EFFECTS OF INTERMITTENT NORMOBARIC HYPOXIC TRAINING ON PULMONARY FUNCTION AT NORMOXIA AND HYPOBARIC HYPOXIA

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Abstract: This study investigates whether 6 weeks of intermittent normobaric hypoxic training (INHT) elicits improvements in maximal oxygen uptake (VO_2max) or various measures of pulmonary function. Training under hypoxic conditions has been suggested as a way to improve endurance performance at sea level and altitude. This is possibly beneficial for the military, as situations occur where personnel are quickly deployed into areas of high altitude without sufficient time for acclimatization. Therefore, 10 highly trained male members of the Army or Air Force Reserve Officers' Training Corps (ROTC) performed maximal treadmill tests in normoxia (FiO_2 - 20.9%, 744.0mmHg, PO_2 - 155.5mmHg) and hypobaric hypoxia (HH) (FiO_2 - 20.9%, 536.7mmHg, PO_2 - 112.2mmHg). Forced vital capacity (FVC), forced expiratory volume in 1 second, (FEV_1), and peak expiratory flow rate (PEF) were also measured prior to treadmill testing with a portable spirometer. After pre-testing subjects were split into experimental (EXP), INHT (n=6) or control (CON), normoxic training (NT) (n=4) groups and started training 3 days per week, 1 hour sessions. The INHT group trained using normobaric hypoxia (FiO_2 - 14.7%, 744.0mmHg, PO_2 - 109.4mmHg). Intensity of training was progressively increased to prevent under or overtraining and was based on maximal heart rate (MHR) achieved during their respective VO_2max test. Post-testing revealed a lack of significant changes in VO_2max, FVC, FEV_1, or PEF between or within groups due to INHT or NT. Although, when percent increase was calculated the EXP group significantly improved in HH VO_2max compared to the CON group ($p < 0.05$). Also, combined VO_2max scores were able to show significant improvements at HH and normoxia. Furthermore, combined spirometric measures for FVC significantly decreased at HH, while PEF increased. These results are favorable for military implementation to increase endurance in personal who are quickly deployed into altitude. In addition, the training program implemented did prove to be effective for using INHT within current physical training. Lastly, despite a change in pulmonary measures due to training, FVC and PEF were sensitive to changes in altitude and may be beneficial in detecting the onset of acute mountain sickness (AMS) or high altitude pulmonary edema (HAPE).

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CHAPTER I

INTRODUCTION

The respiratory system encompasses three separate but related functions, including pulmonary ventilation, gas exchange, and internal respiration (24). Pulmonary function consists of the mechanics for inspiration and expiration. Gas exchange focuses on the transport and exchange of oxygen (O_2) and carbon dioxide (CO_2) throughout the body. Lastly, internal respiration concentrates on the utilization of oxygen by tissues and ultimately the mitochondrial processes at a cellular level. The current study concentrated primarily on the first mentioned, pulmonary function.

Altitude elicits many physiological responses such as, an increase in heart rate, increased release of erythropoietin (EPO) to stimulate greater production of red blood cells (RBC), and an increase in ventilation due to a decrease in the partial pressure of oxygen (PO_2) (14). These changes occur due to a decrease in atmospheric pressure. As altitude increases, the atmospheric pressure decreases, causing the PO_2 to decrease as well. For example barometric pressure at sea level is 760 mmHg, PO_2 in the air is 159 mmHg, and arterial PO_2 is 100 mmHg. These measures differ greatly when compared to an altitude of 3,048m (10,000 ft), where the barometric pressure is 523 mmHg, PO_2 in the air is 109 mmHg, and arterial PO_2 is 69 mmHg. However, the actual percent of O_2 always remains constant at 20.9%.

Since the air decreases in pressure the air also becomes molecularly less dense. This has major effects on all three aspects of the respiratory system (23, 29, 60). Parameters the current study focused on were forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and peak expiratory flow rate (PEF). FVC is the total amount of air that can be forced from the lungs via maximal exhalation. The amount of air forced out in the first second is known as the FEV_1 and the PEF is an individual's maximal rate of expiration. These measures are useful in diagnosing lung diseases, detecting obstructions, as well as checking to see if a medication or pulmonary rehabilitation is having a positive effect.

Training at altitude has traditionally included traveling to a high altitude region and staying for a period of 2-3 weeks. Due to the high cost and time commitment, this method was typically only utilized by elite athletes. To create similar conditions while still being close to home hypoxic conditions can be simulated, either by hypobaric chambers or normobaric generators. Hypobaric hypoxia (HH) chambers work by pumping air out of an airtight chamber until the chamber reaches a pressure similar to a desired altitude, while the fraction of inspired oxygen (FiO_2) remains constant at 20.9%. Normobaric hypoxia generators (NH) simply dilute the ambient air with nitrogen so users are breathing in air with a lower FiO_2, while the pressure is unaffected. Due to these key differences HH chambers are considered to be a closer simulation to actual altitude when compared to NH. However, HH chambers are expensive, require a large building for storage, and a trained technician to run the chamber. Additionally, while the HH chamber is in use it is typical to have medical personnel on hand. Whereas, NH generators are lightweight, easy to run, and can be used at any location for sleeping and living in, or for use while training. The extent of the body's physiological adaptations to these stressors is still highly debated due to the different ways HH and NH simulate hypoxic conditions (50).

The primary concept behind training at altitude is to further increase the body's oxygen-carrying capacity (45). The process begins in the kidneys, where production of EPO rises significantly, generating an increase of RBC production (hematopoiesis) in the red marrow of flat bones (19). The increase in RBC improves the amount of O_2 that can be carried throughout the body, thus increasing maximal aerobic capacity. This is all a result of the decreased PO_2 which reduces the amount of oxygen available to the body. However, hematopoiesis is not the only change that occurs due to hypoxic stress. Since air at altitude is less dense and there are less molecules of O_2 for utilization the respiratory system mechanics must adapt as well. When at altitude or HH the decrease in air density has been shown to decrease airway resistance, as a result maximal inspiration and expiration flow rates may be greater than at sea level (23). Conversely, mechanics of the lungs are under additional stress striving to increase the intake of O_2. Since the accessory respiratory muscles are working at a greater rate with lower efficiency, pulmonary endurance may be decreased (29).

Due to the many changes that occur at altitude a period of acclimatization is typically utilized before performing high efforts of physical work. Historically, experienced mountain climbers after passing 3,048m typically only climb 305m per day and rest for a day every 915m. Slowly ascending into extreme altitudes is important for proper acclimatization and minimizing adverse events. However, present day military personal may be rapidly deployed into high altitude situations without adequate time to acclimate (53). The initial decline in physical performance may compromise the ability for military personal to complete an operation successfully and at full physical potential. Additionally, it may put personnel at an increased risk of suffering from acute mountain sickness (AMS) to the even more dangerous high altitude pulmonary edema (HAPE) or high altitude cerebral edema (HACE) (3). NH generators may be a possible method to acclimatize soldiers before they are deployed into mountainous regions. Intermittent NH training (INHT), involves performing physical work under hypoxic stress to stimulate exercising at altitude. The NH generators ease of use and portability make this one possible way the military could implement INHT to acclimatize soldiers while still living at their low-altitude base.

Considering the amount of physiological changes that occur at altitude, the differing methods for simulating altitude, and the importance of performing at altitude the purpose of this study was to examine the effects of a 6 week aerobic INHT intervention on pulmonary function and aerobic fitness at sea level and HH using current members of the Army and Air Force Reserve Officers' Training Corps (ROTC).

CHAPTER II

LITERATURE REVIEW

Altitude Training Benefits

Beneficial effects of training at altitude are highly debated amongst scientists, coaches, and athletes (15, 36) . Furthermore, the time spent at altitude, how to train at altitude, the level of altitude, and when to come down from altitude add even more controversy to the topic (8, 44). Regardless, altitude training is still frequently used with elite endurance athletes of all types to supplement sea level training and to optimize both altitude and sea level performance (1, 4, 76). The interest in training at altitude greatly increased after the world watched athletes from areas of high altitude dominate endurance events at the 1968 Olympic Games held in Mexico City at an altitude of 2,300 m (7,544 ft) (2, 8, 36). The basic theory of altitude training is to improve the body's ability to transport and utilize oxygen, thus increasing their maximal oxygen uptake (VO_2max) (36, 68).

Levine and Stray-Gunderson (45) report the most commonly accepted theory as to why high altitude increases VO_2max. Suggesting that exposure to hypoxic conditions stimulates an increase in the production and the release of erythropoietin (EPO) from the kidney. This in turn increases the production of erythrocytes in red bone marrow. Overall, this escalates red blood cell (RBC) volume, which increases the body's ability to transport oxygen to the working muscles. Gore and Hopkins (33) later scrutinize this theory by constructing a review of the current literature considering any alterative causes, but derived the same conclusions as Levine and Stray-Gunderson. If an athlete is exposed to the correct amount of altitude exposure, which varies individually, erythropoietic changes will occur, increasing the body's RBC volume, thereby increasing their VO_2max. However, these are not the only changes the human body undergoes after being exposed to hypoxic conditions (32). Physiological adaptations to the pulmonary system, cardiovascular system, exercise economy, muscle buffering capacity, and even changes at a genetic level have all been documented (25, 51, 63, 70, 78). Svedenhag (70) was able to report an increase in left ventricle muscle mass after 1 month of training and living at an altitude of 1,900 m in elite Swedish cross-country skiers. Saunders et al. (63) found a decrease in the total VO_2 over the last 60 seconds of a four-minute submaximal trial, demonstrating a decrease in the submaximal use of oxygen, thus an increase in running economy. This decrease was after elite runners underwent a 20-day program of simulated live high (2,550 m) and train low (600 m). Mizuno (51) et al. utilized muscle biopsies of the gastrocnemius in well-trained cross-country skiers to suggest an increase in muscle buffering capacity due to two weeks of training (2,700 m) and living (2,100 m) at altitude. Zhu and Bunn (78) focused on a transcription factor known as hypoxia inducible factor-1 (HIF-1). This factor is present in every cell in the body and is signaled when the body detects hypoxic conditions. HIF-1 levels increase due to hypoxia and are responsible for simulating the body's responses to the decrease in oxygen. These studies demonstrate the extensiveness of changes occurring due to hypoxic exposure.

Nevertheless, adaptations occurring in the pulmonary system due to training at altitude were the primary focus of the current study. One of the initial changes that occurs due to exposure to altitude, is an increase in ventilation due to a decrease in the arterial partial pressure of oxygen (PO_2). After a study subjecting mice to hypoxia, Zhang et al. (77) determined proper lung function to be the most crucial factor for surviving severe hypoxia. Hence, demonstrating why high altitude pulmonary edema (HAPE) is one the primary causes of death at altitude. Dempsey and Johnson (12) go on to challenge the general view that the lungs are overbuilt for respiratory demands brought about by short-term high-intensity or endurance exercise, stating that the respiratory demands of highly trained endurance athletes may push the pulmonary system to near failure. Dempsey et al. (13) later investigated respiratory adaptations to hypoxia and found there to be detriments in exercise capacity of approximately 5 to 10 % per every 304.8 m. The authors concluded that this occurs because highly trained endurance athletes already push their respiratory system towards failure, so when at altitude performance declines in order to protect the body. These articles clarify the importance of the pulmonary system for performance and survival when experiencing hypoxia.

Pulmonary Adaptations Due To Exercise Training

"Athlete's heart", cardiac adaptations due to consistent rigorous exercise training, is well established and highly accepted among scientists in the field (57, 59). The adaptations of the lungs however, specifically mechanical pulmonary function, are less agreed upon. The current study focused on three main pulmonary function measures; forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and peak expiratory flow rate (PEF). Nourry et al. (54) and Fatima et al. (20) conducted studies that focused on short-term exercise training plans and the acute effects of exercise on pulmonary function. Nourry et al. (54) investigated the effects of an 8-week high-intensity training plan in 24 children. They were able to report significant increases in FVC, FEV_1, and PEF in the training group, compared to the control group who showed no change. Fatima et al. (20) focused on 292 college-aged students performing 30 minutes of aerobic cycle ergometry at a moderate intensity for eight weeks, five days a week. After the eight weeks subjects exhibited a significant increase in FEV_1, but oddly a significant decrease in FVC. The authors were surprised as well with the decrease in FVC, but attributed it to the participants' lack consistent effort while performing spirometry. Measures taken via spirometer are always dependent on the instructions given and patient's effort. Further, Hulke et al. (29) and Thaman et al. (71) focused on similar training methods but extended the training periods over longer periods of time, all using college age populations. Hulke et al. (29) implemented different intensity levels, splitting their college age subject pool into three groups, high intensity (75% max heart rate (HR), low intensity (60% max HR), and sedentary control, while extending their protocol to 20 weeks. This resulted in a significant increase in FVC and PEF for the high intensity group, while the low intensity group saw fewer changes with only a significant increase in PEF, leading the investigators to conclude that high intensity training is more efficient at developing the cardiopulmonary system. Thaman et al. (71) conducted a study using 100 of India's Border Security Force (BSF) trainees and sedentary students both groups aged 18 to 23. The BSF trainees underwent 9 months of demanding physical training consisting of moderate to severe workouts 7 days a week. Due to the rigorous training FVC, FEV_1, and PEF all increased significantly both within group and compared to the sedentary control group. The authors concluded that structured physical training greatly improved lung function parameters in the BSF trainees.

The articles above demonstrated the ability for exercise training to improve pulmonary function acutely. In a similar manner, studies below attempted to provide evidence of the positive benefits of participating in athletics long-term. Ghosh et al. (31) attempted to determine the differences in pulmonary capacities between different types of sports and compared to a sedentary group. Their findings showed significant differences in FEV_1 for soccer, field hockey, swimming, and volleyball compared to the sedentary group. While basketball, boxing, cricket, soccer, and field hockey all showed significant differences in FVC compared to the sedentary group. They concluded the athletes' physical training may improve their lungs accessory muscles for inspiration and expiration. Vedala et al. (72) conducted a similar study, but only focused on well-trained distance runners. They found similar results; runners had significantly higher numbers for FVC, FEV_1, and PEF compared to a sedentary control group. The authors of this study built on the previous conclusions, stating physical activity will lead to improved pulmonary function, thus decreasing the risk for future pulmonary diseases. Lastly, the study by Doherty and Dimitriou (16) had the largest sample size (n = 459) and focused on pulmonary function differences between swimmers, land based athletes, and the sedentary. They concluded that male swimmers significantly differed in FVC, FEV_1, and PEF when compared to the sedentary group and land-based athletes. While the land-based athletes only showed an upward trend compared to the sedentary group. This may have been due to the authors including all land-based athletes into one group instead of separating them by type of sport, aerobic versus anaerobic. Interestingly, the authors continued analyzing the swimmers and found those that competed for the national team, (i.e. the better swimmers) had superior FEV_1 compared to the non-national swimmers. When they controlled for years of training the difference was no longer apparent, suggesting the number of years trained has a significant impact on FEV_1. These articles provide evidence to show the positive influence long-term training provides to improving lung function.

Conversely, despite all of the previously mentioned studies' finding, significant positives in the impact of exercise training on pulmonary function there are many studies that oppose such findings. Koch and Eriksson (41) used a 4-month period of endurance training in 11 to 13 year-old children and found no significant differences in FVC and FEV_1. However, this study only had 9 participants who completed the study and did not utilize a control group to rule out effects from the boys' maturation process. Another study conducted by Hamilton and Andrew (35) focused on children aged 13-17 who played ice hockey. Ventilatory tests were conducted before and after the 28-week hockey season. Results were compared to age matched controls and showed no significant difference for any pulmonary measure. However, they later suggest that the training the boys underwent may not have been rigorous enough to cause changes in lung function. Additionally, Hulke and Phatak (38) utilized a 12-week 30 minutes daily of unsupervised, moderate intensity aerobic work. Their results showed significant increases in PEF, but no other parameters significantly changed post exercise training. The lack of improvements led them to believe the intensity and duration were not high enough to cause changes in FVC and $FEV_{1.}$ The findings of the aforementioned studies demonstrate that with the correct intensity, duration, and type of exercise improvements in pulmonary function are probable, particularly with long-term training.

Importance of Lung Health

The following studies support the importance of the pulmonary variables measured in the current study, as well as investigate the relationship between physical activity and its influence on long-term health and delaying lung aging. In a study conducted by Pelkonen et al. (56) data on pulmonary function and physical activity was collected on 890 adult males for 25 years. The authors concluded high levels of physical activity are associated with a slower decline in pulmonary function and a decrease in risk of mortality. Another study by Schunemann et al. (67) demonstrated that FEV_1 is the most crucial variable when used as a long-term predictor for survival rates. This was based on a 29-year follow up study of 554 men and 641 women. The most complete study conducted by Nystead et al. (55) covered 8,047 subjects for 10 years, monitoring physical activity and lung function. Their conclusion while similar went into more detail, stating regular physical activity corresponds to a reduction of 3-5 years of normal decline in $FEV_{1.}$ Another variable crucial to measuring lung health is FVC, which is able to predict the compliance of lungs and the chest wall (20). Together these create the FEV_1/FVC ratio and in healthy adults this percentage should be greater than 70%. Lastly, a decrease in PEF at hypoxia has shown a correlation with severity of acute mountain sickness (AMS) (60, 69). These two studies found those with a significant drop in PEF compared to sea level suffered from the most severe cases of AMS. Pollard et al. (60) explains this well, stating the AMS symptoms decrease the subject's ability to produce a maximal expiration

Effect of Altitude on Pulmonary Function

After covering the importance of physical trainings positive effect on lung function acutely and long-term, the following will focus on the influence of altitude on pulmonary function. Reductions in airway resistance occur due to a decrease in air density, increasing the altitude only further increases the effect. This has been known to increase maximal inspiratory and expiratory flow rates (23). As a result, FEV_1 and PEF have generally been shown to increase (7, 23, 29, 47, 62), PEF more so than FEV_1. Forte Jr. et al. (23) demonstrated this in a study, of 18 healthy young men at environmental altitude and hypobaric hypoxia (HH). In both situations FEV_1 significantly increased while PEF was not reported in this study. Another study by Wolf et al. (61) found PEF to increase significantly after testing 21 healthy subjects at environmental altitude of 1,560 m. On the other hand, FEV_1 was not deemed significantly different, but showed an upward trend. At an altitude of 3,457 m 9 subjects showed an increase in FEV_1 and PEF in a study by Gautier et al. (29). They attributed this to the changes in ventilatory rate due to a decrease in arterial partial pressure of oxygen (PO_2) and decrease in airway resistance.

Conversely, FVC has generally been shown to decrease with exposure to hypoxic conditions (22, 23, 29, 47, 60). Edward Schneider (65) noted a decrease in vital capacity in the early 1930s after studying mountaineers ascend Pikes Peak, CO. He noted that the decrease in FVC may be due to an enlargement of the pulmonary blood vessels that consequently occupy alveolar space normally available for air (17, 65). Two other common concepts for the fall in FVC are respiratory muscle fatigue and subclinical pulmonary edema (47, 74). More current studies by Forte Jr. (23) and Pollard (60) demonstrate further the decrease in FVC. The study by Forte Jr. et al. (23) found significant decreases in FVC at an environmental altitude of 4,300 m and HH set to 4,200 m. Pollard et al. (60) studied subjects at an even more extreme altitude of 5,300 m at the Mount Everest base camp. 51 members of the British Mount Everest Medical expedition were studied using a hand held turbine spirometer as used in the current study. Their results demonstrated a decrease in FVC, as well as an increase in PEF, but no change in FEV_1. Furthermore, an early study conducted by Consolazio (7) et al. for U.S. Army Medical Research used 24 Army volunteers. After traveling to an altitude of 4300 m, a decrease in FVC and increase in FEV_1 in the soldiers was noted. As shown by the information in studies reviewed the general consensus is a slight increase to change FEV_1, a large increase in PEF, but a decrease in

FVC. However, these results are still somewhat unexplained physiologically and not entirely agreed upon.

Hypobaric versus Normobaric Hypoxia

Due to the majority of these studies utilizing environmental altitude, even less is known about the adaptions to HH or normobaric hypoxia (NH). The aim of both of these altitude simulations is to decrease the inspired partial pressure of oxygen (PiO_2) either through a decrease in barometric pressure (HH) or a reduction in the fraction of inspired oxygen (FiO_2) (NH). Since the end result of both methods is a reduction of PiO_2 either method should produce the same physiological response. However, the literature commonly reports differences in all aspects including pulmonary function (46, 50, 64). Savourey et al. (64) and Loppky et al. (46) both investigated the effects of NH and HH measuring various cardiopulmonary variables. Authors from both studies had similar findings; respiratory rate increased, tidal volume decreased, and there was a reduction in ventilation during HH compared to NH. The key difference as stated by Kayser (40), is that the lower pressure produced in HH leads to a lower air density, whereas NH just decreases the physical amount of O_2 available. Millet et al. (50) later concluded in their review article that HH is a more severe environmental condition, which causes different physiological adaptations compared to NH. However, the differences in NH and HH are not completely agreed upon in the literature. Mounier and Brugniaux (52) believe it all centered around the body sensing the amount of available oxygen and centers on the hypoxia-inducible factor-1 (HIF-1) transcription factor. Further stating the differences in HH and NH are too small to be clinically relevant. In the current study NH was used for training purposes due to the portability and practicality of use in military personal training. While HH was used for pre and post testing due to the majority of literature agreeing that HH is a closer simulation of environmental altitude.

Military Applications & Acclimatization

The current study utilizes members from a Midwest University's Reserve Officer' Training Corps (ROTC) program. Reasoning behind the choice of this population is due to the military's current need to rapidly deploy soldiers in to mountainous regions (3, 42, 53). Military duties commonly consist of lifting or carrying loads for long periods at submaximal intensities with quick periods of high intensity action (58). These bouts of physically demanding work may result in a decline in performance due to fatigue or injury. Consequently, adding altitude to the equation only exacerbates the issue. Soldiers based at sea level typically experience a large decrease in aerobic endurance performance and only a slight to no decrease in anaerobic performance upon arriving to altitude (26). AMS also commonly occurs to unacclimatized soldiers who ascend too quickly to high altitude, resulting in symptoms of headache, nausea, fatigue, and sleep disturbances, further reducing their ability to perform physical work (3). A study by Hackett and Roach (34) found an astonishing 80% of individuals developed some symptoms of AMS within a few hours and reach peak severity at 36 hours. Additionally, Fulco et al. (27) found that endurance performance can decrease 60% or greater at altitude compared to sea level. Due to the high prevalence and severity of detriments a rapid acclimatization method would prove to be highly beneficial to the military.

Acclimatization is traditionally achieved by a slow progression, living a few days at various stages, gradually ascending to the desired altitude (26, 53). However, this lengthy process defeats the purpose of the military being able to utilize a rapid response mission on short notice. Therefore, various studies have investigated the effectiveness of using simulated intermittent altitude exposure prior to being deployed to high altitude regions (3, 5, 6, 26, 53, 66). Review articles by Muza (53), Fulco et al. (26), and Burtscher et al. (5) highlight the extensive scope of different methods authors have implemented to attempt rapid acclimatization, from 0.4 h to 16 h a day for 3 to 42 days at elevations ranging from 2250 m to 6000 m either utilizing exercise or at rest. The various doses, level of altitude, and different types of simulated altitude make it difficult to compare the results of different studies. Muza (53) closes his review stating protocols utilizing altitudes greater than 4000 m and a duration greater than 1.5 h have a high probability of promoting altitude acclimatization, but studies under 4000 m and 1.5 h were not well documented and further research should be conducted in this area. Fulco et al. (26) concluded that HH was more effective than NH for preacclimatization, however all NH studies included in this review used protocols where subjects were at rest or sleeping while using NH. Lastly, Burtscher et al. (5) concluded there is lack of studies to make a firm conclusion on recommendations using intermittent hypoxia for preacclimatization. Authors also stated that longer protocols including exercise may further enhance endurance performance at altitude.

Finally, studies conducted by Schommer et al. (66), Dunfor et al. (18), Ventura et al. (73), Fulco et al. (20), and Debevec et al. (11) were found to have very similar methodologies to the current study. Schommer et al. (66) randomly split 40 healthy volunteers, who then performed 4 weeks of intermittent normobaric hypoxic training (INHT) or normoxic training (NT) using a bicycle ergometer. INHT altitudes progressed from 2500 m to 3500 m over weeks 1-3 for a total of 9 h of training, while the last week was spent passively for 90 m sessions. Once training was completed, subjects ascended to 3611 m for and overnight stay then proceeded to the peak at 4559 m. Testing for AMS and cardiopulmonary measures were conducted at both sites. Results showed no significant differences at 4559 m, but AMS was significantly less prevalent in the INHT group compared to the NT group. Due to the rarity of altitudes around 4559 m, the positive result at 3611 m would prove to be beneficial for the military. Dufour et al. (18) implemented a similar 6 weeks of INHT at 3,000 m, using varying intensities with 18 highly trained endurance athletes. After training only the INHT group improved their VO_2max significantly in normoxia and hypoxic conditions. They attributed these improvements to muscle adaptations occurring during hypoxic training. Further, Debevec et al. (9) and Ventura et al. (73) both utilized healthy males who performed 18 to 20 INHT sessions on a cycle ergometer. Debevec et al. pre and post-tested subjects at normoxia and HH similar to the current study, while Ventura et al. only tested at normoxia. Both studies came to the same conclusion, INHT did not significantly improve performance at sea level or HH compared to NT. However, Ventura et al. implemented training sessions only 30 m long most likely resulting in an undertraining effect. Whereas, Debevec et al. trained subjects at 4500 m for 70 m a day, five days a week for 4 weeks straight. This level of extreme altitude without sufficient rest between sessions may have resulted in overtraining or an inability for the subjects to perform maximally at post-testing. Lastly, Fulco et al. (20) studied time-trial results for 6 healthy men after using NH for 6 straight days. Two hours a day were spent at rest at 4500 m and 1 hour a day at 3000 m on a cycle ergometer. Interestingly, time trial performance improved significantly in hypoxic conditions, but not at normoxia. These results are ideal for the military, as the protocol is quick (6 d) and effective for soldiers performing work at altitude. The current study looks to build off the previously implied methods of INHT, furthering the research in the area.

CHAPTER III

METHODOLOGY

Participants

Highly trained male members of the Oklahoma State University (OSU) Army or Air Force Reserve Officers' Training Corps (ROTC) were solicited to participate in this study. Before participating, the study protocol, risks, and benefits were carefully explained to potential subjects. Participants gave their voluntary consent agreeing to participate in the protocol approved by the Oklahoma State University (OSU) Institutional Review Board (IRB). Subjects were aged 19.6±0.4 further, all anthropometric and physiological measures are shown in Table 1. In the weeks before and throughout the study, subjects lived at an altitude just below 300 m above sea level.

Prior to screening for eligible participants, all members of the Army and Air Force ROTC were presented protocol information, time required, risks, and benefits of the study. Those who were interested were required to complete a maximal aerobic capacity treadmill test (VO_2max) as a part of the pre-screening process. A total of 191 cadets were informed of the study, of these 27 responded with interest. Of the initial 27, 20 participants were able to report for the VO_2max test conducted in normoxic conditions (VO_2-Npre). Seventeen cadets exceeded the minimum fitness level and did not report any previous medical problems. Two were excluded due an inability to achieve the required fitness level of a VO_2max greater than 49.2 $ml{\cdot}min^{-1}{\cdot}kg^{-1}$ and one was excluded due to a prior lower leg injury. The 17 participants were then informed of their qualification for the study and scheduled for pre VO_2max testing in hypobaric hypoxic (HH) conditions (VO_2-Hpre). Between the VO_2-Npre and VO_2-Hpre 5 cadets decided to no longer participate in the study, thus a total of 12 participants completed both Vo2-Npre and VO2-Hpre tests. The remaining subjects were split into two groups experimental (EXP) n = 7 and control (CON) n = 5 via pair-wise selection, based on VO_2-Npre scores. Averages of 61.6 $ml \cdot min^{-1} \cdot kg^{-1}$ (EXP) and 62.0 $ml \cdot min^{-1} \cdot kg^{-1}$ (CON) were determined to be not significantly different based on a t-test via SPSS ($p > .05$). All additional anthropometric data and initial VO_2max test scores are listed in Table 1. No significance differences were found between groups for any measured variable.

Experimental Design

The study was organized into four stages, as illustrated in Figure 1. Pre-screening, pre-testing in HH conditions, exercise training, and finally the post-testing.

Pre-screening was completed in the laboratory under normoxic conditions. Pre-screening consisted of completing a medical history questionnaire, assessments of body weight and height, and a VO_2max test. Criteria for inclusion consisted of a minimum VO_2max of 49.2 $ml \cdot min^{-1} \cdot kg^{-1}$, no history of chronic obstructive pulmonary disease (COPD) or bronchial asthma (BA), and a non-smoker. A VO_2max score of 49.2 $ml \cdot min^{-1} \cdot kg^{-1}$ was chosen due the ACSM guidelines, stating 49.2 $ml \cdot min^{-1} \cdot kg^{-1}$is the minimum to be considered excellent amongst males of similar age (48). If the minimum fitness requirement was met and there were no abnormities found in the medical questionnaire participants were scheduled for the next phase of testing.

Pre-testing in HH conditions were performed in a hypobaric chamber adjusted to resemble approximately 3,048 m (10,000ft) above sea level. Lung function assessments consisting of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and peak expiratory flow rate (PEF) were measured prior to the treadmill test with a portable spirometer. Also, the same VO_2max test was performed but in HH conditions. Peripheral capillary oxygen saturation (SpO_2) was measured via a finger pulse oximeter before and after testing. Due the acute exposure of hypoxia a board certified doctor of osteopathic medicine (D.O.) monitored participants closely while performing the VO_2max test. To support maximal effort, a minimum recovery of 48 h was allowed between the normoxic and HH VO_2max tests.

Prior to the first training session, baseline measures for normoxic conditions were recorded. These assessments included a spirometry test, heart rate (HR), $SpO_{2,}$ and a 7-site body fat measurement. The exercise training spanned six weeks and involved three weekly sessions consisting of an hour each session. A warm-up and cool down was integrated into the hour sessions, resulting in 18 hours as the maximum training time for each subject. All training sessions were based on a percentage of their maximum HR (MHR) achieved in their respective VO_2-pre-test. For example, if assigned to the EXP group, training heart rates were based on the MHR achieved in theVO_2-Hpre test, vice versa for the CON group. Before beginning training each participant received their exercise prescription card and was briefed on the day's workout. Subjects were allowed to control their own speed throughout training, but required to stay within the instructed HR range. The treadmill grade remained constant at 2%.

The first week served as an acclimatization period, where subjects walked/jogged at 50-60% of MHR throughout the hour of training. The second week increased the intensity to 60-70% of MHR for 40 min, with a 10 min warm up and cool down at 50-60% MHR. Weeks 3 and 4 further increased the intensity to 70-77% of MHR, with the same warm up and cool down. Finally, weeks 5 and 6 consisted of subjects jogging 10 minutes at 60-70% Max HR, followed by 20 minutes running at 78-85% MHR, then another 10 minutes at 60-70%, again with the same warm up and cool down. This workout was performed so participants could become comfortable running at a moderately hard intensity, but preventing a maximal effort. All participants were required to have their training scheduled at least 24 hours apart for recovery purposes. Cardiopulmonary resuscitation (CPR) certified investigators supervised cadets throughout each training session.

Post-testing involved the same VO_2max test, spirometry, and pulse oximetry at normoxic and HH conditions. The first post-test was separated by at least 48 hours from the final training session and again, the normoxic and HH tests were separated by 48 hours.

Procedures

Hypobaric Hypoxia Simulation

The environmental conditions for normoxic and HH testing are illustrated in Table 5. The fraction of inspired oxygen (FiO_2) remained constant at 20.9% throughout all pre and post-tests. However, normoxia averaged a barometric pressure of 744.0 mmHg, (PO_2 - 155.5 mmHg), while the HH chamber was able to attain an average of 536.7 mmHg (PO_2 - 112.7 mmHg). This is achieved by pumping air out of the airtight chamber until the chamber reached a pressure similar to an altitude of 3,048 m. A hypobaric chamber technician was responsible for constantly maintaining and monitoring the pressure in the main chamber. Prior to entering the hypobaric chamber each participant was instructed on the methods for clearing pressure from the ears via yawning or swallowing on the ascent and the Valsalva maneuver on the decent to ensure minimal pain. To ensure each subject was exposed to the same acute dose of hypoxia, participants were brought up and taken down from HH individually accompanied by a registered nurse (RN), in a small, connected chamber. Two primary investigators and a D.O. remained inside the main chamber throughout the testing duration to conduct and closely supervise all testing.

Spirometry

Lung function was measured following the standard testing procedures set by the American Thoracic Society (ATS) and European Respiratory Society (ERS) (49). The same portable spirometer, Contec SP10, was used in all pre and post-tests (Nature Spirit, Dallas, TX). Guidelines for recording an accurate FVC call for three different stages during the test, starting with a maximal inspiration, followed by a blast of exhalation, and exhalation is continued until the end of the test (EOT). Emphasis was placed on the blast of exhalation, not holding the maximal inspiration too long, and continuing to exhale until the EOT. All subjects were given detailed explanation and shown an example to make sure there procedures were fully understood. Each test was completed in a standing position with a nose clip in place and the subject holding the spirometer in one hand. The investigator made certain the lips were sealed around the mouthpiece and provided verbal encouragement until the EOT criteria was met. EOT criteria consisted of the participant exhaling for at least six seconds or the volume-time curve must have not shown any change for one second. Every participant was given one practice test then was asked to record three acceptable spirograms. To be considered acceptable the two largest values for FVC and FEV_1 must be within 0.150L of each other. The largest values were used for analysis. In order to account for race, age, and height differences FVC and FEV_1 was also interpreted into a percent predicted using the spirometric references presented by Hankinson et al. (37).

Normoxic and HH Maximal Aerobic Capacity Tests

VO_2max tests were all conducted with a consistent protocol and under the same instructions. Additionally, the same equipment, a Trackmaster motorized treadmill (TMX 425C, Newton, KS), TrueOne 2400 metabolic cart (Parvo Medics, Salt Lake City, UT), and Polar HR monitor (Polar Electro FT1 and T31, Lake Success, NY) was used throughout all testing to ensure reliable results. The modified Astrand-Saltin protocol was used to achieve a true VO_2max. This protocol consisted of a 5-minute warm-up at treadmill speed of 9.7 km/h (6.0 mph) with a 0%

slope. The initial testing stage began a 14.5 km/h (9.0 mph) with a 0% slope. As the participant progressed, the speed remained constant, but the slope increased by 2% every two minutes until the subject experienced complete exhaustion. Each participant was verbally encouraged throughout to give maximal effort. Criteria for attaining a true VO_2max was a respiratory exchange ratio greater than 1.10 or a MHR greater than 90% of their predicted MHR. The expired gases were analyzed through the mixing chamber and reported using a 4 breath average. Calibration of the metabolic cart was performed with a 3-liter syringe and gas analyzers were calibrated according to the manufacture specifications.

Intermittent Normobaric Hypoxic Training

Training at normobaric hypoxia was achieved using Hypoxico (Hypoxico Altitude Training Systems (New York, NY) equipment which diluted the ambient air with nitrogen. In a single blind approach, all participants were required to wear a High Altitude Training Mask (mask) throughout the training sessions. However, only the subjects in the EXP group had their masks connected to the Everest Summit II Altitude Generator (altitude generator) breathing in hypoxic air. Subjects in the control group were not connected to the altitude generator allowing them to exercise under normoxic conditions, but their masks were attached to a machine, an unused metabolic cart, to further resemble training in the INHT group. This was an attempt to lessen the placebo effect and control for the effect of simply wearing a mask during training. An altitude of 3,085 m (10,120 ft) was simulated during all INHT sessions for the EXP group. To achieve this, the FiO_2 of the inspired air was lowered to approximately 14.7% (PO_2 - 155.5 mmHg). SpO_2 was measured via an Oxi-Go finger pulse oximeter (Oximeter Plus, Inc., Roslyn, NY) before and after each VO_2max test as well as the 50 m mark of training sessions for safety. HR was also monitored throughout all training so that the participant and investigator could monitor their effort level and safety.

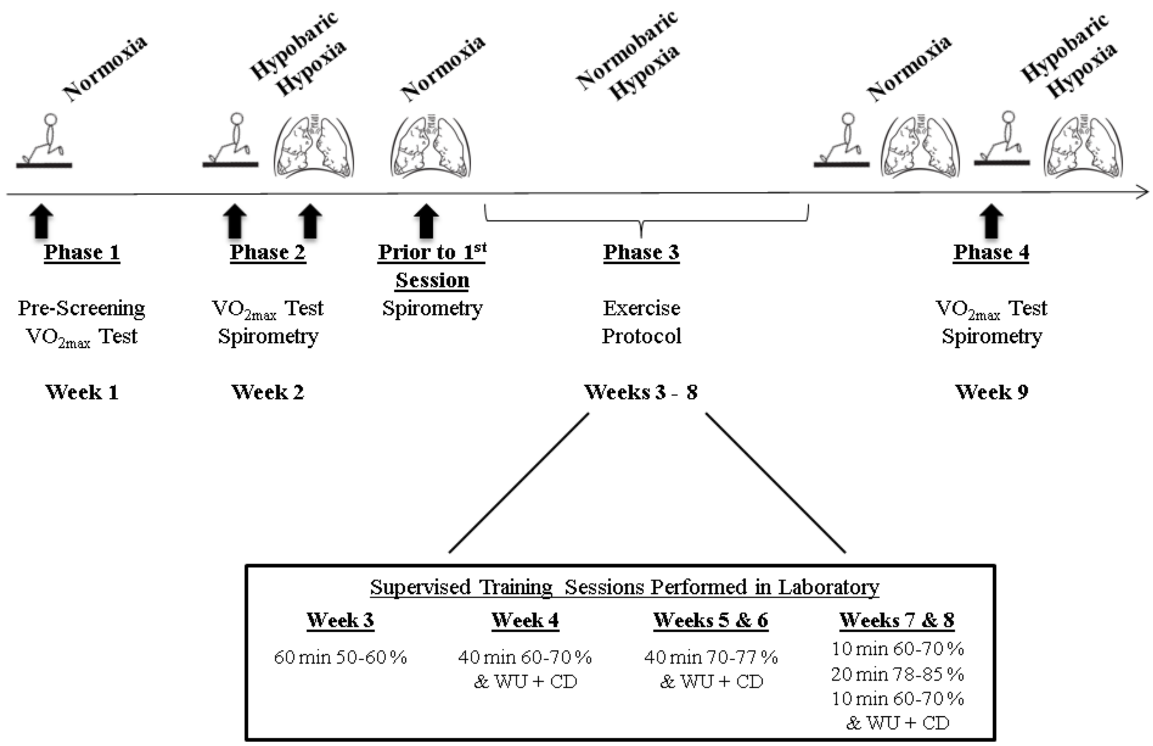
Anthropometrics

Body weight and height were measured during the pre-screening process using a Detecto Weigh Beam Eye-Level physician scale (Detecto Scale Company, Webb City, MO). Body fat percentage was calculated following the American College of Sports Medicine (ASCM) guideline for a 7-site skinfold measurement (48). Measurements were taken by the same investigator using a Lange Skinfold Caliper (Beta Technology Incorporated, Cambridge, MD) at the triceps, pectoral, midaxillary, subscapular, abdomen, suprailiac, and thigh locations and in that same order. The investigator performed a minimum of two measurements at each site and the average of the two measurements was recorded. A third measurement was taken if there was a difference greater than ± 1mm between the first and second measurements.

Statistics

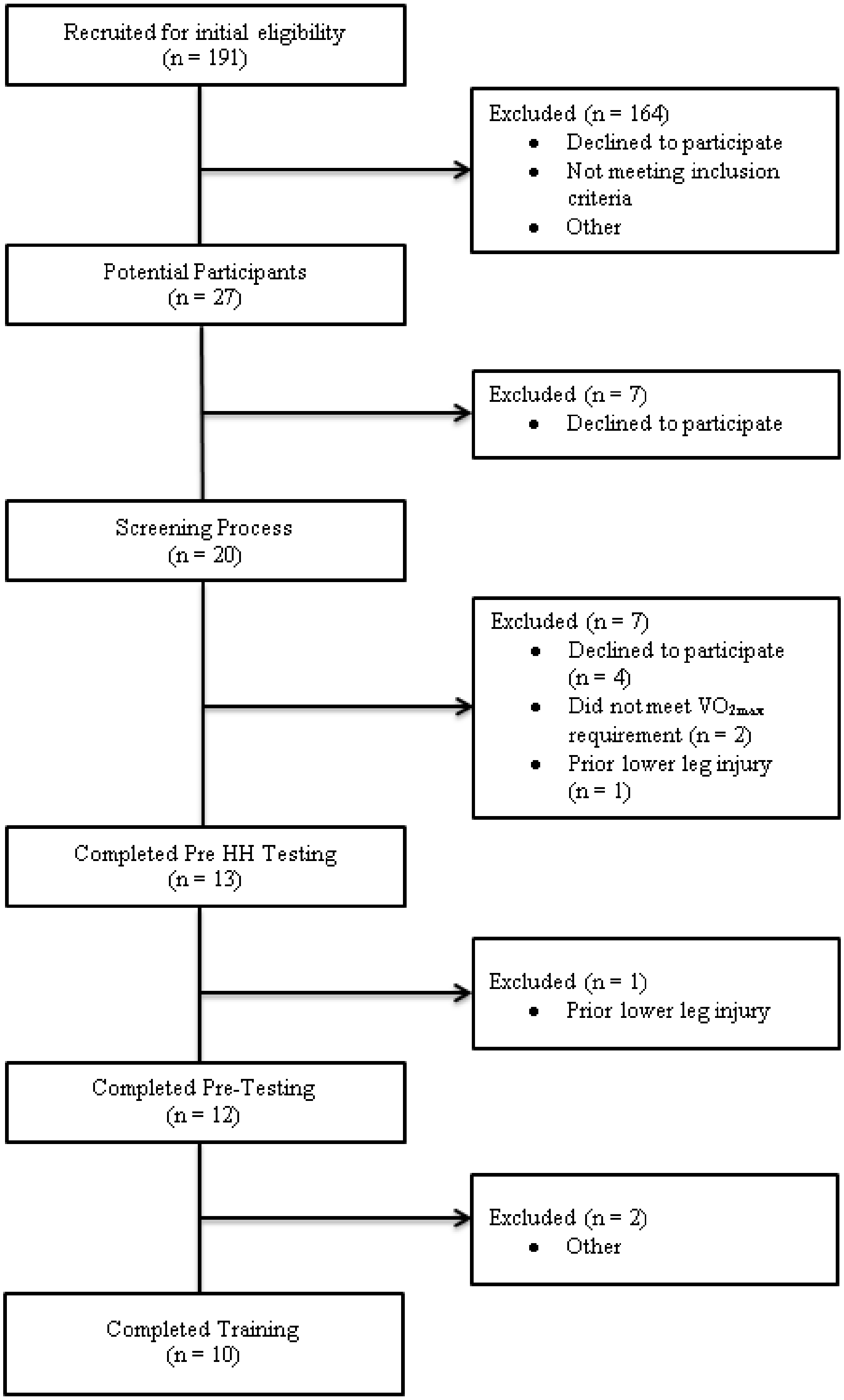
Repeated measures analysis of variance (ANOVA) were used to measure between group (INHT versus normoxic training) and within group (pre and post-testing) effects. A Newman Keuls post hoc test was used to determine the source of significant difference. All other data were compared using a T-Test. A value of $p < 0.05$ was considered statistically significant. Values are presented as means ± standard error (SE). All statistical analyses were performed using SPSS version 21.0.

Figure 1. Study design



Complete study design, including training program. Training percentages are based on maximum heart rate archived during respective VO_2-pre-test. WU = Warm Up, CD = Cool Down, always at 50-60% of maximum heart rate.

Figure 2. Subject Participation



Flowchart describing participants, from recruiting to our final subjects who completed the entire study.

CHAPTER IV

FINDINGS

The purpose of this study was to determine the effects of aerobic INHT for 6 weeks, investigating VO_2max and pulmonary function at normoxia and HH. Of the 12 subjects who started, final participant count after two pre-tests, 6 weeks of training, and two post-tests was 10 (EXP = 6, CON = 4) male ROTC members. Age, baseline VO_2max, and all other anthropometric information is listed in Table 1, none of these parameters different significantly between groups. The EXP and CON groups completed an average of 16 and 17 sessions respectively, out of the maximum 18. There were no adverse events, complaints of injury, or severe discomfort during all training sessions. Over the 6 weeks the EXP group covered an average distance of 95.9 km (59.6 miles) per person, ran at an average pace of 10:00 min/km, climbed 10,129.8 m, and recorded an average SpO_2 of 82.4% throughout training. Whereas the CON group averaged a total of 109.7 km (68.2 miles) per person, average pace was 9:17 min/km, climbed 11,552.8 m, and average SpO_2 was 97.2. Weekly averages and all other training information are listed in Table 2.

Table1. Anthropometrics and maximum oxygen uptake

	INHT Group (EXP)	NT Group (CON)	Significance
Number of Subjects, n	6	4	
Age, yr	20.2±0.3	19.0±1.0	NS
Height, cm	176.4±2.3	181.9±2.3	NS
Body Weight, kg	73.5±2.2	81.8±5.5	NS
Body Fat, %	9.4±1.5	9.3±1.5	NS
Normoxic VO_2max	61.6±2.5	62.0±3.0	NS
Hypoxic VO_2max	34.7±1.9	37.7±2.3	NS

Values are means ± SE. VO_2max in $ml \cdot min^{-1} \cdot kg^{-1}$. NS = $p > 0.05$.

Table 2. Training Sessions

		Week 1 Per Session	Week 2 Per Session	Weeks 3 & 4 Per Session	Weeks 5 & 6 Per Session	Total Per Person
Distance Covered (km)	**E**	5.13	5.76	6.30	6.22	95.9
	C	5.08	6.23	6.71	6.79	109.7
SpO_2 (50 min)	**E**	84.8	82.4	82.3	84.2	82.4
	C	97.1	96.9	97.1	97.5	97.2
Average Pace (min/km)	**E**	11:41	10:25	9:31	9:40	10:00
	C	11:49	9:38	8:57	8:50	9:17
Meters Climbed (m)	**E**	541.7	608.6	665.4	656.8	10,129.8
	C	536.6	657.8	708.1	716.8	11,552.8

Average 16 sessions per person for EXP and 17 sessions per person for CON out of a possible 18.
E = experimental group, C = control group.

VO_2max Tests

When both EXP and CON groups were combined, a t-test showed a significant increase in VO_2max from pre to post-testing in both conditions, normoxia and HH. VO_2max in normoxia improved from 61.7±1.8 $ml \cdot min^{-1} \cdot kg^{-1}$ to 65.0±1.7 $ml \cdot min^{-1} \cdot kg^{-1}$ ($p = 0.031$) while, in HH VO_2max improved from 35.9±1.5 $ml \cdot min^{-1} \cdot kg^{-1}$ to 39.6±1.3 $ml \cdot min^{-1} \cdot kg^{-1}$ ($p = 0.005$) (Fig. 3 &

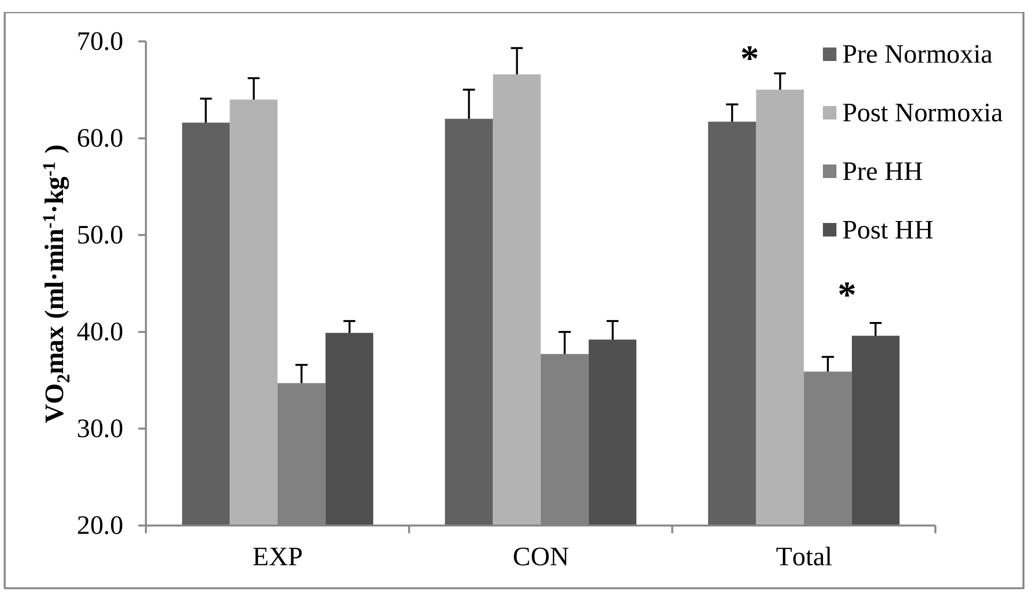
Table 3). When splitting the total population into their respective EXP and CON groups there were no significant differences seen between pre and post VO_2max measures (Table 3). However, the EXP group did improve an average of 4.5% at normoxia and 15.5% at HH, while the CON group improved 7.3% at normoxia and 4.2% at HH. The percent increase after training in the EXP group of 15.5% at HH was found to be a significant increase when compared to the CON group ($p=0.046$). Altitude did have a significant impact on VO_2max scores ($p < 0.001$), demonstrating a 40.4% decrease at HH compared to normoxia. In addition to VO_2max, maximum heart rate and SpO_2 are listed in table 4. Environmental conditions such as, temperature, percent humidity, and barometric pressure are listed in table 5.

Table 3. VO_2max Measures

	Normoxia			HH		
	Pre	**Post**	**% Increase**	**Pre**	**Post**	**% Increase**
EXP	61.6±2.5	64.0±2.2	4.5±3.2	34.7±1.9	39.9±1.2	15.5±4.2 **
CON	62.0±3.0	66.6±2.7	7.3±1.8	37.7±2.3	39.2±1.9	4.2±1.8
Total	61.7±1.8	65.0±1.7*	5.6±2.0	35.9±1.5	39.6±1.3*	11.0±3.1

Values are means ± SE. VO_2max in $ml \cdot min^{-1} \cdot kg^{-1}$. * = $p < 0.05$ when comparing total pre-test to post-test. ** = $p < 0.05$ when comparing % increase between EXP and CON group.

Figure 3. VO_2max



Values are means ± SE. * = $p < 0.05$ when comparing total pre-test to post-test.

Table 4. Maximum Heart Rate and SpO_2

	Heart Rate				SpO_2		
	Normoxia		HH		Normoxia	HH	
	Pre	Post	Pre	Post	Resting	Pre	Post
EXP	199.5±1.3	196.8±3.1	193.7±2.6	187.0±3.5	98.6±0.3	90.7±0.5	92.8±0.3
CON	192.3±1.7	192.0±3.8	182.0±3.2	184.5±4.2	98.3±0.3	92.0±0.6	88.9±0.4

Values are means ± SE.

Table 5. Environmental Conditions

Pre/ Post Test & Oxygen Concentration	Barometric Pressure, mmHg	Temperature, C°	Humidity, %
Pre-Test Normoxic	744.0	22.5	42.2
Pre-Test HH	537.2	26.0	54.9
Post-Test Normoxic	744.0	22.0	31.3
Post-Test HH	536.2	24.0	22.5

Values are means.

Pulmonary Function Tests

Table 6 and 7, as well as figure 4 show pulmonary function results taken with the spirometer. The combined results of the EXP and CON groups pre and post-tests were evaluated to compare the effect of altitude versus normoxia. Starting with FVC, when combined there was a significant decrease in FVC at HH compared to normoxia ($p = 0.037$). In addition, when body size was accounted for FVC% also showed a significant decrease at HH ($p = 0.040$) compared to normoxia. FEV_1 and FEV_1% showed no significant change, however PEF demonstrated a significant increase at HH compared to normoxia ($p = 0.001$). Due to the decrease in FVC there was also a significant increase in the FEV_1/ FVC ratio at HH when compared to normoxia ($p = 0.017$).

Once measures were split into separate EXP and CON groups for pre and post-tests there were significant differences in 3 of the 6 variables. FVC showed significant differences within the EXP group due to testing at normoxia compared to HH. The pre-test at normoxia was significantly higher than the pre-test at HH ($p = 0.041$) and the post-test at HH ($p = 0.016$). Further, the post-test at normoxia was also significantly higher than the pre-test at HH ($p = 0.004$) and the post-test at HH ($p = 0.001$). FVC% results displayed a significant decrease in the EXP group from the normoxic pre-test to hypoxic post-test ($p = 0.032$), along with the post-test at normoxia to pre-testing at HH ($p = 0.009$) and post-testing at HH ($p = 0.001$). Additionally, in the CON group there was a significant decrease from the pre-test at HH to the post-test at HH. Lastly, FEV_1/FVC increased significantly in the EXP group from the pre-test at normoxia to the pre-test at HH ($p = 0.006$) and the post-test at HH ($p = 0.004$). In the CON group there was a significant increase from the pre-test at normoxia to the post-test at normoxia. No other measures, including FEV_1, PEF, and FEV_1%, for the EXP or CON group differed significantly when comparing pre-test to post-test within or between groups.

Table 6. Spirometry Measures

		EXP Group		**CON Group**	
		Pre	**Post**	**Pre**	**Post**
FVC, L	**N**	4.99±0.3	5.07±0.3	5.01±0.4	5.10±0.4
	HH	4.89±0.3**	4.85±0.3**	5.14±0.3	5.00±0.4
FEV_1, L	**N**	4.16±0.3	4.28±0.3	4.27±0.2	4.53±0.3
	HH	4.27±0.3	4.25±0.3	4.47±0.2	4.37±0.3
PEF, L/s	**N**	8.92±0.7	9.27±0.6	9.63±0.8	10.38±0.8
	HH	9.58±0.7	10.25±0.8	10.80±0.9	11.41±0.9
FEV_1/FVC	**N**	83.1±0.9	84.4±1.0	85.3±1.1	88.8±1.3*
	HH	87.1±1.2**	87.5±0.8	86.8±1.5	87.2±1.0
FVC, %	**N**	90.8±3.0	92.2±2.9	89.5±3.7	91.9±3.1
	HH	89.0±2.6	88.1±2.8**	91.2±3.5	89.5±3.4*
FEV_1, %	**N**	89.8±4.6	92.5±4.8	91.1±5.6	96.8±5.9
	HH	92.2±4.7	91.8±4.7	95.5±5.7	93.4±5.8

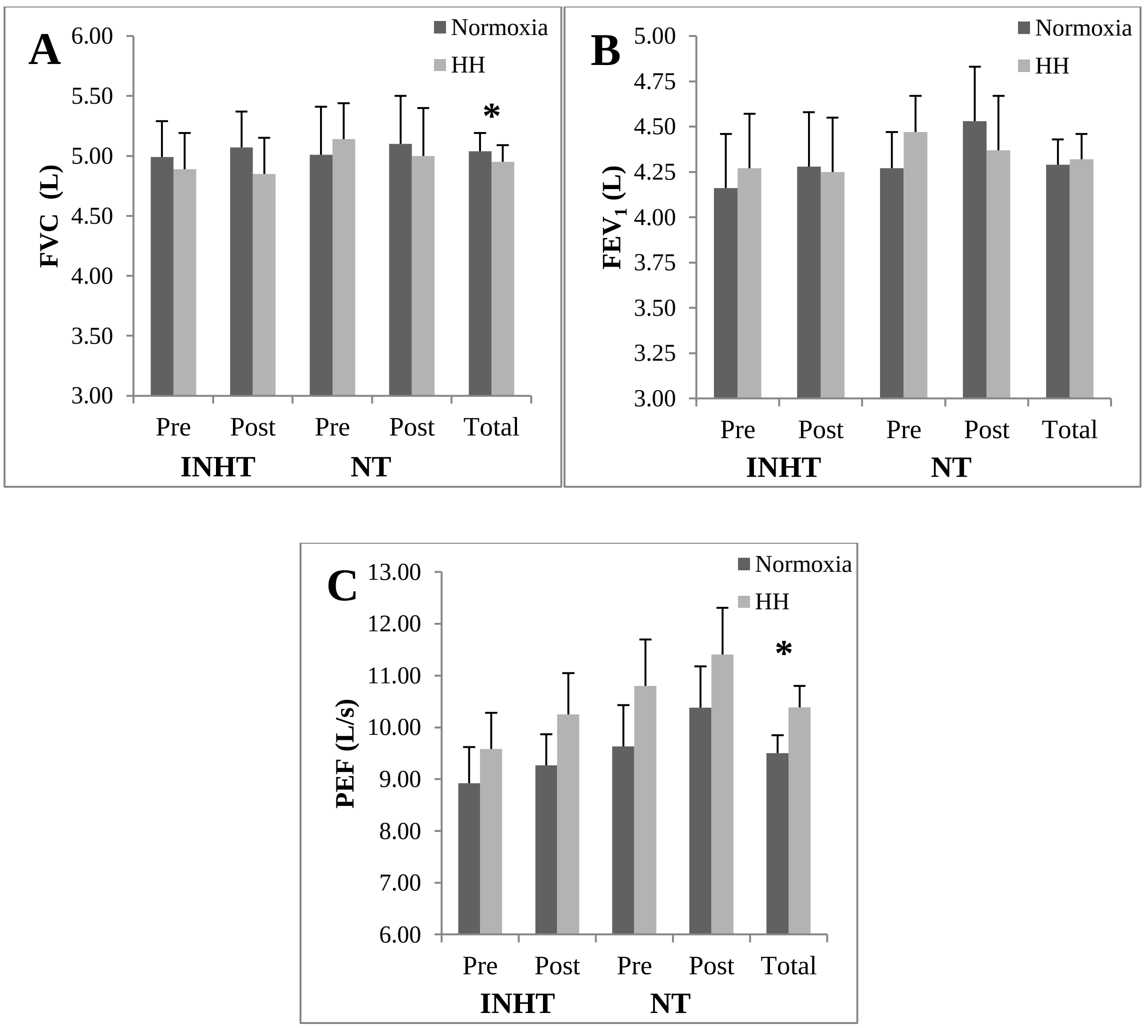
Values are means ± SE. N = normoxia, HH = hypobaric hypoxia. * = $p < 0.05$ when comparing within group pre-test and post-test. ** = $p < 0.05$ when comparing within group normoxia versus HH.

Table 7. Study Total Pulmonary Function

	Normoxia	HH	p-Value
FVC, L	5.04±0.2	4.95±0.1	0.037*
FEV_1, L	4.29±0.1	4.32±0.1	0.465
PEF, L/s	9.50±0.4	10.39±0.4	0.001*
FEV_1/FVC	85.1±0.9	87.2±0.8	0.017*
FVC, %	91.0±2.2	89.4±2.0	0.040*
FEV_1, %	92.2±2.4	93.0±2.4	0.461

Values are means ± SE. * = $p < 0.05$ when comparing study totals at normoxia versus HH.

Figure 4. Pulmonary Function Pre-test versus Post-test & Normoxia versus HH



Values are means ± SE. * = $p < 0.05$ when comparing study totals at Normoxia versus HH. A = FVC, B = FEV_1, C = PEF.

CHAPTER V

CONCLUSION

Training & VO_2max

Six weeks of INHT integrated into ROTC members normal physical training did not elicit any greater improvements when compared to the NT group in VO_2max or pulmonary function measures after testing in normoxic and HH conditions. Although, when percent increase in VO_2max was analyzed there was a significant improvement at HH in the INHT group compared to the NT group. Further, when groups were combined there was a significant increase in VO_2max from pre to post-testing at normoxia and HH, demonstrating the training methods were effective in improving fitness. When comparing the EXP and CON groups overall training distances, pace, and SpO_2 major differences can be noted. Overall the CON group covered 14.4% more distance and trained at a pace 43 seconds faster, but maintained an average SpO_2 of 97.2 compared to the EXP group average of 82.4. These differences are in part due to the way training intensities were established. Running based on MHR was implemented so each group would see an equal relative workload. However, while the heart was accounted for the lungs and skeletal muscle may have seen different workloads between groups. Interestingly, in the CON group an increase of 7.3% was seen in normoxic VO_2max compared to 4.5% in the EXP group. This difference may have been due to the CON group experiencing a greater absolute workload, increasing their fitness to a greater extent. In contrast, the EXP group while training at a lower absolute workload was adjusting to running while breathing in hypoxic air and exercising with greatly decreased SpO_2 levels. These training adaptations may have been responsible for the 15.5% increase in VO_2max at HH compared to 4.2% in the CON group.

Our results are consistent with the Fulco et al. (28) study that found INHT improved time trial performance at hypoxia, but not in normoxia. However, their study only included 6 subjects and was able to achieve these results in only 6 days, but lacked a control group. Furthermore, when looking at percent increase after training, our results are similar to a study by Geiser et al. (30). Their study utilized the same method of INHT at an altitude of 3850 m, but used two different training intensities within the INHT and NT groups. After 6 weeks of training the INHT groups, both high intensity and low intensity, demonstrated a 7.2 % VO_2max improvement in hypoxia, compared to around a 3.0% increase in the NT groups. On the other hand, all four groups improved around 3.5% when tested in normoxia. In addition, Dufour et al. (18) noted an increase in VO_2max at hypoxia with the INHT group compared to the NT group, but also saw a similar increase in normoxia. The authors did not attribute these improvements to an increase in the oxygen carrying capabilities of the blood, but rather beneficial adaptations at a muscular level. In contrast, when only looking at the lack of significant between group and within group results our study is in agreement with Debevec et al. (11) and Ventura et al. (73). Their studies both found a lack of significant improvements due INHT, however Debevec et al. shows similar trends to the current study. As the NT group improved to a greater extent when tested in normoxia, while the INHT group showed greater improvements in hypoxic conditions. Ventura et al. did not show improvements in either group, but this may have been due to a lack of time training in hypoxia, as their training total only included 9 hrs over 6 weeks. Since the current study did not involve blood testing, we conclude the improvement is due to adaptations of the skeletal muscle. In detail, training in hypoxic conditions may cause transformations within skeletal muscle, allowing the muscle to be more efficient at utilizing limited amounts oxygen (18, 30, 61).

Mechanical Pulmonary Function Changes

There were no significant pre to post-test differences in FVC, FEV_1, or PEF due to either INHT or NT. These findings are in disagreement with Nourrey et al. (54) and Fatima et al. (20), yet major differences are seen in study populations from the current study compared to these studies. Nourrey et al. (54) investigated training effects in 24 children. Since the children are still growing their lungs may have more plasticity for adjusting to the stressors of exercise training. Fatima et al. (20) used a much larger population of 292 subjects and stated some variability in their results was due to lack of effort during spirometry. A spirometry test is completely user dependent and it is important each subject is motivated to give maximal effort. Hulke et al. (39) and Thaman et al. (71) also noted significant changes in pulmonary function, but were able to use much longer study designs of 20 weeks and 9 months, respectively. Due to their findings, along with positive findings from other previous studies analyzing the long term effects of training on pulmonary function, led us to believe our study did not have a long enough duration to cause mechanical changes within the pulmonary system. Further strengthening this suggestion are the findings of Debevec's (10) study that found no differences in FVC, FEV_1, and PEF after 4 weeks of rigorous aerobic training.

Hypoxia versus Normoxia

Hypoxic testing conditions did cause a significant decline in VO_2max, FVC, and FVC%, while causing an increase in FEV_1/FVC and PEF in comparison to normoxia. The average decline of 40% in VO_2max was greater than initially expected. However, studies by Lawler et al. (43) and Ferretti et al. (21) were able to demonstrate that highly trained individuals, such as the subjects in our study, demonstrated a greater loss in VO_2max when exposed to acute hypoxia, compared to untrained individuals. Dempsey et al. (13) alluded to the mechanisms that may be responsible for this variability in loss of performance at altitude. Their estimation was a reduction of 5 to 10% for every 304.8 m, based on their concept in which trained endurance athletes are already pushing their respiratory systems towards failure. Therefore, when altitude becomes a factor performance suffers to a greater extent. Whereas, an untrained subject is not stressing the limits of their respiratory system, so altitude does not have as large of an impact. Further, the authors declare the hyperventilatory response to hypoxia leads to increased work for the respiratory muscles leading to fatigue.

Fatigue and an increase in workload for respiratory muscles has been reported as a possible method to explain the decrease in FVC, which was reported in the current study as well as many other studies (22, 23, 47, 60, 65). Another possible explanation for this decrease as stated by Drinker et al. (17), is an enlargement of pulmonary blood vessels. Further supporting this explanation, Welsh et al. (74) noted an acute decrease in FVC after hypoxic exposure that resolved slowly after 48 hours. They also attributed the fall in FVC to an increase in pulmonary blood volume, which slowly decreased over time. Providing other alternative explanations, Mason et al. (47) reported a decrease in FVC, no change in FEV_1, and an increase in PEF during an ascent to 5300m. They proposed a reduction of inspiratory force, subclinical pulmonary edema, increase in pulmonary blood flow, or changes in airway closure were four possible methods causing the decline in FVC. The authors concluded that multiple factors may play a role in the decrease of FVC but the main factor is subclinical pulmonary edema. Due to the increase in PEF, muscular fatigue is unlikely to be the main cause, unless measures were taken post exercise. Subsequently, the current study lacks a chest x-ray or measures of pulmonary blood flow, therefore we are unable to build further on the other two proposed concepts.

Our outcome on FEV_1 of no significant change, but a slight increase at HH is also in agreement with most current studies (7, 29, 47, 60, 75). Forte Jr. et al. (23) was able to report a significant increase in FEV_1, but was surprised by this finding and proposed no reasoning behind the increase. Authors were much more in agreement with the general findings of the literature, stating FEV_1 measures are relatively insensitive to altitude. Since FEV_1 is the flow of the first second, it benefits from the higher PEF, however the later part is influenced by the same mechanisms that affect FVC. Due to FEV_1 being a combination of the two, a slight increase to no change at altitude is largely agreed upon throughout the literature.

As in this study, the common findings of PEF are a significant increase when at altitude (22, 23, 29, 47, 60, 75). The increase in PEF is due to the changes in gas density because of the decrease in barometric pressure. Dawson and Elliot (9) were able to come up with a formula to predict PEF change based on the difference in barometric pressure : $PEF = \sqrt{(SLBP/ABP)}$. SLBP represents the sea-level barometric pressure and ABP is the barometric pressure at altitude. However, it has been found this formula only works in ideal, identical conditions at sea-level and altitude. In actual mountain ascent situations there is typically a decrease in temperature as the environment normally becomes much colder with an increase in altitude. The cold environment causes an increase in airway obstruction that may counteract the reduction in gas density. Despite, environmental factors, PEF remains highly agreed upon throughout numerous studies and is the only predictable variable tested.

Military Applications

The current study revealed encouraging results for the implication of INHT training into normal military training. While mostly ineffective at sea-level, increased endurance at altitude would be very beneficial for quick personal deployment into a high altitude region. Due to the increased effort required to exercise in hypoxic conditions care must be taken not to overtrain participants. This was the reasoning behind our 6 week training plan. By using low intensities the first two weeks participants were able to become accustomed to the mask and decrease in available oxygen. However, undertraining is just as ineffective, therefore at weeks 3 and 5 intensity was increased. We believe this method of slowly building up training intensity is more effective than setting one intensity for the entire training period as seen in other studies (11, 73). Further supporting this model are the positive results from the study by Dufour et al. (18), which also increased intensities midway through training. Additionally, care must be taken not to interfere or reduce their normal training load outside of INHT. Modifying all training just before being deployed may cause more detrimental outcomes rather than benefits. It is important to remember this is supplemental training not the centerpiece.

While spirometry measures proved to be unaffected by 6 weeks of training under either training conditions, differences at simulated altitude compared to sea-level may be beneficial for evaluating AMS and acclimatization. As reported, FVC and PEF were significantly affected by the change from sea-level to simulated altitude. Multiple studies have found a significant correlation between AMS symptoms and decreased PEF scores (60, 69). This was explained by Pollard et al. (60) as a reduction of effort due to the symptoms that occur with AMS. Thus, a quick spirometric measure may be able to detect the severity of AMS an individual is experiencing. Furthermore, considering one of the main reasons FVC is decreased at altitude is thought to be subclinical pulmonary edema, hence FVC may be more beneficial at detecting AMS or HAPE. This is backed by findings from the study by Fischer et al. (22), where one of their subjects suffered from HAPE. The subject showed a 51% reduction of FVC from baseline when suffering from HAPE, however after residing in low altitude for two days HAPE normalized and FVC increased to 95% of baseline. This is only one case, but due to the severity of HAPE and the simplicity of spirometry, a reduction of FVC may serve as an early indicator for impending pulmonary edema. Further support for this concept are results from a study by Welsh et al. (74) that found FVC returns to near normal levels after 48-72 hrs at altitude.

Limitations and Future Studies

A major limitation is the lack of participants completing the study, which greatly reduces the statistical power. While there was an abundant number initially willing to participate, once they learned of the time commitment many dropped out of the study despite being interested. Although it was only three hours a week, our population of current students participating in ROTC were likely to already have full schedules. The other studies similar to ours were able to have anywhere from 12 to 18 subjects (11, 18, 73). Future studies, as hard as it may be, must aim for 18-20 subjects in order to detect changes with greater precision.

This study was the first of our knowledge to incorporate a sham treatment, allowing both groups to train while wearing masks and completely unaware of which condition they were in. Wearing a mask while training can be awkward, uncomfortable, and may restrict breathing. Therefore, accounting for this we felt this eliminated an important confounding variable. Additionally, this was able to limit any placebo effect the participants may have felt. Future studies should implement similar methods in order to control for these dissimilarities, increasing the probability that changes in measured variables were caused by INHT alone.

Future studies focusing on changes to FVC and PEF after being exposed to altitude are needed to determine the process of pulmonary acclimatization. Studies would need to be longer in duration in order to track the time at which FVC approaches baseline measures. However, if there is strong correlation, spirometry may be a quick way to estimate ones level of acclimatization and risk for adverse symptoms.

Conclusions

The results of the current study showed that an INHT protocol of varying intensities spanning 6 weeks was able to provide a significant percent increase in VO_2max for the EXP group at HH, but not at normoxia. However, spirometric measures were unaffected by either training method at HH or normoxia. FVC and PEF were affected by the change from sea-level to simulated altitude, possibly proving to be beneficial in detecting early indications of AMS or HAPE. The present study was also able to provide an effective training design for introducing INHT into regularly scheduled outside physical training. Lastly, a unique method in limiting the difference in opposing experimental groups was carried out, by suggesting that all subjects wear masks while training.

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APPENDICES

Oklahoma State University Institutional Review Board

Date:	Monday, July 20, 2015
IRB Application No	ED15101
Proposal Title:	The effects of normobaric hypoxic training on hypobaric performance
Reviewed and Processed as:	Expedited

Status Recommended by Reviewer(s): Approved Protocol Expires: 7/19/2016

Principal Investigator(s):

John Sellers	Taylor Monaghan	Bert Jacobson
226 Hartford Street	1001 W Will Rogers Dr	180 CRC
Stillwater, OK 74078	Stillwater, OK 74075	Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1.Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval. Protocol modifications requiring approval may include changes to the title, PI advisor, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms
2.Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3.Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of the research; and
4.Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Dawnett Watkins 219 Scott Hall (phone: 405-744-5700, dawnett.watkins@okstate.edu).

Sincerely,

Hugh Crethar, Chair
Institutional Review Board

Oklahoma State University Institutional Review Board

Date:	Tuesday, August 11, 2015	Protocol Expires:	7/19/2016
IRB Application No:	ED15101		
Proposal Title:	The effects of normobaric hypoxic training on hypobaric performance		

Reviewed and Processed as: Expedited

Modification

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s):

John Sellers	Taylor Monaghan	Bert Jacobson
226 Hartford Street	1001 W Will Rogers Dr	180 CRC
Stillwater, OK 74078	Stillwater, OK 74075	Stillwater, OK 74078

The requested modification to this IRB protocol has been approved. Please note that the original expiration date of the protocol has not changed. The IRB office MUST be notified in writing when a project is complete. All approved projects are subject to monitoring by the IRB.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

The reviewer(s) had these comments:

Modification to use the Astrand-Saltin protocol instead of the Bruce protocol and to add a spirometry test.

Signature :

Hugh Crethar, Chair, Institutional Review Board

Tuesday, August 11, 2015
Date

Dear Potential Participant,

My colleagues and I are currently interested in investigating the effects of intermittent hypoxic training on hypobaric performance. In other words, we are interested in learning about the efficacy of simulated altitude exercise training on aerobic performance at altitude. We are doing so with the hope of gaining more insight in this area in order to assist those responsible for the physical training programs in the U.S. Armed Forces.

This study will involve the completion of the following testing procedures:

- Once you have read through and signed the informed consent document you will be asked to schedule a day to begin the study. Once you provide your consent you will be scheduled to participate.

- Depending on the group you are randomly selected to participate in, you will be asked to attend testing on either four or five separate days, the pre-assessment testing days, the

- Post-assessment testing days approximately 6 weeks following the initial testing day, and if you are selected to participate in the experimental group, the final testing day approximately 2 weeks following the post-assessment testing day.

On the first day of both the pre- and post-assessment testing days you will be asked to complete the following at the OSU Health & Human Performance Laboratory located in the Colvin Recreation Center:

- Complete height, weight, and body composition (7-site skinfold) testing
- Complete a 5 minute warm-up on a stationary bicycle
- Complete a spirometry test.
- Complete a maximal oxygen uptake (VO2max) treadmill test. This test consist of 2-minute stages with increases in intensity every stage until exhaustion is reached and the participant ends the test.
- Complete fingertip point-of-care (POC) blood lactate measurements immediately and 5 min after completion of VO2max test
- Complete a blood draw performed by an Oklahoma State University Health Sciences Center medical professional

On the second day of both the pre- and post-assessment testing days you will be asked to complete the following at the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric Medicine:

- Complete a 5 minute warm-up on a stationary bicycle
- Complete a spirometry test.
- Complete a maximal oxygen uptake (VO2max) treadmill test in a simulated altitude chamber. This test consist of 2-minute stages with increases in intensity every stage until exhaustion is reached and the participant ends the test.
- Complete fingertip point-of-care (POC) blood lactate measurements immediately and 5 min after completion of VO2max test

On the fifth testing day, which will only apply if you are selected to participate in the experimental training group, you will be asked to complete the following at the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric Medicine:

- Complete a blood draw performed by an Oklahoma State University Health Sciences Center medical professional
- Complete a 5 minute warm-up on a stationary bicycle
- Complete a spirometry test.
- Complete a maximal oxygen uptake (VO2max) treadmill test in a simulated altitude chamber. This test consist of 2-minute stages with increases in intensity every stage until exhaustion is reached and the participant ends the test.
- Complete fingertip point-of-care (POC) blood lactate measurements immediately and 5 min after completion of VO2max test

If you decide to participate, and you complete all phases of this research study, you will be compensated in the amount of $100. If you are willing to participate in the aforementioned study, please email me indicating your willingness to participate.

Thank you in advance for your time.

Sincerely,

John Sellers

(918) 625-9945

john.sellers@okstate.edu

Taylor Monaghan

(918) 520-8954

taylor.monaghan@okstate.edu

RESEARCH PARTICIPANT CONSENT FORM

Project Title: The effects of normobaric hypoxic training on hypobaric performance.

Investigators: John Sellers, Health & Human Performance, Oklahoma State University

Taylor Monaghan, Health & Human Performance, Oklahoma State University

Dr. Bert Jacobson, Health & Human Performance, Oklahoma State University

Purpose:

The primary aim of the research project will be to determine the efficacy to improve maximal aerobic capacity in well-trained individuals in a hypobaric hypoxia setting using normobaric hypoxia intermittent hypoxia training (IHT). A secondary aim of this investigation will be to determine if the proposed intervention protocol (6 wk x 1 hr x 3 d per week) is sufficient to elicit significant changes in the oxygen carrying capacity measures in the blood. Another purpose of this study will be to determine if significant changes in body composition as evaluated through skinfold measurements occur as a result of IHT when compared to the control group.

Procedures:

The tasks required if you volunteer for this study are to:

- Once you have read through and sign this informed consent document you will be scheduled to participate.

Upon granting consent to participate in this study, you will perform the initial base-line testing. Testing will take place in either the Applied Musculoskeletal and Human Physiology Research Laboratory in 192 Colvin Recreation Center at Oklahoma State University or the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric Medicine. As part of the pre-screening process, you will complete the following:

- Height and weight measurements
- A 7-site skinfold measurement test to analyze body composition
- A blood draw performed by an Oklahoma State University Health Sciences Center medical professional
- Complete a spirometry test.
- Complete a maximal oxygen uptake treadmill test (aka VO2max test). This test consist of 2-minute stages with increases in intensity every stage until exhaustion is reached and the participant ends the test
- Complete fingertip point-of-care (POC) blood lactate measurements immediately and 5 min after completion of VO2max test

- In order to be considered for continued participation in the study, participants will have achieved a VO2max of at least 49.2 ml/kg/min. As there is a minimum fitness standard that must be met prior to participation in the training intervention, it is possible that you will complete the first day of pre-testing at the Applied Musculoskeletal and Human Physiology Research Laboratory and will not meet the minimum standards and will thus no further participation will be asked of you. If you meet the VO2max requirement from the initial pre-tests, you will then perform the same VO2 treadmill test in an altitude chamber at the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric Medicine. If selected for participation, you will be asked to maintain your current dietary and sleeping habits as well as complete weekly physical activity logs.

Upon selection to this study, you will then perform a treadmill ergometer training protocol, with the control group performing the training sessions in a normoxic (normal partial pressure of Oxygen in the air) setting and the experimental group will perform all training sessions in normobaric hypoxia (simulated altitude of 3,000 m). The training intervention will be 6 weeks in duration, with 3 1-hr training sessions per week. Each training session for all participants will consist of a 10-min warm-up at approximately 50% of the participant's pre-screening VO2max, a 40-min interval workout between 60-80% of the participant's pre-screening VO2max, and a 10-min cool-down at approximately 40-50% of the participant's pre-screening VO2max. There will be a 24-48 hr recovery period between each training session. All training sessions will take place in the Oklahoma State University Applied Musculoskeletal and Human Physiology Research Laboratory in 192 Colvin Recreation Center under the supervision of the primary investigator or other OSU Health & Human Performance graduate assistants who have completed the necessary CITI Responsible Conduct of Research Training.

Upon the completion of the 6-wk intervention period, you will perform post-testing assessments. Post-testing will take place in either the Applied Musculoskeletal and Human Physiology Research Laboratory in 192 Colvin Recreation Center at Oklahoma State University or the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric Medicine. As part of the post-testing process, you will complete the following:

- Weight measurement
- A 7-site skinfold measurement test to analyze body composition
- A blood draw performed by an Oklahoma State University Health Sciences Center medical professional
- Complete a spirometry test.
- Complete a maximal oxygen uptake treadmill test (aka VO2max test) in both the Oklahoma State University Applied Musculoskeletal and Human Physiology Research Laboratory in 192 Colvin Recreation Center and the altitude chamber at the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric

Medicine. This test consist of 2-minute stages with increases in intensity every stage until exhaustion is reached and the participant ends the test.

- Complete fingertip point-of-care (POC) blood lactate measurements immediately and 5 min after completion of VO2max test

If you are selected to participate in the experimental training group, you will be asked to return approximately two weeks after the completion of the post-testing sessions. During this fifth and final testing day, you will be asked to complete the following:

- A blood draw performed by an Oklahoma State University Health Sciences Center medical professional

- Complete a spirometry test.

- Complete a maximal oxygen uptake treadmill test (aka VO2max test) in the altitude chamber at the Oklahoma State University Center for Health Sciences Center for Aerospace and Hyperbaric Medicine. This test consist of 2-minute stages with increases in intensity every stage until exhaustion is reached and the participant ends the test.

- Complete fingertip point-of-care (POC) blood lactate measurements immediately and 5 min after completion of VO2max test

Risks of Participation:

As this study will be utilizing highly-trained individuals, the risks associated with the study are minimal and with no greater physical demands than your current exercise program. In case of injury or illness resulting from this study, emergency medical treatment will be available; responders are CPR – 1st responder certified along with access to 911 will be available. No funds have been set aside by Oklahoma State University to compensate you in the event of illness or injury.

Benefits:

Participants will benefit from being able to see if increases in aerobic capacity occur as a result of completing 6 weeks of intermittent hypoxic training. Participants will also be compensated in the amount of $100 if they complete all phases of this study.

Confidentiality:

Due to multiple testing sessions to be completed during various times over more than 2 months, all testing results will be tracked by a random subject ID number issued to each participant. The subject ID number will only be known by the primary investigator and the respective participant. Upon completion of the testing period subject ID numbers will be destroyed immediately. Only aggregate data will be reported. Aside from the original data all references will only contain subject ID number references as each participant will be assigned an ID number. We are interested in reporting data reflective of how the group(s) as a whole responded to the intervention program. Only aggregate data will be published. No individual data will be published. Testing results will not be shared with supervisors of any nature. Research records will be stored securely for 3 years and only researchers and individuals responsible for research oversight will have access to the records. These forms will be kept in a locked file cabinet in the Applied Musculoskeletal and Human Physiology Laboratory which only the researchers will have access to. The signed consent forms will be kept for 3 years after the research is complete per federal guidelines.

Compensation:

Financial compensation in the amount of $100.00 will be provided to each participant that completes all phases of this research study; further, individual results of the study can be obtained by all participants following analysis by contacting John Sellers at john.sellers@okstate.edu.

Contacts:

If you need additional information concerning the study contact advisor Dr. Bert Jacobson, 101 CRC, Oklahoma State University, Stillwater, OK 74078, 405-744-2025, bert.jacobson@okstate.edu, or John Sellers, 192 CRC, Oklahoma State University, Stillwater, OK 74078, 405-744-9373, john.sellers@okstate.edu.

If you should have questions about your rights as a research volunteer, you may contact Dr. Hugh Crethar, IRB Chair, 223 Scott Hall, Oklahoma State University, Stillwater, OK 74078, 405-744-3377 or irb@okstate.edu.

Participant Rights:

Participation in this research is voluntary and there is no penalty for your refusal to participate. You are free to withdraw from the study at any time and revoke your consent to participate at any time without penalty.

Signatures:

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy of this form has been given to me.

____________________________ ______________

Signature of Participant Date

I certify that I have personally explained this document before requesting that the participant sign it.

____________________________ ______________

Signature of Researcher Date

VITA

Taylor Patrick Monaghan

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF INTERMITTENT NORMOBARIC HYPOXIC TRAINING ON PULMONARY FUNCTION AT NORMOXIA AND HYPOBARIC HYPOXIA

Major Field: Health and Human Performance

Biographical:

Education:

Completed the requirements for the Master of Science in Health and Human Performance, option in Applied Exercise Science at Oklahoma State University, Stillwater, Oklahoma in December, 2015.

Completed the requirements for the Bachelor of Science in Physiology at Oklahoma State University, Stillwater, Oklahoma in December, 2013.

Experience:
Graduate Research Assistant, Oklahoma State University
January 2013 - December 2015

Professional Memberships:
American College of Sports Medicine