Theophylline and Ambrisentan combination: Effects on human performance, AMS and physiological responses to altitude

Hilary Linda Palakovich

The University of Montana

Follow this and additional works at: http://scholarworks.umt.edu/etd

Recommended Citation

THEOPHYLLINE AND AMBRISENTAN COMBINATION: EFFECTS ON HUMAN PERFORMANCE, AMS, AND PHYSIOLOGICAL RESPONSES TO ALTITUDE

By

HILARY LINDA PALAKOVICH

B.S. Health and Human Performance, University of Montana, Missoula, MT, 2011

Thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Health and Human Performance, Exercise Science

The University of Montana
Missoula, MT

May, 2014

Approved by:

Sandy Ross, Dean of The Graduate School
Graduate School

Dr. Brent Ruby, Chair
Health and Human Performance

Dr. Charles Dumke
Health and Human Performance

Dr. Holly Thompson
Chemistry and Biochemistry
Theophylline and Ambrisentan combination: Effects on human performance, AMS and physiological responses to altitude

Chairperson: Dr. Brent Ruby, Ph.D., FACSM

Recent research efforts have attempted to determine the effectiveness of the combination of a pharmacological cardiac stimulant (theophylline) and pulmonary vasodilator (ambrisentan) in attenuating performance losses and mountain sickness at altitude. This combination has been observed to improve hypoxic exercise performance in rats, but never tested in human subjects. In the current study, 30 male participants were tested in a paired fashion to assess whether this drug combination would improve exercise performance and/or decrease symptoms of AMS. The experimental group (EXP, n = 15) received a treatment of 400 mg theophylline and 5 mg ambrisentan, while the placebo group (PLA, n = 15) received a placebo combination. Data collection was performed in the field at an altitude of 4,267 m. Participants completed two 2-mile time trials (TT) on subsequent days, between which they spent the night at 3,048 m. Treatment with theophylline and ambrisentan was not associated with improved 2-mile TT performances or AMS symptoms. The EXP group had significantly lower measures of diastolic blood pressure and mean arterial pressure compared to PLA while at altitude (69.2 ± 7.9 vs 77.7 ± 8.0 mmHg and 90.8 ± 8.9 vs 97.2 ± 7.9 mmHg, respectively), main effect for group, p<0.05. Significantly, this study was the first to assess this drug combination in human subjects at altitude in a field setting. Additionally, the observed reduction in vascular pressures as a result of treatment may have positive implications for the health state of sojourners to altitude.

Key Words: pharmaceutical, hypoxia, vascular pressure, endothelin, stimulant
Acknowledgments

First, I would like to thank my committee chair and mentor, Dr. Brent Ruby. Your work is inspirational and I feel honored to be receiving my Master’s degree under your guidance. Thank you for allowing this research study as my thesis. It has been an amazing experience. I have gained innumerable skills throughout my laboratory and field work with WPEM and will be forever grateful for the experience.

This thesis would never have been possible without the incredible support of John Cuddy and Walt Hailes. Thank you for patiently and selflessly supporting all of us who work with you in the lab and the field. Your work, including your consistent organization, positive attitudes, and proactive natures, oftentimes goes unnoticed and underappreciated. I can’t even begin to explain your significance to our department. Most of us cannot appreciate the thought, time, and energy you put into the studies you successfully complete. Thank you for going above and beyond to make all of this possible.

Thank you to the research assistants who were involved in this project, Kyle Cochrane and Michael Cramer. This was a very intensive and consuming project, but data collection could not have gone much smoother. The two of you played enormous roles and I am grateful to have worked with you. Good luck to you both in the completion of your Master’s degrees.

This research project was funded with a grant received from the Defense Advanced Research Projects Agency (DARPA). The researchers would like to thank DARPA for their continued support of our research.
# Table of Contents

**Chapter One: Introduction**

- Introduction ........................................ 1
- Problem .............................................. 3
- Purpose ............................................. 3
- Hypothesis ........................................ 3
- Significance of Study ................................ 4
- Limitations ........................................... 4
- Delimitations ........................................ 4
- Definition of Terms ................................ 4

**Chapter Two: Review of Literature** ........................................ 6

**Chapter Three: Methodology** ........................................ 11

- Subjects and Setting ................................ 11
- Preliminary Testing ................................ 11
- Experimental Trials ................................ 13
- Statistical Analysis ................................ 14

**References** ........................................ 16

**Chapter Four: Manuscript for High Altitude Medicine and Biology** ........................................ 19

**Appendix: IRB-Approved Subject Information and Consent Form** ........................................ 41
Chapter 1: Introduction

Introduction

Many individuals are required to exercise or carry out work tasks at altitude, which makes them prone to decreased submaximal aerobic performance, decreased maximal oxygen consumption, (VO_{2max}), [13, 20, 28, 39] and risk for acute mountain sickness (AMS) [28, 39, 55, 60]. Impairment of performance begins at an altitude of 1500 meters [51]. AMS, the most common form of altitude illness, occurs in situations of non-acclimatized exposure to altitudes of 2,500 meters or greater [18, 31, 46]. AMS is diagnosed when headache plus at least one of the following symptoms is present: gastrointestinal distress, fatigue or weakness, dizziness or lightheadedness, and difficulty sleeping [32]. High altitude pulmonary edema (HAPE), another altitude related illness, occurs at more drastic altitudes, rarely below 4000 meters [18, 41] and is the result of hypoxia-driven pulmonary vasoconstriction, pulmonary hypertension and capillary stress [46]. In addition to numerous athletic and recreational events, occupations such as mountain rescue and military personal are expected to perform at high altitudes, increasing the demand for treatment strategies to combat the performance decrements associated with altitude [20, 51].

Athletes wishing to improve performance and decrease incidence of AMS have employed several strategies over the years. Classic high altitude training involves training and living at altitudes of 2000-2800 meters for 2-4 weeks. [9] This method has transitioned to minimizing the time spent at altitude, substituting houses and/or tents to simulate altitude exposure with hypoxia [9, 62]. Several pharmaceutical treatments have also been utilized, including supplemental oxygen, and medications such as dexamethasone, acetazolamide and tadalafil. These treatments have been shown to improve exercise performance and decrease risk for AMS, [25, 38, 53] but come with high expense, possible side effects, and ethical issues [58]. Other classes of medications, such as those used to treat hypertension and pulmonary obstructive disorders, and cardiac stimulants are also of interest to researchers for the role they may play in the prevention of AMS and improvement of performance at altitude [2, 23, 24, 35, 49].
Ambrisentan is a selective endothelin type A (ET\textsubscript{A}) receptor antagonist commonly used to treat pulmonary arterial hypertension [19]. Endothelins are class of proteins responsible for various vascular functions [1] of which endothelin-1 is the most abundant type. Endothelin-1 is secreted by vascular endothelial cells and is a potent vasoconstrictor [1]. Endothelin-1 generation is increased by many factors, including hypoxia and free radical production [1, 40]. Endothelin-1 acts through two receptors, ET\textsubscript{A} and ET\textsubscript{B} [64, 65]. ET\textsubscript{A} is more abundant in the lungs and mediates vasoconstriction [3, 16, 64], while ET\textsubscript{B} mediates vasodilation and clearance of endothelin [26]. Thus, specific ET\textsubscript{A} antagonists are useful in the treatment of pulmonary hypertension and situations of excessive endothelin production [19]. Administration of ambrisentan is associated with relaxation and vasodilation of vascular smooth muscle tissue, evidenced by reduced pulmonary vascular resistance and mean arterial pressure [29]. Recently it has been suggested that hypoxia triggers the production of reactive oxygen species (ROS), responsible for cell dysfunction and vascular permeability and astrocyte swelling [4-8], which can result in AMS in hypoxic conditions [4, 40]. In situations of oxidative stress, the body produces nuclear related factor 2 (Nrf2) which regulates expression of more than 90% of antioxidant genes, thus acting as a defense mechanism against cellular damage from ROS [14, 40]. In a study performed in rats, ambrisentan significantly increased Nrf2 activation and reduced high-altitude-induced cerebral vascular leak by nearly 40%, suggesting that ambrisentan has the ability to protect against the damage caused by hypoxia-induced ROS [31].

Theophylline is a non-specific phosphodiesterase inhibitor, with therapeutic effects on the lungs, cardiac system and vasculature [21]. Theophylline also acts as an adenosine receptor antagonist, [49] stimulating heart rate. Originally reported effective as a bronchodilator, it has since been used as a treatment of COPD and asthma [43, 52] and has been shown to significantly improve FEV\textsubscript{1} and FVC in COPD patients [43]. Several pharmaceutical effects of theophylline would be beneficial in the prevention of altitude-related illness, including decreased vascular permeability in the brain and lungs, [47] bronchodilation [48], central respiratory stimulation [35, 36], decreased pulmonary arterial pressure [37],
and induction of mild diuresis [24]. Kupper et al. found ingestion of theophylline resulted in significantly decreased AMS severity at moderate altitude (3,440 meters) during ascent and during a five day stay at high altitude (4,559 meters). Theophylline also resulted in a significantly decreased respiratory disturbance index (respiratory events per hour of sleep, RDI) at high altitude [35].

Combining a cardiac stimulant, such as theophylline with a vasodilating agent, such as ambrisentan would seemingly be beneficial in the prevention of AMS and improvement of performance at altitude, but little research has been conducted on this combination [49]. Radiloff et al. assessed the efficacy of theophylline and ambrisentan in concert to improve exercise capacity in rats under simulated high altitude (hypobaric chamber at an altitude equivalent of 4,267 meters). The combination significantly improved run-to-fatigue time in female rats over the control trial as well as the theophylline and ambrisentan treatments alone [49]. The results of this study encourage the further investigation of this drug combination in human subjects.

Problem

Individuals who are acutely exposed to high altitude are prone to acute mountain sickness and decreased physical performance resulting from decreased partial pressure of oxygen and adverse physiological responses to hypoxia.

Purpose

The purpose of this study is to determine if an oral dose of ambrisentan and theophylline taken in concert can alleviate symptoms of acute mountain sickness and improve physical performance at altitude.

Hypotheses

1. Ingestion of theophylline and ambrisentan will decrease symptoms of AMS at high altitude.

2. Ingestion of theophylline and ambrisentan will decrease completion time of a two mile run trial at high altitude.
3. Ingestion of theophylline and ambrisentan will not affect blood oxygen saturation, though altitude will decrease blood oxygenation saturation.

4. Ingestion of theophylline and ambrisentan will decrease mean arterial pressure.

5. Ingestion of theophylline and ambrisentan will increase forced vital capacity and forced expiratory volume in one second.

6. Ingestion of theophylline and ambrisentan will increase heart rate.

**Significance and Rationale**

The findings of this study have implications on individuals who compete, recreate, or work at high altitude. If this medication combination is found to significantly improve performance, its use among athletes and in the occupational setting could be encouraged as a safe alternative to other performance enhancing treatments used at altitude.

**Limitations**

1. To control for extraneous variables, participants will be medicated, fed, and tested at the same time of day for both altitude trials.

2. To minimize the effect of a learning curve on the results of the study, participants will undergo a familiarization session on the treadmills to be used in the data collection prior to testing.

3. Human error can occur with the use of any instrumentation. To limit the occurrence of error, all researchers will be trained and equipment will be carefully calibrated.

4. Participants will not be randomly sampled, but sampled by convenience. Random sampling of treatments will be utilized.

**Delimitations**

All participants in this study will be recreationally active. They must have no history of serious acute mountain sickness (HAPE and HACE).

**Definition of Terms**

*Acute Mountain Sickness:* illness affecting individuals at altitudes usually above 2,500 meters, results in symptoms such as headache, disturbed sleep, dizziness, appetite loss, nausea, increased heart rate [24]
*Theophylline*: a phosphodiesterase inhibitor, results especially in bronchodilation and increased heart rate, medically used to treat COPD and asthma [43]

*Ambrisentan*: an endothelin receptor antagonist, prevents pulmonary vasoconstriction, medically used to treat pulmonary arterial hypertension [15]

*VO₂*: rate at which an individual’s metabolism utilizes oxygen, measured in L/min or ml/kg/min

*Recreationally active individuals*: individuals who exercise on a regular basis but who may work indoor during the week and/or exercise outdoors on a varying basis throughout the week
Chapter 2: Review of Literature

There are several inherent decrements to performance at altitude, among them decreased arterial oxyhemoglobin saturation, ($%\text{SaO}_2$) [13, 22] and muscle oxygenation, [42, 55] ultimately resulting in decreased submaximal aerobic performance, decreased maximal oxygen consumption, ($\text{VO}_{2\text{max}}$), [13, 20, 28, 39] and risk for acute mountain sickness (AMS) [28, 39, 55, 60]. According to a review by Bartsch and Saltin [9], possible impairment of performance begins at an altitude of 1500 meters, particularly in highly trained athletes, while others may observe decreases in $\text{VO}_{2\text{max}}$ at altitudes as low as 600 meters [51]. With increasing altitude, performance further deteriorates and at very high altitudes, (between 3000 and 5000 meters) athletic performance is decreased not only acutely but even after acclimatization [9, 61]. Numerous athletic and recreational events are held at varied altitudes, including skiing, mountaineering, cycling, and skating, as well as occupational settings such as military operations and mountain rescue. Thus, the demand for treatment strategies to combat the performance decrements involved with the hypoxic conditions of altitude is always present [20, 51].

AMS is a result of non-acclimatized exposure to altitude. The most effective form of prevention is slow ascent, [18, 31, 46] which is not practical in many settings. AMS is the most common form of altitude illness, which encompasses high altitude cerebral edema (HACE) and high altitude pulmonary edema (HAPE). AMS affects 25% of travelers at moderate altitudes and 50-85% of travelers at altitudes >4000 meters [18, 32, 33]. The Lake Louise survey has been developed as a screening tool for diagnosing AMS. According to the survey, in order to diagnose AMS, headache plus at least one of the following symptoms must be present: gastrointestinal distress, fatigue or weakness, dizziness or lightheadedness, and difficulty sleeping [32]. HAPE occurs at more drastic altitudes, rarely below 4000 meters and occurs in .1 – 4% of travelers [18, 41]. Though rare, HAPE is a life-threatening disorder that must be considered and monitored in high altitude travelers.
Athletes desiring to improve performance and decrease AMS at altitude have employed several strategies over the years. Classic high altitude acclimatization involves training and living at altitudes of 2000-2800 meters for 2-4 weeks [9]. Acclimatization improves athletic performance at altitude [11] and ideally, should be utilized when training for performance at altitude. However, the time commitment and decreased work capacity at altitude make these protocols impractical. Intermittent hypoxia has been shown, in some situations, to result in signs of acclimation [10] and may be a more appropriate training tool than longer programs. Intermittent hypoxia protocols may vary from daily hour-long sessions of alternating normoxia/hypoxia exposure to daily four hour sessions of hypoxic exercise [10, 34]. The ideal intermittent hypoxia protocol has not been determined and it has not demonstrated conclusive benefits. After the completion of a nine-day protocol of seven sessions of hypoxia (12.9% O₂) exposure, Faulhaber et al. found no improvement in a 30-minute time trial at moderate altitude [20]. Elite athletes can more easily fit acclimation strategies into their training with the use of houses and/or tents to simulate altitude exposure. This is accomplished by displacing ambient oxygen concentrations by increasing ambient nitrogen [9, 62]. However, due to the expense, time commitment, and less-than-certain performance improvement, this approach to acclimation is not practical for most athletes and recreationalists. Several pharmaceutical treatments have also been utilized, including supplemental O₂, and medications such as dexamethasone, acetazolamide and tadalafil [9, 38], which have been shown to improve exercise performance and decrease risk for AMS, [25, 38, 53]. Other classes of medications, such as those for pulmonary hypertension and obstructive disorders are also of interest to researchers for the role they may play in the prevention of AMS and improvement of performance at altitude [2, 23, 24, 35, 49]. The main cause of HAPE, for example, is hypoxic pulmonary vasoconstriction and pulmonary hypertension [31, 46, 56] and thus vasodilating agents are often explored as possible means of preventing and/or treating HAPE [31, 45, 46].

Ambrisentan is a medication commonly used to treat pulmonary arterial hypertension. Approved by the Food and Drug Administration in 2007, it is a selective endothelin type A (ETₐ) receptor antagonist [19].
There are three endothelin isoforms, of which endothelin-1 is most abundant in the human body [19, 64]. Endothelin-1 has strong vasoconstrictive properties and acts through two receptor subtypes (ET\textsubscript{A} and ET\textsubscript{B}) with differing functions [19, 50]. ET\textsubscript{A} is more common in the human lung [27] and mediates the vasoconstrictive effects of endothelin-1 [3, 16, 64] while ET\textsubscript{B} promotes vasodilation through the release of nitric oxide and mediates the clearance of circulating endothelin-1 [26]. It is therefore theoretically beneficial to utilize a selective ET\textsubscript{A} receptor antagonist to block the vasoconstrictive activity while preserving the vasodilation activity of ET\textsubscript{B} activation of endothelin. It is known that the vasoconstrictive activity of endothelin-1 affects the peripheral vasculature as well as pulmonary but little is known on the influence of ambrisentan on vascular resistance and oxygen transport to the muscle [12, 49]. Blood pressure measurements could be a useful tool for assessing the effects of ambrisentan treatment on peripheral vasculature resistance. Administration of ambrisentan is associated with relaxation and vasodilation of vascular smooth muscle tissue, evidenced by reduced pulmonary vascular resistance and mean arterial pressure [29]. After ingestion, ambrisentan reaches its maximal concentration in two hours and has an elimination half-life of nine hours. The recommended dose is ~5 mg daily [19].

Recently it has been suggested that hypoxia triggers the production of reactive oxygen species (ROS), responsible for cell dysfunction, vascular permeability and astrocyte swelling [4-8]. AMS could be the result of hypoxia-induced cerebral vascular leak and astrocyte swelling in the trigeminal area [4, 40]. Activation of nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) is a defense mechanism the body enacts to protect against the production of ROS and the resulting cellular damage thought to be responsible for AMS. Nrf2 is involved in a sequence known as the antioxidant-responsive element (ARE), which regulates gene expression of detoxification enzymes and antioxidant proteins [14, 40]. In situations of oxidative stress, Nrf2 enters the cell nucleus and activates transcription of ARE genes [40]. Lisk et al. hypothesized that medications used for AMS symptoms could activate Nrf2 and decrease high altitude cerebral vascular leak. Through in vivo and in vitro experiments, Nrf2 activation and cerebral vascular leak during hypoxia in rats was analyzed. The researchers compared the actions of several
medications, of which six (protandim, methazolamide, nifedipine, amlodipine, ambrisentan, and sitaxentan) successfully activated Nrf2. Ambrisentan resulted in a 13-fold increase in Nrf2 activation and reduced high-altitude-induced cerebral vascular leak in vivo by nearly 40%. In vitro, ambrisentan decreased hypoxia-induced hydrogen peroxide production and cell permeability. These data suggest that endothelin receptor antagonist drugs, such as ambrisentan, have the ability to protect against the damage of ROS in hypoxia [31].

Theophylline is a non-specific phosphodiesterase inhibitor, with therapeutic effects on the lungs, cardiac system and vasculature [21]. Theophylline also acts as an adenosine receptor antagonist, [49] stimulating heart rate. Originally reported effective as a bronchodilator, it has sense been used as a treatment of COPD and asthma [43, 52]. In a meta-analysis of 18 studies regarding theophylline’s efficacy in stable COPD patients, theophylline was found to consistently improve FEV₁ and FVC significantly over placebo [43]. Voduc et al. assessed exercise capacity (duration at 75% max) and pulmonary function in clinically stable COPD patients given theophylline vs. placebo. There was a trend among theophylline-treated participants towards improved exercise duration (28.9% mean improvement vs. 2.8% for placebo). This difference was not statistically significant, possibly due to the small sample size (Placebo arm n=11, Theophylline arm n=10) and large standard deviation in the theophylline group [57].

Several pharmaceutical effects of theophylline would be beneficial in the prevention of altitude-related illness, including decreased microvascular permeability in the brain and lungs, [47] bronchodilation [48], central respiratory stimulation [35, 36], decreased pulmonary arterial pressure [37], and induction of mild diuresis [24]. In a study by Kupper et al., subjects were randomly assigned to one of two groups, given either theophylline (300 mg) or placebo during ascent and during a stay at high altitude. Ingestion of theophylline resulted in significantly decreased severity of AMS at moderate altitude (3,440 meters) during ascent and during the five day stay at high altitude (4,559 meters). Theophylline also resulted in a significantly decreased respiratory disturbance index (respiratory events per hour of sleep, RDI) at high altitude [35].
The combination of pharmaceutically-driven cardiac stimulation and vasodilation is a concept of increasing interest in the prevention of AMS and improvement of performance at altitude [49]. It may be possible to counteract tissue hypoxia and performance loss by improving blood flow and increasing heart rate [44, 49]. A study by Radiloff et al. assessed the efficacy of the combination of theophylline and ambrisentan to improve exercise capacity in rats under simulated high altitude [49]. Female rats performed a two-hour long ramp protocol on a motorized wheel at an altitude equivalent of 4,267 meters. Theophylline was administered at a dose of 15-30 mg/kg body weight and ambrisentan at .1 mg/kg body weight. These dosages were chosen based upon those previously researched in humans [17, 29, 30]. The rats continued to run until showing signs of fatigue. After two hours, more than half the animals were still running with the combination treatment, compared to the control, theophylline alone and ambrisentan alone trials (median run time 52.5, 113, 93 min respectfully). Thus, the combination was the only treatment to significantly improve run-to-fatigue time compared to control (p < .005) [49]. The results of this study encourage the further investigation of this drug combination in human subjects.
Chapter Three: Methodology

Participants and Setting

Participants in this research study will be 30 recreationally active males from the Missoula, MT area, be between the age of 18 and 40 years, and have a VO\textsubscript{2} max \( \geq 45 \) ml/kg/min. Participants must have no history of serious acute mountain sickness. Participants also must be able to fly from Missoula to Denver and back at their scheduled times. Half of the 30 participants will be administered a combination of the medications theophylline and ambrisentan and half will be administered a placebo. Participants will be recruited on a volunteer basis and pass the PAR-Q health/exercise questionnaire to screen for known risk factors of coronary heart disease. Additionally, an informed consent form approved by the Institutional Review Board of the University of Montana in Missoula, MT will be signed by the participant agreeing to partake in the research study. Data collection will occur in the Montana Center for Work Physiology and Exercise Metabolism at the University of Montana in Missoula, MT and in the field at Mt. Evans, CO.

Experimental Design

Preliminary Testing

PAR-Q

Preliminary testing will include a pre-screening assessment, which involves a health/exercise questionnaire (PAR-Q). Prior to any testing the participants will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease.

Hydrodensitometry:

Body composition will be assessed using hydrodensitometry using estimated residual volume. Participants will arrive at the lab fasted for \( \geq 3 \) hours prior to body composition assessment. Body weight will be recorded on a dry weight scale (Befour Inc., Cedarburg, WI) and height will be measured. Body composition will be determined using an underwater weighing tank with digitalized and calibrated weight
scales (Exertech, Dresbach, MN). Participants will be submerged underwater on the scale to determine underwater weight. Underwater weighing will continue until consistent measurements, within 100 grams, are obtained. Underwater weight will be used to calculate body density to further calculate percent body fat using estimated residual volume and the Siri equation [54].

*Maximal Aerobic Capacity (VO$_{2\text{max}}$)*

Participants will arrive at the lab fasted for $\geq$ 3 hours prior to VO$_{2\text{max}}$ testing. VO$_{2\text{max}}$ testing will be performed on a treadmill ergometer (Fullvision, Inc., Newton, KS). Following a 5-minute warm-up, the Bruce Protocol will be performed. This test will begin at the first stage: 1.7 mph and a 10% grade for 3 minutes. Upon completion of the first stage, the speed and intensity will continue to a consecutive increased workload. After the first stage the workload will be raised to 2.5 mph and 12% grade, 3.4 mph and 14% grade, 4.2 mph and 16% grade, and 5 mph and 18% grade, respectively [63]. In order to measure VO$_{2\text{max}}$, the participants’ expired gas will be collected and analyzed every 15 seconds by a metabolic cart (Parvomedics, Inc., Sandy, UT). VO$_{2\text{max}}$ will be considered to be met when one of following criteria are met: 1) there is a plateau in VO$_2$ despite an increased workload; 2) Respiratory Exchange Ratio (RER) is greater than 1.10; 3) a heart rate within 10 beats of the participants’ predicted maximal heart rate is reached; and 4) volitional fatigue occurs in combination of a RPE $> 17$.

*Treadmill Familiarization*

Participants will perform 30 minutes of varied exercise (10 minutes walking 2 mph, 10 minutes walking 4 mph, and 10 minutes self-selected running pace) in order to become familiar with the function and feel of the manual treadmill that will be used for the remaining exercise trials (Curve, Woodway USA, Waukesha, WI)
Experimental Trials

Participants will perform two exercise trials: one in Missoula, MT, (WPEM) and one in Colorado (Mt. Evans).

Missoula Trial

Participants will run a scripted warm up followed by a two-mile time trial on the manual treadmill (Curve, Woodway USA, Waukesha, WI). Participants will be instructed to run at the fastest pace they can maintain for two miles.

Colorado Trial

Day One: Participants will be shuttled to the Missoula airport by the research staff to board a flight to Denver, Colorado. Two hours prior starting exercise at 14,000 ft (Mt. Evans summit) the participants will be given their first dose of medication (either placebo or medication combination). Once the participants have arrived in Denver, they will be shuttled to the campsite at 10,000 ft on Mt. Evans, and within one hour of arrival be shuttled to 14,000 ft for exercise testing. The test will be performed in the same manner as the Missoula trial, a two mile time-trial following warm up on the manual treadmill (Curve, Woodway USA, Waukesha, WI). After completing the exercise trial, participants will be shuttled back to the campsite at 10,000 ft where they will remain for the night.

Day Two: The timing of the events of day one will be replicated. At the appropriate times, participants will receive medication, food and be shuttled up to perform their exercise trial. Upon completion of exercise testing on this day, all participants will board a flight back to Missoula.

Lake Louise AMS Questionnaire

The Lake Louise questionnaire is used to assess symptoms and severity of acute mountain sickness (AMS). It contains five questions for self-report of symptoms such as sleep disorder, headache, and
nausea, given on a four-point scale [59]. Participants will complete this questionnaire four times throughout the CO trial, (1) upon arrival at the campsite, (2) after completion of the exercise trial, (3) upon awakening on day 2, and (4) after exercise completion on day two.

**Blood Oxygen Saturation**

Blood oxygen saturation (SaO$_2$) will be measured at rest in Missoula, at rest at the campsite (10,000 ft), at rest at 14,000 ft and during exercise testing at 14,000 ft. SaO$_2$ is assessed by a sensor (Nonin Medical, Inc. Plymouth, MN) placed on the fingertip which emits infrared light to measure the arterial hemoglobin oxygen saturation.

**Pulmonary Function Testing**

Pulmonary function, specifically FEV$_1$ and FVC, will be measured with a spirometer at rest before each exercise trial and immediately post each exercise trial. Participants will be instructed to maximally inhale and maximally exhale as quickly as possible.

**Blood Pressure Testing**

Blood pressure will be measured with a sphygmomanometer at the brachial artery and be taken at rest before each exercise trial and immediately post each exercise trial.

**Heart Rate Monitor**

A chest belt sensor will be worn to measure heart rate (Polar USA, Lake Success, NY) resting before, and during exercise trials.

**Statistical Analysis**

Lake Louise Survey will be expressed as a numerical value (1-15) and be analyzed with a 2 x 4 mixed design ANOVA (trial x time).
SaO$_2$ will be expressed as a percentage and be analyzed with a 2 x 4 mixed design ANOVA (trial x time). A t-test will be used to analyze resting campsite (10,000 ft) SaO$_2$ values between trials.

Heart rate will be expressed as beats per minute (bpm) and be analyzed with a 2 x 4 mixed design ANOVA (trial x time)

Blood pressure will be expressed as mmHg (mean arterial pressure) and be analyzed with a 2 x 4 mixed design ANOVA (trial x time)

FVC and FEV$_1$ will be expressed as liters and be analyzed with a 2 x 4 mixed design ANOVA (trial x time)

Time trial will be expressed as seconds and be analyzed with a 2 x 3 mixed design ANOVA (trial x time)

VO$_2$ max will be expressed in “ml/kg/min” and reported as a descriptive.

Body mass will be expressed in “kg” and reported as a descriptive.

Body fat will be expressed as a percentage and reported as a descriptive.

The level of statistical significance will be achieved at p<0.05 and all descriptive data will be reported as mean ± SD and all physiological graphed data will be reported as mean ± SEM.
References


Title

Theophylline and Ambrisentan combination: Effects on human performance, AMS and physiological responses to altitude

Abstract

Recent research efforts have attempted to determine the effectiveness of the combination of a pharmacological cardiac stimulant (theophylline) and pulmonary vasodilator (ambrisentan) in attenuating performance losses and mountain sickness at altitude. This combination has been observed to improve hypoxic exercise performance in rats, but never tested in human subjects. In the current study, 30 male participants were tested in a paired fashion to assess whether this drug combination would improve exercise performance and/or decrease symptoms of AMS. The experimental group (EXP, n = 15) received a treatment of 400 mg theophylline and 5 mg ambrisentan, while the placebo group (PLA, n = 15) received a placebo combination. Data collection was performed in the field at an altitude of 4,267 m. Participants completed two 2-mile time trials (TT) on subsequent days, between which they spent the night at 3,048 m. Treatment with theophylline and ambrisentan was not associated with improved 2-mile TT performances or AMS symptoms. The EXP group had significantly lower measures of diastolic blood pressure and mean arterial pressure compared to PLA while at altitude (69.2 ± 7.9 vs 77.7 ± 8.0 mmHg and 90.8 ± 8.9 vs 97.2 ± 7.9 mmHg, respectively), main effect for group, p<0.05. Significantly, this study was the first to assess this drug combination in human subjects at altitude in a field setting. Additionally, the observed reduction in vascular pressures as a result of treatment may have positive implications for the health state of sojourners to altitude.

Key Words: pharmaceutical, hypoxia, vascular pressure, endothelin, stimulant
Introduction

There are several inherent decrements to performance at altitude, beginning as low as 1,500m [43]: decreased arterial oxyhemoglobin saturation, \((\text{SaO}_2)\) [8, 15], decreased muscle oxygenation [32, 48], decreased submaximal aerobic performance [19], decreased maximal oxygen consumption \((\text{VO}_{2\text{max}})\) [8, 13, 20, 29], and increased risk for AMS [20, 29, 48, 51]. The most common form of AMS, occurs in situations of non-acclimatized exposure to altitudes of 2,500m or greater [11, 22, 38]. In contrast, high altitude pulmonary edema (HAPE), occurs at more drastic altitudes, rarely below 4,000m [11, 31] and is the result of hypoxia-driven pulmonary vasoconstriction, pulmonary hypertension and capillary stress [38]. In addition to recreational expedition climbers, those in occupations such as mountain rescue and military are often expected to perform at high altitudes, increasing the demand for treatment strategies to combat the associated performance and health decrements [13, 43].

Several pharmaceutical treatments have been utilized to combat the harmful effects of altitude on performance, including supplemental oxygen, and medications such as dexamethasone, acetazolamide and tadalafil [18, 28, 46]. These treatments have been shown to improve exercise performance and decrease risk for AMS and HAPE, but come with high expense, possible side effects, and ethical or legal issues pertaining to rules of competition [50]. Other classes of medications, such as those used to treat hypertension and pulmonary obstructive disorders, and cardiac stimulants are of interest to researchers for the role they may play in the prevention of AMS and concomitant improvement of performance at altitude [1, 16, 17, 25, 41].

Ambrisentan is a selective endothelin type A (ET\(_A\)) receptor antagonist commonly used to treat pulmonary arterial hypertension [12]. Administration of ambrisentan is associated with relaxation and vasodilation of vascular smooth muscle tissue, evidenced by reduced pulmonary vascular resistance and mean arterial pressure [21]. Since the main driver of HAPE is hypoxia-driven pulmonary vasoconstriction, Ambrisentan may reduce symptoms of HAPE. It has been recently suggested that
hypoxia triggers the production of reactive oxygen species (ROS) and is responsible for cell dysfunction, vascular permeability and astrocyte swelling [2-6], which increase AMS symptoms under hypoxic conditions [2, 30]. In situations of oxidative stress, the body produces nuclear related factor 2 (Nrf2) which regulates expression of more than 90% of antioxidant genes, thus acting as a defense mechanism against cellular damage from ROS [9, 30]. In a study performed in rats, ambrisentan significantly increased Nrf2 activation and reduced high-altitude-induced cerebral vascular leak by nearly 40%, suggesting that ambrisentan has the ability to protect against the damage caused by hypoxia-induced ROS [30].

Theophylline is a non-specific phosphodiesterase inhibitor, with therapeutic effects on the lungs, cardiac system and vasculature [14]. Theophylline also acts as an adenosine receptor antagonist, [41] stimulating heart rate. Originally reported as an effective bronchodilator, theophylline has since been used to treat chronic obstructive pulmonary disorder (COPD) and asthma [33, 44] and has been shown to significantly improve FEV1 and FVC in COPD patients [33]. Several pharmaceutical effects of theophylline would be beneficial in the prevention of altitude-related illness, including decreased vascular permeability in the brain and lungs, [39] bronchodilation [40], central respiratory stimulation [25, 26], decreased pulmonary arterial pressure [27], and induction of mild diuresis [17]. Kupper et al. found ingestion of theophylline resulted in significantly decreased AMS severity at moderate altitude (3,440m) during ascent and during a 5-day stay at high altitude (4,559m) [25]. Theophylline also resulted in a significantly decreased respiratory disturbance index (respiratory events per hour of sleep) at high altitude [25], improving sleep quality at altitude.

Combining a cardiac stimulant, (theophylline) with a vasodilating agent (ambrisentan) may alleviate AMS oriented symptoms while preserving exercise performance at altitude. However, little research has been conducted on this combination [41]. Radiloff et al. assessed the efficacy of theophylline and ambrisentan in concert to improve exercise capacity in rats under simulated high altitude (hypobaric chamber at an altitude equivalent of 4,267m). The combination significantly improved run-to-fatigue time
in female rats compared to the placebo trial as well as compared to the theophylline and ambrisentan treatments alone. [41]. The purpose of the current study was to determine the effects of oral doses of theophylline and ambrisentan taken together on symptoms of AMS and exercise performance in human subjects during approximately 27 hours of acute altitude exposure.

Methods

Participants

Participants for the current study included 30 recreationally active males from the local community. Prior to data collection, participants completed the Physical Activity Readiness Questionnaire (PAR-Q) and an informed consent form approved by the University Institutional Review Board.

Experimental Procedures

For the first visit to the laboratory, participants completed an informed consent form, PAR-Q, and preliminary testing. Preliminary testing included measurement of body composition and maximal aerobic capacity (VO\textsubscript{2max}). The second visit consisted of a 3.2km time trial completed on a manual treadmill in the laboratory (975 meters). The time trial was preceded by a 15-minute warm-up (5-min: 0.45m/s, 5-min: 0.89m/s, 5-min: 1.34m/s), in which heart rate and SaO\textsubscript{2} were measured. Participants were stratified into either a placebo (PLA, N=15) or experimental (EXP, N=15) group in a paired fashion based on their time trial times (18.27 ± 1.77 min, 18.36 ± 1.93 min for PLA and EXP, respectively) and anthropometric characteristics, Table 1. On the third visit, participants met at the laboratory and were transported to the airport to be flown to Denver. Upon landing in Denver (1,560m), the participants received either the placebo or the experimental treatment in a double-blind fashion. The EXP group received a treatment of 400mg theophylline and 5mg ambrisentan, while the PLA group received a similar-looking placebo. After consuming the pills, participants were driven from the airport to the summit of Mt. Evans (4,267m; approximately 2-hour drive). Upon arrival to the summit, resting blood pressure (BP) and pulmonary function (PFT) were measured. Following this, participants completed a time trial in the same manner as the 975m laboratory trial (15-minute warm-up followed by 5-minute break and 2-mile time trial). After
participants completed their time trial, they were shuttled to camp at Echo Lake (3,048m) where they remained for the night. At camp, at 17:00, resting PFT and BP measures were taken in the same manner as at 4,267m. Participants were in bed by 22:00 and awakened at 06:00 the following morning. Participants ate breakfast shortly after awakening, at the same time they had eaten the previous morning. They were then allowed to relax around the campsite or go back to bed to mimic the activity of the previous morning as closely as possible. Medications were administered at 09:00 and participants were shuttled to reach the summit at the same time as the previous day. Once at the summit, data collection was performed in the exact order and manner as the previous day, Figure 1. At the completion of the time trial, participants were shuttled back to the airport to return home.

Data Collection

Preliminary Testing

Participants arrived at the lab fasted for ≥ 3 hours prior to body composition and VO$_{2\text{max}}$ testing. Body density was assessed using hydrodensitometry with a digitalized and calibrated weight scale (Exertech, Dresbach, MN). Body weight was recorded on a dry weight scale (Befour Inc., Cedarburg, WI) and height was measured using a stadiometer (Narragansett Machine Co, Providence, RI). Body composition was calculated from body density using estimated residual volume using the Siri equation \[47\]. Participants performed VO$_{2\text{max}}$ testing on a treadmill ergometer (Fullvision, Inc., Newton, KS) utilizing the standard Bruce protocol. At the end of each three-minute stage RPE was measured using the Borg 6-20 Rating of Perceived Exertion Scale. Expired gas was measured every 15 seconds from a metabolic cart (Parvomedics, Inc., Sandy, UT) to determine VO$_{2\text{max}}$.

Time Trial

Time trials were performed on a manual treadmill (Curve, Woodway, WI). The manual treadmill is comprised of individual slats which glide over a ball bearing system of roller guides. Roughly speaking, the treadmill works in the same manner as a hamster wheel. The belt is propelled and speed is controlled by the runner on the treadmill. Participants completed a scripted 15-minute warm-up (0.45m/s for 5-min,
0.89m/s for 5-min, 1.34m/s for 5 min) followed by a 5-minute break before commencing the 3.2km time trial. During each of the 3.2km time trials (one performed at 975m, two performed at 4,267m), participants were blinded to treadmill speed and time, but were able to view the distance completed. Participants were instructed to complete two miles as fast as possible.

Lake Louise Survey

The Lake Louise Survey (Roach, 1993) was administered to monitor symptoms of acute mountain sickness. Participants completed the survey at 3,048m in the evening of Day 1 (17:00) and in the morning of Day 2 (09:00).

Heart Rate and Oxyhemoglobin Saturation

Heart rate and SaO₂ was measured at the fingertip using pulse oximetry (WristOx₂, Nonin, Plymouth, MN). Measurements were recorded for each 5-minute stage of the standardized warm-up and throughout the 3.2km time trial.

Blood Pressure

Blood pressure was measured after ten minutes of seated rest at the right brachial artery using an automatic blood pressure cuff (Omron Healthcare, Lake Forest, IL). Mean arterial pressure was calculated as 1/3(SBP-DBP) + DBP. Blood pressure was measured once at 3,048 m and at 4,267 m prior to each of the 2 time trials (Day 1 and Day 2).

Pulmonary Function

Pulmonary function was assessed immediately following resting blood pressure measurement using a handheld spirometer (Microloop, CareFusion, San Diego, CA). Participants were instructed to place their mouth directly around the mouthpiece, then to perform a maximal inhalation followed by a maximal exhalation as long as possible. Three inhalation/exhalation cycles were performed and the best measures were used for data analysis. Measures included forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), and estimated maximal voluntary ventilation (MVV). Pulmonary function was measured once at 3,048m and twice at 4,267m, prior to each of the two time trials (Day 1 and Day 2).

Statistical Procedures
All descriptive data are expressed as means ± SD. Time trial performance, blood pressure, and pulmonary function were analyzed using a 2 x 3 mixed design ANOVA for treatment and time. AMS scores and heart rate and SaO₂ at each speed (0.45m/s, 0.89m/s, 1.34m/s, and TT) were analyzed using a 2 x 2 mixed design ANOVA for treatment and time. All data was analyzed using SPSS version 13.0, and statistical significance was established using an alpha level of p < 0.05.

**Results**

**Time Trial**

The trial-by-time interaction was not significant for time trial performance, Table 2. The 975 m. time trial (18.32 ± 1.88 minutes) was significantly faster than Day 1 and Day 2 time trials at 4,267 m., Table 2 for 4,267 m. trials, p < 0.05, main effect for time.

**Acute Mountain Sickness - Lake Louise Survey**

The trial-by-time interaction was not significant for the Lake Louise Survey scores, Table 2. The evening scores on Day 1 were higher compared to the Day 2 scores, main effect for time, p<0.05.

**Heart Rate**

There was a trial-by-time interaction for heart rate at 0.45m/s, with the experimental group having a higher heart rate on Day 2 compared to Day 1, Table 2, as well as higher than the placebo group on Day 2 p<0.05, Table 2. The trial-by-time interaction was not significant for heart rate at 0.89 m/s, 1.34 m/s, or during time trials.

**Oxyhemoglobin Saturation**

The trial-by-time interaction was not significant for SaO₂. For 0.45m/s, 0.89m/s, 1.34m/s SaO₂ was higher on Day 2 compared to Day 1, main effect for time, p < 0.05, Figure 2. During the time trials, there was a trend for main effect for time (76.41 ± 3.53 vs 77.52 ± 2.79 % for Day 1 and Day 2 respectively, p = 0.058).

**Blood Pressure**
The trial-by-time interaction was not significant for DBP, SBP, or MAP. Resting diastolic blood pressure (DBP) was lower in the EXP compared to the PLA, main effect for group, p < 0.05, Figure 3. DBP was lower at 3,048m compared to 4,267m on Day 1 and Day 2, main effect for time, p<0.05, Figure 2. Mean arterial pressure was significantly lower in the EXP compared to PLA (90.8 ± 8.9 vs 97.2 ± 7.9 mmHg, respectively), main effect for group, p<0.05, and was lower at 3,048 m compared to 4,267 m (91.2 ± 6.7 vs 95.4 ± 9.6), main effect for time, p<0.05. There was no difference between groups or between time points in resting systolic blood pressure.

Pulmonary Function

No significant differences in resting FEV₁, FVC, or MVV existed between groups or among time points, Table 3.

Discussion

The current study included a relatively rapid ascent to high altitude (975 to 4,267m above sea level in approximately 4 hours in a field setting to test the efficacy of a combined oral dose of theophylline and ambrisentan on symptoms of AMS and time trial performance. The current data suggest that the drug combination did not alleviate symptoms of AMS or provide an enhanced 2-mile time trial performance compared to placebo while exercising in hypobaric hypoxia. Several physiological parameters related to performance (heart rate, SaO₂, and pulmonary function) were unchanged with drug treatment, possibly contributing to the minimal differences in running performance.

Physiological Parameters and Performance

A limitation of performance at altitude is decreased oxygen delivery to muscles, a result of both decreased atmospheric pressures and impaired lung diffusion [8]. Past research has shown limited lung diffusion to be predictive of decreased SaO₂ [24, 36]. In the current study, no difference existed in SaO₂ between the experimental and placebo groups, aligning with the lack of performance difference between groups. De Bisschop et al. observed increased exercise performance after the ingestion of sitaxsentan, which works
via the same mechanism as ambrisentan, blocking the activity of $\text{ET}_A$ [10]. Participants were administered the medication (100 mg sitaxsentan or placebo) each day of their week-long ascent to 5,050 m. Compared to the placebo, sitaxsentan intake improved VO$_2$max at altitude. This improvement in exercise capacity was attributed to decreased pulmonary vascular resistance, associated with improved lung diffusing capacity [10]. However, the results of the current study indicate that diffusion at the alveolar level is not improved with the ingestion of ambrisentan during a relatively acute altitude exposure period of 27 hours. It is possible that an extended stay at altitude, such as in the De Bisschop study may be necessary to exacerbate the symptoms of AMS and/or to demonstrate positive pharmacological impacts on lung function and exercise capacity. Additionally, as such little data exists on acute benefits of ambrisentan, our participants may have needed a longer treatment to elicit improvements in lung diffusion at altitude.

Although less studied and understood than other consequences of hypoxia, previous research has determined that exposure to altitude of less than 20 hours decreases forced vital capacity (FVC) and consequently limits performance in healthy individuals [52]. In the current study, no differences existed between groups in FVC or any other measures of pulmonary function throughout the duration of the altitude exposure. In patients with COPD, theophylline acts as a bronchodilator and significantly improves FVC [33]. According to a meta-analysis by Molfino and Zhang, theophylline improves peak FVC in COPD patients by an average of 186 mL over placebo. However, most of the analyzed studies involved a treatment duration of close to a month [33]. Very few studies analyzed treatment durations as short as 2 days [7, 45]. Shivaram et al. observed a single dose of theophylline (400mg, the same dose as the current study) significantly improved FVC in patients with COPD within 60 min, however the single dose was not able to improve exercise capacity [45]. None of the participants in the current study suffer from pulmonary disorders and thus, even with the altitude decrement, did not develop a sufficient deficit for the treatment to elicit physiological impacts. Even if improvements in pulmonary function existed, they may not have improved exercise performance, as indicated by the results of Shivaram et al.
In the only other existing study of this drug combination on performance at altitude, Radiloff et al. found significant improvements in exercise capacity in female rats under the influence of the ambrisentan/theophylline combination. Specifically, the medication combination increased time-to-fatigue in a 2 hour running trial compared to each medication alone and the placebo group [41]. Administration of the medication combination occurred 30 minutes prior to exposure. The exposure period only included the 2-hour exercise test. No difference was observed between groups in SaO₂ and performance improvements were attributed to an increase in blood flow, accomplished through accelerated heart rate and vasodilation. Similar to Radiloff et al., participants in the current study were exposed within a short time period of taking medication, assessing the acute effects. A surprising lack of difference in heart rate between groups in the current study assists in explaining the discrepancy in performance between the two existing studies on this drug combination. In short, the lack of difference in SaO₂, FVC, and heart rate helps to explain the finding that performance was not improved with drug treatment.

*Acute Mountain Sickness*

Lake Louise surveys were taken on each of the two days spent at altitude. Administration of the medication treatment began on the morning of initial ascent and was repeated at the same time on Day 2, after a night spent at altitude. Participants had been at altitude for 5 hours when taking the first survey and 21 hours at the time of the second. No difference in AMS symptoms existed between groups on either day. Previous research demonstrated that theophylline (300 mg once daily) was successful in decreasing AMS symptoms when administered for 5 days prior to ascent and during a 48 hour ascent to 4,559 m [25]. In another study measuring AMS symptoms during acute hypoxic exposure (3.5 hours), theophylline (375 mg twice daily 3 days prior to hypoxic exposure) successfully decreased AMS symptoms [17]. These findings, compared to that of the current study, suggest that theophylline’s ability to reduce AMS symptoms is a gradual process, requiring buildup of the medication in the participant’s system. A several-day dosage period was not the intent in the current study, which sought to determine if an acute dose would have the same effects as those shown in a several-day dosage protocol. In some
instances, such as a rescue, fire suppression, or military situation, individuals are required to ascend to altitude with little notice and without time to partake in a several-day protocol. As such, the simplicity and convenience of an acute dose versus several days was worthy of pursuing.

**Blood Pressure**

Upon exposure to altitude, the sympathetic nervous system is activated to improve oxygen delivery, via elevated heart rate, muscle blood flow, and cardiac output, which increases systemic blood pressure [23, 34, 37]. In the current study, DBP was higher at 4,267m compared to 3,048m, indicating that the sympathetic response to altitude [37] was higher while at altitude. Additionally, in response to hypoxia, endothelin type A (ET$_A$) is stimulated, producing increased pressures in both pulmonary and peripheral vasculature [42], which occurs immediately with exposure and can persist for several days [37]. Ambrisentan targets this response, and blocks the vasoconstrictive effects of ET$_A$ [41]. Blockage of ET$_A$, with administration of the similar medication sitaxsentan, was shown to improve exercise capacity, and decrease symptoms of AMS while significantly reducing MAP, and pulmonary artery pressures at an altitude of 5,050 m. This response was achieved after week-long sitaxsentan treatment and altitude exposure. Interestingly, a second portion of the study assessed the effects of the same treatment after a 1-hour hypobaric hypoxic exposure at an altitude of 4,500m. MAP and pulmonary artery pressures were again decreased, indicating that the benefits can be achieved in an acute exposure, given a sufficient prior treatment time [35]. The current study found lower vascular pressures (DBP and MAP) in the EXP treated with ambrisentan and theophylline, but not in the PLA, suggesting the drug was inducing an effect on blood pressure. It is important to note that past research has indicated ET$_A$ blockage through pharmaceutical treatment reduces pulmonary hypertension in a similar fashion to peripheral pressures [35]. In the current data collection, we did not possess the resources to assess pulmonary arterial pressures and assume, based upon past research, that the pulmonary pressure response mimics the observed peripheral response (decreased DBP and MAP). It is worth noting that we observed this effect after only 1 day of treatment. Our findings contribute to the literature by indicating that an acute dose may be just as beneficial in the prevention of HAPE as a weeklong treatment. Hypoxia-induced pulmonary
vasoconstriction and the resulting fluid leakage are the causes of HAPE and significantly decrease performance during longer periods of altitude exposure [22, 38, 49]. Our findings present treatment with ambrisentan combined with theophylline as promising in the alleviation of altitude illness and thus beneficial to performance over sustained altitude exposure.

Conclusion

The current study is the first to assess the effects of an ambrisentan and theophylline combination in hypobaric hypoxia in humans. Fortunately, we observed no negative side effects of the drug combination, supporting its safety for use in humans. A previous study in animals (female Sprague-Dawley rats) found the combination to improve performance within two hours of treatment and altitude exposure, which was not seen in the current study. Although the current study found no improvements in running performance and symptoms of AMS compared to placebo, decreased vascular pressure emerges as a notable benefit of the drug combination. The application of this drug combination offers a potentially novel approach during extended stays at higher altitudes where increased vascular pressure may result in HAPE and decreased performance capacity.
References


Table 1. Descriptive information for study participants. Data are expressed as means ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Body Fat (%)</th>
<th>VO$_2$max (ml·kg$^{-1}$·min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (N = 15)</td>
<td>25.1 ± 3.1</td>
<td>180.8 ± 4.6</td>
<td>77.5 ± 7.4</td>
<td>11.6 ± 5.0</td>
<td>59.6 ± 4.6</td>
</tr>
<tr>
<td>Experimental (N = 15)</td>
<td>25.1 ± 4.0</td>
<td>181.7 ± 6.6</td>
<td>80.7 ± 10.8</td>
<td>14.1 ± 3.6</td>
<td>56.3 ± 7.0</td>
</tr>
<tr>
<td>All (N = 30)</td>
<td>25.1 ± 3.6</td>
<td>181.3 ± 5.6</td>
<td>79.1 ± 9.3</td>
<td>12.8 ± 4.5</td>
<td>57.9 ± 6.0</td>
</tr>
</tbody>
</table>
Table 2. Measures of exercise heart rate (BPM), time trial performance (minutes), and Lake Louise Survey score for AMS at two days spent at altitude. Data are expressed as means ± SD.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>0.45m/sec</th>
<th>0.89m/sec</th>
<th>1.34m/sec</th>
<th>TT (min)</th>
<th>0.45 m/sec</th>
<th>0.89m/sec</th>
<th>1.34m/sec</th>
<th>TT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>Experimental</td>
<td>86 ± 10</td>
<td>100 ± 12</td>
<td>116 ± 13</td>
<td>143 ± 18</td>
<td>95 ± 10*†</td>
<td>103 ± 14</td>
<td>119 ± 15</td>
<td>138 ± 19</td>
</tr>
<tr>
<td>(BPM)</td>
<td>Placebo</td>
<td>83 ± 8</td>
<td>99 ± 5</td>
<td>113 ± 11</td>
<td>143 ± 10</td>
<td>84 ± 10</td>
<td>98 ± 7</td>
<td>112 ± 10</td>
<td>142 ± 18</td>
</tr>
<tr>
<td>Time trial</td>
<td>Experimental</td>
<td>25.67 ± 2.63</td>
<td></td>
<td></td>
<td></td>
<td>25.85 ± 2.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25.27 ± 1.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS Score</td>
<td>Experimental</td>
<td>1.9 ± 1.1</td>
<td></td>
<td></td>
<td></td>
<td>1.4 ± 1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3,048 m.)</td>
<td>Placebo</td>
<td>1.9 ± 1.0</td>
<td></td>
<td></td>
<td></td>
<td>0.7 ± 1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 vs. Day 1; † p < 0.05 vs. placebo.
Table 3. Measures of resting pulmonary function at three time points. Data are expressed as means ± SD.

<table>
<thead>
<tr>
<th></th>
<th>3048 m.</th>
<th>4267 m. Day 1</th>
<th>4267 m. Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ (L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>4.5 ± 0.5</td>
<td>4.5 ± 0.5</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>Experimental</td>
<td>4.4 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td>FVC (L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>5.4 ± 0.8</td>
<td>5.3 ± 0.8</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>Experimental</td>
<td>5.1 ± 0.9</td>
<td>5.1 ± 0.8</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>MVV (L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>168.5 ± 20.0</td>
<td>170.1 ± 18.2</td>
<td>166.7 ± 21.8</td>
</tr>
<tr>
<td>Experimental</td>
<td>165.7 ± 25.4</td>
<td>169.0 ± 24.8</td>
<td>169.0 ± 28.1</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1.** Schematic illustrating timeline of data collection in Colorado

**Figure 2.** Oxyhemoglobin saturation during exercise trials at 4,267m meters.
* Day 2 higher than Day 1, main effect for time, $p < 0.05$

**Figure 3.** Resting diastolic blood pressure at three time points.
* 3048 meters lower compared to Day 1 and Day 2 at 4267 meters feet, main effect for time, $p < 0.05$
† experimental group lower than placebo group, main effect for group, $p < 0.05$. 
Figure 3

Diastolic Blood Pressure (mmHg)

Altitude (meters)

- 3,048
- 4,267 (Day 1)
- 4,267 (Day 2)

- Control
- Experimental

* Statistical significance
† Higher than control
SUBJECT INFORMATION AND CONSENT FORM

PROJECT IN BRIEF: Enhancing physical performance and mitigating acute mountain sickness via pharmaceutical intervention while at altitude

SPONSOR: Defense Advanced Research Projects Agency (DARPA)

RESEARCHERS: Brent Ruby, PhD (406) 243-2117
              John Cuddy
              Walter Hailes
              Hilary Pavalovich
              Kyle Cochrane

The University of Montana
Montana Center for Work Physiology and Exercise Metabolism (WPEM)
32 Campus Drive
McGill Hall – HHP
Missoula, MT 59812
(406) 243 – 2117 (Dr. Brent Ruby, PhD)

Please read the following information carefully and feel free to ask questions. Only sign the final page when you are satisfied procedures and risks have been sufficiently explained to you.

REQUIREMENTS
This research study requires that you meet the following criteria:

- Participants must be males between the ages of 18 and 40 ______(initial)
- Participants must have no history of serious acute mountain sickness ______(initial)
- Participants must have a VO₂ max greater than 45 ml•kg•min⁻¹
- Participants must fly to Denver from Missoula at their scheduled times
PURPOSE OF THE STUDY
The study is designed to address the issue in current hypoxia research of the effects of an oral dose of AMBRISENTAN and THEOPHYLLINE taken in concert on well-being and physical performance at altitude.

TEST PROCEDURES
2 VISITS TO THE LABORATORY AND 1 TRIP TO COLORADO WILL BE REQUIRED, AS SUMMARIZED BELOW

Anthropometric Testing (Visit 1, Missoula, MT)

1. A pre-screening assessment, which involves a health/exercise questionnaire (Par-Q) and question regarding prior acute mountain sickness.
   a. Prior to any testing, you will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease.
   b. Prior to any testing, you will be EXCLUDED from the study if you have previously had acute mountain sickness or ARE CURRENTLY TAKING ANY MEDICATIONS INCLUDING OVER-THE-COUNTER MEDICATIONS ______________(initial).

2. If you successfully complete the PAR-Q, you will then provide written informed consent following the reading of this document.

3. A measure of percent body fat obtained using underwater weighing
   This test session will require that you do not eat for a minimum of 3 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighing tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided upon request. This test will take approximately 20 minutes.

4. A maximal treadmill test to measure aerobic fitness
   This test will consist of walking and running on a laboratory treadmill to volitional fatigue. The workload will increase every three minutes and will progress to fatigue. You will be encouraged to continue until volitional fatigue, the point at which you can no longer continue exercise. During this test you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during exercise. Heart rate will be measured using an elastic chest strap that is
worn on the skin under your shirt around your chest. This test will take approximately 30 minutes. You will be asked to fast for approximately 3 hours prior to this test.

5. **Treadmill familiarization**

Following your treadmill test to measure aerobic fitness you will perform 30 minutes of varied exercise (10 minutes of walking at 2 mph, 10 minutes of walking at 4 mph, and 10 minutes at self-selected running pace) on the manual treadmill that will be used for the remaining exercise trials to become familiar with its function and feel.

**Pre-experimental trial commitments**

**48-24 Hours Preceding Experimental Trial**

48-24 hours prior to your first experimental trial you are free to exercise, but the intensity must be moderate and the duration cannot be longer than 2 hours.

**24 Hours Preceding Experimental Trial**

In the 24 hours prior to your experimental trial, you cannot exercise.

**Missoula Trial (Visit 2, Missoula, MT)**

During this visit you will run 2 miles as quickly as possible, after a scripted warm up, on a treadmill in the laboratory.

**Colorado Trial (Visit 3, Travel to Mt Evans, CO):**

*Day 1:* You will be shuttled to the airport by a research staff in the morning and will board a flight to CO. You will consume your first dose of medication 2 hours prior to starting exercise at 14,000 ft. Upon arrival in CO you will be shuttled by research staff to a campsite at approximately 10,000 ft on Mt Evans. Within 1 hour of arriving at the campsite you will be shuttled to a testing area at approximately 14,000 ft for your first exercise testing. Exercise testing will consist of a standardized warm up followed by a 2 mile run to be completed as quickly as possible. After exercise testing you will be shuttled back to the campsite where you will relax for the rest of the evening. The timing of the events during this day will be recorded and replicated on day 2 of testing.

*Day 2:* A member of the research staff will awaken you. At the appropriate times you will repeat all the activities of Day 1, including consumption of food, medication, and exercise testing. Upon completion of the exercise testing on this day you will be shuttled back to the airport and board a flight back to Missoula.

**Travel accommodations**

You will be provided with a tent, bathroom facilities and all food during your travel. You will be responsible for your personal clothing, sleeping and hygiene supplies.
TIME REQUIREMENTS

The medication tolerance testing will require approximately 2 contiguous hours. Anthropomorphic testing will require approximately 1 hour. The Missoula time trial will require approximately 1 hour. The CO trial will require approximately 60 contiguous hours. The total time commitment to this research project will be approximately 64 hours over the course of 3 weeks.

TRIAL EXPENSES: All expenses related to this data collection effort, which include but are not limited to airfare, meals, ground transportation and living accommodation, will be directly paid for by WPEM.

DATA COLLECTION PROCEDURES

Lake Louise AMS questionnaire

You will be asked to complete a questionnaire that asks about the presence and severity of several symptoms associated with acute mountain sickness, including headache, nausea, and sleep quality. You will be asked to complete this questionnaire 5 times while on Mt Evans; 1) Upon arrival at the campsite, 2) after completion of the exercise trial, 3) upon waking on Day 2, 4) at your arrival time to the campsite on Day 1, 5) after completion of your Day 2 exercise trial.

Blood Oxygen Saturation

Blood oxygen saturation will be measured at rest in Missoula, at rest at the campsite (10,000ft) on Mt Evans and during all exercise testing in CO. This is done by attaching a sensor to the tip of your finger. This device emits an infrared light into your finger to measure saturation; no finger sticks are required for this measure.

Pulmonary Function Testing

Pulmonary function will be assessed by spirometry. The spirometer measures how much air you can breathe into your lungs and how much air you can quickly blow out of your lungs. This test is done by having you take in a deep breath and then, as fast as you can, blow out all of the air. You will be blowing into a tube connected to a machine (spirometer). You will be asked to complete several maximal inspirations and expirations and breathe as fast as you can for a 15 second period. It takes effort to do this test and you may become tired. This is expected. If you become light-headed or dizzy during this test, immediately stop blowing and let the technician know.

Physiological Monitor

We will fit you with a chest belt sensor (similar to a heart rate monitor) that measures heart rate, respiration rate, skin temperature, body motion, and body position. This system has been certified by the Food and Drug Administration (FDA) for use for these purposes.

Core Temperature

You will consume a radio equipped temperature monitor the night before and the morning of your exercise trial. The thermometer pill allows us to continuously measure your body temperature. The pill is
about the size of a large vitamin pill. Although the pill is made of strong food-grade plastic, you will be asked not to chew the pill as you swallow it. A member of the research team who has been trained in administering these pills will watch you as you swallow the pill. The pills are disposable and are only used once. You will be given a standardized amount of water to ingest with the temperature sensor. Within 24 to 48 hours, it will pass through your system. You will wear a small data logger in a small fanny pack belt that will receive wireless signals from the temperature sensor. A second temperature sensor will be placed on your chest to collect skin temperature. These sensors will send data continuously to the data logger. Once you have swallowed the thermometer pill we will check to make sure that it is functioning correctly.

Thermometer Pill

Core Temperature Monitor

Urine

You will be asked to void your bladder before each trial. After the initial void, urine will be collected in a disposable plastic container and urine volume will be measured for the duration of each trial.

EXERCISE RISKS and DISCOMFORTS

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.
3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. The laboratory has standard emergency procedures should any potential problems arise.
4. Symptoms of dehydration such as headache and general fatigue may result during and after the exercise.
5. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to
determine when a test should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.

6. During recovery at Mount Evans, CO, you may experience acute mountain sickness and may experience the following symptoms: headache, fatigue, dyspnea, hyperventilation, gastrointestinal distress, and decreased thirst.

7. There are potential discomforts and risks with wearing the chest strap system that include some itching and during prolonged use, and a rash in some people. If you have highly sensitive skin you should not participate in this study. When heart rate, respiration rate, and skin temperature are collected from the chest strap system it is painless and you do not actually feel it being collected. This device has also been certified for use by the FDA and there is little or no risk of shock from these battery powered systems. All of the systems will have had safety checks performed on them prior to this test.

**BLOOD DRAW RISKS**

There is a minor risk of infection and bruising associated with blood sampling.

**DRUG SIDE EFFECTS/POTENTIAL DISCOMFORTS**

Theophylline may cause none, some or all of the side effects listed below:

More likely

- upset stomach
- stomach pain
- diarrhea
- headache
- restlessness
- trouble going to sleep or staying asleep (insomnia)
- irritability

Less Likely But Serious

- vomiting
- increased or rapid heart rate
- irregular heartbeat
- seizures
- skin rash
- fine muscle tremors
- increased production of urine (diuresis)

Ambrisentan may cause none, some, or all of the side effects listed below:

More likely

- Swelling of hands, legs, ankles and feet (peripheral edema)
- Stuffy nose (nasal congestion)
- Inflamed nasal passages (sinusitis)
- Hot flashes or getting red in the face (flushing)

**Less Likely But Serious**

- Swelling all over the body
- Sperm count reduction. Reduced sperm counts have been observed in some men taking a drug similar to LETAIRIS.
- Low red blood cell levels (anemia)
  
  Some medicines that are like LETAIRIS can cause liver problems. Please tell a researcher if you get any of these symptoms of a liver problem while taking LETAIRIS:
  - loss of appetite
  - nausea or vomiting
  - fever
  - achiness
  - generally do not feel well
  - pain in the upper right stomach (abdominal) area
  - yellowing of your skin or the whites of your eyes
  - dark urine
  - itching

**DRUG INTERACTION SIDE EFFECTS**

There are no known negative interactions/side-effects when using Theophylline and Ambrisentan at the same time.

You should discuss these medications with Dr. Brent C. Ruby, PhD and your regular health care provider if you choose.

**UNFORSEEN RISKS**

All drugs have potential risk of an allergic reaction, which, if not treated promptly, could become life threatening. You should tell your study doctor about any side effect or new health problems that develop while you are participating in this study.

**NEW INFORMATION**

You will be informed of any new findings that may affect your decision to remain in the study as they arise.

**PAYMENT FOR PARTICIPATION**

Payment will be according to the following schedule:

After visit 2 to the lab (Missoula trial): $25
Upon return from CO: $425

**BENEFITS OF PARTICIPATION**

1. The information from these tests will provide you with an accurate assessment of your aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.
2. There is no promise that you will receive any benefit as a result of taking part in this study.
3. The scientific benefit includes elucidating the effects of pharmaceutical intervention on enhancing physical performance and mitigating acute mountain sickness while at altitude.

**CONFIDENTIALITY**

1. Your records will be kept private and not be released without consent except as required by law.
2. Only the researcher and his research assistants will have access to the files.
3. Your identity will be kept confidential.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, names will not be used.
5. All data, identified only by an ID #, will be stored in our laboratory.
6. The signed consent form and information sheet will be stored in a locked cabinet separate from the data and destroyed after 10 years.

**COMPENSATION FOR INJURY**

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. *In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University’s Claim representative or University Legal Counsel.*

If you feel you have been injured by this research, including any unusual redness, swelling or drainage at the blood sampling sites you should seek medical attention and then notify Dr. Brent C. Ruby, PhD, study director at (406) 243-2117.

**VOLUNTARY PARTICIPATION AND WITHDRAWAL**

It is important that you realize that you are free to withdraw from the study at any time. As mentioned above, even if you decide to drop out of the study, you will receive compensation for all the test sessions you initiate. A copy of this consent form will be provided for you at your request. In addition, the data collected during this study will be done at no cost to you.
QUESTIONS

You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Dr. Brent C. Ruby, PhD at (406) 243-2117 (office) or (406) 396-4382. If you have any questions regarding your rights as a subject, you may contact the chair of the IRB through the University of Montana Research Office at (406) 243-6670.

STATEMENT OF CONSENT

I have read the above statements and understand the risks involved with this study. I authorize Dr. Brent C. Ruby, PhD and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Dr. Brent C. Ruby, PhD at home (406) 542-2513, cell (406) 396-4382 or at the Human Performance Laboratory (406) 243-2117.

Participant (print)  ______________________________

Signature  ______________________________

Date  ______________________________

Disclosure of Personal Health Information

My individual health information that may be used to conduct this research includes:

Age, height, weight, %body fat, VO₂ max, blood oxygen saturation, and blood markers in response to exercise/hypoxia.

I authorize Dr. Brent C. Ruby, PhD and the research staff to use my individual health information for the purpose of conducting the research project entitled “Enhancing physical performance and mitigating acute mountain sickness via pharmaceutical intervention while at altitude.”
Since I receive compensation for participating in this study, identifying information about me may be used as necessary to provide compensation.

Signature ___________________________   Date ________

STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION

During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names and physiological data will not be associated with them.

Signature ___________________________   Date ________