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ORIGINAL ARTICLE

Hypoxia and Hypoxic Exercise Induced Systemic Ros Disrupts the Redox Homeostasis in the Brain

EDA AĞAŞCIOĞLU[1], RIDVAN ÇOLAK[2], MEHMET CAN ATAYIK[3], AHMET ÇEVIK TUFAN[4], UFUK ÇAKATAY[5]
[1]*Assistant Professor Department of Recreation, Faculty of Sports Sciences, Lokman Hekim University, Sogutozu, 06510, Ankara, Turkey*
[2]*Assistant Professor Department of Physical Education and Sports, Ardahan University, Ardahan Üniversitesi Kampüsü, Çamlıçatak Mevkii, Merkez-Ardahan, 75002, Turkey*
[3]*Medical Student Medical Program, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, 34098, Turkey.*
[4]*Professor Department of Histology and Embryology, School of Medicine, Yildirim Beyazit University, Ankara, Turkey.*
[5]*Tenure Professor Department of Medical Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Istanbul, 34098 Turkey*
Correspondence to: Eda Ağaşcıoğlu, Email: eda.agascioglu@lokmanhekim.edu.tr, edaagascioglu@gmail.com, GSM: +90 532 726 95 54, Orcid ID: https://orcid.org/0000-0001-7550-8245

Abstract

Aim: *We aimed to investigate the overall effects of hypoxic/normoxic exercise and hypoxia on redox status in both systemic circulation and brain, and to prove whether the variations in plasma redox status could affect the brain's own redox homeostasis, vice versa.*
Methods: *We designed hypoxic, normoxic exercise groups with their respective controls. We studied on redox status biomarkers i.e., hydroperoxide, low molecular weight thiols, protein thiols, total thiols, and advanced oxidation protein products in frontal cortex; total antioxidant and total oxidant status in the plasma.*
Results: *There is no statistically significant difference observed in redox homeostasis of the brain after hypoxic and/or normoxic exercise or hypoxia itself with an increased systemic oxidant status.*
Conclusions: *Live in hypoxia and exercise at normoxia might diminish the hazardous effect of ROS on the brain at hypoxia. From our findings, thiols, which are the indicators of the antioxidant power of the brain, are found to be protected in groups that are exposed to long-term hypoxia and exercise at normoxia. It might be possible that people who are exposed to hypoxia will be least affected by this damage with normoxic exercise, or even will not be affected at all.*
Keywords: *Hypoxic exercise, Redox homeostasis, Brain, Plasma*

INTRODUCTION

Hypoxic exercise is a major challenge to whole-body redox homeostasis by triggering acute and adaptive responses on the systemic and neurological basis [1]. It is commonly known that exercise stimulates neurogenesis through neurotrophic factors, increases perfusion rate, reduces oxidative damage, and enhances repair of oxidatively modified macromolecules. Range and results of overall effects of exercise on the brain varies according to the type and duration [2].

Exercise at hypoxia has been used conventionally for many years to improve endurance exercise performance [3]. Endurance exercise is closely related by the oxygenation capacity of the active muscles [4]. Improved oxygenation capacity increases the efficiency of aerobic energy production and, therefore, advances exercise performance by increasing exercise intensity [5]. Also, hypoxic exercise stimulates athletic performance in aerobic events by increasing erythropoiesis and improving the oxygen-transporting and utilizing capacities [6]. When exposed to hypoxia, moderate intensity exercise may increase cerebral blood flow and compensate for decreased SpO2. Notable findings also support that strengthening endurance training has a major impact on improvement of cognitive abilities [7].

The blood-brain barrier (BBB) establishes a both physical and chemical barrier between the brain and systemic circulation. Its vessels contain pores which allow rapid exchange of molecules, have few pinocytic vesicles to minimize uptake of extracellular substances, and have extensive tight junctions that severely restrict cell permeability [8]. Restricted permeability limits the flow of substances to the brain circulation which also buffers the brain from rapid changes in ionic or metabolic conditions. Selective BBB permeability plays an important role in central nervous system redox homeostasis. Blood-borne/mediated reactive oxygen species (ROS) have been shown to penetrate BBB to enter cerebrospinal fluid and interstitial fluid spaces of the brain and spinal cord [9].

Hypoxia and reoxygenation is associated with an increased production of ROS which are prone to cross through the blood brain barrier and pass into cerebrospinal fluid [9 ,]. ROS overproduction is supposed to affect the brain as a result of hypoxic exercise due to interactions between various ROS-related signal transduction pathways [10]. The brain is highly sensitive to oxidative damage due to reliance on oxidative metabolism for energy. Relative to other organs, the brain consumes more oxygen and has relatively limited antioxidant defense capacity [11]. Using free radicals and non-radical derivatives to signal sensitizes the brain to oxidative stress when redox signaling goes awry [12].

Because of the enhanced sensitivity of brain to ROS mediated oxidative damage, it is especially important to maintain an optimal redox state in neuronal cells. Following hypoxia-associated neurotoxicity and other neurodegenerative insults, mitochondria will take up calcium which leads to an increased production of ROS. Neuronal cells expose excessive amounts of ROS, and thus, ROS formation rate significantly affects brain functions and hypoxic exercise performance [,13].

Due to regulation of brain redox homeostasis during exercise is crucial, we hypothesized that exercise performance at various hypoxia-normoxia cycles may depend on the redox status of peripheral circulation, blood-brain barrier, and brain itself. The aim of the current research is to investigate the overall effects of hypoxic exercise, normoxic exercise and hypoxia on redox status in in both systemic circulation and brain, and to prove whether the variations in plasma redox status could affect the

brain's own redox homeostasis, vice versa. In this context, we classified two groups with and without exercise at selected regimens (e.g., hypoxic exercise, normoxic exercise), then examined the redox status changes in redox status biomarkers of the brain and plasma oxidant status after hypoxic and/or normoxic exercise or hypoxia itself. Therefore, to meet the purpose of research, we investigated possible correlations to clarify whether the hypoxic/normoxic exercise-induced plasma redox status changes were related to changes in brain redox status.

MATERIAL AND METHODS

Chemicals: All reagents used were of analytical grade, stored at +4°C and equilibrated at ambient temperature for 30 mins before the biochemical analysis. Analytical grade water was used for the preparation of the reagents.

Apparatus: Separation procedures were performed with a Z 323 K cooled centrifuge (Hermle, Germany). The absorbance values of the redox biomarkers such as t-SHs, LMWTs, AOPP, and LOOH were analyzed with the PowerWave XS, (BioTek Instruments, VT, USA) spectrophotometer. The RBC and Hb levels were determined by a Cell-Dyn 3700 hematology autoanalyzer (Abbott Diagnostics, Illinois, USA). Normobaric hypoxia was provided by 8850 SUMMIT oxygen generator (Altitude Tech, Bristol, UK). The indoor air of the hypoxic tent was continuously monitored by MX6 iBrid O_2 detectors (Industrial Scientific Co, PA, USA). The CO_2, temperature and humidity inside the tent were regularly checked by a device (Testo 435; Testo AG, Germany). A CO_2 cleaner device (Altitude Tech. Co.) was used to keep the environmental CO_2 at an optimum level. The rats were exercised with a MAY-TME 9805 motorized treadmill (Commat, Ankara, Turkey). Brain samples were homogenized in 2mL glass homogenizator (Thomas Scientific, NJ, USA).

Animals Subjects and Procedures: 8-week-old male Sprague-Dawley rats were supplied from Experimental Animal Production and Research Center of Pamukkale University, Denizli, Turkey. Experimental animals were initially recruited by running inclination on a motorized treadmill at 0.3 km/h up to 0.5 km/h, 0% incline, 10 minutes/day, for 5 days. The preselected animals were initially divided into two groups: training groups and non-training control groups. The aim of this classification was to alleviate all possible effects of the exercise in environmental variables, and also to indicate the sole effect of the environment on redox balance. Afterwards, training groups were randomly divided into three experimental groups: LHTL (live high train low), LHTH (live high train high), and LLTL (live low train low). Non-training control groups were also randomly set as LH (live hypoxia), LL (live normoxia), and LHL (live hypoxia and normoxia). N values are equal to 6 for each group. LHTL was kept at hypoxia and exercised at normoxia. LHTH was kept at hypoxia and exercised at hypoxia. LLTL was kept at normoxia and exercised at normoxia.

The final body weights of the rats were found to be unchanged before starting familiarization (Table 1). The rats eating an unrestricted diet were fed a standard rat chow, including 20% protein, 6% cellulose and 2% fat. Tap water was available to all groups ad libitum. Animals were housed in conventional wire mesh cages, two rats per cage, in a temperature-regulated room (21 ± 2 °C), humidity at 45%–55% and 12-hours light–dark cycles. All the procedures in the present research were conducted with the ethical standards of the Pamukkale University Ethics Committee of Animal Care and Usage (PAUHDEK-2009/014).

Experimental Procedures:

Exercise protocol: All experimental animals in the established training groups were given a familiarization exercise for 4 weeks, 4 days a week (2 + 2), and 15–30 min/day at the laboratory conditions, which exists at an altitude of about 350m above the sea level (Denizli/Turkey), to ensure all the rats are trained at the same altitude.

Outline of the exercise regimen is given in figure 1. At the end of the first training period, all the groups of rats were exercised for 30 minutes to be able to run at a speed of 1.5 km/h. To supervise the training intensities for the following 4 weeks with precision, a maximal aerobic velocity test was evaluated for all the training groups, after a resting period of 2 days. The test obtained at both normoxia and hypoxia was estimated by using a treadmill during a continuous and progressive maximal exercise test. At normobaric hypoxia (3000 m ≈ 15% O_2, LHTH), the treadmill was set at a speed of 0.3 km/h at an incline of 0%. Then, the running speed was gradually increased by 0.3 km/h every 3 minutes until the maximal intensity was obtained for each rat, till the animal could not maintain its running position. The test at normoxia (350m ≈ 20.9% O_2) was evaluated by following the same protocol, but with a starting speed of 0.6 km/h. The exercise sessions were performed for 4 days in the 1st week, then 5 days in a week in the following weeks. The running speed was adjusted to 60% of the test speed, for 20 minutes in the 1st week; 65% of the test speed, for 25 minutes in the 2nd week; 70% of the test speed, for 30 minutes in the 3rd week; and 70% of the test speed, for 35 minutes in the 4th week. All exercises were performed on successive days (2+2 and 2+3 days of exercise and a day rest in between) of the familiarization and hypoxic training period at 06.00–09.00 a.m.

During the exercise protocol, the second test was evaluated for the training groups after a resting period of 2 days. A normobaric hypoxia condition was performed by using a hypoxic tent and an oxygen generator. Current approach was established to provide a condition of normobaric hypoxia. Indoor air ingredients (O_2 and CO_2) of the hypoxic tent were continuously monitored throughout the whole experiment, and a CO_2 cleaner was also kept in run inside. CO_2 levels were constantly monitored and kept under 0.03%. Normoxic conditions were ensured with room air (20.9% O_2) at 350m altitude above the sea level [14].

The exposure of hypoxic and/or normoxic conditions to all groups were separately managed for 4 weeks. LHTH, LHTL, and LLTL were exposed to 24-hour hypoxia, 12-hour hypoxia/normoxia, and 24-hour normoxia, respectively.

Sacrification and sample collection: Animals were anesthetized in normoxia by intraperitoneal injection of Ketamine hydrochloride (Ketasol 10%; Richter-Pharma, Wels, Austria) and Xylasine (Alfazyne 2%; Alfasan, Woerden, The Netherland.) After drug administrations, blood samples were withdrawn from the abdominal aorta

and anticoagulated with heparin and EDTA. The anticoagulated blood samples were centrifuged at 3000 g for 20 min within 30 min following sampling and the separated plasma samples were stored at –80°C up to the day when measurements were done. Total antioxidant status (TAS) and total oxidant status (TOS) were investigated in heparinized plasma samples. Plasma samples with EDTA were instantly tested for the hematological parameters.

All rats were decapitated between 9 and 10 a.m., and the whole brain was quickly removed, washed in a chilled isotonic solution, placed on an ice-cold plate. Frontal lobe was immediately separated and frozen in liquid N_2 until homogenization. Brain tissue (200 mg) was manually homogenized in 2mL of homogenizing buffer (100 mM KH_2PO_4–K_2HPO_4, pH 7.4, plus 0.1% (w/v) digitonin) in a glass homogenizer to avoid possible nucleic acid contaminations. Brain homogenates were centrifuged at 5000 x g, in +4 ºC, for 10 mins, and various redox biomarker determinations were performed in the supernatant fraction [15].

Analytical Methods:

Lipid hydroperoxide groups: Lipid hydroperoxide (LOOHs) levels were assessed spectrophotometrically based on the method of ferrous oxidation with xylenol orange version 2 (FOX2) which binds ferric ions (Fe^{3+}) and forms a blue-purple chromogen complex [16]. Thus, the chromogen complex formation rate is directly correlated with the absorbance and thereby with the total LOOHs concentrations.

Simply put, the supernatant fractions of the cortical tissue homogenate were added to the 96-well microplates along with the FOX2 reagent. Incubated for about 30 minutes at room temperature in the dark. Then, centrifuged for 10 minutes at 3,000 g. Finally, the absorbance values of the final samples were recorded at the wavelength of 560 nm.

Assessment of the thiol groups: Low molecular weight thiol (LMWT) and total thiol groups (t-SH) were assessed by using 5,5'-dithio-bis (2-nitrobenzoic acid / DTNB) reagent, also known as Ellman's chromogenic reagent. DTNB is reduced by free thiol groups, and thus, mixed disulfide and 5-thio-2-nitrobenzoic acid (TNB) products are released.

The absorbance value of the coloreds, which is TNB, was read at wavelength of 412 nm for the assessment of t-SHs content. For LMWT analyses, the supernatant fractions were initially incubated with trichloroacetic acid for an hour. Then, centrifuged for 10 minutes at 3,000 g. Finally, DTNB reagent was added to the remaining supernatant [17]. Then, the absorbance was read at 412 nm.

Protein thiol groups (p-SH) were calculated by subtracting LMWT from t-SH.

Advanced oxidation protein products: Advanced oxidation protein products (AOPP) were assessed spectrophotometrically, according to the volumetrically modified colorimetric method of Witko-Sarsat. The supernatant sample and related chloramine-T standards (0-100µM) are mixed with citric acid and potassium iodide reagents, respectively. The absorbance value of the final reaction mixture was read at wavelength of 340 nm against a reagent blank. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents [18].

Plasma Redox Status: Plasma redox status was assessed by commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). TOS assay results calculations are performed with hydrogen peroxide (H_2O_2) standard curve by interpolation method, and plasma TOS concentrations are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Eq/L). Trolox, water-soluble analogue of vitamin E, was used as a TAS calibrator. Plasma TAS results are expressed as µmol Trolox Eq/L.

The plasma redox status index was defined as the ratio of the TOS level to TAS level. Redox status index (arbitrary unit) = TOS (µmol H_2O_2 Eq/L)/TAS (µmol Trolox Eq/L).

Hematological parameters: All hemogram values were determined by using Cell-Dyn 3700 hematology autoanalyzer (Abbott Diagnostics, Illinois, USA).

Statistical Analysis: All raw data were statistically processed by SPSS V.22 (IBM, Chicago, IL). When not specified, $p < 0.05$ was considered significant.

Descriptive statistics of the studied parameters were expressed with mean and standard error. Kolmogorov-Smirnov test was applied due to check if all the data were in concordance with Gauss distribution.

Statistical significance was evaluated by using the analysis of variance. Pearson correlation analyses were applied for normally distributed data.

RESULTS

In order to fulfill the main aim of the current research, we studied various redox status biomarkers in both systemic circulation and brain tissue to investigate whether the variations in plasma redox balance could affect the brain's own redox status directly or indirectly during the exercise with/without in hypoxic conditions.

Table 1: Descriptive parameters of the exercised groups and their respective controls.

Parameters	Training Groups			Control Groups		
	LHTH	LHTL	LLTL	LH	LHL	LL
Food consumption (g/day/rat)	20.01 ± 1.25 ^{a3*}	22.54 ± 0.84	22.46 ± 0.58	20.74 ± 1.01^{d*}	22.05 ± 0.73 ^{e*}	24.85 ± 0.87^{a3,d,e*}
Initial body weights (g)	233.57 ± 4.63	228.42 ± 3.18	233.33 ± 3.72	238.16 ± 2.88	237.50 ± 4.38	241.33 ± 1.99
Final body weights (g)	232.36 ± 5.02	235.28 ± 4.22	244.16 ± 5.86	241.00 ± 4.87	243.50 ± 8.69	253.02 ± 3.80
Erythrocyte count (10^6/µL)	10.15 ± 0.19 ^{a1*,a3*}	9.60 ± 0.31 ^{b3*}	9,16 ± 0.30^{a1*}	10.01 ± 0.23^{d*}	9.82 ± 0.58 ^{e*}	8.54 ± 0.38^{a3,b3,d,e*}

LHTH, live high train high; LHTL, live high train low; LLTL, live low train low; LH, live hypoxia; LHL, live hypoxia and normoxia; LL, live normoxia.

[a1]LHTH vs LLTL; [a2]LHTH vs LH; [a3]LHTH vs LL
[b1]LHTL vs LH; [b2]LHTL vs LHL; [b3]LHTL vs LL
[c]LLTL vs LHL
[d]LH vs LL
[e]LHL vs LL

Values are given as means (± SEM). ***** p<0,05.

All animals in the study groups were meticulously examined throughout the entire experimental process, they were ensured to be survived during the exercise protocol, and no rats were lost before euthanizing them.

The initial and final body weights, erythrocyte counts, and food consumptions of all groups, which were recorded and statistically compared between in all groups, are shown in Table 1. There was no significant difference between initial and final body weights of the animals. A significant decrease was found in the food consumptions of the LH, LHL, and LHTH when compared to LL. Also, a significant elevation was observed in the erythrocyte counts of the LH, LHL, LHTH, and LHTL when compared to LL. Apart from these significant differences, erythrocyte counts were found to be significantly higher in LHTH when compared to LLTL.

Table 2: Redox biomarkers of the exercised groups and their respective controls

Redox Biomarkers	LH	LHL	LL	p values
$LOOH_b$ (µmol/L)	7.71±0.91	7.40±1.78	5.51±0.87	x:0.663; y^{**}:0.004; z^{*}:0.012
$LMWT_b$ (nmol/mg pr.)	10.95±3.48	9.68±0.55	11.28±1.98	x:0.212; y:0.741; z:0.118
p-SH_b (nmol/mg pr.)	33.42±2.66	35.19±4.65	31.82±6.09	x:0.398; y:0.445; z:0.113
t-SH_b (nmol/mg pr.)	44.37±2.78	44.87±4.27	43.10±7.37	x:0.823; y:0.575; z:0.434
$AOPP_b$ (nmol/mg pr.)	48.02±2.74	45.97±1.55	44.02±1.56	x:0.356; y^{*}:0.047; z:0.378
TAS_p (µmol Trolox Eq/L)	0.85±0.17	0.92±0.23	1.09±0.43	x:0.565; y^{*}:0.037; z:0.189
TOS_p (µmol H_2O_2 Eq/L)	21.59±0.52	19.25±3.12	15.46±0.75	x^{**}: 0.006; y^{***}: 0.001; z^{***}: 0.001
TOS/TAS (Arbitrary Unit)				

LOOHs, lipid hydroperoxide; LMWT, low molecular weight thiols; p-SH, protein thiols; t-SH, total thiols; AOPP, advanced oxidation protein products; pr., protein; TAS, total antioxidant status; TOS, total oxidant status. LH: Live hypoxia, LHL: Live hypoxia and normoxia, LL: Live normoxia. Subscripts explains where to be investigated, b for brain, p for plasma. To compare groups, x for LH versus LHL; y for LH versus LL; z for LHL versus LL. Values are given as means (± SEM). *, p<0,05 ; **, p<0,01; ***, p<0,001

Table 3: Pearson correlation values between plasma and brain redox status biomarkers in all groups.

Redox Status Biomarkers	LH		LHL		LL		LHTH		LHTL		LLTL	
	TAS_p	TOS_p	TAS_p	TOS_p	TAS_p	TOS_p	TAS_p	TOS_p	TAS_p	TOS_p	TAS_p	TOS_p
$LOOH_b$	,487	,774*	-,641	-,087	,197	,099	,274	,298	,880*	,313	-,628	-,298
$LMWT_b$	,625	-,853*	,131	,700	-,085	,567	,465	-,845*	-,095	-,337	-,431	,434
p-SH_b	-,085	,217	-,192	-,680	-,159	,236	,648	-,747*	-,341	-,157	,692	-,598
t-SH_b	,704	-,867*	-,192	-,650	-,154	,347	,599	-,784*	-,391	-,284	,659	-,537
$AOPP_b$	,515	-,759*	-,330	-,345	-,154	,188	-,448	-,389	-,394	-,576	-,365	-,408
TOS/TAS (Arbitrary Unit)	-,991***	,313	-,780	,486	-,936***	,620	-,869*	,877*	-,908**	-,105	-,843*	,191

LOOH, lipid hydroperoxides; LMWT, low molecular weight thiols; p-SH, protein thiols; t-SH, total thiols; AOPP, advanced oxidation protein products; TAS, total antioxidant status; TOS, total oxidant status.

LH: Live hypoxia, LHL: Live hypoxia and normoxia, LL: Live normoxia. Subscript defines where to be investigated, b for brain, p for plasma.

Correlation coefficients (r) are given for each group. *, p<0,05; **, p<0,01; ***, p<0,001.

Values are given as means (± SEM).

Figure 1: Experimental design of the training groups

α: Four weeks of familiarization exercise at normoxia (20.9% of 0_2; ~350 m) for the training groups. **β:** Two days of resting, and MAV test at hypoxia for LHTH group and at normoxia for the rest at the end of a 2-day rest. **γ:** Four Weeks of Hypoxic or Normoxic Exercise: LHTH at hypoxia (15% of 0_2; ~3,000 m); LHTL and LLTL at normoxia.

δ: Blood collection and tissue extraction. LLTL, live low train low; LHTH, live high train high; LHTL, live high train low; MAV, maximum aerobic velocity test.

The statistical comparison of the redox status biomarkers of the hypoxic/normoxic exercised groups and their respective controls were given in Table 2. LOOH levels were found to be significantly higher in LH and LHL when compared to LL. However, no significant difference was found between the LH and LHL control groups and all other experimental groups. There is only statistically significant elevation in AOPP levels found in LH when compared with LL, while there are no statistically significant changes in the rest of groups in the brain. In accordance,

plasma TAS was only found to be lower in LH when compared with LL. All data obtained from studied plasma TOS comparisons were found to be statistically significant except for the experimental groups of LHTL and LLTL groups. Plasma TOS values, from highest to lowest, were found as LH, LHL, LHTH, LL, LHTL and LLTL respectively. There was no significant difference found between all groups when they compared in biomarkers of LMWT, p-SH, and t-SH. Arbitrary units, which refer to the ratios of the TOS level to TAS level, are described. The arbitrary unit values were found from highest to lowest as LH, LHL, LHTH, LL, LHTL and LLTL. All statistical data are presented in Table 2.

The correlation analysis expressing whether there is a relationship between redox status biomarkers in the brain and TOS/TAS studied in plasma, and if there is a correlation, whether it is in an increasing or decreasing trend are given in Table 3. In LH, the most significant correlation was observed between TAS_p and TOS/TAS $Index_p$. Also, other significant negative correlations were in LH when comparing TOS_p with LMWT, p-SH, and AOPP, respectively one by one. Only significant positive correlation was between TOS_p and LOOH. In LL, we observed a statistically significant negative correlation between TAS_p and $Index_p$ ($p<0,001$). In LHTH, negative correlations were observed when comparing TOS_p with LMWT, p-SH and t-SH. Moreover, when comparing TOS/TAS $Index_p$, we observed a significant negative correlation with TAS_p and a significant positive correlation with TOS_p. In LHTL, we obtained a significant positive correlation between TAS_p with LOOH ($p<0,05$), and a significant negative correlation between TAS_p and TOS/TAS $Index_p$ ($p<0,001$). In LLTL we received a significant negative correlation between TAS_p and TOS/TAS $Index_p$. There were no significant correlation results found in LHL. All correlation analysis results of the studied redox biomarkers with significance values in training groups are given in Table 3.

DISCUSSION

Recently, exercise has been growing interest in its effects on the brain and cognition [19]. Hypoxic exercise affects brain functions and leads to structural, biochemical, and physiological adaptations via redox status-related pathways [20]. Redox balance of the brain with exercise is closely related with muscular activity and the intensity and duration of training. Inadequate ROS production causes insufficient signal transduction and gene expression, and its over concentrations result in significant oxidative damage, apoptosis, and necrosis [21].

The purpose of the current research was to investigate the overall effects of hypoxic and/or normoxic exercise and hypoxia on redox status in both systemic circulation and brain, and to prove whether the variations in plasma redox status could affect the brain's own redox homeostasis, vice versa.

It is widely known that the brain is prone to oxidative damage and its energy and oxygen needs are very high, but its antioxidant capacity is much more limited compared to other post-mitotic tissues [11,22]. After hypoxic and/or normoxic exercise, detection of no or trivial changes in favor of oxidant in brain redox balance along with increased oxidative stress in the whole plasma, lessens the possibility that increased oxidants in plasma penetrated the blood-brain barrier and passed into the brain. However, higher negative relations between thiols fractions of the brain and TOS level in plasma of the hypoxic control LH and hypoxic lived and exercised LHTH groups, strengthened the possibility of plasma oxidant penetration through the blood brain barrier. Higher positive correlation between plasma TOS level and LOOH level of the brain supports this approach.

It has been shown that after long-term hypoxic conditions, oxidative stress provokes the activation of microglia and astrocytes leading to the great elevations in the levels of inflammatory mediators and the loss of cell integrity in the brain and eventually results with permanent ROS mediated brain cell damage [23-]. An increase in oxidant levels affect these synaptic dynamics directly and induce an incessant increase in the activity of redox signaling cascades to influence neuronal functions. For example, an oxidized redox state is likely an early marker of neurodegenerative diseases by contributing to the emergence of synaptic malfunction before histopathological evidence [24]. On the other hand, we observed no correlations between plasma oxidant status and oxidant parameters of the brain in the hypoxic lived and normoxic exercised LHTL, the normoxic lived and exercised LLTL groups, and their respective controls. This means exercise might be diminishing the detrimental oxidant's effect of hypoxia by augmenting expression of antioxidant enzymes and mitochondrial biogenesis [25]. Indeed, repetitive aerobic type of swimming decreased mutation of the mitochondrial DNA and increased the number of mitochondrial DNA copies in rats [26]. Repetitive moderate intensity exercise upregulates some repair mechanism and various antioxidants [27].

However, there need to be more studies conducted on this issue to fully evaluate the overall effects of increased plasma oxidant radicals on the brain during hypoxic and/or normoxic exercise to understand whether systemic oxidative radicals leading to damage on the brain.

As a widely accepted nonpharmacological strategy, physical exercise improves overall health and enhances mental capacity. Albeit, there is controversial evidence showing impaired brain redox status after high-intensity exercise, which presumably worsens cognitive performance [28]. Our hypoxic and/or normoxic exercise regime is considered as a long duration medium-intensity exercise. Therefore, contrary to high intensity exercise, in our exercise protocols it is not possible to consider lactate induced ROS formation. High intensity exercise leads to ROS formation due to higher levels of lactate [29].

CONCLUSION

Further studies need to be conducted with hypoxic and/or normoxic exercised rats groups in order to clarify the beneficial effects of low intensity exercise on the brain tissue during hypoxic and/or normoxic exercise regimens.

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