ABSTRACT

Aim: though modification to intermittent hypoxia (IH) improve exercise performance by increasing oxygen delivery and utilization, the effectuate of high intensity interval intermittent in normobaric hypoxic chamber on CBC interaction induced by exhaustion exercise remain undecipherable. The goal of this investigation is to compare the effect of high intensity interval training in hypoxic (O₂:14%) and normoxic (O₂:21%) conditions on CBC, lactate thresholds, WVO₂max, WLT and blood cells in response to exhausting exercise.

16 male athletes were shared randomly into 2 groups (hypoxic and normoxic groups). They did exhaustive exercise (ergometric cycle) and blood samples were taken before and after the exhaustive exercise. These 16 athletes accomplish 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. Hemoglobin, leukocytes, (eosinophil, lymphocyte, monocyte and neutrophil) lactate thresholds, WVO₂max, WLT and blood cells were evaluated and analyzed by SPSS software (version 19).

There were no significant differences between the increases in any of the above-mentioned performance parameters in either training environment (p≤ 0.05). In addition, neither hemoglobin concentration nor hematocrits were significantly changed in no groups (p≤0.05). It is concluded that acute exposure of moderately trained subjects to normobaric hypoxia during a short-term training program consisting of moderate to high-intensity intermittent exercise has no enhanced effect on the degree of improvement in neither aerobic nor anaerobic performance. These data suggest that if there are any advantages to training in hypoxia for sea level performance, they would not arise from the short-term protocol employed in the present study.

Keywords: Normoxia, Hypoxia, Altitude, CBC, WLT, VO₂max.

INTRODUCTION

The conception of training at altitude to ameliorate sea level performance is now an accomplished and highly researched area within the sport and exercise sciences. However, no unequivocal evidence exists to propose any of the altitude training strategies about 30 years of research (i.e. live high–train low, constant exposure or intermittent hypoxic training (IHT)) can amend sea level functioning over and above that of equivalent weight sea level training12. Exercise is linked with diverse stressors various metabolic disturbance, heighten temperature, higher concentration of reactive oxygen species (ROS), mechanical damage, and hormonal changes1. It is thought that the stress of hypoxic exposure, in add-on to training stress, will compound the training adjustments experienced with normal endurance training, which, in turn, will lead to heavier improvements in performance13. 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 state2. Some sport scientists conceived that extreme and continuous physical activity heightens free radicals which lead to cell injury and aging3. Cytokine yield is induced by tissue damage incurred by physical activity or increased reactive oxygen4. 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-65 Physical activity,
Participants had to take a 48 hr rest period. Thereafter, 12 athletes took 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. Moreover, IH can enhance pulmonary ventilation capacity and alters homeostasis and causes specific changes in body. High attitude is a stressor that affects physiological function of athletes and can alter immune system function. It is shown that continuous exercise in IH enhances aerobic capacity and it does not damage mucosal immune system. 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. 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.

The aim of this investigation is to examine the effect of strenuous exercise in intermittent hypoxia on leukocyte-platelet accumulation, lactate thresholds, WVo2 max, WLT and blood cells via exercise training in hypoxia hypoxic (O2:14%) and normoxic (O2:21%) conditions.

### MATERIALS AND METHODS

16 athletes with at least a 2-years account of activity in sports took part in this study voluntarily; those who smoked cigarettes, applied supplements or took any medication were missed from the study. In order collect 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.

<table>
<thead>
<tr>
<th>Variable Groups</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Age (year)</th>
<th>Subcutaneous fat (%)</th>
<th>Wmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>176.21 ± 4.06</td>
<td>75.89 ± 9.14</td>
<td>23.14 ± 2.85</td>
<td>20.14 ± 4.46</td>
<td>262.85 ± 18.89</td>
</tr>
<tr>
<td>Normoxia</td>
<td>179.81 ± 6.91</td>
<td>75.89 ± 9.14</td>
<td>23.38 ± 2.85</td>
<td>19.39 ± 2.84</td>
<td>255.00 ± 20.00</td>
</tr>
</tbody>
</table>

Participant's body composition was attempted on the first day at 7:00 a.m. while fasting using bio-electric resistance system (made in South Korea). After a 30 min rest they performed the exhaustive test. Using this test Wmax was determined for the participants and they were at random divided to two groups (hypoxia and normoxia groups). Blood samples were taken before and immediately afterward the test finishing. Then the participants had to take a 48 hr 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 48 hr and then they experienced the same exhaustive test the same as the first day (fig.1).

**Training program:**

The training program contain 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 (O2:14%) and had an air refreshment rate of 1000 l.min71. The normoxi group performed the same training protocol in the laboratory at (O2:21%) conditions. The temperature in both atmospheric condition during training was about 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 person training intensities were compounded by 5% after six training sessions.

**Exhaustive test:**

(Maximal O2 consumption (V O2 max) and maximum work load were measured on an electronically braked cycle ergometer (monark 1860)). Minute ventilation, O2 uptake, and CO2 production were incessantly monitored via open-circuit spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT). Heart rate was assessed always (Accurex Plus, Polar Electro, Woodbury). The test began with a 3-min warm-up at 75 W. After the warm-up, the workload was enhanced 25 W every minute until volitional fatigue. Subjects were verbally recommended to keep on for as long as possible. The criterion used to measure VO2 max included 1) a heart rate in excess of 90% of age anticipated maximum (220 – age), 2) a respiratory exchange ratio of ≥1.10, and 3) recognition 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 carried out for 1 min during the test was called Wmax and was used in the computation of relative workloads for the training program.

-722630168275Blood sample test pre00Blood sample test pre180340168275blood sample test post00blood sample test post498919574295blood sample test post413512057785blood sample test pre00blood sample test pre378841033147048 rest0048 rest4478019166500-93981149860003808730567055029527501504956 sessions training in hypoxia006 sessions training in hypoxia11366504810695060038533655048 rest0048 rest1483360704856 sessions training in hypoxia006 sessions training in hypoxia52406542159000479869564262006521441651000012738102006600127381019621500-331470371475Exhaustion test00Exhaustion test241173091440003846195316250041052751422400029667202000256 sessions training in normoxia006 sessions training in normoxia11512552997200820420172000014738351974856 sessions training in normoxia006 sessions training in normoxia4312285535305Exhaustion test00Exhaustion test261683439179500207645127000-17995032258024 rest0024 rest

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).

Blood samples were gathered from a peripheral vein in forearm with a plastic syringe bearing 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 examined at Sophia Laboratory by Sysmex XT-1800i Hematology Analyzer. 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.

RESULTS

16 athletes were randomly apportioned to two normoxic and hypoxic conditions, mean ages of who were 23.14±2.85 and 23.38±2.85, severally (table 1). In table 2 serum level of blood cell counts are expressed as mean ± SD.

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

<table>
<thead>
<tr>
<th>Groups/ Variables</th>
<th>Hypoxia</th>
<th>Normoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st exhaustive exercise</td>
<td>2nd exhaustive exercise</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>16.58±0.65</td>
<td>17.73±0.72</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>49.93±1.35</td>
<td>53.9±1.59</td>
</tr>
<tr>
<td>Eosinophil (10³/dl)</td>
<td>0.155±0.07</td>
<td>0.187±0.09</td>
</tr>
<tr>
<td>Neutrophil (10³/dl)</td>
<td>2.965±0.55</td>
<td>4.2±1.3</td>
</tr>
<tr>
<td>Lymphocyte (10³/dl)</td>
<td>2.483±0.66</td>
<td>4.43±0.83</td>
</tr>
<tr>
<td>Monocyte(10³/dl)</td>
<td>0.652±0.14</td>
<td>1.091±0.23</td>
</tr>
</tbody>
</table>

Table 2: Interleukin serum levels and blood cell counts of the participants in normoxic and hypoxic groups (Mean ± SD)
Also it was observed that high interval training led to significant increase in hemoglobin, hematocrit level, VO$_2$max ($t_{14} = 0.398, P = 0.397$), wvO$_2$max ($t_{14} = 1.000, P = 0.334$) and Wmax ($t_{14} = 1.161, P = 0.872$), also the training caused an elevation in Wlt, VE max. Nevertheless hypoxia condition led to significant increase in hemoglobin, hematocrit level, VO$_2$max, WVO$_2$max and Wmax.

**DISCUSSION**

The objective of the present study was to determine whether short-term IHT would increase sea level aerobic and modulate leucocyte-aggressions and and blood cells in response to exhaustive exercise over and higher up that happening with equivalent sea level training. It was showed that 2 weeks of moderate- to high intensity IHT (i.e. intensities corresponding to 80 –85% Wmax) in moderately trained subjects led in like increases in aerobic performance when equated to equivalent sea level training. Specifically, VO$_2$max, WvO$_2$max and Wmax, Wlt, VE max., exhibited similar increases in response to hypoxic and sea level training. The increase in VO$_2$max and Wmax arising from training was not increased by acute exposure to normobaric hypoxia, in agreement with previous studies (e.g. Terrados et al. 1988, Levine et al. 1992, Engfred et al. 1994, Emonson et al. 1997, Geiser et al. 2001, Masuda et al. 2001). Of the previously cited research, Levine et al. (1992), Engfred et al. (1994) and Geiser et al. (2001) used protocols that were most like to that of the present design, whereas training was executed at the same absolute intensity across. It therefore appears that repeated short-term exposures to hypoxia during acute physical training does not significantly lead to the mechanisms responsible for the improvements in aerobic performance detected with sea level endurance training.

Also our study proposed that high intensity interval training at intermittent hypoxia reduction lymphocytes, neutrophils, and monocytes in response to exhaustive exercise. Also it was noted that high interval training led to significant enhance in hemoglobin, hematocrit level, vo2max, wvO2max and wmax, also the training caused an elevation in WLT, VE max. Nevertheless hypoxia condition led to significant increase in hemoglobin, hematocrit level, vo2max, wvO2max and wmax. Previous study manifested indicated that after 8 weeks of aerobic fitness eosinophil, IL-1β, and platelet-related thrombosis had reduced although serum levels of IL-6 and IL-10 had increased. Our study revealed that exhaustive exercise in hypoxia could increase neutrophils and neutrophils.

Eosinophilic reaction is the main cause of allergic diseases. Not only eosinophils have a role in bacterial transmission but also they have part in respiratory distress syndrome and also asthma. Stimulated eosinophils and neutrophils produce oxygen metabolites as super-oxidase 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 exhaustion exercise (with gradual increase of strength up to exhaustion at VO$_2$ max and Wmax). Extreme exercise increases eosinophil-platelet aggregation with shear forces and inflammatory factors like N-formyl-methionyl-leucyl-phenylalanine (MLP) and lipopolysaccharide (LPS). Heterotypic attaching responses are increased with exercise and could enhance inflammation and thrombosis formation in microcirculation. Former data show that oxidative stress increases adherence of leucocytes to platelets and enhances heterotypic cell aggregation capacity to cope with shearing force of blood flow in pathologic and physiologic conditions.

It is well known that blood has oxidative stress in hypoxia that develops free radicals which causes rapid inflammatory response in microcirculation, conducting to increased migration of endothelial leucocytes and vascular permeability. Yet, adaptation to long term hypoxia causes production of more anti-oxidants. Systemic inflammation lets inphagocytosis and atherosclerosis and other cardiovascular diseases. Several studies have shown that some cytokines like IL-1β increase platelet’s activity and their capacity to inhere in leucocytes. Strenuous, resistant or eccentric exercise could increase IL-1β in muscles. IL-1β has no role in aerobic physical activity, the conception of which we also observed in current study. Exhaustive physical activity increases 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 eosinophil aggregation, RBC, hemoglobin and neutrophils. Since intermittent hypoxia increases neutrophils and eosinophil aggregation might be decreased in response to exhaustion exercise.

Hypoxic condition could increase IL-6, which might be induced by catecholamines that can stimulate the production and secretion of IL-6 into plasma. In mice epinephrine secretion could increase IL-6 level. B-adrenergic pathway might mainly stimulate production of IL-6 at hypoxic conditions.

**CONCLUSION**

It is ended that short-term moderate- to high-intensity IHT performed 6 times/week for 2 weeks in moderately trained subjects resulted in similar increases in aerobic performance to those occurring with equivalent normoxic training. This is the case, even when training in hypoxia can be achieved at the same absolute intensity as sea level controls. The present data propose that if there are any advantages to training in hypoxia for sea level performance they would not arise from the short-term training protocol used in the present study. But The present study showed that high intensity interval training in intermittent hypoxia condition can decrease eosinophil-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 condition can also simultaneously suppress eosinophil- and platelet-related thrombosis caused by exhaustion exercise. These experimental findings can help to determine effective IH regimens to increase aerobic performance and minimize the risk of inflammatory and thrombotic disorders associated with exhaustion exercise. These experimental findings can help to determine effective IH regimens to increase aerobic capacity and minimize the risk of inflammatory and...
thrombotic troubles associated with exercise. As in numerous other investigations, one limitation of the present work is that the subjects used attended be young and healthy, and thus further clinical evidence is required to extrapolate the present results to patients with abnormal or diseased cardiovascular systems.

REFERENCES


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