The effect of cetirizine HCl on exercise-induced arterial hypoxemia in highly-trained swimmers

Robert F. Chapman, PhD, FACSM
Department of Kinesiology
Indiana University - Bloomington

Co-Investigator

Curtis Goss, MS
Department of Kinesiology
Indiana University - Bloomington

Support Provided by:
General research funds (Chapman)
University Graduate School Doctoral Research Grant

Table of Contents:

1.0 Background & Rationale

A constant supply of oxygen is critical for the maintenance of oxygen uptake ($\dot{V}O_2$) during exercise and increasing volumes of oxygen are needed to accommodate increases in exercise intensity. In healthy humans during exercise, the oxygen-carrying molecule hemoglobin is almost fully (>94%) saturated with oxygen, and a difference between the oxygen tension in the alveoli of the lung and the arterial blood exists (20-25 mmHg) but does not necessarily compromise $\dot{V}O_2$. However, a subset of otherwise healthy, highly-trained endurance athletes demonstrate a phenomenon termed exercise-induced arterial hypoxemia (EIAH), which refers to a decline in arterial oxyhemoglobin saturation (<92%) during exercise at sea level and is marked by a widened alveolar-arterial oxygen difference (25-40 mmHg) (Dempsey and Wagner, 1999). Over the past half-century, this phenomenon has been well-chronicled in highly-trained and elite athletes across numerous sporting disciplines, especially running (Chapman and Stager, 2008; Dempsey et al., 1984; Gavin and Stager, 1999; Harms and Stager, 1995) and cycling (Derchak et al., 2000; Rice et al., 1999; Tanner et al., 2013).

There are four recognized mechanisms for the development of EIAH: inadequate compensatory hyperpnea, diffusion limitation, alveolar ventilation-perfusion ($\dot{V}_A/\dot{Q}$) mismatch, and pulmonary veno-arterial shunt (West and Luks, 2016). EIAH is of concern to athletes because oxyhemoglobin saturation is associated with oxygen transport and, by extension, $\dot{V}O_{2MAX}$ (Dempsey and Wagner, 1999). Many researchers have investigated the relative contributions of inadequate compensatory hyperpnea, $\dot{V}_A/\dot{Q}$ mismatch, and veno-arterial shunt to the development of EIAH; however, the contribution of a diffusion limitation has not been as thoroughly examined, despite the fact a diffusion limitation is a viable mechanism by which athletes may ultimately experience EIAH (Prefaut et al., 2000).

There are two theorized routes by which a diffusion limitation could occur during exercise and ultimately lead to EIAH. One mechanism is that EIAH can develop due to an inflammatory response secondary to injury at the interface of the alveoli and pulmonary capillaries (West and Mathieu-Costello, 1995). Numerous studies (Groves et al., 1987; Reeves et al., 1990; Wagner et al., 1986; West and Mathieu-Costello, 1999) have estimated that pulmonary capillary pressure rises high enough to cause injury at the very thin (2-3 μm) diffusive barrier, which has been observed in rabbits, dogs, and Thoroughbred racehorses (Birks et al., 1994; Tsukimoto et al., 1991; West et al., 1991). The other mechanism by which a diffusion limitation could occur is through a subclinical pulmonary edema, where fluid from the capillaries leaks into the alveolar space (Hodges et al., 2006). Each of these mechanisms would cause a widening off the diffusive barrier through inflammation following injury or through a thickening of the aqueous barrier between the air in the lung and the blood, respectively, and are not necessarily mutually exclusive and could occur in the same individual.

Histamine is a compound that has been implicated in adding to the diffusion limitation associated with EIAH (Prefaut et al., 1997; Prefaut et al., 2000). Exercise (especially intense exercise) is a well-documented initiator of acute-phase inflammatory processes, including stimulating the action of granulocytic cells (Camus et al., 1993;

Suzuki et al., 2002). Indeed, various compounds that appear in greater concentrations in the blood of athletes who experience EIAH have been implicated in signaling and modulating histamine release from its progenitor cells (Horio et al., 2010; Mucci et al., 2000, 2001; Prefaut et al., 2000; Suzuki et al., 2002). Once released from basophils (blood-derived) and mast cells (tissue-derived), histamine is available to bind to the large quantity of H_1-receptors available in the pulmonary circuit (Panula et al., 2015). The binding of histamine to pulmonary H_1-receptors causes microvascular permeability edema and tissue inflammation characteristic of several pathological conditions (Borriello et al., 2017; Brigham and Owen, 1975; Probst et al., 1978) which, even at subclinical levels not nearly considered anaphylaxis, would ostensibly impair gas exchange during exercise. Furthermore, compounds that inhibit the release of histamine from mast cells (Coyle and Stager, 2001; Prefaut et al., 1997) and competitively inhibit histamine (Coyle and Stager, 2001) have been shown to attenuate EIAH in athletes.

Nearly all of the studies involving EIAH mechanisms and athletes have used either running or cycling as the mode of exercise. However, competitive swimming is an interesting, yet understudied, athletic arena in which to examine EIAH. There is limited evidence (Holmer et al., 1974; Labreche, 2012; Spanoudaki et al., 2004) to suggest that competitive swimmers might experience EIAH. However, it is difficult to draw definitive conclusions from these studies, as they likely either did not have an adequate subject population (Holmer et al., 1974) or did not utilize an exercise protocol with sufficient intensity or duration (Labreche, 2012; Spanoudaki et al., 2004) in which to determine the presence of EIAH. There are numerous plausible mechanisms by which EIAH could develop in swimmers, especially those that are highly-trained. First, swimming occurs 1) in water, with 2) a horizontal body position, and both of these conditions cause an increase in venous return of blood to the heart which would theoretically increase pulmonary capillary pressure (Pendergast et al., 2015). Second, swimmers breathe at incredibly high flow rates (Leahy et al., 2019) with their lungs almost completely inflated (Li, 2016), both of which serve to increase pulmonary capillary pressure (Fu et al., 1992). Third, breathing in competitive swimming is “phase-locked,” meaning swimmers can only breathe when their face is out of the water “in-phase” with their swimming stroke, and this ultimately leads to a relative hypoventilation compared to land exercise (Li, 2016). Finally, there is myriad literature linking swimming and pulmonary edema, and this phenomenon is well-chronicled in swimmers of many ages and abilities (Adir et al., 2004; Hohmann et al., 2018).

Therefore, the goals of the present study are twofold:

1. To examine whether EIAH occurs in highly-trained swimmers, and
2. To examine histamine release as a mechanism by which EIAH occurs in highly-trained athletes.

Objective(s)

2.1 Primary Objective

Aim 1. To confirm whether that highly-trained swimmers experience EIAH

It is hypothesized that highly-trained swimmers will experience EIAH, as evidenced by a decrement in peripheral capillary oxyhemoglobin saturation (S_pO_2) of 5% or greater from rest to submaximal and/or maximal exercise.

Aim 2. To test whether a pharmacological agent that is a competitive inhibitor to histamine (cetirizine HCl) can improve nadir S_pO_2 during submaximal (70 and/or 85% $\dot{V}O_{2max}$) and/or maximal (100% $\dot{V}O_{2max}$) exercise.

It is hypothesized that the pharmacological agents will significantly ($p < 0.05$) increase nadir S_pO_2 during all exercise intensities. This will be assessed using a linear mixed model including random effects for subject, subject within-treatment, and subject within-intensity to account for the repeated measures design. Treatments (placebo and cetirizine HCl), intensity (70, 85, and 100% of $\dot{V}O_{2max}$), and the interaction between treatment and intensity will be included. Main effects and interactions will be tested using *F*-tests from a two-way ANOVA.

2.2 Secondary Objective

Aim 3. To test whether degree of desaturation from rest to maximal exercise ($\Delta S_pO_{2,rest}$-$S_pO_{2,100\%}$) is correlated with the magnitude of histamine release from rest to maximal exercise ($\Delta\%H_{rest}$-$\%H_{100\%}$).

It is hypothesized that degree of saturation and histamine release will be significantly ($p < 0.05$) correlated. Correlational strength will be assessed via Pearson correlation coefficient.

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures

Plasma histamine, whole blood histamine, histamine release, peripheral capillary oxyhemoglobin saturation, oxygen consumption ($\dot{V}O_2$)

3.2 Secondary Outcome Measures

Basophil count, IL-1β, IL-8, exercise ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (f_B), carbon dioxide production ($\dot{V}CO_2$)

4.0 Eligibility Criteria

4.1 Inclusion Criteria

- Men and women
- 18-35 years old
- Current collegiate or professional swimmer
- Currently training at least 300 minutes per week
- Self-reported to be healthy

4.2 Exclusion Criteria

- Not within defined age range
- Current diagnosis of or using medication for:
    - Severe allergies
    - Asthma
    - Exercise-induced asthma
    - Exercise-induced bronchoconstriction
- Pulmonary function test considered to be abnormal (defined as forced vital capacity (FVC) <80% of predicted, forced expiratory volume in one second (FEV1) <80% predicted, and/or FEV1/FVC ratio >5% of the predicted ratio)
- Hypertension during screening (systolic blood pressure >139 or diastolic blood pressure >89)
- Current tobacco or electronic cigarette use or consistent use within the last 2 years
- A contraindication for use of cetirizine HCl:
    - Previous adverse reaction to cetirizine HCl or a similar medication
    - Allergy to the food additives E218 or E216
    - An intolerance to or inability to absorb some sugars, such as lactose or sorbitol
    - Liver or kidney failure
    - Epilepsy or similar condition
    - A condition that makes urinating difficult
    - Use of midodrine or ritonavir
- Are pregnant or could possibly be pregnant by self-report
- Subjects with current, irregular menstrual cycles (amenorrhea) or who had irregular menstruation patterns for greater than one year
- People who answer ‘yes’ to any of the pre-participation screening questions on page one of the PAR-Q+ questionnaire.

5.0 Study Design

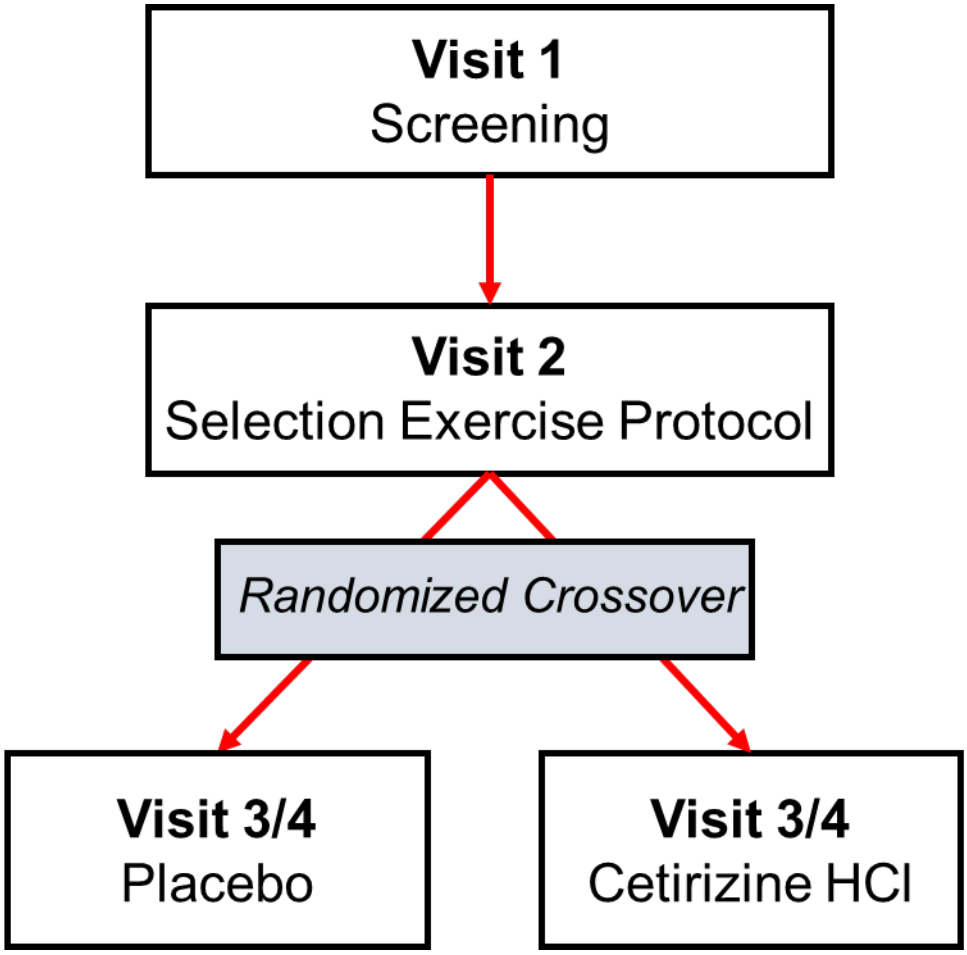

Figure 1: Experimental design for proposed study protocol.

The general study protocol is outlined in Figure 1. For the first testing session, participants will report to the Indiana University Human Performance Laboratory to complete a medical screening and, if willing, consent to the procedures of the study. The medical screening will include measurements of height, weight, resting heart rate, resting blood pressure, and resting pulmonary function along with a health history questionnaire. The second testing session consists of an assessment of risk factors for development of EIAH, specifically changes in blood biomarkers pre- and post-swimming exercise. Participants will report to the Counsilman-Billingsley Aquatics Center and complete an exercise protocol designed to elicit an inflammatory response. Venous blood draws will be performed to assess participants' pre-exercise complete blood count and pre- and post-intense exercise concentrations of plasma IL-1β, IL-8, plasma histamine, whole blood histamine, and histamine release. Measurements of hemoglobin concentration and hematocrit will also be performed pre- and post-exercise in order to correct biomarker

concentrations for fluid loss during exercise. Twelve swimmers (n = 6 men, n = 6 women) will be selected for further trials.

Those selected for further study will visit the Human Performance Laboratory on two occasions separated by at least 48 hours and no more than 60 days. Apart from receiving a placebo (PL) or drug treatment (cetirizine HCl, CH) prior to exercise, participants will perform identical protocols on each visit to the laboratory. Participants will report to the lab and consume either a placebo or CH pill, followed by a health history update questionnaire and their resting pulmonary function will be measured. Participants will then complete a self-selected warm-up that will be standardized across all testing sessions, followed by instrumentation. The exercise protocol beings with a progressive swimming test to maximum aerobic capacity ($\dot{V}O_{2max}$) in a swimming flume, followed by two constant load work bouts at approximately 70 and 85% of the previously recorded HRmax while peripheral capillary oxyhemoglobin saturation (S_pO_2) is continuously monitored. Participants will receive a 20-min break between each work bout. Drug treatments will be assigned in a double-blind, randomized crossover fashion such that each participant receives each treatment. Concentrations of plasma histamine, whole blood histamine, and histamine release will be assessed from pre- and post-exercise blood samples.

6.0 Enrollment/Randomization

We plan to recruit up to 30 participants for this project to ensure that at least 24 participants will complete the first two visits of the study.

Participants will be recruited from the Indiana University varsity swim team and the associated professional training group. Indiana University is home to a NCAA Division I swim team that is perennially among the nation's top-10 for both men and women. Participants will be recruited from this group of over 50 swimmers using a standard email script.

Potential participants will be given the basic information about the study and schedule an appointment for screening if they indicate that they would be interested in participating. Participants will be provided a with detailed description of study procedures prior to study enrollment. Participants will be asked to read the consent form at this time and be encouraged to ask any questions. Participants can then: 1) sign the consent form; 2) take it home for further consideration; or 3) decide not to participate. Participants will be screened once written informed consent has been given.

Experimental trials 3 and 4 will be completed in a double-blinded randomized crossover design. The randomization order will be determined using a customized program (Excel; Microsoft Corporation, Redmond, WA, USA). Subjects will be randomized using the randomization list by order of enrollment in the study by a researcher listed on the IRB personnel. This researcher will also be responsible for drug administration but will otherwise not participate in any data collection or analyses.

7.0 Study Procedures

All of the below mentioned procedures are being conducted for purposes of the research. Please see Table 1 for information regarding which visit to the laboratory these procedures will take place.

- Facilities: Visit 2 will take place at the Counsilman-Billingsley Aquatics Center, a 44,651 square foot aquatics center used by Indiana's varsity swimming and diving programs. It contains an eight-lane Olympic-sized pool spanning 30,512 square feet with depth ranging from seven to eight feet to allow for greater speed. A photo depicting the Counsilman-Billingsley Aquatics Center is below (Figure 3).

Figure 2: Photo of the Counsilman-Billingsley Aquatics Center where Visit 2 will take place.

Visits 3 and 4 will take place in the swim flume (Endless Pool). This pool works like a "swimming treadmill," pushing water from the front (near side in Figure 4) to the back at various flow rates to simulate various swimming speeds. A standard pulley is affixed to the back of the flume to add a tethered load to the swimmer, like changing the "grade" on a treadmill. This is a familiar training environment for high-level competitive swimmers. A photo depicting the swim flume in the IUB School of Public Health is below (Figure 4).

Figure 3: Photo of the swim flume where Visits 3 and 4 will take place.

- Height: Body height will be measured using a stadiometer.
- Weight: Weight will be measured using a scale.
- Heart rate: Resting heart rate will be obtained by taking a radial pulse for 15 seconds during the screening procedure. Exercising and pre-exercise heart rate will be continuously recorded using a specialized waterproof swimming heart rate monitor (Cardio Swim HR monitor; Freelap, Switzerland).
- Urine specific gravity: Urine specific gravity will be measured via refractometry.
- Blood pressure: Blood pressure will be measured using standard brachial artery manual auscultation techniques.
- Health history questionnaire: Participants will complete a health history questionnaire to ensure they meet the inclusion and exclusion criteria.

- Health history update questionnaire: Upon each visit to the laboratory participants will complete the heath history update questionnaire to ensure no changes in their health history has occurred.

- Pulmonary function: Pulmonary function will be examined using procedures described by the American Thoracic Society. Following 10 min seated rest, participants will be fitted with a previously disinfected non-rebreathing mouthpiece. Following 30 s of normal breathing the participants will be instructed to take a maximal inhalation followed by a maximal forced exhalation. This very large, very fast breath will be repeated three times. From this test peak expiratory flow rate (PEFR), forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and the maximal expiratory flow at 50% vital capacity (MEF50) will be measured. These tests will be used to determine if the participants classify as having normal pulmonary function (Barreiro and Perillo, 2004).

- Venous blood sample: Venous blood will be drawn by a trained researcher from an antecubital vein using standard aseptic techniques, as defined by the World Health Organization. Each blood draw will be ~10 mL (Experimental Trials) or 15 mL (Selection Exercise Protocol) total. Measurements include:

    - Complete blood count: Samples will be collected into EDTA-coated Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). The samples will be immediately stored on ice and transported to a local medical facility (Student Health Center, Indiana University, Bloomington, Indiana) that will perform a complete blood count using an automated hematology analyzer (SX-1000i; Sysmex, Kobe, Hyogo, Japan). The complete blood count will assess hemoglobin, hematocrit, white blood cell count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelets, neutrophil percentage, lymphocyte percentage, monocyte percentage, eosinophil percentage, basophil percentage, neutrophil number, lymphocyte number, monocyte number, eosinophil number, basophil number, red cell distribution width standard deviation, red cell distribution width coefficient of variation, and mean platelet volume.

    - Plasma volume correction: In order to account for loss of plasma volume during exercise, all plasma-derived post-exercise measures will be corrected via the methods of Dill and Costill (1974). First, post-exercise blood volume (BV_A) is determined via the equation:

$$BV_A = BV_B \times \frac{Hb_B}{Hb_A}$$

      where Hb_B is the pre-exercise hemoglobin concentration, BV_B is the pre-exercise blood volume and is 100 for the purpose of this equation, Hb_A is the post-exercise hemoglobin concentration. Whole blood histamine concentration will then be corrected based on the differences in the blood

volume pre- and post-exercise (ΔBV_A-BV_B%). BV_A is then used to determine post-exercise red cell volume (CV_A) via the equation:

$$CV_A = BV_A \times Hct_A$$

where Hct_A is the post-exercise hematocrit value. CV_A is then used to determined post-exercise plasma volume (PV_A) via the equation:

$$PV_A = BV_A - CV_A$$

Concentration of plasma-derived biomarkers will then be corrected based on the differences in plasma volume pre- and post-exercise (ΔPV_A-PV_B%). Hemoglobin and hematocrit will be assessed in triplicate from a venous blood sample.

- Plasma IL-1β: Samples will be collected into EDTA-coated Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). The samples will be immediately stored on ice and transported to the laboratory where they will be centrifuged at 4°C at 1,300 *g* for 10 minutes and 2000 *g* for 15 minutes (model X-22R; Beckman, St. Louis, MO). Two-hundred microliters of plasma will be aliquoted into 0.5 mL cryogenic storage vials (CryoKING®, Biologix, Jinan, Shandong, China) and stored immediately at −80°C. Samples will be later diluted accordingly and analyzed in duplicate using a commercially-available enzyme-linked immunoassay (ELISA) kit (Invitrogen #BMS224HS; Thermo Fisher Scientific, Waltham, MA, USA) with detection by spectrophotometry (Powerwave XS™ Spectrophotometer, Bio-Tek Instruments, Winooski, VT). Based on a previous study, it is expected that plasma IL-1β in this cohort will be 1-15 $pg \cdot mL^{-1}$ (Mucci et al., 2000). The sensitivity of this assay is 0.05 $pg \cdot mL^{-1}$ and the range is 0.16-10.0 $pg \cdot mL^{-1}$.

- Plasma IL-8: Samples will be collected, processed, and stored as previous. Samples will be later analyzed in duplicate with a commercially-available ELISA kit (Invitrogen #KHC0084; Thermo Fisher Scientific, Waltham, MA, USA) with detection as previous. Based on a previous study, it is expected that plasma IL-8 will be 0-10 $pg \cdot mL^{-1}$ (Mucci et al., 2000). The sensitivity of this assay is <100 $fg \cdot mL^{-1}$ and the range is 0.39-25 $pg \cdot mL^{-1}$.

- Plasma histamine: Samples will be collected as previous. The samples will be immediately stored on ice and transported to the laboratory where they will be centrifuged at 4°C at 900 *g* for 15 minutes (model X-22R; Beckman, St. Louis, MO). Samples will be analyzed in duplicate with a commercially-available ELISA kit (AB213975; Abcam, Cambridge, United Kingdom) with detection as previous. Based on a previous study, it is expected that plasma histamine will be 1-6 $ng \cdot mL^{-1}$ (Mucci et al., 2000). The sensitivity of this assay is 0.03 $ng \cdot mL^{-1}$ and the range is 0.098-25 $ng \cdot mL^{-1}$.

- Whole blood histamine: Samples will be collected into heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Fifty microliters of whole blood will be pipetted into 950 µL of distilled water and stored at −80°C in 1 mL vials (Eppendorf Tubes®; Eppendorf, Hamburg, Germany) until analysis. The diluted blood will be fully thawed and frozen twice more to ensure cell lysis. For analysis, thawed samples will be centrifuged as previous and the supernatant will be analyzed using a commercially-available ELISA kit and detected as previous. Based on a previous study, it is expected that whole blood histamine will be between 600-1400 $ng \cdot mL^{-1}$ (Mucci et al., 2000). The sensitivity of this assay is 0.03 $ng \cdot mL^{-1}$ and the range is 0.098-25 $ng \cdot mL^{-1}$.

- Histamine release: Histamine release (%H) will be calculated via the equation:

$$\%H = \frac{PH}{WH} \times 100$$

  where PH is the plasma histamine concentration and WH is the whole blood histamine concentration.

- Selection exercise test: This test will take place in a standard 25-yard swimming pool (Counsilman-Billingsley Aquatics Center). Following a standardized 1000 yds (914.4 m) warm-up, an intense exercise protocol will be used to elicit release of inflammatory markers. At his or her fastest sustainable pace, each swimmer will complete 6 x 100 yards (91.4 meters), 6 x 50 yds (45.7 meters), and 6 x 25 yards (22.9 meters) of freestyle swimming with 10 seconds of rest between each of the 18 total bouts. This is a common exercise paradigm that is typically completed by collegiate and professional swimmers during their training season. Blood draws for assessment of inflammatory markers will be completed 1 hour prior to commencement of the warm-up and immediately following completion of the last intense exercise bout.

- Peripheral capillary oxyhemoglobin saturation (S_PO_2): This measurement will take place during the experimental trials in the swimming flume in the IU Human Performance Laboratory. A pulse oximetry sensor (Covidien/Medtronic, Dublin, Ireland) will be placed on the subject's forehead after application of a topical vasodilator (Finalgon; Boehringer Ingelheim, Ingelheim am Rhein, Germany). Though the probe has its own adhesive, an adhesive bandage patch (Tegaderm; 3M, Saint Paul, MN) will be placed over the top to further secure and waterproof the probe below a standard swimming cap. S_PO_2 will be monitored through a standard bedside pulse oximetry unit (Nellcor N600x; Covidien/Medtronic, Dublin, Ireland) outputting an analog signal to a customized A/D board (Hector Engineering, Ellettsville, IN) and recorded at a 1 Hz sampling frequency by a customized computer program (DasyLab 13.0; Measurement Computing Corporation, Norton, MA).

- Indirect calorimetry: During the experimental trials in the swimming flume, resting and exercise metabolic ($\dot{V}O_2$ and $\dot{V}CO_2$) and ventilatory ($\dot{V}_E$, f_B, and V_T) variables will be

measured breath-by-breath using a portable metabolic computer (K5; Cosmed, Rome, Italy) suspended above the Endless Pool and adapted for swimming exercise (Aquatrainer, Cosmed, Rome, Italy). This portable system has been determined to be valid and reliable while using breath-by-breath mode (Guidetti et al., 2018) and will be calibrated according to the manufacturer's instructions prior to each test. The swimmer will breathe through a customized two-way, nonrebreathing snorkel mouthpiece (Hans Rudolph, Shawnee, KS).

- Graded exercise test: After a self-determined warm-up and a rest period of at least 2 minutes, a progressive maximal exercise test will be performed in a specially constructed, customized variable water flow pool (Endless Pools, Aston, PA, USA), with subjects swimming against a flow rate of approximately 1.5 $m \cdot s^{-1}$ for men and 1.31 $m \cdot s^{-1}$ for women tethered to a pulley with a 1.2 and 1 kg mass, respectively. After two minutes, the tethered mass will be increased by 0.2 kg every two minutes until each subject reaches volitional exhaustion. Metabolic and ventilatory variables and S_PO_2 will be measured as described below. Subjects' heart rates during the tests will be continuously recorded (Cardio Swim HR Monitor; Freelap, Switzerland). $\dot{V}O_{2MAX}$ will be considered as the maximal value attained during the test, provided at least two of three criteria are met, which will include an RER of greater than 1.05, achievement of 90% of age-predicted maximal heart rate, and an increase in $\dot{V}O_2$ of less than 0.15 L/min over the previous workload. Plasma histamine, whole blood histamine, and histamine release will be measured before and after this test as previous.

- Constant load tests: Following the maximum exercise test, subjects will perform constant load swimming exercise at approximately 70 and 85% of subjects' previously recorded HR_{MAX} during that session. The tethered mass used to elicit the workloads will be estimated during the first 20-minute rest period by performing a linear regression between HR and tethered mass to approximate the tethered mass that would elicit 70 and 85% of maximum HR. Subjects will swim for 5 minutes. Metabolic and ventilatory variables and S_PO_2 will be measured as described below. Plasma histamine, whole blood histamine, and histamine release will be measured following these tests as previous.

- Drug administration - An opaque sugar-free gelatin capsule (Capsuline, Dania Beach, Florida, USA) will be administered 60 minutes prior to testing and will either be empty (placebo) or contain CH (10 mg; Zyrtec®; Johnson & Johnson, New Brunswick, NJ, USA) according to the visit condition.

Timeline of procedures:

- *Screening Visit (Visit 1):* Once participants arrive at the laboratory, an investigator will obtain written informed consent after all procedures and study risks are fully explained and all questions answered. Participants will then complete a pre-exercise health screening questionnaire and a health history questionnaire. The PI and/or approved study team member will administer these questionnaires/assessments. These individuals can fully explain any technical terms and answer any inquiries related to

the questionnaires. Participants will also have their resting heart rate and blood pressure, height, and weight in a private room. They will then complete a pulmonary function test. This visit will take approximately 1 h. If the participant is female, they will be asked to self-identify when their last menses (period) occurred. To prevent sex differences caused by fluctuations in hormone across the menstrual cycle, experimental visits (2-5) for female participants will only be tested during the first 7 days following menstruation when estrogen and progesterone hormones are at their lowest levels.

- *Selection Exercise Protocol (Visit 2):* Participants will arrive to the Counsilman-Billingsley Aquatics Center after abstaining from allergy medication for 48 hours, intense exercise for 24 hours, caffeine and alcohol for 12 hours, and food for 2 hours. They will complete the health history update questionnaire to ensure there have been no changes in their health history. Following a standardized 1000 yds (914.4 m) warm-up, an intense exercise protocol will be used to elicit release of inflammatory markers. At his or her fastest sustainable pace, each swimmer will complete 6 x 100 yards (91.4 meters), 6 x 50 yds (45.7 meters), and 6 x 25 yards (22.9 meters) of freestyle swimming with 10 seconds of rest between each of the 18 total bouts. A venous blood draw will be completed 30 minutes prior to commencement of the warm-up and immediately following completion of the last intense exercise bout.

- *Experimental Trials (visits 3 and 4*): Prior to all testing sessions, participants will be asked to abstain from allergy medication for 48 hours, intense exercise for 24 hours, caffeine and alcohol for 12 hours, and food for 2 hours. Prior to the sessions 3-4, participants will also be asked to consume a similar diet the night before and day of each testing session. Women participants will be asked to self-identify when their last menses occurred. In order to prevent sex differences caused by fluctuations in hormone across the menstrual cycle, women participants will only be tested during the first 7 days following menstruation (when estrogen and progesterone hormones are at their lowest levels) for sessions 2-4 (Allen et al., 2016). Apart from receiving a placebo (PL) or drug treatment (CH) prior to exercise, participants will perform identical protocols on each visit to the laboratory. Participants will report to the lab and consume either a placebo or CH pill, followed by a health history update questionnaire and their resting pulmonary function will be measured. Participants will then complete a self-selected warm-up that will be standardized across the two testing sessions, followed by instrumentation. The exercise protocol beings with a progressive swimming test to maximum aerobic capacity ($\dot{V}O_{2max}$) in a swimming flume, followed by two constant load work bouts at approximately 70 and 85% of the previously recorded HR_{max} while peripheral capillary oxyhemoglobin saturation (S_pO_2) is continuously monitored. Participants will receive a 20-min break between each work bout. Drug treatments will be assigned in a double-blind, randomized crossover fashion such that each participant receives each treatment. Concentrations of plasma histamine, whole blood histamine, and histamine release will be assessed from a venous blood draw one hour prior to commencement of the aerobic capacity test, and immediately following the aerobic capacity test and each of the two constant load bouts.

8.0 Study Calendar

The following procedures will be undertaken during the visits.

Table 1: Description of Study Procedures that will take place on each visit to the laboratory.

STUDY PROCEDURES	Screening (Visit 1)	Selection Exercise Test (Visit 2)	Experimental Trials – Randomized Crossover (Visits 3-4)	
			Placebo	Cetirizine HCl
Height	X		X	X
Weight	X		X	X
Heart rate	X		X	X
Urine specific gravity		X	X	X
Blood pressure	X			
Health history questionnaire	X			
Health history update questionnaire		X	X	X
Pulmonary function	X		X	X
Complete blood count		X		
Plasma IL-1β		X		
Plasma IL-8		X		
Plasma histamine		X	X	X
Whole blood histamine		X	X	X
Histamine release		X	X	X
Selection exercise test		X		
Peripheral capillary oxyhemoglobin saturation			X	X
Indirect calorimetry			X	X
Graded exercise test			X	X
Constant load tests			X	X
Placebo pill			X	
Cetirizine HCl pill				X

9.0 Reportable Events

Safety will be constantly monitored during all data collection activities. This will occur on a participant-by-participant basis. If a suspected adverse event occurs, the principal investigator will immediately report to the IRB and cooperate with the IRB in any necessary investigation. All safety data will also be reviewed in weekly lab meetings, particularly as it relates to changes to the risk-benefit ratio.

If any of the following adverse events, which are specific to the procedures conducted in this study, were to occur, they will be reported within 5 business days to the IRB using standard operating procedures.

- Infection at venous blood draw site
- Loss of consciousness or fainting
- Adverse drug reaction
- Loss of confidentiality
- Injury requiring hospitalization
- Death

Other events deemed as events occurring outside the criteria above will be reported to the IU IRB reported within 5 business days using standard operating procedures. If any event occurs that would potentially impair the health of the subject, the experiment will be halted, and the IRB will be notified.

Any incident, experience, or outcome that meets all the following criteria will also be reported:

(1) unexpected (in terms of nature, severity, or frequency) given
   a. the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and
   b. the characteristics of the subject population being studied;

(2) related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

(3) suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Subjects will be informed of possible risks before consenting to participate through the informed consent document. Details of risks and discomforts for the procedures, and steps taken to lessen the probability and/or magnitude of these risks are noted below.

- Bacterial/Viral Transmission: There is a risk of germ transmission through shared face masks, despite cleaning and treatment in an anti-bacterial solution following each use. Researchers will thoroughly scrub all shared equipment with anti-bacterial detergent and warm water immediately following use.

- Exercise: Submaximal (low and moderate effort) and maximal (all-out effort) exercise tests of healthy individuals, as described by the American College of Sports Medicine, present little risk and do not require medical clearance for individuals under the age of 40. Potential risks and/or discomforts can include episodes of temporary light-headedness, chest discomfort, leg cramps, occasional irregular heartbeats, and abnormal blood pressure responses. The risk of heart attack, although minor, (approximately 1 to 2 in 10,000) does exist. One death occurs for roughly every 880,000 man hours of submaximal exercise in apparently healthy individuals.

- Drowning: There is a risk of drowning during swimming exercise. All participants have undergone many years of swimming training. During the Selection Exercise Protocol, lifeguards from Campus Recreational Sports will be present. During the Experimental Trials, swimmers will be closely monitored by researchers, at least one of whom has completed safety training in accordance with USA Swimming coaching standard.

- Drug administration:

    - Cetirizine HCl: Serious side effects of cetirizine HCl ingestion are exceedingly rare, and in very rare cases subjects can experience anaphylaxis. Cetirizine HCl can have adverse reactions with midodrine (low blood pressure medication), ritonavir (an HIV treatment), and could exacerbate symptoms of any medication that can cause drowsiness, dry mouth, or infrequent urination. Mild side effects of cetirizine HCl include drowsiness, headaches, dry mouth, nausea, dizziness, stomach pain, diarrhea, sore throat, cold-like symptoms of the nose, itching, paresthesia in the hands or feet, and feeling agitated. We have previously used cetirizine HCl in the following projects in our lab:

    - IRB #1706871260: The Individual Exercise Performance Response to Altitude: Implication for the Warfighter (PI: Chapman; Publication in progress)

  In order to help further mitigate the risk of drug administration, a board-certified physician, Dr. Andrew K. Watters, MD, will acquire the pharmaceuticals for the project and will instruct researchers on best practice for drug administration. He will not oversee any other aspects of the study.

- Venous blood sample: Participants will likely experience discomfort during venous blood draws and in rare circumstances may feel lightheaded or faint. This risk will be minimized by the venipuncture always occurring in the semi-recumbent or supine position. There is a rare risk of infection following venous blood draws. This risk will be minimized by blood draws only being conducted by trained laboratory personnel.

- Loss of confidentiality: There is a potential risk for loss of confidentiality. This risk will be alleviated by only using de-identified information.

- Electricity and Water: There is a risk of operating electrical equipment in proximity to water. All equipment that is connected to an electrical outlet will never be suspended above the pool and will be secured with safety straps in a fashion that will not allow their entry into the pool. The Cosmed K5 is battery-powered and will be the only device suspended above the water (during Experimental Trials only). The device will be connected by multiple metal components to a steel guide wire connected to a steel frame over the pool (see Figure 3).

- Other risks: There may be other risks that we cannot predict.

10.0 Data Safety Monitoring

The primary investigator is responsible for the data and safety monitoring. In addition, Dr. Zac Schlader, a faculty member within the Department of Kinesiology, will serve as an independent data safety monitor for the study. Data quality, subject recruitment, accrual, retention, outcome and adverse event data will be monitored on a weekly basis.

Data will be monitored by subject number. Thus, analysis of data will be performed without any identifying features to individuals. Statistical software will be used to analyze data for significance. Results will be reported through traditional scientific outlets such as peer-reviewed manuscript and presentations at national and international meetings. Communication with the IRB regarding safety will be reported directly by the principal investigator.

11.0 Study Withdrawal/Discontinuation

Subjects can voluntarily withdraw from the study at any time by contacting any of the researchers on the study via any available means (personal contact, email, phone, etc.). If a researcher on the project wishes to withdraw a subject from the study, they will contact the subject directly (personal contact, phone, email, etc.). Possible indications for withdrawal by a researcher include a change in their health history that excludes them from participation and an inability to keep appointments or follow or understand the rules of the protocol. Data collected up to the time of withdraw (either voluntary or by the study team) may be used for analysis.

12.0 Statistical Considerations

Sample size justification was conducted using data from a study with a similar design (Coyle and Stager, 2001) which compared the effect of diphenhydramine HCl (a competitive inhibitor to histamine similar to CH) to a placebo on S_aO_{2ear} in male cross-country runners running at 70, 80, 90, and 100% of $\dot{V}O_{2max}$. A depiction of the study's data can be found in Table 2 below.

Table 2: S_aO_{2ear} data from Coyle, 2001

	Intensity (% $\dot{V}O_{2max}$)			
Treatment	**70**	**80**	**90**	**100**
Placebo	93.7 ± 0.7%	93.4 ± 2.5%	93.0 ± 2.5%	89.8 ± 3.1%
Diphenhydramine HCl	96.5 ± 1.2%	96.2 ± 1.0%	95.8 ± 1.2%	94.2 ± 1.8%

A two-way (treatment x intensity) ANOVA using these data produced a Cohen's *f* of 1.22 for the treatment main effect. Therefore, it was determined that using an a priori α value of 0.05, an estimated twelve subjects are necessary to achieve a power of 0.80. The sample size calculation was conducted using G*Power 3.1.9.7 (ANOVA: Fixed effects, special, main effects and interactions). Due to the randomized crossover design, all twelve subjects (n = 6 men, n = 6 women) will complete all experimental treatments (PL and CH) at all intensities (70, 85, and 100% of $\dot{V}O_{2max}$).

Previous investigations (Anselme et al., 1994; Mucci et al., 2001) have observed a strong, positive, and significant ($r > 0.7$; $p < 0.05$) correlation between Δ%H ($\dot{V}O_{2max}$ - rest) and ΔP_aO_2 (rest - $\dot{V}O_{2max}$). Using a regression equation (Δ%H = 0.0444(ΔP_aO_2) - 0.1137; r = 0.80) from Anselme and colleagues (1994), a conservative estimate that a ΔP_aO_2 > 10 mmHg represents EIAH (Prefaut et al., 2000), we intend to recruit participants for the experimental trials who experience a Δ%H > 0.33 during the Selection Exercise Protocol. However, should this inclusion criterion not be met by at least eight subjects, those experiencing the highest Δ%H during the Selection Exercise Protocol will be recruited instead.

For proof of principle that EIAH can be prospectively identified using Δ%H, a small control group (n=2 men, n=2 women) will also be recruited. For this group, we intend to recruit participants who exhibit the lowest Δ%H during the Selection Exercise Protocol. These participants will complete both conditions of the experimental trials.

Insufficient data exist to establish a prevalence rate of EIAH among endurance athletes. Attempts have been made to establish prevalence rates in cyclists (Powers et al., 1988) and runners (Constantini et al., 2017), however no such data exist for swimmers. Estimates of prevalence rates range from approximately 70% (Constantini et al., 2017) in highly-trained endurance runners to 32% (Coneys et al., 2011) in untrained individuals. As prevalence rates of EIAH have been demonstrated to increase with aerobic training status (Dominelli and Sheel, 2019), it is likely that the true prevalence of EIAH in highly-trained swimmers is between these values. Therefore, to ensure enrollment of an adequate number of swimmers who experience EIAH, a prevalence estimate of 50% will be utilized. Using a binomial distribution and a target of 8 EIAH-afflicted highly-trained swimmers, recruiting 26 swimmers (n = 13 men, n = 13 women) for the Selection Exercise Protocol provides a >95% probability that the population will contain at least 8 EIAH-afflicted highly-trained swimmers. In order to account for potential participant withdrawal, we anticipate recruiting no more than 30 individuals.

Prior to conducting statistical analysis all data will be visually inspected for normality and potential outliers using kernel density and quantile-quantile plots. Summary statistics of the mean, standard deviation, median, and range will be calculated for all variables of interest.

Hypothesis 1. EIAH has been previously defined by a decrement in S_pO_2 of ≥ 5%. Should a participant experience this during submaximal or maximal exercise, the hypothesis will be confirmed.

Hypothesis 2. Prior to analyses, any carry-over effect will be identified using a two-way linear mixed model (visit order x time), independent of the drug treatment (placebo, or cetirizine HCl). If a carry-over effect is observed, a regressor for visit order group will be included in all subsequent analyses.
In order to test for potential differences in the dependent variables of interest, linear mixed models will be used including random effects for subject, subject within-treatment, and subject within-intensity to account for the repeated measures design. Treatment (PL and CH), intensity (70, 85, and 100% of $\dot{V}O_{2max}$) and the interaction between treatment and intensity will be included. Main effects and interactions will be tested using *F*-tests from a two-way ANOVA. Statistical significance will be assessed using a pre-determined α level of 0.05. Where significant main effects or interactions are observed, estimated marginal means will be used to test for differences in the drug conditions and adjusted for multiple comparisons using Dunnett's method.

Hypothesis 3. The correlation between the change in histamine release ($\Delta\%H_{pre}$-$\%H_{post}$) and the change in S_PO_2 at $\dot{V}O_{2max}$ (ΔS_PO_{2rest}-$S_PO_{2@100\%}$) will be assessed in the pooled experimental and control cohorts (n=12) by a Pearson correlation coefficient using a pre-determined α level of 0.05.

13.0 Statistical Data Management

Primary data will be collected via direct data capture from measurement instruments and stored electronically on an electronic spreadsheet on a password protected computer. The storage location will be backed up manually every week. Quality assurance steps will be confirmed by plotting the data prior to formal analyses. Outliers will be identified and checked for authenticity in the database and other original data documents. Extraction and cleaning of data that will be used for analysis will be carried out after each block of subjects used in the randomization of visits 3, 4, and 5 have completed all laboratory visits.

14.0 Privacy/Confidentiality Issues

Informed consent will be obtained, and the testing procedures will be completed in a closed, private laboratory setting at the School of Public Health (SPH), with only the participant and investigators present.

Aside from the Screening Exercise Protocol, all testing will take place in a closed, private laboratory setting in the School of Public Health. All participants will be assigned an identification number, which will then be used when recording and analyzing data. A code list containing the participants names and identification numbers will be stored separately in a locked filing cabinet and will only be available to the investigators. The reports generated as a result of this investigation will not identify individual subjects. Data will be collected and stored on password protected computers located in the Human Performance Laboratories, which are always kept locked. Any paper data will be stored in a locked filing cabinet inside a locked office. Primary data are backed up by manual download to an encrypted flash drive once per week, which is then stored in a separate locked office in a locked filing cabinet accessible only to the investigators. Access to all data from this study will be strictly limited to the investigators which will be listed on the personnel section of the IRB. This includes the PI, graduate student investigators, and laboratory technicians who have all completed the appropriate training to handle this type of data.

While testing is in progress, a sign will be outside the laboratory door to prevent people not associated with the study from entering.

15.0 Follow-up and Record Retention

We anticipate that all participants will be enrolled within 36 months. Any identifiable data (e.g., signed consent forms) will be maintained for a minimum of 3 years. De-identified data will be stored indefinitely. Only investigators will have full access to data. All electronic data will be kept on password protected computers, while paper data will remain in locked file cabinets.

16.0 References

Adir, Y., Shupak, A., Gil, A., Peled, N., Keynan, Y., Domachevsky, L., and Weiler-Ravell, D. (2004). Swimming-induced pulmonary edema: clinical presentation and serial lung function. Chest *126*, 394-399.

Allen, A.M., McRae-Clark, A.L., Carlson, S., Saladin, M.E., Gray, K.M., Wetherington, C.L., McKee, S.A., and Allen, S.S. (2016). Determining menstrual phase in human behavioral research: a review with recommendations. Exp Clin Psychopharmacol *24*, 1-11.

Anselme, F., Caillaud, C., Couret, I., Rossi, M., and Prefaut, C. (1994). Histamine and exercise-induced hypoxemia in highly trained athletes. J Appl Physiol *76*, 127-132.

Barreiro, T.J., and Perillo, I. (2004). An approach to interpreting spirometry. American family physician *69*, 1107-1116.

Birks, E.K., Mathieu-Costello, O., Fu, Z., Tyler, W.S., and West, J.B. (1994). Comparative aspects of the strength of pulmonary capillaries in rabbit, dog, and horse. Respir Physiol *97*, 235-246.

Borriello, F., Iannone, R., and Marone, G. (2017). Histamine release from mast cells and basophils (Cham, Switzerland: Springer International Publishing).

Brigham, K.L., and Owen, P.J. (1975). Increased sheep lung vascular permeability caused by histamine. Circ Res *37*, 647-657.

Camus, G., Deby-Dupont, G., Deby, C., Ferir-Juchmes, A., Pincemail, J., and Lamy, M. (1993). Inflammatory response to strenuous muscular exercise in man. Mediators Inflamm *2*, 335-342.

Chapman, R.F., and Stager, J.M. (2008). Caffeine stimulates ventilation in athletes with exercise-induced hypoxemia. Med Sci Sports Exerc *40*, 1080-1086.

Coneys, G., Lu, P., Labossiere, C., Arneja, A., and Sharma, S. (2011). Epidemiology of exercise-induced hypoxemia in athletes and the role of expiratory flow limitation. Am J Respir Crit Care Med *183*, A5195.

Constantini, K., Tanner, D.A., Gavin, T.P., Harms, C.A., Stager, J.M., and Chapman, R.F. (2017). Prevalence of exercise-induced arterial hypoxemia in distance runners at sea level. Med Sci Sports Exerc *49*, 948-954.

Coyle, M.A., and Stager, J.M. (2001). Exercise-induced hypoxemia is ameliorated by nedocromil sodium and diphenhydramine HCl (Abstract). Med Sci Sports Exerc *33*, S58.

Dempsey, J.A., Hanson, P.G., and Henderson, K.S. (1984). Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. J Physiol *355*, 161-175.

Dempsey, J.A., and Wagner, P.D. (1999). Exercise-induced arterial hypoxemia. J Appl Physiol *87*, 1997-2006.

Derchak, P.A., Stager, J.M., Tanner, D.A., and Chapman, R.F. (2000). Expiratory flow limitation confounds ventilatory response during exercise in athletes. Med Sci Sports Exerc *32*, 1873-1879.

Dill, D.B., and Costill, D.L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydtration. J Appl Physiol *37*, 247-248.

Dominelli, P.B., and Sheel, A.W. (2019). Exercise-induced arterial hypoxemia; some answers, more questions. Appl Physiol Nutr Metab *44*, 571-579.

Fu, Z., Costello, M.L., Tsukimoto, K., Prediletto, R., Elliott, A.R., Mathieu-Costello, O., and West, J.B. (1992). High lung volume increases stress failure in pulmonary capillaries. J Appl Physiol *73*, 123-133.

Gavin, T.P., and Stager, J.M. (1999). The effect of exercise modality on exercise-induced hypoxemia. Respir Physiol *115*, 317-323.

Groves, B.M., Reeves, J.T., Sutton, J.R., Wagner, P.D., Cymerman, A., Malconian, M.K., Rock, P.B., Young, P.M., and Houston, C.S. (1987). Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. J Appl Physiol *63*, 521-530.

Guidetti, L., Meucci, M., Bolletta, F., Emerenziani, G.P., Gallotta, M.C., and Baldari, C. (2018). Validity, reliability and minimum detectable change of COSMED K5 portable gas exchange system in breath-by-breath mode. PLoS One *13*, e0209925.

Harms, C.A., and Stager, J.M. (1995). Low chemoresponsiveness and inadequate hyperventilation contribute to exercise-induced hypoxemia. J Appl Physiol *79*, 575-580.

Hodges, A.N.H., Mayo, J.R., and McKenzie, D.C. (2006). Pulmonary oedema following exercise in humans. Sports Med *36*, 501-512.

Hohmann, E., Glatt, V., and Tetsworth, K. (2018). Swimming induced pulmonary oedema in athletes - a systematic review and best evidence synthesis. BMC Sports Sci Med Rehabil *10*, 1-10.

Holmer, I., Stein, E.M., Saltin, B., Ekblom, B., and Astrand, P.-O. (1974). Hemodynamic and respiratory responses compared in swimming and running. J Appl Physiol *37*, 49-54.

Horio, S., Fujimoto, K., Mizuguchi, H., and Fukui, H. (2010). Interleukin-4 up-regulates histamine H1 receptors by activation of H1 receptor gene transcription. Naunyn-Schmiedeberg's Arch Pharmacol *381*, 305-313.

Labreche, J.M. (2012). The pulmonary profile of competitive swimmers. In Kinesiology (Vancouver: The University of British Columbia), pp. 85.

Leahy, M.G., Summers, M.N., Peters, C.M., Molgat-Seon, Y., Geary, C.M., and Sheel, A.W. (2019). The mechanics of breathing during swimming. Med Sci Sports Exerc *51*, 1467-1476.

Li, T. (2016). Ventilatory strategies of competitive swimmers performing maximal and submaximal swimming and cycling exercise (Beijing, China: Beijing Sport University).

Mucci, P., Durand, F., Lebel, B., Bousquet, J., and Prefaut, C. (2000). Interleukins 1-beta, -8, and histamine increases in highly trained, exercising athletes. Med Sci Sports Exerc *32*, 1094-1100.

Mucci, P., Durand, F., Lebel, B., Bousquet, J., and Prefaut, C. (2001). Basophils and exercise-induced hypoxemia in extreme athletes. J Appl Physiol *90*, 989-996.

Panula, P., Chazot, P.L., Cowart, M., Gutzmer, R., Leurs, R., Liu, W.L.S., Stark, H., Thurmond, R.L., and Haas, H.L. (2015). International Union of Basic and Clinical Pharmacology. XCVIII. Histamine receptors. Pharmacol Rev *67*, 601-655.

Pendergast, D.R., Moon, R.E., Krasney, J.J., Held, H.E., and Zamparo, P. (2015). Human physiology in an aquatic environment. Compr Physiol *5*, 1705-1750.

Powers, S.K., Dodd, S., Lawler, J., Landry, G., Kirtley, M., McKnight, T., and Grinton, S. (1988). Incidence of exercise induced hypoxemia in elite endurance athletes at sea level. Eur J Appl Physiol *58*, 298-302.

Prefaut, C., Anselme-Poujol, F., and Caillaud, C. (1997). Inhibition of histamine release by nedocromil sodium reduces exercise-induced hypoxemia in master athletes. Med Sci Sports Exerc *29*, 10-16.

Prefaut, C., Durand, F., Mucci, P., and Caillaud, C. (2000). Exercise-induced arterial hypoxaemia in athletes. Sports Medicine *30*, 47-61.

Probst, K., Millen, J.E., and Glauser, F.L. (1978). The effects of endogenous and exogenous histamine on pulmonary alveolar membrane permeability. Am J Respir Crit Care Med *117*, 1063-1068.

Reeves, J.T., Groves, B.M., Cymerman, A., Sutton, J.R., Wagner, P.D., Turkevich, D., and Houston, C.S. (1990). Operation Everest II: cardiac filling pressures during cycle exercise at sea level. Respir Physiol *80*, 147-154.

Rice, A.J., Thornton, A.T., Gore, C.J., Scroop, G.C., Greville, H.W., Wagner, H., D., W.P., and Hopkins, S.R. (1999). Pulmonary gas exchange during exercise in highly trained cyclists with arterial hypoxemia. J Appl Physiol *87*, 1802-1812.

Spanoudaki, S.S., Maridaki, M.D., Myrianthefs, P.M., and Baltopoulos, P.J. (2004). Exercise induced arterial hypoxemia in swimmers. J Sports Med Phys Fitness *44*, 3442-3348.

Suzuki, K., Nakaji, S., Yamada, M., Totsuka, M., Sato, K., and Sugawara, K. (2002). Systemic inflammatory response to exhaustive exercise: cytokine kinetics. Exerc Immunol Rev *8*, 6-48.

Tanner, D.A., Duke, J.W., and Stager, J.M. (2013). Ventilatory patterns differ between maximal running and cycling. Respir Physiol Neurobi *191*, 9-16.

Tsukimoto, K., Mathieu-Costello, O., Prediletto, R., Elliott, A.R., and West, J.B. (1991). Ultrastructural appearances of pulmonary capillaries at high transmural pressures. J Appl Physiol *71*, 573-582.

Wagner, P.D., Gale, G.E., Moon, R.E., Torre-Bueno, J.R., Stolp, B.W., and Saltzman, H.A. (1986). Pulmonary gas exchange in humans exercising at sea level and simulated altitude. J Appl Physiol *61*, 260-270.

West, J.B., and Luks, A.M. (2016). West's respiratory physiology: the essentials, Tenth edn (New York: Wolters Kluwer).

West, J.B., and Mathieu-Costello, O. (1995). Stress failure of pulmonary capillaries as a limiting factor for maximal exercise. Eur J Appl Physiol *70*, 99-108.

West, J.B., and Mathieu-Costello, O. (1999). Structure, strength, failure, and remodeling of the pulmonary blood-gas barrier. Annu Rev Physiol *61*, 543-572.

West, J.B., Tsukimoto, K., Mathieu-Costello, O., and Prediletto, R. (1991). Stress failure in pulmonary capillaries. J Appl Physiol *70*, 1731-1742.

17.0 Appendix

17.1 Payment schedule

Participants will be compensated with $100 for completing the Selection Exercise Protocol (n=26). Participants selected for the experimental trials (n=12) will receive $100 for each trial (Visits 3 and 4) completed, for a total of $300. Compensation will be paid by VISA gift card.

17.2 Attachments

The following Questionnaires have been included as attachments:

- Heath History Questionnaire
- Health History Update Questionnaire