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Abstract

Acute high altitude (HA) exposure compromises cognitive function thus posing a significant risk to personnel safety in a HA environment, particularly when performing tasks that requires cognitive vigilance. Normal cerebral function, and thus cognitive function, is dependent upon oxygen supply. At HA there is reduced oxygen availability results in compensatory increases in cerebrovascular blood flow which may be related to nitric oxide (NO), a primary signaling molecule that acts to increase blood flow and ensure an optimal neurovascular coupling (NVC). Upon initial ascent to HA, however, there may be reductions in NO production which may play a role in acute decrements in cerebrovascular/cognitive function at HA. Dietary nitrate may serve as a means to replenish NO availability. Increasing NO in this manner could have positive effects on NVC during increased cognitive demand. **Purpose**: To investigate the effects of acute nitrate supplementation on 1) cognitive and 2) cerebrovascular function compared to an inert placebo at HA. **Hypotheses**: It was hypothesized that compared to placebo at HA, nitrate supplementation would 1) increase cognitive function, and 2) increase cerebral blood flow. **Methods**: 20 healthy men (23 ± 3 yrs, BMI 24.3 ± 3.0 $kg \cdot m^{-2}$) participated in this randomized, double-blind, crossover design study on two separate days. Following sea level (SL) cognitive/NVC testing, participants consumed either nitrate (NIT) or a NIT-depleted placebo (PLA). Participants then underwent 120 minutes of HA (11.5 ± 0.2% O_2) and all cognitive/NVC testing was repeated. NVC was assessed by measuring the change in mean middle cerebral artery (MCA) and common carotid artery (CCA) blood flow during a cognitive challenge (incongruent Stroop task) using Doppler ultrasound. Brachial artery flow-mediated dilation (FMD), salivary nitrite, and exhaled NO (in a subset of participants) were assessed as systemic proxies of NO-metabolism. A computerized testing battery was used to assess cognitive function

across a variety of cognitive domains including memory, executive function, cognitive flexibility, sensorimotor, and attention. **Results**: Salivary nitrite and exhaled NO significantly increased following supplementation at HA for NIT compared to PLA ($p < 0.05$). FMD significantly decreased and MCA and CCA blood flow increased at HA in both conditions ($p < 0.05$). Measures of NVC were unchanged at HA in both conditions. Memory performance significantly decreased at HA in both conditions ($p < 0.05$), while all other domains were unaffected. **Conclusions**: NIT significantly increased markers of NO-metabolism at HA compared to PLA. Cerebrovascular blood flow increased at HA compared to SL in both conditions at rest. NIT, however, was unable to prevent reductions in FMD or memory at HA nor was NIT able to augment NVC at HA compared to SL.

Effects of Nitrate Supplementation on Cognitive and Cerebrovascular Function at Simulated High Altitude

by

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B.S. Skidmore College, 2011

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Thesis

Submitted in partial fulfillment of the degree of Master of Science in Exercise Science

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Glossary of terms and abbreviations

Abbreviation	Term
HA	High altitude
PO_2	Partial pressure of oxygen
Hb	Hemoglobin
RT	Reaction time
NVC	Neurovascular coupling
CBF	Cerebral blood flow
MCA	Middle cerebral artery
CBFv	Cerebral blood flow velocity
TCD	Transcranial Doppler
HR	Heart rate
MAP	Mean arterial pressure
PP	Pulse pressure
AIx	Augmentation index
RBC	Red blood cell
NO	Nitric oxide
ROS	Reactive oxygen species
NOS	Nitric oxide synthase
n-n-NO	Nitrate-nitrite-nitric oxide

Chapter I: Introduction

Numerous populations are exposed to physiologically taxing high altitude (HA) environments including mountaineers,[1] HA natives,[2] aircraft pilots,[3] and military personnel.[4] Acute HA exposure compromises cognitive function across several areas; specifically, it results in visual impairment,[5] impaired complex reaction time,[6] loss of motor skills,[7] and decreased marksmanship.[8] Moreover, performance decrements cannot be attenuated with cognitive-specific training prior to ascent. These impairments pose a significant risk to personnel safety in a HA environment, particularly when performing tasks that requires cognitive vigilance.

Normal cerebral function, a critical determinant of cognitive function[9], is dependent upon oxygen supply.[10] At HA, the partial pressure of oxygen (PO_2) decreases, leading to reduced oxygen availability, and hypoxemia[10] at the expense of critical organs such as the brain. This results in a compensatory increase in cerebral blood flow (CBF),[10-12] to offset drops in blood oxygen saturation. A primary signaling molecule that may help regulate blood flow in this setting is nitric oxide (NO). NO is released from the vascular endothelium and acts to relax vascular smooth muscle, eliciting vasodilation and increased blood flow.[13] In addition to its central role as a regulator of blood flow,[14] NO ensures optimal hyperemic response to neural activity (neurovascular coupling between central and cerebrovasculature),[15] and is necessary for hypoxia-induced cerebral vasodilation.[16] Moreover, NO appears to play an integral role in adaptation to HA.[14] Specifically, NO production is elevated following acclimatization to HA,[14] and HA natives have been shown to have drastically greater levels of circulating NO products compared to lowlanders, which was further associated with increased blood flow.[17] These chronic and acclimatization-based adaptations most likely serve to increase oxygen delivery[18] and may be related to the improvement in cognitive function with acclimatization.[19,20] Some

populations, such as military fighting units or rescue teams, are often rapidly deployed to HA[4] permitting little or no time to properly acclimatize to HA-hypoxia and increase NO production. This is important since upon initial ascent there may be reductions in exhaled NO,[21] indicative of decreased NO production. This reduction in bioactive NO may play a role in acute decrements in cerebrovascular/cognitive function at HA. Finding a method to increase NO upon initial ascent to HA might present a means to positively augment blood flow, neurovascular coupling, and ultimately attenuate decreases in cognitive performance that occur with sudden acute exposure.

NO can be synthesized endogenously by NO synthases (NOS),[22] or from dietary nitrate,[23] a natural ingredient of beetroots, vegetables, and leafy greens.[24] Nitrate is subsequently reduced to circulating nitrite through reactions occurring in the saliva and stomach.[25,26] Increasing circulating plasma nitrite has been shown to increase NO formation[27] and peripheral vascular blood flow[27,28] in humans. Furthermore, bioactive levels of plasma nitrite can be attained through increased intake of dietary nitrate.[29] The NOS pathway requires the presence of oxygen, however the conversion of dietary nitrite to NO takes place preferentially in hypoxic conditions,[29] making nitrate supplementation of particular interest to populations exposed to hypoxia. Acute dietary nitrate supplementation increases arterial/muscle oxygenation during high simulated altitude (5,000m).[23] Thus, it has been proposed that increased nitrite concentration via dietary nitrate consumption could enhance NO production during neuronal activity, augmenting cerebral oxygenation and subsequent neurovascular coupling during increased cognitive demand.[30] In fact, increased dietary nitrate intake increases regional cerebral perfusion in areas involved in executive functioning (the ability to perform complex, goal-oriented tasks)[31] in older adults.[25] To date, no research has directly investigated the effects of

nitrate supplementation on cerebral perfusion and cognitive function in hypoxic conditions observed at HA. Thus, an acute nitrate supplement may be able to attenuate cognitive decrements following rapid ascent to HA in lieu of proper acclimatization and could be of value to personnel in HA hypoxic conditions.

The specific aims of the proposed study are as follows:

Aim 1: To investigate the effects of acute nitrate supplementation on cognitive function compared to an inert placebo at HA.

Hypothesis 1: It is hypothesized that compared to placebo, cognitive function will increase following acute nitrate supplementation.

Aim 2: To investigate the effects of acute nitrate supplementation on central and cerebrovascular function compared to an inert placebo at HA.

Hypothesis 2: It is hypothesized that compared to placebo, central and cerebral blood flow (CBF) will increase following nitrate supplementation.

Chapter II: Review of Literature

Exposure to high altitude (HA) is associated with a myriad of environmental and physiological challenges that can impair physical and cognitive performance and function. This ultimately compromises the health and safety of personnel exposed to HA without proper acclimatization. This review will focus on the effects of HA on oxygen kinetics, cognitive function, cerebrovascular and central hemodynamics, acclimatization to HA, and the role of nitric oxide in adaptation to HA.

High altitude effects on oxygen diffusion.

Barometric pressure decreases exponentially with increasing altitude, resulting in a decreased partial pressure of oxygen (PO_2) known as hypoxia.[10,32] PO_2 at a given altitude is calculated as (PB – 47 mmHg) x 0.2093, where PB is barometric pressure, 47 mmHg is the water vapor pressure at 37ºC, and 0.2093 is the fractional concentration of O_2 in the air (which is unchanged with altitude).[33] This equation clearly details the relationship between a decrease in barometric pressure and the resulting drop in PO_2. This altered PO_2 has severe effects on the human body because of altered O_2 cascade gradients. The movement of O_2 from the alveoli to the pulmonary capillaries and circulation occurs via passive diffusion[32] where the rate of diffusion is directly proportional to tissue cross sectional area, partial pressure gradient, and gas solubility.[34] At HA a decrease in PO_2 leads to a decreased diffusion rate into the pulmonary vasculature, this results in greater time for hemoglobin (Hb) to become fully saturated.[34] This can result in diffusion limitation at HA, where the rate of diffusion has slowed to the point that red blood cells and Hb cannot fully saturate as they pass through the pulmonary capillaries.[34] Ultimately, the reduced diffusion gradient results in hypoxemia (decreased arterial O_2

saturation), which reduces O_2 delivery to tissues and impairs cognitive function and disrupts cerebrovascular hemodynamics.[32]

High altitude effects on cognitive function.

Cognitive domains

Cognitive function is generally divided into 7 domains including: attention/concentration, language, visuospatial skills, psychomotor skills, executive functions, memory and orientation.[35] Executive function is an expansive term describing the high-level interrelated cognitive abilities, dependent on lower-level functions, which are necessary to complete goal-directed behavior.[36,37] There is some debate as to the specific components that comprise executive function, but information processing, attentional control, cognitive flexibility, and working memory have all been acknowledged as playing a role.[35-37] Description of the cognitive domains and tests that have been used to target them are displayed in Table 1.

Acute effects of high altitude on Cognitive domains

Ascent to HA results in severe impairments in cognitive function[38] with more pronounced effects occurring at more extreme hypoxia.[39] Of note, decrements occur independent of acute mountain sickness[32] and can occur within 30 min of exposure.[40] Decrements often develop in memory, motor skills, language, and executive function[32] at altitudes greater than 2000-4500 m,[5] although some decrements have been documented to occur as low as 1500 m.[41] For a brief summary table of HA effects on divisions of cognition see Table 1. The threshold to observe memory dysfunction is posited to be approximately 3,500 m.[32] There are, however, marked decreases in spatial memory in both rat models[42] and humans[43] above 5000 m. Short-term memory decreases between 3,658 m and 4,600 m in both simulated[43,44] and expedition based[45,46]

designs. Typically, memory assessment at HA has used number recitation, digit span, and digit symbol tests.[32]

Psychomotor impairment at HA often manifests as decreased motor speed and precision during complex tasks and decreases with hypoxia.[47-50] These decrements in motor skill are believed to be indirect results from HA hypoxia and are not responsible for changes in reaction time (RT) with HA.[32] RT is comprised of simple (timed response to single unvarying stimuli) and complex (timed response to multiple stimuli and respective responses) and is a measure of higher cognitive function.[6] There appears to be an inverse relationship between increasing altitude and RT performance[6,51-54] when above a threshold of 4000 m.[6] Furthermore, complex RT appears to be more affected than simple RT, as 2-choice RT does not change with ascent to 8,848 m.[49] HA exposure has been linked with impaired cognitive flexibility (as assessed using the Stroop color test) months after an ascent in world class mountain climbers. Hyperventilation-induced brain hypoxia (hyperventilation as a means to induce cerebral vasoconstriction) has also resulted in impaired cognitive flexibility at an arterial O_2 saturation of 90%, roughly equivalent to 2,500 m.[55]

Numerous experimental factors can impact cognitive function at HA such as simulated HA vs. hypobaric HA, rate of ascent, previous cognition-based training, and difficulty of the cognitive task. Expedition or transit based ascent to hypobaric hypoxic HA differs from hypobaric and normobaric simulated HA.[32] Studies using expedition-based ascent to HA have a large number of external environmental factors that may affect cognitive performance; including fatigue, dehydration, environmental temperature, rate of ascent, and sleep quality.[32] Poor sleep quality at HA has been shown to greatly affect the cognitive decline at altitude[56] and rate of ascent can alter neuropsychological performance at HA, as slower ascent may permit more

acclimatization compared to rapid ascents.[57] The brain is characterized by neural plasticity and the ability to improve cognitive functioning through training.[58,59] Populations that perform in significant operational work (such as military personnel) require greater cognitive skills than other workers, which may lead to better cognitive performance compared to their lesser developed counterparts.[60] Importantly, rather than attenuate decreases in cognitive function at HA, cognitive-training prior to HA ascent results in more substantial decrements in performance compared to untrained personnel.[60] Moreover, task difficulty plays a large role in the detection of hypoxia-induced decrements, such that tasks that require higher memory capacity[45] or greater complexity[38,50,54] are more affected by hypoxia than simpler tasks. Tasks that document response time are more useful for highlighting differences in cognitive processing speeds than non-timed tasks since performance slows at HA, a strategy that may aim to minimize mistakes.[5] This suggests that detecting changes in cognition would be most sensitive using complex time-limited tasks following a rapid ascent to an altitude greater than 4000 m.

Neurovascular coupling

Brain metabolism accounts for 20% of the body's energy and nutrients.[61] Proper brain function is dependent on continuous, adequate perfusion and oxygen delivery provided by the cerebrovasculature.[10,39,61] Blood flow and neural activity are strongly linked such that insufficient glucose and oxygen delivery to a region of the brain can result in glial cell injury or death.[15] Functional hyperemia is a compensatory increase in blood flow to brain regions where neurons are active, ensuring adequate blood flow to support neural activity.[15] As brain activity increases, blood flow must increase to meet neural metabolic demands.[62] Therefore, optimal coupling must be established between the brain and blood perfusion, a process known as neurovascular coupling (NVC).[15] The ability of the vasculature to respond to stimuli,

appropriately vasodilate and increase blood flow is dependent on endothelial function. In this manner, cognitive function is linked to the cerebrovascular endothelial function in order to support neural activity.

The cerebrovascular endothelium, neurons and non-neuronal cells combine to form a functional unit that is responsible for regulating hemodynamic NVC in addition to other interactions.[63] Since neural activity is dependent on blood flow matching neural metabolic demand, deleterious changes in vascular and endothelial function can impact NVC and thereby impair neural and cognitive function. Endothelial dysfunction has been associated with cognitive impairments,[64] with one study reporting worse flow-mediated dilation in participants with multiple-domain cognitive impairment.[65] Vascular cognitive impairment (VCI), is an umbrella term that has been proposed to represent the spectrum of cognitive decrements associated with vascular dysfunction and disease (ranging from mild cognitive impairment to dementia)[66] that profoundly affects NVC through changes in blood flow to neurons.[63]

Hypertension, a risk factor of VCI that contributes to endothelial dysfunction, is characterized by accelerated atherosclerotic development and decreased endothelium-dependent vasodilation,[67] which may be manifested in the peripheral and cerebral vasculature. Indeed, hypertension and its sequela are associated with dementia,[68] mild cognitive impairments and disorders,[69] and impaired executive function.[70] Cerebrovascular disease is linked to greater declines in cerebral blood flow (CBF)[71-73] and decreased hyperemic reserve capacity,[74] suggesting impaired NVC as the vasculature cannot adapt to the increased demands, resulting in blunted blood flow responses to neural activity.[75] This has been shown in older adults who have been reported to have depressed NVC compared to younger adults.[76-78] Contrastingly, beneficial changes in vascular function have been reported to attenuate and even reverse this process.

Specifically, as vascular function improves, as reported with exercise training, measures of cognitive function (executive function and working memory) similarly improve[79,80] at least in part through beneficial adaptations in the cerebrovasculature. In this manner, cognitive performance and neural activity are dependent on the vasculature (through NVC) for proper function and any dysfunction or abnormalities in the cerebrovasculature, resulting from environmental challenges or perturbations, may compromise the integrity of brain function.[61]

Effects of high altitude on cerebrovascular hemodynamics

Cerebral blood flow

Cerebral hemodynamic responses to HA-induced hypoxia result from two competing compensatory reactions. The development of hypoxemia at HA results in a compensatory increase in CBF[10,39,81,82] via increased flow velocity,[57] and relaxation of the cerebral arterioles and/or release of vasodilator substances.[39,81] Perfusion-based increases in oxygen delivery are necessary because there is no capillary recruitment in the cerebrovasculature, meaning increased brain metabolism is dependent on enhancing the diffusion gradient.[39] Ventilation increases with increasing altitude in order to positively augment arterial saturation of oxygen.[83,84] However, although arterial PO_2 increases, there is a concomitant decrease in arterial PCO_2, which potentiates cerebral vasoconstriction, acting to decrease CBF.[85,86] In this manner, the given CBF response to HA-hypoxia is a result of the balance between a compensatory vasodilation response, seeking to increase oxygen delivery, and vasoconstriction responding to decreased arterial PCO_2.[39,87] These competing responses can result in unaltered,[86] increased[10,39,81] or decreased[88] CBF depending on external factors such as the magnitude of hypoxia, as well as cerebrovascular and ventilatory oxygen and carbon dioxide sensitivity.[87] External stimuli such as exercise or

cognitive tasks may be required to create perturbation sufficient to quantify changes in CBF at HA.

Cerebral perfusion is largely dependent on changes in vessel diameter and blood flow velocity. It has generally been shown that CBF velocity (CBFv) increases with HA-hypoxia,[57,81,89] although this finding is not universal.[10,90] Recent data has suggested that cerebral vessels vasodilate in response to HA.[10] Wilson et al (2011) documented changes in the middle cerebral artery (MCA) during an Everest expedition to 7,930 m in addition to a simulated HA condition using magnetic resonance imaging to quantify changes in the MCA.[10] Their results demonstrated no change in CBFv during the expedition, while diameter increased drastically above 6,400 m. In the simulated HA study, MCA diameter similarly increased following 180 min at 4,400 m, although CBFv also increased compared to baseline.[10] This suggests that vasodilation plays a larger role in the response to HA-hypoxia than originally believed, although these changes in diameter were principally seen at extreme HA and after three hours of simulated-HA.[10] Moreover, the changes in MCA diameter were reversed under supplemental oxygen use, suggesting that increased CBF is a direct hypoxic effect.[10] More recently, however, CBFv was shown to increase during a 6-day HA sojourn and remain significantly elevated 6-hr post descent.[89] Which may suggest that changes in cerebral hemodynamics rely mechanisms other than vasodilation since CBF remained elevated after returning to normoxic conditions.[89]

CBFv measured via transcranial Doppler (TCD) has been widely used to document CBFv across rest, exercise, and cognitive perturbations in normal and pathological populations,[91] as well as in different environmental conditions, including HA.[10,16,57] This method assumes that diameter of cerebral vessels are relatively unchanged during different physiological stimuli, and thereby indirectly estimates blood flow.[91] It has been recently suggested that cerebral

vasodilation occurs frequently at HA to increase CBF,[10] which would not be quantifiable using TCD. Importantly, there are additional noninvasive methods to indirectly assess changes in cerebrovascular tone and altered CBF. Doppler ultrasound can be used to document changes in carotid hemodynamics, which feed into the MCA and other cerebral arteries and provide information on blood flow to the brain.[92-96]

High altitude acclimatization

Upon ascent to HA, there are decrements in both cognitive and physical function.[97] Fortunately, human physiology undergoes a series of adaptations and adjustments to compensate for the hypoxic environment.[97] Adaptations to prolonged hypoxic exposure (days to weeks) are beneficial and paramount in the ability to survive at HA, although they do not return bodily functions to sea level performance.[33] Importantly, these adaptations take time to develop before performance is positively affected. The rate of adaptation varies based on rate of ascent, peak altitude, and individual physiology.[98] Adaptations can occur within the first days to weeks or can occur from chronic exposure (such as the native populations of the Tibetan high plains). Ultimately, acclimatization-based adaptations can serve as a model to elucidate how best to design interventions intended to maintain performance during acute exposure to HA without proper acclimatization.

Oxygen transport

Initial key adaptations to HA-hypoxia pertain to augmenting oxygen saturation. Hyperventilation, achieved through deeper and more frequent breathing, increases alveolar ventilation by as much as 5-fold and is often referenced as the most important HA adaptation.[33,99] This occurs through the hypoxic ventilatory response and leads to greater blood oxygen

saturation, and a concomitant decrease in CO_2 in the blood.[97] As discussed previously, the hyperventilatory response to HA may affect cerebrovascular responses, as hypocapnic conditions can precipitate cerebral vascoconstriction.[85,86] The decrease in CO_2 causes a shift in acid-base balance, leading to respiratory alkalosis, a process by which excess CO_2 exhalation decreases hydrogen ion concentration in the blood, resulting in an increase in pH. Within a week the body compensates by lowering the bicarbonate concentration in the blood via urinary excretion, acting to balance pH while maintaining elevated ventilation and oxygen saturation.

Hematological adaptions occur in parallel to respiratory adaptations to maximize oxygen transport.[100] There is an increase in red blood cells (RBC), the primary oxygen transporter in the body, which initially results from reduction in plasma volume, but later from erythropoiesis (the production of new RBCs). Erythropoiesis can begin as early as 24-48 hours after ascent, evident by elevated plasma erythropoietin, the glycoprotein responsible for stimulating RBC production.[101] Importantly, when increased RBC production is coupled with dehydration-induced decreases in plasma volume, a frequent occurrence at HA,[102] there is a marked increase in RBC concentration[97] and blood viscosity. These changes, when combined with small shifts in the oxygen-dissociation curve, facilitate greater oxygen unloading and delivery to tissues, which improve function at HA although do not permit a full return to sea level performance.

Oxygen delivery

The effects of HA acclimatization on oxygen delivery and cardiovascular adaptations remain somewhat less clear. There are conflicting results regarding the cardiac acclimatization response, with some findings suggest decreased cardiac output[100] and others finding a return to baseline[103] or no change[104] following prolonged exposure. Ultimately, heart rate remains

elevated following acclimatization in order to compensate for the decreased plasma volume and ventricular filling time.[103]

Increased oxygen transport, elicited from the aforementioned adaptations would be expected to favor brain-tissue oxygenation and potentially attenuate decrements in cognitive performance. Indeed, a study by Pagani et al reported that acclimatized mountaineers out performed non-acclimatized participants at 5,350 m in cognitive function.[20] Notably, these results are limited by the cross-sectional design, but none the less suggest that acclimatization may improve cognitive function. Another study showed no difference in saccadic eye movement, a marker for impaired cerebral performance, following prolonged stay at very high altitudes.[1] Consistent with these findings, HA natives do not appear to have severe decrements in cognitive function. Cognitive function in adolescent HA natives has been reported to be similar to their lowland counterparts, indicating no evidence of compromised function while being tested at their native altitudes.[2] Moreover, event-related potentials in the brain electrical activity were similar between HA and lowland natives during cognitive testing.[2] Despite increased oxygen carrying capacity of the blood following acclimatization, cognitive performance still depends on NVC, and the ability of the vasculature to respond and increase blood flow to the active areas of the brain. Therefore, any increase in cognitive function with acclimatization must depend on the matching of blood flow by the vascular system to neural activity.

Role of nitric oxide in vascular adaptations

There are rather limited data on vascular responses to HA acclimatization, as much of the literature focuses on cardiorespiratory adaptations. However, some of the most insightful

research into vascular adaptations to HA has been derived from studying native populations to HA, specifically from Tibet. Tibetan HA natives have been shown to have less saturated Hb,[105] and lower concentrations of Hb and erythropoietin[106] compared to their Andean HA counterparts. These adaptations, or lack thereof, result in lower arterial oxygenation content, which would suggest their HA performance would be impaired. In fact, Tibetan's cardiovascular adaptations may target the oxygen delivery through the vascular system and blood flow's dependence on vasodilation, rather than oxygen transport.[107]

Tibetan HA natives have been reported to have extremely high levels of exhaled nitric oxide (NO),[108] a potent vasodilator and modulator of blood flow produced by the vasculature,[13] compared to lowlanders and other HA natives. This up-regulation of NO production and bioavailability likely plays a large role in increased blood flow for oxygen delivery. Indeed, rather than vasoconstrict pulmonary vessels, Tibetan HA natives display vasodilation of the pulmonary vasculature, normal blood flow, and essentially no pulmonary hypertension.[109,110] This is in contrast to the typical lowlander response to HA which includes a down-regulation of NO synthesis,[21,111] and the development of severe HA performance limitations. This enhanced NO bioavailability is related to increased systemic blood flow (indicated by forearm blood flow),[17] lung blood flow,[110] CBF during exercise,[95] and greater hyperemic responses to temporary occlusion.[112] In this manner, higher blood flow may offset the low arterial oxygen content observed in this HA population,[113] and the key adaptation may revolve around the up-regulation/ bioavailability of NO.

The decrease in NO bioavailability experienced by lowlanders during acute HA exposure is thought to be caused by reduced enzymatic production, and reaction oxygen specie (ROS) interference.[114,115] Enzymatic production depends on oxygen availability and thereby decreases

in hypoxia, while the production of ROS inactivates NO.[14,115] Importantly, levels of bioactive NO increase as acclimatization proceeds and relative function improves in lowlanders.[14] Levett et al demonstrated that plasma biomarkers of NO production (nitrate/nitrite) are increased following acclimatization, with increases evident as low as 1,300 m and with the largest gains noted following the greatest rate of ascent.[14] This suggests that NO-based adaptations to HA are not unique to Tibetan HA natives,[14] although their adaptions are to a far greater extent compared the lowlander acclimatization response. Moreover, it offers the possibility that increasing NO upon initial ascent to altitude may attenuate the initial, large decrements in performance prior to hematological adaptations that occur with acclimatization.

Nitric oxide and the nitrate-nitrite-nitric oxide pathway

Nitric oxide can be synthesized endogenously by NO synthases (NOS),[22] or from dietary nitrates via the nitrate-nitrite-nitric oxide (n-n-NO) pathway.[23] It is an autocrine and paracrine signaling molecule[29] that plays a role in regulation of endothelial function.[114] Once created, NO diffuses into the vascular smooth muscle, activating guanyl cyclase and in turn, causing relaxation of the vascular smooth muscle (vasodilation).[13,114] NO has been implicated in the systemic blood flow response to mental stress (induced via cognitive challenges),[116] as well as blood flow responses to HA,[16] hypoxic vasodilation of cerebral vessels,[16,117] and proper acclimatization/adaptation to HA,[14] as discussed previously. Importantly, research has suggested that NO plays a role in optimizing NVC.

The appropriate hyperemic vascular response to neural activity, discussed previously, is believed to occur via feed-forward mechanisms.[15] Specifically, neuron signals potentiate the release of vasoactive agents **(**Figure 1**)**. With increased neural activity there is increased synaptic

release of glutamate, which binds to neuron receptors, leading to a calcium influx, activation of neuronal NOS, and increased NO production.[15] Glutamate can also bind to active astrocytes, resulting in increased arachadonic acid formation which can increase both vasodilatory and vasoconstrictor messenger production.[15] Importantly, NO concomitantly inhibits vasoconstrictor substances that may be derived from arachadonic acid[118] in addition to its vasodilatory functions, in attempt to optimize the vascular hyperemic response. Research has demonstrated that NO has an integral role in establishing NVC. If neuronal NOS is inhibited, thereby decreasing NO bioavailability in the cerebrovasculature, functional hyperemia is decreased by 37-60% in the somatosensory cortex,[119,120] and 50-90% in the cerebellum.[121-124] NOS activity is dependent on oxygen, and NO synthesis is thus limited by oxygen availability.[15] Importantly, NOS activity and creation of NO depends on reacting L-arginine with oxygen, whereas the n-n-NO pathway does not require oxygen for reaction and, in fact, gradually increases activation as oxygen tension decreases.[29] In this manner, the n-n-NO pathway serves as a NOS-independent NO formation pathway that can increase NO production as NOS reactions are attenuated.[29] This may be of greater importance when operating in hypoxic conditions (such as HA), when NOS-dependent formation may be impaired.

Once NO has been formed, its lifetime and diffusion capabilities depend on scavenging radicals, that can quickly react with circulating NO.[29] NO oxidation to nitrate (NO_3^-) or nitrite (NO_2^-) may present a more stable form that can circulate and be converted back to NO under hypoxic conditions.[29] This suggests that nitrite may be a primary modulator of hypoxic vasodilation and blood flow at HA. Research has demonstrated that increased plasma nitrite is associated with greater forearm blood flow,[125] peripheral blood flow,[27,28] and NO formation.[27] Nitrate/nitrite must be reduced to the bioactive from of NO and research suggests that Hb may

act as an allosterically-regulated nitrite reductase, generating NO and contributing to hypoxic signaling and vasodilation.[27,126,127] Moreover, it appears that maximal nitrite reduction rates occur when Hb is 40-60% saturated with oxygen,[127] meaning that if plasma nitrite is available in such conditions there will be increased NO formation and vasodilation.

Nitrates can be readily consumed through diet, as they are natural ingredients in of beetroots, vegetables, and leafy greens,[24] and can increase bioactive levels of nitrite adequate for increased NO formation.[29] Ingested nitrate is quickly absorbed from the upper gastrointestinal tract[29] and is transported to the salivary glands where it is reduced to nitrite via anaerobic bacteria before re-entering the gut.[128] Some of the nitrite is converted to nitrous acid, which dissociates to form NO,[129,130] while some nitrite is absorbed through the intestines and enters circulation.[26] Indeed, following a dietary nitrate bolus, plasma, salivary and urine levels of nitrite increase, indicating greater storage pools for NO formation.[131] Increased nitrite could aid in the vascular adjustments attempting to increase oxygen delivery during acute exposure to a hypoxic environment through increased NO formation.

High dietary nitrate intake in older adults has been shown to increase regional cerebral perfusion to the frontal cortex compared to a low dietary nitrate group.[25] Moreover, nitrate supplementation has been shown to result in shorter hyperemic lag times to visual stimulation, indicative of improved NVC in healthy males.[30] At both low and HA, administration of a sublingual NO donor resulted in cerebral vasodilation (evident through decreased CBFv) in two different HA native populations.[16] Dietary nitrate consumed prior to a rapid ascent to 5000 m resulted in significantly higher arterial/muscle oxygenation during submaximal and maximal exercise compared to placebo.[23] This study concurrently noted no change cerebral oxygenation and estimated flow which may be a direct result of the exercise stimulus. Specifically, exercise

at HA causes a marked increase in ventilation, that concomitantly decreases the partial pressure of CO_2, causing vasoconstriction of cerebral vessels. Therefore, the insignificant changes in cerebral perfusion may have resulted from an intense, compensatory reaction to vasoconstrict vessels. The effect of nitrate supplementation on cognitive responses to acute HA exposure has yet to be elucidated.

Proposed study

Dietary nitrate supplementation may serve as a means to mimic Tibetan HA natives' adaptations to acute HA-hypoxia prior to compensatory acclimatization. Tibetan natives accommodate large changes in blood flow and oxygen delivery through increased NO bioavailability, rather than up-regulation of oxygen transport.[108] Increased nitrate availability during HA exposure may potentially reduce hypoxic performance decrements. High concentration of nitrates would result in greater ability of the n-n-NO pathway to augment NO formation, especially in the face of hypoxic deactivation of the NOS pathway.[29] This would in turn act to increase vasodilation, increasing blood flow[16,117] and oxygen delivery, as well as optimizing NVC by facilitating more rapid hyperemic responses to neural activity.[30] In this manner, nitrate supplementation could act to improve or attenuate decrements in cognitive performance following rapid ascent to HA. Therefore, the purpose of this study is to document the effects of acute nitrate supplementation compared to a placebo on 1) cognitive function at HA and 2) cerebrovascular function at HA. It is hypothesized that cognitive function will improve and that cerebrovascular blood flow will increase compared to the placebo at HA.

Chapter III: Methodology

Participants

20 Recreationally active men, age 18-30 yrs, were recruited from the local University community for this randomized, double-blind, crossover-design study. Exclusion criteria included self-reported (health history questionnaire) smoking, hypertension, diabetes mellitus, hyperlipidemia, pulmonary disease, renal disease, neurological disease, or peripheral artery disease. All participants provided written informed consent prior to study initiation. Testing was conducted at the same time of day in a temperature-controlled laboratory. Participants were instructed to fast for $\geq$ 3 hours and avoid vigorous exercise and avoid consuming caffeine and alcohol the day of testing. Additionally, participants were given a list of high-nitrate foods to avoid for the 2 days prior to experimental testing. Height and weight was assessed via wall-mounted ruler and electronic scale, respectively, and body composition was estimated via air displacement plethysmography (BodPod; COSMED, Concord, CA).

Design

Participants rested in the supine position for 10 minutes upon arrival before baseline (normoxic) vascular and cognitive measures were assessed. Participants consumed either a) a 0.45 g nitrate bolus (Beet It Sports Shot; NIT) or b) an inert placebo (PLA) prior to exposure to HA in a randomized order. This single dose of nitrate has been previously reported to significantly increase plasma nitrite concentration at HA (2,500 m).[26] Participants remained at HA for 105 minutes before undergoing HA vascular and cognitive testing (Figure 1). This timeline was chosen so that cognitive testing would occur at approximately 2 hrs post-nitrate ingestion since previous literature suggests peak plasma nitrite levels occur between 2-3 hrs post-

ingestion (and remain elevated for approximately 2 additional hours).[25,132] HA testing was conducted in a normobaric hypoxic chamber (Hypoxico Systems) at simulated altitude conditions approximately equivalent to 4,500 m (achieved by lowering the fractional concentration of inspired oxygen (FiO_2) to approximately 11.5 ± 0.2% O_2). Oxygen concentration was measured using an oxygen monitor (PureAire Monitoring Systems Inc., Lake Zurich, IL) secured inside the hypoxic chamber. This altitude was chosen based on previous research that established 4000-5000 m as the critical altitude for changes in cognitive function.[133]

Vascular Measures

Arterial Hemoglobin Concentration and Saturation

Arterial oxygen saturation was assessed using a reflectance pulse oximeter placed forehead (Nonin Medical, Plymouth, MN) at baseline, and during vascular testing. The forehead reflectance sensor was secured using adhesive and a flexible head band to the forehead just above the brow. Hemoglobin concentration was assessed at baseline via finger-stick blood sample and microcuvette (The Hemocue Hemoglobin System, Hb201+; Angelholm, Sweden).

End-tidal CO_2

End-tidal CO_2 ($EtCO_2$) was measured (Nellcor OxiMax, Covidien, Mansfield, MA) at sea level and HA baseline with sampling lines secured directly under the nostrils. Participants rested for 10 minutes prior to assessment to ensure resting values. Data was collected over a 5-minute period with triplicate measures taken during minutes 2-4 and averaged.

Nitrite and Exhaled Nitric Oxide

Salivary nitrite was qualitatively assessed using salivary test strips (Berkeley Test, Berkeley, CA). A salivary absorbent pad was placed under the tongue for 3-5 s and then pressed against a reagent strip. The resulting color was compared to a colored scale to qualitatively assess nitrite availability. Exhaled NO was measured in a subset of participants using a NIOX MINO (Aerocrine AB Solna, Sweden). This method has been described in detail previously.[134] Briefly, participants were instructed to empty their lungs and fully inhale through the NIOX MINO mouthpiece before beginning a paced exhalation (approximately 5 seconds). The participants exhalation was guided by a visual cue program that provided immediate feedback allowing the participant to adjust the exhalation force to the target level (i.e. too forceful an exhalation would push the needle farther right on the computerized display dial). Nitrite/NO availability was assessed at sea level and approximately 2 hours after nitrate ingestion/HA exposure. This timeline was chosen based on previous findings that report nitrite/NO availability to peak approximately 2 hours post-ingestion.[25,132]

Brachial Blood Pressure

Systolic blood pressure (SBP) and diastolic brachial blood pressure (DBP) were measured prior to each set of vascular measures (baseline, HA) using a validated, automated oscillometric cuff (EW3109, Panasonic Electric Works, Secaucus NJ). Pressures were taken in duplicate and averaged. If values were different by more than 5 mmHg a third measure was obtained and the average of the 2 closest measures were used for subsequent analyses.

Doppler Ultrasonography

Images of the left common carotid artery (CCA) and brachial artery (BA) were obtained using Doppler ultrasound (ProSound α7, Aloka, Tokyo, Japan) and 7.5-10.0 mHz linear-array

probe. The CCA was imaged distal to the carotid bulb. CCA and BA intima-media thickness (IMT) were assessed using a longitudinal view with both near wall and far wall lumen-IMT boundaries visible. Wall thickness was measured across a 5 mm region of interest via semi-automated digital calipers during systole and diastole (indicated by the R-wave and end of the T-wave from simultaneous ECG gating). The distance from the lumen-intima interface to the media-adventitia interface was measured as the IMT. IMT was measured across the cardiac cycle and at each time point given findings that 1) IMT may change during the cardiac cycle; and 2) IMT may be altered with changes in vascular tone.[135] Systolic and diastolic diameters were measured from inside the near-wall IMT to far-wall IMT. Mean blood velocities (Vm) were measured using Doppler-ultrasound and calculated as: $Vm = \int V(t)\, dt/FT$, where $\int V(t)\, dt$ is the velocity-time integral of the velocity waveform and FT is flow time. Flow and shear rate were calculated as π x (1/3 systolic radius + 2/3 diastolic radius)^2 x Vm x 60 and 4 x (Vm/systolic diameter), respectively. Pulsatility index (PI) was calculated with a semi-automated flow tracing software using the following equation: $(V_s\text{-}V_d)$/mean V, where V_s is the peak systolic velocity, V_d diastolic velocity and mean V the mean velocity.

Wave intensity analysis (WIA) combined with eTracking was used to derive forward and reflected wave intensity as measures related to pulsatile cerebrovascular burden and arterial stiffness in the CCA. WIA was performed immediately after carotid applanation tonometry on the left CCA. The distance from the near wall to far wall lumen-intima interface was continuously traced using eTracking software, creating a distension waveform almost identical to pressure waveforms.[136,137] WIA distension waveforms were calibrated against carotid systolic and diastolic pressures obtained via applanation tonometry described below. Flow waveforms were measured using range gated color Doppler signals averaged along the Doppler beam. An

insonation angle $\leq$ 60° was maintained for all measures and sample volume was manually adjusted to encompass the entire vessel. At least 8 carotid waveforms were averaged to gain a representative average waveform. Wave intensity was calculated using time derivatives of blood pressure (P) and velocity (U), where wave intensity = (dP/dt x dU/dt); the area under the dP/dt x dU/dt curve represents the energy transfer of the wave.[138] W_1 represents a forward compression wave produced by the left ventricle that can travel into the cerebral circulation via the CCA which acts to accelerate flow and increase pressure. The negative area (NA) occurring immediately after W_1 is a backward travelling compression wave due to reflected waves from the periphery (cerebral circulation) that decelerate flow but increase pressure. Measures of CCA wave reflection (i.e. NA) have been reported to be related to altered cerebrovascular tone, as changes in cerebral resistance would affect the magnitude and timing of pressure waves being reflected from the brain as they travel down the CCA.[92,93] The time interval between the R-wave on the ECG and W_1 is analogous to the pre-ejection period[136] and has been used as a proxy of peripheral sympathetic activation.[139]

Arterial stiffness measures include beta stiffness index (β), and Peterson's pressure-strain elastic modulus (Ep) and were calculated as:

$$\beta = \ln(P_{Max}/P_{Min})/[(D_{Max} - D_{Min})/D_{Min}]$$

$$Ep = (P_{Max} - P_{Min})/[(D_{Max} - D_{Min})/ D_{Min}]$$

where P and D correspond to pressure and diameter respectively, and Max and Min refer to maximum (systolic) and minimum (diastolic) values during the cardiac cycle.

Flow mediated dilation

BA flow mediated dilation (FMD) was assessed in the non-dominant arm during two separate protocols; cuff occlusion and mental stress. BA FMD has been previously reported to be a largely endothelial and nitric oxide-dependent process,[140] with impaired vasodilation occurring from impaired/inhibited nitric oxide synthesis (as occurs in hypoxic conditions or drug blockade). The BA was imaged using ultrasonography described above with diameter and velocities measured as previously described. For the cuff-occlusion method, blood flow was occluded using a rapid inflator cuff (Hokanson, Bellevue, WA,) placed just below the olecranon process, inflated to suprasystolic pressure (>200 mmHg) for 5-minutes. Blood flow velocity was assessed for the first 30 seconds following cuff deflation to obtain peak shear rates. BA diameter was assessed using semi-continuous image capturing synced to the QRS complex from simultaneous ECG gating until two minutes post cuff deflation. Additionally, FMD was also measured during the Stroop task (described below) to measure mental stress BA vasodilation, which has previously been reported as primarily NO-mediated.[116] FMD was calculated as peak percent change from baseline for both methods.

Carotid Blood Pressure Waveform Analysis

Carotid pressure waveforms were obtained from a 10 s epoch and measured in duplicate using applanation tonometry (SphygmoCor, AtCor Medical, Syndey, Australia) on the left CCA. Carotid pressure waveforms were calibrated to brachial MAP and DBP. Pulse pressure (PP) was calculated as SBP minus DBP. Augmentation index was calculated as the difference between the early- and late systolic peaks of the pressure waveforms to the total PP expressed as a percentage ($P2 - P1/PP \times 100$) and standardized to a heart rate of 75 beats per min (AIx75).

Cerebral Blood Flow

Middle cerebral artery (MCA) blood flow velocity was assessed using a 2-mHz transcranial Doppler (TCD) ultrasound probe (DWL Doppler Box-X, Compumedics, Germany) applied to the left temporal window. Mean blood flow velocity (MnV) and pulsatility index were measured at a depth of 50-65mm, as is commonly reported for MCA measurements.[141,142] All repeated measurements within each participant were taken at the same depth and position to ensure recapture of the same cerebral artery. The envelope of the velocity spectrum and mean velocity was calculated by a standard algorithm implemented on the instrument with use of a fast Fourier transform. MCA PI was calculated via an automated waveform tracking function using the same equation described for CCA PI. Cerebrovascular conductance (CVC) was calculated as CVC = V_{mean}/MAP.

Vascular Reactivity to Mental Stress

A computerized, modified incongruent Stroop color-word interference task (E-Prime, Psychology Software Tools Inc, Sharpsburg PA) was used as a means to manipulate cognitive load. This task has been used previously in HA cognitive,[32] and cardiovascular stress research.[143-145] All participants were familiarized with the Stroop task prior to experimental testing in order to control for learning effects. The Stroop task was completed in the supine position with the head tilted slightly back, thereby optimizing the imaging window of the carotid artery. The viewing display for sea level testing was a specialized wall-mounted 107-cm flat screen television that extended over the participant. Font was displayed approximately 102-cm above the participant with 3.0-cm font on a black background. For HA testing, the Stroop task was projected onto the ceiling of the chamber using a computer-interfaced projector (INFO) that

displayed the task approximately 160-cm above the participant with 4.5-cm font. Despite the task being displayed farther away from the participant at HA, the ratio between viewing distance (cm) and font size (cm) was comparable to sea level (34:1 sea level, 36:1 HA).

Each trial began with participants presented with a white crosshair in the center of the viewing window for approximately 3-seconds. A target word was displayed in incongruous colors (e.g. the word "blue" written in the color red; Figure 2), with four names of response colors presented similarly (e.g. the word "red" written in the color blue). The task was to use a response clicker to identify the color that the target word was displayed in as fast as possible. The response colors (1-4) corresponded to the remote clicker buttons (1-4) which the participant manipulated using the digits on their dominant side (index finger – pinky finger). This task lasted 4-minutes in duration, which has been previously been shown to elicit marked changes in HR and BP.[145]

Participant's identification accuracy was titrated to 60% in order to produce equivalent hemodynamic responses across sea level and HA stroop testing. This was achieved via manipulation of the inter-trial timing intervals (ITI). For every three consecutive trials answered correctly the ITI was decreased by 300 ms (shortest ITI of 400 ms). Similarly, three consecutive missed trials would increase the ITI by 300 ms (longest ITI of 5,000 ms). If the participant did not respond in time, a large "TOO LATE!" prompt was displayed before the next trial was displayed. Percent correct and mean reaction times (RT) for correct response were recorded for analysis. This test has been previously used as a mental stressor, and measure of executive function.

Vascular measures, described above (carotid tonometry, WIA, CBF), were also assessed during the 4-minute Stroop task. Previous data from this laboratory (unpublished) has revealed that there are no significant differences between primary outcome measures across three time

points during the Stroop task. Thus, vascular measures were assessed once during the Stroop task in the following order; carotid blood pressure, carotid blood flow/WIA, and mental stress-mediated brachial vasodilation beginning 30-seconds after task initiation. CBF was averaged across two measurements during the task. This provides a novel means of performing vascular measures while concomitantly manipulating cerebrovascular reactivity. Psychological stress from cognitive tasks causes arteries to dilate. Change in cerebrovascular blood flow measured during increased brain activity with sensori-motor/cognitive stimulation has been reported to reflect changes in cerebral metabolism and was used as a measure of neurovascular coupling.[146] Additionally, mental stress-mediated dilation of the CCA was used as a measure of CCA endothelial function.[116,147-149] Cerebrovascular reactivity metrics were calculated as percent change from baseline.

The Student Opinion Scale was administered after each Stroop task to measure participant motivation for each trial (two subscales: [a] the importance of doing well on the task; [b] perceived degree of effort/mental taxation put forth to complete the task). Scores for each subscale can vary from 5-25, with higher scores indicative of full effort/engagement to do well.

Cognitive Measures

Computer-based cognitive assessment

Additional measures of cognitive function were assessed through a computer-based program (WebNeuro; Brain Resource, San Francisco CA). The cognitive tests have been described in detail previously,[150] brief summaries of the tests are provided below.

Sensorimotor domains

Simple motor tapping test. Participants were required to tap the space bar on the keyboard with their dominant index finger as fast as possible for 30 sec. The total number of taps was recorded.

Choice reaction time test. Participants were required to use the left/right arrow keys to respond as fast as possible to two target circles that were illuminated in pseudorandom sequence over a series of trials. Trials were administered with a random delay of 2–4 sec between trials. Mean RT was recorded from the trials.

Memory Domain

Memory recognition/verbal learning task. 20 words were presented for memorization and later recognition from memory. The list contained 20 concrete words from the English language (matched for word length and frequency). The list was repeated four times. After each trial, the participant was instructed to recognize as many words as possible by deciphering between 20 sets of words on the screen. In each set, one word was correct and the other 2 were distracter words. Approximately 10-minutes after the fourth trial (6 test batteries), a delayed memory recognition trial was completed. The number of words correctly recognized during the four trials and memory recognition trial were averaged and used to calculate verbal memory index[151] (immediate verbal memory recognition + delayed verbal memory recognition), and verbal intrusion index (immediate verbal memory intrusions + delayed verbal memory intrusions).

Social Cognition Domain

Emotion perception test. Participants were presented with a succession of faces with diverse emotional expressions in order to test emotion recognition. The mouse was used to identify the

emotion that best described the facial expression by clicking on one of 6 emotion words below the image (happy, sad, fear, disgust, neutral, angry) as quickly as possible. The total number of correct responses, and reaction time was recorded. Approximately 10 minutes after completion, the participant was asked to recall the faces they had been presented before. Target faces (from the previous trial) were presented next to a new face. The participant was instructed to select the face that they have been presented with before by clicking with the mouse as fast as possible. The immediate and delayed emotion recognition accuracy scores were summed, similar to memory task performance, to form the emotion recognition index (out of a perfect score of 200), and RT's were averaged for analysis purposes.

Attention Domain

Digit span test. Participants were presented with a series of digits, separated by a one second intervals. The participant was required to enter the digits in the correct forward order using the mouse and a number pad displayed on the screen. The number of digits in each sequence increased from 3 to 7 with the outcome being the maximum number of digits the participant correctly recalled.

Continuous performance test. To assess sustained attention, a series of letters similar in appearance (B, C, D, or G) were presented on the screen (200 msec), separated by 2.5 sec intervals. The participant was instructed to press the space bar as fast as possible if the same letter appeared twice in a row. There were 125 stimuli presented in total (85 non-target letters, 20 target repeated letters). The number of errors and false positives were recorded.

Executive Function Domain

Switching of attention test. This is a computerized adaptation of the "Trail Making Test" Part B[152] where the participant was presented with a distribution of 13 numbers (1–13) and 12 letters (A–L) on the screen. The mouse was used to click on the circles for numbers and letters in and alternating, ascending sequence (e.g. 1, A, 2, B, 3, C etc.). Each correct number/letter that selected was connected to the preceding number by a line. This task aims to assess the ability to switch attention between mental tasks (number versus letter sequence). The time to completion was recorded.

Verbal interference test. This task is similar to the Stroop described above and is intended to assess the ability to inhibit automatic and irrelevant responses. A target color word (red, yellow, green, and blue) was presented one at a time. Below each target word there were four possible responses displayed in black and in fixed format. The first part of this task required the participant to identify the name of each word, ignoring the color of the word, as quickly as possible. The second portion required the participant to name the color of each word as quickly as possible. The number of correct responses in the minute allotted for each test was recorded.

Maze test. The participant was presented with an 8 x 8 grid of circles on the screen. The goal of the task was to correctly identify the hidden path through the grid, from the start point (bottom of the grid) to the end point (top of the grid). Grid navigation was controlled via pressing the arrow keys (up, down, left, right). An incorrect move was denoted by a red cross at the bottom of the screen, a green tick signified a correct move. The task aims to quantify how quickly the correct route is learned and the ability to remember that route. A single maze was presented until it was completed twice without error. The total maze time was recorded. This task measures aspects of executive function and memory through requiring the correct path to be repeated without errors.

Go–no-go test. Participants were instructed to press spacebar as quickly as possible every time the word "press" was displayed in green (go) and not press spacebar if it was colored red (no-go). Green was repeated frequently and red repeated less frequently. Stimuli were repetitively presented approximately once per second. This requires inhibition of responses when the target is colored red, thereby assessing the capacity to suppress automatic responses (known as inhibition). This task measured errors of commission and omission, rate of target detection, and response time.

Statistical analyses

Averaging effect size values from previous literature for cerebral blood flow (0.65)[81], reaction time (0.48)[60] and memory (0.89)[7] at HA suggested an effect size of 0.67. Therefore, for a power of 0.80 with alpha set as 0.05 for a two-tailed T-test, approximately 20 participants was determined to be sufficient to observe similar changes in blood flow and cognitive function at HA.

All data are presented as mean ± standard deviation. Normality of distribution for variables was assessed qualitatively using histograms and Q-Q plots as well as quantitatively using the Shapiro-Wilk test. The effect of altitude exposure was tested using paired T-tests between SL and HA. The effect of NIT was tested using paired T-tests between NIT and PLA at HA. Significance was set *a priori* at $p < 0.05$. Stroop RT's greater than 2.5 standard deviations above or below the mean RT were removed as outliers prior to analyses. Reactivity scores (absolute Δ) were calculated as mental-stress – baseline, for each treatment (PLA, NIT) and condition (SL, HA).

Chapter IV: Results

Participants were young men (23 ± 3 yrs) in overall good health (BMI 24.6 ± 2.8 $kg \cdot m^{-2}$, body fat 13.3 ± 6.8%, hematocrit 43 ± 3%, hemoglobin 14.7 ± 1.5 g/dL; Table 3). Twenty-four participants were recruited for this study: two participants were lost to follow-up and of the 22 remaining, 21 completed the trials. One participant experienced a syncopal episode upon ascent to HA and could not complete the study. One participant was excluded from data analysis due to excessive time between trials (>4 weeks), leaving 20 participants with for final analyses (Figure 2). The duration of hypoxic exposure (165 ± 8 min, PLA; 161 ± 8 min, NIT) and percent oxygen in the hypoxic chamber (11.6 ± 0.1%, PLA; 11.7 ± 0.1%, NIT) were not significantly different between treatments (PLA vs NIT; Table 4). Participants had similar measures of hematocrit and hemoglobin at baseline between both PLA and NIT treatments. HA exposure resulted in similar significant decreases in SaO_2 and ET-CO_2 in both PLA and NIT treatments compared to SL ($p < 0.05$). Symptoms of AMS were greater for NIT (3 ± 2) compared to PLA (2 ± 2) after approximately 110 min of hypoxia ($p = 0.053$), although these differences subsided after approximately 137 min of hypoxia ($p > 0.05$). Salivary nitrite was significantly greater at HA for NIT compared to PLA (Table 11; $p < 0.05$). Likewise, exhaled NO (collected in a subset of participants, $n = 9$) was significantly greater at HA for NIT compared to PLA ($p < 0.05$). In combination, these data indicate that levels of nitrate/nitrite/NO bioavailability were different between treatments at HA.

Effect of high altitude on cognitive function

There were no differences in cognitive function at SL baseline between PLA and NIT treatments ($p > 0.05$). A significant effect of HA was detected within the memory and

information processing domains (Table 5). Memory recognition was significantly lower at HA compared to SL in both PLA and NIT treatments ($p < 0.05$). This was driven by significantly greater intrusion errors and significantly lower memory recognition performance in both PLA and NIT treatments at HA ($p < 0.05$). Information processing performance (accuracy and RT) during the verbal interference task was significantly improved in both PLA and NIT treatments at HA compared to SL ($p < 0.05$). Emotion recognition index was significantly lower in both PLA and NIT treatments at HA compared to SL ($p < 0.05$). Cognitive performance in the remaining cognitive domains were not affected by HA exposure in either PLA or NIT treatments ($p > 0.05$). There were no differences in cognitive function between PLA and NIT at HA ($p > 0.05$), indicating NIT supplementation was not effective in altering cognitive function at HA.

Effect of high altitude on neurovascular coupling

By design, there were no differences in percent correct and percent incorrect between treatments (PLA vs NIT) or conditions (SL vs HA) during the Stroop protocol (Table 6). This was achieved by adjusting inter-trial intervals based on task performance to elicit ≈60% accuracy (described in the methodology). Additionally, the total number of questions answered, and mean reaction times were similar between both treatments at SL and HA ($p > 0.05$). Qualitative data on mood state self-reported via questionnaire suggested that participants in both PLA and NIT treatments felt they exerted less effort, had less control, and were less happy during the mental-stress protocol at HA compared to SL ($p < 0.05$). There were no differences in perceived arousal or importance of the Stroop task between PLA or NIT treatments or between SL and HA ($p > 0.05$).

Cerebrovascular reactivity scores during mental stress were calculated as a measure of NVC during a cognitive perturbation. For both PLA and NIT treatments, cerebral reactivity to mental stress (ΔPI, ΔmV, ΔConductance; Table 7), were not different at HA compared to SL ($p > 0.05$). Carotid diameter reactivity during mental-stress was significantly lower in both PLA and NIT treatments at HA compared to SL ($p < 0.05$); the carotid artery dilated less during mental stress at HA compared to SL. There were no differences in carotid blood pressure, blood flow, blood flow pulsatility, or stiffness reactivity to mental stress with ascent to HA compared to SL ($p > 0.05$; Table 8). Likewise, measures of wave intensity (W_1, NA), global wave reflection (AIX75), and carotid blood pressure changed similarly during mental-stress at SL and HA in both PLA and NIT treatments ($p > 0.05$). There were no significant differences in cerebrovascular reactivity to mental-stress at HA between NIT and PLA treatments ($p > 0.05$), indicating NIT did not significantly alter NVC at HA compared to PLA.

Effect of high altitude on cerebrovascular and peripheral vascular function at baseline

There were no differences in cerebrovascular or vascular function between PLA or NIT treatments at SL baseline ($p > 0.05$). Cerebral conductance and cerebral blood flow pulsatility in both PLA and NIT treatments were not different at HA compared to SL ($p > 0.05$; Table 9). Mean MCA blood flow velocity for both PLA and NIT treatments was greater at HA compared to SL ($p < 0.05$).

Carotid DBP in both PLA and NIT treatments was greater at HA compared to SL ($p < 0.05$; Table 10). SBP and MAP were similar in both treatments at HA and SL ($p > 0.05$). Mean carotid artery diameter and carotid blood flow were greater in both PLA and NIT treatments at HA compared to SL ($p < 0.05$). Carotid stiffness tended to be lower in both PLA and NIT

treatments at HA compared to SL but this was not significant (β-stiffness, $p = 0.051$; Ep, $p = 0.20$). Carotid blood flow pulsatility, measures of wave reflection (NA, AIX75), and forward wave magnitude (W_1) were not different at HA compared to SL. Brachial FMD was significantly lower in both PLA and NIT at HA compared to SL ($p < 0.05$; Table 11). There were no differences in reactive hyperemia stimulus, assessed as area under the hyperemic curve for PLA and NIT at HA compared to SL. There were no significant differences in peripheral or cerebrovascular measures between PLA and NIT at HA ($p > 0.05$), indicating NIT supplementation did not alter vascular responses to hypoxia.

Chapter V: Discussion

This study investigated the effect of acute dietary nitrate supplementation in the form of beetroot juice on cerebrovascular and cognitive function at HA. The overarching hypothesis was that nitrate supplementation would result in greater NO formation and act to increase vasodilation, blood flow, and oxygen delivery to the cerebrovasculature, thereby optimizing NVC and attenuating decrements in cognitive performance following rapid ascent to HA. The primary findings of the study were as follows: although acute nitrate supplementation improved markers of NO metabolism (i.e. increased salivary nitrate and exhaled NO), acute nitrate supplementation 1) did not prevent reductions in cognitive function at HA; 2) did not prevent reductions in NO-mediated vascular reactivity at HA; 3) did not augment carotid or cerebral blood flow at HA. Thus, targeting NO metabolism with acute dietary nitrate supplementation may not be efficacious in maintaining cognitive or cerebrovascular function at HA in young, healthy men.

Ascent to HA and the resulting hypoxemia impairs cognitive function,[38] with more pronounced effects occurring at more severe altitudes.[39] This study was conducted at a simulated altitude of ≈4,700m based on previous reports that the critical altitude to observe changes in cognitive function is between 4,000-5,000m,[133] although some studies have observed changes well below this threshold.[3,41,153] The current study observed significant decreases in memory function following approximately 2.5 hours of hypoxia. Specifically, there were reductions in verbal memory and intrusion indexes, resulting from decrements in immediate and delayed memory accuracy and greater error rates, respectively. These decrements in the memory domain are consistent with previous reports[6,7,52,60,154,155] across a variety of altitudes ranging from 2,800m[153] to 9,449m.[154] There was also significant dysfunction with regards to emotion

recognition. Emotion recognition has not previously been assessed at HA, but has recently been reported to be fundamentally related to general cognition and may play an integral role in the organization of information processing.[156] Other domains of cognitive function may not be affected by HA. Performance on simple tasks, such as 2-choice RT and finger tapping, is maintained at HA below 6,000m.[32] Indeed, we observed no significant changes in finger tapping speed, choice RT, or go-no-go tasks. We also observed no effect of HA on verbal learning rate and executive function and this too is consistent with several previous reports.[157-159] Recent data, obtained after 30-minutes at 5,334m, indicated no significant differences in performance or complex RT during the Stroop task,[160] consistent with our findings. This may suggest that executive function as a domain may not be sensitive to the acute hypoxia,[161] or perhaps requires longer exposure or more extreme hypoxia to observe effects. Taken together and these findings confirm general cognitive decrements in select domains under hypoxic conditions.

Proper cognitive function requires continuous, adequate perfusion and oxygen delivery provided by the cerebrovasculature.[10,39,61] NVC is a compensatory increase in blood flow to brain regions where neurons are active, ensuring adequate blood flow to support neural activity during cognitive engagement.[15] The ability of the vasculature to respond to stimuli, appropriately vasodilate and increase blood flow is partially dependent on NO, a vasoactive metabolite released from the endothelium that causes vascular smooth muscle relaxation and vasodilation.[162] There is a decrease in NO bioavailability during acute HA exposure and this may impair the vasculatures ability to vasodilate, thereby affecting NVC.[114,115] In support of this, our study documented reductions in peripheral endothelial function (brachial FMD) at HA compared to SL, consistent with one recent publication.[163] The carotid artery also dilated significantly less during mental-stress at HA compared to SL in both treatments, suggesting

carotid endothelial dysfunction.[164] Thus the goal of this study was to enhance NO availability during cognitive tasks via acute consumption of dietary nitrate as a means of improving NVC and cognitive function at HA. Participants consumed a 0.45 g nitrate bolus, a dose which has previously been used in HA research and reported to significantly increase plasma nitrate and nitrite.[26] Under normoxic conditions, nitrate ingestion has been reported to increase regional cerebral perfusion in older adults,[25] and reduce hyperemic lag times during visual stimulation,[30] indicative of improved NVC. Additionally, normoxic data would suggest that nitrate supplementation can acutely reduce blood pressure,[165] and increase brain perfusion[25] (although this is not a universal finding).[30,166] Using semi-quantitative measurements of salivary nitrite, we documented significant increases in nitrite approximately 137 minutes after ingestion and HA exposure. This finding was corroborated with measures of exhaled NO in a subset of participants (n = 9). Acute beetroot juice consumption significantly increased nitrite bioavailability and exhaled NO, although it was not able to prevent reductions in cognitive function that occurred at HA, nor was it able to prevent reductions in NO-mediated vascular reactivity or augment NVC.

Despite aforementioned reductions in vascular endothelial function at HA, the results of this study suggest that NVC was largely maintained in this hypoxic setting. Blood flow is ultimately determined by changes in vessel diameter and/or flow velocity with optimal flow delivery (i.e. laminar flow versus pulsatile flow) being affected by regional vascular stiffness. Impaired mental-stress-mediated carotid dilation did not attenuate carotid or cerebral blood flow responsiveness during mental-stress since blood flow reactivity was similar at SL and HA between treatments. Similar mental-stress vascular-hemodynamic reactivity scores were also observed for carotid stiffness (β-stiffness, Ep) and cerebral vascular tone as indicated by similar

changes in carotid wave reflections[92,93] (AIX75, W_1, NA) at SL and HA. Our data revealed a trend ($p = 0.062$) for attenuated reductions in carotid mean blood flow velocities during mental-stress. That is, although flow velocity was reduced during mental-stress, it was reduced less at HA. Slightly higher flow velocities may have compensated for the reduced dilation in maintaining carotid flow and NVC. Nearly 80% of the common carotid artery blood flow feeds the internal carotid, which in turn provides approximately 80% of the blood supply to the brain at rest.[167] The carotid reactive hyperemia would beneficially direct blood flow to the cerebral vessels and maintain oxygen delivery to the brain in hypoxic conditions. Indeed, the carotid hyperemic response likely fed upstream to the cerebral vessels (i.e. increased CBFv), consistent with previous findings.[57,81,89]

One reason NVC may have been maintained at HA may be due to compensatory blood flow augmentation during the initial hypoxic exposure. Oxygen availability is a fundamental factor in determining blood flow to target organs such as skeletal muscle or the brain. Hypoxic conditions result in a hyperemic response,[168] the degree of which is proportional to the degree of hypoxemia with arterial oxygen content as the primary factor eliciting compensatory dilation[169] and augmented blood flow.[170] Ultimately, the compensatory vasodilation ensures oxygen delivery is matched to demand in the face of arterial hypoxemia.[169] We noted significant increases in carotid artery dilation and concomitantly increased blood flow during baseline at HA in PLA and NIT treatments. These findings are congruent with recent, comprehensive studies by Lewis et al. (2014) aiming to document the acute effects of hypoxia on measures of peripheral vascular[163] and cerebrovascular function at HA.[171] In line with our findings, they reported significant increases in carotid dilation under both normobaric[171] and hypobaric hypoxia.[163] Although Lewis et. al (2014) assessed carotid function 72-96 hr after ascending to 5,050m during

an expedition,[163] our study was conducted using an acute bout of hypoxia in a normobaric setting, without potential confounding variables (i.e. fatigue, dehydration or disturbed sleep) and noted similar vascular changes that were documented under normobaric hypoxic conditions.[171] Previous simulated HA studies have reported no dilation of the internal carotid artery after only 15 minutes of hypoxia.[172] Together, these data suggest that the carotid vasodilatory response to hypoxia may not occur until after 15 minutes of exposure[172] but before 120 minutes (as measured in this study) and persists following acclimatization (12-14 days after ascent).[163]

Overall, our findings suggest that acute nitrate supplementation may not be an effective therapy to combat cerebrovascular and cognitive responses to HA; NO may not be the sole modulator of peripheral vascular function under hypoxic conditions. Numerous vasoactive mediators have been implicated in regulating vasodilation beyond NO including prostacyclin, endothelium-dependent hyperpolarizing factor, and substance P,[173] while reactive hyperemia may be primarily determined by inward-rectifying potassium channels.[174] Additionally, NO inhibition does not affect FMD responses to prolonged hyperemia.[140] This suggests that sustained hyperemia, comparable to the prolonged hypoxemia-driven hyperemia at HA, is not NO-dependent. Ascent to HA is associated with increased sympathetic drive and catecholamine release[175,176] which may modulate the cardiovascular response to hypoxia.[177] Indeed, recent findings have indicated that reductions in peripheral endothelial function at HA may be the direct result of HA-induced sympathoexcitation.[163] Lewis et al. exquisitely demonstrated that an α_1-adrenoreceptor blockade reverses reductions in brachial FMD under normobaric hypoxic conditions,[163] likely explaining the peripheral endothelial dysfunction observed in our study at HA. Consistent with previous literature, we found significant indications of enhanced sympathetic activation including, increases in diastolic blood pressure,[178] heart rate,[179-181] and R-

W_1 (a proxy measure of pre-ejection period)[136] at HA compared to SL in both treatments. These data suggest that the peripheral endothelial dysfunction observed at HA may be the result of increased sympathetic drive rather than reduced NO-availability. The increase in sympathetic drive may explain why we documented no change in blood pressure, or FMD with nitrate supplementation, despite the normoxic data supporting these hypotheses mentioned previously.

Although peripheral vascular dysfunction at HA appears to be largely mediated by sympathetic activation, the factors responsible for the central vascular dysfunction we documented at HA have yet to be clearly defined. One possibility is that the carotid dysfunction observed at HA (evident by reduced mental-stress-mediated dilation) may share common mechanisms with brachial dysfunction, such as increased sympathetic activation or oxidative stress.[163] Recent data, however, submits that the elastic and muscular arteries may respond differently to the sympathetic activation that occurs with mental-stress and HA. With mental-stress-induced sympathoexcitation muscular arteries (i.e. radial artery) may increase in stiffness[182] while recent unpublished data from our lab indicates elastic arteries such as the CCA do not change. At HA, administration of a α_1-blockade does not alter larger, extracranial vessel (i.e. internal/common carotid, vertebral arteries) dilation or blood flow,[171] suggesting that there may be differential effects of sympathetic activation on peripheral versus central vessels at HA. Importantly, although sympathoexcitation does not appear to affect carotid function at rest during hypoxia, it may still play a role in modulating carotid responses to mental-stress and NVC. Ultimately, the mechanisms responsible for hypoxia-induced cerebrovascular vasodilation and hyperemia may be multi-factorial and rely on multiple, redundant pathways similar to those observed with the vasodilatory responses to exercise.[183] A recent review has suggested these

mechanisms may involve NO, adenosine, PaO_2, NVC responses to decreased tissue oxygen, and anaerobic neuronal metabolism.[170]

Methodological Considerations

The current study utilized acute ingestion of a nitrate bolus and documented changes in nitrate-nitrite-NO availability using measures of salivary nitrite and exhaled NO. Exhaled NO has been reported to be related to AMS symptoms, with lower exhaled NO prevalent in AMS positive compared to AMS negative individuals.[184,185] In the current study, exhaled NO was collected in a subset (n=9) of participants as an exploratory measure to observe the effect of nitrate ingestion on pulmonary NO measures at HA. Relating exhaled NO to symptoms of AMS was outside the scope of the current study. Consistent with previous literature, our data suggests that exhaled NO does increase acutely following the ingestion of a nitrate bolus, which may be reflective of salivary nitric oxide formation[186] and provide insight into changes in plasma nitrate.[187]

Salivary nitrite, which we used as a proxy measure of plasma nitrate availability, significantly increased following nitrate ingestion at HA. Although this suggests there were changes in plasma nitrate, it was not directly measured and could be viewed as a limitation since recent reports suggest concentrations of nitrate may reach 10-fold higher in the saliva than plasma.[188] The dosage used in our study however has been previously used in acute exercise at HA[26] and reported to significantly increase plasma nitrite and nitrate concentrations.[26] It is possible that a greater dose of nitrate might elicit a greater vasoactive response, however it should be noted that even the acute dose used in the current study elicited undesirable side effects. Three participants reported severe nausea following consumption of the nitrate, while no

reports occurred in the PLA treatment group. This resulted in one participant vomiting during the trial, and one vomiting after leaving the laboratory post-testing. For these reasons, higher doses of nitrate may have a greater chance of eliciting beneficial effects but should be approached cautiously as it may make side effects more common or severe. Additionally, nitrate loading studies have revealed no effects on cerebral oxygenation status,[23] indicating that increased doses may not be more effective.

The timing of measures after nitrate ingestion could also impact findings. The timeline utilized in the current study was chosen so that cerebrovascular and cognitive testing would occur at approximately 2 hrs post-nitrate ingestion since previous literature suggests peak plasma nitrite levels occur between 2-3 hrs post-ingestion (and remain elevated for approximately 2 additional hours).[25,132] We believe our dose was successful in manipulating nitrate bioavailability based on previous research using a similar method of nitrate supplementation[26] and the well-documented plasma nitrate/nitrite responses to beetroot juice.[165] We do not believe the ineffectiveness of nitrate in modulating cardiovascular and cognitive function at HA can be explained by an insufficient nitrate dose or the timing of outcome measures.

This study was conducted under simulated-hypoxia in order to conduct a highly-controlled investigation on the effects of nitrate on cerebrovascular and cognitive function. Specifically, this study utilized a normobaric hypoxic chamber, which may have impacted the effectiveness of nitrate supplementation. Recent data may indicate that nitrate availability may be different between normobaric and hypobaric hypoxia.[189] Faiss et al. (2013) found that plasma nitrate and nitrite availability decreased in hypobaric hypoxia, whereas they were unchanged under normobaric hypoxia.[189] This suggests that the method of achieving hypoxia may alter the availability of nitrate/nitrite/NO and thereby the efficacy of nitrate supplementation. If there is

no decrease in NO-availability in normobaric hypoxic conditions, additional nitrate/NO availability may not impact blood flow, vascular function, NVC, or cognitive function. Moreover, some data suggest hypobaric hypoxia and normobaric hypoxia are not equivalent stimuli[190] and hypobaric hypoxia may lead to greater reductions in oxygen saturation and greater hypoxemia.[3] Therefore, different findings may be revealed if this study were repeated under hypobaric hypoxic conditions.

An alternate explanation may be related to Stroop task training effects.[191] Our study utilized a separate Stroop task as a mental-stress stimulus to assess NVC prior to cognitive testing. The repeated, additional exposure to the Stroop task may have masked any decrements in executive function and cognitive flexibility at HA.

The current study was largely powered to detect changes in cerebral blood flow and memory at HA based on previous literature.[7,60,81] The *a priori* sample size estimations for these variables resulted in sufficient power as we observed significant differences at HA in both cerebral and carotid blood flow and memory function. It is, however, possible that some of our insignificant findings with the ascent to altitude or effect of nitrate may be due to insufficient statistical power. In order to adequately address this, we conducted post-hoc power calculations on select cognitive and vascular measures to obtain measures of effect size and power. Effect size is a statistical measure of the magnitude of difference, or strength of a difference within a two groups. An effect size of 0.20, 0.50, 0.80 would be considered evidence of a small, moderate, and large magnitude of difference, respectively.[192] Effect size can be combined with sample size and error probability to estimate power, defined as the probability that the null hypothesis is correctly rejected. Measures for post-hoc power analyses were selected based on

1) *a priori* hypotheses that the particular variable would change with altitude or nitrate and 2) values appeared to differ between conditions or treatments although not statistically.

Although measures of executive function have been reported to decrease with high altitude,[32] this is not a universal finding.[160,161] In the current study, the ability to accurately navigate a maze (a measure of executive function) was not significantly affected by hypoxia. Moreover, effect size (PLA, 0.11; NIT, 0.21) and power (PLA, 0.08; NIT, 0.14) were low when interrogating the effect of altitude on this measure of executive function (Table 12). This suggests either 1) altitude exposure may not effect executive function, at least in the duration and degree of hypoxia used in this study; or 2) altitude may not effect this specific executive function task. Similarly, performance on switching of attention tasks, such as the Trails A-B task, were expected to change with altitude, however we noted no significant changes in the average connection time during the Trails task. Post-hoc power analyses indicated that although PLA had a moderate effect size and power (0.59 and 0.71, respectively), NIT had a small effect size and low power (0.24 and 0.18, respectively). The data appears to indicate that performance during the Trails task improved at HA for PLA but was impaired for NIT. Indeed, the effect of nitrate at altitude on this task was found to have somewhat moderate power (0.45), suggesting that differential performance between NIT and PLA may have been revealed if we had a larger sample size ($n \approx 50$ for a power of 0.80). The differences in effect size between NIT and PLA may have been related to the high variability often observed with reaction times.

Nitrate supplementation was expected to enhance blood flow and NVC at HA, although no such effects were observed in this study. Post-hoc power analyses indicated that the magnitude of difference between PLA and NIT was minimal with respect to MCA blood flow reactivity during mental stress (a measure of NVC), with an effect size of 0.07 and power of

0.06. Additionally, the effect of nitrate supplementation on carotid blood flow at rest at HA was found to have a small effect size (0.38) and low power (0.37). This small effect size suggests that even with a larger sample (n≈55 to achieve a power of 0.80) the effects would likely lack clinical or physiological relevance. Thus, nitrate supplementation appears to have minimal effects on blood flow at HA and insignificant differences observed in this study are likely not related to lack of statistical power.

The cognitive findings of this study may have been affected by: 1) the order in which the tasks were presented and 2) practice effects that accompany multiple exposures to a single task. The cognitive tasks used in this study were presented in the same order across all trials and thus may have altered our cognitive findings and compromised internal validity. Task performance may be confounded by time when tasks are presented in the same order, such that fatigue or boredom could affect performance on a given task later in the battery. Although this is a possibility since the order of tasks was not randomized within this study, we do not believe it played a large role since post-hoc analyses suggested that performance on tasks in the second half of the battery was not different from performance in the first half. None the less, future studies in this area should adopt the proper psychological methodology of randomizing task order.

Practice effects may have also played a role in our cognitive findings at HA. Practice effects occur due to repeated exposure to a task and generally improve task performance. These effects manifest as improved reaction times and accuracy, along with reduced effort to complete the task.[193] The effect of practice on task performance is multifaceted, affecting information processing speed, response caution, and nondecisional processing time to both repeated and new stimuli.[193] Moreover, it is currently unknown whether these effects are related to familiarity with

the task itself or the task stimuli, although it is likely combination of both.[193] We attempted to reduce practice effects with the mental-stress Stroop task (used during the assessment of NVC) by familiarizing participants with the Stroop task prior to experimental testing. We did not, however, familiarize participants with the cognitive battery prior to experimental trials, thus it is possible that practice effects may have effected task performance during the cognitive battery. Specifically, practice effects may explain the significant increase in visual-interference performance and insignificant change in verbal-interference performance with the ascent to HA. If a learning effect were present it would have resulted in stepwise increases in performance and different baseline values between the first and second visit. This, however, was not observed in the current study as there were no differences between visits at baseline. The Stroop task used during the mental-stress protocol has been reported to be vulnerable to practice effects,[191] thus repeated exposure to the mental-stress Stroop task may 1) have masked decrements in cognitive battery verbal-interference performance or 2) explain the improved performance on the cognitive battery visual-interference task at HA because of their strong similarity to the Stroop task. Future HA research should attempt to control for practice effects whenever possible by allowing ample familiarization prior to experimental trials.

Limitations and Future Directions

This study was conducted under simulated normobaric hypoxia in order to design a highly controlled investigation to isolate the effect of acute nitrate supplementation on cognitive and cerebrovascular function. This design brings with it some inherent limitations since participants were not exposed to sleep disturbances, dehydration, physical fatigue, or mental-stress that may accompany expedition-based ascents to HA. Additionally, the potential differences between normobaric hypoxia and hypobaric hypoxia highlighted previously[3,190]

poses a threat to the applicability of our findings to real-life HA scenarios. The computer-based cognitive battery used in this study has been used in over 180 scientific publications, validated against traditional paper-and-pencil tests,[194] and shows sound test-retest reliability.[195] Despite these strengths, it is possible the specific tasks selected to interrogate each domain (i.e. Maze task, forward digit span etc.) may not have been sensitive to hypoxia and therefore may not have detected hypoxia-induced decrements in specific domains. A recent review by Petrassi et al. (2012) on cognitive function at HA suggests that cognitive and psychomotor deficits reported in literature are difficult to quantify and reliably reproduce.[3] These inconsistencies may be explained by the subtlety of cognitive decrements at HA, differences in compensatory mechanisms, methodology, test sensitivity, inter-individual variation, and the role of exercise which generally compounds the cognitive decrements seen at HA.[3]

Our study utilized males which limits the results applicability to other populations. Results may differ if this study was conducted in females based on hormonal differences introduced by the menstrual cycle and the subsequent timing of measures during the cycle. Estrogen has been reported to increase endothelial function, assessed by brachial FMD,[196] principally through enhanced expression of NO synthase.[197] Females, however, may experience similar decrements in peripheral endothelial function as their male counterparts since dysfunction appears sympathetically-mediated at HA,[163] rather than low-NO mediated. In the cerebrovasculature, estrogen has been associated with neuroprotective, anti-inflammatory, and vasodilatory effects, which may also be related to NO production.[198] If NO-production is attenuated in hypoxic environments as previously reported,[14,115] females may experience greater disruption in cerebrovascular hemodynamics because of the loss of estrogen's protective effects that are elicited through NO-mediated mechanisms. The effects of estrogen on cognitive

function appear less defined, as estrogen therapy does not appear effective in reducing incidence of mild cognitive impairment in postmenopausal women.[199] The role of estrogen in cognitive function at altitude, however, has not yet been investigated and is most likely complex, owing to estrogens intricate interactions with neurotransmitters systems and dopamine.[200] The participants in the current study were generally young (23 ± 3 yrs) and healthy. However, investigating the effects of HA on the cognitive and cerebrovascular function is important across a large range of ages because vascular response to hypoxia may change with age,[3] warranting further investigations. Future research should attempt to elucidate the mechanism responsible for the reductions in carotid endothelial function at HA, evident in reduced dilation to mental-stress compared to SL.

Strengths of this study should also be noted. This is the first study to investigate the effect of acute nitrate supplementation on cerebrovascular and cognitive function at HA. Our study is also the first to apply novel measures of NVC assessed via simultaneous measurements of TCD, carotid blood flow, and carotid WIA during mental-stress (elicited by a cognitive challenge) in a highly-controlled hypoxic chamber. The change in hemodynamics during a mental-stress task that requires cognitive engagement serves as a novel means to assess NVC in the cerebrovasculature.

Implications

Military deployment in high altitude (HA) environments have increased in mountainous terrain such as Afghanistan and Northern Iraq.[4] These fighting units are often rapidly deployed to HA for combat operations[4] permitting little time to prepare the body for exposure to HA-hypoxia. Our data demonstrates that an acute dose of nitrate prior to ascent to HA does not

significantly alter cognitive function or novel measures of cerebrovascular function and may not be efficacious as a means to reduce hypoxia-induced cognitive decrements on military personnel, at least not in the dosage used in this study. Importantly, previous HA nitrate administration studies have noted significant effects on muscular oxygenation status but not cerebral status,[23] and on time trial performance and steady state oxygen consumption,[26] suggesting perhaps differential effects of nitrate supplementation on the skeletal muscle and brain. Recent findings implicate sympathetic-drive as a contributing factor to the vascular dysfunction observed at HA[163] which may suggest that adrenergic blockades may alter NVC and cognitive function at HA.

In conclusion, both the NIT and PLA treatments experienced significant increases in cerebrovascular blood flow, reductions in brachial endothelial function, and decrements in memory performance at HA compared to SL. Our novel assessment of NVC revealed carotid artery endothelial dysfunction in both treatments, manifesting as an attenuated vasodilator response to mental-stress at HA compared to SL. These data provide new insight into endothelial dysfunction that occurs not only in the periphery but the central vasculature as well while at HA. Ultimately we found that acute nitrate supplementation does not alter cognitive or cerebrovascular function at HA compared to a nitrate-depleted placebo.

Acknowledgments

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Illustrative Materials

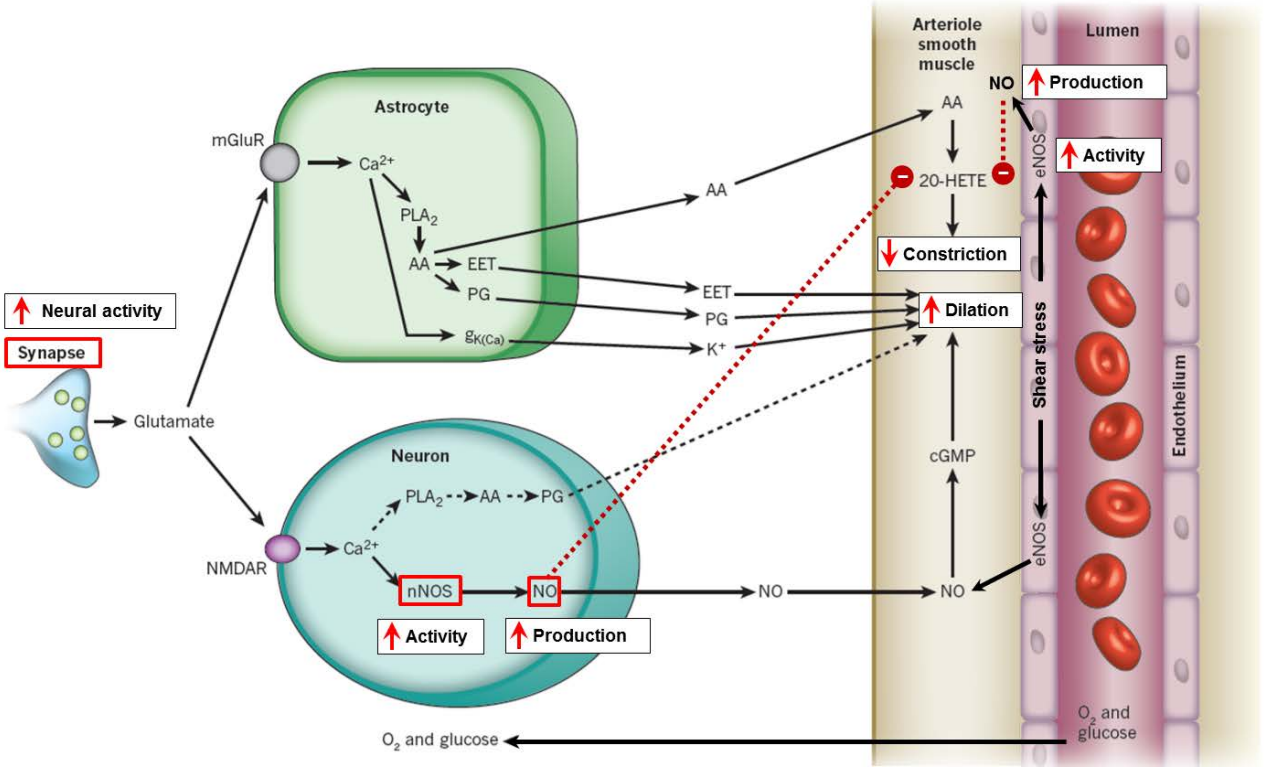
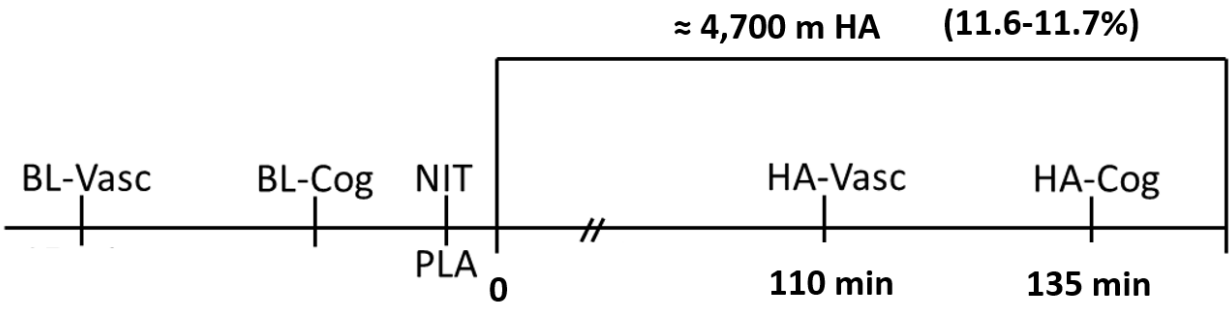


Figure 1: Neurovascular coupling under normal conditions (normoxia)

Adapted from Attwell et al. (2010).

nNOS, neuronal nitric oxide synthase; NO, nitric oxide



BL, baseline; Vasc, vascular testing; Cog, cognitive testing; NIT, nitrate; PLA, placebo; HA, high altitude

Figure 2: Study design.

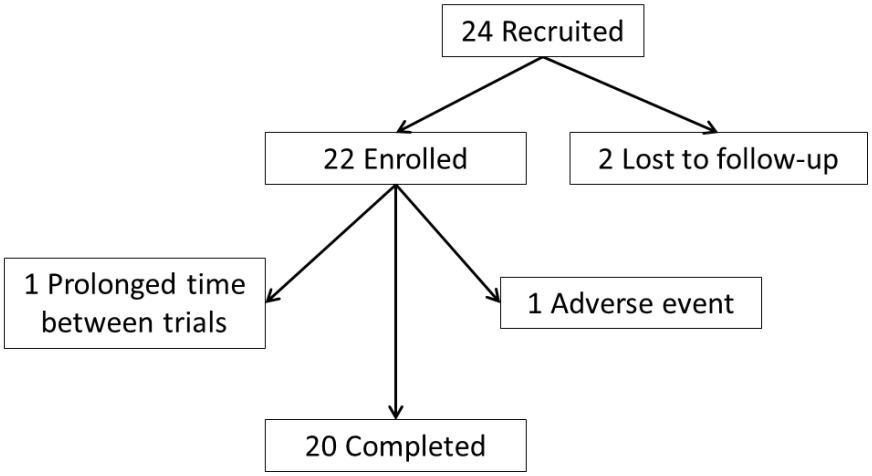


Figure 3: Enrollment and participant drop-out.

Table 1: Cognitive domains.

Domain	Description	Example test
Attention/concentration	Ability to focus on/accomplish goal-oriented task	Calculations/ visual search
Language	Ability to understand and use oral/written language.	Word search/ fluency
Visiospatial skills	Ability to comprehend shapes/forms and their interpretation	Reproduction of shapes/ images
Psychomotor skills	Ability to perform gross/fine motor skills	Peg board, finger tapping test
Memory	Ability to store/retrieve information (short/long term/semantic memory)	digit span/free recall
Orientation	Ability to correctly orient date, place, name	Orientation task
Executive function	Ability to conceptualize, evaluate, and complete goal-oriented tasks	
	Components	
	Information processing	Reaction time
	Attentional control	Stroop, PASAT
	Cognitive flexibility	Stroop, Trails B
	Working memory	Digit span forward

Adapted from Davis et al. 2013, Logue et al. 2013, Burnett et al. 2013.

Table 2: Effects of HA on cognitive performance.

Study	Design	Tests	Cognitive domain	Results
Crow et al. (1971)	S-HA; 610 m, 2438 m, 3658 m	Digit recall	Memory	↓ Accuracy at peak
Bolmont et al. (2000)	S-HA; sea level to 8,848 m in 31 days	Binary visual RT	Executive function	No change
		Peg board task	Psychomotor skills	↓ Motor skills
Dykiert et al. (2010)	E-HA; 76 m to 5,565 m in 17 days	4-Choice RT	Executive function	↓ CRT
Wang et al. (2013)	E-HA; 402 m to 3,561 m in 1 day	PASAT	Executive function	↓ Performance
		DSST	Executive function	↓ number correct (*ns*)
		Free recall	Memory	↓ Word recall
		OST	Executive function	No change
McCarthy et al. (1995)	S-HA; 2134 m, 3658 m	RT	Executive function	↑ RT, ↓ accuracy
Du et al. (1999)	S-HA; 300 m, 2800 m, 3600 m, 4400 m	Memory tasks	Memory	↓ Accuracy at 4,400 m
van der Post et al. (2002)	S-HA; SaO_2 97%, 90%, 80%	Word recognition	Memory	↓ Accuracy at SaO2 80%
		Corsi block tapping	Memory	No change
		Visual search	Executive function	No change
		Binary visual RT	Executive function	↓ Accuracy, ↑ RT at SaO_2 80%
Li et al. (2012)	E-HA; <300 m to 3,900 m within 5 days	Auditory memory	Memory	↓ Performance
		Dexterity	Psychomotor skills	↓ Performance
		4-Choice RT	Executive function	↓ Accuracy, ↑ RT
		Visual perception	Visiospatial skills	No change

S, simulated; E, expedition; HA, high altitude; SaO_2, arterial O_2 saturation; RT, reaction time; PASAT, paced auditory serial addition test; DSST, digital symbol substitution test; OST, operation span task; *ns*, non-significant

Table 3: Descriptive characteristics (n = 20).

	Mean ± SD
Age (yrs)	23 ± 3
Height (cm)	181.1 ± 5.8
Weight (kg)	79.82 ± 9.72
BMI (kg/m^2)	24.3 ± 3.0
Body fat (%)	13.3 ± 6.8
Hematocrit (%)	43 ± 3
Hemoglobin (g/dl)	14.7 ± 1.5

Table 4: Time at high altitude, blood measures, and AMS across condition (mean ± SD).

	Placebo	**Nitrate**
Total time at HA (min)	165 ± 8	161 ± 8
$\%O_2$ at cognitive testing	11.6 ± 0.1	11.7 ± 0.1
Baseline hemoglobin (g/dL)	14.5 ± 0.9	14.3 ± 1.1
Baseline hematocrit (%)	42 ± 3	43 ± 3
AMS, 110 min	2 ± 1	3 ± 2
AMS, 137 min	3 ± 2	3 ± 2

HA, high altitude; vasc, vascular testing; cog, cognitive testing; AMS, acute mountain sickness score.

Table 5: Cognitive performance and reaction times by cognitive domain across condition and altitude (mean ± SD).

			Placebo		Nitrate	
Domain	Task	Construct	SL	HA	SL	HA
Memory	Memory Recognition	Learning Rate	0 ± 1	0 ± 1	0 ± 1	0 ± 1
		Verbal Memory Index	75 ± 9	70 ± 10*	75 ± 5	71 ± 10*
		Verbal Intrusion Index	5 ± 9	10 ± 10*	5 ± 5	9 ± 10*
Working Memory Capacity	Digit Span (Forward)	Recall Span (forwards)	7 ± 1	7 ± 2	7 ± 1	6 ± 1
		Trials Correct (forwards)	9 ± 2	9 ± 3^	9 ± 2	7 ± 3^
Attention and Concentration	Continuous Performance Test	RT (ms)	560.29 ± 200.25	518.56 ± 127.83	467.20 ± 69.27	538.91 ± 185.50
		Commission Errors	2 ± 5	2 ± 2	1 ± 2	3 ± 6
		Ommission Errors	1 ± 1	1 ± 1	0 ± 1	1 ± 1
Information Processing Efficiency	Switching of Attention (Part 2)	Duration (ms)	38783 ± 6742	35235 ± 6463	38765 ± 7160	40163 ± 11671
		Connection Time (ms)	1508 ± 285	1382 ± 258	1483 ± 274	1580 ± 455
		Accuracy	0 ± 1	1 ± 1	1 ± 2	1 ± 1
Response Speed	Motor Tapping	Tapping Speed	200 ± 25	199 ± 23	206 ± 23	197 ± 22
Information Processing Efficiency	Choice RT	Choice RT (ms)	343.35 ± 73.64	346.52 ± 31.85	328.72 ± 34.56	350.52 ± 26.52
	Visu-I (Word)	# Correct w/ Visu-Int	17 ± 5	20 ± 4*	17 ± 5	19 ± 5*
		RT w/ Visu-Int (ms)	1186 ± 321	969 ± 156*	1108 ± 214	1056 ± 306*
	Verb-I (Color)	# Correct w/ Verb-Int	17 ± 4	17 ± 3	18 ± 5	17 ± 5
		RT w/ Verb-Int (ms)	1156 ± 278	1146 ± 212	1139 ± 285	1124 ± 208
Executive Function	Maze	Trials Completed	7 ± 3	8 ± 3	8 ± 2	8 ± 2
		Completion Time (ms)	114988 ± 42270	113379 ± 52528	107712 ± 32708	117845 ± 42424
		Path Learning Time (ms)	94322 ± 38855	94159 ± 53061	88668 ± 30494	97255 ± 38098
		Accuracy	30 ± 14	28 ± 11	29 ± 12	31 ± 10
		Number of Overruns	14 ± 8	12 ± 5	13 ± 7	15 ± 7
Impulsivity	Go/No-Go	Speed	292.02 ± 44.96	282.58 ± 60.81	287.57 ± 44.13	274.77 ± 44.03
		Commission Errors	5 ± 4	5 ± 3	5 ± 2	7 ± 3
		Ommission Errors	1 ± 2	1 ± 2	1 ± 3	2 ± 3
Emotion Identification	Emotion Recognition	Emotion Recognition Index	175.42 ± 8.96	172.77 ± 11.06*	176.35 ± 6.23	170.18 ± 11.20*
		Average RT (ms)	1671.38 ± 318.10	1593.84 ± 518.05	1620.17 ± 281.33	1528.67 ± 256.00

SL, sea level; HA, high altitude; scr, score; int, intrusions; RT, reaction time; Visu-Int, visual interference; Verb-Int, verbal interference

* $p < 0.050$ vs within-treatment SL; ^ trend, $p < 0.100$ vs within-treatment SL

Table 6: Stroop task performance and emotional responses across treatments and altitude (mean ± SD).

	Placebo		Nitrate	
	SL	**HA**	**SL**	**HA**
Total questions	110 ± 8	110 ± 9	112 ± 8	112 ± 8
Correct (%)	67 ± 13	64 ± 12	67 ± 15	67 ± 13
Incorrect (%)	33 ± 13	36 ± 12	33 ± 15	33 ± 13
Mean correct RT	1044 ± 165	1033 ± 172	998 ± 141	992 ± 132
Happiness	7 ± 1	6 ± 2*	7 ± 1	6 ± 1*
Perceived control	7 ± 1	7 ± 2*	7 ± 1	6 ± 2*
Arousal	4 ± 2	4 ± 2	3 ± 1	4 ± 2
Effort	23 ± 2	22 ± 4*	23 ± 2	21 ± 5*
Importance	24 ± 3	22 ± 3	24 ± 3	22 ± 4

SL, sea level; HA, high altitude; RT, reaction time

* $p < 0.05$ vs within-treatment SL

Table 7: Cerebral mental-stress-mediated reactivity across treatments and altitude (mean ± SD).

	Placebo		**Nitrate**	
	SL	**HA**	**SL**	**HA**
Mean velocity (m/s)	+ 5 ± 6	+ 5 ± 9	+ 7 ± 7	+ 6 ± 7
PI	-0.01 ± 0.06	-0.01 ± 0.10	-0.04 ± 0.08	-0.03 ± 0.07
Conductance	-0.02 ± 0.08	+ 0.01 ± 0.10	-0.04 ± 0.10	+ 0.02 ± 0.12
HR (bpm)	+ 8 ± 11	+ 7 ± 8	+ 6 ± 8	+ 5 ± 7
SaO2 (%)	+ 0 ± 2	+ 0 ± 5	+ 0 ± 2	+ 1 ± 4

SL, sea level; HA, high altitude; PI, pulsatility index; HR, heart rate; SaO_2, arterial oxygen saturation.

Table 8: Carotid artery mental-stress-mediated reactivity across treatments and altitude (mean ± SD).

	Placebo		**Nitrate**	
	SL	**HA**	**SL**	**HA**
SBP (mmHg)	+ 6 ± 9	+ 5 ± 10	+ 1 ± 8	+ 4 ± 11
DBP (mmHg)	+ 10 ± 6	+ 4 ± 7	+ 6 ± 6	+ 7 ± 8
MAP (mmHg)	+ 9 ± 6	+ 5 ± 6	+ 4 ± 4	+ 5 ± 8
Mean diameter (mm)	+ 0.16 ± 0.15	+ 0.00 ± 0.20*	+ 0.17 ± 0.19	+ 0.07 ± 0.26*
MnV ($cm \cdot s^{-1}$)	- 0.8 ± 3.1	- 0.4 ± 3.6^	- 2.0 ± 3.6	+ 0.5 ± 3.1^
PI	- 0.01 ± 0.17	- 0.01 ± 0.25	- 0.09 ± 0.24	- 0.07 ± 0.24
Blood flow ($ml \cdot s^{-1}$)	+ 16.3 ± 66.7	- 10.9 ± 91.6	- 0.8 ± 67.6	+ 28.3 ± 90.2
β-stiffness	- 0.7 ± 1.0	- 0.2 ± 1.6	- 0.7 ± 1.2	- 0.4 ± 1.2
Ep (kpa)	- 4 ± 14	- 2 ± 21	- 6 ± 15	- 3 ± 15
AIX75 (%)	+ 9 ± 12	+ 5 ± 16	+ 7 ± 10	- 3 ± 15
W_1 ($mmHg \cdot m \cdot sec^{-3}$)	+ 0.69 ± 7.06	+ 0.03 ± 5.11	- 1.15 ± 4.40	+ 0.61 ± 7.17
NA ($mmHg \cdot m \cdot sec^{-2}$)	- 17.605 ± 45.738	+ 5.748 ± 22.545	- 17.326 ± 43.514	- 14.390 ± 51.243

SL, sea level; HA, high altitude; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PI, pulsatility index; Ep, elastic modulus; AIX75, augmentation index at 75 bpm; W1, forward wave magnitude; NA, reflected wave magnitude.

* $p < 0.050$ vs within-condition SL; ^ trend $p = 0.062$ vs within-condition SL

Table 9: Cerebral hemodynamics at baseline across treatments and altitude (mean ± SD).

	Placebo		**Nitrate**	
	SL	**HA**	**SL**	**HA**
Mean velocity ($m \cdot s^{-1}$)	66 ± 15	74 ± 20†	68 ± 16	75 ± 18†
PI	0.83 ± 0.14	0.78 ± 0.11	0.84 ± 0.17	0.79 ± 0.13
Conductance	0.80 ± 0.19	0.87 ± 0.26	0.82 ± 0.19	0.90 ± 22
HR (bpm)	56 ± 9	64 ± 8†	54 ± 7	64 ± 11†
$ETCO_2$ (mmHg)	39 ± 2	33 ± 2†	39 ± 3	34 ± 2†
SaO2 (%)	98 ± 2	75 ± 6†	98 ± 2	75 ± 7†

SL, sea level; HA, high altitude; PI, pulsatility index; HR, heart rate; $ETCO_2$, end-tidal CO_2; SaO_2, arterial oxygen saturation

† $p < 0.05$ vs within-treatment SL

Table 10: Carotid measures of vascular function during baseline and mental stress across condition and altitude (mean ± SD).

	Placebo		Nitrate	
	SL	**HA**	**SL**	**HA**
SBP (mmHg)	106 ± 11	108 ± 11	107 ± 7	106 ± 11
DBP (mmHg)	68 ± 6	74 ± 7†	70 ± 6	71 ± 8†
MAP (mmHg)	83 ± 7	87 ± 6	84 ± 6	85 ± 9
Mean diameter (mm)	5.91 ± 0.46	6.60 ± 0.47†	5.75 ± 0.48	6.54 ± 0.45†
PI	1.99 ± 0.33	1.92 ± 0.42	2.09 ± 0.31	1.96 ± 0.31
Blood flow ($ml \cdot s^{-1}$)	621.0 ± 89.9	786.5 ± 139.7†	602.0 ± 88.1	746.5 ± 126.3†
β-stiffness	4.4 ± 1.2	3.7 ± 1.4^	3.9 ± 1.0	3.5 ± 1.1^
Ep (kpa)	50 ± 14	45 ± 19	45 ± 12	41 ± 15
R-W1 (ms)	112 ± 22	106 ± 19	119 ± 28	106 ± 17
AIX75	-27 ± 12	-29 ± 11	-28 ± 13	-29 ± 13
W_1 ($mmHg \cdot m \cdot s^{-3}$)	10.61 ± 5.50	12.51 ± 8.19	9.87 ± 4.88	11.64 ± 5.39
NA ($mmHg \cdot m \cdot s^{-2}$)	66.532 ± 62.625	54.054 ± 68.902	54.335 ± 46.900	55.940 ± 42.460

SL, sea level; HA, high altitude; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PI, pulsatility index; Ep, elastic modulus; AIX75, augmentation index at 75 bpm; W_1, forward wave magnitude; NA, reflected wave magnitude.

† $p < 0.05$ vs within-treatment SL; ^ $p = 0.051$ vs within-treatment SL

Table 11: Measures of nitric oxide bioavailability and brachial vascular measures across treatments and altitude (mean ± SD).

	Placebo		**Nitrate**	
	SL	**HA**	**SL**	**HA**
Salivary nitrate (AU)	0.1 ± 0.2	0.2 ± 0.3	0.1 ± 0.2	2.7 ± 1.0 §*
Ex NO (ppb; n = 9)	19 ± 5	20 ± 5	16 ± 6	35 ± 9 §*
Brachial SBP (mmHg)	114 ± 10	114 ± 10	113 ± 9	112 ± 12
Brachial DBP (mmHg)	68 ± 6	74 ± 7*	70 ± 6	72 ± 8*
Brachial MAP (mmHg)	83 ± 7	86 ± 6	84 ± 6	84 ± 8
Average BL diameter (mm)	4.00 ± 0.48	4.07 ± 0.43	3.95 ± 0.47	4.08 ± 0.45
FMD (%)	6.22 ± 3.62	4.87 ± 3.22*	8.13 ± 3.14	5.52 ± 3.14*
Reactive hyperemia AUC	697.14 ± 221.81	736.59 ± 161.74	675.09 ± 194.39	726.36 ± 249.41

SL, sea level; HA, high altitude; Ex NO, exhaled nitric oxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; BL, baseline; FMD, flow mediated dilation; AUC, area under the curve.
§ $p < 0.05$ vs Placebo-HA; * $p < 0.05$ vs within-treatment SL

Table 12: Effect sizes and statistical power for the effect of altitude and nitrate across select variables.

	Effect of HA				**Effect of nitrate at HA**	
	Placebo		Nitrate		Placebo vs Nitrate	
Variable	Effect size	Power	Effect size	Power	Effect size	Power
Switching-attention connection time	0.59	0.71	0.24	0.18	0.43	0.45
Maze accuracy	0.11	0.08	0.21	0.14	0.29	0.24
MCA blood flow	0.77	0.90	0.96	0.98	0.07	0.06
CCA blood flow	1.49	1.00	1.25	1.00	0.38	0.37
ΔMCA blood flow	0.03	0.05	0.14	0.09	0.07	0.06
ΔCCA blood flow	0.23	0.16	0.26	0.20	0.26	0.20

Appendix 1

EXERCISE SCIENCE
820 COMSTOCK AVENUE
201 WOMEN'S BUILDING
SYRACUSE, NY 13210
(315)-443-2114

EFFECTS OF BEETROOT JUICE SUPPLEMENTATION ON HIGH ALTITUDE COGNITIVE AND VASCULAR FUNCTION

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IRB Protocol #: 13-370

We are inviting you to participate in a research study. Involvement in the study is voluntary, so you may choose to participate or not to participate. This sheet will explain the study to you and please feel free to ask questions about the research if you have any. I will be happy to explain anything in detail if you wish.

Purpose

Normal brain function relies on oxygen supply. At high altitudes the amount of oxygen you inhale and deliver to your body decreases, impairing your body's ability to function normally. This negatively affects your ability to think and process problems. Research has shown that at high altitude your reaction time slows down, you lose some of your fine motor skills and your ability to remember things is decreased. This may be the result of decreased blood flow and oxygen delivery to the brain when you are thinking or processing information. These effects are very problematic for people who are exposed to high altitude, such as military personnel or mountaineers.

The purpose of this study is to investigate whether or not supplementing with beetroot juice before ascending to high altitude will increase oxygen delivery to the brain when you are thinking and performing cognitive tasks, resulting in increased brain function. Beetroot juice is a natural, vegetable juice that comes from beets, a vegetable commonly found in salads. It has high amounts of nitrate, a molecule that can be broken down into the body into a compound that can cause your blood vessels to expand and deliver more blood and oxygen. In this study, we will have you perform a series of cognitive tests and will measure your blood vessels both in a normal

environment (i.e. no change in altitude) and in a simulated high altitude chamber after consuming beetroot juice. This will be done over 3 visits that are described below. Understanding if a natural supplement, like beetroot juice, can help brain function at high altitude has important benefits for the health and safety of people who work at high altitude.

Who can participate?

- Men and women between the ages of 18-30.

Do I have to participate?

- Your participation in this study is voluntary, which means you get to decide whether or not you want to participate
- Make sure that you read this entire form before making a decision and take as much time as you need.
- Feel free to ask as many questions about the study as you want. If you do not understand a term in the form, ask, and a researcher will explain it for you.
- If you decide to participate in the study you will be asked to sign a consent form.
- Do not sign the consent form until all of your questions have been answered and you understand what will happen in the study.
- Your signature means that you agree to participate in this study.
- You can ask for a copy of this form whether or not you agree to take part in the study.
- Your decision not to be in this research study will not result in any loss of benefits to which you are otherwise entitled.

Can I Withdraw From The Study Once It Has Started?

- At any time you may remove yourself from the study without giving any reason.
- If you are a student, withdrawing from the study will not affect your grade in courses in any way.

What Can I Expect From Participating?

For this study, you will need to visit the Human Performance Laboratory and Altitude Simulation Laboratory, located in the Women's Building at Syracuse University *once* for study screening and *twice* for the study. The screening will take about 45 minutes and each study visit will take approximately 4 hours.

- At the ***screening visit*** you will be asked to fill out and sign this consent form, Health History Questionnaire, and a vision and colorblindness examination. Additionally, we will measure your height using a large ruler that is mounted against a wall. With your shoes still off, we will ask you to stand upright with your back against this wall for a few moments as we measure your height.

- We will ask you to give us a small urine sample so that we can check the function of your kidneys. We will provide a small sample container and escort you to the restroom.
- We will then estimate your body composition (percent body fat) using a BodPod that will require you to wear tight fitting, minimal clothing for greatest accuracy in estimations. You will be asked to sit quietly in a chamber that resembles a giant egg for approximately two, 60-second intervals. This machine measures your body volume to estimate body fat.
- We will also have you perform a brief cognitive test to measure basic brain function. We will also have you practice one on the computer in order to become familiar with it.
- We will take you down to the altitude chamber (it will not be on, so we will still be at normal altitude) to familiarize you with the chamber and answer any questions you might have. In total, the screening visit will take approximately 45 minutes.
- We will send you home with a 3-day dietary recall survey and a list of certain foods that we will request you avoid for the 3-4 days before each of your next scheduled visits.
- For the two ***study visits*** we will ask you to arrive not having eaten within the past 3 hours (we will provide a snack (granola bar and juice) at the end of testing). Blood pressure can be affected by exercise and consuming food, caffeine or alcohol. Therefore, we will please ask you to refrain from exercising or consuming alcohol or caffeine (including caffeinated coffee, tea, soda or energy drinks) on the day that you will come into the lab.
- You will be asked to lie down and rest for 10 minutes. Following rest, we will place a blood pressure cuff around upper arm (bicep). We will also measure your heart rate using ECG. Three electrodes (stickers) will be placed on you. One will be placed on your left shoulder, one on the lower left rib and one on the lower right rib. These stickers can be easily peeled off when the study is over. Following this we will check blood pressure in your wrist (radial artery), neck (carotid artery) and upper leg (femoral artery). To do this, we will use a very sensitive blood pressure machine that looks like a pen with a little watch battery at the end. We will gently place this pen on top of your skin over wrist followed by your neck and upper leg. This measurement is non-invasive (no needles/no blood) and will take less than 10 minutes. From this information, we can estimate artery elasticity.
- We will measure how reactive your vessels are by imaging the artery in your arm using an ultrasound probe which is a small device (about the size of a deodorant stick) that we will set on the surface of your arm. We will inflate a cuff around your forearm to about 200 mmHg, similar to the highest pressure used when your blood pressure is measured at the Doctor's office, for 5 minutes. We will then release your artery and measure how it responds to the release in blood flow.
- Next we will measure your neck blood flow and brain blood flow using two non-invasive (no needles, no blood) techniques, Doppler ultrasound and blood flow sensors. Ultrasound probes will be placed on your neck and on the side of your face (between eye and ear) to assess neck artery stiffness and blood flow and brain blood flow. Blood flow sensors will be placed on the forehead to assess brain blood flow.
- Then we will give you a cognitive task to complete, which will be displayed on a monitor. You will use hand clickers to respond to questions on the monitor for 5-10 minutes. During these cognitive tasks we will continue to measure artery stiffness and blood flow using the ultrasound

probes and blood flow sensors. This test will give us information about how your arteries react to the thinking required to answer the cognitive test. This 4 minute test will be repeated after a short break, requiring a total of about 15 minutes.

- We will also measure your hemoglobin and hematocrit by obtaining a small drop of blood from your fingertip (finger prick).
- We will also have you stick a small test strip under your tongue for 3-5 seconds to absorb a small amount of saliva. This test is designed to give us a general idea of how much of a certain molecule you have present in your saliva. We believe this molecule (nitrite) may have an effect on your blood vessels.
- We will have you exhale into a mouthpiece for a brief amount of time. The mouthpiece will be attached to a small machine (approximately the size of a mini gum ball machine) that measures the amount of a certain molecule (nitric oxide) in your lungs. We think this molecule may affect your blood vessels.
- You will complete a computer-administered cognitive and mental status tests. These tests may require between 25-30 minutes for completion. You will be seated at a computer for these tests, which are designed to assess your memory, attention, reflexes and problem solving skills. This test covers a wide range of brain function because we want to see what part of your brain function might change with exposure to high altitude.
- Before beginning the next portion of the visit you will be permitted a bathroom break. You will then be given a small, approximately 2.3 ounces (70 mL) juice drink to consume before entering the altitude simulation chamber. You will relax in the altitude chamber at an approximate altitude of 14,000 ft for 1 hour and 45 minutes. At high altitudes there is less oxygen available for your body, so your body compensates by increasing the rate of your breathing and how quickly your heart beats. Understand that if you begin to feel these symptoms, they are natural and are not dangerous.
- During this time we will attach a clip to your finger and will also attach a small cord to your forehead using a headband in order to measure blood oxygen saturation. Every 30-minutes you are in the altitude chamber we will give you a short survey to see how you are feeling physically.
- After 1 hour and 45 minutes, we will conduct the same measures we did outside of the chamber, finger stick blood sample, saliva strip testing, blood pressure and blood flow measures, and cognitive testing.
- Upon completion of the testing we will escort you out of the altitude chamber and will take a few final blood pressures on your upper arm and monitor your oxygen saturation until it has reached your pre-chamber levels. At this point you will be permitted to leave.
- If you wish to withdraw from the study at any time you are free to do so.

Estimated study timeline for participants.

Visit 1 (≈ 45 min)		Visit 2-3 (≈ 4 hours each)	
Item	Time (min)	Item	Time (min)
Consent form	15	Blood pressure	10
Color vision testing		Blood flow	
Height	5	Vascular reactivity	10
Urine sample		Finger stick	
Body composition	5	Expiration test	5
Cognitive test practice	10	Saliva test strip	
Cognitive testing	10	Cognitive testing	45
		Consume juice beverage	
		Enter altitude chamber	105
		Repeat above procedures	65

Can I be excluded from participation for any reason?

- Based on answers to the above mentioned health history questionnaire, you may be excluded from the study. If you regularly experience any signs or symptoms that suggest you may have a medical condition and your health care provider is not aware that you are experiencing these symptoms, we will exclude you from the study and ask that you contact your health care provider. We also will exclude you if you have known allergies to beets, a primary ingredient of the juice drink we ask you to consume, or if you have a history of losing consciousness.
- If you are experiencing any signs or symptoms of a serious/significant health condition *at the time of consent* (i.e. severe chest pain, leg pain, dizziness, feelings of heart palpitations) we will contact emergency medical services immediately and you will not be able to participate in the study.

What Benefits Can I Expect From Participating?

- A benefit from this study is helping us understand if we can increase the cognitive performance of those who work in high altitude conditions.
- You may feel good about helping others with their research study by participating in this research study.

- You will receive information on your blood pressure, body composition, hemoglobin/hematocrit and cognitive function.
- ***These tests are not being used to diagnose a problem (NOT for medical/clinical purposes). These tests are for research purposes only.*** If you have high blood pressure we will inform you to go the university health center or go see your health care provider.

Are There Any Potential Risks From Participating In This Study?

- There are some risks associated with portions of this study.
- We will use a small amount of gel to help us measure your brain blood flow. There is a small risk that this gel may get in your eye. To protect against this happening, we will use the least amount of gel possible to obtain our measurements. Also, all measures will be made with you in a stationary position, lying down. We will remind you to remain still as we take this measure to ensure that the gel does not come into contact with your eye. If gel does come into contact with your eye, it may cause slight discomfort (slight drying) but it is not permanent. The gel is water soluble and actually designed to be used for eye exams therefore it rinses out easily. We will escort you quickly to an eye fountain to rinse out your eye.
- Communicating with the researcher throughout the protocol will reduce risks.
- If at any point you are uncomfortable or feel pain anywhere, please tell us immediately.
- You may experience discomfort from the finger stick to test your blood lipids, hematocrit and hemoglobin. This will only be done two or three times and no more than that. We will use different fingers each time to reduce discomfort. If desired we can also place ice on the finger prior to the finger stick to reduce discomfort from the pinch.
- There is a small risk of infection associated with the finger stick. However, we will reduce this risk by ensuring that equipment is clean and sterile and the finger stick technician will wear lab coat, gloves, will clean the finger with alcohol swabs and will clean the area with a disinfectant wipe afterwords.
- There is some risk of discomfort with the measurement of vessel reactivity. This technique uses a blood pressure cuff inflated around the forearm to pressures around 200 mmHg. This pressure may become uncomfortable over the 5 minute duration. You may feel as though your arm is "falling asleep," and may feel a numb or tingling sensation in the hand. This feeling will subside almost immediately when the cuff is released from the forearm.
- There is some risk of developing a headache and nausea at high altitude, known as acute mountain sickness (AMS). Importantly, this occurs more frequently with prolonged exposure to high altitude and intense exercise, the duration of the current study is short enough (and does not include exercise) so we do not anticipate the development of these complications. As a precautionary measure, however, we will monitor your blood oxygen saturation and administer a survey to document any symptoms of acute mountain sickness. If you end up presenting multiple symptoms based on the survey we may remove you from the altitude chamber. Additionally, we will monitor your blood oxygen saturation until it returns to normal values after exiting the chamber.
- There is a small risk of losing consciousness after entering the altitude chamber. We will minimize this risk by having you sit upon entering the chamber while your body initially

adjusts to the changes in altitude and by keeping a technician nearby during any major changes in body position (i.e. moving from lying down to standing). Additionally, we will investigate whether you have a history of losing consciousness prior to testing. If you have a history of losing consciousness you may be excluded from participating.

- There is minimal risk with consuming beetroot juice. It is possible you may experience gastric distress if you are allergic to beetroot juice. Beetroot juice has been shown to lower blood pressure after consuming it. This could potentially result in light-headedness or dizziness upon changes in body position (such as lying to standing). These risks are minimized because you will not be exercising while in the altitude chamber. As a precautionary measure, we will measure and monitor your blood pressure following the conclusion of testing and exiting the altitude chamber. You will be released once blood pressure has returned to normal values to pre-chamber values.

- In the event of illness or physical injury resulting from taking part in this research study, medical treatment will not be compensated for. You will be responsible for any costs not paid by your insurance company. No other compensation is offered by Syracuse University. You have not waived any of your legal rights by signing this form.

Are There Any Costs?

- There will be no costs to you for participating in this study.

Who Can See Information About This Study?

- ***The research records from this study will be confidential.*** Confidentiality means that it is our responsibility to keep any information you provide private and safe.
- Only members of the trained research staff for this study with training in research ethics may look over your research records.
- The paperwork, results and records will be kept in a locked filing cabinet that only the researchers with training in research ethics will have access to.
- You will be given a study identification number (coded numbers, known only by primary researchers) and this will be entered into all research computers used to collect your blood pressure and blood flow. Your name will not appear anywhere on these computers or the data output from these computers.
- All information stored on computers requires a password access it. Only members of the research team with training in research ethics will have this password.
- The data and research record will be stored for up to 10 years.
- ***Your individual results will not be used in any way (we will average all results and display group averages only when presenting findings in papers and presentations)***

What Are My Rights In This Study?

- If at any point you wish to withdraw yourself from the study you may.
- You do not give up any of your legal rights by participating in this study.

Who Can I Contact For Questions Or More Information?

- If there are research related injuries or if you have any questions, concerns, or complaints about this study at any time, please feel free to contact:
  - Dr. Kevin Heffernan at ksheffer@syr.edu or call his office at 315-443-9801.
- If you have any questions about your rights as a research participant, you have questions, concerns, or complaints that you wish to address to someone other than the investigator, if you cannot reach the investigator, or have experienced research related injuries, contact the Syracuse University Institutional Review Board at 315-443-3013.

By signing below you indicate that you have read and fully understood this informed consent form. You are fully aware of the purpose and procedures of this study as well as the risks, discomforts, and benefits associated with the experimental protocol and that you sign this document freely and voluntarily.

All of my questions have been answered, I am 18 years of age or older, and I wish to participate in this research study. I have received a copy of this consent form.

_____ _____

Signature of participant Date

_____

Printed name of participant

_____ _____

Signature of researcher Date

_____

Printed name of researcher

Appendix 2

Human Performance Lab Health Screening Form

Date_____

Age _____

Gender _____

Please answer the following questions as honestly as you can. Your patterns of responses will determine whether you may participate in the study.

Known Diseases (Medical Conditions)

1. List the medications and dietary supplements you take on a regular basis. (Include prescription and non-prescription, aspirin, vitamins/minerals, nutrition supplements [Ensure, Boost, etc.])

   _____

   _____

   _____

2. Has your health care provider ever told you have diabetes? No Yes
3. Do you have acute or terminal illness (if so, please explain below)? No Yes

   -

_____

_

4. Have you ever had a stroke, heart attack or heart trouble? No Yes
5. Has your health care provider ever told you that you have a heart murmur? No Yes
6. Have you had a head injury in the past 3 months? No Yes
7. Do you have asthma /take asthma medication? No Yes
8. Has your health care provider ever told you that you have kidney or liver disease? No Yes
9. Has your health care provider ever told you that you have chronic pulmonary or respiratory disease? No Yes
10. Has your health care provider ever told you that you have peripheral artery disease? No Yes

11. Has your health care provider ever told you that you have high blood pressure? No Yes

12. Has your health care provider ever told you that you have high cholesterol? No Yes

13. Do you smoke cigarettes on a daily basis? No Yes

If yes to #13, how many packs per day _____

If yes to #13, how long have you been smoking _____

14. Have you lost or gained weight in the previous 6 months? No Yes

If yes, how much weight? _____

15. Has a first degree relative (e.g. father, mother, sister, brother, or child) suffered from a heart attack or diagnosed cardiovascular disease? No Yes

Relative	Age	Did they pass away?

16. Do you often have pains in your heart, chest, neck, jaw, arms or other areas especially during exercise? No Yes

17. Do you regularly get pains in you calves or lower legs during exercise which are not due to soreness or stiffness? No Yes

18. Do you experience swelling or accumulation of fluid in or around your ankles? No Yes

19. Do you often feel faint or have spells of severe dizziness during exercise? No Yes

20. Do you often get the feeling that your heart is beating faster, racing, or skipping beats, either at rest or during exercise? No Yes

21. If you answered YES to question(s) 17-21, does your health care provider

know that you have this/these symptom(s)? No Yes

22. If you answered YES to question(s) 16-20, are you currently experiencing this/these symptom(s) RIGHT NOW? No Yes

23. With which hand do you write? Left Right

24. How do you define your race/ethnicity? _____

25. What is the highest grade/level of schooling/education completed?

8th Grade Some HS HS some college college graduate school

26. Do you have a known allergy to beets? No Yes

27. Do you know of any reason you should not travel to high altitude? No Yes

If you answered YES to question 27, please explain below.

_____

28. Have you traveled to any location above >2,500 m (8,200 ft; i.e. higher than Denver, CO) in the past 2 years? No Yes

If you answered YES to question 28, please explain below.

_____

29. About how many hours of sleep did you get last night?

_____

30. Please describe your current physical activity:

Mode: resistance exercise, running, cycling, swimming, other _____

_____

Sports: _____

Days per week _____ Minutes per day _____

31. Additional:

Please circle all that apply

Allergies	Fibromyalgia	Polio	Flu
Anemia	Attention Deficit Hyperactivity Disorder	Reflux or Ulcers	Seizures
Anxiety	Glaucoma	Liver Disease	Concussion
Arthritis	Lupus	Bone Disease	Eczema
Asthma	Meningitis	Leg/foot Ulcers	Epilepsy
Cataracts	Chronic Lyme Disease	Diverticulitis	Headaches/Migraine
Chronic Bronchitis	Gout	Infection	Urinary Tract Infection
Lung Disease	Thyroid (underactive/overactive)	Cold	Kidney Stones

Menstrual Status (answer these questions only if you are a female)

32. At what age did you have your first menstrual period? _____

33. What was the date of your last menstrual period?_____

34. Have you ever been amenorrheic (only 1-2 periods in a year)? _____ If yes, for how long?_____

35. If your last menstrual cycle was greater than 28 days ago and/or you have a history of amenorrhea are you currently under the care of a health care provider? _____

36. Do you use oral contraceptives or hormone replacement therapy? _____

Which kind? _____ What dose?_____ If yes, for how long? _____

Do you take the withdrawal/Placebo pills?_____

37. Do you use Depo-Provera for birth control? _____

If yes, for how long have you used this method?_____

38. Have you ever experienced menstrual irregularity?_____ Please describe (i.e. number of skipped menses, or prolonged menses): _____ How long did this occur?_____

39. Do you currently experience a menstrual cycle? _____

If yes, how many periods in a year do you have? _____ and how many days between periods? _____

If no, how many years ago did you have a regular menstrual cycle (10-12 a year)?_____

Answer question 40 if you are a male

40. Do you regularly use erectile dysfunction medication (i.e. Viagra, Levitra)? No Yes

Appendix 3

Nitrate Food Frequency Questionnaire

Item	Serving	Never	once per week	2-4 per week	5-6 per week	Daily	Once per month	Once per 3 months	Once per year
Broccoli	5 florets								
Cauliflower	1/2 cup								
Spinach	1 cup								
Beets	1 cup								
Beetroot juice	1 cups								
Radishes	1 cup								
Turnips	1 cup								
Carrots	1 normal/ 10 baby								
Lettuce	1 cup								
Kale	1 cup								
Arugula	1/2 cup								
Cabbage	1 cup								
Fennel	1 bulb								
Parsley	1 cup								
Leeks	1 cup								
Processed meats (hot dogs)	1 link								
Cured meats (sausage/cold cuts)	1 slice								

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Publications

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National Presentations

- Ives SJ, **Lefferts WK**, Wharton M, Fehling PC, Smith DL. "Exercise-induced heat stress disrupts the shear-dilatory relationship in the brachial artery." Presented at Experimental Biology, Boston, MA, March 28-April 1, 2015.

- Heffernan KS, Spartano NL, **Lefferts WK**, Augustine JA, Hughes WE, Gump BB. "Buffering of carotid artery pressure and flow pulsatility during cognitive engagement in healthy adults." Presented at the North American Artery Society 4th Annual Meeting, Chicago, IL, September 5-6, 2014.

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*Slide presentation; †Award recipient

- Haller JM, Hultquist EM, **Lefferts WK**, Smith DL, Fehling PC. "Influence of Clothing Layers Under Firefighting Protective Clothing on Physiological/Perceptual Responses to Intermittent Work." Presented at the American College of Sports Medicine's 59th Annual Meeting, San Francisco, CA, May 29-June 2, 2012.

- Hultquist EM, Arena L, **Lefferts WK**, Storer TW, Cooper CB, Fehling PC, Smith DL. "The Effect of Pre-cooling on Cardiovascular and Metabolic Strain During Incremental Exercise." Presented at the American College of Sports Medicine's 59th Annual Meeting, San Francisco, CA, May 29-June 2, 2012.

- Smith DL, Hultquist E, Arena L, **Lefferts WK**, Fehling PC "Effect of Heat Acclimation on Cardiac and Vascular Function, Oxygen Consumption, and Body Fluids." Presented at the American College of Sports Medicine's 58th Annual Meeting, Denver, CO, May 31-June 4, 2011.

Regional Presentations

- ***† Lefferts WK**, Hughes WE, White CN, Brutsaert TD, Heffernan KS. "Effect of Nitrate on Cognitive Function and Neurovascular Coupling at High Altitude" Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, October 31-November 1, 2014.

- Babcock MC, **Lefferts WK**, Heffernan KS. "Relation Between Exercise Central Hemodynamic Load and Resting Cardiac Structure and Function in Young Men" Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, October 31-November 1, 2014.

- Augustine JA, **Lefferts WK**, Spartano NL, Hughes WE, Gump BB, Heffernan KS. "Physical Function, Cognitive Function, and Aortic Stiffness in Older Adults" Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, October 31-November 1, 2014.

- Martin E, Augustine JA, Spartano NL, **Lefferts WK**, Heffernan KS. "No Association between Body Fat and Arterial Stiffness in Non-obese Women" Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 1-2, 2013.

- Augustine JA, **Lefferts WK**, Martin E, Spartano NL, Heffernan KS. "Vascular Function in Exercise-Trained Women." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 1-2, 2013.

- Spartano NL, Augustine JA, **Lefferts WK**, Hughes W, Morse B, Martin E, Bill K, Gump B, Heffernan KS. "Carotid blood pressure reactivity is associated with carotid intima-media thickness independent of central adiposity." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 1-2, 2013.

*Slide presentation; †Award recipient

- Hughes WE, Spartano NL, **Lefferts WK**, Augustine JA, Heffernan KS. "Sex differences in arterial stiffness and left ventricular pressure energetics." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 1-2, 2013.

- ***Lefferts WK**, Augustine J, Heffernan K. "Resistance exercise, carotid artery stiffness, and cerebral blood flow pulsatility" Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 1-2, 2013.

- ***Lefferts WK,** Hultquist E, Heffernan K, Fehling P, Smith D. "Vascular changes following exercise-induced hyperthermia." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 2-3, 2012.

- Tarzia BJ, Kasprowicz AG, **Lefferts WK**, Heffernan KS. "Physical Activity, Sedentary Behavior and Blood Pressure in Young Adults." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 2-3, 2012.

- ***† Lefferts WK**, Hultquist E, Arena L, DeBlois J, Fehling PC, Smith DL. "Effect of Base Layers on Physiological and Perceptual Strain during Exercise in Personal Protective Equipment." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 5-6, 2010.

- Hultquist E, Arena L, DeBlois J, **Lefferts WK**, Fehling PC, Smith DL. "Effect of Heat Acclimation on Cardiac and Vascular Function, Oxygen Consumption, and Body Fluids." Presented at the Mid-Atlantic Regional Conference American College of Sports Medicine, Harrisburgh, PA, November 5-6, 2010.

Grants/Support

1. American College of Sports Medicine Foundation Research Grant ($4,983.93) – PI
   The effect of Dietary Nitrate on Cognitive and Cerebrovascular Function in Hypoxia (2014)
2. Syracuse University School of Education Travel Grant ($400) – PI (2014)
3. Syracuse University Graduate School Organization Travel Grant ($200) – PI (2014)

Professional Organizations

American College of Sports Medicine (ACSM), 2010 – present.

Mid-Atlantic American College of Sports Medicine (MARC), 2010 – present.

Community Service Activities

Invited Talks

- **Lefferts WK**, Spartano N, Augustine J. "Cardiovascular function and health implications: research and application." Presented to the Syracuse University School of Education Board of Visitors, Syracuse, NY, September 21, 2013.

*Slide presentation; †Award recipient

- **Lefferts WK** "Summer Collaborative Research Experience." Presented at the Skidmore College Board of Trustees Reception and Dinner, Saratoga Springs, NY, February 24, 2011.

Invited Presentations

- **Lefferts WK**, Hultquist E, Arena L, Fehling, PC, Smith, DL. Effects of Altered Core Temperature on Cardiovascular Strain, Thermal Strain and Performance. Presented at Undergraduate Research Conference, Saratoga Springs, NY, October 1, 2011.

- **Lefferts WK**, Hultquist E, Arena L, Fehling PC, Smith, DL. Effects of Altered Core Temperature on Cardiovascular Strain, Thermal Strain and Performance. Presented at Skidmore College Academic Festival, Saratoga Springs, NY, May 4, 2011.

- **Lefferts WK**, Hultquist E, Arena L, DeBlois J, Fehling PC, Smith DL. Effect of Base Layers on Physiological and Perceptual Strain during Exercise in Personal Protective Equipment. Presented at Skidmore College Celebration Weekend Spotlight on Collaborative Research, Saratoga Springs, NY, October 15, 2010.

- **Lefferts WK**, Hultquist E, Arena L, DeBlois J, Fehling PC, Smith DL. Effect of Base Layers on Physiological and Perceptual Strain during Exercise in Personal Protective Equipment. Presented at Final Summer Collaborative Research Meeting, Saratoga Springs, NY, August 6, 2010.

Invited Lectures

- **Lefferts WK**, Cardiovascular Physiology: Form and Function. Presented to PPE 497: Exercise Physiology, Syracuse University, Syracuse, NY, October 28, 30, 2014.

- **Lefferts WK**, Novel Measures of Body Composition. Presented to PPE 500: Body Composition, Syracuse University, Syracuse, NY, April 22, 2014.

- **Lefferts WK**, Cardiovascular Responses to Exercise. Presented to PPE 497: Exercise Physiology, Syracuse University, Syracuse, NY, February 11, 2013.

Certification/Honors/Awards

- MS Research Award, Mid-Atlantic Regional Chapter, ACSM, 2014
- American College of Sports Medicine (ACSM) Environmental and Occupational Physiology Interest Group MSc/BSc Research Award, 2012
- Graduated Magna Cum Laude, Skidmore College, Health and Exercise Sciences, 2011
- Margaret Paulding Award in Exercise Science, Skidmore College, 2011
- Matthew Kerner Undergraduate Research Award, Mid-Atlantic Regional Chapter, ACSM, 2010
- American Red Cross CPR