

A Simulated Altitude Device can Improve Endurance Performance without Mucosal
Immune System Compromise.

Thesis

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By

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Abstract

"Live High-Train Low" (LHTL) altitude acclimatization has been shown to increase athletic endurance performance; however, this type of training has been associated with decreases in mucosal immune system function as measured by salivary IgA antibodies (Tiollier et al., 2005). Physiological effects of altitude, strenuous training, and isolation from family could be additive stresses that depress the immune system (Walsh et al., 2006). Babcock and Kirby (2008) have shown that a simulated intermittent altitude exposure (IE) device utilized for short durations has the same performance enhancing effects as LHTL; however, the effect of IE via re-breathing on running performance and maintenance of immune system functionality has not been established. **PURPOSE:** To determine the effects of IE on running performance and the mucosal immune system. **METHODS:** Sixteen well-trained male runners/triathletes were exposed to the hypoxic stimulus for fifteen days. IE was administered using a simple device (Alto2Lab, Pharma Pacific, Inc.) consisting of a breathing tube attached to an open-ended silo containing carbon-dioxide absorptive soda lime. Oxygen saturation was monitored with a pulse oximeter, and progressively reduced (90% on Day 1 to 77% on Day 6-15; equivalent altitudes equal 3600-6200 m) for treatment group 1 (LON) or held constant for treatment group 2 (SHO, 90%; Day 1-15); the control group (CON) was not exposed to the IE. Time to complete a 10K race was tested at baseline and post-treatment. Salivary IgA was

compared from baseline to post-treatment using ELISA. Comparisons between groups were made using the non-parametric unpaired Mann-Whitney test. RESULTS: Time to complete the 10K race significantly decreased for the LON treatment group compared to CON ($p < 0.05$). There was a non-significant improvement in run performance for the SHO group. No significant decrease in salivary IgA antibodies was detected in any of the groups ($p < 0.05$). CONCLUSION: A simple, cost-effective, device can elicit the same performance enhancing effects as LHTL in a much shorter time, without mucosal immune system compromise. Such a method to simulate altitude exposure while remaining at sea-level is considered highly desirable among competitive endurance athletes.

This document is dedicated to David.

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Chapter 1: Introduction

Background

Much interest has been focused on the use of altitude training to enhance athletic performance in competitive sports. For example, to follow the live high-train low method (LHTL), the athlete lives at a high altitude to gain the beneficial physiological effects of altitude training, but trains at sea level to maintain appropriate training intensity (Levine & Stray-Gundersen, 1997; Chapman, Stray-Gundersen, & Levine, 1998).

However, this type of training has been shown to lead to decreases in mucosal immune system function as measured by IgA antibodies in the saliva (Tiollier et al., 2005). In addition, the physiological effects of the altitude itself, harsh training, and isolation from family are thought to be additive stresses that depress the immune system (Walsh & Whitham, 2006).

Recently it has been shown that a simulated altitude device worn for a brief period of time has the same performance enhancing effects as LHTL (Babcock & Kirby, 2008).

The purpose of the present study was to determine whether this device would impact the immune system in the same manner as LHTL. It was hypothesized that this device would have the performance enhancing effects of LHTL while having no impact on the mucosal immune system, as measured by IgA antibodies.

Intermittent altitude exposure holds promise as an alternative to the more expensive and time consuming methods of altitude and simulated altitude training currently in use. The present study attempted to remove the added stresses of this type of training using intermittent exposure devices. Additionally, this study attempted to determine if the devices could reduce or negate the negative effects of LHTL on the mucosal immune system.

Definition of Terms and Significance to Study

Antibody: protein found in the body that is used to defend against foreign materials, such as viruses or bacteria

B cells: produce immunoglobulin (Ig) and are important for humoral or antibody mediated immunity. B cells generally increase during exercise, but studies on circulating Ig are conflicting; some authors report a decrease, no change, or even an increase (Rowbottom & Green, 2000). Intense exercise may reduce serum and mucosal Ig levels (Mackinnon, 2000) including salivary IgA, which has been shown to decrease during periods of intense training, potentially increasing the incidence of upper respiratory tract infections (Rowbottom & Green, 2000). It has thus been presumed that overtraining primarily influences immunity of the upper respiratory tract.

IgA: type of antibody most common in the mucous membranes of the body

Mucosal Immunity: the components of the human immune system present in the mucous membranes of the body

Neutrophils: a type of leukocyte that contains granules composed of bactericidal enzymes. Neutrophils also ingest foreign material through phagocytosis and kill bacteria through the “respiratory burst:” a chain of reactions involving hydrogen peroxide and hypochloride ions (Tizard, 1992). Although *in vitro* studies have been inconclusive, generally the number, degranulation, and phagocytic function of these cells increase after exercise (Rowbottom & Green, 2000). The degranulation, or increase in release of cellular granules, increases plasma proteolytic enzymes and leads to increased expression of surface antigens. Contradictory studies suggest that a “refractory period” in these cells after exercise may mean less responsiveness to microbes; conversely, long moderate duration exercise may increase function. Since neutrophils also play a role in responding to skeletal muscle damage from exercise, changes in their response may have no relation to extracellular microbe threats (Rowbottom & Green, 2000).

Natural Killer (NK) Cells: responsible for killing tumor and virus infected cells. NK cells are a type of lymphocyte cell that originate in the bone marrow and are found in some lymphoid organs (Tizard, 1992). They function to destroy tumor cells and have effects on some bacteria by secreting toxic factors (Tizard, 1992). Studies are consistent that there is an increase in the function proportional to the intensity of the exercise, and then a decrease in the function after long duration exercise (Rowbottom & Green, 2000).

VO₂ max: also, maximal oxygen consumption; measure of maximum amount of oxygen that can be transported and used during exercise; used to gauge aerobic fitness.

Submaximal Running Economy: reduced VO₂ max during submaximal exercise (Truijens et al., 2008).

T cells: coordinate cell mediated immunity and release cytokines (signaling molecules). Most of the literature reviewed corroborates that all intensities and durations of exercise have been shown to reduce the number of proliferating T cells (Rowbottom & Green, 2000).

Limitations of the Study

The study will not be able to directly compare the LHTL method (subjects actually living at altitude) to the simulated altitude devices due to limited budget and safety concerns for the study participants. This study uses only male participants; therefore, any results are specific to that population and may not be generalized to females.

Basic Assumptions

This study assumes that all the subjects are healthy, non-smoking, low-risk athletes with no immune system dysfunction or deficiency. It is also assumed that participating athletes are not using any other types of ergogenic aids. Finally, it is assumed that participants are residents of Ohio and have not lived at high altitude within the past six months.

Chapter 2: Literature Review

Altitude adaptation and its effects are well documented (Grover, Weil, & Reeves, 1986). Reduced atmospheric pressure and reduced oxygen partial pressure occurs at altitude (Grover, Weil, & Reeves, 1986). The body detects these environmental changes through stimulation of peripheral chemoreceptors by reduced arterial oxygen content, leading to hyperventilation and sympathetic activation in an attempt to decrease the hypoxemia (Robbins, 2007; Hansen & Sander, 2003). Hyperventilation leads to a respiratory alkalosis which is corrected by the renal system (Ge et al., 2006). The body attempts to maintain adequate oxygenation by increasing cardiac output initially (heart rate increases but stroke volume remains unchanged), and then later adaptations include hemoconcentration and smaller blood volume. According to Sawka, Convertino, Eichner, Schnieder, and Young (2000) blood volume adjustments at altitude occur in two phases. The first phase is marked by a plasma volume decrease, hemoglobin decrease, and blood volume decrease. The initial plasma volume reduction may result from an oncotic mechanism of protein loss from the blood or from increased erythropoietin (EPO) that reduces renin/angiotensin/aldosterone through negative feedback, but the mechanism remains unclear (Sawka et al., 2000; Gore, Clark, & Saunders, 2007). The second phase results from continued altitude exposure and is characterized by increased erythrocyte (RBC) volume (Sawka et al., 2000). This increase most likely occurs because lowered

oxygen pressure in renal tissue induces the kidneys to produce EPO which induces RBC production in bone marrow (Gore et al., 2007; Ge et al., 2002). Another mechanism contributing to increased heart rate, compensation in cardiac output, and increases in RBC content includes the increased stimulation of the sympathetic nervous system (Grover et al., 1986; Favret & Richalet, 2007; Mazzeo, 2005). It logically follows that a reduction in VO_2 max occurs (Sawka et al., 2000) at altitude due to the reduction in cardiac output resulting from the decreased plasma volume (leading to reduced ventricular filling) and increased blood viscosity and vascular resistance; however, Favret et al. (2007) also believe that alterations in control by the autonomic nervous system is the most likely explanation based on experimental evidence.

Ge et al. (2006) showed that there is a threshold for EPO increase, but that there is some variability among subjects. Blood volume increases have been documented previously in athletes, and this increase in blood volume may contribute to the eventual increases in VO_2 max observed with aerobic training as well as with altitude training (Sawka et al., 2000). Although the main mechanism for improvement in sea level performance with altitude training is believed to be the increase in RBC mass, Gore et al. (2007) argue that most of the statistical improvement in VO_2 max could be attributed to other factors and that the adaptations in response to altitude exposure have been inconsistent. As an example, native highlanders do not always have larger than average blood hemoglobin (Hb) concentration. Some other possible mechanisms for the physiological changes include: cell growth and survival factors, glycolytic enzymes used for energy

metabolism, transporters for glucose uptake and lactose metabolism, vasodilators, and pH regulation. Gore et al. (2007) suggests that observed improved exercise economy in some studies may result from increased mitochondrial efficiency, muscle pH regulation, or increased muscle buffer capacity.

Clearly from the literature, physiological changes occur with altitude training, but the exact mechanisms for change and improvement in performance are controversial and of debate. The prevailing theory appears to be that initial hyperventilation and hemoconcentration and later increase in blood volume due to increased RBC mass lead to altitude acclimatization (Muza, 2007). Whatever the mechanism, it is evident that physiological adaptations are occurring that could lead to increases in sea level performance, and in fact, altitude training is used and widely accepted to increase aerobic performance (Gore et al., 2007).

In competitive sports, performance differences are so small that athletes will go to great measures to gain a competitive edge (Wilber, 2007). One aspect of concern, however, is that potential negative consequences of training at altitude outweigh the possible benefits. In an early review, Bailey and Davies (1997) indicated that there can be negative consequences to training at altitude including possible detraining, mountain sickness, pulmonary edema, cardiac arrhythmias, and immune system dysfunction. They further found no increases in aerobic or anaerobic performance after a return to sea level. They noted that altitude dosage must be carefully monitored: too low will not result in

adaptations, and too high could result in altitude sickness or detraining due to lower sustainable work rate during training at altitude. By decreasing training intensity, athletes would effectively be detraining because training at altitude results in a decrease in VO_2 max of 1% for every 500 m over 1500 m (Levine & Stray-Gundersen, 1997). More importantly, altitudes greater than 2350 m can cause altitude sickness, cerebral edema, and pulmonary edema (Luks & Swenson, 2008). Symptoms of acute mountain sickness include shortness of breath, palpitations, headache, insomnia, tinnitus, mental confusion, appetite loss, and altered sensation in the digits; mountain sickness can progress to pulmonary hypertension and eventually congestive heart failure (Rivera-Ch, Leon-Velarde, & Huicho, 2007; Moore, Niermeyer, & Vargas, 2007). To prevent these issues, ascension to altitude should be gradual (Luks & Swenson, 2008).

To moderate the detrimental effects of training at high altitude, Levine and Stray-Gundersen (1997) proposed the live high-train low (LHTL) model which entails living at a moderate altitude (above 2500 m), but training at a low altitude (below 1500 m) to theoretically improve oxygen transport without decreasing training intensity. As a guideline, altitude ranges can be categorized as follows: sea level = 0–1000 m, low altitude = 1000–2000 m, moderate altitude = 2000–3000 m, high altitude = 3000–5000 m, and extreme altitude = 5000–8848 m (Gore et al., 2007). The LHTL concept involves living/sleeping at a moderate/high altitude (such as 2000 to 2700m/6560 to 8856ft) while training at sea level ($\leq 1000\text{m}/3280\text{ft}$) in order to maintain desired exercise intensity and performance levels (Wilber, 2001). Alternatively, some athletes use hypobaric

apartments, chambers or other devices to simulate living at altitude. Over time, LHTL altitude exposure causes the body to adapt to a lower oxygen pressure by increasing EPO release from the kidneys which in turn increases production of new RBCs. The oxygen carrying capacity of the blood is thereby increased. VO_2 max, an indicator of aerobic capacity, has been shown to increase in these athletes (Rusko et al., 1999).

Indeed, Levine and Stray-Gundersen (1997) found significant increases in RBC volume, VO_2 max, and performance in running time trials, and postulated that it was most likely the increase in RBC volume that led to the increase in performance time; however, they noted that other adaptations, including alveolar oxygenation and skeletal muscle oxygen extraction and substrate utilization, have been observed in animal models (and could be happening in humans as well) that could contribute to acclimatization to altitude. Schmitt et al. (2006) showed that LHTL increased VO_2 max and power output, and Wehrin, Zuest, Hallen, and Marti (2006) found that LHTL led to higher VO_2 max and increased Hb and red cell volume. The performance enhancing effects were apparent even in elite athletes who may have already been near their aerobic capacity (Stray-Gundersen, Chapman, & Levine, 2001). Chapman et al. (1998) showed that maintenance of training intensity at low altitude during the training periods and sufficient altitude during the “live high” phase are required to be able to see the effects of LHTL. They reiterated suspicions from an earlier study that there are other adaptations besides the RBCs that may lead to the performance improvements, and showed that athletes need to have adequate iron levels when implementing this type of training.

When a LHTL group was compared to LLTL and LHTH groups, only the LHTL group improved sea level performance, but there was variation in the improvement (Stray-Gundersen & Levine, 1999). Maintaining oxygen transport to the tissues at altitude was shown to be a key factor in improved performance at sea level; i.e., according to Stray-Gundersen & Levine, it is the increase in EPO resulting in increased erythropoiesis that leads to increases in performance and not other possible metabolic adaptations.

However, the exact mechanism is still a topic of debate (Wilber, 2007). Additionally, the performance enhancing effects of LHTL are apparent even in elite athletes who may already be near their aerobic capacity (Stray-Gundersen, Chapman, & Levine, 2001).

Recently, Wilber (2007) reviewed altitude training to date. He discussed the importance of optimum dose, responders versus non responders including the possibility that genetic factors could play a role, and “accelerated erythropoiesis” as proposed by Levine and Stray-Gundersen. Interestingly, he reviewed that adaptations without the erythropoiesis had been observed, and suggested that mitochondrial efficiency and improved muscle buffering could be important factors not related to increased EPO that lead to increases in sea level performance.

The negative consequences of training at high altitude and promising results of LHTL suggest that LHTL is the optimum method for altitude training. However, cost, logistical issues, and disruption to family can be deterrents to implementing this type of training. Intermittent altitude exposure (IE) has been proposed as a way to train at altitude without

these issues. IE involves a brief exposure to hypoxia over a period of time, with the assumption that the positive physiological adaptations of living at altitude will be acquired without the negative physiological effects, time, cost, etc. of actually living at altitude. This type of training can be achieved using chambers or other devices that simulate high altitude. IE also can be useful for decreasing acute mountain sickness by acclimatizing individuals before ascension, especially military personnel that must rapidly deploy to high altitude areas (Beidleman et al., 2004; Muza, 2007). IE is administered using either normobaric or hypobaric hypoxia; hypobaric hypoxia involves decreasing the atmospheric pressure while normobaric hypoxia is achieved by nitrogen dilution of expired air so that the pressure of inspired oxygen is decreased (Muza, 2007).

Julian et al. (2004) described an approach developed in the former Soviet Union that involved breathing normobaric hypoxic gas for about 70 minutes at rest to simulate an altitude of about 5000 m. Subjects were given four weeks of IE, five times per week using a mask [GO2Altitude Hypoxic Training System (Hypoxicator), Biomedtech], with a progressive decrease in the fraction of inspired oxygen over the course of the study. Although no increases in performance or erythropoiesis were detected, the authors hypothesized that a specific dose was necessary to affect change, or that an increase in RBC destruction (neocytolysis) was occurring during the non-exposure periods of the intermittent treatment.

In fact, IE has been controversial and not all studies have demonstrated increases in exercise performance, either aerobic or anaerobic (Morton & Cable, 2005; Tadibi, Dehnert, Menold, & Bartsch, 2007). Additionally, an erythropoiesis paradox was reported by Gore et al. (2006) who found that IE could increase serum EPO without an increase in erythropoiesis. Hypobaric and normobaric hypoxia in swimmers and runners were studied. The authors hypothesized that the total dose of altitude exposure was not sufficient to affect change in erythropoiesis. According to Levine and Stray-Gundersen (2006), the IE “dose” is characterized by degree of altitude, hours per day, and length of treatment. Conversely, Robach et al. (2006) found that IE stimulated erythropoiesis without increasing VO_2 max or RBC volume with a progressive LHTL protocol.

Generally, the literature has shown that IE leads to improvements in aerobic performance and ventilatory and blood parameters (Meeuwssen, Hendriksen, & Holewijn, 2001; Rodriguez et al., 2000; Rodriguez, Murio, & Ventura, 2003; Ojiri, Inoue, Nohara, & Sunagawa, 2003; Beidleman et al., 2008; Casas et al., 2000; Knaupp, Khilnani, Sherwood, Scharf, & Steinberg, 1992). The IE exposure period required could be as short as nine days (Rodriguez et al., 1999). This lab has shown previously that IE using a re-breathing device improves cycling time trial performance and may improve VO_2 max (Babcock & Kirby, 2008).

Reasons cited for lack of increases in performance include dosage (Rodriguez et al., 2004; Rodriguez et al., 2007), individual variation in response (Muir, Myhre, Salazar, &

Dahms, 2008), and degree of hypoxic stimulus (i.e., normobaric versus hypobaric hypoxia) (Truijens et al., 2008). Wilber, Stray-Gundersen, and Levine (2007) claim that the optimal IE dose is 2000-2500 m for 12-16 hours per day, repeated for 3-4 weeks. Muza (2007) reviewed that most IE studies look at effects of IE treatment within 24 hours of the last treatment, indicating lack of information on how long physiologic adaptations remain after the treatment period has ended. Only a fifth of the studies reviewed used normobaric hypoxia, and the question of whether to use normobaric versus hypobaric remains. Muza agreed that simulating higher altitudes is more effective, and that in 40 of 59 metrics (including blood parameter changes, increased Hb and RBC number, and VO₂ max and time trial increases), the IE method led to acclimatization. Concerning dosage, daily exposure of about 1.5 hours was just as effective as 4-8 hours, but the treatment should be repeated for at least 5-6 days. Interestingly, blood changes (like increased Hb concentration and increased erythropoiesis) did not necessarily correspond with duration and frequency of IE. However, IE may be more effective than continuous exposure when increases in VO₂ max are concerned. Clearly, more research on IE exposure should be done to determine optimal dosage, but as Muza suggests, IE has been shown to be an effective tool to increase altitude acclimatization as well as increase performance.

Based on the available literature and experience of this lab, the current study used IE devices that simulate a hypoxic environment to increase aerobic capacity [Alto2Lab-Portable Altitude Simulator, Pharma Pacific]. These devices remove expired carbon

dioxide and prevent ventilatory reflexes from increasing breathing rate, simulating a hypoxic hypoxia environment. Each device consists of “silos” that contain stacks that absorb expired carbon dioxide. Each stack simulates an altitude of 5000 ft. According to the manufacturer, the physiological benefits last for 15 days when a 15 day treatment protocol is followed. Time to complete a 10 K race was analyzed for each study subject to determine aerobic capacity enhancement effects.

It is known that LHTL can improve aerobic capacity, but it has been suggested that there are negative consequences to using this training method. Zhang, Hu, and Wang (2007) observed changes in T lymphocyte subsets in soccer players after LHTL. Tiollier et al. (2005) reviewed that IgA concentration is decreased after periods of intense training. These authors hypothesized that the LHTL regimen could stress the immune system in addition to the stress of exercise itself. Salivary IgA was studied in elite cross country skiers who underwent an 18-day training camp. The study group followed a LHTL regimen, while the control group maintained living and training at 1200 m altitude. IgA levels decreased in both groups throughout the training period, but significantly so only in the LHTL group and decreases in IgA tended to correlate with the higher altitude. Additionally, the IgA levels remained low, even after a two-week recovery period. Robach et al. (2006) also observed a decrease in salivary IgA with LHTL. So, it appears that LHTL is having some effect on the immune system.

It has been known for some time that exercise itself affects the immune system, but the consequences of these effects remain unclear. During exercise, immune system cells and chemicals are modified from the resting state (Rowbottom & Green, 2000; Mackinnon, 2000; Nieman, 2007). Modifications and responses can be attenuated based on training, age, fitness level, and type, intensity, and duration of exercise. The immune system consequences of training at altitude present another variable in the complex equation linking exercise and the immune system.

Research on the beneficial effects of exercise on the immune system is extensive (Surgit, Ersoz, Gursel, & Ersoz, 2001; Friedrich, 2008; Bacurau et al., 2007). While the benefits of moderate exercise on health are apparent, the effects of strenuous exercise are less well defined. In fact, decreases in the immune system after continuous exercise have been observed (Takahashi et al., 2007). In 2000, Mackinnon (2000) and Rowbottom and Green (2000) separately reviewed and critically evaluated the available literature regarding the effects of chronic exercise on the immune system. They summarized that intense physical training or competitions appear to increase the risk of upper respiratory tract infection (URTI); however, moderate exercise appears to decrease this risk. Neiman's "Open Window Theory," (2007) refers to a time after intense exercise when the immune system is thought to be depressed and infection can result.

Exercise has been shown to change all aspects of the immune system including cells and chemical messengers. Rowbottom and Green (2000) described a phenomenon called the

“biphasic response.” In general, circulating leukocytes (white blood cells) increase immediately after (or during, depending on exercise intensity and duration) exercise. After exercise, circulating lymphocytes and monocytes decrease to below resting levels, but circulating neutrophils continue to increase. Relative lymphocyte subsets, i.e., number of T, B and NK cells, change also. Both aerobic and resistance training affect these changes.

Quadrilatero, Boudreau, and Hoffman-Goetz (2003) found a decrease in T cells, but significant increase in B and NK cells 24 hours after a single bout of intense exercise in mouse submandibular lymph nodes (SLN). SLN are important to the upper respiratory tract and the anterior ocular chamber, as well as for synthesis of IgA (Boudreau & Hoffman-Goetz, 2006). Quadrilatero et al. (2003) reasoned that the mice could be more susceptible to URTIs due to the changes in percentages and distribution of these cells. The drop in the particular type of T cell also implied less help for B cell differentiation, IgA production, and humoral (antibody-mediated) immunity at mucosal surfaces.

Generally, the immune system changes in the reviewed studies appear to fit the biphasic model. Training appears to mediate the effect somewhat (Boudreau & Hoffman-Goetz, 2006), but the literature is conflicting (Davidson & Hoffman-Goetz, 2006; Hong et al., 2005). It appears that training does have an effect on lymphocyte subsets: Compared to non-athletes, athletes express more immune activation markers (Rowbottom & Green, 2000). Repeated or long term exercise bouts as well as acute exercise affect immune

changes that fit the biphasic model; however, the changes 48 hours post exercise correlated negatively to subject VO_2 max (Malm, Ekblom, & Ekblom, 2004). Therefore, training level appears to influence the effects of strenuous exercise on the immune system. Type of exercise may be a factor in immune system change as well, and different types of athletes may be more susceptible to these changes than others. For example, B cells decrease after plyometric training but increase after eccentric training (Malm, Ekblom, & Ekblom, 2004).

Various mechanisms have been proposed for the changes in the immune system during exercise. The decreases in immune cell number could result simply from increased turnover of cells or migration out of circulation (Mackinnon, 2000). Heat shock proteins produced by the body during exercise and expressed on the surface of immune cells have been implicated in affecting immune response during exercise (Whitham & Fortes, 2008; Horn et al., 2007). The release of stress hormones such as catecholamines and corticosteroids during exercise could, over time, lead to decreased receptors, decreased receptor sensitivity, or depletion of catecholamines (Mackinnon, 2000). Training could reduce stress hormones which mediate immune responses (Mackinnon, 2000).

Adrenergic input during exercise influences expression of adhesion molecules and leads to altered cell migration (Kruger & Mooren, 2007). These stress-induced effects might serve to enhance immune function; however, studies on the importance of stress hormones in mediating the immune response are conflicting (Quadrilatero et al., 2003).

The inflammatory cytokine IL-6 is produced in fat tissue, and appears to have an inhibitory role on the B-1 cell population (Elphick et al., 2003). Elphick et al. reasoned that running rats had less fat tissue and therefore less inhibition of B cell production. This led to an increase in B cell number. Elenkov (2004) reviewed that glucocorticoids could cause a shift from cellular immunity to humoral immunity. Another mechanism for this shift could be decreased glutamine, which is required by lymphocytes for metabolism (Mackinnon, 2000). Low levels of glutamine have been observed in athletes (Lakier Smith, 2003). In summary, the mechanisms for the changes in immune system function during and after exercise are currently being studied. The most promising potential mediators include catecholamines, corticosteroids, and glutamine. Regardless of the changes in the immune system that occur, it remains to be determined if these effects are important to immune function and if the changes lead to increased risk of illness.

There are plenty of studies on immune system changes in response to exercise, but few studies show that a clinical correlation between these changes and infection or illness exists. In fact, some of the observed URTIs may not be infections, but inflammation (Bermon, 2007). Few articles conclusively show an increase in infection as a result of immune system change due to exercise. Most research appears to indicate that exercise, especially intense and long duration, does indeed lead to changes in the immune system; however, the mechanisms for these changes in response are varied and may involve a combination of factors. These mechanisms and the connection between immune change and illness are important areas of emerging research that merit additional study.

Altitude exposure affects the immune system similarly to exercise (relative lymphopenia and neutrophilia) (Thake, Mian, Garnham, & Mian, 2004), and effects of exercise and hypoxia on the immune system appear to be additive (Pedersen & Steensberg, 2002; Klokker et al., 1995; Mazzeo, 2005). According to Mazzeo (2005), immune system changes due to acute altitude exposure include reduction in CD4+ T cells and impairment of T cell activation and proliferation, decreased lymphocytes, increased neutrophils, no change in B cells, increase in NK cell number and activity, and increase in some pro-inflammatory cytokines. However, there is some debate about the effects of exercise and altitude and the immune system. It can be difficult to determine if symptoms result from altitude sickness or clinical infection (Bailey et al., 2003). Schobersberger et al. (2000) actually found increased immune system activation markers after long exercise at moderate altitude. Pyne et al. (2000) showed that changes from exercise at altitude were secondary to the effects from the altitude alone. Blegen et al. (2008) claim that exercise in hypoxic environments does not affect the immune system; however, IgA was not measured. Plasma IgA and IgM were increased at altitude in one study, but IgG levels remained unchanged (Shephard, 1998). Clearly, studies are conflicting, but it appears that altitude exposure does affect the immune system. It remains to be determined whether these changes have a positive or negative effect on the body and susceptibility to illness. Further research is needed to clarify these issues.

Mechanisms for a potentially decreased immune system with altitude are varied and include decreases in nutrients required by immune system cells (because the fuel is being

used for exercise and survival at altitude), and tissue hypoxia leading to oxidative stress (Winzer et al., 1999). Bailey et al. (2003) reviewed that reduced glutamine may be a mechanism for decreased cell mediated immunity. Mazzeo (2005) reviewed that the increase in sympathoadrenal activity due to altitude exposure leads to potential changes in the immune system, and implied that acclimatization may be the key to reducing these changes. In fact, adrenergic mechanisms may be responsible for the changes in IgA secretion observed during exercise (Tiollier et al., 2005; Winzer et al., 1999; Ring et al., 2000).

It is apparent that both exercise and altitude exposure alter the immune system; however, in competitive sports, it is important to reduce stresses on the athlete to maintain an efficiently functioning immune system. Methods to reduce this risk include acclimatization through LHTL (Pyne et al., 2000) or IE. Wang, Lin, Cheng, and Wong (2007) used normobaric IE on a sedentary population to increase VO_2 max and decrease immune system changes that occur with vigorous exercise. Beidleman et al. (2006) recommend the use of IE to “pre-acclimatize” individuals to altitude because no hormonal stress response and no activation of the immune system were detected at IE of 4300 m. The current study also used IE with the expectation that subjects would increase aerobic performance while maintaining immune system functionality, particularly humoral.

The present study analyzed secretory IgA since this antibody is the first line of defense on mucosal surfaces (Libicz et al., 2006; Davison, Allgrove, & Gleeson, 2009), and deficiencies may correspond to infection (Winzer et al., 1999). Tiollier et al. (2005) were the first to look at salivary IgA instead of circulating plasma IgA in response to the physiological altitude stress of LHTL. The present study also examined salivary IgA using similar methodology as Tiollier et al., but used a simulated altitude exposure device that was administered intermittently to the study group. The IE devices were expected to improve running performance, but have no effect on salivary IgA levels. The present study also attempted to remove the added stresses of altitude, such as isolation, because study subjects were able to continue their normal daily routines (Walsh & Whitham, 2006). Additionally, the devices eliminated the potential for altitude sickness.

Chapter 3: Procedures

This study required 15 sessions of hypoxic exposure (AltO2Lab) for either a short-term continuous period (SHO, 15 minutes) or a long-term intermittent period (LON, 6 minutes hypoxia, 4 minutes normoxia over 60 minutes). The duration of the study was approximately 3.5 weeks after commencement. Exercise protocols (10 K running time-trials) were completed prior to and after the 15 day treatment period. At these same time points, salivary IgA was measured. Rest days were provided prior to each of the exercise testing procedures, and weekends were “no altitude treatment” days.

Research Design

A pre-test/post-test control group design was used to address the hypotheses: 1) SHO and LON altitude exposure will improve aerobic exercise performance; 2) neither SHO nor LON altitude exposure will have an effect on measured immunological characteristics (salivary IgA).

Subjects

Subjects consisted of 22 well-trained male triathletes or distance runners. “Well-trained” was defined as no less than 1 month experience in triathlon/duathlon training (typically intense running, biking, and/or swimming) or distance running training, and sustained

training practices (moderate to high intensity) of no less than 6 hours per week for the previous month. Subjects were required to maintain current training starting prior to baseline testing and ending with the last performance trial. Fitness training, for the purpose of this study, consisted of moderate to high intensity running, biking, and/or swimming. These conditions were set to ensure proficiency and to ensure that a change in performance was not attributable to learned mechanical efficiency. Each participant kept a training log during the duration of the testing that ended with the last performance trial. The exercise logs tabulated exercise training based on type, duration, and intensity of each exercise session. Intensity was quantified using an average rate of perceived exertion scale (RPE, Appendix A) or heart rate. Subjects were also requested to maintain a consistent diet during the study. Only subjects categorized as “low risk” according to the American College of Sports Medicine (ACSM) Risk Stratification were eligible to participate in the proposed study.

The American Heart Association/American College of Sports Medicine (AHA/ACSM) Health/Fitness Facility Preparation Screening Questionnaire (Appendix B) was utilized for subject symptom and risk factor screening. Subjects chosen for the study were determined to be apparently healthy and free from medical or enhancement preconditions (acclimatization to altitude). Informed consent procedures as required by the Institutional Review Board (IRB) were adhered to in the study and participation was voluntary.

Exclusion Criteria

Subjects who were stratified as moderate or high risk by ACSM Risk Stratification criteria were not eligible for this study. Female subjects were not recruited due to performance related differences related to the menstrual cycle. The length of time between performance measurements is 23 days. For the majority of females, the menstrual cycle is likely to re-occur every 28 days. Therefore it would be impossible to test females during the same phase of their menstrual cycle. Previous research has shown that females exercising at workloads corresponding to 80% VO_2 max have shown varying levels of oxygen consumption dependent upon the stage of the menstruation cycle (Williams & Krahenbuhl, 1997). Finally, potential subjects who had been acclimatized to altitude within four months prior to the start of the study were not eligible to participate in the study.

Sample Size Justification

Bonetti, Hopkins, and Kilding (2006) utilized two groups of sub-elite kayakers and were able to detect differences in rowing power and hematological variables after employment of a 6-week hypoxic breathing protocol. A priori analysis previous completed by Babcock and Kirby (2008) indicated that a minimum of 9 subjects per group was necessary for detecting statistical differences in power output during a time-trial event in sub-elite cyclists. Due to the similarities in the dependant variables under investigation, it was expected that 30 potential subjects would be needed for statistical analysis for appropriate power. Tiollier et al. (2005) were able to find significant differences in

salivary IgA levels using a total of 11 cross country skiers using a LHTL method; therefore, the 30 subjects were more than sufficient to detect statistical differences. To account for attrition due to ineligibility or subject withdrawal, the accrual goal was 60 subjects; however, due to recruitment difficulties, the final number of subjects participating in the study was 22. Two consented subjects dropped out due to illness at the beginning of the study, two others dropped out due to injury sustained while training, and results from three other subjects were discounted due to lack of adherence to study protocol. Subjects were randomized into one of the three groups by choosing a slip of paper marked with the group name. The group breakdown was as follows: 6 CON, 7 SHO, and 9 LON.

Measurements

The specific parameters examined in this investigation included: running performance (time to complete a 10 K run) and immune system parameters (salivary IgA). The measured characteristics have been previously shown to be objective, repeatable measurements in well-conditioned endurance athletes (Tiollier et al., 2005).

Testing Protocol

The exercise testing was performed on a relatively flat, 10K trail mapped on The Ohio State University (OSU) campus (Olentangy Bike Trail). Subjects ran the course with instructions to run to the best of their abilities. Time to complete the course was used as the measure of exercise performance. Subjects performed the exercise test both before

and after the simulated altitude treatment. Rest periods of at least two days occurred between the altitude treatments and exercise testing. The performance testing sessions were held during typical spring and summer weather conditions, with the exception of one group which was tested in early winter. Inclement weather (rain, snow, lightning, sleet, etc.) or large fluctuations in temperature and humidity precluded testing for that day, and tests were rescheduled as necessary. Every attempt was made to control for issues such as weather, traffic, etc. that could impair performance on the testing course. The course was free from automobile traffic, and weather was monitored by the study investigators (via local radar websites) to ensure an appropriate testing date was made. Testing dates were proposed with “rain dates” also determined.

Salivary IgA Levels

Salivary IgA and total salivary protein content were measured using commercially available kits (Alpco Immunoassays and Pierce Protein Research products, respectively), and secretion rate was calculated based on the methods of Libicz et al. (2006).

Interlaboratory differences in salivary IgA have been noted in the literature, possibly due to secretion rate and total protein content in the saliva (Libicz et al., 2006); therefore, secretion rate and total protein also were measured. Tiollier et al. (2005) described baseline levels of IgA >200 mg/L, and the kit used in this study had a reference range of 102-471 mg/L. The change from baseline in salivary IgA levels from baseline to post-test was expected to be within this reference range.

Subjects were asked to rinse their mouths with sterile water, and then were asked to provide a saliva sample without forcing. Saliva samples were prepared for analysis per assay kit instructions. Two replicates of each saliva sample were tested per IgA assay. A standard curve provided with the kit was included with each assay plate for IgA quantitation, and provided positive and negative controls also were included with each plate.

Initial Meetings and Informed Consent

Recruitment was initiated through contact with the OSU Triathlon Club and local triathlon and running organizations. Additionally, a study flier was provided to community health/recreation centers, local race organizers, and the local science center, COSI (Center of Science and Industry). Interested subjects received study criteria, the informed consent form, and AHA/ACSM Health/Fitness Facility Preparation Screening Questionnaire. Only subjects that met the “well trained” criteria as previously described were eligible for study inclusion. Data from the initial Study Information Sheet was kept with all other study data until the end of the study, regardless of subject follow up, participation, or exclusion from the study.

Subjects were screened for participation requirements after verbal consent was obtained (via script). Screening included questions pertaining to pre-existing medical conditions and acclimatization to altitude in the previous month (Appendix C). The ACSM Risk Stratification was utilized to determine subjective risk to exercise testing (Appendix D).

Only subjects categorized as “low risk” were eligible to participate in the proposed study, as described hereafter in subject inclusion and exclusion criteria. A description of the testing and simulated altitude procedures were fully explained to each subject. Subjects were asked to perform maintenance training only during the study period, and to refrain from strenuous physical activity 24 hours prior to each performance test.

Exercise Performance Baseline Visit

On day 1, subjects performed an exercise performance protocol (10K run performance time-trial). The data collected from day 1 served as baseline performance data. Each subject was instructed to perform to the best of their ability, and was given race numbers for identification purposes during the run. Race numbers were assigned based on availability, and had no bearing on data outcome. The testing session consisted of a 10 K run, a typical running event for the given population.

Studies investigating time trial efforts, similar to those described above, have shown no significant test-re-test group differences (Ingham, et al., 2001). Therefore, although performance changes due to simulated altitude exposure may be relatively small (1-3%), the protocol was sufficiently capable and reliable to detect changes in aerobic performance. The benefit of this exercise protocol was that it accommodated the type of efforts commonly associated with training and racing for triathletes and it examines the potential effects on actual performance.

Exercise Performance Post Visit

Exercise performance was conducted in the exact manner as described for day 1 during the post-treatment time.

ALT O2 Hypoxic Exposure

Treatments were provided in the Exercise Science Laboratory at OSU or at Labs in Life at COSI. Six subjects received no altitude exposure and served as controls (CON). The remaining subjects were divided into short (SHO) and long-term (LON) exposure groups. To quantify the effects of two intermittent altitude exposure protocols, the LON and SHO groups used the Alto2Lab re-breathing device for 15 sessions over a 3 week period. For the LON group, each day's exposure (M-F) consisted of alternating between a re-breathing device and atmospheric air for 6 min and 4 min respectively over 1 hour, as previously described to be safe and effective (Babcock & Kirby, 2008). Oxygen saturation was monitored with a pulse oximeter and dropped progressively from 90% on Day 1 to 77% on Day 6-15 (equivalent altitudes equal to 3600-6200m). Subsequent training occurred in this range (saturation: 77-82%; altitude: 6200-5600m). The SHO group was provided a shorter duration of hypoxia totaling 15 continuous minutes per session (M-F). Oxygen saturation was again monitored with a pulse oximeter and was maintained within a saturation of 90% during all sessions (equivalent altitude equal to 3600m). All subjects remained passive (in a sitting position) during exposure. Subjects were encouraged to report any feelings of discomfort during the study. Additionally, any

feelings of illness such as runny nose, sniffing, sneezing, coughing or other symptoms of upper respiratory illness that occurred during the course of the study was recorded.

Biological Specimen Collection and Processing

Subjects submitted pre- and post-treatment saliva samples which were stored at -80°C until analysis for IgA levels. Samples were collected using the method of Tiollier et al. (2005) for the majority of the subjects. Briefly, subjects fasted overnight and saliva was collected before the races in the morning. Subjects were requested to refrain from eating at least 30 minutes prior to sample collection as per ELISA kit instructions. To collect saliva, subjects were asked to rinse their mouths with sterile water, and then were asked to collect saliva in their mouths for 2 minutes without forcing. Subjects discharged the saliva into a conical tube, and the volume of saliva was measured to the nearest 0.2 mL.

Two of the control subjects were treated somewhat differently, but the procedures for the pre- and post-collection remained the same. These subjects did not fast overnight, and saliva was collected during the evening. However, they did fast 30 minutes prior to saliva sample collection. Subjects were requested to keep their eating regimes consistent between pre- and post-race days.

Prior to analysis, the samples were centrifuged at 3000 x g to spin down particulates. The supernatant was removed to a new tube, the volume was measured, and any necessary dilutions were prepared. Salivary IgA was measured using a commercially available

Enzyme-Linked Immunosorbent Assay (ELISA) kit (Alpco). Total protein content was measured using a commercial kit (Pierce, BCA Protein Assay Reagent). Each sample was assayed in duplicate. Concentration of salivary IgA, total protein concentration, and IgA secretion rate were determined as described previously (Libicz et al., 2006).

Race time and IgA ELISA results were analyzed to determine: 1) Was run performance time significantly decreased by the simulated LHTL treatment? (i.e., did the devices produce the desired physiological training adaptation), and 2) Was salivary IgA significantly decreased or otherwise affected by the simulated LHTL treatment?

Biostatistical Design and Analysis

Mann-Whitney non-parametric tests were used to examine the variables of interest using Minitab version 15 statistical software. Significance was set a priori at 0.05.

Chapter 4: Results

Subjects

Subjects were male, between the ages of 18-44. All were low risk for exercise testing as defined by the ACSM. Subjects were training an average of 9.7 hours per week at the beginning of the study. The CON group trained an average of 9.9 hours per week, the SHO group trained an average of 8.6 hours, and the LON group trained an average of 10.5 hours per week. These values were self-reported. Examination of the training records indicated that subjects maintained their training consistently throughout the duration of the study.

Performance Testing Results

Mean race results by group are presented in Table 1 and Figure 1. Figure 1 shows the mean race times for each group and compares baseline times to post-race times. Race times ranged from 34.30 - 67.22 min. Data from two participants, 166 CON and 65 SHO, were removed as these values were greater than two standard deviations from the mean times. Both of these participants had claimed that they felt ill prior to the scheduled post-race. Additionally, data from three participants were removed due to non-compliance to race procedures. Overall, mean time to complete the race tended to

increase from baseline in the CON group, but decreased in both treatment groups (Table 1, Figure 1). Neither trend was significant within groups.

Group	Mean Baseline Race Time (Min)	Mean Post-test Race Time (Min)	Mean Difference (Post-Pre) (Min)
CON	49.52 ± 9.1	51.21 ± 10.0	1.69 ± 1.8
SHO	42.82 ± 6.2	42.41 ± 5.8	-0.41 ± 1.1
LON	43.80 ± 4.4	43.22 ± 4.4	-0.58* ± 1.7

Table 1. Change in Race Performance (± SD)

*Compared to CON mean performance difference, the mean performance difference from baseline to post-race significantly decreased for the LON group (p<0.05).

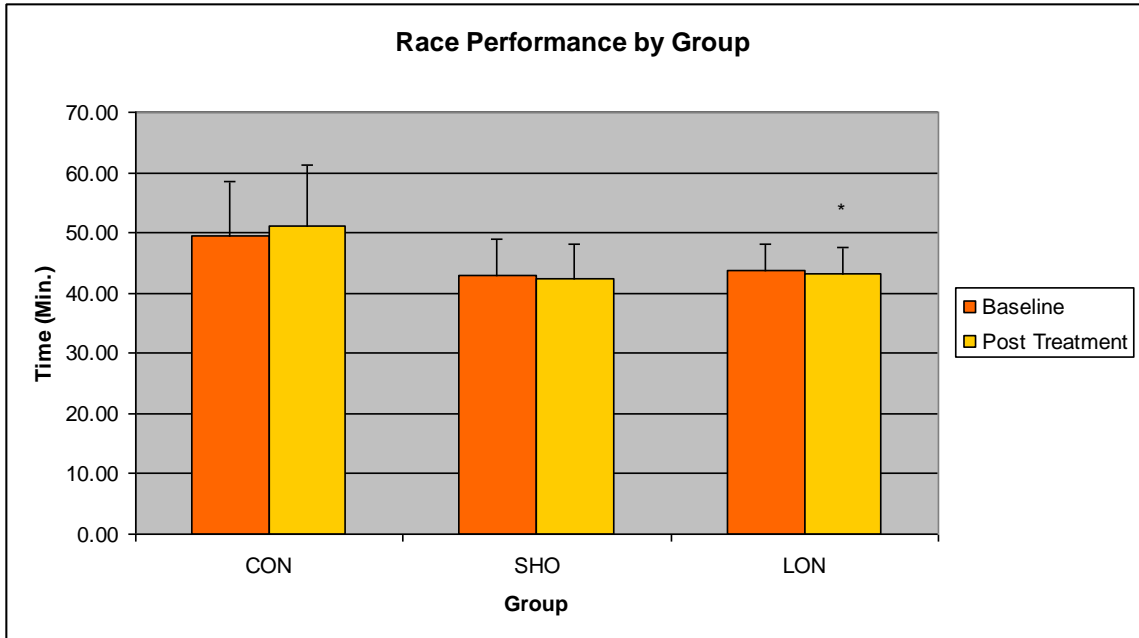


Figure 1. 10 K Race Performance by Group

Mean race times for each group for baseline and post-races. Time to complete the 10 K race only increased non-significantly from baseline to post-test in the CON group.

* LON significantly improved difference in race performance compared to CON ($p < 0.05$).

The mean performance differences from baseline to post-race were then compared across the groups (Fig. 2). Time to complete the 10K race was significantly shorter for the LON group compared to the CON group ($p < 0.05$, Figure 2) when the mean group differences were compared. There was no significant mean difference in performance between the SHO and LON treatment groups ($p > 0.05$), or between the SHO and CON groups ($p > 0.05$).

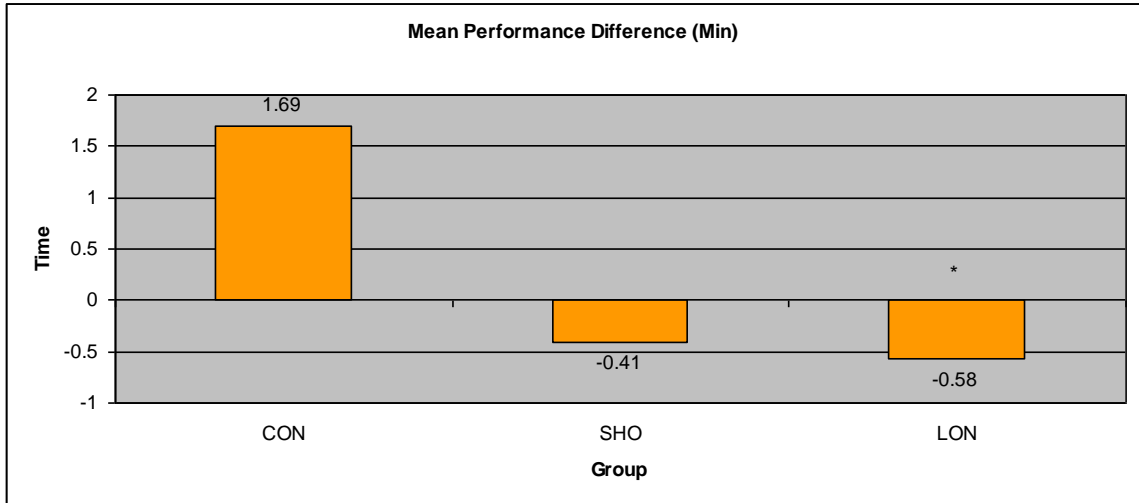


Figure 2. 10 K Race Performance Differences among Groups

* LON significantly improved difference in race performance compared to CON (p<0.05).

Every attempt was made to keep weather conditions consistent between baseline and post-races (Table 2). In total, eleven races were held, with five separate groups of racers. The temperature ranged from 29 to 74.5°F, and wind was minimal along the tree-lined path. The greatest temperature differences between pre- and post-races were during the spring months (Races 1 and 2) and winter (Race 5). Humidity did differ for some of the baseline versus post-races, but the difference did not appear to affect performance, as some racers performed just as strongly in humid weather (data not shown). Races were conducted either first thing in the morning or during the early evening, depending on subject schedules, and starting times were kept consistent for both baseline and post-races.

Race	Temp. at Start (°F)	Wind Speed (mph)	Start Time (am)	Date	Comments
Pre 1	59.8	0.0	6-6:15	5/6/09	
Post 1	68/67.1	2-5/0.4	6:10-6:25	6/3/09	Light rain at start
Post 1 makeup	70	0.3-0.4	7:00	6/10/09	
Pre 2	65.3	0-2.4	6:40	7/14/09	
Post 2	74.3	0.0	6:40	8/12/09	
Pre 3	71.3	0.0	6:32	7/14/09	Muggy
Post 3	71.8	0.0	6:40	8/26/09	Slightly humid, nearly dark
Pre 4	70.2	0-0.1	6:40	7/27/09	Slightly humid
Post 4	74.5	0.0	7:00	8/17/09	Very humid
Pre 5	56.9	0-4.6	6:10 p.m.	11/23/09	Dark, occasional raindrops
Post 5	29	0	6:10 p.m.	12/21/09	Dark

Table 2. Race Environmental Conditions

IgA Results

Mean salivary IgA concentrations are presented in Table 3 and Figure 3. Salivary IgA concentration did not significantly decrease from baseline to post-race ($p < 0.05$), and in most cases (15 of 22 subjects), the concentration non-significantly increased from baseline to post-race. The increase from baseline to post-race was nearly significant ($p = 0.059$) within the CON group.

When the group mean differences (from baseline to post-test) were compared, there were no significant differences among any of the groups ($p < 0.05$). Because some of the data points were higher than the highest point on the standard curve and saliva samples could not be re-analyzed due to sample degradation, these points were assigned the value of the

highest point on the curve when calculating statistical differences (samples 166, 17A, 60, 246, and 86).

All concentrations were higher than expected (minimum of 71.91 $\mu\text{g/mL}$ to maximum of 1034.70 $\mu\text{g/mL}$), and an adjustment factor was made to all samples for probable pipette calibration error. After this adjustment, 13 of 22 baseline values were within the normal adult range provided by the kit manufacturer (102-471 $\mu\text{g/mL}$) while nine remained high. All positive and negative controls provided with the ELISA kit performed acceptably, and the recovery values provided by the manufacturer were consistently high. The intra-assay and inter-assay coefficients of variation for the ELISA test were 9% and 8%, respectively.

Group	Mean Baseline IgA Conc. (µg/mL)	Mean Post IgA Conc. (µg/mL)	Mean IgA Conc. Difference (µg/mL)
CON	494.95 ± 274.5	720.10 ± 346.0	225.15 ± 283.9
SHO	393.62 ± 176.6	431.85 ± 148.3	38.22 ± 97.4
LON	541.74 ± 248.6	587.42 ± 266.8	45.69 ± 227.0

Table 3. Change in Salivary IgA Concentration (± SD)

Mean IgA concentration differences were not significantly different among groups.

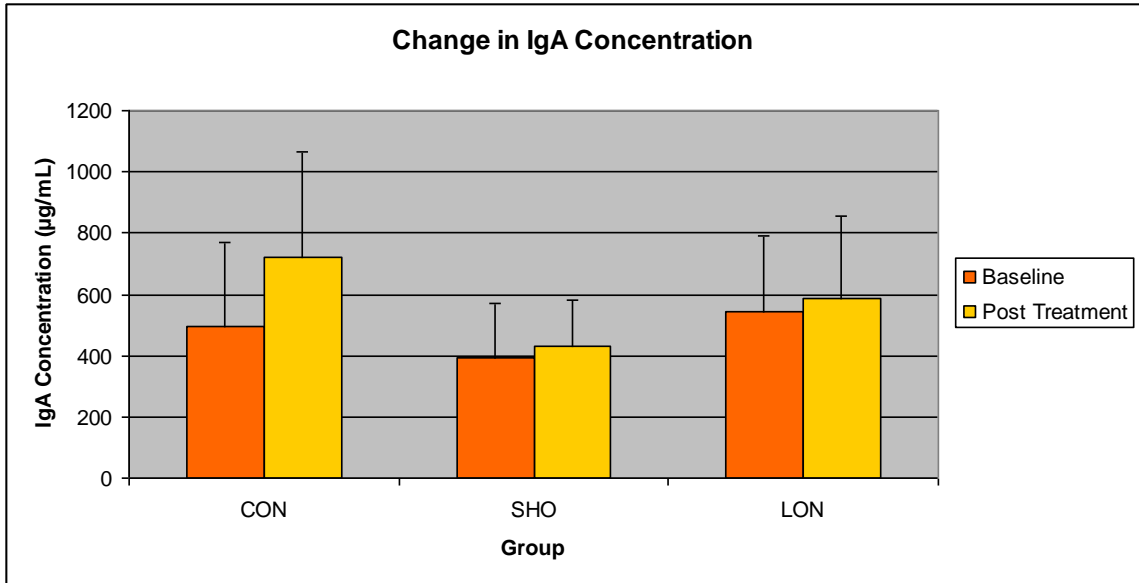


Figure 3. Change in Salivary IgA Concentration

Increase in mean IgA concentration from baseline to post-test approached significance in the CON group. The treatment groups showed non-significant increases from baseline. Mean group differences for the treatment groups were not significantly different from the CON.

Mean IgA secretion rate was calculated and compared from baseline to post-test (Table 4, Figure 4). In 15 of 22 subjects, secretion rate increased non-significantly from baseline to post-test, but the rate of increase was less for the treatment groups than the CON. Additionally, the increase in secretion rate was not as high for the LON group as the SHO group. Differences in secretion rates from baseline to post-test were averaged within groups, and the group means were compared. No significant differences between the three groups were detected; however, statistical significance for these measurements

could not be made with accuracy, as the saliva volumes measured for this calculation were not accurate. Transfer of saliva between tubes resulted in potential measurement error.

Group	Mean Baseline IgA Flow Rate (µg/min)	Mean Post IgA Flow Rate (µg/min)	Mean IgA Flow Rate Difference (µg/min)
CON	456.20 ± 323.0	532.31 ± 204.9	76.11 ± 329.2
SHO	297.03 ± 187.1	324.85 ± 173.9	27.82 ± 107.3

Table 4. Change in Salivary IgA Flow Rate (± SD)

Mean IgA flow rate differences were not significantly different when groups were compared.

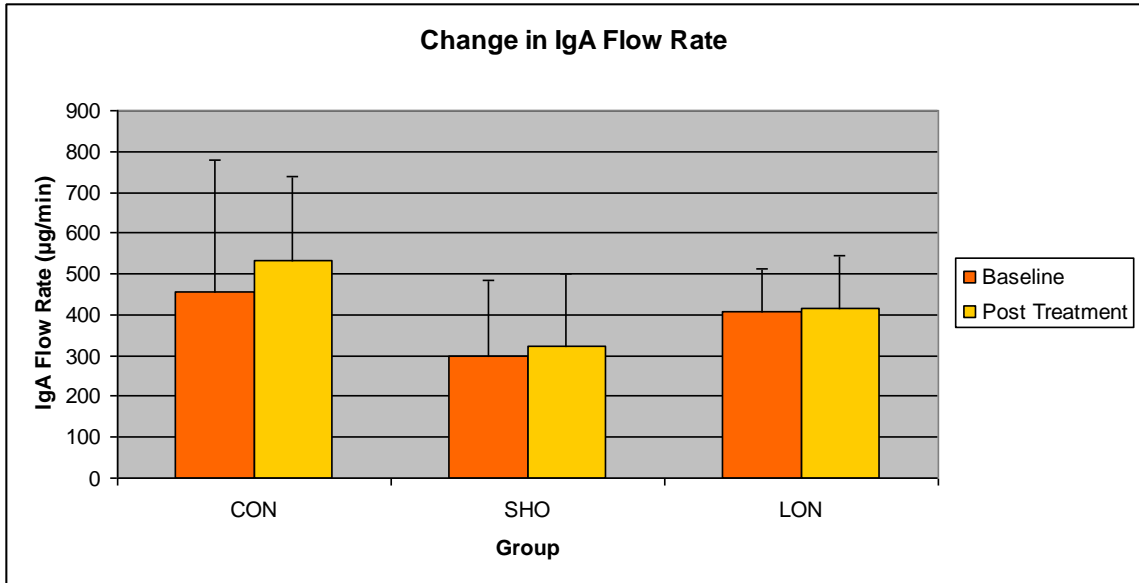


Figure 4. Change in Salivary IgA Secretion Rate

All groups showed non-significant increases in IgA flow rate from baseline. Mean group differences for the treatment groups were not significantly different from the CON.

Protein Concentration Results

Salivary protein concentration is presented in Table 5 and Figure 5. Because four of the data points were higher than the highest point on the standard curve and saliva samples could not be re-analyzed due to sample degradation, these points were assigned the value of the highest point on the curve when calculating statistical differences (samples 67 baseline and post, and 128 baseline and post). The salivary protein concentration non-significantly decreased in 12 subjects and increased in seven. In the remaining two subjects, an increase or decrease could not be quantitated because of the points being above the highest point on the standard curve, although the raw values estimate a

decrease. Average protein concentration within all groups decreased from baseline to post-test; this decrease from baseline to post-test was significant ($p < 0.05$) only within the SHO group. The mean protein concentration differences were not significantly different among any of the groups. When salivary IgA was expressed as a ratio of IgA to total protein, average group differences were 0.27 for CON, 0.12 for SHO, and 0.11 for LON.

Group	Mean Baseline Protein Conc. ($\mu\text{g/mL}$)	Mean Post Protein Conc. ($\mu\text{g/mL}$)	Mean Protein Conc. Difference ($\mu\text{g/mL}$)
CON	1143.08 \pm 367.8	1125.29 \pm 348.0	-17.79 \pm 306.9
SHO	1176.80 \pm 375.4	975.54 \pm 398.8	-201.26 \pm 161.0*
LON	1106.06 \pm 367.8	1052.22 \pm 301.8	-53.84 \pm 312.5

Table 5. Change in Salivary Protein Concentration (\pm SD)

Mean protein concentration differences were not significantly different when the three groups were compared; however, the within group mean decrease for the SHO group was significant ($p < 0.05$).

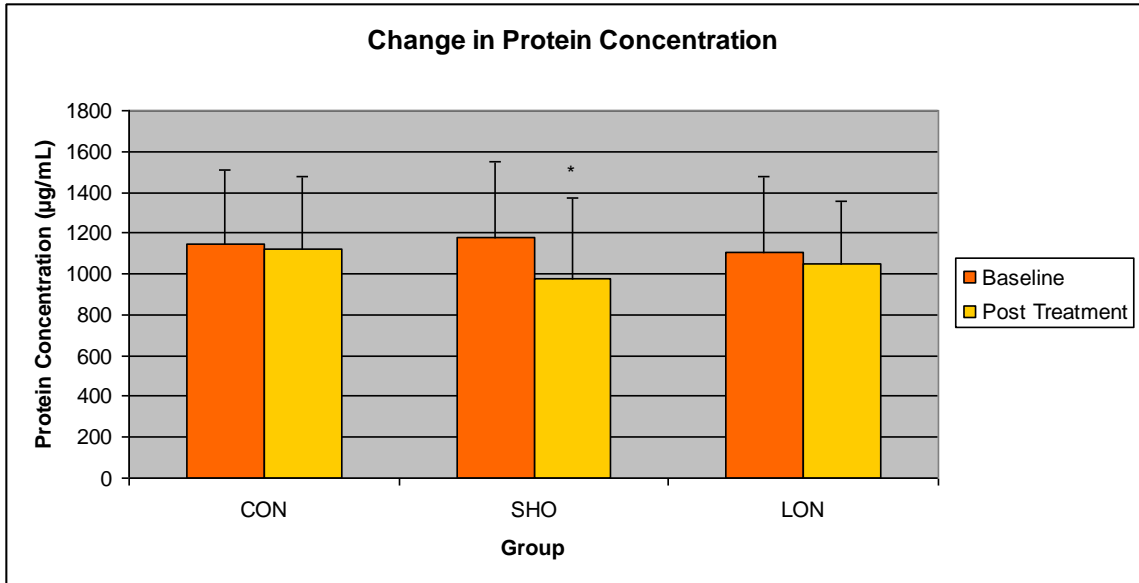


Figure 5. Change in Salivary Protein Concentration

All groups showed decreases in salivary protein concentration from baseline; this decrease was significant only in the SHO group ($p < 0.05$). Mean group differences for the treatment groups were not significantly different from the CON.

Chapter 5: Discussion

IE Can Increase Running Performance

Simulated altitude treatment significantly shortened time to complete the 10 K race for the LON treatment group compared to the CON. This finding supports current research which indicates that IE influences improvements in aerobic performance and ventilatory and blood parameters (Meeuwssen, Hendriksen, & Holewijn, 2001; Rodriguez et al., 2000; Rodriguez, Murio, & Ventura, 2003; Ojiri, Inoue, Nohara, & Sunagawa, 2003; Beidleman et al., 2008; Casas et al., 2000; Knaupp, Khilnani, Sherwood, Scharf, & Steinberg, 1992). The manufacturer of the device [Alto2Lab-Portable Altitude Simulator, Pharma Pacific] used in the current study recommends the LON protocol, and a previous study performed on cyclists by this lab found improvement of aerobic performance while using the Alto2Lab devices (Babcock & Kirby, 2008). The current study used the protocol employed previously (LON, one hour at progressively increasing simulated altitude from 3600-6200 m for 15 days) as well as a new protocol (SHO, 15 minutes at simulated altitude of 3600 m per day for 15 days). Pilot study data in rowers and cyclists suggested that the SHO protocol was just as effective in improving endurance performance as the longer protocol, and the current study attempted to determine a “minimum” dose of effective altitude training. The current study did not support use of the SHO protocol to improve running endurance performance; however,

the improvement in performance for the SHO group approached significance ($p=0.08$) and may have been significant if subject number had not been so low in the group ($n=6$ versus $n=9$ for LON group). Further studies using the SHO protocol to test VO_2 max as well as hematological parameters are warranted. The LON protocol is still convenient in comparison to LHTL, and may be very appealing to competitive athletes. The use of such a protocol can improve performance while minimizing time and exposure to potentially negative physiological consequences of LHTL training.

Although most of the previous research has focused on increases in VO_2 max as a measure of altitude training effectiveness, the current study used a 10 K race to measure performance. In fact, several recent studies have begun to look at time trial performance improvements. Saunders, Telford, Pyne, Gore, and Hahn (2009) found a 1.9% improvement in middle distance race performance after simulated LHTL training plus moderate natural altitude training. Race distances ranged from 800-3000 m. Robertson et al. (2009) exposed runners to LHTL for two 3-week periods after which the subjects ran a 4.5 km time trial on a road course. The LHTL group was faster than the control group after the first round of training, but not after the second, suggesting that improvement in performance may not be reproducible. The LHTL group showed an increase in Hb mass, but performance differences were attributed to fatigue, training status, and motivation. Bonetti, Hopkins, Lowe, and Kilding (2009) found an improvement in cycling time trial performance with IE using devices and an hour long protocol very similar to those used in the current study. Finally, a meta-analysis by

Bonetti and Hopkins (2009) showed that IE was one of the best altitude protocols for increasing aerobic power in sub elite athletes when compared to various other altitude training protocols.

Although the mean difference in race performance for the LON group was significantly shorter than the CON, it is interesting to note that the race times within the LON group were not significantly decreased from baseline. Chapman et al. (1998) and Mazzeo (2008) described individual variation in response to altitude acclimatization that included genetic factors, differences in bone marrow sensitivity to EPO, and differences in serum ferritin. Differences in sampling times might also play a role; it could take additional time for the physiological changes to occur for some subjects. A limitation of the current study may be that serum ferritin was not measured, nor did subjects receive iron supplementation. The possibility exists that a statistically significant improvement in performance may not always occur with LHTL training; however, a practical improvement may be more important to individual athletes. The runners in the LON group improved by 1.3%, which is consistent with current studies using intermittent simulated altitude (Saunders et al., 2009). Although a 1% improvement may not appear to be a large difference, even 30 seconds of improvement could be important during a race or triathlon, and could mean the difference between placing or not.

Races for the current study were conducted outside in order to mimic as closely as possible a realistic race scenario. All race subjects wore numbers, were provided with

race time at specific mile markers, and were started in groups of 3-6 in order to increase competition. Groupings were consistently maintained baseline to post-race with the exception of the two racers who were ill. While it is true that conducting the tests in the laboratory would have allowed for more controlled environmental conditions, the variable this study intended to measure was the effect of altitude training on *performance*. Additionally, a laboratory test would have removed the elements of competition and strategy, which are important contributors to race performance. It is already known that altitude training provides an increase in VO_2 max; this study aimed to provide evidence that altitude training could enhance a real-world race situation.

It is interesting to note whether altitude can increase performance in spite of changing weather conditions as well as participants not feeling their “best.” Indeed, one of the true tests of an ergogenic aid such as altitude training is: Can it improve performance in the face of common race obstacles like suboptimal rest, diet, weather, etc.? Some of the subjects acknowledged their lack of performance could be attributed to various other factors such as staying up late the night before, final exams, other competitions, gastrointestinal disturbances, etc. Altitude training may not be a significant enough factor to improve performance if subjects are not motivated, as indicated by the race results for subject SHO 65. This subject ran poorly during the post-test (baseline time 44.88 min versus 50.75 for post-test), and his results were discarded because they were an outlier. He noted that his poor performance could have been attributed to lack of conditioning during the week immediately prior to the post-race, but his training logs

indicated that he was able to sustain sub six-minute miles during training. He indicated that this was something he was not able to do before the altitude training. The altitude treatments may have contributed to his increased ability during training, but lack of motivation during the race could not be overcome by the treatments.

The influence of motivation cannot be regarded lightly, as this could have had a substantial impact on the performance of the CON. Bonetti and Hopkins (2009) proposed a nocebo effect in which runners in a control group perform worse than the treatment group because they know they are in the control group. An additional limitation of the current study is the small sample size and low number of controls. Further research should be conducted on a large number of athletes. The opposite phenomenon could be observed with the treatment groups; whether due to a placebo effect or a true physiological adaptation, the subjects maintained that they “felt better” during their normal training than they did previously. For example, subjects claimed to be able to run faster during training, while a number of others stated that they could hold their breath underwater longer. These subjective feelings have not been mentioned in other studies, but might be an interesting aspect of altitude training to catalogue for further research.

One trend noticed in recent studies is that sub elite athletes responded more favorably to IE than elite athletes (Bonetti et al., 2009; Bonetti & Hopkins, 2009). Although the current study used sub elite athletes only, the runners able to perform the 10 K race in

less than 40 minutes were observed for such a trend. No such trend was observed in the current study, but sample number was most likely too small (four runners) to detect this tendency.

It is important to note that training records were maintained by these athletes (95% compliance) and were examined for any conspicuous changes in training during the study period. Although no glaring changes were observed in this study, future studies would benefit from keeping all training regimes consistent between athletes. The current study aimed to do so by using the OSU Triathlon Club as subjects. These student athletes train together as a team and maintain a reasonably consistent training schedule. Unfortunately, due to low recruitment numbers, this subject pool was too small to use exclusively and was widened to include runners and triathletes from the community. Training status varied widely and could have contributed to the variability in performance (Robertson et al., 2009). The CON group appeared to be less conditioned than the other groups (mean baseline race time of 49.52 minutes compared to SHO 42.82 min. and LON 43.80 min.); however, race times were compared baseline to post-race and any changes in performance should have been detectable.

Athletes may not have been able to sufficiently integrate their typical training regimen with the new altitude training regimen (Robertson et al., 2009). Anecdotal reports from some of the athletes suggested that they did not always have the time, especially during the first week of the study, to maintain their normal training due to the time demands of

the study. It is unknown whether this slight decrease in regular training was a detriment or an advantage to final performance. It is unlikely that a large detraining effect could have occurred in this short amount of time, but it is possible that the added rest may have been physiologically beneficial. Conversely, runners who participated in multiple competitions during the study period may have been adversely impacted by fatigue, although no such trend was observed, and in fact, some of the subjects who competed during the study performed better during the post-race (e.g., SHO144, LON504, LON86).

Rest days were incorporated into the study schedule. Weekends were no altitude treatment days, and runners had between approximately 2-5 days after their last altitude treatment before performance of the post-race. Bonetti and Hopkins (2009) found that post-exposure test day positively affected live-low train high subjects, but results were unclear with other types of altitude training protocols. This topic merits further research because the physiological adaptations of altitude training may take time to develop.

These physiological adaptations that could result in improved exercise performance are still of debate. The mechanism for increases in performance are still being debated and include increase in hematological capacity, ventilatory adaptation, neural adaptation, increased muscle buffering capacity, and increased economy. Additionally, these factors may be influenced by hypoxic dose (Millet, Roels, Schmitt, Woorons, & Richalet, 2010). The recent literature meta-analysis by Bonetti and Hopkins (2009) found unclear evidence that an increase in red cell mass is responsible for the changes, and more data

was needed for conclusive substantiation of such a mechanism. Furthermore, changes in VO₂ max and hemoglobin mass did not correspond conclusively to changes in performance. The current study does not provide additional information on these issues, and further research is warranted. Use of molecular biology techniques such as microarrays for gene expression profiling could potentially provide insight into such mechanisms.

IE Does not Decrease Mucosal Immunity

It is believed that both altitude exposure and intense training can alter the immune system, and that effects from both can be additive (Pedersen et al., 2002; Klokker et al., 1995; Mazzeo, 2005). Since altitude is widely accepted as a training method, a need exists for altitude exposure to increase performance, while having no or little effect on the immune system. The current study thus attempted to quantitate the change in salivary IgA levels during IE training. Salivary IgA is thought to be a marker of stress (Rammal, Bouayed, Falla, Boujedaini, & Soulimani, 2010), and is the major mucosal immunoglobulin (Kugler, Hess, & Haake, 1992; Miletic, Schiffman, Miletic, & Sattely-Miller, 1996).

Salivary IgA concentration did not significantly change in each of the CON, SHO, and LON groups from baseline to post-test (Figures 3 and 4). The same effect was observed whether IgA was expressed as an absolute concentration, secretion rate, or percent

difference. Protein concentration also did not significantly change over time in all three groups (Figure 5).

The highest concentrations of IgA determined in this study were somewhat larger (baseline of 71.91-1034.70 $\mu\text{g/mL}$) than those typically seen in the literature (Booth, Dwyer, Pacque, & Ball, 2009) (range of 14.7-483 $\mu\text{g/mL}$). However, the controls provided with the Alpco ELISA kit performed properly, indicating that values for this assay were within normal range. Accuracy of future studies involving saliva could be improved with the use of positive displacement pipettes. Additionally, it is difficult to compare values in the literature because of methodological differences. The use of updated assay methods, such as immunonephelometry, may be recommended for future studies (Booth et al., 2009; Neville, Gleeson, & Follans, 2008). The protein concentrations and salivary flow rates determined in this study were reasonable values compared to the literature (Michishige et al., 2006; Fahlman & Engels, 2005; Li & Gleeson, 2005).

Generally, the literature reports highly variable within and between subject IgA levels, and comparisons between studies are difficult (Neville et al., 2008; Bishop & Gleeson, 2009). In fact, there are various issues in the literature that confuse IgA assay results. For example, a diurnal variation with IgA flow is believed to exist. Li and Gleeson (2004) reported a high IgA concentration in the morning and lowest concentration in the evening, but also found higher saliva flow rate and IgA secretion rate in the evening.

They also found no significant circadian variation in saliva flow rate or IgA secretion rate at rest. A recent study collected samples first thing in the morning (and in the fasted state), similar to the current study (Neville et al., 2008).

Additionally, saliva collection methods differ. Because studies report that cotton collection methods could interfere with total protein, IgA, (Michishige et al., 2006) and immunoassay results, (Shirtcliff, Granger, Schwartz, & Curran, 2001) the current study was performed on whole, unstimulated saliva. Collecting whole saliva results in a significant negative correlation between saliva flow and concentration, but variance in saliva flow is small in unstimulated saliva (Kugler et al., 1992).

Furthermore, most studies do not report the length of storage for the IgA samples collected. Although some samples are analyzed immediately, others are stored in the frozen state for a period of time. One study found no decrease in IgA after 48 hours at 4° C, but did find a decrease after 68 weeks at -80° C, (Booth et al., 2009) while another study determined that IgA in saliva was stable only until 3 months storage at -30° C (Ng, Koh, Fu, & Chia, 2003). The samples collected in the current study were analyzed in a sufficient amount of time to prevent this degradation at -80° C, but were then thawed and left at 4° C. Because sample degradation likely occurred during this time, repeated analysis for quantitation was not possible for high concentration samples.

A lack of control over various other factors including nutritional status and residual exercise effects as well as few saliva samplings during the course of a study also can lead to variation in IgA values (Neville et al., 2008). The lack of repeated measurements is a limitation of the current study. The results of a few individuals could have strongly impacted the data. Future studies should find an average baseline in IgA concentration before treatment begins, and then sample at many points in time over the course of the study. Additionally, post-test sampling should be done to determine return of IgA to baseline values. The current study was limited in budget and scope, and therefore only compared the two sampling points.

Finally, it is difficult to compare literature IgA values because of the differences in IgA concentration expression. Not all studies account for the IgA secretion rate, salivary flow rate, saliva osmolality, or total salivary protein concentration when expressing IgA concentration values. The current study expressed IgA using multiple methods for completeness and ease of comparison to literature values (Figures 3-5). It is generally believed that a decrease in salivary flow rate, such as that observed during exercise, increases IgA concentration (Li & Gleeson, 2004). In fact, the hydration status of subjects may be a factor in the current study, although only resting samples was collected. Subjects were fasted overnight, which could have increased IgA concentration (decreased salivary flow rate) (Walsh, Bishop, Blackwell, Wierzbicki, & Montague, 2002). Miletic et al. (1996) believed that salivary flow rate was important for determination of salivary IgA concentration, but that repeated measurements should be

made to account for the great variability in IgA secretion rates observed within an individual. Miletic et al. also found no correlation between IgA secretion rate and total protein. Another study preferred to express salivary IgA as a secretion rate, and it appears that this is the most current accepted practice (Booth et al., 2009). A positive correlation between secretion rate and resting IgA concentration levels has been observed (Novas, Rowbottom, & Jenkins, 2003).

Lower saliva flow has been shown to correlate with increased plasma adrenaline and may be related to vasoconstriction effects of α 2-adrenergic receptors; however, a specific threshold of adrenergic stimulation may need to be reached before this effect is observed (Li & Gleeson, 2005). Changes in IgA concentration appeared to result from change in salivary flow rate because IgA secretion rate didn't change (Li & Gleeson, 2005).

Fahlman and Engels (2005) claimed that monitoring absolute IgA concentration as well as secretion rate is adequate without the need to analyze salivary protein or osmolality, and that secretion rate was the best predictor of URTI. Their study found that an IgA concentration less than 40 mg/L and IgA secretion rate less than 40 μ g/min leads to increased risk of URTI.

The argument can be made that because subject samples in the current study were collected only at rest, there is no need to account for changes in salivary flow rate; however, even salivary flow rates at rest cannot be assumed to be completely constant (Bishop & Gleeson, 2009). In fact, there were differences in salivary flow rates from

baseline to post-test in the current study (raw values only; 12 decreased, 7 increased, and 3 did not change from baseline to post-test) that did not appear to correlate in any way with IgA concentration. Because of the error associated with saliva volumes, an accurate estimate of the salivary IgA flow rate cannot be determined from this study; however, it appeared that salivary IgA flow rate did not significantly change (Fig. 4).

Interestingly, the IgA concentration within the CON group nearly significantly increased ($p=0.059$) from baseline to post-test (Fig. 3). This may have simply been a factor of the small sample size and the fact that exact quantitation could not be obtained for three of the six CON samples. When IgA was expressed as a secretion rate (Fig. 4), this dramatic difference was not observed, although it was still higher than the change from baseline to post-test in the other two groups. It is possible that the altitude treatments were having a detrimental effect on the IgA levels in the treatment groups, as it may have been expected that IgA levels would increase within these groups as well without the treatments.

Perhaps a decrease in IgA would have been observed in the treatment groups if treatment period had been extended beyond three weeks. If this is the case, the LON protocol used in this study may be an optimal dose of altitude to increase endurance performance while having no significant effect on mucosal immunity.

A recent longitudinal study by Neville et al. (2008) determined that because of the lack of a consensus for expressing IgA as well as differences in methodology and study subject control (dehydration status, diet, training status, etc.), it is preferable to track relative

decline of IgA than absolute. Baseline IgA levels were determined to be unique for each individual and changes in concentration level were tracked over time. A 30% decrease in IgA concentration from baseline correlated with URTI. Again, although limitations of the current study included a lack of repeated measurements and small sample sizes, the pre-test values were assumed to be the baseline. When these baseline values were compared to the post-test values, there was no decrease in IgA antibodies that could increase the risk of URTI.

Although the current study found no significant change in IgA with altitude, there are inconsistencies in the literature with regard to IgA and exercise, and few studies that report the influence of altitude on IgA. Reviews have found increased, depressed, or no change in IgA levels with exercise in literature reports (Li & Gleeson, 2004; Fahlman & Engels, 2005). Interestingly, higher concentrations of IgA and greater variability were found in elite athletes versus recreational and sedentary populations (Francis, Gleeson, Pyne, Callister, & Clancy, 2005). Both an early as well as a recent article shows an increase in IgA concentration in serum or in vitro cultures with altitude or hypoxic exposure (Lopez et al., 1975; Baylor et al., 2003) while Tiollier et al. (2005) showed a decrease. The recent, in vitro study showed that hypoxia increased transcytosis of IgA across the epithelial cell when compared to normoxia and hyperoxia, both of which did not affect transcytosis (Baylor et al., 2003).

Studies describing the change in IgA with altitude training are few. The findings from the current study are contrary to Tiollier et al. (2005) who observed a decrease in IgA; however, the previous study collected saliva 30 minutes after return from altitude. Saliva samples were assessed in the current study after a brief rest period (2-5 days) commencing after the last treatment was conducted. Results from the current study suggest that the change in IgA that may occur after acute bouts of altitude may not change significantly over time. Future studies are warranted to determine the time required for salivary IgA to return to baseline levels after altitude treatment.

To understand what may be happening to IgA concentration with exercise and/or altitude, an understanding of the mechanism for salivary IgA concentration change must be conclusively determined. Both parasympathetic and sympathetic nerves control the salivary glands, and both types of input as well as adrenaline can increase transport of IgA into saliva (Bishop & Gleeson, 2009). Potential mechanisms for exercise induced changes in salivary IgA include removal of parasympathetic vasodilation from salivary gland blood flow which reduces the saliva flow rate (Bishop & Gleeson, 2009). An increase in IgA secretion with exercise may be a result of sympathetic stimulation (Li & Gleeson, 2004), i.e., more transport (transcytosis) of IgA from plasma cells through epithelial cells, or may be due to an upregulation of the Ig receptor on the basolateral surface of the epithelial cell (Bishop & Gleeson, 2004). Differences in IgA secretion in response to exercise could result from changes in adrenergic stimulation and receptors (Li

& Gleeson, 2005). Studies have been performed only in mice, and the exact mechanism is speculative. More research is clearly needed.

Other studies report a decrease in IgA with increased stress such as physical exercise as a result of glucocorticoid secretion which is thought to decrease immune cell function (Li & Gleeson, 2004), although other studies showed no close relationship between salivary cortisol or salivary catecholamines and secretion of salivary IgA (Tiollier et al., 2005; Kugler et al., 1992). Tiollier et al. equated a decrease in IgA concentration at altitude with α -adrenergic mechanisms in response to stressors (an active decrease); hypoxia activates the sympathetic nervous system, and increased salivary protein could reflect this increase. In contrast to Tiollier et al., the current study observed no significant change in salivary protein except in the SHO group (significant decrease from baseline). Again, Tiollier et al., assessed protein levels immediately after a return to altitude. The subjects in the current study may have had an opportunity to recover from the affects of altitude, as the saliva samples were collected at least two days after the last altitude treatment had been administered. Additionally, the exposure period was much less in the current study than in the Tiollier study (maximum of 15 hours over the course of three weeks versus eleven hours per day, respectively). It is possible that the hypoxic stimulus was strong enough to elicit positive change, but low enough to avoid potentially negative physiological consequences.

One person in the LON group, two people in the SHO treatment group, and one person in the CON group reported feelings of illness during the course of the study consistent with an upper respiratory tract infection. IgA levels were not significantly altered in these individuals, except for the two runners in the SHO group. A LHTL protocol utilized by Bonetti et al. (2009) with cyclists affected an increase in hemoglobin concentration without a clear increase in markers of inflammation (C-reactive protein, IL-1 β , and erythrocyte superoxide dismutase). This finding is consistent with the findings of the current study in which no decrease in mucosal immunity was observed with IE. Bonetti et al. (2009) hypothesized that the hypoxic stimulus afforded by the IE was not enough to elicit elevations in inflammation, but was sufficient for improvement in performance. Again, the major difference between the current study and Tiollier et al. (2005) was that Tiollier looked at acute markers of the immune system. While there may be transient changes in immune status during LHTL altitude exposure, it is possible that no long term change transpires. Due to budget and time limitations with the current study, IgA was sampled only before and after the treatment period. Additional research should investigate the pattern of change over time by sampling multiple time points.

Only a few well-conducted studies show a conclusive link between decreased IgA (lowered immune system) and clinical URTI (Neville et al., 2008; Fahlman & Engels, 2005; Gleeson et al., 1999; Spence et al., 2007). Most studies rely upon self report of symptoms (Novas et al., 2003), but those using physician diagnosis and microbial cultures are more credible (Fahlman & Engels, 2005; Gleeson et al., 1999; Spence et al.,

2007). Generally, the amount of exercise and the J shaped curve (intense exercise enhances URTI risk, while moderate exercise decreases URTI risk) are supported (Bishop & Gleeson, 2009; Spence et al., 2007).

The effects of training on other markers of mucosal immunity besides salivary IgA need to be determined to be able to show that mucosal immunity is indeed depressed (Bishop & Gleeson, 2009; Gleeson, Pyne, & Callister, 2004; Davison, Allgrove, & Gleeson, 2009). It is unknown whether mucosal immunity reflects overall immune status. Factors such as training intensity and volume, consistency in saliva collection and analysis, influence of dietary deficiency, and use of objective measures of illness need to be controlled in future studies (Gleeson et al., 2004). Further questions that need addressed include the influence of nutritional supplements and quantitation of the amount and intensity of exercise that decreases immunity. Additional research on altitude training and IgA is certainly needed; only two other studies to date have addressed this issue (Tiollier et al., 2005; Robach et al., 2006). Although there is much work to be done, the field of exercise immunology shows promising growth and impact even beyond URTI risk (Bishop, 2005).

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APPENDIX A: RATE OF PERCEIVED EXERTION SCALE

Rate of Perceived Exertion Scale

6 (No exertion at all)

7 Very, very light

8

9 Very light - (e.g., easy walking slowly at a comfortable pace)

10

11 Fairly light

12

13 Somewhat hard

14

15 Hard

16

17 Very hard

18

19 Very, very hard (e.g., subject cannot continue for long at this pace)

20 (Maximal exertion)

**APPENDIX B: AHA/ACSM HEALTH/FITNESS FACILITY PREPARATION
SCREENING QUESTIONNAIRE**

AHA/ACSM Health/Fitness Facility Preparation Screening Questionnaire*

Assess your health status by marking all true statements

History

You have had:

- a heart attack
- heart surgery
- cardiac catheterization
- coronary angioplasty (PTCA)
- pacemaker/implantable cardiac
- defibrillator/rhythm disturbance
- heart valve disease
- heart failure
- heart transplantation
- congenital heart disease

Symptoms

- You experience chest discomfort with exertion.
- You experience unreasonable breathlessness.
- You experience dizziness, fainting, or blackouts.
- You take heart medications.

Other health issues

- You have diabetes.
- You have asthma or other lung disease.
- You have burning or cramping sensation in your lower legs when walking short distances.
- You have musculoskeletal problems that limit your physical activity.
- You have concerns about the safety of exercise.
- You take prescription medication(s).
- You have/or do take ergogenic aids or otherwise known competition associated banned substances

Cardiovascular risk factors

- You are a man older than 45 years
- You smoke, or quit smoking within the previous 6 months.
- Your blood pressure is > 140/90 mm Hg.
- You do not know your blood pressure.
- You take blood pressure medication.
- Your blood cholesterol level is >200 mg/dL.
- You do not know your cholesterol level.
- You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
- You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 days per week).
- You are >20 pounds overweight.

None of the above *You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.*

*Modified from American College of Sports Medicine and American Heart Association. ACSM/AHA Joint Position Statement: Recommendations for cardiovascular screening, staffing, and emergency policies at health/fitness facilities. Med Sci Sports Excer 1998:1018. **Professionally qualified exercise staff refers to appropriately trained individuals who possess academic training, practical and clinical knowledge, skills, and abilities commensurate with the credentials defined in Appendix F. <According to Exercise Testing and Prescription, 7th ACSM's Guidelines for edition, 2005>

I have read, understood, and checked appropriately, the proceeding document.

Subject Signature _____ Date _____

APPENDIX C: CONSENT FORM

STUDY INFORMATION SHEET and Preliminary Participant Screening Questionnaire

As a participant in this study, you will be exposed to simulated altitude over a period of 15 days. You will be required to maintain detailed records of training on a daily basis. You will perform exercise tests on two occasions: a baseline 10 K performance race (the day prior to simulated altitude exposure), and a 10 K performance race post-treatment. You will provide saliva samples one day before and one day post-treatment.

To participate in this study, you will be required to maintain your existing fitness training beginning 2 weeks prior to baseline testing and ending with the last performance trial. Your fitness training is to consist of moderate to high intensity training.

All subjects will be screened by a study questionnaire for pre-existing medical conditions or ergogenic aid use. To participate in this study you must be apparently healthy.

If you would like to be considered for inclusion in this study, please complete the form and return to the address given below. If you have questions, please contact me directly at (614) 292-0458 or Email at blazek.16@osu.edu.

Thank You,

Primary Investigator: Carmen Babcock Swain, Ph.D.

Co-Investigator: Alisa Blazek

Return to:

Alisa Blazek

The Ohio State University

A12 PAES Building

Name: _____ Date: _____

Email: _____ Phone: _____

Number of years participating in competitive triathlons: _____

Number of competitions in the last 12 months: _____

Average number of hours spent training per week in last month: _____

Have you been acclimatized to altitude within the last 4 months? _____

APPENDIX D: ACSM RISK STRATIFICATION

American College of Sports Medicine Risk Stratification

Once responses have been given to the AHA/ACSM Health/Fitness Facility Preparation Screening Questionnaire, symptom and risk factor screening can occur by using Table 1-3.

Knowing the risk factor for each individual, a decision can be made regarding the medical coverage for the exercise stress test using Table 4.

Coronary Artery Disease Risk Factor Thresholds for Use with ACSM Risk Stratification	
Risk Factors	Defining criteria
Positive:	
Family history	Myocardial infarction, coronary revascularization, or sudden death before 55 years of age in father or other male first-degree relative (i.e., brother or son), or before 65 years of age in mother or other female first-degree relative
Cigarette smoking	Current cigarette smoker or those who quit within the previous 6 months
Hypertension	Systolic blood pressure of ≥ 140 mmHg or diastolic ≥ 90 mmHg, confirmed by measurements on at least 2 separate occasions, or on anti-hypertension medication.
Dislipidemia	Low-density lipoprotein cholesterol (LDL) is available use > 130 mg/dL (3.4 mmol/L) or high-density lipoprotein cholesterol (HDL) of < 40 mg/dL (1.03 mmol/L) or on lipid-lowering medication. If total serum cholesterol is all that is available use > 200 mg/dL (5.2 mmol/L) rather than low-density lipoprotein (LDL) > 130 mg/dL.
Impaired fasting glucos	Fasting blood glucose of ≥ 100 mg/dL (5.6 mmol/L) confirmed by measurements on at least two separate occasions.
Obesity+	Body mass index of ≥ 30 kg/m ² or waist girth of > 102 cm for men and > 88 cm for women or waist/hip ratio: ≥ 0.95 for men and ≥ 0.86 for women.
Sedentary lifestyle	Persons not participating in a regular exercise program or meeting the minimal physical activity recommendations* from the US Surgeon General's report.
Negative:	
High serum HDL cholesterol [^]	> 60 mg/dL (1.6 mmol/L)

+ Professional opinions vary regarding the most appropriate markers and thresholds for obesity; therefore exercise professionals should use clinical judgment when evaluating risk factor.

*Accumulating 30 minutes or more of moderate physical activity on most days of the week.

[^] It is common to sum risk factors in making clinical judgments. If high-density lipoprotein (HDL) cholesterol is high, subtract one risk factor from the sum of the positive risk factors because high HDL decreases CAD risk.

<According to ACSM's Guidelines for Exercise Testing and Prescription, 7th edition, 2005>

American College of Sports Medicine Risk Stratification

ACSM Risk Stratification Categories
<p>Low risk: Men < 45 years of age; women < 55 years of age who are asymptomatic and meet no more than one risk factor threshold from Table 1.</p>
<p>Moderate Risk: Older individuals (men \geq 45 years of age; women \geq 55 years of age) or those who meet the threshold for two or more risk factors from Table 1.</p>
<p>High risk: Individuals with one or more signs/symptoms listed in Table 3. For known cardiovascular (cardiac, peripheral vascular, or cerebrovascular disease), pulmonary (chronic obstructive pulmonary disease, interstitial lung disease, or cystic fibrosis), or metabolic disease (diabetes mellitus (types 1 and 2), thyroid disorders, renal or liver disease).</p>

<According to ACSM's Guidelines for Exercise Testing and Prescription, 7th edition, 2005>

American College of Sports Medicine Risk Stratification

Major signs or symptoms suggestive of Cardiovascular and Pulmonary Disease

- Pain, discomfort (or other anginal equivalent) in the chest, neck, jaw, arms, or other areas that may be result from ischemia
- Shortness of breath at rest or with mild exertion
- Dizziness or syncope
- Orthopnea or paroxysmal nocturnal dyspnea
- Ankle edema
- Palpitations or tachycardia
- Intermittent claudication
- Known heart murmur
- Unusual fatigue or shortness of breath with usual activities

<According to ACSM's Guidelines for Exercise Testing and Prescription, 7th edition, 2005>

ACSM Recommendations for (A) Current Medical Examination* and Exercise Testing Prior to Participation and (B) Physician Supervision of Exercise Tests

	Low Risk	Moderate Risk	High Risk
A.			
Moderate exercise	NN	NN	R
Vigorous exercise	NN	R	R
B.			
Submaximal test	NN	NN	R
Maximal test	NN	R	R
NN - Not Necessary		R - Recommended	

<According to ACSM's Guidelines for Exercise Testing and Prescription, 7th edition, 2005>