Journal of Exercise Physiologyonline

December 2014
Volume 17 Number 6

Official Research Journal of the American Society of Exercise Physiologists

ISSN 1097-9751

JEPonline

# Effects of a Simulated Altitude Device on Endurance Performance and Mucosal Immunity

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## ABSTRACT

**Blazek AD, Anderson PJ, Brichler JG, Slawinski MK, Rose MT, Kirby TE, Swain CB.** Effects of a Simulated Altitude Device on Endurance Performance and Mucosal Immunity. **JEPonline** 2014;17 (6):45-57. The purpose of this study was to determine the effects of a simulated intermittent altitude device on endurance performance and mucosal immunity. Twenty well-trained male runners and/or triathletes were exposed to the hypoxic stimulus for 15 days. Time to complete a 10K race and salivary IgA antibodies were measured at baseline and post-treatment. Comparisons between groups were made using the non-parametric unpaired Mann-Whitney test. Time to complete the 10K race significantly decreased for the treatment group compared to the control group ($P<0.05$). No significant decrease in salivary IgA antibodies was detected in either group ($P\geq0.05$). The simulated altitude from the handheld Alto2Lab device produced the same performance enhancing effects as a live high train low environment in a short time and without the added stress of live high train low training that could compromise the mucosal immune system. The simulated altitude device may be attractive to competitive endurance athletes to enhance training while decreasing the potential for upper respiratory tract infection that often occurs with rigorous endurance or altitude training.

**Key Words**: Altitude, Immunity, Mucosal, Endurance

## INTRODUCTION

Performance differences in competitive sport are so small that athletes will go to great measures to gain a competitive edge (44). Altitude training, in particular, is a commonly used and widely accepted aerobic performance enhancement approach (14,18). However, the concern is that the effect of altitude training on the immune system is unclear (43). Altitude training can be physiologically stressful to athletes, and the negative consequences of training at altitude can include mountain sickness, pulmonary edema, cardiac arrhythmias, detraining, and immune system dysfunction (1).

Furthermore, cost, logistical issues, and disruption to family life are socioeconomic deterrents to implementing this type of training (41). Additive stressors on the athlete can impede performance as well as increase the potential for illness, leading to missed training days (41,43). Intense prolonged exercise or exposure to environmental extremes could increase athlete susceptibility to illness due to chronic exposure to stress hormones (43). A need exists for well controlled studies of the effect of altitude training on the immune system.

Intermittent altitude exposure (IE) may be a less stressful altitude training option than living and training at altitude. The IE option has been useful for decreasing acute mountain sickness by acclimatizing individuals before ascension, especially military personnel that must rapidly deploy to high altitude areas (2,26). It involves a brief exposure to hypoxia over a period of time to acquire the positive physiological adaptations of living at altitude without the negative physiological and socioeconomic effects of living at altitude. Research has shown that IE leads to improvements in aerobic performance, ventilatory responses, and blood parameters (3,7,16,23,34,35) in a relatively short period of time (33). This type of training can be achieved using chambers or other devices that simulate high altitude.

One challenge of using IE to simulate living high and training low (LHTL) is the resulting alteration of the immune system. Zhang, Hu, and Wang (47) observed changes in T lymphocyte subsets in soccer players after LHTL, but the health impact of this effect was unknown. Tiollier et al. (40) showed that salivary IgA antibody concentration decreased after an 18-day period of intense LHTL training in elite cross country skiers. Although IgA levels decreased in both the training group and a sea level control group, the difference reached significance only in the LHTL group, which indicates that the stress of altitude and intense training was a cumulative influence. IgA levels remained low, even after a 2-wk recovery period. Since the IgA antibody is the first line of defense on mucosal surfaces (9,20), deficiencies may increase chances of upper respiratory tract infection (URTI) (42,45). Indeed, although the level of salivary IgA varies between individuals, it has generally been shown that the higher the incidence of URTI the lower the level of IgA (42). It is possible that IE using simulated altitude versus actual ascension may be a better training option when maintenance of the immune system function is concerned. But to our knowledge, no studies of this nature have been published.

The observed cumulative effects of exercise and hypoxia on the immune system (15,21,30) indicate a need for vigilance in reducing the impact of the stressors on the athlete to maintain an efficiently functioning immune system. Elimination or minimization of long term travel, disruption of social life and schedule, acclimatization to altitude, and harsh, long-term training at altitude could impact the health and performance of an athlete (41). Therefore, the purpose of this study was to determine the effects of a simple, handheld, simulated intermittent altitude exposure device used at rest for short time periods and during times convenient to the athletes on running performance and mucosal immunity. The device simulated a hypoxic environment by removing expired carbon dioxide and preventing ventilatory reflexes from increasing breathing rate. It was hypothesized that this device would improve running performance while having no effect on salivary IgA antibodies.

## METHODS

### Subjects

Twenty well-trained male runners and/or triathletes were recruited from The Ohio State University (OSU) campus area and surrounding Columbus, Ohio community. Well-trained was defined as no less than one month experience in triathlon/duathlon training (i.e., intense running, biking, and/or swimming) or distance running training, and sustained training practices (moderate to high intensity) of no less than 6 $hr \cdot week^{-1}$ for the previous month. All subjects were required: (a) to maintain training during the study period, and each participant kept a training log during the duration of the testing that was monitored by study personnel; and (b) to maintain a consistent diet during the study. These conditions were set to ensure that a change in performance was not attributable to changes in training or diet. Only subjects categorized as low risk according to the American College of Sports Medicine (ACSM) Risk Stratification and apparently healthy according to the American Heart Association/American College of Sports Medicine (AHA/ACSM) Health/Fitness Facility Preparation Screening Questionnaire were eligible to participate. None of the subjects had been acclimatized to altitude within 4 months prior to the start of the study. All subjects confirmed that they were not taking any nutritional supplements or ergogenic aids. Each subject provided a written informed consent as required by the OSU Institutional Review Board (IRB).

### Simulated Intermittent Altitude Exposure

The subjects were randomized into three groups. Each group was exposed to 15 sessions of hypoxic exposure via the Alto2Lab re-breathing device for either a short-term continuous period (N=6, SHO, 15 min) or a long-term intermittent period (N=9, LON, alternating 6 min hypoxia exposure and 4 min normoxia via atmospheric air over 60 min), or no altitude exposure, which served as the controls (N=5, CON). The Alto2Lab device consisted of open-ended silos containing carbon-dioxide absorptive soda lime attached to a breathing tube (Figure 1). The subjects breathed through the tube while sitting. By removing the carbon dioxide from the subjects' expired breath, the re-breathing device prevented the physiological response to increase ventilation, thereby producing hypoxia.

**Figure 1. The Subjects Receiving Simulated Altitude Treatment.**

Foam-filled silos could be added to the canister to progressively increase the hypoxic stimulus by increasing respiratory dead space. The altitude treatment sessions were scheduled at times convenient to accommodate the athlete's work, training, and school activities. The subjects could choose from two locations that were most convenient to obtain treatment. Most were able to schedule sessions with teammates and friends to minimize social isolation during treatment. Oxygen saturation was monitored with a pulse oximeter. Oxygen saturation dropped progressively from 90% on Day 1 to 77% on Day 6 to 15 (i.e., the equivalent to altitudes of 3600 to 6200 m) for the LON group. The SHO group maintained an oxygen saturation of 90% during all sessions (3600 m altitude). Any symptoms of potential illness (e.g., runny nose, sniffling, sneezing, coughing, or other symptoms of upper respiratory illness) that occurred during the course of the study were recorded.

**Research Questions**

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

***Running Time Trials***

Ten kilometer running time-trials were completed prior to and after the 15-day treatment period, and saliva was collected before each race. Recovery days (i.e., no altitude simulation) were incorporated into the protocol prior to each of the time trials and during weekends. Time trials were performed on a relatively flat, 10K trail mapped on the OSU campus (Olentangy Bike Trail). Subjects ran the course with instructions to run to the best of their abilities. They were provided with race numbers, and were provided split times throughout the course in order to simulate an actual race event.

***Salivary IgA Analysis***

After an overnight fast, saliva was collected first thing in the morning before each time trial race. After the subjects rinsed their mouths with sterile water, they were asked to collect saliva in their mouths for 2 min without forcing. Subjects discharged the saliva into a conical tube, and the volume of saliva was measured to the nearest 0.2 mL. Samples were frozen at -80°C until analysis. Immediately prior to analysis, 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 in duplicate using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Alpco), and total protein content was measured using the BCA Protein Assay (Pierce). Calculated concentration of salivary IgA, total protein concentration, and IgA secretion rate were determined as described previously (20).

**Statistical Analyses**

Tiollier et al. (40) reported significant differences in salivary IgA levels after altitude training with a total subject number of 11 cross country skiers. Due to the similarities in the dependant variables under investigation, and using GPower Statistical Software v3.1 to perform a power analysis, it was surmised that 6 subjects per group would be sufficient to detect statistical differences. Mann-Whitney non-parametric tests were used to examine the effects of LON and SHO altitude exposure protocols on running time and salivary IgA antibody concentration using Minitab version 15 statistical software. Significance was set at a statistical probability of $P \leq 0.05$.

## RESULTS

**Subject Characteristics**

The subjects were between the ages of 18 to 44. Self-reported training logs indicated that the subjects had been training an average of $9.7 \pm 2.9$ $hr \cdot wk^{-1}$ prior to the study. The CON group trained

an average of 9.9 ± 2.7 hr·wk$^{-1}$, the SHO group averaged 8.6 ± 3.7 hrs, and the LON group averaged 10.5 ± 2.6 hr·wk$^{-1}$. Training logs confirmed maintenance of training throughout the duration of the study.

**Time Trial Results**

The LON treatment group completed the 10K time trial race in a significantly shorter period of time than the CON group (P<0.05, Table 1, Figure 1) when mean differences in running time 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). Two non-significant within group trends were noted: mean time to complete the post-test race tended to increase from baseline in the CON group, and decrease in both treatment groups (Table 1).

**Table 1. Change in Race Performance Times in Response to Simulated Intermittent Altitude.**

| Group | Mean Baseline Run Time (min) | Mean Post-Test Run Time (min) | Mean Difference (Post-Pre) (Min) |
|---|---|---|---|
| **CON** (N=5) | 49.52 ± 9.1 | 51.21 ± 10.0 | 1.69 ± 1.8 |
| **SHO** (N=6) | 42.82 ± 6.2 | 42.41 ± 5.8 | -0.41 ± 1.1 |
| **LON** (N=9) | 43.80 ± 4.4 | 43.22 ± 4.4 | -0.58 ± 1.7‡ |

‡LON performance mean difference decreased from baseline to post-test compared to CON (P<0.05).

**Results of Salivary IgA Analysis**

Salivary IgA concentration did not significantly change from baseline to post-race between or within groups (P<0.05, Figure 2). Both treatment groups showed a trend for increasing IgA with altitude treatment, and an increase in mean IgA concentration approached significance in the CON group (P=0.059).

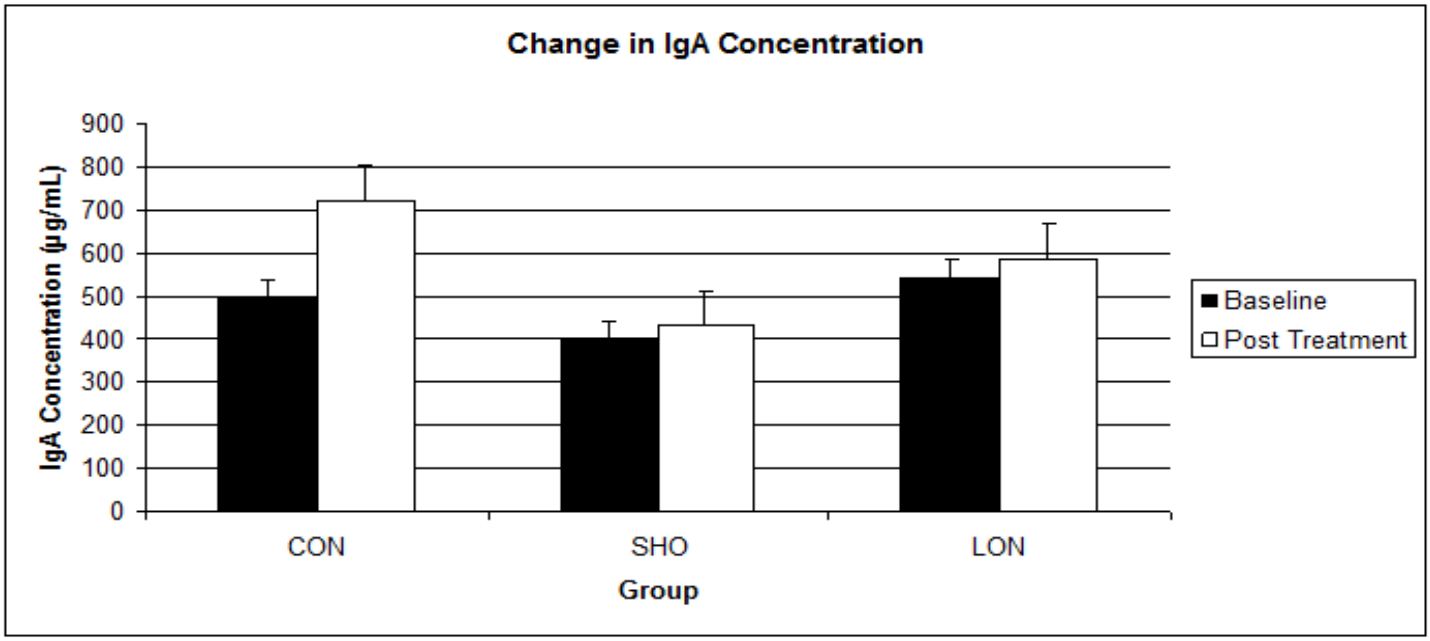


**Figure 2. Change in Salivary IgA Concentration in Response to Simulated IE ± SEM.** Mean salivary IgA concentration changes between baseline and post-IE treatment. IE treatment groups showed non-significant increases in mean IgA concentration from baseline, and the CON group showed increased IgA that approached significance. IE treatment mean group differences in IgA concentration were not significantly different from the CON group.

Mean IgA secretion rate did not significantly change from baseline to post-test between or within groups (Figure 3). 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. This trend paralleled the observed increase in IgA concentrations.

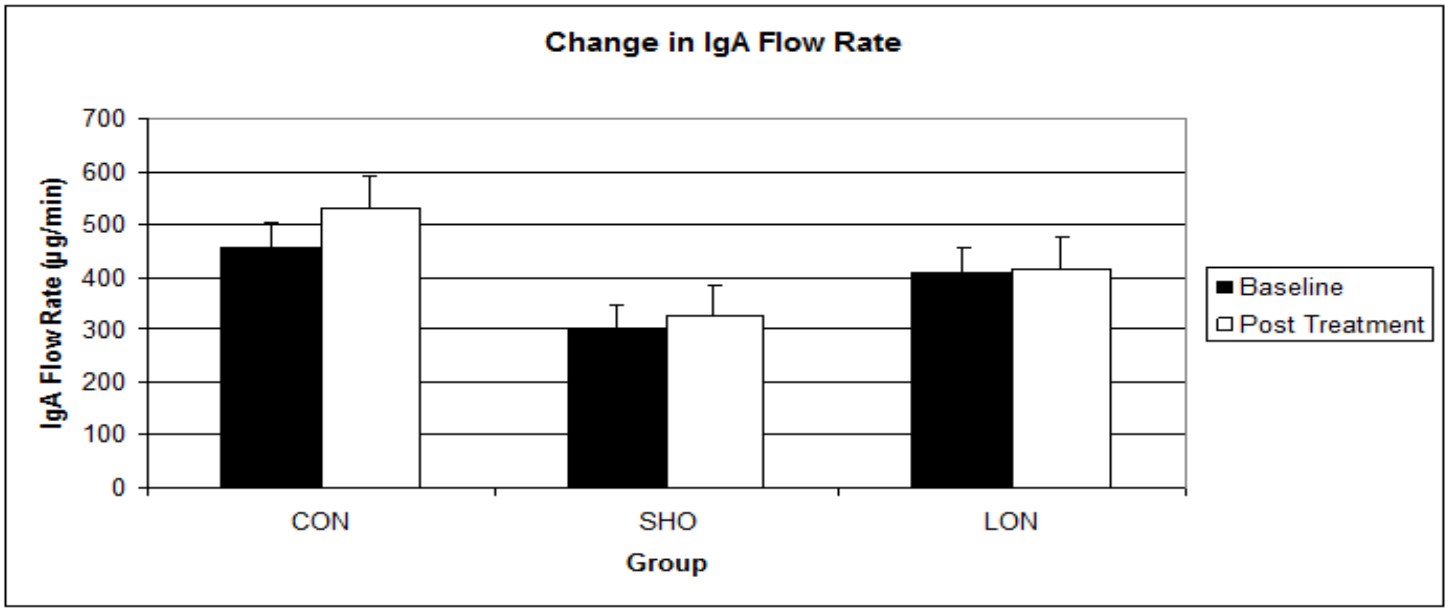


**Figure 3. Change in Salivary IgA Flow Rate in Response to Simulated IE ± SEM.** Mean IgA flow rate change from baseline to post-treatment was non-significantly increased in any group. The mean group differences for the treatment groups were not significantly different from the CON.

Although all groups trended towards a decrease in salivary protein concentration from pre- to post-test, this decrease only reached significance in the SHO group ($P<0.05$, Figure 4). Between group mean protein concentration differences were not significant. 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.

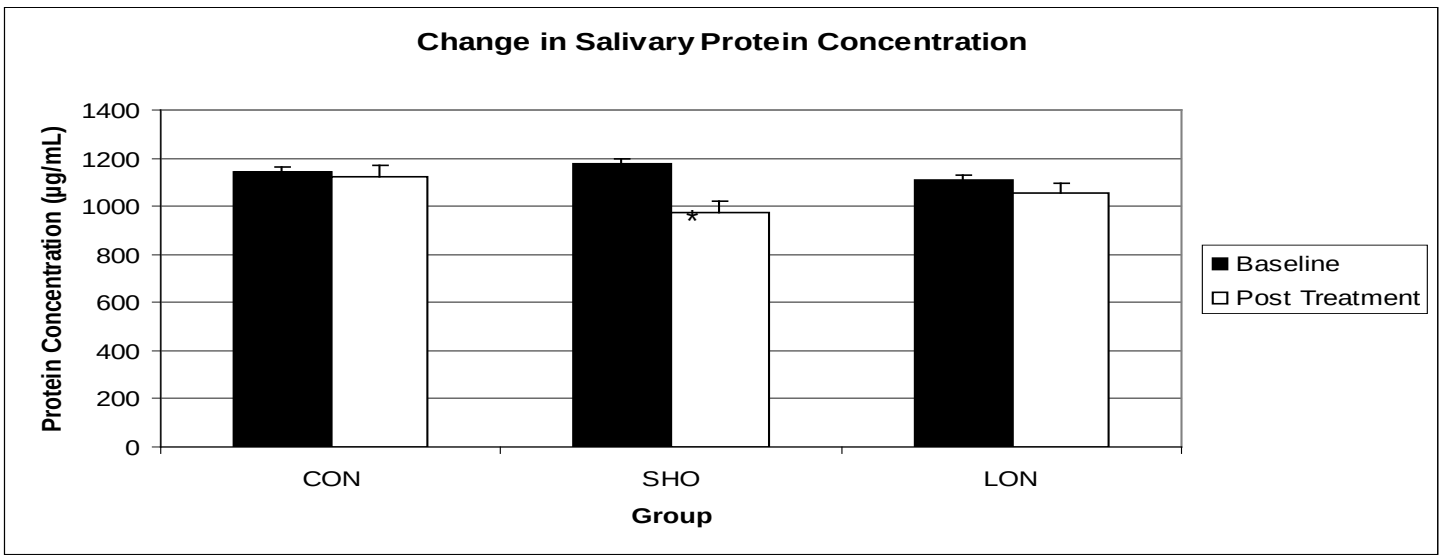


**Figure 4. Change in Total Salivary Protein Concentration in Response to Simulated IE ± SEM.** Although all groups showed a post-treatment decrease in salivary protein concentration from baseline, this decrease was significant only in the SHO group (*$P<0.05$). Mean group differences for the treatment groups were not significantly different from the CON mean group difference.

## DISCUSSION

### Simulated IE Can Improve Running Performance

Simulated IE altitude treatment using the LON protocol significantly shortened time to complete the 10K race compared to the CON. This finding is in agreement with research that indicates IE results in a significant improvement in aerobic performance, ventilatory function, and blood parameters (3,7,16,23,34,35). The findings in the present study are also supported by a previous study from the same laboratory that found improvement in aerobic cycling performance while using the Alto2Lab device (39).

The subjects were exposed to either the LON protocol, which is recommended by the device manufacturer, or the SHO protocol in an attempt to determine a minimum effective dose of altitude. The use of the SHO protocol to improve running endurance performance was not supported in this study. However, the improvement in SHO performance approached significance (P=0.08). Further studies characterizing the influence of the SHO protocol on $VO_2$ max and hematological parameters with a larger group of subjects are warranted. Nonetheless, the LON protocol requires negligible time in comparison to LHTL, and it may be very appealing to athletes seeking to minimize time at altitude. The use of such a protocol improves performance while minimizing time and exposure to potentially negative physiological consequences of LHTL training.

Similar to a previous study using intermittent simulated altitude (36), the runners in the LON group achieved a performance improvement of 1.3% over the CON group. This seemingly small performance improvement of approximately 30 sec could determine the difference between placing or not in a race or triathlon where performance differences are small and every second is critical (44). A study using the same device found a 1.5% increase in sprint speed (46). It should be noted that within group means for the LON group were not significantly different from baseline to post-test, but individual variation in response to altitude acclimatization including genetic factors, differences in bone marrow sensitivity to EPO, and differences in serum ferritin have been described (8,22). Serum ferritin was not measured, nor did the subjects receive iron supplementation in the current study. While the physiological mechanism of altitude adaptation is controversial, Friedmann et al. (11) have shown differences in response to altitude training in spite of adequate iron.

Initial training status varied with the CON group appearing to be less conditioned than the other groups at baseline (e.g., mean baseline race time of 49.52 min compared to SHO 42.82 min and LON 43.80 min). However, the race times were compared baseline to post-race and any changes in performance would have been detected. Further, training records indicated no changes in training frequency, intensity, or duration by any of the subjects during the course of the study.

It is already known that altitude training provides an increase in $VO_2$ max. This study differs from many altitude based studies in that the purpose was to provide evidence that altitude training could enhance performance under race conditions. Although laboratory $VO_2$ max tests would have allowed for controlled environmental conditions, one of the true tests of an ergogenic aid (such as altitude training) is its ability to improve performance in the face of common race obstacles such as suboptimal rest, diet, and weather. Additionally, a laboratory test would have removed the elements of competition and strategy, which are important contributors to race performance. Altitude training in the amateur athlete may not be a potent enough performance enhancement stimulus to overcome these common life interferences as well as that of intrinsic motivation. The influence of motivation could have had an impact on the performance of the CON in the current study, as nocebo effects in control groups and placebo effects on altitude exposure groups have been noted (5).

**Simulated IE does not Decrease Mucosal Immunity**

Both altitude exposure and intense training can alter the immune system, and additive effects of the two have been observed (15,21,30). Since altitude is widely accepted as a performance enhancement method, a need exists to decrease the physiological stress of altitude and its associated factors so that an optimal immune response is maintained. The present study attempted to quantitate the change in salivary IgA levels as a marker of mucosal immunity (31) during simulated IE training (17,24).

Salivary IgA concentration did not significantly change from baseline to post-test in any of the study groups. This result 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 any of the groups.

Although the highest concentrations of IgA observed were higher (baseline of 71.91 to 1034.70 $\mu g \cdot mL^{-1}$) than typically observed in the literature (6) (range of 14.7 to 483 $\mu g \cdot mL^{-1}$), it is difficult to compare with accuracy because of differences in collection method (25,37), time of day of collection (27), sample storage conditions (6,28), and differences in IgA concentration expression between studies. Not all studies account for IgA secretion rate, salivary flow rate, saliva osmolality, or total salivary protein concentration when expressing IgA concentration values. The present study expressed IgA using multiple methods for completeness and ease of comparison to literature values (Figures 2 to 4). In general, highly variable within and between subject IgA levels have been reported, and comparisons between studies are difficult (4,27). The controls provided with the Alpco ELISA kit performed as expected, thus indicating that values for this assay were within normal range. Naturally, it would be ideal to find an average baseline IgA concentration for each subject and, then, take repeated samples throughout the study (27). Although the lack of repeated measurements due to budget is a limitation of this study, the observed protein concentrations and salivary flow rates were reasonable values compared to the literature (10,19,25).

Only a few well-conducted studies show a conclusive link between decreased IgA (lowered immune system) and clinical URTI (10,12,27,38). Most studies rely upon self-report of symptoms (29), but those using physician diagnosis and microbial cultures are more credible (10,12,38). Fahlman and Engels (10) found that an IgA concentration <40 $mg \cdot L^{-1}$ and IgA secretion rate <40 $\mu g \cdot min^{-1}$ leads to increased risk of URTI. No subjects in the current study displayed pre- or post-treatment IgA values that would indicate risk for URTI. Neville et al. (22) determined that a 30% decrease in IgA concentration from baseline correlated with URTI. 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. One LON group subject, two SHO group subjects, and one CON group subject reported feelings of illness during the course of the study that is consistent with an upper respiratory tract infection. IgA levels were not significantly altered in these subjects, except for the two subjects in the SHO group.

Interestingly, the increased IgA concentration from baseline to post-test within the CON group nearly approached significance (P=0.059) (Figure 2). When IgA was expressed as a secretion rate (Figure 3), this dramatic difference was not observed, although it was still higher than the change from baseline to post-test in the other two groups. This result could indicate that the IE treatments were in fact decreasing IgA levels in the treatment groups, and that a decrease in IgA may have been observed in the treatment groups if the IE exposure period had extended beyond 3 wks. If this trend was in fact occurring, the LON protocol used in the present study may be an optimal dose of altitude to increase endurance performance while having no significant effect on mucosal immunity.

The findings contrast with those of Tiollier et al. (40) who observed a decrease in salivary IgA and significant increases in salivary protein with LHTL altitude training. However, Tiollier and colleagues collected subject saliva 30 min after return from altitude. Post-test saliva samples for the current study were assessed after a brief recovery period (2 to 5 days) commencing after the last simulated IE treatment was administered. 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. Due to budget limitations with the current study, IgA was sampled only before and after the treatment period. Future research should investigate the changes in IgA over time by sampling at multiple time points. Future studies also are warranted to determine the time required for salivary IgA to return to baseline levels after altitude treatment.

The current study also differs from Tiollier et al. (40) in that the total altitude exposure period was much shorter in duration (maximum of 15 hrs over the course of 3 wks versus 11 hrs per day, respectively). It is possible that the simulated IE hypoxic stimulus in the current study was strong enough to elicit positive performance changes, but low enough to avoid potentially negative physiological consequences.

An understanding of the mechanisms of IgA change in response to exercise and altitude is warranted. A likely candidate for the changes in IgA seen with exercise is sympathetic stimulation (19), which could increase transcytosis of IgA from plasma cells through epithelial cells. Or, it may be due to an upregulation of the Ig receptor on the basolateral surface of the epithelial cell (4). A decrease in IgA with increased physical stress could result from glucocorticoid secretion which decreases immune cell function (19), although other studies indicate no close relationship between salivary cortisol or salivary catecholamines and secretion of salivary IgA (17,40). Tiollier et al. (40) equated a decrease in IgA concentration at altitude with α-adrenergic mechanisms in response to stressors.

The effects of training on other markers of mucosal immunity besides salivary IgA need to be determined to show that mucosal immunity is indeed depressed (4,9,13), as it is unknown if 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 (13). 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, especially since only two other studies to date have addressed this issue (32,40).

## CONCLUSIONS

The findings of this study demonstrate that a handheld simulated IE altitude training device may provide an optimal dose of altitude exposure to improve 10K running performance while having no significant impact on mucosal immunity. As altitude training is commonly used for exercise performance enhancement, concern for decreasing stressors on the athlete as a result of altitude training should not be regarded lightly. Future studies should continue to find methods for decreasing athlete stress as well as define other physiological parameters (salivary IgA concentration over time, mechanisms of IgA change as a result of altitude and strenuous training, effects of training on other markers of mucosal immunity) as a result of the IE training device and altitude training in general. Such studies could improve training outcomes and decrease the possibility of illness, missed training, and diminished physical performance.

## ACKNOWLEDGMENTS

The authors express their sincere appreciation to Dr. Noah L. Weisleder at The Ohio State University for his review of the manuscript. Funding for Open Access was provided by The Ohio State University Open Access Fund.

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