Effects of hypobaric Endurance Training on Graded Exercise Induced Lymphocyte Mobilization, Senescence and Their Surface Thiol Levels in Elite Male Athletes

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DOI: 10.22631/ijaep.v7i1.227

ABSTRACT:

The effects of each hypoxemic exercise session or overall training period still remains to be more elucidate in elite athletes. Therefore, we investigated the effects of hypobaric endurance training on lymphocytes mobilization and senescence and also their surface Thiol levels following to graded exercise test (GXT) in elite male athletes. Forty six volunteer subjects were randomized into normobaric control (NC), hypobaric control (HC), normobaric exercise (NE) and hypobaric exercise (HE) groups. The NE and HE groups were exposed to homelanod (700 mmHg) and 2800 meters above sea level (570 mmHg) simulated barometric pressures respectively, while HC and NC groups were remained sedentary at the same conditions. The training was included on treadmill running for four weeks, five sessions/week, 45 min/session. Each session was consisted of three-min warmed up period, three cycles of 10-min running at 65% maximal heart rate reserve (HRRmax) interspersed with a three-min active recovery and three-min cool-down running period. Two GXTs were performed before (baseline) and after the interventions and blood samples were collected three times at both occasions. In all groups, mobilization of CD8+ lymphocytes and senescent phenotype population of the ir both CD4+ and CD8+ subsets were increased after both GXTs, however; the changes were reversed following to recovery period (P<0.05). Moreover, HE were decreased lymphocytes surface thiol levels before and after the second GXT (P<0.05). It can be concluded that HE has no additional benefits for elite athletes regarded to lymphocytes mobilization and senescence, however; it may render them to oxidative stress.

KEY WORDS: Altitude, Exercise, Lymphocytosis, Cell Aging, Oxidative Stress

INTRODUCTION

Three to four weeks of moderate altitude training is the minimal recommended period required to maximize the competitive performance at sea level [1, 2]. However, during severe hypoxia oxidative stress (OS) occurs in the blood [3] and excessive exposure to OS facilitates the programmed death of lymphocytes[4-7] and causes immune cell senescence[6, 7] which increases the risk of infectious diseases and also autoimmune disorders.

OS-induced lymphocyte apoptosis is also linked to hypoxemic individuals suffering from cardiopulmonary disorders or exposed to hypoxic environments. However, what kind of the exercise strategy under hypoxic condition minimizes lymphocyte dysfunction caused by OS has not yet been established [8].

Acute aerobic exercise sessions can increase the numbers and also phenotypes of lymphocytes during and immediately after exercise and then a lymphocytopenia occurs during the recovery period [9].

The killer cell lectin-like receptor G1 (KLRG1) expression on human T lymphocytes has been identified as a marker of replicative senescence [10, 11], which generally couples with a lower expression of CD28 (its co-stimulatory molecule) for the activation and proliferation
of naïve T cells [11]. Senescent T lymphocytes cannot enter the cell division cycle and this process impairs immune function [10]. Moreover following to an acute bout of exercise, the CD4+ and CD8+ lymphocyte subsets not expressing CD28 (CD28-) can mobilized into the peripheral blood compartment [12, 13]. The higher KLRG1+ and CD28-lymphocytes levels could be increased lymphocyte apoptosis by elevated consumption of body antioxidants in a hypoxic dosage dependent manner [5].

On the other hand, hypoxic exercise training is reported to be capable of reducing senescent T-lymphocyte subsets in young sedentary subjects [14]. In another study, five weeks of high-intensity-interval(HIIT) and moderate intensity-continuous exercise training(MICT) reduced CD4 lymphocyte apoptosis in sedentary males caused by hypoxic exercise [15]. Tsai et al [16] have concluded that exercise training alleviates hypoxia-induced mitochondrial dysfunction in the lymphocytes of sedentary males which seems to could alleviate lymphocyte apoptosis. In another study exercise training with/without hypoxic exposure were effectively alleviates lymphocyte apoptosis induced by oxidative stress following strenuous exercise in sedentary males [8].

However, the clinical significance of these findings needs to be more elucidates in other populations e.g. old subjects, chronic altitude dwellers, elite athletes and cardiopulmonary patients.

Furthermore, although hypoxia can affect lymphocytes surface Thiol molecules level (-SH), as their major antioxidant source [17, 18], far less attention has been directed to this area in well trained athletes. Pylouix et al (2009) demonstrated that thirteen days of ‘live high–train low’ model (training at 1,200 m and living in 2,500–3,000 m simulated altitude) does not affect prooxidant/antioxidant balance in elite swimmers [19]. Six day training on 3160 m also reported to not change prooxidant-antioxidant balance in alpine skiing competitors [20]. Some evidences on the other hand have shown high altitude expedition had harmful effects which were still detectable in the following recovery period [21-24].

It seems the training patterns improving both physical fitness and the resistance to lymphocyte death and senescence induced by moderate hypoxia has not yet been identified. The effects of hypoxic endurance training on acute exercise induced lymphocyte apoptosis still has not elucidated in elite athletes. Therefore, the goal of this study was to clarify the effects of endurance training with or without systemic hypoxia by elite athletes on CD4+ and CD8+ lymphocyte subsets mobilization and senescence and also their surface Thiol levels after acute exercise.

METHODS

The Ethics Committee of Tabriz Islamic Azad University approved the study protocol, which followed institutional guidelines according to the principles of the Declaration of Helsinki. 46 volunteer healthy elite athletes (Table 1) who were nonsmokers, non-medication/vitamin users, participated in this study. No subject had experience high altitude (> 3000 m) 12 months before the experiment. The subjects were randomized into normobaric control (NC, n = 11), hypobaric control (HC, n = 11), normobaric exercise (NE, n = 12) and hypobaric exercise (HE, n = 12) groups, after an informed consent was signed.

The graded exercise test (GXT) was performed by training groups (NE ad HE) at two occasions; 48 hours before (Baseline) and 48 hours after the last training session (post intervention) by the method explained elsewhere [25] and maximal heart rate (HRmax) was determined at maximal work level [26].

The hypobaric groups (HE and HC) were respectively exposed to simulated barometric pressure equal to 2800 meters above sea level (barometric pressure: 570 mmHg) or 730 meters (homeland altitude, barometric pressure: 700 mmHg) within an air conditioned barometric adjustable altitude chamber [14], while training on a treadmill for 4 weeks, 45 min/day, 5 days/week at 16:00 pm. within the altitude chamber(air temperature was maintained at 21 (+1.3)°C and relative humidity at 55 (+6) %) [14]. Subjects warmed up for 3-min at 30% of maximal heart rate reserve (HRmax which was calculated from subtraction of resting heart rate from HRmax at GXT endpoint [26]) before continue to three exercise cycles, each 10-min at 65% HRmax interspersed with a 3-min active recovery at 40% of HRmax [15]. The exercise sessions were terminated by 3-minute cool-down running at 30% of HRmax. Each subject used a heart rate (HR) monitor (Polar: Finland) to obtain the assigned intensity of exercise. The work-rate of running was adjusted continuously to ensure that the intensity of exercise matched the target HR throughout the training period[15].

Moreover a CO2 scrubber apparatus was eliminating air CO2 (63500 ppm) while training [5].

The normobaric groups (NE and NC) were sedentarily experienced the same conditions without physical activity were watching TV or reading newspapers and/or their favorite texts). It should be noted that none of the groups were inquired about the chamber’s barometric pressure in a simple blind order. All participants were instructed to refrain from extra regular exercise until the end of the study.

Table. 1. About here

Arterial O2 saturation (SaO2) level was controlled by a finger pulse oximeter; blood pressure (BP) and heart rate (HR) were monitored by an automatic apparatus (model 412; Quinton).

For safety reasons, it was necessary to terminate the test if the level of O2 saturation reached to 70% or an unordinary discomfort observation in the subjects.
All subjects were instructed to fast for at least 8 hours and to refrain from strenuous physical exercise for at least 48 hours before sampling and were arrived at the testing center at 8:30 AM to eliminate any possible diurnal effect.

Blood samples were collected from an antecubital vein three times [1] resting, 2) immediately after GXT and 3) two hours recovery following GXT] at both baseline and post intervention occasions Blood cell count was analysed using a cell counter (GMI, Inc., Ramsey, MN).

Lymphocytes isolation, determination of their phenotypes and measuring of their surface thio molecules level were done according to the method described elsewhere [5].

Statistical analyze: By using repeated measure ANOVA we tested the effects of exercise training over the time between each of two barometric dosage groups (Hypobaric vs Normobaric, reffered as p value “Barometric Status × Time”) and between the two trial groups (Exercise vs Control, reffered as p value “Activity Type × Time”). Post hoc analysis for within group time effect was applied by using the Bonferroni test. All statistical analyses were conducted at the 95% level of significance.

**STATISTICAL RESULTS**

All the subjects completed the study. Adherences to training were 87.14±4.85% for NE and 91.37±2.41% for HE groups respectively.

CD8+ lymphocytes counts were markedly increased at both baseline and post intervention conditions in all groups in response to GXT (Fig1), which was followed by a rapid lymphocytopenia of both CD4+ and CD8+ subsets during post 2h recovery period (P<0.05). The GXT markedly increased CD28+ (Fig2) and KLRG1+ (Fig3) forms of the both CD4+ and CD8+ subsets mobilization into the peripheral blood compartments in all groups, however, the their count fell below resting values 2 hours after both GXT (P<0.05). Only HE significantly decreased lymphocytes surface Thiol levels at before and after GXT2 occasions (Fig 4) (P<0.05).

**Fig. 1. Blood CD4+ and CD8+ lymphocytes count**
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NC: Normobaric control, HC: Hypobaric control, NE: Normobaric exercise training, HE: Hypobaric exercise training. *P<0.05 before GXT vs. after GXT or after recovery, †P<0.05, after GXT vs. after recovery.

**Fig. 2.** CD4+ and CD8+ subsets expressing CD28

**Fig. 3.** CD4+ and CD8+ subsets expressing KLRG1
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DISCUSSION

A novel finding from this study was that although mobilization of lymphocyte subsets was elevated immediately after GXT in elite athletes, however; hypoxic endurance training did not provide additional benefits. Promoted mobilizations of CD4+ and CD8+ lymphocytes in our study are similar to those observed in sedentary subjects [14]. According to a recent position statement [9], typically a lymphocytosis occurs during and immediately after an acute exercise in the case of T cells (and their subpopulations) and to a lesser extent, B cells and this pattern is quickly reversed during the recovery period by falling their numbers even below baseline values [27, 28]. The lower numbers of CD4+ and CD8+ T cells alone could not necessarily lead to immunosuppression. Whether these changes in T cell numbers are due to apoptosis or their redistribution to other compartments has still not fully elucidated [9].

The quick migration of lymphocytes from peripheral compartments during the 2-h recovery results in lymphocytopenia. We thought that blood hypoxia induced by hypobaric exercise could be responsible for lymphocytopenia by elevation of lymphocyte apoptosis via elevated OS.

It is also interesting to note that although an increased mobilization of both CD4+ and CD8+ subsets were observed following to GXT at both baseline and post intervention conditions in all the groups, only the amount of CD8+ subsets were statistically significant. The more pronounced effect of acute bouts of aerobic exercise for the CD8+ and NK cell populations also has been previously verified [27, 29, 30].

The different rates of T lymphocytes mobilization after intensive exercise have been thought to reflect the differential density of adrenergic receptor expression [31, 32]. Anyway, we failed to demonstrate any benefits for hypobaric exercise training in elite athletes to modify acute exercise induced lymphocytes mobilization into bloodstream, while we have investigated a relatively short intervention period, no nutritional control were done and the study design did not completely resembled the real training conditions of elite athletes.

It is also of note that the intervening hypoxic dosage reported to be an important factor impacting exercise-mediated lymphocyte apoptosis [5]. Accordingly, one can speculate that HE might be too stressful for the athletes and not to provide additional positive exercise training. No between group differences were observed (P>0.05).
adaptsions related to antioxidant capacity and therefore lymphocyte senescence by apoptosis mediated mechanisms. On this basis, the effects of hypoxic exposure in elite athletes by different dosage merits to be more investigated in the future studies.

Figure 1 about here

Other findings show an elevated mobilization of the CD28+ (Fig.2) form of the both CD4+ and CD8+ lymphocytes into the peripheral blood compartments after GXT in all the groups, in spite of our expectation to observe an augmented response by HE rather than those obtained by NC or HE. The detailed mechanisms underlying CD28+ subsets up-regulation induced by GXT, is unclear, however, could be indicated on a diminished lymphocyte senescence as a reduced expression of CD28 subsets which is found to be as a marker of replicative senescence [10, 11]. Possible mechanisms would be the changes in reactive oxygen species and pro-inflammatory cytokines production [14, 33].

Figure 2 about here

The GXT were also up regulated the CD4+ and CD8+ subsets that were expressing KLRG1 at both baseline and post intervention conditions. Although similar findings have verified previously in the sedentary population [14, 34, 35], we were also observed their rapid fall down during a two hour recovery period to values even lower than the resting level. It should be noted that the elevated KLRG1+ T-lymphocytes count caused by GXT could suppress the capacity of these subsets for clonal expansion [5, 14]. It was demonstrated that T and B cell functions in well-trained athletes are more sensitive, rather than sedentary counterparts, to increases during a period of intensified training [36-38]. A recent position statement about immune function and exercise has suggested that T cell functionality could be compromised in athletes participating in longer intensive training periods caused by an increased circulatory stress hormones and alterations in the pro/anti-inflammatory cytokine balance [9]. Therefore, we speculated that a remarkable decreased senescence type lymphocyte levels during the recovery period in our subjects to the values lower than baseline levels, regardless to the type of intervention, could be indicated on more efficient mechanisms responsible for cell extravasation in well trained athletes, e.g. marginalization or tissue migration [9].

Figure 3 about here

In this study, only the HE remarkably reduced lymphocyte surface Thiol levels in response to GXT at both baseline and post intervention conditions (Fig.4) which is thought to cause the more OS-induced lymphocyte apoptosis [5], the promoted risk of immune suppression and an increased risk of infection. However, the consequences of the reduced lymphocyte antioxidant capacity on their functional properties remains to be clarified in the future studies with a longer examination period.

Figure 4 about here

One limitation of this study was that we have not equalized the total work undertaken in both normobaric and hypobaric conditions. On the other hand, our results might not to be generalized to the actual elite athletic conditions and further investigation is required to studying the effects of hypobaric exposure as a supplementary procedure simultaneously with specialized training protocols. We have not controlled the confounding effects from between group nutritional differences throughout the study and also acute exercise induced plasma shift or lymphocyte aggregation. The subjects were not allocated into groups according to their basal Vo2 level or other baseline characteristics. The subjects were not from the same sport field and the indices resembling exact lymphocyte apoptosis level or the overall immune system functionality and also total anti oxidative capacity were not measured. Future studies should consider all the aforementioned shortcomings and hypobaric exercise training along with the administration of anti-oxidative supplements remains to be investigated. However, the available evidences were failed to support any positive role for some nutritional supplements to prevent exercise-induced immune suppression [39].

Conclusion: Hypobaric endurance training has no additional benefits for well-trained athletes to affect lymphocyte mobilization and senescence. The HE is capable of reducing lymphocyte surface Thiol levels which is thought to can facilitate oxidative stress-induced lymphocyte senescence. However, well controlled studies are still required to be done because the lack of similar evidences and the considerable methodological limitations in this study.

ACKNOWLEDGEMENT
Gratitude is expressed to the subjects that participated in this study as well as to each of the assistants who were instrumental in the collection of the data. This study was funded by a product grant from the Islamic Azad University, Tabriz Branch, Tabriz, Iran.
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