Table 4: examining pre-test and post-test values of attention levels of control group students participated in the research.

variables			N	avarage	Std. Dev.	t	P
control	female	Pre-test number of true ones	18	45,67	8,296	3,384	0,004*
		Post-test number of true ones	18	50,33	7,515		
	male	Pre-test number of true ones	22	47,45	8,382	1,870	0,076
		Post-test number of true ones	22	49,41	9,970		
	female	Pre-test number of wrong ones	18	,39	,850	0,960	0,350
		Post-test number of wrong ones	18	,67	,907		
	male	Pre-test number of wrong ones	22	,45	,596	0,000	1,000
		Post-test number of wrong ones	22	,45	,739		

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EFFECTS OF EXERCISE AT HIGH ALTITUDE ON MICRONUCLEUS FREQUENCY

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Ovidius University Annals, Series Physical Education and Sport / SCIENCE, MOVEMENT AND HEALTH,
Vol. 10 ISSUE 2, 2010, Romania
Our JOURNAL is nationally acknowledged by C.N.C.S.I.S., being included in the B+ category publications, 2008-2010.
The journal is indexed in: 1. INDEX COPERNICUS JOURNAL MASTER LIST. 2. DOAJ DIRECTORY OF OPEN ACCES JOURNALS, 2009, 3. SOCOLAR

Abstract

Objective: The aim of this work is to study effects of acute hypoxia on micronucleus frequency during exercise.

Research methods and subjects: Study group was formed with students of Erciyes University Vocational School of Physical Education and Sports. Students were within similar age and fitness range, mean age 23.35 ± 1.66 year, mean height 168.20 ± 7.32 cm, mean body mass 60.05 ± 8.76 kg, body mass index 21.12 ± 2.17 kg/m^2 , 10 female and 10 male totally 20 students were included in the study. All students were stayed at Mount Erciyes (2200-2500m) and exercised ski, 3 hours a day for 5 days. 1st day and 5th day oxygen saturation, systolic and diastolic blood pressures, heart rate were measured and blood samples were collected. In order to analyze heart rate, systolic and diastolic blood pressures, oxygen saturation between male and female groups Independent Sample Test was used. Paired sample test was used to compare 1st and 5 th day data. Linear regression analyses were used to analyze micronucleus frequency between male and female students.

Results: In the first day and fifth day no significantly difference was observed before and after exercise in micronucleus frequency (p>0.,05). However after 5 days exercise at high altitude, micronucleus frequencies when compared to 1st day pre and post exercise micronucleus frequencies showed very significant increase (p<0.001). In the first day no statistically significant difference was observed before and after exercise in systolic and diastolic blood pressures between male and female groups (p>0.05), oxygen saturation decreased after exercise, heart rate increased after exercise (p<0.,05). In the fifth day between male and female groups systolic and diastolic blood pressures, heart rate and oxygen saturation showed no significant difference compared to 1st day (p > 0.05), after exercise in female group in 5th day systolic blood pressure and oxygen saturation increased compared to 1st day post exercise period (p<0.05), in male group 5th day systolic blood pressures and heart rate increased compared to 1st day post exercise period (p<0.05).

Discussion and conclusion: Results of our study clearly shows that high altitude causes DNA damage and may have mutagenic effects.

Keywords: High altitude, hypoxia, DNA damage, micronucleus.

Introduction

Intense and tiring sports like mountain and nature sports has important systemic and local acute effects on humans (P. Moller et al., 2005; J.A. Jefferson et al., 2004). Although due to lack of oxygen and low oxygen demand, production of reactive oxygen derivatives expected to be low, high altitude exposure (due to reactive oxygen derivatives production and changes in antioxidant activity) may cause oxidative damage. (P. Moller et al., 2005; J.A. Jefferson et al., 2004; Z. Radak et al., 2000). Although reactive oxygen derivatives have important role in regulating normal physical activities such as muscle contraction dramatic increase in their concentration may damage normal cell function, biomolecules (proteins and lipids) and celluler DNA (H. Orhan et al., 2000, R.J. Bloomer et al., 2006). Micronucleus formation is accepted as an indicator for DNA damage. Measurement of micronucleus frequency in peripheral blood lymphocytes is for evaluation genome instability and a common method testing mutagenicity (A. Harman et al., 1997; M. Fenech, 2006, Z. Hamurcu et al., 2005).

There is no study in scientific literature for effects of high altitude under hypoxic conditions on micronucleus frequency during exercise. Because of this reason, by analysing frequency of micronucleus at high altitude in mitogen induced lymphocytes, if high altitude hypoxia or exercise at high altitude have mutagenic effect or not is expected to come to conclusion.

Experimental Methods. Subjects: Our study was included volunteers from our school. Their mean age were 23,35±1,66, mean height 168,20±7,32 cm, mean body mass 60,05±8,76 kg, mean body mass index 21,12±2,17 kg/m^2, 10 female, 10 male students were included in the study. There were no significant difference were present between their age and physical condition.

Exercise Program. Volunteers involved in the study were moved from 1055m. to 2200m. at Mount Erciyes and stayed at the mountain hut for 5 days. Before exercise their blood samples were collected and they did basic interval ski exercise for 3 hours between 2200m. and 2500m. and kept their heart rthym between 140-160 beat/minute. After exercise also their blood samples were collected and same procedure was repeated during 5 days.

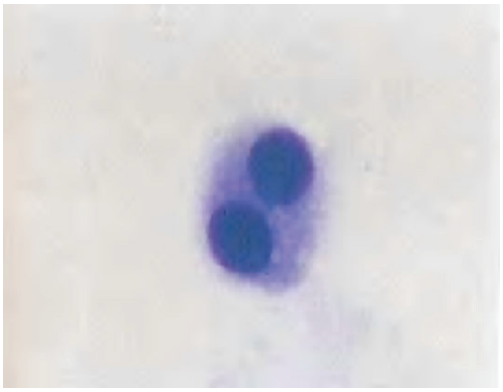
Whole-blood cultures for human lymphocytes

Heparinized 3 ml blood samples were taken after informed consent had been obtained from volunteers at 1st and 5th day before and after exercise. Approximately 0.4 ml of whole blood samples was cultured for 72 hours at 37 ºC in 5 ml of the Peripheral Blood Caryotyping Medium that was supplemented with 1.5 % phytohemagglutinin-M to stimulate the T-lymphocytes (all from Biological Industries, Kibutz Beit Haemek, Israel). To determine intra-individual differences, two parallel cultures of each person were made (M. Fenech, 2006; M. Fenech, 2008)

Micronucleus assay

At 44 hours of incubation, 3 µg/ml (final concentration) cytochalasin-B (Sigma-Aldrich Co, St. Lois, MO, USA) was added to cultures in order to block cytokinesis, according to the method of Fenech and Morley [20]. The cultures were stopped at 72 hours, treated with hypotonic solution (0.1 M KCl) for 4 minutes and fixed in two changes of methanol-acetic-acid (3:1) [21]. The fixed cells were spread onto glass slides and stained with 5% Giemsa for 10 minutes (M. Fenech, 2008; M. Fenech, A.A. Morley, 1985; M. Fenech, A.A. Morley, 1986; M. Fenech, 1980)

Published criteria for micronuclei determinations were followed [22] and for each subject at least 1000 binucleated cells were analyzed.



3.1. Binuclear Cell

3.2. A micronucleus in a Cytokinesis Inhibited Binuclear Cell

Statıstical Analysıs

Statistical analysis of micronucleus frequency from volunteers before and after exercise in 1st and 5th days were done using T-test.

Results

In high altitude at 1st day before and after 3 hours exercise there were no statistically significant difference in micronucleus frequency were found. (p>0.05, Table1). 5th day before and after 3 hours exercise there were no statistically significant difference in micronucleus frequency were found (p>0.05, Table 1). 5th day before and after exercise micronucleus frequency when compared to 1st day before and after exercise values were increased significantly (p<0,001, Table 1).

Table 1. Micronucleus Frequencies

n=20	1st Day	5th Day	p	t
Before Exercise Micronucleus (%) Mean ±SD	0.84±0.088	2.21±0.62	0.001*	7.029
After Exercise Micronucleus E.S. MN (%) Mean ±SD	0.99±0.11	2.07±0.60	0.001*	8.435

*p<0.001

Results and discussion

Due to deep respiration, increased heart rate, circulating red blood cells and hemoglobin concentration camping in high altitude is a training method for professional athletes. However, at high altitude due to hypoxia although reactive oxygen derivatives production expected would be low, recent studies have shown oxidative stress is related to high altitude and oxidative stress increases with high altitude (C. Lundby et al., 2003; J.A. Jefferson et al., 2004). Besides hypoxia at high altitude, intense UV light and environmental factors such as cold climate triggers oxidative stress and cellular macromolecules such as proteins, lipids and damage to DNA reported. (C. Lundby et al., 2003; J.A. Jefferson et al., 2004; M.C. Schmidt et al., 2002). Micronucleus is formed due to misrepaired or unrepaired DNA anomalies and defects of chromosomes during cell division (M.A. Kayani, J.M. Parry, 2008). Micronucleus formatin is triggered by oxidative stress, defects during cell cycle and defects of DNA repair genes. (S. Bonassi et al., 2006; C. Schiffl, C. Zieres, H. Zankl, 1997). We have observed an increase in micronucleus formation at moderate altitude (2200-2500m) during a ski training camp at 5th day when compared to 1st day. Effects of high altitude on DNA of various cells shown an increase in broken DNA strands. (C. Lundby et al., 2005; P. Moller et al., 2001). In our study we did not study breaks in DNA strands but at high altitude increase in micronucleus frequency may be due to increase in broken DNA strands. (M.C. Schmidt et al., 2002, C. Lundby et al., 2005; P. Moller et al., 2001).

We have observed an increase in micronucleus frequency after 5 days exercise at high altitude. Reason for this increase whether due to exercise or high altitude is not known. Further studies are encouraged in order to determine the reason for the increase

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