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INTERMITTENT EXERCISE IN HYPOXIA MODULATES BOTH CYTOKINES LEVELS AND AEROBIC PERFORMANCE

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ABSTRACT

Although acclimatization to intermittent hypoxia (IH) ameliorate exercise performance by increasing oxygen delivery and usage, the effect of high intensity interval intermittent innormobaric hypoxic chamber on platelet-leukocyte interaction and inflammation-related cytokine secretion induced by exhaustion exercise remain indecipherable. The aim of this investigation is to compare the effect of high intensity interval training in hypoxia (O_2: 14%) and normoxic (O_2: 21%) conditions on pre-inflammatory cytokines (IL-6, TNF-α, IL-1β) and blood cells in response to exhaustion exercise.

16 male athletes were divided up randomly into 2 groups (hypoxic and normoxic groups). They did exhaustive exercise (ergometric cycle) and blood samples were taken before and after the exercise. These 16 athletes carry out Ergometer maximum work rate (Wmax) in 12 sessions during two weeks. Once more, afterward two weeks, all athletes experienced exhaustive exercise before and after which blood samples were taken from them. IL-6, TNF-

α, IL-1β, hemoglobin, leukocytes, (eosinophil, lymphocyte, monocyte and neutrophil) were evaluated in the blood samples and analyzed by SPSS software (version 19).

This study demonstrated, exhaustion exercise increased IL-1β , IL-6 leukocyte concentration in plasma before IH intervention but after 2 weeks high intensity interval training in hypoxic condition significantly increased IL-6 (53.5%) and caused a 5.1% drop in IL-1β in response to exhaustive exercise but did not change serum TNF-α level compared to normoxic condition. Training in hypoxic condition leads to insignificant diminished in lymphocytes, monocytes and neutrophils count. On the other hand, exhaustive exercise in normoxia caused 18.3% increase in eosinophil count while in hypoxic condition it caused a 3.6% diminution which was neither significant.

Present data indicated that high intensity interval training in hypoxic condition down influences pro-inflammatory IL-1β secretion and eosinophil count in response to exhaustion exercise. Moreover, IH simultaneously increases circulatory anti-inflammatory IL-6 concentrations. These findings can help to develop effective IH regimens that amend aerobic fitness and minimize risk of thromboinflammation.

Keywords: Hypoxia, Exhaustive Training, Cytokines, Blood Cells

INTRODUCTION

Exercise is linked with various stressors various metabolic disturbance, heighten temperature, higher concentration of reactive oxygen species (ROS), mechanical damage, and hormonal change **[1]**.

Strenuous exercise induces immunologic changes including expelling of interleukins, acute phase proteins, increased activity of dissimilar subtypes of leukocytes, pre- and anti-inflammatory cytokines. It causes a mid-inflammatory state **[2]**.

Some sport scientists conceived that extreme and continuous physical activity heightens free radicals which lead to cell injury and aging **[3]**. Cytokine yield is induced by tissue damage incurred by physical activity or increased reactive oxygen **[4]**. This tissue damage heighten inflammatory cascade. Initially TNF-α and IL-1β are released, so they start the inflammatory response and stimulate the release of IL-6 **[5]**. Physical activity, particularly if it is of extreme type, could induce inflammation since it increases inflammatory mediators (IL-1, IL-6, and CRP, creatinine kinase), and Delayed Onset Muscle Soreness (DOMS). IL-6 is mostly synthesized and freed to circulation by contracting muscle fibers during exercise. Several studies have bringed out a negative connection between amount of regular activity and basal plasma concentration of

IL-6 **[6]**. today athletes take part into strenuous exercises and extreme continuous training programs to hit their peak. They have dissimilar training programs to get there, like training in intermittent hypoxic (IH) condition. The chief reason of using IH method is based on the theory that adaptation to one type of stress assists coping with other forms of stress **[7, 8]**. Moreover, IH can enhance pulmonary ventilation capacity **[9]** and also hemoglobin, mitochondrial oxidative capacity, capillary density. It also raises erythropoietin **[10]**.

Hypoxia mostly takes place at high attitude where air O_2 content is low. In such conditions O_2 pressure of inhaled air (paO_2) is partly reduced and consequently alveolar O_2 pressure, arterial O_2 pressure and arterial O_2 content is decrease. Lowered blood O_2 content alters homeostasis and causes specific changes in body **[11]**. High attitude is a stressor that affects physiologic function of the body and it could ameliorate aerobic capacity of the athletes **[12]**.

The available data show that neuroendocrine can alter immune system function **[13-16]**. It is shown that continuous exercise in IH enhances aerobic capacity and it does not damage mucosal immune system **[17]**. The effect of IH at rest for one hour per day on eosinophils, neutrophils and cytokines in response to strenuous exercise was studied in 2007 **[18]**. The results demonstrated that 8 weeks of IH condition at rest could depressed eosinophil activity, neutrophils, IL-1β, TNF-α and lipid per-oxidation in response to exhaustive exercises which finally conducts to inhibition of thrombosis and inflammation caused by exhaustive exercise. Some inflammatory cytokines might gain platelet activity which could lead to enhanced adherence to leukocytes **[18]**.

The aim of this investigation is to examine the effect of strenuous exercise in intermittent hypoxia on pre-inflammatory cytokines (IL-6, TNF-α, IL-1β) and blood cells in hypoxic (O_2:14%) and normoxic (O_2:21%) conditions.

SUBJECTS AND METHODS

16 athletes with at least a 2-years history of activity in sports participated in this study voluntarily; those who smoked cigarettes, applied supplements or took any medication were omitted from the study.

In order compile the data a demographic form was formulate and filled out. it included age and anthropometric features. Weight was measured by a digital scale (100 grams accuracy) with minimum clothes and barefoot, height was measured by a wooden tape-meter with accuracy of 0.1 cm.

Participant's body composition was assayed on the first day at 7:00 a.m. while fasting

using bio-electric resistance system (made in South Korea). After a 30min rest they performed the exhaustive test. Using this test W_{max} was determined for the participants and they were arbitrarily divided to two groups (hypoxia and normoxia groups). Blood samples were taken before and immediately afterward the test winding up. Then the participants had to take a 48hr rest period. Thereafter, 12 sessions of high intensity interval training was started in 2 weeks.

After the two-week high intensity interval training period was finished, all the participants had a rest period of 48hr and then they underwent the same exhaustive test the same as the first day **(Figure 1)**.

Training Programme

The training programme comprise of 40 min of cycling exercise occurring six times per week for 2 weeks. All subjects exercised on the same mechanically braked cycle ergometer model (860E, Monark, Sweden). The HT group trained in a normobaric hypoxic chamber (O_2:14%) and had an air refreshment rate of 1000 l.min71. The normoxi group performed the same training protocol in the laboratory at (O_2:21%). conditions. The temperature in both conditions throughout training was approximately 21C. Each training session consisted of 5-min warm up, ten 1-min bouts at 80%Wmax separated by 2-min active recovery at 50% Wmax and 5-min cool down. The individual training intensities were heightened by 5% after six training sessions.

Exhaustive Test

(Maximal O2 consumption ($V^{\cdot}$ O2 max) and maximum work load were assessed on an electronically braked cycle ergometer (monark 1860). Minute ventilation, O2 uptake, and CO2 production were continuously monitored via open-circuit spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT). Heart rate was appraised unremittingly (Accurex Plus, Polar Electro, Woodbury). The test began with a 3-min warm-up at 75 W. After the warm-up, the workload was increased 25 W every minute until volitional fatigue. Subjects were verbally encouraged to keep on for as long as possible. The criterion used to assess VO2 max included 1) a heart rate in excess of 90% of age predicted maximum (220 _ age), 2) a respiratory exchange ratio of _1.10, and 3) identification of a plateau (_150 ml increase) in O2 uptake despite a further increase in workload. In all tests, at least two of three criteria were met**)** and The highest work intensity that could be executed for 1 min during the test was called Wmax and was used in the calculation of relative workloads for the training programme.

Blood samples were collected from a peripheral vein in forearm with a plastic syringe having a metal needle, then slowly poured (to prevent hemolysis) in a TDA tube and sealed with parafilm. Four blood samples were taken from each person, before and after the first and the last exhaustive tests.

Each sample was centrifuged at 10000 rpm for 30 minutes using laboratory centrifuge U-32or (made in Germany). Complete Blood Count of each athlete was analyzed at Sophia Laboratory by Sysmex XT-1800i Hematology Analyzer. Interleukin levels of the participants were measured by ELISA Reader (Radim, made in Italy) with an accuracy of 90%, using RayBio® sandwich ELISA kits (made in USA).

Serum level of TNF-α, IL-1β, and IL-6 were measured; blood neutrophils, eosinophils, lymphocytes, and monocytes were counted.

Statistical analyses were carried out using SPSS software for Windows, version 19 (SPSS, Chicago, IL, USA). Data is expressed as means ± SD. Statistical analysis was determined using Mann-Whitney Test and Wilcoxon Test. Significant level was consented when P-values were less than 0.05.

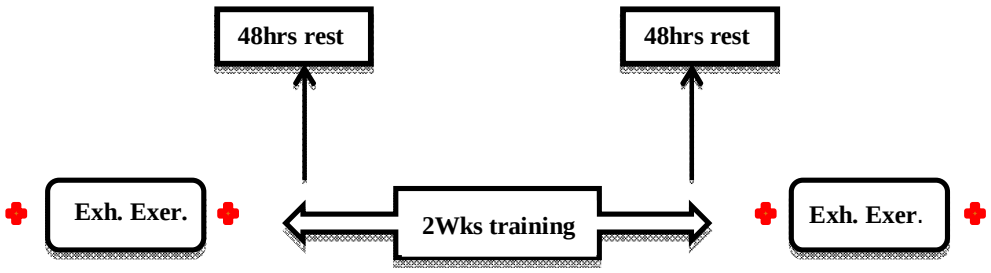

Figure 1: Study Design Diagram: Each Group Underwent Two Exhaustive Exercises (Exh. Exer.) And a Two-Week-Period of Training (2Wks Training) in Normoxic and Hypoxic Conditions. (: Blood Sampling)

RESULTS

16 athletes were randomly allocated to two normoxic and hypoxic conditions, mean ages of who were 23.14±2.85 and 23.38±2.85, respectively **(Table 1)**. In **Table 2** serum level of interleukins and blood cell counts are expressed as mean ± SD. The results revealed that there was no significant difference between TNF-α ($p \geq 0.05$) before and after exercise in either group.

There was a 5.1% reduction in IL-1β level after the trainings in hypoxic group, though not significant ($p \geq 0.05$), however there was a 53.5% increase in IL-6 level in hypoxic group after the training, which was significantly higher than values obtained before the training program ($p < 0.05$).

In hypoxic group eosinophil count had a 3.6% increase while normoxic group had an 18.3% elevation in response to exhaustive exercise; however these two groups should not be compared since they had significant difference based on eosinophil count before entering the training program. This study also exposed that training at hypoxic condition causes insignificant reduction in lymphocytes, monocytes, and neutrophils; there was no difference in cell counts between hypoxic and normoxic groups, after exhaustive exercise ($p \geq 0.05$).

Training in hypoxic and normoxic conditions caused no significant change in hemoglobin; or in RBC count. There was a 0.9% increase in hematocrit in hypoxic group after the training which was neither significant ($p \geq 0.05$).

Table 1: Anthropometric Data of Participants in Each Group (Mean±SD)

Variable Groups	Height (cm)	Weight (kg)	Age (year)	Subcutaneous Fat (%)	W_{max}
Hypoxia	**176.21 ± 4.06**	**75.89 ± 9.14**	**23.14 ± 2.85**	**20.14 ± 4.46**	**262.85 ± 18.89**
Normoxia	**179.81 ± 6.91**	**75.89 ± 9.14**	**23.38 ± 2.85**	**19.39 ± 2.84**	**255.00 ± 20.00**

Table 2: Interleukin Serum Levels and Blood Cell Counts of the Participants in Normoxic and Hypoxic Groups (Mean ± SD)

Groups Variables	Hypoxia				Normoxia			
	1st Exhaustive Exercise		2nd Exhaustive Exercise		1st Exhaustive Exercise		2nd Exhaustive Exercise	
	Before	After	Before	After	Before	After	Before	After
RBC (10^6/dl)	6.17 ± 0.29	6.58 ± 0.39	5.99 ± 0.27	6.36 ± 0.24	5.64 ± 0.42	6.04 ± 0.40	6.53 ± 0.49	7.02 ± 0.46
Hemoglobin (g/dl)	17.08 ± 0.65	18.23 ± 0.72	16.75 ± 0.81	17.73 ± 0.96	17.01 ± 0.92	18.27 ± 0.72	17.36 ± 0.74	18.67 ± 0.75
Hematocrit (%)	49.93 ± 1.35	53.9 ± 1.59	50.08 ± 2.52	54.16 ± 3.01	48.22 ± 3.60	51.47 ± 2.23	50.90 ± 2.17	55.69 ± 3.09
Eosinophil (10^3/dl)	0.1550 ± 0.07	0.1875 ± 0.09	0.1587 ± 0.93	0.2200 ± 0.13	0.1210 ± 0.03	0.1812 ± 0.06	0.0975 ± 0.03	0.1462 ± 0.05
Neutrophil (10^3/dl)	2.965 ± 0.55	3.950 ± 1.30	3.354 ± 0.77	4.141 ± 0.92	2.763 ± 0.54	0.692 ± 0.89	2.683 ± 0.93	3.806 ± 0.87
Lymphocyte (10^3/dl)	2.483 ± 0.66	2.483 ± 0.83	2.116 ± 0.42	4.207 ± 0.93	2.143 ± 0.31	2.523 ± 0.42	1.866 ± 0.46	3.920 ± 0.55
Monocyte(10^3/dl)	0.0652 ± 0.14	1.0910 ± 0.23	0.5680 ± 0.15	0.9480 ± 0.21	0.5620 ± 0.09	0.9510 ± 0.24	0.4070 ± 0.13	0.8060 ± 0.13
TNF-α (Pg/ml)	1.22 ± 0.191	1.27 ± 0.254	1.17 ± 0.254	1.19 ± 0.312	1.26 ± 0.140	1.32 ± 0.135	1.41 ± 0.166	1.443 ± 0.175
IL-1β (Pg/ml)	1.88 ± 0.23	2.16 ± 0.28	1.82 ± 0.28	1.88 ± 0.26	1.81 ± 0.20	2.24 ± 0.24	1.55 ± 0.10	1.92 ± 0.32
IL-6 (Pg/ml)	2.21 ± 0.35	2.25 ± 0.38	2.17 ± 0.22	2.45 ± 0.33	2.27 ± 0.20	2.29 ± 0.19	2.11 ± 0.08	2.22 ± 0.01

DISCUSSION

To clear the relationship between intermittent hypoxia and immune system, this investigation was designed to study the effect of high intensity interval trainingat intermittent hypoxia on pre-inflammatory

cytokines (IL-6, TNF-α, IL-1β) and blood cellsin response to exhaustive exercise.

Our study indicated thathigh intensity interval training at intermittent hypoxia reduces lymphocytes, neutrophils, and monocytesin response to exhaustive exercise. There was a minimal increase in serum IL-1β level; however it did not alter TNF-α level and it increased IL-6 level.

Previous studies have shown that restinintermittent hypoxic condition could increase IL-6 **[6, 18, 19]**. Another study showed no difference in levels of IL-6 and IL-10 at simulated attitudes **[12]**; one of these studies indicated that after 8 weeks of aerobic fitness eosinophil, IL-1β, and platelet-related thrombosis had decreased while serum levels of Il-6 and Il-10 had increased **[18]**. Our study revealed that exhaustive exercise in hypoxia could increase IL-1β and IL-6.

Eosinophilic reaction is the main cause of allergic diseases **[20]**. Not only eosinophils have a role in bacterial infection but also they have part in respiratory distress syndrome and also asthma **[21]**. Stimulated eosinophils and neutrophils produce oxygen metabolites as super-oxidase **[22]** which work as disinfectant and also cause damage to the inflamed area.

The above mentioned data is in concordance with results of this study about exhastion exercise (with gradual increase of strength up to exhaustion at $VO_{2max}$and w_{max}). Extreme exercise increases eosinophil-platelet aggregation with shear forces and inflammatory factors like N-formyl-methionyl-leucyl-phenylalanine (fMLP) and lipopolysaccharide (LPS) **[22]**. Heterotypic attaching reactions are increased with exercise and could enhance inflammation and thrombosis formation in microcirculation. Previous data show that oxidative stress increases attachment of leukocytes to platelets and enhances heterotypic cell aggregation capacity to cope with shearing force of blood flow in pathologic and physiologic conditions **[22]**.

It is well known that blood has oxidative stress in hypoxia **[22]** that produces free radicals which causes rapid inflammatory response in microcirculation, leading to increased migration of endothelial leukocytes and vascular permeability **[23]**. Yet, adaptation to long term hypoxia causes production of more anti-oxidants **[24, 25]**. Systemic inflammation includes phagocytosis and atherosclerosis and other cardiovascular diseases **[26]**. Several studies have shown that some cytokines like IL-1β enhance platelet's activity and their capacity to attach to leukocytes **[27-29]**. Strenuous, resistive or eccentric exercise could increase IL-1β in muscles. IL-1β has no role in aerobic physical activity (30- 32), the concept of which we also observed in

current study. Exhaustive physical activity increases IL-1β and IL-6 and formation of eosinophil-platelet aggregation in shearing stress. Taking extreme continuous exercise (in hypoxic condition, O_2: 14%, for two weeks, six sessions at each), increases IL-6, RBC, hemoglobin and IL-1β. Since intermittent hypoxia increases IL-6 and IL-10, both anti-inflammatory cytokines, pre-inflammatory cytokines and eosiniphil aggregation might be decreased in response to exhaustionexercise.

Hypoxic condition could increase IL-6 **[19, 30-33]**, which might be caused by catecholamines that can stimulate the production and secretion of IL-6 into plasma **[13, 15, 16]**. In mice epinephrine secretion could increase IL-6 level **[13, 16]**. B-adrenergic pathway might mainly stimulate production of IL-6 at hypoxic conditions.

Recent studies have shown that exposure to attitude alters immune system by a strong α- and β-adrenergic component. The β-adrenergic pathway elevates serum level of IL-6 primarily and then the α-adrenergic component keeps it high for several weeks **[14]**. Moreover the increase in IL-6 (via hypoxia induced factor-1, HIF-1) might enhance angiogenesis or production of vascular endothelial growth factor (VEGF) which also increases vascular permeability **[34]**. One important role of IL-6 is to decrease or inhibit the production of pre-inflammatory cytokines like TNF-α and IL-1β in response to exhaustive exercise; IL-6 increases IL-1ra which is an inhibitor of production of pre-inflammatory cytokines **[17, 21, 33]**. In our study IL-6 was increased in hypoxic group but there was no drop in IL-1β serum level.

It is previously indicated that combined hypoxia and exercise might suppress immune system at least in short term **[19]**. A single 30-second-period of maximal exercise is sufficient to induce muscle damage **[35]**.

The present study showed thathigh intensity interval trainingin intermittent hypoxia condition can increase anti-inflammatory cytokines and decreaseeosinophil and IL-1β aggregation in healthy subjects,

In conclusion,high intensity interval training in hypoxic condition compare normoxic for 2 wk improved the aerobic fitness of subjects by enhancing RBC and hemoglobin. Moreover, the training in hypoxic conditioncan also simultaneously suppress eosinophil- and platelet-related thrombosis andproinflammatory cytokine IL-1β production caused by exhastion exercise. Additionally, hypoxic condition is associated with increased contents of anti-inflammatory cytokines IL-6 in circulation. These experimental findings can help to determine effective IH regimens to increase aerobic capacity and minimize the risk of

inflammatory and thrombotic disorders associated with exhaustion exercise.

As in numerous other investigations, one limitation of the present work is that the subjects used tended to be young and healthy, and thus further clinical evidence is required to extrapolate the present results to patients with abnormal or diseased cardiovascular systems.

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