrically.

Measurement of SOD Activity

Tissue SOD activity was measured by the modified method of Sun *et al*. (34). The activity of SOD was measured in an alkaline medium that is titrated to pH: 10.2 during the experiment. This assay for superoxide dismutase SOD (EC 1.15.1.1) activity involved inhibition of nitro blue tetrazolium (NBT) reduction, with xanthine-xanthine oxidase used as a superoxide generator. One unit SOD was defined as the amount of protein that inhibited the rate of NBT reduction by 50%. Reaction mixture was consisted of 40 ml of 0.3 mM xanthine solution, 20 ml of 0.6 mM EDTA solution, 20 ml of 150 μM nitro blue tetrazolium solutions, 12 ml of 400 mM Na_2CO_3 solution and 6 ml of bovine serum albumin. The final concentration of xanthine oxidase was then 167 U/l. The production of formazon was determined spectrophotometrically at 560 nm.

Analysis of Data

The results were given as means ± SEM (standard error of the means). One-way ANOVA with Tukey post-hoc HSD (Highest Significant Difference) test was used to compare group means. A level of $P < 0.05$ was regarded as statistically significant.

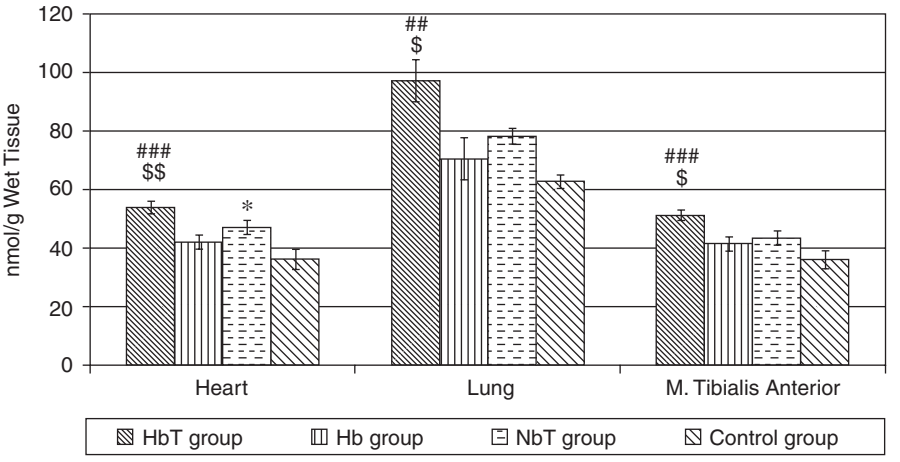

Fig. 1. TBARS levels of tissues in different groups (nmol/g wet tissue). (###): HbT group *vs*. control group ($P < 0.001$); (##): HbT group *vs*. control group ($P < 0.01$); ($$): HbT group *vs*. Hb group ($P < 0.01$); ($): HbT group *vs*. Hb group ($P < 0.05$); (*): NbT group *vs*. control group ($P < 0.05$).

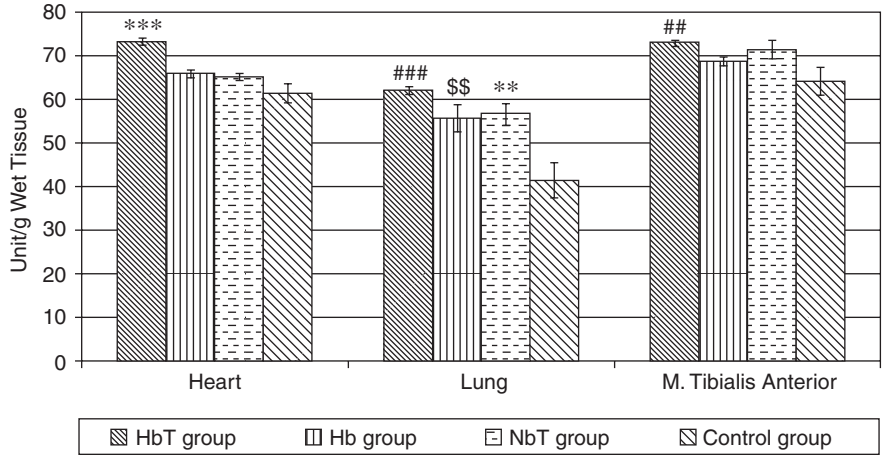

Fig. 2. SOD activity of tissues in different groups (Unit/g wet tissue). (***): HbT group *vs*. others ($P < 0.001$); (###): HbT group *vs*. control group ($P < 0.001$); (##): HbT group *vs*. control group ($P < 0.01$); ($$): Hb group *vs*. control group ($P < 0.01$); (**): NbT group *vs*. control group ($P < 0.01$).

Results

Heart tissue TBARS level of NbT group was significantly higher than that of the control group ($P < 0.05$). TBARS level of heart tissue of HbT group was significantly higher than that of the control and Hb groups ($P < 0.001$, $P < 0.01$ respectively). In lung tissue, TBARS level of HbT group was statistically higher than the control and Hb groups ($P < 0.01$, $P < 0.05$, respectively). In muscle tissue, TBARS level of HbT group was statistically higher than both sedentary groups (control; $P < 0.001$ and Hb; $P < 0.05$, respectively). In addition, there were no significant differences in tissue levels of TBARS between the Hb group and control group (Fig. 1).

SOD activity of heart tissue of HbT group was significantly higher than other groups ($P < 0.001$). Lung tissue SOD activity of HbT, NbT and Hb groups were significantly higher than that of the control group ($P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively). In muscle tissue, SOD activity of HbT group was significantly higher ($P < 0.01$) than that of the control group (Fig. 2).

Discussion

In our study, among LPO results in NbT group, only heart tissue levels are found significantly higher when compared to the control group. This finding supports the view that the increase in oxygen (O_2) consumption leads to the increase of the production of ROS in heart tissue, which paves the way for LPO (24).

Heart is one of the organs which have a high mass specific O_2 consumption. In other words, with

an aerobic characteristic, it is natural that it confronts with more pro-oxidant production and hence oxidative stress. As a result, LPO can appear in the hearts of rats doing chronic exercise (33).

In one of the studies conducted by Metin *et al*., by appling a similar training method as that in our study, they did not find significant changes in heart LPO levels of female rats (27). This finding can be explained that the rats were female, and estrogen played a protective role in terms of antioxidant effect (17). In the same study, Metin *et al*. reported an increased LPO in vastus lateralis which is an aerobic muscle during swim training in muscle tissue of rats (27). In our study, the LPO levels of anterior tibialis muscles which have an important glycolytic property (22), remained unchanged. The contradiction between the results of the two studies may be related to the assessment in different muscle types. It is known that skeletal muscles are highly heterogeneous. Each muscle fiber type has distinct metabolic characteristics and oxidative potential (19).

When examining the tissue levels in our study, in all of the tissues in rats of NbT group the measured SOD activity was higher than the control group. However, the statistical significance was established in only lung tissue. In literature, while there are studies that showed an increase in SOD activity of the organism after training period (16, 29), there are also studies claiming that it did not change after training (13, 20). According to studies focusing on SOD and LPO levels in heart, muscle and lung tissues which we examined in our study as well, there are contradictory findings. When Burneiko *et al*. investigate a decrease in heart SOD enzyme activity after 9 weeks of swimming training (6), Gul *et al*. find out that there is no change in heart tissue SOD activity, as we did in our study (13). Consistent with those findings, Atalay *et al*. report that there are no changes in muscle and heart SOD levels of rats which do 6 weeks of treadmill exercise (3). Anuradha and Balakrishnan determine an increase in LPO levels in heart tissue after 6 weeks of treadmill exercise (1). While Ravi *et al*. find out that LPO levels were decreased in heart tissue, they determined an increase in SOD activity (31). Consistent with our findings, Hatao *et al*. find that exercise increases the SOD activity in lung tissue (15). They conclude that this condition may be caused by the lungs, being exposed to O_2 directly (15). There is a great diversity in the results of the studies about whether exercise increases oxidative stress or not. This fact can be explained by the different types and periods of exercise being applied to subjects, and by the variety in the oxidative stress measurement methods.

In our study, SOD activity of the Hb group only in lung tissue was found significantly higher than that of the control group. As reported, being exposed to serious hypoxia can result in cell injury, but recurrence of short hypoxia can initiate some adaptive responses (37). In one of the studies, during adaptation to intermittent hypobaric exposure, an elevation of antioxidant activity without changes in LPO levels is reported (26). Xanthine dehydrogenase, which normally utilizes NAD^+ as electron acceptor, is converted under the conditions of ischemia/reperfusion into xanthine oxidase which uses O_2 as substrate. During the ischemic period, excessive ATP consumption leads to the accumulation of the purine catabolites hypoxanthine and xanthine, which upon subsequent reperfusion and influx of O_2 are metabolized by xanthine oxidase to yield massive amounts of superoxide and hydrogen peroxide (12). In light of these, it is possible to maintain that the antioxidant defense system, focusing on SOD activity, in lung tissue of Hb group, may be activated by factors such as hypoxanthine and xanthine in the anoxy/reoxygenation period during intermittent hypobaric exposure.

In literature, various studies applying different periods of intermittent hypobaric exposure have investigated parameters of LPO and antioxidant defense system. Nakanishi *et al*. find out that LPO production is high in heart and lung tissues of rats exposed to 5,500 m altitude for 12 h a day, but they did not observe a significant change in muscle tissue (28). During intermittent hypobaric exposure, they determine biphasic changes in lung SOD activity measured in different days. Radak *et al*. observe LPO elevation in soleus muscle of rats which are exposed to intermittent hypobaria for 6 months equivalent to 4,000 m, but they can not identify any significant change in tibialis muscles (30). Singh *et al*. find increased LPO in muscle tissue of rats exposed to intermittent hypobaric exposure for 21 days, 6 h a day, equivalent to 7,620 m altitude (32). According to the results of these studies, being exposed to intermittent hypobaria, depending on different altitude and periods of application, both LPO and SOD activity of the tissues may be affected. However, depending on the property of the muscle type where measurements are made, LPO may change.

While doing the biochemical analysis of the rats in HbT group which experienced both application factors in our protocol, all tissues were found to be influenced by the oxidative stress. LPO levels of the three organs in HbT group were significantly higher when compared to the two sedentary groups (Hb and control). While the SOD activity of this group was significantly higher in heart tissue than that of the other three groups, it was higher in muscle and lung tissues when compared to the control group. In a recent study (11), in muscle tissues of rats which are

exposed to hypoxia with certain training intervals, the LPO levels are obtained lower than those of the group which is trained in normobaric conditions. In our study, there was no difference in LPO levels in tissue between HbT and NbT groups. In the same study (11), SOD activity is identified to be higher than both the control and the training group. However, we identified significantly higher SOD activity when compared to the control group. The difference between the two studies may result from the application of distinct experimental protocols. The fact that the rats do the exercise on the last day and are then sacrificed on the same day following the training period may influence the findings in the previous study (11). The discrepancies may also be related to the differences in the exercise mode, intensity, duration of training program, and muscle fiber type. Each type has different antioxidant defense capacity (19). Therefore, it is possible to expect fiber specific adaptive responses to intermittent hypoxia. When compared to the control group, in terms of the increase in LPO and SOD activity, in all of the groups the highest significance was obtained in HbT group. In this group, applying regular exercise training and intermittent hypobaric exposure plays a more crucial role on the results when compared to the groups taking the hypobaria or exercise training separately (Hb and NbT, respectively). It is known that hypobaric hypoxia and exercise are separate potential sources leading to oxidative stress. It is possible that these above-mentioned factors are able to potentiate each other mutually. Joanny *et al*. suggest that oxidative stress is induced by prolonged hypobaric hypoxia and is maintained even under the condition by the sea level, in a similar way to the post-hypoxic reoxygenation process. It was increased by physical exercise (21).

In our study there were three groups (HbT, Hb, NbT): a statistically significant SOD activity response was obtained only in lung tissue. It can be explained that this organ is the first station facing O_2 directly (15), and that there is a high increase in the respiration load in the above-mentioned processes. The results of our study suggested that intermittent hypobaric exposure can elevate exercise-induced oxidative stress in heart, lung and muscle in trained rats. It can be concluded that antioxidant supplementation might be beneficial to the improvement of the antioxidant defense machinery. Devi *et al*. report that supplementation of vitamin E, vitamin C, and L-carnitine alone, not in a combined way, can be useful for attenuating the oxidative stress relevant to intermittent hypobaric hypoxia when compared to the unsupplemented rats that are exposed to different altitudes (8). Dietary antioxidants can be consumed to prevent or reduce oxidative stress due to long-term exercise and hypobaric conditions. In conclusion, it would be a great contribution to the health and performance of the sportsmen who prefer this *live high train low* training model when providing them with dietary antioxidants.

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References

1. Anuradha, C.V. and Balakrishnan, S.D. Effect of training on lipid peroxidation, thiol status and antioxidant enzymes in tissues of rats. *Indian J. Physiol. Pharmacol*. 42: 64-70, 1998.
2. Askew, E.W. Work at high altitude and oxidative stress: antioxidant nutrients. *Toxicology* 180: 107-119, 2002.
3. Atalay, M., Seene, T., Hanninen, O. and Sen, C.K. Skeletal muscle and heart antioxidant defences in response to sprint training. *Acta Physiol. Scand*. 158: 129-134, 1996.
4. Brugniaux, J.V., Schmitt, L., Robach, P., Jeanvoine, H., Zimmermann, H., Nicolet, G., Duvallet, A., Fouillot, J.P. and Richalet, J.P. Living high-training low: tolerance and acclimatization in elite endurance athletes. *Eur. J. Appl. Phsiol*. 96: 66-77, 2006.
5. Buege, J.A. and Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol*. 52: 302-310, 1978.
6. Burneiko, R.C., Diniz, Y.S., Faine, L.A., Galhardi, C.M., Padovani, C.R., Novelli, E.L. and Cicogna, A.C. Impact of the training program on lipid profile and cardiac health. *Biol. Res*. 37: 53-59, 2004.
7. Demple, B. and Harrison, L. Repair of oxidative damage to DNA; enzymology and biology. *Annu. Rev. Biochem*. 63: 915-948, 1994.
8. Devi, S.A., Vani, R., Subaramyan, M.V., Reddy, S.S. and Jeevaratnam, K. Intermittent hypobaric hypoxia-induced oxidative stress in rat erythrocytes: protective effects of vitamin E, vitamin C, and carnitine. *Cell. Biochem. Func*. 25: 221-231, 2007.
9. Di Meo, S. and Venditti, P. Mitochondria in exercise-induced oxidative stress. *Biol. Signals Recept*. 10: 125-140, 2001.
10. Forster, H.P., Emanuel, E. and Grady, C. The 2000 revision of the Declaration of Helsinki: a step forward or more confusion? *Lancet* 358: 1449-1453, 2001.
11. Gonchar, O. Muscle fiber specific antioxidative system adaptation to swim training in rats: influence of intermittent hypoxia. *J. Sports Sci. Med*. 4: 160-169, 2005.
12. Granger, D.N. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol*. 255: 1269-1275, 1988.
13. Gul, M., Demircan, B., Taysi, S., Oztasan, N., Gumustekin, K., Siktar, E., Polat, M.F., Akar, S., Akcay, F. and Dane, S. Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart. *Comp. Biochem. Physiol. A Mol. Integr. Physiol*. 143: 239-245, 2006.
14. Hahn, A.G., Gore, C.J., Martin, D.T., Ashenden, M.J., Roberts, A. D. and Logan, P.A. An evaluation of the concept of living at moderate altitude and training at sea level. *Comp. Biochem. Physiol. A Mol. Integr. Physiol*. 128: 777-789, 2001.
15. Hatao, H., Oh-ishi, S., Itoh, M., Leeuwenburgh, C., Ohno, H., Ookawara, T., Kishi, K., Yagyu, H., Nakamura, H. and Matsuoka, T. Effects of acute exercise on lung antioxidant enzymes in young and old rats. *Mech. Ageing Dev*. 127: 384-390, 2006.
16. Higuchi, M., Cartier, L.J., Chen, M. and Holloszy, J.O. Superoxide dismutase and catalase in skeletal muscle: adaptive response to

exercise. *J. Gerontol.* 40: 281-286, 1985.
17. Ide, T., Tsutsui, H., Ohashi, N., Hayashidani, S., Suematsu, N., Tsuchihashi, M., Tamai, H. and Takeshita, A. Greater oxidative stress in healthy young men compared with premenopausal women. *Arterioscler. Thromb. Vasc. Biol*. 22: 438-442, 2002.
18. Ilavazhagan, G., Bansal, A., Prasad, D., Thomas, P., Sharma, S.K., Kain, A.K., Kumar, D. and Selvamurthy, W. Effect of vitamin E supplementation on hypoxia-induced oxidative damage in male albino rats. *Aviat. Space. Environ. Med*. 72: 899-903, 2001.
19. Ji, L.L. Antioxidants and Oxidative Stress in Exercise. *Proc. Soc. Exp. Biol. Med*. 222: 283-292, 1999.
20. Ji, L.L., Stratman, F.W. and Lardy, H.A. Antioxidant enzyme response to selenium deficiency in rat myocardium. *J. Am. Coll. Nutr*. 11: 79-86. 1992.
21. Joanny, P., Steinberg, J., Robach, P., Richalet, J.P., Gortan, C., Gardette, B. and Jammes, Y. Operation Everest III (Comex'97): the effect of simulated sever hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. *Recuscitation* 49: 307-314, 2001.
22. Laughlin M.H. and Armstrong, R.B. Muscular blood flow distribution patterns as a function of running speed in rats. *Am. J. Physiol*. 243: 296-306, 1982.
23. Levine, B.D. and Stray Gundersen, J. The effect of altitude training are mediated primarily by acclimatization, rather than by hypoxic exercise. *Adv. Exp. Med. Biol*. 502: 75-88, 2001.
24. Liu, J., Yeo, H.C., Overvik-Douki, E., Hagen, T., Doniger, S.J., Chyu, D.W., Brooks, G.A. and Ames, B.N. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J. Appl. Physiol*. 89: 21-28, 2000.
25. Maiti, P., Singh, S.B., Sharma, A.K., Muthuraj, S., Banerjee, P.K. and Ilavazhagan, G. Hypobaric hypoxia induces oxidative stress in rat brain. *Neurochem. Int*. 49: 709-716, 2006.
26. Meerson, F.Z., Arkhipenko, I.Vb., Rozhitskaia, I.I., Didenko, V.V. and Sazontova, T.G. Opposite effects on antioxidant enzymes of adaptation to continuous and intermittent hypoxia. *Biull. Eksp. Biol. Med*. 114: 14-15, 1992.
27. Metin, G., Atukeren, P., Gumustas, M.K., Belce, A. and Kayserilioglu, A. The effect of vitamin E treatment on oxidative stress generated in trained rats. *Tohoku. J. Exp. Med*. 198: 47-53, 2002.
28. Nakanishi, K., Tajima, F., Nakamura, A., Yagura, S., Ookawara, T., Yamashita, H., Suzuki, K., Taniguchi, N. and Ohno, H. Effects of hypobaric hypoxia on antioxidant enzymes in rats. *J. Physiol*. 489: 869-876, 1995.
29. Pushpalatha, K., Nishanth, K. and Sathyavelu, R.K. Myocardial antioxidant status and oxidative stress after combined action of exercise training and ethanol in two different age groups of male albino rats. *Acta Biol. Hung*. 58: 173-185, 2007.
30. Radak, Z., Lee, K., Choi, W., Sunoo, S., Kizaki, T., Oh-ishi, S., Suzuki, K., Taniguchi, N., Ohno, H. and Asano, K. Oxidative stress induced by intermittent exposure at a simulated altitude of 4,000 m decreases mitochondrial superoxide dismutase content in soleus muscle of rats. *Eur. J. Appl. Physiol. Occup. Physiol*. 69: 392-395, 1994.
31. Ravi-Kiran, T., Subramanyam, M.V., Prathima, S. and Asha Devi, S. Blood lipid profile and myocardial superoxide dismutase in swim-trained young and middle-aged rats: comparison between left and right ventricular adaptations to oxidative stress. *J. Comp. Physiol*. 176: 749-762, 2006.
32. Singh, S.N., Vats, P., Kumria, M.M., Ranganathan, S., Shyam, R., Arora, M.P., Jain, C.L. and Sridharan, K. Effects of altitude (7,620 m) exposure on glutathione and related methabolism in rats. *Eur. J. Physiol*. 84: 233-237, 2001.
33. Somani, S.M., Frank, S., and Rybak, L.P. Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacol. Biochem. Behav*. 51: 627-634, 1995.
34. Sun, Y., Oberley, L.W. and Li, Y.A. A simple method for clinical assay of superoxide dismutase. *Clin. Chem*. 34: 497-500, 1988.
35. Sunderasan, Y.L.H., Ferrars, V.J., Irani, K. and Finkel, T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270: 296-299, 1995.
36. Thannickal, V.J. and Fonburg, B.L. Reactive oxygen species in cell signaling. *Am. J. Physiol. Lung Cell. Mol. Physiol*. 279: L1005-L1028, 2000.
37. Wenger, R.H. Mammalian oxygen sensing, signalling and gene regulation. *J. Exp. Biol*. 203: 1253-1263, 2000.
38. Yu, B.P. Cellular defences against damage from reactive oxygen species. *Biol. Rev*. 74: 139-162, 1994.