THE ACUTE AND CHRONIC EFFECTS OF AN ELEVATION TRAINING MASK ON AEROBIC CAPACITY, ANAEROBIC ENDURANCE, AND PULMONARY FUNCTION

A Thesis

by

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ABSTRACT*

Elevation training masks (ETM) have become popular in professional & recreationally active populations to enhance performance via purported adaptations associated with high elevation training (HET) and respiratory muscle training (RMT). The purpose of this study was to compare the effect of training with (TM) to without (CON) wearing the ETM. 22 healthy recreationally active adults (TM: M=5, F=6; 27.64±0.86 yr; 23.17±0.88 kg·m^{-2} | CON: M=5, F=6; 29.91±1.63 yr; 24.75±1.03 kg·m^{-2}) were recruited & provided consent for this study. VO_2max and time to exhaustion (TTE) were assessed (Bruce protocol GXT, with (M-GXT) & without (U-GXT) ETM). Anaerobic endurance was assessed using two consecutive 300-yard shuttle sprints (separated by 5min). Pulmonary function was assessed using a metabolic cart (FVC, MVV, FEV1). Following group assignment (TM and CON), subjects trained 3d/week for 12-weeks alternating between steady state running (Progression: 65->85% VO_2max, 30->45min) and intense sprint conditioning every other session with VO_2max reassessment following week 6. The TM group performed all sessions wearing the ETM at manufacturer reported simulated altitude of 9,000 ft. A (2)group x (2)time ANCOVA followed by a Tukey's post-hoc test was used to detect within group and between group differences following training. Type I error set at α=0.05.

Results indicate that CON displayed significantly greater ($p<0.05$) improvements in VO_2max (CON=↑6.8±1.2ml•kg^{-1}•min^{-1} | TM=↑4.3±1.0ml•kg^{-1}•min^{-1}), U-GXT TTE (CON=↑83±9sec. | TM=↑63±7sec.) and unmasked sprint performance

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(CON=↓8.43±1.3sec. | TM=↓4.66±0.9sec.). Additionally, %Fat decreased significantly more ($p<0.05$) among CON subjects (CON=↓2.2±0.5% | TM=↓1.0±0.3%). There was no significant change between groups in pulmonary function.

Training with the ETM does not enhance either aerobic or anaerobic endurance beyond standard training and may produce adaptations that are less favorable in comparison. However, under conditions of restricted breathing (i.e. GXT performed while wearing the ETM), the TM group showed greater improvement. While the ETM may not provide benefits to those whose breathing is not typically restricted, further study is required to determine if there may be adaptive benefits for those who typically perform under restricted breathing conditions.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Acute Physiology of Running Exercise

Before beginning the topic of training with a mask purported to mimic altitude, the effects of normal training, specifically endurance running or sprint-based training, must be explored. During exercise, the organ systems must coordinate to provide adenosine triphosphate (ATP) for both endurance and strength. This process can be performed aerobically (oxygen-dependent), or anaerobically (oxygen-independent). Furthermore, the accumulation of metabolic byproducts must be avoided to sustain performance. Acutely, the body must rapidly respond to immediate energy demands of exercise. Chronically, the body must make physiologic adaptations to manage the constant strain for energy. The following is an overview of these processes.

Respiratory Effects

Respiration is one important component of ATP production for exercise. To maintain energy homeostasis, the flow and transport of oxygen-rich air must be intact. During exercise, central and peripheral mechanisms (detailed below) lead to the contraction of the diaphragm, intercostal, and abdominal muscles [1]. These muscles produce an increase in thoracic volume which creates a negative pressure within the lungs. Oxygen-rich air flows through the mouth and nasal cavity, into the respiratory tree, ending in millions of air-sacs called alveoli. The alveolar epithelial cells, basement membranes, and the endothelial cells of the surrounding pulmonary capillaries serve as the exchange membrane for blood and alveolar gasses. Sufficient exchange of

gasses between the pulmonary capillaries and the atmosphere is essential for exercise, particularly aerobic training [1].

At the exchange membrane, a diffusion gradient between alveolar and blood gasses produces a flow of gasses [2]. This gradient depends on the partial pressures of alveolar and blood gasses. With each expiration, a volume of air remains in the alveoli called the residual volume. This volume is mixed with subsequent inhaled air, causing the alveolar air partial pressure of oxygen (P_{O2}) to be ~13.3 kPa (100.0 mm Hg) at rest, despite a sea level atmospheric P_{O2} of 20 kPa (150 mm Hg) [2]. In contrast, the P_{O2} at rest in the pulmonary arterioles is normally around 5.3 kPa (40.0 mm Hg) [2]. Oxygen travels down its concentration gradient, from the alveolar air to the capillaries. In healthy individuals, oxygenated blood entering pulmonary venules has a P_{O2} equal to that of the alveolar air, or ~13.3 kPa at sea level while resting [2]. Similarly, the partial pressure of carbon dioxide (P_{CO2}) in the alveolar air is slightly greater than atmospheric P_{CO2} due to the mixing of atmospheric air with the residual volume. Despite sea-level atmospheric P_{CO2} of ~0.04 kPa (0.30 mmHg), alveolar air P_{CO2} is ~5.3 kPa (40mmHg) [2]. Pulmonary arteriole CO_2, at ~ 6.0 kPa (45mmHg), diffuses down its concentration gradient into the alveolar air. In health individuals, blood entering the pulmonary venules has a P_{CO2} equal to that of alveolar air, ~5.3 kPa [2]. During exercise, pulmonary arteriole P_{O2} and P_{CO2} are altered due to greater O_2 extraction and CO_2 production by active muscles, increasing the diffusion gradient at the exchange membranes in the alveoli-pulmonary capillary interface and maintaining arteriole O_2 and CO_2 homeostasis [2].

To maintain O_2 supply and CO_2 clearance, ventilation increases during exercise via stimulation by several mechanisms [1]. Prior to initiating exercise, the respiratory control center in the pons of the brain increases the tidal volume in anticipation of the approaching energy

demands [1]. During exercise, respiration is primarily controlled by chemoreceptors throughout circulation [1]. These receptors sense the increases in carbon dioxide and proton concentration, which are metabolic byproducts of exercise [1]. Breathing rate and tidal volume increase in proportion to exercise intensity [1]. After exercise, the body enters a recovery phase during which fuels are restored and energy byproducts are metabolized [1].

To sustain ventilation and tidal volume at increasing exercise intensities, respiratory muscles must be adequately conditioned [3]. Measures of respiratory muscle function include maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and maximal voluntary ventilation (MVV) [3]. MIP is the negative pressure produced during maximum inspiratory effort, while MEP is the positive pressure produced during maximum expiratory effort [3]. MVV is the total volume of air exhaled during a 12 second interval of deep, rapid breathing [3]. MVV is generally compared to a predicted MVV based on the forced expiratory volume in 1 second (FEV_1) [3]. Difference between predicted and actual MVV may indicate respiratory muscle dysfunction [3].

Cardiovascular Effects

The cardiovascular system acts as a connection between the respiratory and muscular systems. Oxygenated blood flows from the lungs to the left side of the heart and is pumped throughout systemic circulation. In anticipation of exercise, heart rate (HR) generally increases due to sympathetic stimulation. As exercise intensifies, HR increases proportionally until a plateau is reached at maximum effort, signifying maximal HR. Combined with HR, stroke volume (SV) increases in response to exercise. Additionally, sympathetic stimulation causes venous constriction, increasing venous return. Sympathetic stimulation also increases ventricular contractility, decreasing end systolic ventricular volume. Contractility is further increased due

the stretching of ventricular walls from increased venous return, known as the Frank Starling mechanism. HR and SV combine to form the cardiac output (Q), which is the volume of blood pumped by the left or right ventricle of the heart in a minute. Like HR and SV, Q increases in proportion to exercise intensity to meet the increased metabolic demands [1].

During the transition from resting state and exercise, systolic and mean blood pressures increase with exercise intensity, while diastolic pressure experiences little change. This increase in blood pressure is primarily due to increases in Q, as outline previously. Additionally, sympathetic stimulation causes vasoconstriction, leading to decreased venous blood volume and decreased visceral organ blood flow. Meanwhile, local metabolites (CO_2, lactate, H^+, inorganic phosphate) cause vasodilation and increased blood flow to activated muscles. These local metabolites are released in response to muscle hypoxia that occurs oxygen is consumed. Another vasodilator, nitric oxide is released in response to excess shear stress that occurs during increased blood pressure. The combination of these local metabolites in muscle lead to an overall decrease in systemic vascular resistance [1].

Muscular System Effects

Exercise intensity and duration determine the recruitment of muscle fibers, ranging from slow oxidative “type I” fibers, to fast glycolytic “type II” fibers [4]. Type I fibers are oxygen dependent and are better suited for endurance exercise, while type II fibers are oxygen-independent (anaerobic) and are designed for the force production of sprint training [4]. General populations have a relative equal balance of fiber types, while sprint and endurance athletes have a higher percentage of type II and type I fibers, respectively [4]. Furthermore, exercise intensity and duration determine the type of energy system used for ATP regeneration. However, the

primary ATP regeneration system being used occurs on a spectrum, meaning the shift among energy systems is gradual and ambiguous [4].

Intense exercise lasting up to ~10 seconds relies on the immediate ATP production of the phosphogen system [5]. ATP is broken down to initiate myosin head cycling, leaving adenosine diphosphate (ADP) and inorganic phosphate (Pi) [5]. An enzyme called creatine kinase (CK) releases energy from cellular phosphocreatine (PCr) [5]. This energy is used to join Pi to an ADP molecule, regenerating ATP for further use[5]. This system is quickly regenerates ATP, but is limited by the amount of PCr and ATP in skeletal muscles [5].

Exercise lasting between ~30 seconds to ~ 90 seconds utilizes glycolysis to regenerate ATP for skeletal muscle [5]. Glucose, stored in muscle as glycogen, is catabolized to form two ATP and pyruvate [6]. When there is insufficient O_2 for oxidative phosphorylation, pyruvate is converted to lactic acid, causing a decrease in pH [6]. This inhibits enzymes and disrupts cellular electrical charges [6]. Lactate is converted to glycogen by the Cori Cycle, or is buffered by the bicarbonate buffering system to form CO_2 and taken through the blood stream to the lungs [6]. However, as the Cori cycle and the bicarbonate buffering systems are exhausted, H^+ levels rise, leading to acidosis [6].

Endurance exercise lasting longer than ~2 minutes relies on oxidative phosphorylation as the predominant energy producing system [5]. Although it is relatively slow, oxidative phosphorylation can produce over 30 ATP molecules from a single glucose molecule [6]. A multistep process known as cellular respiration generates ATP from glucose and involves glycolysis, the Krebs cycle, and oxidative phosphorylation via the electron transport chain [6]. Both the Krebs cycle and the electron transport chain are located within mitochondria of cell [6]. Type I fibers contain a higher concentration of mitochondria compared to type II fibers [6].

During cellular respiration, CO_2 is released and transported by blood to be exhaled in the lungs [6]. Furthermore, O_2 is required for the electron transport chain as the final electron accepter in ATP production [6]. This requirement hinders the use of oxidative phosphorylation when the rate of required ATP is greater than the supply of oxygen. In these circumstances, the phosphogen system and glycolysis must be utilized but are limited as previously described [6].

In addition to stored glycogen, triglycerides may be used as a major energy source for aerobic exercise [6]. Triglycerides are catabolized into free fatty acids and glycerol. Fatty acids are transported to the mitochondria, where acetyl-CoA is produced through beta-oxidation[6]. The acetyl-CoA can continue though oxidative phosphorylation to form ATP [6]. Fat catabolism can form as many as 130 ATP from a single fatty acid, making it a considerable source of energy in endurance events [6].

Predominant fuel substrate utilization can be estimated by calculating the respiratory exchange ratio (RER), calculated by dividing the rate of CO_2 expired (VCO_2) by the rate of oxygen consumed (VO_2) during exercise [1]. When glucose is used as the fuel source, six molecules of oxygen are used to produce six CO2 molecules:

$$6\ O_2 + C_6H_{12}O_6 = 6\ CO_2 + 6\ H_2O + 32\ ATP$$

By comparing the amount of O_2 consumed to the amount of CO_2 released, the RER can be calculated to be 1.0. Due to their larger number of carbon and hydrogens, while having less oxygen, the RER from free fatty acid utilization approaches 0.71 [1]. Additionally, RER is affected by lactic acid accumulation as anaerobic mechanisms of energy production are utilized during more intense exercise regiments [1]. CO_2, used as a buffering agent, is exhaled disproportionate to O_2 consumption, causing RER to exceed 1.0 [1].

Oxygen Delivery

Oxygen must be transported through the blood stream to the mitochondria within active muscle cells to be used in oxygen phosphorylation [7]. Among other factors, the oxygen carrying capacity of blood and the diffusion of oxygen from blood to muscle determine the effectiveness of oxygen delivery [7]. Due to oxygen's low plasma solubility, it must be carried by hemoglobin within RBCs [7]. For this reason, hemoglobin concentration and hematocrit are major determinants of the oxygen carrying capacity of blood [7].

Once transported to active muscles, oxygen must be transferred from hemoglobin within RBCs to myoglobin within myocytes. Myoglobin has a greater oxygen binding affinity than hemoglobin, allowing the transfer of oxygen to myoglobin [7]. Furthermore, as CO_2 increases during cellular respiration, it forms bicarbonate and protons, decreasing the pH. This reduces the oxygen binding affinity of hemoglobin, allowing further oxygen transfer to exercising muscle for aerobic respiration [7]. In the lungs, oxygen binding of hemoglobin causes the release of protons, which react with the previously formed bicarbonate to form CO_2 to be exhaled [8] . These events combine for the homeostasis of blood pH and for efficient exchange of oxygen and CO_2 in the tissues and the lungs [8].

Aerobic Capacity

As an assessment of maximum aerobic capacity, VO_2max (mL/kg/min) is a large component of aerobic fitness [9]. VO_2max is dependent on several elements, including pulmonary diffusion capacity, O_2 carrying capacity, muscle diffusion capacity, mitochondrial enzymes, and capillary density [9]. Therefore,VO_2max correlates with the capability of these elements and determines the efficiency of O_2 uptake, delivery, and utilization for physical

activity involving oxidative phosphorylation [9]. As previously explained, oxidative phosphorylation, though present at all exercise durations, is the largest component of energy supply in durations longer than ~2 minutes [5]. Additionally, VO_2max is an element in sports composed of combination of power and endurance components, like football, soccer, lacrosse, basketball, tennis, boxing, and many others [9].

Anaerobic Threshold

While aerobic capacity is an indicator of endurance performance, of similar importance is a person's ability to maintain activity at a high percentage of their VO_2max. To sustain performance, metabolic acidosis due to lactate and CO_2 accumulation must be attenuated [9]. The point at which the rate of acid buildup is markedly increased is known as the anaerobic or lactate threshold [9]. Corresponding to this point, there are abrupt increases in ventilation (ventilatory threshold), end tidal oxygen, and expired fraction of oxygen (FEO_2) [9]. This marks the onset of muscle hypoxia, which causes a decrease in oxidative phosphorylation and a compensatory increase in glycolysis to sustain ATP production [9]. However, decreased stored glycogen and pH limit the duration of performance at intensities greater than anaerobic threshold [9].

As exercise intensity increases, ventilation increases linearly until the events at the anaerobic threshold lead to a nonlinear increase in H^+ concentration and exhaustion of the bicarbonate buffering system [9]. The H^+ concentration stimulates an increase in ventilation greater than the initial linear relationship would predict to increase the exhalation of CO_2 (VCO_2) [9]. This helps to compensate for the inadequate bicarbonate buffering system [9]. Ventilation increases at a greater rate than alveolar oxygen diffusion, causing the FEO_2 to increase [9]. Therefore, assessing the changes in gas-exchange ratios is a method to assess anaerobic threshold

[9]. Additionally, VO_2 at anaerobic threshold, velocity at anaerobic threshold, and velocity at the onset of blood lactate accumulation have been used as assessments of aerobic performance [9]. Accordingly, increases in both VO_2max and anaerobic threshold are key variables to increase running performance [9].

Chronic Adaptations to Running Exercise

Aerobic training lead to adaptations that allow more efficient oxygen delivery to muscles. The magnitude of these changes is dependent on the intensity, duration, length, and mode of training [10]. Additionally, the initial fitness of a person will affect the magnitude of possible improvement [10]. Lastly, genetics play a role in determining the maximum degree of adaptations [11].

Respiratory Adaptations

Prolonged periods of aerobic training are associated with adaptations to the lung capacities and respiratory muscles to support the demands that are placed [12]. The total lung capacity is increased over time, allowing potential for larger gas exchange as more air can flow in and out of the lungs [12]. Respiratory muscles adapt by increasing endurance, increasing the ability to breath during longer duration exercises [12]. The above adaptations lead to increased maximal ventilation allowing greater oxygen binding of hemoglobin for transport to activating muscles [12]. Furthermore, the adaptations to skeletal muscles lead to enhanced oxygen extraction from circulating blood [11]. This causes a larger oxygen gradient between the pulmonary capillary blood and the atmosphere, facilitating gas exchange [11].

Since ventilation likely limits anaerobic performance to a lesser degree when compared to aerobic performance, lung adaptations may be less pronounced. However, future investigation is needed to assess this.

Cardiovascular Adaptations

Cardiac myocytes respond to the challenges of exercise with hypertrophy, increasing contractile potential of the heart [11]. Furthermore, hematological adaptations lead to increases in plasma volume, increasing availability of blood for return to the heart [11]. As ventricles receive this increased volume, they are stretched, increasing the internal dimensions of the ventricles and contributing to contractility via the Frank Starling mechanism [11]. The combination of increased contractility and increased ventricular filling leads to increased SV at rest and during all stages of exercise [11]. HR decreases at rest and at submaximal exercise intensities, causing Q to remain constant at these intensities [13]. However, maximum HR remains constant, allowing Q to increase at maximum exercise intensities following adaptation to training [11].

There is limited literature on the chronic cardiovascular effects of anaerobic or sprint-based exercise. It is suspected that cardiovascular adaptations are depend on the magnitude and duration of increased heart rates during exercise [11]. Further, since less skeletal muscle vascularization occurs during anaerobic training, it is suspected that peripheral vascular resistance and blood pressures may remain unchanged [11].

Muscular System Adaptations

Aerobic exercise causes metabolic and structural adaptations in the muscles involved in training. Mitochondrial size and content increases, allowing greater oxidative phosphorylation potential [11]. Mitochondrial enzymes are adapted to increase capacity to oxidize fatty acids, ketones, and pyruvate [11]. Furthermore, there is an increased ability to store glycogen to be used in glycolysis [11]. However, the enhanced fatty acid oxidation ability allows glycogen stores to be reserved while fat is used as an energy source [11]. The chronic adaptations from

sprint-based training is slightly different. Sprint-based training generates adaptations that increases enzymes involved in the phosphogen and glycolytic pathways [11]. Although to a lesser degree compared to aerobic training, mitochondrial enzymes are increased after chronic anaerobic training despite the reduced role of oxidative phosphorylation and beta oxidation [11].

As previously explained, type I muscle fiber types are more suitable for aerobic activity. The demands of chronic aerobic exercise lead to an increase in cross-sectional area of type I fibers, which have increased mitochondrial content, high capillary density, high oxidative capacity, and are more fatigue resistant when compared to type II fibers [14]. Contrasting to endurance training, sprint-based training involves a shift to type II muscle fibers [4]. Compared to aerobic training, there is an increase in muscle cross-sectional area with corresponding increases in sarcoplasmic reticulum volume to allow the release of calcium into the thicker muscles [4]. As expected, the conduction velocity of muscle is increased with increases in type II muscle fibers and sarcoplasmic reticulum volume, allowing quicker muscle activation [4].

Oxygen Delivery

The volume percentage of RBCs, or hematocrit, is increased through erythropoietin (EPO) production [15]. In adults, EPO is synthesized by interstitial fibroblasts of the renal cortex [15]. The location of these fibroblasts near peritubular capillaries allow hypoxemia to induce production of EPO, which stimulates RBC production in the bone marrow [15]. This hypoxemia may be caused by a high oxygen demand, low oxygen supply, or both [16]. Oxygen demand is increased during strenuous aerobic exercises in which oxygen is extracted from the blood into muscle for oxidative phosphorylation [16]. Oxygen supply is decreased in hypoxic environments (high elevation) or by an obstruction of oxygen from the atmosphere to the alveolar sacs (asthma, chronic obstructive pulmonary diseases, pneumonia, etc.) [15]. The effects of anaerobic exercise

on production of EPO are not well documented, but are suspected to be less correlated compared to aerobic exercise [15].

Coupled with the increased oxygen-carrying capacity of blood, aerobically trained muscle has augmented oxygenation capacity. Myoglobin content is increased, allowing for more oxygen storage [17]. Additionally, increased capillary density allows improved blood flow throughout the exercising muscle [11]. These adaptations lead to greater oxygen extraction from blood to myoglobin, and greater oxidative phosphorylation capacity of aerobically trained muscle [11]. As previously described, this increase in oxygen extraction leads to an increase oxygen diffusion gradient in the lungs, facilitating diffusion of oxygen in the alveoli [11].

Aerobic Capacity

It has been well established that chronic aerobic training is correlated with dose-dependent increases in VO_2max [9]. This is due to the combination of the previously mentioned adaptations, involving Q, ventilation capabilities, oxygen carrying capacity of blood, and oxygen extraction by muscles [9]. Though there are other factors determining VO_2max, the rate limiting element of aerobic capacity is speculated to be oxygen delivery, not oxygen extraction by muscle [9]. This is due to the capability of the remaining factors in exceeding the oxygen delivery potential [9]. The oxygen delivery system may be increased through previously mentioned mechanisms [9]. Despite the importance of oxygen delivery, inadequacy in oxygen transport at any stage will reduce VO_2max [9]. Nevertheless, all stages of oxygen transport should be addressed when looking to increase aerobic capacity.

Sprint-based training has been found to improve VO_2max to a similar degree to endurance training [18]. The physiologic mechanisms leading to improvements in VO_2max from sprint-based training is not well documented [18]. Additionally, the translation of increased

VO_2max to improved aerobic or anaerobic performance depends on the integration of many other factors, including but not limited to anaerobic threshold, strength and power output, running economy, and specificity of training [18].

Anaerobic Threshold

In conjunction with increasing VO_2max, increasing anaerobic threshold is desired for performance purposes due to the previously explained relationships of anaerobic threshold and sustained performance [9]. These adaptations occur by training near or above one's anaerobic threshold, and include changes in muscle ultrastructure, capillarization, oxidative capacity, and substrate utilization [9]. These adaptations lead to decreased lactate accumulation and decreased glycogen depletion at equal power outputs, allowing aerobic performance intensity to be increased and aerobically sustained [9]. Therefore, training intensity should be considered when designing a training program due to its link to improvements in aerobic performance through increases in the anaerobic threshold [9].

Physiology Of Elevation Training

The general goal of training at elevation is to induce additive physiological adaptations associated with both exercise and altitude, leading to performance improvements. This concept has been accepted and applied by professional and recreational athletes [19]. However, the precise effects of training at high elevations are vague. Though there are benefits to hematological variables noted previously [15], it is important to assess the effects of other limiting factors of performance [9]. Aerobic performance improvement is restrained if there are discrepancies anywhere along the pathway of oxygen from the environment, through the pulmonary, circulatory, or skeletal muscle systems [9]. Within these systems, appropriate

improvements of Q, mitochondrial enzyme levels, capillary density, and anaerobic threshold must be maintained with the improvements in oxygen carrying capacity brought about by the effects of elevation training [9]. Additionally, training specificity for a sport is worthy of attention. It has been found that exercise designed to improve performance in a sport should be structured like that particular sport [20]. Previously explained adaptations in molecular, morphological, and capillary characteristic occur primarily in the activated muscles during exercise [20]. Likewise, research on increasing performance should be designed so that it is applicable to specific sports, while maintaining controlled variables (diet, environmental factors, exercise intensity, etc.) to preserve internal validity. This may allow better application of the determined concepts into specific training programs. The literature on the physiological effects of elevation and elevation training is examined below.

Acute and Chronic Elevation

The physiological effects of increased elevation can be attributed to decreases in partial pressures of oxygen [21]. This causes a decreased alveolar oxygen availability, leading to a decreased oxygen saturation of hemoglobin. Beginning at altitudes of 1500 m, peripheral chemoreceptors sense hypoxia, which induces an increase in ventilation to counteract the low atmospheric oxygen content [21]. Accordingly, hypocapnia and alkalosis lead to an eventual reduction in ventilation and increase renal bicarbonate excretion to restore pH [21].

Systemically, the increased secretion of bicarbonate decreases plasma volume, which decreases stroke volume [22]. However, there is an initial increase in HR, Q, and blood pressure [22]. Within a week, normalization of HR, Q, and blood pressure occurs with acclimatization[21]. At sea level, the lungs respond to localized hypoxic regions with localized vasoconstriction to shunt blood from hypoxic to oxygen-rich regions [22]. However, global lung

hypoxia leads to global pulmonary hypertension, which may be exacerbated with exercise [22]. Prolonged hypoxia leads to fibrosis and angiogenesis through molecular mechanisms [22]. This can create chronic pulmonary hypertension that may lead to additional cardiac stress [22]. However, cardiovascular disease has not been shown to result from this added stress [22].

Similar to pulmonary vascular changes, chronic hypoxia induces compensatory systemic vascular changes [21]. To supply oxygen to tissues, angiogenesis occurs through stimulation of hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) production [21]. Additionally, mitochondria and myoglobin have been found to increase in response to chronic altitude exposure, facilitating O_2 extraction by tissues [21].

In the blood, changes occur to augment tissue oxygenation despite low blood oxygen levels. Within hours there is an increased release of EPO from the kidneys, which will lead to chronic increases in red cell volume and hemoglobin [22]. Furthermore, hematocrit increases acutely due to the previously explained decreases in plasma volume, and chronically due to increases in red cell volume [23]. To allow better extraction of oxygen from blood to tissue, 2, 3 diphosphoglycerate (2-3, DPG) is increased, which causes an effect similar to the Bohr Effect explained previously [21]. Much like the effects of CO_2 and H^+, the affinity of oxygen to hemoglobin is decreased by 2-3, DPG, allowing more efficient tissue oxygenation [21].

Elevation Training

The aerobic effects of altitude training have been researched with fluctuating results depending on living/training elevation, and on baseline performance values. VO_2max has been found to increase when living at high altitudes (2500m), regardless training altitude [24]. However, maximal steady state VO_2 (indicator for ventilatory and anaerobic threshold) and velocity at VO_2max were found to have greater improvements in subjects who trained at lower

sea-level while living at high altitudes [24]. One study found that training and living at high elevation (2650m) did not provoke improvements [25]. However, the subjects' mean baseline VO_2max in this study was much higher, at 81.4 ml/kg/min, compared to the study by Levine et. al, which had a mean baseline of less than 65 ml/kg/min. The subjects with a high baseline VO2max may have already attained their natural physiologic maximum, limiting the opportunity for improvements. For comparison, it has been found that high altitude training, coupled with low altitude living, caused an increase in VO_2max when tested at high altitude [26], but not when tested at low altitude [26-28]. It is likely that short term hypoxic exposure does not lead to adequate physiologic responses that increase VO_2max.

To parallel the improvements of VO_2max, it was found that altitude living resulted in increases in red cell mass [24], likely due to erythropoietin production in response to altitude. However, as was found with VO_2max, training and living at high elevation was found to have no change in RBC production despite prolonged altitude exposure [25]. Again, this was likely due to the high baseline value, with initial hemoglobin measurements being 14.7 g/kg [25]. Subsequent investigations have found results that support the initial finding that prolonged altitude exposure with altitude training have a positive effect on hemoglobin mass, as well as the expected increase in hematocrit and EPO production [29]. When training in hypoxic altitude conditions, while living in sea-level conditions, it was found that hemoglobin and RBC mass was unchanged [26-28]. It appears stimulation of erythropoiesis is directly correlated with the duration of hypoxic exposure.

In conjunction with the above findings, muscle and molecular adaptations have been discovered in various studies [26]. Intriguingly, it was found that intense hypoxic training

induced a larger increase in muscle volume, capillary length density, and mitochondrial volume density when compared to normoxic training at equal intensity [26].

Training with an Altitude-Simulating Mask

To make it more convenient to capture the possible advantages of training at high altitudes, a mask was developed to mimic the oxygen restricting effects of elevation. However, the efficacy of this mask has been debated and current research is limited.

Mixed results have been found when assessing the aerobic effects of wearing an altitude simulating mask. Using ROTC cadets, it was found that there was a decrease in magnitude of improvement in VO2max and time to fatigue [30]. However, due to the outdoor nature of the study, there were inconsistencies with regards to environmental weather conditions[30]. Additionally, participants frequently removed the mask during resting periods and during swimming [30]. Furthermore, pre- and post-performance was measured using Monark Ergomedic 828E stationary bicycle, which was considerably different from the ROTC training, which involved sit-ups, pushups, dips, running, and a multitude of other exercises [30]. This makes improvements less likely due to lack of specificity of exercises [20]. An additional study found no difference in magnitude of improvements in VO_2max after training and assessing using a stationary bicycle ergometer [31], supporting the lack of advantage in wearing an altitude simulating mask.

There is conflicting research on the effects altitude-simulating mask and anaerobic or ventilatory threshold. The study by Sellers et al.[30] found that masked groups held no advantage over unmasked groups in improving anaerobic threshold. Meanwhile, Porcari et al.[31] found greater improvements in the masked group as opposed to unmasked group in ventilatory threshold and power output at ventilatory threshold, which are both indicators of increased

anaerobic threshold. This may allow individuals to train and perform at greater percentages of their VO_2max, implying that there may be some performance benefits to wearing an altitude-simulating mask.

Jagim et al.[32] assessed the acute effects of an elevation training mask on muscle strength. They found that although there was no significant difference in strengths, there was a difference in peak velocity in the back squat, bench press, and sprint test in those who were wearing the elevation training mask [32]. This indicated that there may be a reduction in strength increases. Additionally, they found that alertness and focus was impaired by the elevation training [32].

Current Study Rationale

My current proposed experiment has several differences from prior research. Opposed to the Sellers, et al.[30] experiment, the environmental conditions and exertion efforts of the subjects will be controlled. Additionally, specificity of training and testing will be near identical. We will have two groups, both of whom undergo 12-weeks of combination endurance and sprint training: a group who undergoes 12-weeks of combination sprint and endurance running. The control group (CON) will train without the use of the TrainingMask 2.0 (ETM) (Training Mask LLC, Cadillac, Michigan). The experimental group (TM) will train while wearing the ETM.

In comparison to Porcari, et al. [31], we will use running as the training and testing method. This may be better translated to various sport training programs due to the commonality of running with many sport training programs. Furthermore, we will monitor diet, have greater training duration and frequency, will involve both sprint and endurance training and performance measurements, and will include body composition measurements to compare to the previously mentioned effects of altitude training on muscle mass.

Aims and Hypotheses of the Experiment

We designed a 12-week training intervention comparing traditional exercise to exercise with the use of ETM. Our objective was to measure the acute responses and chronic adaptation to exercise both with and without the ETM. Therefore, this thesis addresses the following aims:

Specific Aim 1: Determine the degree to which acute performance is affected by wearing the ETM, regarding submaximal endurance treadmill running speed, submaximal sprint performance, and rates of perceived exertion.

Specific Aim 2: Compare improvements between TM and CON groups regarding VO_2max, ventilatory threshold, time to exhaustion, and maximal sprint performances with and without the ETM. Additionally, changes in body composition and pulmonary function between TM and CON groups will be compared.

Specific Aim 3: Compare ventilation rates between masked and unmasked endurance running among all subjects.

Our hypotheses were:

Hypothesis 1*:* The acute maximal and submaximal sprint and endurance performance would be hindered with the use of the ETM, leading to reduced sprint times and treadmill time to exhaustion. We predicted rates of perceived exertion would be greater with the use of the ETM.

Hypothesis 2: The CON group would undergo greater improvements in VO_2max, ventilatory threshold, time to exhaustion, and maximal sprint performances when compared to TM group. Due to the specificity of training and respiratory muscle adaptations, we hypothesized the TM group would witness greater improvements in masked maximal sprint/endurance performances

and pulmonary function. Due to the predicted hinderance in training performances, we hypothesized the TM group would have inferior decreases in percent body fat and increases in lean mass.

Hypothesis 3: Submaximal ventilation rates will be reduced under masked conditions.

CHAPTER II

THE ACUTE AND CHRONIC EFFECTS OF AN ELEVATION TRAINING MASK ON AEROBIC CAPACITY, ANAEROBIC ENDURANCE, AND PULMONARY FUNCTION

Introduction

Athletes and coaches are frequently seeking methods to increase athletic performance in competitive sports. High altitude training and respiratory muscle training have become popular in the professional and recreationally active populations to further enhance athletic performance through assumed additive physiological adaptations associated with both exercise and altitude [33]. In general, high elevation exposure is known to cause erythropoiesis through production of erythropoietin[15, 23]. This leads to increased red cell mass, which enhances the oxygen carrying capacity of blood, allowing for greater oxygenation of aerobically active muscles[15, 23]. The optimal duration of exposure for erythropoiesis has been found to be 4 weeks, with the minimum daily dose requirement of 12 hours/day [34]. Nevertheless, intermittent altitude training has continued to be used as a method of improving aerobic and anaerobic fitness.

The aerobic effects of altitude training have been researched with diverse results. The concept of "Live High – Train Low" is a commonly referred method of increasing aerobic fitness through elevation-induced erythropoiesis without the intensity-limiting effects of altitude training [24, 26, 35, 36]. VO_2max, a measure of aerobic fitness and an indicator of endurance performance, has been found to increase when living at high altitudes (2650m) [24]. However, maximal steady state VO_2 (indicator for ventilatory and anaerobic threshold) and running velocity at VO_2max (measure of running performance and running economy [37]) were found to have greater improvements in subjects who trained at lower altitudes (sea-level) while living at

high altitudes [24]. Additionally, it has been found that high altitude training, coupled with low altitude living, caused an increase in VO_2max when tested at high altitude[26], but not when tested at low altitude[26-28] indicating that specificity of training for a given performance condition plays a critical role as to whether or not beneficial training outcomes are achieved [38, 39]. In conjunction with these findings, muscle and molecular adaptations have been observed in various studies [26, 40-42]. Intriguingly, it was found that intense hypoxic training induced a larger increase in muscle volume, capillary length density, and mitochondrial volume density when compared to normoxic training at equal intensity [26].

Respiratory muscle fitness is another determining factor for performance in a variety of sports [43-45]. Verges et al. (2007)[45] found that prior respiratory muscle fatigue decreased average running speed by 0.13 m/s. Additionally, the extent of respiratory fatigue correlated with the degree of reduction in running performance [45]. Respiratory muscle training has successfully been used as a method of preventing respiratory fatigue and improving exercise performances, but improvements did not correlate with changes in endurance cycling performance [43, 44, 46]. Furthermore, Verges et al. (2007)[43] reported that respiratory muscle fatigue was only improved in those with lower baseline pulmonary function tests. In a study on the effects of RMT in swimmers, Lemaitre et al. (2013) [46] found that forced vital capacity (FVC) and strength and endurance of respiratory muscles were increased after 8 weeks when compared to a group without RMT. Importantly these improvements in lung functions correlated with better time trials during swimming competition [46].

To capture the possible advantages of training at high altitudes and RMT, the TrainingMask 2.0 (ETM) (Training Mask LLC, Cadillac, Michigan) was developed and purported to mimic the oxygen restricting effects of elevation. The ETM (Figure 1)[47] consists

of a neoprene sleeve with a rubber insert designed to cover the nose and mouth. An inaccuracy in naming, the ETM acts more like a respiratory muscle trainer than an elevation simulator by resisting air-flow rather than decreasing the partial pressure of oxygen [31]. It has inspiratory and expiratory valves, which can be altered to achieve advertised different levels of altitude simulation ranging from 3000 to 18000 feet. However, the efficacy of this mask has been debated and current research is conflicting. A study by Sellers, et al. (2016) [30] found a smaller degree of VO_2max improvement after training with the ETM, with no statistical difference in anaerobic threshold improvements. However, training was not well monitored, and there was discordance between training and testing specificity. Meanwhile, Porcari et al. (2016)[31] found that when compared to traditional training, the ETM may lead to greater improvements in ventilatory threshold (VT) and power output at ventilatory threshold. This may allow individuals to train and perform at greater percentages of their VO_2max, implying potential performance benefits to ETM intervention training.

In light of previous findings, the purpose of this study was to determine the effects of training while wearing the ETM on anaerobic and aerobic endurance performance and pulmonary function in healthy young men and women. We hypothesized that contrary to the manufacturer's claims, the ETM would lead to smaller improvements in aerobic and anaerobic fitness when compared to traditional training. Since the ETM has resistance valves that may exercise muscles of respiration, we hypothesized that ETM training would produce greater improvements in pulmonary function.

Figure 1: TrainingMask 2.0. Reprinted from muscleandfitness.com[47].

Methods

Twenty-two healthy, untrained recreationally active volunteers were recruited from the surrounding community in Houston, TX to participate in the study. Two groups were formed: an experimental group (TM) that trained while wearing the ETM (Male=5, Female=6, Age=27.6±0.86yrs, Weight=68.7±3.7kg), and a control group (CON) that trained without the ETM (Male=5, Female=6, Age=29.9±1.6yrs, Weight=72.5±3.7kg). Potential subjects were recruited through email and by word of mouth. Each subject was provided written informed consent. The study was approved by the Houston Methodist Hospital Institutional Review Board. Volunteers were screened to ensure that they did not currently perform planned exercise (>2 bout/wk) and that they were healthy enough for exercise. Subjects with contraindications to exercise or those taking medications known to affect metabolism, blood clotting, blood pressure, or heart rate were excluded from participation. Screening data is provided in Table 1.

Table 1: Screening Data

	Number of Subjects
Expressed Interest	42
Met Questionnaire Screening Criteria	26
Completed Baseline Testing	24
Completed Study	22

General Study Protocol

Following initial screening via a phone survey, all subjects provided informed consent. Baseline screening tests, body composition, and physiological assessments were completed

during the first week (methods to follow). All baseline data was reassessed during the week following the final training session. Final performance measurements were conducted within 2 hours of baseline measurements. Order of sprint and endurance testing under masked and unmasked conditions was randomized aside from the initial screening Bruce protocol stress test[48] (details below). All testing and training sessions were conducted in a temperature-controlled facility approved by the Houston Methodist Hospital Institutional Review Board. During testing and training sessions, the ETM was always set at the manufacture-defined setting of 9000 feet.

Diet and Activity

Prior to participation, subjects were instructed to maintain their current diet habits. No attempt was made to modify their diet in any way. To verify compliance, diet logs were completed during baseline and final testing weeks. Subjects were asked to complete the diet logs on consecutive or nonconsecutive days that represent their normal diet, including two weekdays and one weekend day. Weekdays were defined as Monday through Thursday, and weekend days were defined as Saturday or Sunday. Baseline and final total kilocalorie (kcal), carbohydrate, fat, and protein consumption within TM and CON groups were assessed using a paired 2-tailed student's T-Test. Changes in total kcal and macronutrient kcal contribution was compared between groups using an unpaired 2 sample 2-tailed student's T-Test.

Baseline Measurements

Body composition was measured with dual-energy x-ray absorptiometry (DEXA), and included measures of body fat percentage, fat mass, lean mass, and bone mineral content. A Bruce protocol graded exercise test (U-GXT) was completed on a motor-driven treadmill with concurrent oxygen consumption measurements using a calibrated metabolic gas-analysis system

(Ultima®, MCG Diagnostics, Minneapolis, MN) [49]. VO_2max (ml•kg^{-1}•min^{-1}) was measured as the maximum 10 second average oxygen consumption during the U-GXT. Time to exhaustion (TTE) was measured as the duration (sec.) of the U-GXT from the start of the treadmill to the time of maximal exertion. Rates of perceived exertion (RPE) was measured using a Borg scale ranging from 6-20 [50]. Manual blood pressures (BP) were measured during the final 60 seconds of each 3-minute stage, and at 1-, 3-, and 5-minute recovery intervals after maximal exertion [50]. Ventilatory threshold (VT) was measured using the V-Slope Method, with VT defined as the VO_2 at which VCO_2 increased disproportionately to VO_2 [51]. The initial U-GXT, providing screening and performance data, included heart rate (HR) and rhythm monitoring from a 12-lead electrocardiogram (ECG) to ensure exercise capability and safety. Achievement of maximum exertion was considered if HR oxygen consumption plateaued with increasing workload, respiratory exchange ratio exceeded 1.2 (VO2 / VCO2), RPE exceeded 17, with HR having to exceed >85% age predicted maximal HR. Subsequent running tests (detailed below) were completed in a randomized order, with > 24 hr rest between testing sessions. Caffeine or food consumption was not permitted 4-hours prior to all testing sessions to avoid abnormal HR deviations.

Masked aerobic performance was measured with an identical protocol to the U-GXT, with the addition of the ETM. Masked graded exercise test (M-GXT) included a measure of TTE in an identical manner as the U-GXT. However, oxygen consumption was not measured due to interference of the oral and nasal airways by the ETM. Since healthy heart rhythm was previously verified via the screening U-GXT, HR was measured with wearable Polar M400 watches and H7 chest straps (Polar Electro, Kempele, Finland), which has been previously utilized [52].

Sprint testing was also assessed both with and without the ETM. The sprint protocol involved a standardized warm-up followed by two consecutive 300-yard shuttle sprints (with 25-yard increments) separated by a 5-minute rest period during which the subjects were required to remain standing. Warm-up was identical among all sprint testing, and consisted of dynamic stretches and a 100-yard light jog. During the masked shuttle sprint, subjects were not permitted to remove the ETM at any point during the run or rest period. HR was monitored with wearable Polar M400 watches and H7 chest straps. Average sprint time for each subject was measured as the average of the two consecutive repetitions. Sprint time drop-off was measured as the difference of repetition 1 and repetition 2 (drop-off = rep 2 time – rep 1 time) in both masked and unmasked sprint tests. Fatigue Index (FI) in both masked and unmasked sprint tests were measured as the ratio of times of Rep 2 and Rep 1.

Baseline and final pulmonary function testing (PFTs) were conducted prior to GXT using a calibrated metabolic gas-analysis system. They included measurements of maximum voluntary ventilation (MVV), forced expiratory volume in one second (FEV1), and forced vital capacity (FVC) [53]. Subjects were given instruction and demonstration of the testing procedures. They were verbally encouraged prior to and during the tests to ensure maximal effort. The best of 3 successful testing attempts was recorded.

Exercise Training

After baseline testing, subjects were selectively randomized to either a masked (TM) or unmasked (CON) group, while ensuring equal group sizes, equal male:female ratios, and matching groups for body composition and VO_2max. Baseline demographics for subjects who completed this study are shown in Table 2. No statistical differences between groups were present in baseline values shown in Table 2. Subjects completed 3 supervised training session

per week, with alternating sprint and endurance sessions and incremental increases in intensity (See Table 3). To deter subjects' inclination to participate in inconsistent strength training, all subjects performed a standardized upper and lower body resistance exercise program using either body weight or light weights as resistance. Subjects were required to participate in ≥ 85% of all training sessions. If rescheduling was needed, training sessions were completed within one week of the subject's scheduled day.

Table 2: Baseline Demographics

	TM	CON
Women	6	6
Men	5	5
Age, yr	27.6 ± 0.86	29.9 ± 1.6
Weight, kg	68.7 ± 3.7	72.5 ± 3.7
BMI, kg•m^{-2}	23.2 ± 0.88	24.8 ± 1.0
% Body fat	30.4 ± 1.8	31.7 ± 2.0
Lean Mass (kg)	46.2 ± 2.9	47.4 ± 2.4
VO_2max (ml•kg^{-1}•min^{-1})	38.2 ± 1.6	36.5 ± 1.7
Data are mean ± standard error of the mean. TM = elevation training mask group, CON = control group. BMI = Body Mass Index.		

Endurance session intensity was based on a percentage of each subject's VO_2max using their individual VO_2 / HR relationship, calculated using linear regression from baseline U-GXT screening VO_2 and HR measurements. TM group relationships were calculated by estimating VO_2 during a given stage of the M-GXT, and by the HR at the corresponding stage of the M-GXT. Weekly endurance training intensity, measured as a percentage of VO_2max, is shown in Table 3. Initial treadmill training speed and grade for each session was estimated using their speed/grade VO_2 equation, and adjusted to be within 5 beats per minute (bpm) of their target HR for the given VO_2max percentage of the workout. Speed and/or grade was reduced if the subject physically could not sustain exercise intensity or if HR exceeded the prescribed training range

for that particular week. Endurance prescriptions were adjusted for increases in VO_2max during week 7 to maintain training intensity.

Table 3: Training Progression

ENDURANCE TRAINING PROGRESSION			
Training Week	**Duration** Minutes	**Intensity** $\%VO_{2max}$	**Frequency** Days/Week
Week 1 - INITIAL ASSESSMENT			
Week 2	30min	65	2
Week 3	35min	70	1
Week 4	40min	75	2
Week 5	45min	75	1
Week 6	45min	80	2
Week 7 - REASSESSMENT AND PROGRAM ADJUSTMENT			
Week 8	35min	70	2
Week 9	40min	75	1
Week 10	45min	75	2
Week 11	45min	80	1
Week 12	45min	85	2
Week 13 - FINAL ASSESSMENT			

SPRINT TRAINING PROGRESSION		
Training Week	**Activity**	**Frequency** Days/Week
Week 1 INITIAL ASSESSMENT		
Week 2	15 x 10yrd sprint | 4 x 100yrd shuttle	1
Weeks 3 & 4	15 x 10yrd sprint | 4 x 5yrd shuffle (30 sec) 4 x 150yrd shuttle	Wk 3 (2 days) Wk 4 (1 day)
Weeks 5 & 6	15 x 10yrd sprint | 4 x 10yrd shuffle (30 sec) 4 x 150yrd shuttle	Wk 5 (2 days) 1
Week 7 - REASSESSMENT AND PROGRAM ADJUSTMENT		
Week 8	15 x 10yrd sprint | 4 x 200yrd shuttle	1
Weeks 9 & 10	15 x 10yrd sprint | 4 x 5yrd shuffle (45 sec) 4 x 250yrd shuttle	Wk 9 (2 days) Wk 10 (1 day)
Week 11	15 x 10yrd sprint | 4 x 10yrd shuffle (45 sec) 4 x 250yrd shuttle	2
Week 12	15 x 10yrd sprint | 2 x 300 yrdshuttle	1

Endurance and sprint training progression was performed equally by both TM and CON groups. A 5-minute rest period was completed between each sprint activity. TM group removed ETM for 1-minute during rest period to allow for rehydration. Sequential 10-yrd sprints began on 40-second intervals. 30- and 45-second shuffles included a 60- and 90-second rest between shuffles, respectively. 100-, 150-, 200-, 250-, and 300-yard shuttles began on 90-, 150-, 180-, and 270-second intervals, respectively.

Each endurance session began with a 3-minute warm-up walk on the treadmill at 2 miles per hour (mph). After the warm-up, speed/grade was adjusted to their prescribed intensity. Every 5-minutes, RPE was assessed using the previously described Borg scale, and speed/grade was adjusted to ensure the subject's target HR was maintained in the prescribed range. Subjects in the TM group were required to wear the ETM for the duration of the endurance session. Following the prescribed session, subjects completed a cool-down walk for 3-minutes at 2 mph. The TM group was permitted to remove their ETM during the cool-down period.

Sprint sessions included a combination of short-sprints, shuffles, and shuttles. A standardized warm-up was conducted prior to each sprint session. Short sprints were 10-yard sprints at maximum effort. Shuffles consisted of 30- to 45-second lateral movements across 5- to 10-yards. Intra-run rest periods between shuffle repetitions were 60-seconds for the 30-second shuffle durations, and 90-seconds for the 45-second shuffle durations. Shuttles were 100- to 300-yard distances (with 25-yard increments). Each subject was given a 5-minute rest period between each of the three sprint activities. To allow subjects to begin each sprint simultaneously, shuttle start times were based on a timer, with each shuttle repetition beginning at pre-determined times. The remaining time until the next scheduled shuttle was used as the "intra-run rest period". The duration of this rest period was dependent on the speed each subject's sprint, with faster sprints leading to a longer rest period. Table 4 outlines average shuttle intra-run rest periods by group. No statistical significant difference was present in intra-run rest periods between groups ($p>0.05$). Subject in the TM group were permitted to remove the ETM for 60-seconds to allow for hydration. Intensity was increased weekly by increasing shuttle distances and shuffle durations. Shuffle performance was measured by the quantity of full-length laps each subject

completed in the 30- to 45-second prescribed shuffle duration. Shuttle times for each subject were recorded to assess the impact of ETM on sprint performance during each session.

Table 4: Sprint Rest Periods

Average Shuffle Intra-Run Rest Period (seconds)		
Training Week	**CON**	**TM**
Week 2	66.43 ± 1.35	67.56 ± 0.70
Week 3	111.57 ± 1.42	111.84 ± 1.00
Week 4	112.63 ± 1.69	112.37 ± 1.38
Week 5	114.13 ± 1.17	113.39 ± 0.92
Week 6	113.46 ± 1.71	112.89 ± 1.21
Week 8	119.27 ± 2.16	118.67 ± 1.74
Week 9	110.40 ± 2.97	108.31 ± 2.02
Week 10	111.78 ± 3.67	111.34 ± 1.83
Week 11	113.13 ± 2.35	109.35 ± 1.93
Week 12	201.74 ± 7.30	193.12 ± 3.73
Data are mean ± standard error of the mean. No statistical difference was present between CON and TM groups ($p>0.05$)		

Statistical Analysis

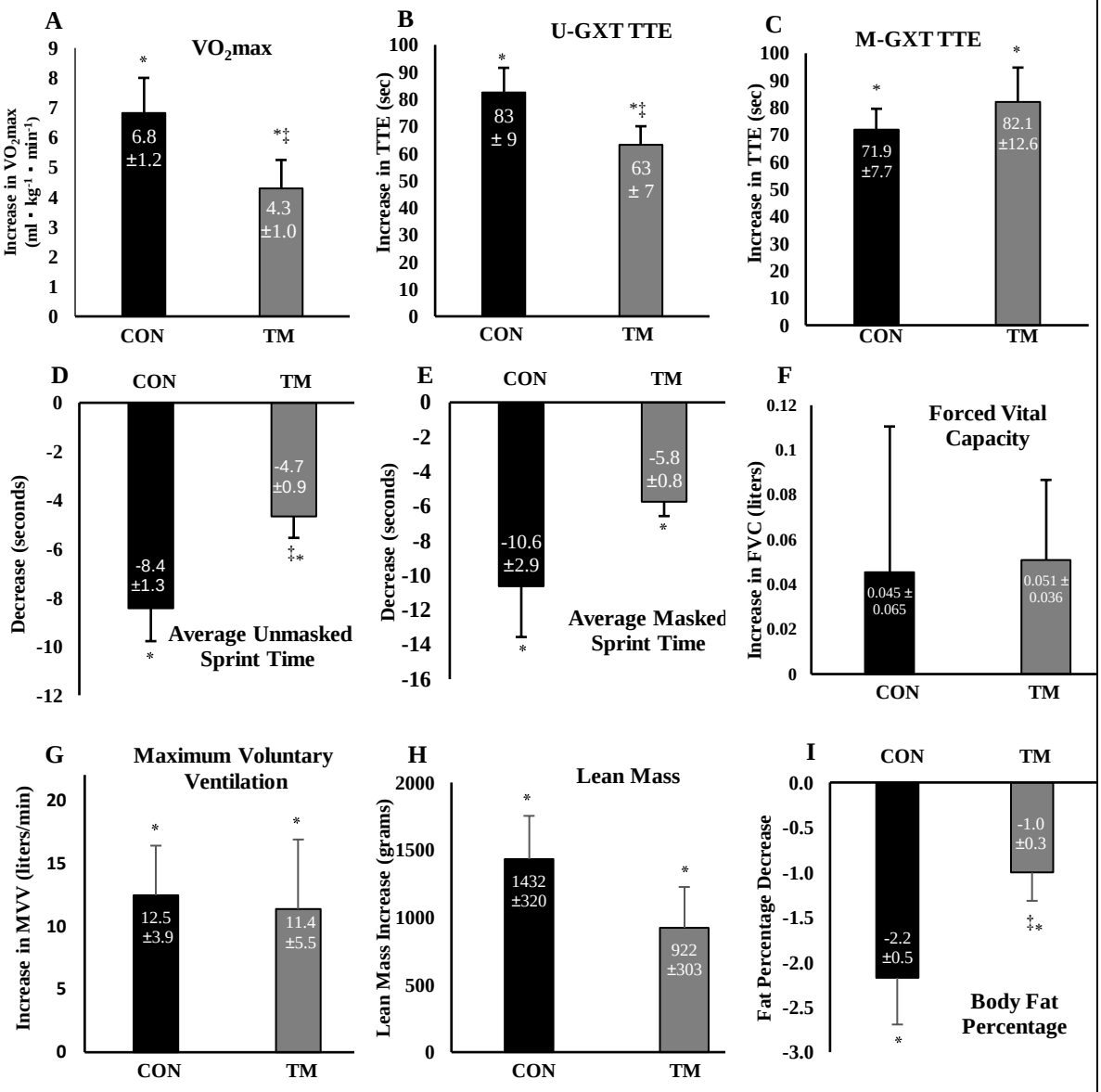
A 2(group) x 2(time) Mixed Model ANCOVA (covaried on baseline measures and gender) repeated across training was used to detect group x time interactions for VO_2max, body composition, pulmonary functions, sprint performance, and dietary recall. All significant interactions were followed by a followed by a Tukey's post-hoc test for pairwise comparisons. A student's T-Test was also used to analyze the above variables for relative change (%change) following training. The comparison-wise error rate, α, was set at 0.05 for all statistical tests.

Results

Baseline data of subjects in each group are shown in Table 1. No significant differences in any of these characteristics were found between CON and TM groups at the beginning of training. No effect of gender was observed in any of the analyses and it was therefore removed

from the models for final analysis. Nutrition analysis revealed no significant difference ($p>0.05$) within or between TM and CON groups with regards to total kcal and percent kcal contribution of carbohydrates, fats, or proteins. Tables 5 and 6 list baseline and final values for all independent variables. Statistical analysis indicated significant group x time interaction of body composition, maximal aerobic capacity, maximal sprint performance, pulmonary function, and RPE ($p<0.05$). Significant group interactions were found for change in body composition, maximal aerobic capacity, and maximal sprint performance.

Figure 2: Physiologic Adaptations to Chronic Exercise



*Data is shown as mean ± SEM for change from baseline for aerobic capacity, sprint performance, pulmonary function, and body composition after 12 weeks of concurrent endurance and sprint training. . U-GXT TTE = Unmasked Graded Exercise Test Time to Exhaustion. M-GXT TTE = Masked Graded Exercise Test Time to Exhaustion. FVC = Forced Vital Capacity. MVV = Maximum Voluntary Ventilation. *= significantly different from baseline (p<0.05). ‡= significantly different between CON and TM groups (p<0.05).*

Table 5: Baseline and Final Testing Data

	CON			TM		
Variable	**Baseline**	**Final**	**Sig.** Within Group	**Baseline**	**Final**	**Sig.** Within Group
Maximal Aerobic Capacity						
VO_2max (ml•kg^{-1}•min^{-1})	36.5 ±1.68	43.3 ±2.44	<0.01	38.2 ±1.55	42.5 ±1.72	<0.01
VT (ml•kg^{-1}•min^{-1})	19.5 ±0.79	23.4 ±1.56	<0.05	21.3 ±0.73	23.6 ±0.67	<0.01
U-GXT TTE (sec)	604 ±27.9	687 ±29.4	<0.01	633 ±21.1	696 ±20.0	<0.01
M-GXT TTE (sec)	569 ±24.2	641 ±27.4	<0.05	589 ±28.3	671 ±19.4	<0.05
Maximal Sprint Performance						
Unmasked Average Sprint (sec/rep)	82.3 ±4.29	73.9 ±3.18	<0.01	75.5 ±2.74	70.8 ±2.11	<0.01
Unmasked Sprint Rep 1 (sec)	80.9 ±5.05	72.4 ±2.98	<0.05	73.1 ±2.71	69.6 ±2.22	<0.05
Unmasked Sprint Rep 2 (sec)	83.8 ±3.82	75.4 ±3.44	<0.01	77.9 ±2.92	72.0 ±2.07	<0.01
Unmasked Sprint Drop-off (sec)	2.89 ±2.52	3.02 ±0.90	NS	4.85 ±1.32	2.35 ±0.81	NS
Unmasked Sprint FI	0.046 ±0.024	0.041 ±0.033	NS	0.068 ±0.019	0.035 ±0.011	NS
Masked Average Sprint (sec/rep)	89.5 ±6.64	78.9 ±4.43	<0.01	78.6 ±2.80	72.9 ±2.44	<0.01
Masked Sprint Rep 1 (sec)	88.8 ±6.83	78.5 ±4.61	<0.05	77.3 ±2.94	71.9 ±2.52	<0.01
Masked Sprint Rep 2 (sec)	91.2 ±6.26	80.1 ±3.95	<0.01	80.0 ±2.82	73.8 ±2.43	<0.01
Masked Sprint Drop-off (sec)	1.50 ±3.23	0.88 ±0.86	NS	1.34 ±4.45	1.94 ±0.79	NS
Masked Sprint FI	0.025 ±0.033	0.014 ±0.012	NS	0.037 ±0.018	0.028 ±0.012	NS
Rates of Perceived Exertion						
U-GXT Stage 1	7.27 ±0.27	8.45 ±0.65	NS	7.55 ±0.25	9.18 ±0.62	<0.05
U-GXT Stage 2	10.36 ±0.53	9.64 ±0.64	NS	9.82 ±0.40	10.73 ±0.57	NS
U-GXT Stage 3	13.80 ±0.79	12.82 ±0.52	<0.05	12.64 ±0.39	13.00 ±0.62	NS
M-GXT Stage 1	8.36 ±0.53	9.18 ±0.57	NS	8.27 ±0.52	9.36 ±0.51	NS
M-GXT Stage 2	11.82 ±0.64	11.64 ±0.78	NS	11.45 ±0.62	11.09 ±0.37	NS
M-GXT Stage 3	15.25 ±0.77	14.64 ±0.56	NS	14.80 ±0.57	14.09 ±0.34	NS

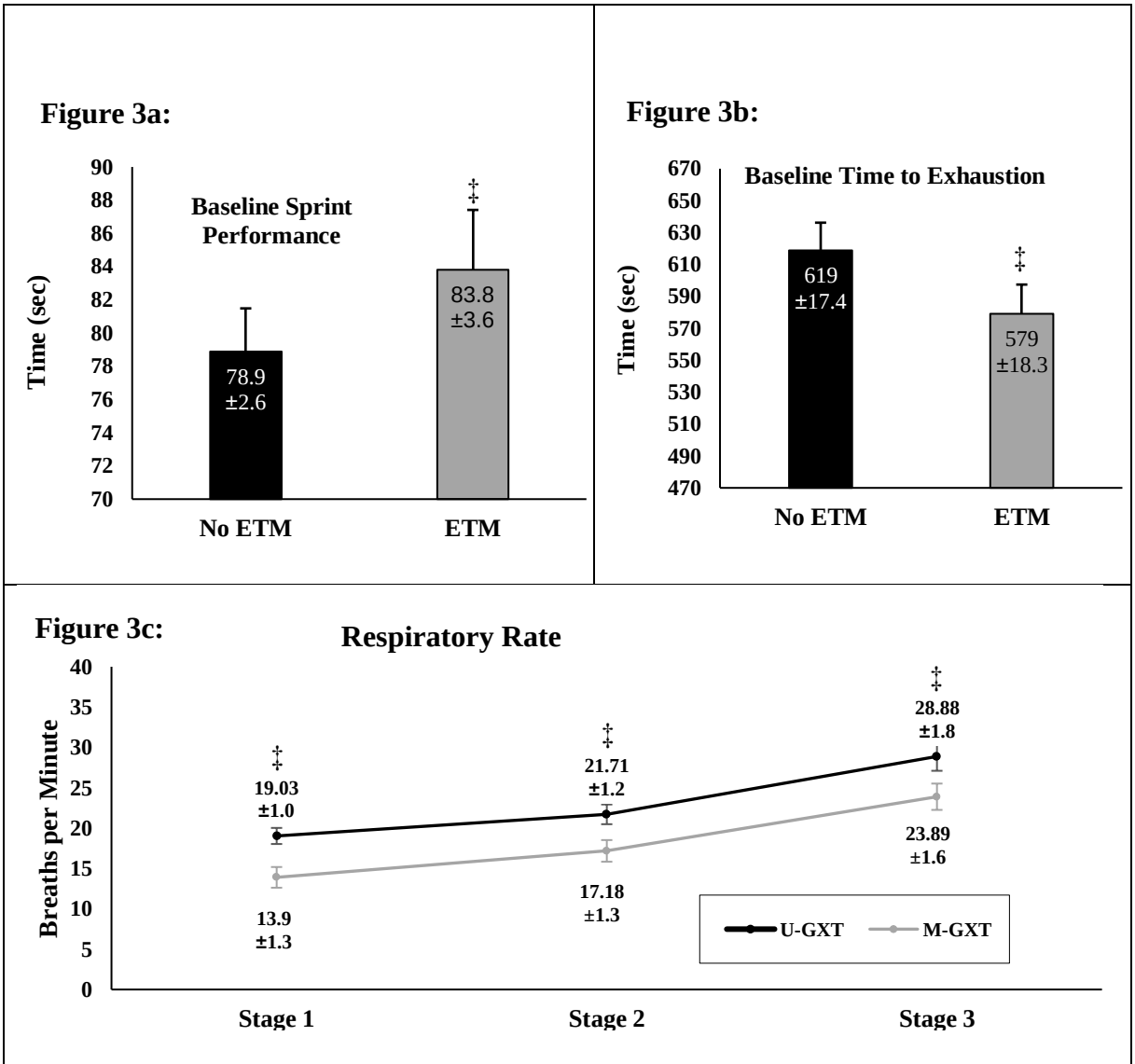
Data are mean ± standard error of the mean. VT = Ventilatory Threshold. U-GXT TTE = Unmasked Graded Exercise Test Time to Exhaustion. M-GXT TTE = Masked Graded Exercise Test Time to Exhaustion. FI = Fatigue Index. NS = Not Significant (p>0.05).

Table 6: Baseline and Final Pulmonary Function and BMI

	CON			TM		
Variable	**Baseline**	**Final**	**Sig.** Within Group	**Baseline**	**Final**	**Sig.** Within Group
Total Body Composition						
BMI	24.8 ±1.03	24.7 ±0.97	NS	23.2 ±0.88	23.4 ±0.90	NS
% Body Fat	31.7 ±2.05	29.5 ±1.83	<0.01	30.4 ±1.79	29.4 ±1.79	<0.05
Lean Mass (kg)	47.4 ±2.41	48.9 ±2.42	<0.05	46.2 ±2.92	47.1 ±2.90	<0.05
Pulmonary Function						
FVC (Liters)	5.16 ±0.33	5.20 ±0.33	NS	4.38 ±0.31	4.43 ±0.30	NS
FVC (% Predicted)	113.4 ±4.66	114.7 ±4.84	NS	95.5 ±2.29	97.7 ±2.11	NS
FEV1	4.13 ±0.28	4.21 ±0.25	NS	3.60 ±0.25	3.65 ±0.24	NS
MVV (Liters)	158.8 ±13.2	171.3 ±13.7	<0.05	152.3 ±10.9	163.6 ±12.5	<0.05
MVV (% Predicted)	112.0 ±4.48	122.64 ±4.32	<0.01	107.5 ±3.80	114.9 ±3.16	<0.05

Data are mean ± standard error of the mean.. BMI = Body Mass Index. FVC = Forced Vital Capacity. FEV1 = Forced Expiratory Volume in 1 second. MVV = Maximum Voluntary Ventilation. NS = Not Significant (p>0.05).

Figure 3: Masked vs. Unmasked Testing Conditions (Groups Combined)

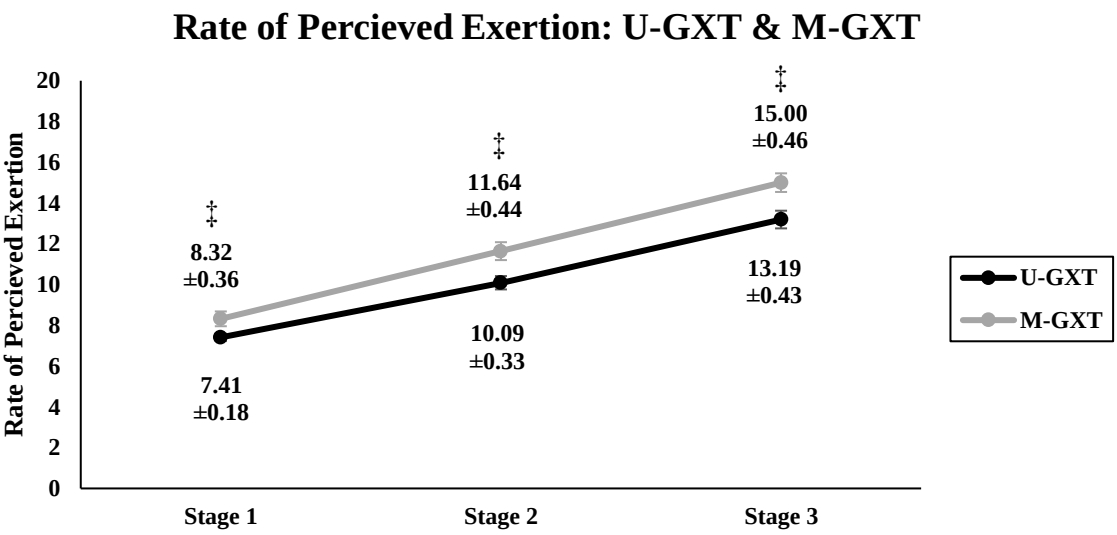


Data is shown as mean ± SEM. Baseline sprint performance (Figure 3a) was measured as the baseline average sprint time of all subject on two consecutive baseline sprints. Baseline Time to Exhaustion (Figure 3b) measured the baseline average maximum achievable duration at of all subjects on the Bruce-protocol treadmill stress test. Respiratory Rate (Figure 3c) is the average breathes per minute of all subjects during the Bruce-protocol treadmill stress tests. ‡ = significantly different between masked and unmasked testing conditions ($p<0.05$). ETM = sprint or endurance performance with the TrainingMask 2.0. No ETM = sprint or endurance performance without the TrainingMask 2.0. U-GXT = Unmasked Graded Exercise Test. M-GXT = Masked Graded Exercise Test

Aerobic Capacity

VO_2max, VT, U-GXT TTE, and M-GXT TTE were increased in both groups following exercise training (Figure 2). The CON group was found to have significantly greater increases in VO_2max (CON = ↑6.8 ± 1.2ml•kg^{-1}•min^{-1} | TM =↑4.3 ± 1.0 ml•kg^{-1}•min^{-1}) and U-GXT TTE (CON = ↑83 ± 9 sec. | TM =↑63 ± 7 sec.), while the TM group was found to have a non-significant trend ($p<0.50$) towards greater increases in M-GXT TTE (CON = ↑72 ± 7.7 sec. | TM =↑82 ± 12.6 sec.). VT was measured to be significantly increased in both groups (CON = ↑4.0 ± 1.30 ml•kg^{-1}•min^{-1} | TM =↑2.3 ± 0.53 ml•kg^{-1}•min^{-1}) with a nonsignificant trend towards greater increases in the CON group ($p<0.25$). We compared U-GXT TTE and M-GXT TTE among all subjects at baseline to determine the ETM's effect on maximal aerobic performance (Figure 3b). We found that masked conditions significantly decreased GXT time by an average of 39.7 ± 9.0 seconds (No ETM = 619 ± 17.4 sec. | ETM =579 ± 18.3 sec.) ($p<0.05$). Respiratory rate during U-GXT and M-GXT were measured during final testing to evaluate for changes. Similar to previous studies [54], respiratory rate was found to be significantly decreased at each GXT stage under masked conditions (Figure 3c) ($p<0.05$), without a difference between TM and CON groups. Regarding RPE we found the average RPE for each stage of the GXT was greater under masked conditions ($p<0.05$) (Figure 4).

Figure 4: Endurance Rates of Percieved Exertion



Mean rates of perceived exertions (RPE) among all subjects during baseline Bruce-protocol treadmill stress tests. RPE was measured with the Borg scale. Values are presented as mean±SEM. ‡= significantly different between masked (M-GXT) and unmasked (U-GXT) conditions ($p<0.05$).

Sprint Performance

Significant improvements were observed in sprint tests in both CON and TM groups. However, significantly greater improvements in average unmasked sprint time were observed in the CON group (CON = ↓8.43±1.3 sec. | TM = ↓4.66±0.9 sec.) ($p<0.05$). Additionally, average masked sprint time results indicate a non-significant trend ($p<0.14$) for greater improvements in the CON group (CON = ↓10.63±2.94 sec. | TM = ↓5.75±0.83 sec.). Similar to aerobic performance, we compared baseline masked to unmasked sprint performance to determine the effect of the ETM on anaerobic performance (Figure 3a). We found that the ETM reduced sprint performance by significantly increasing sprint time by an average of 5.1 ± 1.4 sec (No ETM = 78.9 ± 2.6 sec. | ETM =83.8 ± 3.6 sec.) ($p<0.05$).

Body Composition

Lean mass was shown to increase significantly in both groups, with the CON group displaying a non-significant trend ($p<0.27$) towards greater increases in lean mass (CON = ↑1.4±0.32 kg | TM = ↑0.92±0.30 kg). Percent body fat significantly decreased in both CON and TM groups, with the CON group achieving statistically significantly greater reductions in percent body fat (CON = ↓2.2±0.5 % | TM = ↓1.0±0.3%) ($p<0.05$).

Pulmonary Function

Pulmonary function test results revealed improvements in MVV in both groups, without a significant difference in magnitude of improvement between groups (CON = ↑12.5±3.9 L. | TM = ↑11.4±5.5 kg). Meanwhile, FVC volume changes were not significant within or between groups.

Discussion

The goal of this study was to compare the effects of ETM usage during training on physiologic variables, including aerobic capacity, sprint performance, and lung function. The findings in the present study indicate that the ETM impedes rather than improves gains in maximal aerobic and anaerobic running performances despite subjects' ability to maintain submaximal exercise.

Aerobic and Sprint Performances

Aerobic performance increased to a greater degree in the CON group, as indicated by changes in VO_2max and U-GXT TTE. However, the TM group showed nonsignificant greater increases in M-GXT TTM. Despite lack of statistical significance, the trend may support the

concept of specificity of training, demonstrating that it may be beneficial to train in air flow restricted conditions if an individual commonly performs in airway restricted conditions. However, the general population does not typically perform under airway restricted conditions and likely are reducing aerobic improvements with the use of the ETM. Special populations of interest that may benefit from the use of the ETM include firefighters, jet pilots, and deep-sea divers due to their common use of respiratory apparatuses. Future studies are needed to evaluate the value to respiratory muscle training on these special populations.

Regarding anaerobic sprint performance, the TM group demonstrated inferior performance improvements when compared to the CON group regardless of masked or unmasked testing conditions. It is likely that training performance was hindered by the ETM, preventing adequate training intensity needed for greater fitness improvements.

Body Composition

Results of changes on body composition indicate that the ETM may have impeded training intensity, leading to inferior decreases in body fat percentage and increases in lean mass. As stated previously, treadmill speed or grade was reduced if the participants physically could not maintain running intensity. Additionally, running speed during sprint training sessions was under the control of each participant. The ETM may have reduced TM groups ability to maintain running intensity during endurance and/or sprint training sessions, reducing the total energy expenditure. The possible reduced sprint training intensity in the TM group may have resulted in less motor unit being activated [55]. Since the quantity of activated muscle units is associated with greater increases in lean mass [55, 56], this may be an explanation for the discrepancy in magnitude of change in lean mass between CON and TM groups.

Rates of Perceived Exertion

Despite equal endurance workloads, we found that RPE during the first three stages of the GXT was greater with the use of the ETM, which is similar to results in previous studies [31, 54]. Accordingly, many people may presume that greater subjective exertion suggests greater kilocalories depletion or quicker fitness improvement. However, the ETM did not improve endurance or sprint performances to a greater extent than traditional training methods. The increased RPE in the TM group likely caused subjects to reduce their workload to continue exercising, reducing the total exercise volume. This is supported by the previously mentioned body composition changes, which show that the mask hindered increases in lean mass and decreases in body fat percentage.

Pulmonary Function

Contrary to our hypothesis and to the manufacturers claims of the ETM being used as a "respiratory muscle trainer", results of pulmonary function tests indicate no added benefit of the ETM, without statistical difference in improvements from baseline between TM and CON groups. Furthermore, the only measured lung function change in both CON and TM groups was in MVV whereby both groups improved to the same degree. This supports the findings of Porcori et. al. who also found no change in FEV1 or FVC [31].

Resembling the effect of obstructive pulmonary diseases (e.g. asthma), the ETM limits inspiration and expiration (Figure 3c). The ETM also increases external dead space brought about by the internal volume of the ETM, which has been observed to be 240mL [54]. Increased external dead space leads to rebreathing of carbon dioxide which has been found to decrease aerobic performance[57]. While these potentially hypoxic conditions occur, previous investigations have found no effect on increases in the oxygen carrying capacity of red blood

cells via increases in hemoglobin [31]. It's important to note that the optimal duration of hypoxic exposure that leads to accelerate erythropoiesis is 4-weeks [34]. Additionally, to prevent the hindrance of hypoxia on training intensity, the live high / train low method has been shown to maximally improve sea-level aerobic performances[24]. However, if improved hypoxic performance is desired, hypoxic training may offer benefit, highlighted by the non-significant trend ($p<0.50$) towards greater performance improvements under masked endurance conditions (Figure 2c). Future studies are needed to determine the effect of the ETM on populations who commonly perform under restricted breathing conditions (eg. Deep sea divers, firefighters, etc.). With these considerations, it is not wise to induce airway obstruction during exercise if maximal or high intensity performance is desired during training.

Limitations

Our study provided a monitored exercise regimen. However, it was not without limitations. Due to the product design as a wearable device, a limitation of this study includes the lack of blinding to subjects and researchers. Additionally, the lack arterial oxygen saturation measurements made it impossible to determine hypoxemia. Lastly, subjects were asked not to participate in any regular anaerobic or aerobic exercise outside of normal daily activity, however as with most human intervention studies, activity and diet noncompliance is a possibility.

Conclusions

This study included a 12-week anaerobic and aerobic combination running regimen with monitoring of caloric intake and running exercises. Supporting our hypothesis, the ETM does not offer an advantage over traditional training and reduces maximal aerobic and anaerobic exercise

performance while hindering improvements in aerobic fitness, anaerobic running performance, and body composition. The ETM may induce hypoxemia through its tendency to decrease respiratory rate and increase external dead space [54]. The hypoxemic effects may be similar to those of high elevation training, but the duration of hypoxemia is not enough for the stimulation of red cell production [34]. Despite being advertised as a respiratory muscle trainer, pulmonary function remains unchanged. Utility may exist in special populations who may benefit by becoming accustomed to airway obstruction during physical exertion.

CHAPTER III

CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

This study was an assessment of an exercise device that has become popular in recent years. Our goal was to provide an exercise regimen that was well monitored to ensure exercise and ETM use compliance, while being applicable to exercises that are commonly used by athletes, coaches, and personal trainers. We opted for a concurrent endurance, strength, and sprint conditioning training model since many training regimens incorporate similar exercises, and an interference effect has been found to be minimal[58-60]. Furthermore, we used running-based training and testing conditions due to the familiarity and prevalence of running among recreational and professional athletes. This allows results to be relevant and applicable to various levels of athletes, personal trainers, and coaches. Within this study, we evaluated the chronic physiologic effects of training with the use of the ETM device, while capturing acute differences in performance variables. Findings from this study provide insight on the importance of an individual's desired training outcomes, and the impact of training specificity on the attainment of performance improvements. Because of the volume of data that was acquired, further data analysis may reveal additional conclusions guided by the results in the current study. While much is still unknown regarding the hormonal and hematological aspects of ETM training, the present study challenges the use of ETM for individuals desiring fitness and performance improvements under traditional, unobstructed-airway conditions.

Conclusion #1

The ETM hinders ability to improve aerobic and sprint running performance. In the present study, we hypothesized that twelve weeks of combined endurance and sprint-based training would lead to greater improvements in aerobic and sprint performances in the CON

group compared to the TM group. VO_2max increased more in the CON group (↑6.8 ± 1.2ml•kg^{-1}•min^{-1}) when compared to the TM group (↑4.3 ± 1.0 ml•kg^{-1}•min^{-1}). Similarly, U-TTE was increased to a greater degree in CON (↑83 ± 9 sec.) when compared to the TM group (↑63 ± 7 sec.). Since fitness improvements are a product of exercise intensity, duration, and adherence [61, 62], the ETM likely impedes exercise intensity since adherence and duration were controlled, hindering aerobic fitness improvements. Though red cell mass and hematocrit were not measured, it is likely that the duration of hypoxia was inadequate in eliciting physiologic adaptations that are desired for aerobic performance improvements [63].

Conclusion #2

The resistance valves of the ETM do not lead to greater improvements in pulmonary function when compared to traditional training, with MVV being the only endpoint that showed significant change among both CON and TM groups. Respiratory muscle training has been suggested in several studies[64-67] to improve athletic performance. However, in our study, pulmonary function was not found to be changed, and athletic performance was not found to be significantly improved in TM when compared to CON.

Of importance, specificity of sport may provide a place for respiratory muscle training with the goal of improving performance[64], in that training under air flow restricted conditions may provide an advantage over traditional training if the particular activity is associated with restricted breathing (eg. Deep sea diving, swimming, firefighting, etc.). Although it wasn't statistically significant, the TM group did improve to a greater degree in the M-GXT TTE, indicating a possible advantage when tested under masked conditions. This may be due to psychological or physiological factors. Regardless of the reason for the improvement in performance, the advantage of ETM training may exist in these populations.

Conclusion #3

The ETM leads to decreased body composition improvements, with inferior increases in lean mass and percent body fat. Similar to the previously stated reasons for impaired fitness improvements, the body composition results are likely due to impaired exercise intensity, decreasing kcal expenditure during exercise. Diet analysis indicates that no significant difference in kcal or percentage protein, carbs, or fat existed among or between TM and CON groups. This suggests differences in body composition changes were the result of exercise, rather than changes in diet.

Conclusion #4

The ETM significantly increased endurance rates of perceived exertion (Figure 4). This supports previous studies which have found statistically significant differences in RPE [31, 54]. The effect on RPE may be due to anxiety associated with restricted breathing[54], rather than a difference in workload. Regardless, this perception may lead people to falsely assume greater kilocalorie expenditure despite equivocal or reduced workloads of training with the ETM when compared to training without the ETM.

Directions for Future Research

Hematological Measurements

Hypoxic and high elevation exposure is known to cause hypoxemia [15]. Though the ETM does not cause a decrease in partial pressure of oxygen [54], the restriction to breathing may lead to hypoxemia similar to asthma or chronic obstructive pulmonary disease [15].

Measuring intra-workout peripheral oxygen saturation with a finger pulse oximeter is a non-invasive technique that can be utilized in future research.

The downstream effect of hypoxemia is the production of erythropoietin, which leads to increases in red cell mass[15]. Pre- and post-intervention measurements of erythropoietin and red cell mass would elucidate whether the possible hypoxemia is sufficient to produce physiologic changes that may improve oxygen carrying capacity of blood, which may improve aerobic capacity[15, 54].

Lactate Measurements

Lactate measurements during testing would have provided more direct assessment of changes in lactate threshold. Additionally, the rebreathing of CO_2 due to increased dead space of the mask may affect buffering capacity, leading to increased lactate build-up and cause increased muscle fatigue [9]. Lactate, pH, and pCO_2 measurements during training would elucidate these possibilities.

Work of Breathing

In our experiment, we measured VO_2max and RER during the Bruce Protocol treadmill stress test, which can be used to assess metabolic expenditure. Previous studies have assessed the metabolic demand of breathing in ventilator-dependent patients [68, 69] using indirect calorimetry. However, due to the physical obstruction of airways in our study, we could not measure metabolic demands of breathing via indirect calorimetry. One way around this would be to measure the force required to open the valves of the ETM. The mask could be secured to a suction device, and the energy (kilojoules) required to open the valves could be converted to kilocalories (1 kilocalorie = 4.184 kilojoules) to estimate the additional energy expenditure associated with exercising while wearing the ETM.

Special Populations

The population included in this study included recreationally active adults. Specified populations who may benefit from this study include firefighters, swimmers, fighter pilots, or others who commonly perform under air-restricted conditions. Training under restricted breathing conditions with the ETM may provide specificity of training that will translate to the air-restricted conditions that these populations are frequently tested under.

Limitations and Delimitations

Limitations

Compliance

Dietary compliance was based on self-reported diet logs, dependent on the subjects' ability to accurately portray their usual nutrition intake. Though we found no statistical difference in baseline and final total kcal or macronutrient intake, there are possibilities of inaccuracies. Additionally, subjects were urged not to participate in exercise outside of the study. It would be impossible to verify this without 24-hour monitoring of subjects. If noncompliance among subjects was present, it is unlikely that group-specific noncompliance occurred.

Subject Population

Since our study relied on volunteer participants, we were unable to control race demographics. Within the study, all subjects currently resided in the same geographical area, and some races were not represented in the study. Despite their local residence, the racial makeup was not homogeneous, as shown in Table 4. Additionally, the ratios of races were notably similar between groups. It is not known whether populations of different race would respond differently than those in the present study.

Table 7: Racial Demographics of TM and CON Groups

	CON	TM
White	7	7
Asian	1	2
Hispanic	3	2

Testing and Training Effort

Subjects were encouraged to give their complete effort in all training and testing sessions, both prior to and during exercises. However, we are unable to completely control the degree of effort each subject puts forth.

Delimitations

Subject Specificity

The results of this study are limited to the parameters of the subject population, which included recreationally active, untrained health adults in the general population. Further study is needed to determine if conclusions can be translated to other populations of interest.

Control Group

In the present study, we did not include an inactive control group. Both of TM and CON groups participated in identical training regimens, with the exception of the utilization of the ETM in the TM group. To compare these groups to an inactive group, an inactive control is needed in future studies.

Significance of Findings and General Summary

With the constant search for methods of improving aerobic and anaerobic fitness among recreational and elite athletes, the ETM has emerged as a popular exercise device. Contrary to previous established benefits of "Living high, training low" the ETM offers a contradictory approach by bringing about training under restricted-breathing conditions while living under

normal conditions. The results presented in the current study confirm a portion of our hypothesis and suggest that the goal of training should be considered when deciding to use the ETM or similar restrictive breathing devices. If the goal is to improve unrestricted aerobic performance, or sprint performance under restricted or unrestricted breathing conditions, training without the ETM may be more beneficial in improving performance. However, more studies must be conducted to further evaluate restricted aerobic performance improvements with the use of the ETM, as indicated by the nonsignificant trend towards a greater improvement in M-GXT TTE in the TM group. Unexpectedly, the ETM offered no benefit to improving pulmonary function despite being marketed as a respiratory muscle trainer. Despite the benefit of unrestricted breathing training in most settings, the ETM may offer a placebo effect by increasing RPE (non-significantly), making people more likely to exercise due to the perception that greater training difficulty leads to larger fitness gains, thereby improving exercise adherence.

Further investigation is needed to determine whether the ETM offers benefit to special populations outside of those tested in the present study, including the elderly, elite athletes, or clinical populations including those with weakened respiratory muscles. Furthermore, other modes of exercise can be evaluated to encompass a broader translation of results. As previously stated, populations who consistently perform under restricted breathing conditions, including but not limited to divers, firefighters, fighter pilots, or swimmers should be studied for a potential benefit.

This thesis represents one of the most applicable and experimentally monitored evaluations of the ETM or related devices. It presents level 1 evidence of the acute and chronic effects of training with an ETM. While room for further research exists, the current investigation

indicates that the ETM does not enhance, but may hinder, aerobic and anaerobic fitness improvements.

REFERENCES

1. W. Larry Kenney JHW, David L. Costill. Physiology of Sport and Exercise. 5th ed. Champaign, IL: Human Kinetics; 2012.
2. Petersson J, Glenny RW. Gas exchange and ventilation-perfusion relationships in the lung. Eur Respir J. 2014 Oct;44(4):1023-41.
3. American Thoracic Society/European Respiratory S. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med. 2002 Aug 15;166(4):518-624.
4. Ross A, Leveritt M. Long-term metabolic and skeletal muscle adaptations to short-sprint training: implications for sprint training and tapering. Sports Med. 2001;31(15):1063-82.
5. LeMura LM, Von Duvillard SP. Clinical exercise physiology : application and physiological principles. Philadelphia: Lippincott Williams & Wilkins; 2004.
6. Poortmans JR. Principles of exercise biochemistry. 3rd, rev. ed. Basel ; New York: Karger; 2004.
7. Pittman RN. Regulation of Tissue Oxygenation. San Rafael (CA); 2011.
8. Satyanarayana U, Chakrapani U. Biochemistry (with clinical concepts & case studies). 4th ed. New Delhi: Elsevier Health Sciences APAC; 2015.
9. Bassett DR, Jr., Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc. 2000 Jan;32(1):70-84.
10. Wenger HA, Bell GJ. The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. Sports Med. 1986 Sep-Oct;3(5):346-56.
11. Rivera-Brown AM, Frontera WR. Principles of exercise physiology: responses to acute exercise and long-term adaptations to training. PM R. 2012 Nov;4(11):797-804.
12. McKenzie DC. Respiratory physiology: adaptations to high-level exercise. Br J Sports Med. 2012 May;46(6):381-4.
13. Blanco E, Galeano P, Palomino A, Pavon FJ, Rivera P, Serrano A, et al. Cocaine-induced behavioral sensitization decreases the expression of endocannabinoid signaling-related proteins in the mouse hippocampus. Eur Neuropsychopharmacol. 2016 Mar;26(3):477-92.
14. Howald H, Hoppeler H, Claassen H, Mathieu O, Straub R. Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. Pflugers Arch. 1985 Apr;403(4):369-76.
15. Jelkmann W. Erythropoietin after a century of research: younger than ever. Eur J Haematol. 2007 Mar;78(3):183-205.
16. Schwandt HJ, Heyduck B, Gunga HC, Rocker L. Influence of prolonged physical exercise on the erythropoietin concentration in blood. Eur J Appl Physiol Occup Physiol. 1991;63(6):463-6.
17. Hickson RC. Skeletal muscle cytochrome c and myoglobin, endurance, and frequency of training. J Appl Physiol Respir Environ Exerc Physiol. 1981 Sep;51(3):746-9.
18. Milanovic Z, Sporis G, Weston M. Effectiveness of High-Intensity Interval Training (HIT) and Continuous Endurance Training for VO2max Improvements: A Systematic Review and Meta-Analysis of Controlled Trials. Sports Med. 2015 Oct;45(10):1469-81.
19. Chapman RF, Karlsen T, Resaland GK, Ge RL, Harber MP, Witkowski S, et al. Defining the "dose" of altitude training: how high to live for optimal sea level performance enhancement. J Appl Physiol (1985). 2014 Mar 15;116(6):595-603.

20. Hawley JA. Adaptations of skeletal muscle to prolonged, intense endurance training. Clin Exp Pharmacol Physiol. 2002 Mar;29(3):218-22.
21. Paralikar SJ, Paralikar JH. High-altitude medicine. Indian J Occup Environ Med. 2010 Jan;14(1):6-12.
22. Riley CJ, Gavin M. Physiological Changes to the Cardiovascular System at High Altitude and Its Effects on Cardiovascular Disease. High Alt Med Biol. 2017 Mar 15.
23. Naeije R. Physiological adaptation of the cardiovascular system to high altitude. Prog Cardiovasc Dis. 2010 May-Jun;52(6):456-66.
24. Levine BD, Stray-Gundersen J. "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. J Appl Physiol (1985). 1997 Jul;83(1):102-12.
25. Gore CJ, Hahn A, Rice A, Bourdon P, Lawrence S, Walsh C, et al. Altitude training at 2690m does not increase total haemoglobin mass or sea level VO2max in world champion track cyclists. J Sci Med Sport. 1998 Sep;1(3):156-70.
26. Geiser J, Vogt M, Billeter R, Zuleger C, Belforti F, Hoppeler H. Training high--living low: changes of aerobic performance and muscle structure with training at simulated altitude. Int J Sports Med. 2001 Nov;22(8):579-85.
27. Morton JP, Cable NT. Effects of intermittent hypoxic training on aerobic and anaerobic performance. Ergonomics. 2005 Sep 15-Nov 15;48(11-14):1535-46.
28. Roels B, Bentley DJ, Coste O, Mercier J, Millet GP. Effects of intermittent hypoxic training on cycling performance in well-trained athletes. Eur J Appl Physiol. 2007 Oct;101(3):359-68.
29. Heinicke K, Heinicke I, Schmidt W, Wolfarth B. A three-week traditional altitude training increases hemoglobin mass and red cell volume in elite biathlon athletes. Int J Sports Med. 2005 Jun;26(5):350-5.
30. Sellers JH, Monaghan TP, Schnaiter JA, Jacobson BH, Pope ZK. Efficacy of a Ventilatory Training Mask to Improve Anaerobic and Aerobic Capacity in Reserve Officers' Training Corps Cadets. J Strength Cond Res. 2016 Apr;30(4):1155-60.
31. Porcari JP, Probst L, Forrester K, Doberstein S, Foster C, Cress ML, et al. Effect of Wearing the Elevation Training Mask on Aerobic Capacity, Lung Function, and Hematological Variables. J Sports Sci Med. 2016 Jun;15(2):379-86.
32. Jagim AR, Dominy TA, Camic CL, Wright G, Doberstein S, Jones MT, et al. Acute Effects of the Elevation Training Mask on Strength Performance in Recreational Weight lifters. J Strength Cond Res. 2018 Feb;32(2):482-9.
33. Lundby C, Millet GP, Calbet JA, Bartsch P, Subudhi AW. Does 'altitude training' increase exercise performance in elite athletes? Br J Sports Med. 2012 Sep;46(11):792-5.
34. Millet GP, Roels B, Schmitt L, Woorons X, Richalet JP. Combining hypoxic methods for peak performance. Sports Med. 2010 Jan 1;40(1):1-25.
35. Humberstone-Gough CE, Saunders PU, Bonetti DL, Stephens S, Bullock N, Anson JM, et al. Comparison of live high: train low altitude and intermittent hypoxic exposure. J Sports Sci Med. 2013;12(3):394-401.
36. Wilber RL. Pro: Live high+train low does improve sea level performance beyond that achieved with the equivalent living and training at sea level. High Alt Med Biol. 2013 Dec;14(4):325-7.
37. Billat LV, Koralsztein JP. Significance of the velocity at VO2max and time to exhaustion at this velocity. Sports Med. 1996 Aug;22(2):90-108.

38. McLean BD, Gore CJ, Kemp J. Application of 'live low-train high' for enhancing normoxic exercise performance in team sport athletes. Sports Med. 2014 Sep;44(9):1275-87.
39. Reilly T, Morris T, Whyte G. The specificity of training prescription and physiological assessment: a review. J Sports Sci. 2009 Apr;27(6):575-89.
40. Hoppeler H. Exercise-induced ultrastructural changes in skeletal muscle. Int J Sports Med. 1986 Aug;7(4):187-204.
41. Engfred K, Kjaer M, Secher NH, Friedman DB, Hanel B, Nielsen OJ, et al. Hypoxia and training-induced adaptation of hormonal responses to exercise in humans. Eur J Appl Physiol Occup Physiol. 1994;68(4):303-9.
42. Mizuno M, Juel C, Bro-Rasmussen T, Mygind E, Schibye B, Rasmussen B, et al. Limb skeletal muscle adaptation in athletes after training at altitude. J Appl Physiol (1985). 1990 Feb;68(2):496-502.
43. Verges S, Lenherr O, Haner AC, Schulz C, Spengler CM. Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training. Am J Physiol Regul Integr Comp Physiol. 2007 Mar;292(3):R1246-53.
44. Illi SK, Held U, Frank I, Spengler CM. Effect of respiratory muscle training on exercise performance in healthy individuals: a systematic review and meta-analysis. Sports Med. 2012 Aug 01;42(8):707-24.
45. Verges S, Sager Y, Erni C, Spengler CM. Expiratory muscle fatigue impairs exercise performance. Eur J Appl Physiol. 2007 Sep;101(2):225-32.
46. Lemaitre F, Coquart JB, Chavallard F, Castres I, Mucci P, Costalat G, et al. Effect of additional respiratory muscle endurance training in young well-trained swimmers. J Sports Sci Med. 2013;12(4):630-8.
47. American Media IFHN. Elevation Training Mask. Muscle and Fitness.
48. Bruce RA, Blackmon JR, Jones JW, Strait G. Exercising Testing in Adult Normal Subjects and Cardiac Patients. Pediatrics. 1963 Oct;32:SUPPL 742-56.
49. Detrano R, Froelicher VF. Exercise testing: uses and limitations considering recent studies. Prog Cardiovasc Dis. 1988 Nov-Dec;31(3):173-204.
50. Myers J, Froelicher VF. Exercise testing. Procedures and implementation. Cardiol Clin. 1993 May;11(2):199-213.
51. Breuer HW. [Cardiopulmonary exercise tests -- proposals for standardization and interpretation]. Pneumologie. 2004 Aug;58(8):553-65.
52. Gillinov S, Etiwy M, Wang R, Blackburn G, Phelan D, Gillinov AM, et al. Variable Accuracy of Wearable Heart Rate Monitors during Aerobic Exercise. Med Sci Sports Exerc. 2017 Aug;49(8):1697-703.
53. Ponce MC, Sharma S. Pulmonary Function Tests. StatPearls. Treasure Island (FL); 2018.
54. Granados J, Gillum TL, Castillo W, Christmas KM, Kuennen MR. "Functional" Respiratory Muscle Training During Endurance Exercise Causes Modest Hypoxemia but Overall is Well Tolerated. J Strength Cond Res. 2016 Mar;30(3):755-62.
55. Schoenfeld BJ. The mechanisms of muscle hypertrophy and their application to resistance training. J Strength Cond Res. 2010 Oct;24(10):2857-72.
56. Mangine GT, Hoffman JR, Gonzalez AM, Townsend JR, Wells AJ, Jajtner AR, et al. The effect of training volume and intensity on improvements in muscular strength and size in resistance-trained men. Physiol Rep. 2015 Aug;3(8).

57. Johnson AT, Scott WH, Lausted CG, Coyne KM, Sahota MS, Johnson MM. Effect of external dead volume on performance while wearing a respirator. AIHAJ. 2000 Sep-Oct;61(5):678-84.
58. Murach KA, Bagley JR, McLeland KA, Arevalo JA, Ciccone AB, Malyszek KK, et al. Improving human skeletal muscle myosin heavy chain fiber typing efficiency. J Muscle Res Cell Motil. 2016 Apr;37(1-2):1-5.
59. Lundberg TR, Fernandez-Gonzalo R, Gustafsson T, Tesch PA. Aerobic exercise does not compromise muscle hypertrophy response to short-term resistance training. J Appl Physiol (1985). 2013 Jan 1;114(1):81-9.
60. Shaw BS, Shaw I, Brown GA. Comparison of resistance and concurrent resistance and endurance training regimes in the development of strength. J Strength Cond Res. 2009 Dec;23(9):2507-14.
61. De Feo P. Is high-intensity exercise better than moderate-intensity exercise for weight loss? Nutr Metab Cardiovasc Dis. 2013 Nov;23(11):1037-42.
62. Fisher G, Brown AW, Bohan Brown MM, Alcorn A, Noles C, Winwood L, et al. High Intensity Interval- vs Moderate Intensity- Training for Improving Cardiometabolic Health in Overweight or Obese Males: A Randomized Controlled Trial. PLoS One. 2015;10(10):e0138853.
63. Wilber RL, Stray-Gundersen J, Levine BD. Effect of hypoxic "dose" on physiological responses and sea-level performance. Med Sci Sports Exerc. 2007 Sep;39(9):1590-9.
64. HajGhanbari B, Yamabayashi C, Buna TR, Coelho JD, Freedman KD, Morton TA, et al. Effects of respiratory muscle training on performance in athletes: a systematic review with meta-analyses. J Strength Cond Res. 2013 Jun;27(6):1643-63.
65. Ker JA, Schultz CM. Respiratory muscle fatigue after an ultra-marathon measured as inspiratory task failure. Int J Sports Med. 1996 Oct;17(7):493-6.
66. Romer LM, McConnell AK, Jones DA. Effects of inspiratory muscle training on time-trial performance in trained cyclists. J Sports Sci. 2002 Jul;20(7):547-62.
67. Griffiths LA, McConnell AK. The influence of inspiratory and expiratory muscle training upon rowing performance. Eur J Appl Physiol. 2007 Mar;99(5):457-66.
68. Savino JA, Dawson JA, Agarwal N, Moggio RA, Scalea TM. The metabolic cost of breathing in critical surgical patients. J Trauma. 1985 Dec;25(12):1126-33.
69. Hoher JA, Zimermann Teixeira PJ, Hertz F, da SMJ. A comparison between ventilation modes: how does activity level affect energy expenditure estimates? JPEN J Parenter Enteral Nutr. 2008 Mar-Apr;32(2):176-83.

APPENDIX A

INFORMED CONSENT DOCUMENTATION

Informed Consent for Observational/Non-Interventional Research

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Participant's Name: **Subject ID Number:**

Study Title: The Effects of Training with and Altitude Mask

Principal Investigator: Dr. Joshua Harris

Funding Source (if applicable): Internally Funded

Study Purpose/ Summary: There is currently an increased interest in evaluating the efficacy and/or risk associated with training in an altitude-simulating mask, which has become increasingly popular in recent years. Briefly, this method of training is supposed to simulate the effects of training in high elevation, causing greater increases in aerobic and anaerobic performances by increasing aerobic capacity, anaerobic threshold, and by strengthening respiratory muscles. Furthermore, altitude training has been found to cause possible beneficial increases in muscle capillarization and muscle growth. However, there is debate among the exercise physiology and orthopedic community on the effectiveness of these masks on simulating training at high elevation, and the translation of this to increases in running performance. Furthermore, there may be risk involved with the product interfering with natural breathing responses to exercise. Therefore, the goal of this study is to evaluate the potential risks verses the previously intended benefits.

Why me: You are being asked to participate in a research study because you are considered a recreationally active healthy volunteer.

Study Purpose/Executive Summary:

This research study aims to assess the effectiveness of an altitude-simulating mask in improving sprint, anaerobic endurance, and aerobic endurance performance to a greater degree than traditional training. We will assess the effects of the altitude-simulating mask on aerobic capacity. We will monitor and evaluate the effects on peak heart rate, anaerobic threshold, and

blood oxygenation during training with and without the mask. Finally, body composition will be measured using dual-energy x-ray absorptiometry (DEXA) before and after the training program.

This study is a randomized controlled trial with two study groups. You will be randomized to participate in either one of the following groups:

1. A standard sprint and endurance training regimen **without** wearing an altitude-simulating mask, or
2. A standard sprint and endurance training regimen **while** wearing and altitude simulating mask.

Both groups will participate in a similar sprint/endurance training regiments. The training regiments will include alternating endurance and sprint based training. Endurance training will involve individualized treadmill running with controlled speed and grade based on your VO_2max (ml/kg/min), which is a measure of your maximum volume of oxygen consumption. During training, intensity and duration will increase week to week. Sprint training will include interval sprints and interval shuttle runs ranging from 100 to 300 yards. Sprints and shuttle runs have pre-determined intra-run rest periods, and a rest period between sprint and shuttle portions. Distance and frequency will increase week to week.

VO_2max of each individual will be determined via Bruce Protocol Graded Exercise Stress Test prior to beginning the training regimen to monitor improvements and to calculate endurance training. VO_2max will be reassessed after 5 weeks of training to account for fitness improvements that may have occurred. This reassessed VO_2max will be used to calculate training intensity for each person for the final 5 weeks of training. Additionally, a warm-up and cool-down session will be included in the training sessions, consisting of a series of dynamic stretches and exercises.

This study involves research and there are certain outcomes that are unknown.

Your participation in this study is voluntary. You can choose to participate at any time without any penalty or loss of benefits to which you are entitled.

What risk will I face by taking part in the study and how will Researchers protect me from these risks?

Both control and experiment groups may have risks associated with exercise. These include, but are not limited to, risks of:

- sudden cardiac death
- myocardial infarction
- arrhythmia
- rhabdomyolysis
- bronchoconstriction
- hyperthermia
- dehydration
- electrolyte imbalances
- nausea
- acute muscle strains
- muscle inflammation
- chronic strains
- stress fractures
- fall injuries
- tendonitis
- bursitis

The experiment group, which will be wearing the mask, may have an increased risk of difficult breathing, stress on the heart (due to potential increase in red blood cells), asthma exacerbation, hypoxemia, dizziness, headache, altitude sickness, high altitude cerebral edema, high altitude pulmonary edema, suffocation sensation.
In an effort to reduce possibilities of the mentioned injuries, exclusion criteria will be explained prior to consent and subjects will monitored throughout screening, testing, and training. Stress testing will be a component of screening, which will include a Bruce Protocol Graded Exercise Stress Test, Cardiovascular evaluation using ECG, and measurements of VO_2max and anaerobic thresholds. Additionally, the presence of asthma will be evaluated using spirometry. If life threatening abnormalities are present, the subject will be excluded from participation in the study.

The researchers have taken steps to minimize the risks of this study. Please tell the researchers in the contact section about any injuries, side effects, or other problems that you have during this study. You should also tell your regular doctors**.**

As with any research study, there may be additional risks that are unknown or unexpected. If these become known, the study team will notify you in a timely manner of any changes that may change your willingness to participate. If new information is provided to you after you have joined the study, it is possible that you may be asked to sign a new consent form that includes the new information.

Research Related Injury: As with any research study, there may be additional risks that are unknown or unexpected. If you are injured as a direct result of this study, medical care is available and can be provided. However, no long-term or short-term medical care for research-related injuries will be financially covered by Houston Methodist. You do not waive (give up) any legal rights by signing this informed consent form.

During the study, subjects will be continuously reminded that the nature of the study is entirely voluntary. Heart rates and ratings of perceived physical exertion (Borg scale) will be monitored during all testing and training activities. Blood pressure will be measured before and after each session involving physical activity. All training and performance testing will only occur after a standardize warm-up. Subjects in either group will be reminded during training that while we are asking for maximal exercise effort, subjects may slow down or stop if they experience symptoms of nausea, dizziness, chest pain/discomfort, or any circumstance of physical injury such as sprains, strains, or fractures. In the event that any of these occur, such instances will be recorded and an adverse event report will be created and submitted to the IRB.

Please tell the researchers listed in Section 10 about any other problems that you have during this study. You should also tell your regular doctors**.**

How could I and others benefit if I take part in this study?

Participants may benefit with the advantage of gaining exercise supervision and accountability. Furthermore, the information gained though the health screening process and performance assessments may be of value to the participants.

However, because this is a research study, there is no guarantee that you will receive any benefits from this study.

Are there any cost or payments?

You and/or your insurance company will be responsible for payment of items and services that you would receive even if you were not participating in the research study. You will be responsible for your normal co-payments and co-insurance/deductibles.

You will not be paid for taking part in the study.

If you have any questions as to what your obligations are for payment for items or services under this study, or would like to see a list of procedures or items for which you are responsible financially, please talk with the study team and/or your insurance company.

The investigator and Houston Methodist Research Institute do not have any financial interest in the outcome of the study.

If commercial products or other valuable discoveries result from this research project, these products and discoveries could be patented, licensed, or otherwise developed for commercial sale by Houston Methodist Research Institute or the study Sponsor or their respective designees. If this should occur, there are no plans to provide financial compensation to you. There are no plans for you to share in the patent rights, other ownership rights, or rights to control the commercial products and discoveries that may result from this research project.

If I want to stop participating in the study, what should I do?

If you wish to stop your participation in this research study for any reason you should let the principal investigator/study coordinator know as soon as possible so that you can stop safely. You may be asked why you are leaving the study and your reasons for leaving may be kept as part of the study record. If you decide to leave the study before it is finished, please tell one of the persons listed in "Contact Information".

What happens if I get hurt, my condition worsens, or have other problems as a result of this research?

If you are injured as a direct result of this study, medical care is available. In general, no long-term medical care or financial compensation for research-related injuries will be provided by Houston Methodist. You do not waive (give up) any legal rights by signing this informed consent form.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.
For questions about your rights as a research participant, or if you have complaints, concerns, or questions about the research, please contact Susan M. Miller, M.D., M.P.H., Chair, Houston Methodist Institutional Review Board for the Protection of Human Subjects, at 713-441-2750 or Ethan Natelson, MD, Chair, Houston Methodist Research Institute Institutional Review Board for the Protection of Human Subjects, at 713-441-5154. You may also contact the Director, HMRI Office of Research Protections at HMRI Office of Research Protections, 1130 John Freeman, MGJ6-016, Houston, Texas 77030. Ph: 713-441-7548

The research team will take proper precautions to ensure that any information regarding your identity obtained in connection with this research will remain confidential.

Authorization to use and disclose protected health information

If you decide to participate in this study, information about your health may be used or disclosed (shared outside of the Hospital) for the purposes of conducting this study. This information may include information from your medical record that is relevant to this study, such as your medical history, medications, test results, diagnoses, treatments, operative reports (reports from operations that you have undergone), and discharge summaries. It may also include information *relating to: Human Immunodeficiency Virus ("HIV") infection or Acquired Immunodeficiency Syndrome ("AIDS"); treatment for or history of drug or alcohol abuse; or mental or behavioral health or psychiatric care.* Information collected by the study doctor and/or research staff specifically for this study, such as test results, blood samples, physical examinations, information about possible side effects, and surveys you might be asked to complete could also be used or disclosed.

Houston Methodist may release your personal health information to other researchers or institutions, or to government agencies for the conduct of this research, for monitoring and safety, or regulatory research. If approved by the Institutional Review Board (IRB), your coded personal information may be released to other researchers or institutions who may wish to conduct their own research. In most cases, the information released to the above listed individuals or entities will not contain your name, social security number, or any other personal information. However, authorized representatives of your study doctor, IRB, FDA, or other government agencies may review records containing personal information to make sure that the study information is correct. Because of the need to provide information to these parties, absolute confidentiality cannot be guaranteed.

Because this information is being disclosed for research use, there is no expiration date for the use of your information. This authorization is valid until you revoke it. You can revoke this authorization at any time by contacting the investigators and if possible any identifiable information will be destroyed. I understand that the revocation will not apply to information that already has been released or actions that have already been taken in response to this authorization. I have a right to request a copy of any of my health information that is released under this authorization.

Other researchers or institutions that receive your information may not be covered by Federal or Texas privacy laws. As such, your information may not be protected under these laws once it is disclosed and, therefore, may be subject to re-disclosure or use by such individuals or institutions.

Where can I get more information?

If you have any questions regarding your participation in this study, please ask us. If you have any additional questions later, please contact the researchers listed below to:

Principal Investigator: Joshua Harris, MD
Mailing Address: 6445 Main St. Suite 2500 Houston, TX 77030
Telephone: 713-441-8393

Study Coordinator: Tyler Heimdal
Mailing Address: 6445 Main St. Suite 2500 Houston, TX 77030
Telephone: 940-390-7466

Optional Participation

USE OF DATA

The researchers would like to use your data for future research. Your samples may be kept by an external third party Sponsor, HMRI, or a collaborative organization designated by HMRI (such as another research organization, university, or a private company). Once you contribute data and samples, they may no longer be in an identified form that would allow them to be located and destroyed, or there may be other reasons why the samples need to be retained for further study and validation of results. Therefore, when you contribute data or samples, you should assume that it will not be possible for you ever to get them back.

Please √ check one: [ ] Yes [ ] No

NOT RECONTACTING PARTICIPANTS

It is possible that, in studying tissue samples and data from you and others, researchers may discover information that would be potentially relevant to your future health. In the event that this occurs, there are no plans to make this information available to you. This is because the tissue samples may have been coded or de-identified in a way that makes it difficult to trace the result back to a specific person, and because the results of research often are too uncertain to be

used as specific medical information. Your signature below indicates that you understand this to be true.

Please √ check one: [ ] Yes [ ] No

Future Contact

Please indicate whether you would or would not be willing to let our researchers get in touch with you in the future, to ask whether you would be willing to contribute more tissue samples or data or participate in another study at that time:

Please √ check one: [ ] Yes [ ] No

Study Participant:

I have read this consent form or had it read to me. I have discussed it with the study team and my questions have been answered. I will be given a signed copy of this form. I agree to take part in this study including any options where I checked 'yes'.

Signature of Study Participant or Legally Authorized Representative:

_____ _____ **Date:**_____ **Time:** _____

Name (Print Legal Name): _____

Legal Representative Information (If Applicable)

Phone: _____

Check Relationship to Subject: □Parent □ Spouse □Child □Sibling □Legal Guardian □Other: _____

Reason subject is unable to sign for self:_____

Person Obtaining Consent:

I have given this research subject (or his/her legally authorized representative) information about this study that I believe is accurate and complete. The subject has indicated that he or she understands the nature of the study and the risks and benefits of participating.

Name: _____ **Title:** _____

Signature: _____ **Date of Signature:** _____

Translation Service: I verbally translated the informed consent process and the conversation between the investigator and the study participant.

Name: _____ **Organization:** _____

Signature: _____ **Date of Signature:** _____

Witness:

_____ I was present as an impartial witness (not a member of the research team or family) for the informed consent process. I observed the above subject (or his/her legally authorized representative, if applicable) indicate consent.

If applicable participant unable to sign, how did he or she indicate consent: : _____
_____

Name: _____

Signature: _____ **Date of Signature:** _____

APPENDIX B

PHONE SCREENING

Date: _____

Phone Screening Altitude Training Mask Study

Name: _____
Sex: M F Age: _____ DOB: _____ Race: _____
Have you ever participated in a Houston Methodist study? Y N
Which study? _____
How did you hear about our study? _____

Home phone: _____ Cell phone: _____
Address: _____
City: _____ State: _____ Zip Code: _____
Email address: _____

I am going to ask you some questions about your health to see if you would be eligible for our study:

Height: _____ Weight: _____ BMI: _____

- Have you experienced significant weight loss in the past year? Y N
- Have you had a stable body weight for at least 1 year? Y N
- Do you have a history of falls (≥2/year)? Y N

Medication	**Condition**	**Dose/Frequency**	**How long?**
OTC Medications	**Condition**	**Dose/Frequency**	**How long?**

Vitamins/Supplements/Herbs	Condition	Dose/Frequency	How long?

Have you ever been diagnosed with:

Heart disease? Y N _____

Liver disease? Y N _____

Kidney disease? Y N _____

Blood disease? Y N _____

Thyroid issues? Y N _____

ASTHMA? Y N _____

Respiratory disease? Y N _____

Peripheral vascular disease? Y N _____

Diabetes or other untreated endocrine disease? Y N _____

Other diseases? Y N _____

Do you have active cancer, or a history of cancer? Y N _____

Have you ever had any major surgeries? Y N _____

_____

Have you been treated with anabolic steroids or corticosteroids in the past six months? Y N _____

Do you struggle with alcohol or drug abuse? Y N _____

Do you smoke or chew tobacco? Y N _____

Do you have any food allergies? Y N _____

Are you allergic to local anesthesia? Y N _____

How many exercise training sessions per week do you participate? _____

What are your current exercise habits? _____

_____

While you are sitting, would you be able to kick your leg out? Y N Maybe

Status of subject: _____Not Eligible _____Not Interested _____Potential Subject

Dates available:_____

Screening date: _____

APPENDIX C

DIET RECORDS

Houston Methodist Orthopedics and Sports Medicine
Altitude Training Mask Study
Three Day Diet Record

Name:_____ Age:_____ Ht:_____ Wt:_____

DIRECTIONS: This Three Day Diet Record is designed to measure your food intake over the course of three days. Please make sure that ONE recorded day is a weekend, and TWO recorded days are weekdays, excluding Friday.

1. Records should be kept over a time period that best represents your "normal" eating patterns for 2 weekdays and one weekend day. For example, if Monday is a work holiday, it is unlikely that you will eat as you normally would.

2. Record **ALL** food and drink (**including water**) that you consume on each day. Record both the type of food or drink and the amounts consumed.

3. Please be as specific on foods and amounts as possible. For example, if you eat a turkey sandwich, please record the type of bread (white, whole wheat, rye, etc), number of slices of meat, and any additional items (cheese, tomato, mayonnaise, etc). Also include brand names of items when possible. For help in determining what is considered a serving, see the serving size chart on page 2 for some common food items.

4. Page 3 shows a sample day of the diet record. Please read this to help you become familiar with the recording format.

5. If you have any questions about filling out the record, please contact laboratory staff for assistance.

6. Return this record to the laboratory staff once it is complete.

Please do not change your diet in any way during the course of the study. Maintain normal eating habits, please do not begin a "diet". If you travel, don't worry, these changes from normal are only temporary.

Serving Size Chart

1 Cup cereal flakes or 1 baked potato = size of a fist

½ cup cooked rice, pasta or potato = size of an ice cream scoop

1 pancake = size of a CD

1 cup of salad greens or 1 medium fruit = size of a baseball

½ cup fresh fruit or vegetables = size of a standard light bulb

¼ cup dried fruit = 1 large egg

3 oz. meat, fish, poultry = size of a deck of cards

2 Tbsp peanut butter = size of a golf ball

1 ½ oz. cheese = 4 stacked dice or 2 cheese slices; 1 tsp margarine, butter and spreads = 1 dice

DAY 1 **Date: _____** **Day of Week:_____**

Food Eaten	# of servings or amount

DAY 2 **Date: _____** **Day of Week:_____**

Food Eaten	# of servings or amount

DAY 3 **Date: _____** **Day of Week:_____**

Food Eaten	# of servings or amount

DAY 1 **Date: SAMPLE** **Day of Week: _SAMPLE DAY__**

Food Eaten	**# of servings or amount**
Breakfast	
coffee (caffinated)	1-8oz cup
w/ half&half	2 Tbsp
w/ Splenda	1 Tbsp
Raisin Bran cereal	1 cup
w/ 1% milk	1 cup
Multivitamin	1 vitamin
Lunch	
Turkey sandwich (homemade)	
w/ turkey deli meat	3 slices
w/ Kraft American cheese	1 slice
w/ Lite mayo	2 Tbsp
w/ whole wheat bread	2 slices
w/ mustard	1 tbsp
apple	1 medium
Lay's potato chips	1 snack bag
Sprite	12oz can
Snacks	
water	20oz bottle
Nature's Own honey granola bar	2 bars
Hershey's Kisses	3 kisses
Lemon-lime Gatorade	32oz bottle
Dinner	
McDonald's Big Mac	
w/ cheese and mayo	
french fries	medium
Diet Coke	medium
Bluebell Vanilla Ice Cream	2 scoops
w/ chocolate syrup	2 Tbsp

Diet Compliance Form

DIET COMPLIANCE

NAME:_____ DATE:_____

1. My diet (**has / has not**) changed from the last diet record submitted.

2. My diet changed as follows:

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Printed Name _____

Signature _____

APPENDIX D

GRADED EXERCISE DATA SHEET

OBRL Graded Exercise Testing Data Sheet

Pre-exercise Data

Subject ID _____ Date of Test_____ Test Protocol: _____

Pertinent Medical History: _____

Pertinent Medications / Doses: _____

Supine HR _____ Supine BP _____

Pre-ex HR _____ Pre-ex BP _____ Predicted max HR _____ 85% Predicted max HR _____

Exercise Data

Time	Speed	Grade	HR	BP	RPE	Comments

Recovery Data

Time	HR	BP	Comments

Maximal Exercise Data

Max. Ex. Time _____ Peak HR _____ Peak BP _____

Reason for Stopping _____n

n

1 ST or 2 mm
2 Chest Pain
3 Induced BB Block
4 Abnormal BP Response
5 V-Tach
6 Sust. A-Tach.
7 2nd or 3rd degree HB
8 Frequent PVC's
9 R on T PVC
10 Multifocal PVC's
11 Light Headedness
12 Dyspnea
13 Claudication
14 General Fatigue
15 Other _____

ECG Technician :_____ BP Technician : _____

APPENDIX E

SPRINT TESTING DATA SHEET

Sprint Testing Data Sheet

Pre-exercise Data

Subject ID: _____ Date of Test: _____ Test Protocol: _____
Pertinent Medical History: _____
Pertinent Medications / Doses: _____
Weight: _____ Sitting HR: _____ Sitting BP: _____

Masked? _____ Start Time on Watch _____
Polar Watch # _____ End Time on Watch _____

Exercise Data

Shuttle	Split	Interval Time	Continuous Time	HR	Comments
#1	100				
	200				
	300				
	Total				
Rest 5 Minutes					
#2	100				
	200				
	300				
	Total				

Recovery Data

Recovery Time	Continuous Time	HR	BP	Comments

BP Technician: _____

APPENDIX F

TREADMILL TRAINING DATA SHEET

OBRL – Treadmill Training Data Sheet

Subject ID # _____
Training Wk# _____
Date _____
Time _____
Polar Watch # _____
Group (N or NM) _____
Weight _____

Exercise Prescription

%VO2 _____
SPEED _____
GRADE _____
METS _____
TARGET HR (unmasked) _____
TIME (min) _____

Exercise Data

RBP _____
RHR _____

INTERVAL	DATA	INTERVAL	DATA
5min HR		30min HR	
5min RPE		30min RPE	
5min SPEED		30min SPEED	
5min GRADE		30min GRADE	
INTERVAL	DATA	INTERVAL	DATA
10min HR		35min HR	
10min RPE		35min RPE	
10min SPEED		35min SPEED	
10min GRADE		35min GRADE	
INTERVAL	DATA	INTERVAL	DATA
15min HR		40min HR	
15min RPE		40min RPE	
15min SPEED		40min SPEED	
15min GRADE		40min GRADE	
INTERVAL	DATA	INTERVAL	DATA
20min HR		45min HR	
20min RPE		45min RPE	
20min SPEED		45min SPEED	
20min GRADE		45min GRADE	
INTERVAL	DATA	POST EXERCISE	DATA
25min HR		5min Post EX BP	
25min RPE		5min Post EX HR	
25min SPEED		PAIN?	Y or N
25min GRADE		Overall RPE	

1 MIN POST-EXERCISE BP _____
3 MIN POST-EXERCISE BP _____
5 MIN POST-EXERCISE BP _____

1 MIN POST-EXERCISE HR _____
3 MIN POST-EXERCISE HR _____
5 MIN POST-EXERCISE HR _____

PAIN? Y OR N
OVERALL RPE _____

COMMENTS: _____
_____
_____

APPENDIX G

SPRINT TRAINING DATA SHEET

Sprint Training Data Sheet

Subject ID # _____ Polar Watch # _____
Training Wk# _____ Group (M or U) _____
DATE _____ Weight _____
Time _____

Exercise Prescription

	Distance	Repetitions	Duration	Rest Period
Sprint				
Shuffle				
Shuttle				

*5-minute rest between activity

Exercise Data

RBP _____
RHR _____

SPRINT		SHUFFLE		SHUTTLE	
Pre HR		Pre HR		Pre HR	
Post HR		Post HR		Post HR	
Pre RPE		Pre RPE		Pre RPE	
Post RPE		Post RPE		Post RPE	
Time Rep 1/2/3		Quantity Rep 1		Time Rep 1	
Time Rep 4/5/6		Quantity Rep 2		Time Rep 2	
Time Rep 7/8/9		Quantity Rep 3		Time Rep 3	
Time Rep 10/11/12		Quantity Rep 4		Time Rep 4	
Time Rep 13/14/15					

*Start and End times need to be recorded based on the Polar Watch

1 MIN RECOVERY HR _____ 3 MIN RECOVERY HR _____
5 MIN RECOVERY BP _____ PAIN? Y OR N
5 MIN RECOVERY HR _____ OVERALL RPE _____

COMMENTS: _____
_____
_____