DISSERTATION

ROLE OF THE SYMPATHO-ADRENAL SYSTEM IN THE REGULATION OF PERIPHERAL VASCULAR TONE IN HEALTHY AGING HUMANS

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ABSTRACT

ROLE OF THE SYMPATHO-ADRENAL SYSTEM IN THE REGULATION OF PERIPHERAL VASCULAR TONE IN HEALTHY AGING HUMANS

The following dissertation is comprised of a series of experiments with the overall aim of determining the role of the sympatho-adrenal system in the regulation of peripheral vascular tone in normal, healthy aging humans. Aging is associated with a reduction in aerobic capacity and elevated risk of cardiovascular disease. The decline in aerobic capacity could be a consequence of impaired O_2 delivery, directly attributed to attenuated skeletal muscle blood flow. Attenuated skeletal muscle blood flow reflects a decline in the ability to adequately regulate peripheral vascular tone, either due to elevated vasoconstrictor signaling or decreased vasodilator signaling.

Understanding the regulation of peripheral vascular tone and what leads to its decline with age could lend itself to the creation of treatments capable of improving oxygen (O_2) delivery by increasing muscle blood flow. Net vascular tone is determined by the balance between vasodilator and vasoconstrictor tone within the vessel. With age, there is both a decrease in the production of local endothelium-dependent dilators as well as an increase in resting sympathetic nervous system (SNS) activity, which can lead to elevations in vasoconstrictor tone, potentially limiting blood flow to skeletal muscle. To better understand the role of the SNS in regulation of peripheral vascular tone with age, we can use established pharmacology and locally inhibit the sympatho-adrenal system and observe the net blood flow response during conditions that challenge the vascular system such as lowered O_2 delivery (systemic hypoxia) and elevated peripheral O_2 demand (exercise) in both young and older adults.

We hypothesize that older adults will exhibit impaired regulation of peripheral vascular tone to both graded systemic hypoxia and graded handgrip exercise. Further, we hypothesize that the age-associated impairment is attributed to elevated vasoconstrictor tone mediated from the SNS.

The primary findings of this dissertation are that older adults exhibit impaired regulation of peripheral vascular tone in response to physiological stressors (systemic hypoxia and handgrip exercise). Contrary to our hypothesis, the age-associated impairment was not directly attributed to elevated vasoconstrictor tone from the SNS and is likely due to attenuated vasodilatory signaling in older adults.

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CHAPTER I – INTRODUCTION AND EXPERIMENTAL AIMS

The primary aim of this dissertation is to determine the role the sympatho-adrenal system plays in the regulation of peripheral vascular tone with advancing age. With respect to the vasculature, homeostasis is maintained when oxygen (O_2) delivery is in balance with peripheral metabolic O_2 demand. Throughout adult aging, aerobic capacity declines and this could be attributed to a decline in O_2 delivery, leading to a state of altered homeostasis whereby a given metabolic demand is accompanied by attenuated peripheral O_2 delivery. In support of this idea, abundant evidence indicates that resting and exercising skeletal muscle blood flow (O_2 delivery) is lower with age.

When examining the factors that determine blood flow through a vessel or a vascular bed, we can apply Poiseulle's equation which states that the primary determinants of blood flow are perfusion pressure, radius of the vessel, length of the vessel, and the viscosity of the blood. Simply stated, blood flow is primarily determined by the perfusion gradient (ΔP) and the vascular conductance within that vascular bed and can be mathematically stated as follows: Blood Flow (Q) = $(\Delta P)*(\pi r^4)/8l\eta$. The ΔP represents the difference in pressure across the vascular bed ($\Delta P = P_{arterial} - P_{venous}$), and reflects the driving pressure of blood flow into the vessel. Vascular conductance is proportional to the radius of the vessel raised to the power of four (r^4) and inversely proportional to vessel length (l) and blood viscosity (η). In an attempt to maintain homeostasis whereby O_2 delivery is matched to O_2 demand, vessel length (l) and blood viscosity (η) are not acutely regulated and therefore alterations in blood flow reflect changes in perfusion pressure (ΔP) and the caliper or radius (r^4) of the resistance vessels due to changes in vasodilator or vasoconstrictor signaling.

In resting skeletal muscle beds, the pressure gradient (ΔP) is stable and therefore blood flow and O_2 delivery are primarily determined by the caliper (r^4) or net vascular tone of the

resistance vessels, which reflects the balance of local vasoconstrictor and vasodilator signaling. The balance or "net vascular tone" of these resistance vessels are the primary determinant of blood flow, such that vessels exhibiting a greater degree of vasoconstrictor influence, exude a greater degree of resistance that needs to be overcome for blood and O_2 to be delivered to tissues. Conversely, vessels with a greater degree of vasodilatory tone exhibit lower resistance to blood flow and O_2 delivery.

Signals that are associated with regulating peripheral vascular tone include circulating humoral factors, local vasodilator and vasoconstrictor signaling, and the degree of sympathetic vasoconstrictor tone. With age, there is an attenuated production and bioavailability of local endothelium-dependent dilators as well as an increase in resting sympathetic nervous system (SNS) activity, which can lead to elevations in vasoconstrictor tone, potentially limiting blood flow to skeletal muscle. To quantify the role of the SNS in any age-associated impairment with the regulation of peripheral vascular tone we can use established pharmacology and locally inhibit the sympatho-adrenal system and observe the net blood flow response during conditions that challenge the vascular system such as lowered O_2 delivery (systemic hypoxia) and elevated peripheral O_2 demand (exercise) in young and older adults.

During the application of physiological stressors that evoke a mismatch in O_2 delivery and demand, net vasodilation in skeletal muscle beds is required to increase O_2 delivery to the tissue and maintain cellular respiration and homeostasis. Vasodilation is attributed to locally released factors and, during exercise, mechanical factors associated with compression of vessels during skeletal muscle contraction. Several locally released factors have been implicated in facilitating increases in blood flow during hypoxia and exercise including nitric oxide, prostaglandins, K^+, adenosine, adenosine triphosphate, and lactate. However, to date, no single factor is considered

wholly responsible for augmenting blood flow, rather there appears to be redundancy such that blood flow is maintained following the elimination of one dilator. In addition, production of many local dilators is dependent upon a healthy endothelium, and this process is often referred to as endothelium-dependent dilation. Traditionally, the measure of endothelial vasodilatory function can occur via two tests; the examination of the vasodilatory responsiveness to intra-arterial infusion of the muscarinic receptor agonist acetylcholine or the degree of brachial artery dilation that occurs in response to an acute increase in shear stress along the vessel wall following five minutes of brachial artery occlusion. These tests can quantify the degree of endothelial dysfunction, which is reported to be significantly correlated with increasing age and risk of cardiovascular disease.

The SNS is tonically active at rest, resulting in norepinephrine release from sympathetic nerve terminals following bursts of nerve activity. Vasoconstriction ensues after norepinephrine diffuses through the synaptic cleft and binds α-adrenergic (α_1- and α_2-) receptors located on vascular smooth muscle. Aging is characterized by a 2-3 fold increase in resting SNS activity and the degree to which this tonic level of elevated SNS activity restrains skeletal muscle blood flow at rest has been quantified through the administration of both α_1- and α_2- adrenergic receptor antagonists. Resting leg blood flow is significantly lower in older adults compared to that in young and following local α-adrenergic blockade, the legs of older adults increase blood flow to a significantly greater degree than young (100% vs. 60% increase), indicating that the SNS actively limits blood flow to the leg in older adults at rest. This elevated vasoconstrictor tone in older adults occurs despite the presence of decreased α-adrenergic receptor responsiveness in the legs of older adults, providing evidence of the high degree of SNS signaling in this population.

In the present study, we utilized the forearm as our model which possesses both similar and different characteristics compared to the findings mentioned in the leg. Resting forearm blood flow is generally not different between young and older adults. Additionally, following sympatho-adrenal blockade we have not observed a greater increase in forearm blood flow at rest in older compared to young adults, suggesting that there is not a greater degree of sympathetic vasoconstrictor tone at rest in older adults. Similar to the findings reported in the leg, there is a significant decrease in α-adrenergic receptor responsiveness in older adults.

In addition to vasoconstriction, SNS activity can also facilitate skeletal muscle vasodilation. Sympathetic nerves innervate the adrenal medulla and cause epinephrine to be released into circulation which then can bind β-adrenergic receptors located on the endothelium and vascular smooth muscle and result in vasodilation (primarily mediated through β_2 adrenergic receptors). The dilatory response to β_2 adrenergic receptor stimulation has been observed to be impaired with age, thus any contribution that β-receptors may have on increasing skeletal muscle blood flow could be attenuated in an aging population.

The body of work that comprises this dissertation determines the role of the sympatho-adrenal system in the regulation of peripheral vascular tone in aging adults during two physiological stressors; attenuated O_2 delivery (graded systemic hypoxia) and elevated peripheral O_2 demand (graded handgrip exercise). Both systemic hypoxia and exercise are strong stimuli to increase peripheral O_2 delivery and by doing so, provided a means for us to examine the regulation of peripheral vascular tone.

The first physiological stressor implemented was systemic hypoxia. Acute systemic hypoxia stimulates chemo-receptors and results in activation of the SNS, leading to elevations in both vasoconstrictor (α-adrenergic receptor) and vasodilatory (local and β-adrenergic receptor)

signaling, the net result in young healthy individuals being peripheral vasodilation. Presently, it is unknown how older individuals respond to graded systemic hypoxia and given that they exhibit impaired local vasodilatory signaling, lower β-adrenergic receptor responsiveness and elevated SNS activity, it is feasible that vasodilation to hypoxia is impaired.

The second physiological stressor implemented was graded handgrip exercise, which requires an increase in O_2 delivery (blood flow) due to augmented metabolic demand. The SNS is tonically active at rest and will become more active once moderate exercise intensity has been attained or a large muscle mass has been recruited. In our forearm model, a small muscle mass is utilized to perform exercise and the intensities chosen for handgrip exercise were mild to moderate, which limits further SNS engagement. Although resting SNS activity is elevated with age and the forearm of older adults does not always exhibit elevated vasoconstrictor tone at rest, recent animal work indicates that in the absence of any difference between resting arteriolar diameter in young and older mice, electrically stimulated muscle contractions revealed a significant blood flow impairment in older mice that was reversed following the application of a non-selective α-adrenergic receptor antagonist (phentolamine). It is feasible that despite young and older adults having similar resting forearm blood flows and a similar degree of SNS vasoconstrictor tone, the elevated basal activity of the SNS in older adults may impede the ability of forearm blood flow to increase during exercise, similar to findings in animal studies. Collectively, these date indicate a potential role for the SNS to actively restrain skeletal muscle blood flow during exercise.

In the present dissertation we assessed endothelium-dependent vasodilation and through the local administration of adrenergic receptor antagonists, we were able to determine the role of the sympatho-adrenal system in the regulation of peripheral vascular tone both in young and older

adults. In addition to quantifying the degree of sympathetic restraint of skeletal muscle blood flow, following sympatho-adrenal blockade we isolated local vasodilatory signaling in both young and older adults.

Overall hypothesis: Age-associated impairments in the ability to increase skeletal muscle blood flow during attenuated O_2 delivery (graded systemic hypoxia) or elevated peripheral O_2 demand (graded handgrip exercise) are present and can be attributed to elevated α-adrenergic mediated vasoconstrictor tone.

Specific Aims

Experiment #1: to determine whether regulation of peripheral vascular tone during acute graded systemic hypoxia is impaired with aging and whether an age-associated impairment is due to elevated α-adrenergic vasoconstrictor tone.

Follow-up Experiment: to determine whether the age-associated impairment in the regulation of peripheral vascular tone during acute systemic hypoxia is ameliorated by augmenting local vasodilatory signaling. Following local sympatho-adrenal blockade, we aimed to determine if acute ascorbic acid infusion would improve local vasodilation in older adults during system hypoxia.

Experiment #2: to determine whether impairments in forearm blood flow during graded handgrip exercise in older adults are attributed to elevated α-adrenergic vasoconstrictor tone.

Follow-up Experiment: to determine if acute ingestion (2 grams) of ascorbic acid improves forearm vasodilation during graded handgrip exercise in older adults.

This collection of work provides novel insight into the role of the sympatho-adrenal system in the regulation of peripheral vascular tone in both young and older adults during physiological and commonly encountered stressors that require elevated O_2 delivery and can increase SNS activity in a graded fashion. Through local pharmacological inhibition of the sympatho-adrenal system we can determine its contribution to any age-associated impairment in the regulation of peripheral vascular tone. To date, the majority of the literature has examined how impaired production of local vasodilators with aging impact net vascular tone and subsequently, blood flow. To our knowledge, these are the first experiments designed to determine whether elevated sympathetically mediated vasoconstrictor tone contributes to impaired regulation of peripheral vascular tone with aging. Specifically, this is the first study to determine if there is an age-associated impairment in the vascular response to graded hypoxia in older adults and what role the sympatho-adrenal system contributes to an impairment. Additionally, this is the first study to determine what role the sympatho-adrenal system plays in restraining skeletal muscle blood flow *during* graded handgrip exercise in both young and older adults.

CHAPTER II – MANUSCRIPT I

Altered regulation of peripheral vascular tone during graded systemic hypoxia in aging humans: role of the sympatho-adrenal system

Summary

Systemic hypoxia is a physiological stressor that activates both vasoconstricting and vasodilatory signaling pathways, and in young adults, the net response is peripheral vasodilation. To date, it is unknown how the vasculature of older adults respond to graded hypoxia. Further, it is unclear what role the age-associated changes in the sympatho-adrenal system influence the net response. We tested the hypothesis that peripheral vasodilation to graded systemic hypoxia (90, 85, 80% SaO_2) was impaired in older healthy adults and that this age-associated impairment was attributed to elevated α-adrenergic vasoconstriction and attenuated β-adrenergic mediated vasodilation. Forearm blood flow was measured (Doppler ultrasound) and vascular conductance (FVC) was calculated in 12 young (24±1 years) and 10 older (63±2 years) adults to determine the local (brachial artery catheter) dilatory response to graded hypoxia in control, following local β-blockade (propranolol), and combined local α+β blockade (phentolamine + propranolol) conditions. Compared to young, older individuals exhibited impaired vasodilation to hypoxia (peak Δ FVC: Older = +4±6%; Young = +35±8%; $P<0.01$) and following β-blockade, older adults actively constricted (peak Δ FVC= -20±7%) whereas the young response was not significantly impacted (peak Δ FVC 28±8%). α+β blockade increased the dilatory response to hypoxia in both groups, however the dilatory response remained significantly greater in young compared with the old (peak Δ FVC 58±11% vs.12±11% ; $P<0.05$). Our findings indicate that hypoxic vasodilation is significantly impaired in older adults and, contrary to our hypothesis, this age-associated impairment is likely due to attenuated local vasodilatory signaling.

Introduction

In normoxic conditions, tissue oxygen (O_2) delivery is sufficiently matched to tissue O_2 demand and upon acute exposure to systemic hypoxia, there is an immediate decline in tissue O_2 delivery. This decline in arterial PO_2 is detected by the aortic and carotid bodies which feedback centrally and activate the SNS in a graded fashion relative to the level of hypoxia [1]. The increased SNS activity leads to sympathetically mediated vasoconstriction that occurs when norepinephrine is released from sympathetic nerve endings and binds vascular smooth muscle α-adrenergic receptors (α_1- and α_2-) causing vasoconstriction. In order for the mismatch between O_2 delivery and demand to be corrected, net vasodilation should result following exposure to systemic hypoxia. Despite the sympathetically mediated increase in vasoconstrictor signaling, young adults do exhibit net vasodilation to systemic hypoxia [2, 3]. Part of this vasodilation is also attributed to activation of the SNS. Elevations in SNS activity lead to epinephrine release into the circulation from the adrenal medulla. Epinephrine can bind β-adrenergic receptors and elicit vasodilation. In addition, locally released factors contribute to the net vasodilation observed (nitric oxide and prostaglandins) [2-5].

Although the peripheral vascular response to hypoxia in young individuals has been well studied, it remains to be determined how healthy older individuals respond to graded systemic hypoxia. Aging is characterized by elevated SNS activity [6] as well as endothelial dysfunction [7], and these changes are exacerbated in many disease conditions including sleep apnea and heart failure [8]. Elevated SNS activity and endothelial dysfunction can directly impact the regulation of peripheral vascular tone and systemic hypoxia is a stress that increases SNS vasoconstrictor signaling [6] and relies upon local β-adrenergic and endothelium-dependent vasodilatory signaling [2, 3] to augment peripheral blood flow and O_2 delivery. Given that aging

is an independent risk factor for cardiovascular disease, as well as the increased likelihood of experiencing an acute hypoxic event with age, it is important to understand how some known age-associated changes in the vasculature (endothelial dysfunction and elevated SNS activity) influence the net peripheral response to a graded hypoxic stimulus. Further, if older adults do exhibit impaired hypoxic vasodilation, it is important to determine the underlying mechanism. In this context, attenuated vasodilation in the older adults could be attributed to elevated α-adrenergic vasoconstriction, impaired β-adrenergic mediated vasodilation, and/or attenuated local vasodilatory signaling. The aim of the present study was to test the hypothesis that compared to young, older adults have an impaired hypoxic vasodilatory response and this impairment is attributed to increased α-adrenergic vasoconstrictor tone and attenuated β-adrenergic vasodilation.

Methods

Subjects

With Institutional Review Board approval and following written informed consent, a total of 12 young (4 female, 8 male) and 10 older (4 female, 6 male) healthy subjects participated in the present study. All participants were non-smokers, non-obese, normotensive, and not taking any medications including over the counter supplements. Young females were studied during the placebo phase of birth control or during the early follicular phase of their menstrual cycle to minimize any potential vascular effects of sex hormones. Older females were post-menopausal (2 + years) and not taking hormonal supplements. Subject characteristics are presented in Table 1. All Studies were performed in the Human Cardiovascular Physiology Laboratory located at

Colorado State University (~1500 m above sea level) following a 12-hour fast with the subjects in the supine position. All studies were performed according to the Declaration of Helsinki.

Arterial Catheterization

The non-dominant arm was chosen to be the experimental arm and after local application of anesthesia (2% lidocaine), a 20-guage, 7.6 cm catheter was inserted into the brachial artery utilizing aseptic technique. The catheter was connected to a pressure transducer for continuous monitoring of mean arterial pressure (MAP) as well as a 3-port connector to allow for drug infusions and blood sampling. Throughout the duration of the study, heparinized saline was continuously infused at a rate of 3 ml minute^{-1}.

Body Composition and Forearm Volume

Dual- energy X-ray absorptiometry (DEXA: Hologic: Bedford, MA, USA) was used to determine body composition. A regional analysis of the experimental forearm area (proximal to distal radio-ulnar joint) from the whole body DEXA scan was performed to determine forearm volume. Drug dosage was normalized according to forearm volume [9]. Body mass index was calculated as body weight (kg) divided by height (meters) squared.

Graded Systemic Isocapnic Hypoxia

To elicit graded systemic hypoxia we utilized a self-regulating partial re-breathe system [10] which allows for constant alveolar fresh air ventilation independent of changes in minute ventilation and enables end-tidal CO_2 ($EtCO_2$) to be clamped [9, 10]. O_2 levels were titrated down by mixing nitrogen with air in a medical gas blender to attain steady arterial O_2 saturations

(SaO_2) of 90, 85, and 80 % as assessed by pulse oximetry (SpO_2) of the earlobe. Nasal breathing was prevented through the use of a nose clip while subjects breathed through a scuba mouthpiece. An anesthesia monitor was used to monitor gas concentrations at the level of the mouthpiece (Cardiocap, Datex-Ohmeda, Louisville, CO, USA) as well as to monitor heart rate (HR; 3 lead ECG). Additionally, ventilation was measured with a pneumotachograph (model 17125 UVM,Vacu-Med, Ventura, CA, USA).

Forearm Blood Flow (FBF) and Vascular Conductance (FVC)

Brachial artery mean blood velocity (MBV) and diameter was determined using a 12 MHz linear-array ultrasound probe (Vivid 7, General Electric, Milwaukee, WI, USA). The probe was placed proximal to the catheter site as previously described [9]. During blood velocity measurements, the probe insonation angle was maintained at less than 60 deg and the frequency used was 5 MHz. A multigon 500M TCD spectral analyzer (Multigon Industries, Mt. Vernon, NY, USA) was used to analyze the Doppler shift frequency and subsequently determine MBV from the weighted mean of the spectrum of Doppler shift frequencies. Brachial artery diameter measurements were made in duplex mode at end-diastole in triplicate during steady state conditions. Forearm blood flow (FBF) was calculated as FBF=MBV x $\times \pi$ (brachial artery diameter/2)2 $\times$ 60, where the FBF is in ml min^{-1}, the MBV is in cm s^{-1}, the brachial diameter is in centimeters, and 60 was used to convert from ml s^{-1} to ml min^{-1}. Forearm vascular conductance (FVC) was calculated as (FBF/MAP) $\times$100, and expressed as ml min^{-1} 100$mmHg^{-1}$ [11, 12].

All studies were performed in a cool temperature-controlled environment with a fan directed toward the forearm to minimize the contribution of skin blood flow to forearm

hemodynamics. Further, during each trial a cuff was inflated around the wrist to 200 mmHg to eliminate blood flow to the hand.

Regional α- and β- adrenergic receptor responsiveness

α- and β-adrenergic receptor agonists were locally infused in three incremental doses at two minutes per dose to determine adrenergic receptor responsiveness. The α-adrenergic agonist was norepinephrine bitartrate (Levophed, Hospira Inc., Lake Forest, IL, USA) (12, 24, 90 pmol dL^{-1} forearm volume (FAV) $minute^{-1}$) [13] and the β-adrenergic agonist was isoproterenol hydrochloride (Isuprel, Hospira Inc., Lake Forest, IL, USA (1, 3, 10 ng dL FAV^{-1} min^{-1}) [14].

Regional β-adrenergic receptor blockade

To determine the contribution of β-adrenergic receptor mediated vasodilation to graded systemic hypoxia, we locally infused propranolol hydrochloride (Baxter, Deerfield, IL,USA), a non-selective β-adrenergic receptor antagonist, for five minutes prior to hypoxia (10 μg dL FAV^{-1} min^{-1}) and continued the infusion at a maintenance rate (5 μg dL^{-1} FAV^{-1}) throughout the hypoxia trial [3, 9]. These doses were chosen based on previous studies in our laboratory demonstrating them to be effective at β-adrenergic receptor blockade [3, 9].

Regional α-adrenergic receptor blockade

To eliminate α-adrenergic mediated vasoconstriction during graded systemic hypoxia, we locally infused phentolamine mesylate (Bedford Laboratories, Bedford, OH, USA), a non-selective *α*-adrenergic receptor antagonist for 10 minutes prior to hypoxia (12 μg dL FAV^{-1} min^{-1}) and maintained the infusion during graded systemic hypoxia (5 μg dL FAV^{-1} min^{-1}). These

doses were chosen based on previous studies in our laboratory demonstrating effective α-adrenergic blockade [3, 9].

Blood Gas Sampling and Catecholamine Analysis

Arterial blood gases and catecholamine (epinephrine and norepinephrine) samples were collected at the end of baseline and each level of hypoxia (90, 85, 80% SpO2) in all conditions (Control, β-blockade, and α+β-blockade). Blood gas samples were analyzed with a clinical blood gas analyzer (Siemens Rapid Point 400 series, Los Angeles, CA USA). Arterial catecholamine samples were analyzed with high performance liquid chromatography electrochemical detection (Mayo Clinic, Rochester, MN, USA).

Experimental Protocol

The overall study timeline is presented in Figure 1. All participants arrived in the morning after an overnight fast. All measurements were performed with the subjects in the supine position within a temperature controlled room with a fan directed towards the arm and a wrist cuff inflated to limit blood flow measures to the forearm. All study drugs were administered via brachial artery catheter with a Harvard infusion syringe pump.

Following placement of the catheter, subjects rested quietly for a minimum of 30 minutes. To begin, α- and β- adrenergic receptor responsiveness were assessed using norepinephrine and isoproterenol, respectively. Following 2 minutes of baseline, three incremental doses of each agonist were locally infused for two minutes at each dose. The last 30 seconds of each dose was used to calculate steady state FBF and FVC. A 10 minute break was given between administration of the α- and β-agonist, the order of which was randomized and

counter-balanced. Following the determination of both α- and β- adrenergic receptor responsiveness, individuals underwent three trials of graded systemic hypoxia. Each hypoxia trial consisted of 5 minutes of baseline where subjects breathed room air through the mouthpiece, followed by 12-15 minutes of hypoxia. Each level of steady state hypoxia (90, 85, 80% SpO_2) was maintained for at least 1 minute before the next level of hypoxia was begun. 20 minutes of rest occurred between hypoxia trials.

During the first hypoxic trial, saline was infused and the normal hypoxic vasodilatory response was assessed. Prior to (5 minutes) and throughout the second hypoxic trial, propranolol was locally infused to eliminate β-adrenergic receptor mediated vasodilation, enabling us to observe the net peripheral vascular response under the influence of α-adrenergic vasoconstriction and local vasodilatory signaling. Prior to and throughout the third hypoxic trial, both phentolamine and propranolol were infused to eliminate both α-adrenergic vasoconstriction and β-adrenergic vasodilation, thus removing sympatho-adrenal influence and enabling us to isolate local vasodilatory signaling. Following completion of the hypoxia trials, we tested the efficacy of our α-adrenergic and β-adrenergic blockade by re-administering agonists for two minutes each. In anticipation that older adults would exhibit attenuated adrenergic receptor responsiveness, we chose to challenge with the high dose of both agonists in the older group and the medium dose of each agonist in the young group.

Data Acquisition/Analysis

Data were collected and stored on a computer at 250Hz and later analyzed off-line with signal-processing software (Windaq DATAQ Instruments, Akron, OH, USA). MAP was determined from the brachial artery pressure waveform. HR, MAP, FBF, FVC, and oxygen

saturations represent an average of the last 30 seconds of each time period. Minute ventilation and end-tidal CO_2 were determined from an average of the data over a minute time period. Blood gas values were obtained during the last minute of each condition.

In the present study, FVC was chosen as the primary variable of interest because it reflects net vascular tone of the resistance vasculature. Systemic hypoxia as a stimulus does not significantly alter the arterial-venous pressure gradient and therefore changes in O_2 delivery are reflected by changes in the caliper of the resistance vessels or vascular conductance. Additionally, baseline blood flows increase following local sympatho-adrenal blockade and therefore to more accurately reflect dilation or constriction we chose to present the data as a percent change in FVC from baseline (Δ FVC (%)) [11].

Utilizing SPSS statistical software (IBM, Armonk, New York) a three way repeated measure ANOVA was used to examine the impact of age, $\%SpO_2$, as well as any drug interaction affects. When appropriate, post-hoc comparisons were made using Tukeys HSD and significance was set at $P<0.05$. All values are presented as means ± standard error of the mean (SEM).

Results

Subject Characteristics

Subject characteristics are presented in Table 1. Older adults had significantly greater total cholesterol (184 vs. 141 mg dl^{-1}) and LDL cholesterol (108 vs. 81 mg dl^{-1}) $P<0.05$ although the elevated cholesterol in the older group (total and LDL) are still considered healthy and are within the normal range (<200 mg dl^{-1} and <129 mg dl^{-1}).

Hemodynamic response to graded systemic hypoxia

There were no significant differences in baseline FBF or FVC between young and older adults (Table 2). During the control hypoxia trial, young individuals exhibited progressive vasodilation (Δ FVC 17±3, 19±5, 35±8 %) in response to graded hypoxia (90, 95, 80% SpO_2) whereas older adults failed to significantly vasodilate at any level of SpO_2 (Δ FVC -4±4, -3±3, 4±7 %) (Figure 2A).

Following local β-adrenergic receptor blockade, there were no significant differences in baseline FBF or FVC between young and older adults (Table 2). Additionally, baseline FBF and FVC in both young and older adults was not different than the control condition. During graded systemic hypoxia, young adults continued to exhibit net vasodilation, and the Δ FVC (%) at each level of hypoxia was only slightly less than that observed in control conditions (peak Δ FVC 28±8 vs. 35±8 %) (Figure 2B). Conversely, during β-adrenergic blockade, older adults actively constricted (Δ FVC -5±5, -11±4, -13±6 %) to graded systemic hypoxia (Figure 2B).

Following local infusion of phentolamine (α-adrenergic receptor antagonist), the increase from baseline was similar in both young and older adults (Δ FVC 43% young, 52% old). During the third hypoxia trial, when both α-adrenergic vasoconstriction and β- mediated vasodilation were inhibited, young adults still exhibited a significant increase in ΔFVC (%) from baseline at all levels of hypoxia (Δ FVC 18±, 34±7, 58±11 % at 90, 95, 80% SpO_2) whereas the older adults failed to significantly vasodilate from baseline at any level of hypoxia (Δ FVC 4±7, 4±8, 12±1 %) and the age-associated impairment in peripheral vasodilation persisted (Figure 2C).

Baseline heart rate was lower in older adults, although not significant (Table 2). During the hypoxia trials, heart rate was greater in young adults compared to older adults and was

significant at 85% and 80% SpO_2 in all hypoxia trials and significant at 90% SpO_2 in the control hypoxia trial and β-adrenergic blockade trial (Table 2). Mean arterial pressure was not different between young and old during baseline in any hypoxia trial. Older adults had significantly high mean arterial pressure at 80% SpO_2 in control (102±4 vs. 93±3 mmHg) and in β-adrenergic blockade hypoxia (105±3 vs. 96±3 mmHg) trials. There were no differences in mean arterial pressure between young and old in the α+β- blockade hypoxia trial.

Effects of graded systemic hypoxia on blood gases, ventilation, and arterial catecholamine concentrations

At rest, there were no significant differences between young and older adults with respect to baseline blood gases (Table 3) and ventilation (Table 4). Further, there was no effect of time (hypoxic bout) or age on blood gas or ventilatory response to hypoxia. There were no significant differences in baseline arterial catecholamine concentrations between young and older adults in any condition (control, β-blockade, and α+β-blockade) (Table 3). Arterial epinephrine concentrations increased with the level of hypoxia in both young and older adults, becoming significantly greater than baseline in some conditions at the lowest level of $\%SpO_2$. There were no significant difference between young and older adults in arterial epinephrine concentrations during any level of the hypoxia trials. Older adults had significantly greater norepinephrine concentrations in the β-blockade condition at 85% (257±20 vs. 192±15 pg ml^{-1} $P<0.05$) and 80% SpO_2 (283±28 vs.185±19 pg ml^{-1} $P<0.05$) and in the α+β-blockade condition at 90% SpO_2 (365±56 vs. 222±23 pg ml^{-1} $P<0.05$), 85% SpO_2 (392±45 vs. 219±19 pg ml^{-1} $P<0.05$) and 80% SpO_2 (461±58 vs. 205±25 pg ml^{-1} $P<0.05$).

FBF and FVC response to α- and β- adrenergic receptor agonists

Baseline FBF and FVC were not different between young and old (Table 5). Compared to young, older individuals exhibited lower α- and β- adrenergic receptor responsiveness. Older adults had significantly lower vasoconstrictor responses to the middle and high doses of norepinephrine (Δ FVC -10±5 vs. -27±4 % and -24 ±6 vs. -40±4 %) (Figure 3A) and significantly lower vasodilatory responses to the middle and high doses of isoproterenol (Δ FVC 61±18 vs. 142±21 % and 154±34 vs. 315±42%) (Figure 3B).

Propranolol and phentolamine efficacy

At the end of the hypoxic trials, the efficacy of the local α+β blockade was challenged with a single dose of norepinephrine and isoproterenol. In anticipation of observing blunted receptor responsiveness in the older group, we chose to administer the medium dose of both agonists in the young group and the high dose of both agonists in the older group. There was no significant change in FBF or FVC in response to the challenge at the end of the trial, indicating effective local α- and β- adrenergic receptor blockade (Figure 3A and 3B).

Discussion

To our knowledge, this is the first study to determine the peripheral vascular response to graded systemic hypoxia in older adults and attempt to determine what role the age-associated changes in the sympatho-adrenal system may play in regulating the net response. The primary finding of the present study is that compared to young, older individuals exhibit impaired peripheral vasodilation during graded systemic hypoxia. Further, contrary to our hypothesis, this

age-associated impairment is not attributed to elevated α-adrenergic vasoconstrictor tone, but rather is likely due to impaired β-adrenergic and local vasodilatory signaling.

Hypoxia trials

In the control hypoxia trial, at the onset of hypoxia (90% SpO_2) young individuals vasodilated (ΔFVC 17±3%) and progressively dilated as the level of saturation declined (ΔFVC 35±8% at 80% SpO_2). Conversely, older adults failed to vasodilate at any level of hypoxia during control conditions. Previous studies that examined the peripheral vasodilatory response to systemic hypoxia in older populations have utilized a single level of hypoxia and the findings vary where some report an age-associated impairment [15, 16] and others do not [17]. The discrepancy among previous publications is likely due to the population examined and method of assessment [18]. The lack of a vasodilatory response in the control condition could be due to elevated α-adrenergic vasoconstrictor tone, attenuated β-mediated vasodilation and/or impaired local vasodilator signaling.

Hypoxia during local β-adrenergic receptor blockade

In the second hypoxia trial, we locally inhibited β-adrenergic mediated vasodilation to determine the contribution of this pathway to the overall net hypoxic vasodilatory response. Previous studies in younger adults have examined the influence of hypoxic β-mediated vasodilation after inhibition of α-adrenergic vasoconstriction and under these pharmacological conditions, it is estimated that β-receptors contribute ~50% to the net vasodilatory response [2]. However, the contribution of β-receptors may have been overestimated due to the pharmacological design of the study. Administering an α-adrenergic receptor antagonist (phentolamine) alone may have led to an augmented hypoxic vasodilatory response.

Phentolamine can bind α_2- adrenergic receptors located on sympathetic nerve endings and evoke NE release, which can bind β-adrenergic receptors and evoke vasodilation [19]. In the present study, inhibition of β-adrenergic receptors alone had no significant impact on the net hypoxic vasodilation in young adults who still exhibited progressive vasodilation to hypoxia, although slightly attenuated from control (Δ FVC 35% vs. 28% at 80% SpO_2). To our knowledge, only two previous studies have examined the role of β-adrenergic receptor mediated vasodilation when α-adrenergic vasoconstriction was intact and the findings were conflicting [5, 20], however the results of the present study appear to be in agreement with Richardson et al. who also only observed a slight, yet non-significant decrease in hypoxic vasodilation following β-blockade (Δ FVC 27% vs. 35%), suggesting that in young adults this pathway is not obligatory for hypoxic vasodilation.

To date, the contribution of β-receptors to graded hypoxic vasodilation in aging had never been assessed. We hypothesized that β-adrenergic receptor responsiveness was impaired in older adults and therefore inhibition of this vasodilator pathway would have little effect on older individuals vascular response to systemic hypoxia. However, we observed that following local β-blockade, older adults actively constricted (Δ FVC -5%, -11%, and -13% at 90, 85 and 80% SpO_2) during graded hypoxia. These findings suggest that despite a lack of vasodilation in the control hypoxia trial, β-mediated vasodilation plays an important role in buffering sympathetic vasoconstrictor signaling. The net vasoconstriction observed in older adults during the second hypoxia trial (β-block) may be due to augmented α-adrenergic vasoconstrictor tone. Indeed, in the present study, older adults had significantly greater arterial plasma norepinephrine concentrations at both 85% and 80% SpO_2 in the second and third hypoxic bout, potentially providing a greater vasoconstrictor stimulus than that observed in control conditions. In

addition, the active vasoconstriction observed in older subjects to systemic hypoxia during local β-receptor blockade could be due to elevated concentrations of locally produced substances such as endothelin which can cause robust vasoconstriction [21], inhibit nitric oxide signaling [22] and is also reported to be elevated with aging [23].

Hypoxia during local α- and β-adrenergic receptor blockade

We hypothesized that any age-associated impairment in hypoxic vasodilation would be partly attributed to elevated α-adrenergic vasoconstrictor tone. However, following local sympatho-adrenal blockade, young and older adults elevated resting FBF to similar levels, indicating there was not a greater degree of α-adrenergic restraint of FBF at rest. Furthermore, during hypoxia young adults exhibited a significant increase in the hypoxic vasodilatory response whereas the older adults failed to significantly vasodilate to the hypoxic stimulus. The lack of a robust increase in hypoxic vasodilation in the older group in this condition suggests that the age-associated impairment is more likely due to attenuated local vasodilatory signaling. In accordance with this, our lab has previously determined that in the absence of sympatho-adrenal influence, the hypoxic vasodilatory response could be abolished in young individuals following combined inhibition of nitric oxide and vasodilating prostaglandins [3]. Given that older adults have impaired endothelial function, less signaling through these pathways in response to a hypoxic stimulus may explain the observed lower hypoxic vasodilation. Additionally, the red blood cell has been proposed to be a sensor of low PO_2 that responds by releasing the vasodilator ATP. Our lab has also reported that compared to young, older adults have attenuated ATP release form their red blood cells during a hypoxic stimulus [24]. Despite these identified impairments with aging, it remains to be determined whether improving local vasodilator

signaling (nitric oxide availability and/or ATP release) would reverse the age-associated hypoxic vasodilatory impairment.

α- and β- adrenergic receptor responsiveness

In the present study, compared to young, older adults exhibited attenuated α- and β-adrenergic receptor responsiveness. Previous work has demonstrated attenuated α-adrenergic receptor responsiveness in older healthy adults in response to both tyramine (evokes endogenous norepinephrine release) and phenyleprhine (α_1- agonist) [25]. Additionally, some groups have also observed attenuated peripheral vasodilation to β-agonists with increasing age [26] where others have not [27, 28] and this discrepancy may be due to administration of different doses and/or the technique utilized to measure FBF (venous occlusion plethysmography or Doppler Ultrasound) as Doppler Ultrasound is observed to be a more sensitive measure of FBF [18]. Whether the decrease in adrenergic receptor responsiveness is cause or consequence of augmented SNS activity at rest is unclear, however, in combination with impaired local vasodilatory signaling, attenuated α-adrenergic responsiveness with age may be protective.

Experimental Considerations

In the present study, we utilized the forearm model to examine the regulation of peripheral vascular tone. In older adults, both the forearm [25] and the leg [29] exhibit attenuated α-adrenergic receptor responsiveness. However, unlike the forearm model, legs of older adults do have lower baseline blood flows [30] and following α-adrenergic blockade, exhibit significantly greater increases in leg blood flow and leg vascular conductance [31] indicating that sympathetically mediated constriction is limiting leg blood flow at rest, despite

attenuated α-adrenergic receptor responsiveness. Therefore, the findings of the present study may not translate to the vasculature of the leg. Despite the findings in the forearm potentially being different from that in the leg, the vascular response observed in the forearm model has significant clinical relevance as impairments in forearm endothelial function are correlated to coronary endothelial function, which is an indicator of cardiovascular disease risk.

Although we observed a significant age-associated elevation in arterial NE concentrations during the second and third bouts of hypoxia, there were no differences in baseline or hypoxic catecholamine concentrations in the control hypoxia bout. Despite similar concentrations between young and older adults in the control trial, previous studies utilizing direct nerve recordings report elevated muscle sympathetic nerve activity in older adults at rest and in response to hypoxia [6] and it is therefore possible that the absolute level of SNS activity was greater in the older adults but differences in catecholamine clearance [32] or secretion [33] may have influenced our arterial catecholamine measures.

In the present study, we chose to use NE as our α-adrenergic receptor agonist to measure receptor responsiveness. When infused intra-arterially NE has a high affinity of α-adrenergic receptors located on the endothelium and vascular smooth muscle. Conversely, NE can also bind β-adrenergic receptors located on the endothelium and vascular smooth muscle and evoke vasodilation. It is possible that our observation of attenuated α-adrenergic receptor responsiveness to NE in older subjects could be due to an enhanced affinity of NE for β-adrenergic receptors and therefore, some vasodilation could mask or off-set vasoconstriction. However, we do not think this is likely given older adults also had attenuated β-adrenergic receptor sensitivity and if anything, we may have underestimated the degree of constrictor response in young adults if NE was able to bind β-adrenergic receptors.

Perspectives – See Chapter VI

Conclusion

The present study demonstrates an age-associated impairment in the ability to vasodilate to an acute systemic hypoxic stimulus. Contrary to our hypothesis, the attenuated vasodilatory response observed in older adults was not due to elevated sympathetically mediated vasoconstrictor tone. The lack of significant improvement in hypoxic vasodilation in older adults following local sympatho-adrenal blockade suggests that impaired local vasodilation is likely contributing to the attenuated response. Previous studies in young adults indicate that nitric oxide and prostaglandins are important vasodilators during systemic hypoxia. Production of both these vasodilators is dependent upon a healthy functional endothelium, and it is observed that endothelial function declines with increasing age. Additionally, previous work from our lab suggests that attenuated release of ATP from erythrocytes of older adults may also contribute to a lower vasodilatory response.

Acute graded systemic hypoxia is physiological stimulus that challenges homeostasis such that in order to match peripheral metabolic demand, O_2 delivery needs to increase. Further, elevated O_2 delivery needs to occur despite elevated sympathetically mediated vasoconstriction. This stimulus provides insight into how well older adults maintain homeostasis and are able to regulate peripheral vascular function and this information is important given that older adults are at an elevated risk for experiencing an ischemic event and that age is an independent risk factor for cardiovascular disease. Understanding the mechanisms contributing to the age-associate impairment in the ability to regulate peripheral vascular tone may lend itself to better treatments for augmenting O_2 delivery.

Table 1. Subject Characteristics. * P<0.05 vs. Young. Although some cholesterol was elevated in older adults, it is still considered to be within a healthy normal range. LDL = low-density lipoprotein, HDL = high-density lipoprotein.

Variable	Young	Older
Male:Female	8:4	6:4
Age (years)	24±1	63±2*
Body mass index (kg m^{-2})	24.2±1.3	25.4±1.5
Body fat (%)	25.4±3.2	30.6±2.2
Forearm volume (ml)	883.2±35.6	879.4±81.8
Total cholesterol (mg dl^{-1})	141.2±7.6	184.5±13.3*
LDL cholesterol (mg dl^{-1})	81.8±4.7	108.1±9.5*
HDL cholesterol (mg dl^{-1})	46.2±4.1	53.0±6.5
Triglycerides (mg dl^{-1})	76.2±9.4	115.9±22.4

Table 2. Hemodynamic response during all hypoxia trials. *P<0.05 vs. Young and † P<0.05 vs. Baseline in respective condition.

	Baseline		90% SaO_2		85% SaO_2		80% SaO_2	
	Young	Older	Young	Older	Young	Older	Young	Older
Control								
Heart Rate (beats min^{-1})	64 ±4	57 ±2	76 ±4†	62 ±2*†	82 ±5†	67 ±2*†	89 ±5†	70 ±2*†
Mean Arterial Pressure (mmHg)	94.6 ±2.5	100.9 ±3.5	96.7 ±2.9	102.0 ±4.0	97.3 ±3.3	101.9 ±3.4	93.4 ±2.6	102.3 ±3.8*
Forearm Blood Flow (ml min^{-1})	29.4 ±2.6	28.8 ±3.2	34.2 ±2.8†	27.1 ±2.9	35.8 ±4.0†	28.0 ±3.4	37.4 ±2.9†	29.6 ±3.8
Forearm Vascular Conductance (ml min^{-1} $mmHg^{-1}$)	30.9 ±2.3	28.3 ±3.2	35.2 ±2.4†	26.6 ±2.9*	36.5 ±3.4†	27.3 ±3.2*	40.1 ±2.9†	29.6 ±3.8*
β- Block								
Heart Rate (beats min^{-1})	62 ±4	58 ±2	74 ±4†	62 ±2*†	80 ±4†	65 ±3*†	83 ±4†	68 ±2*†
Mean Arterial Pressure (mmHg)	95.6 ±2.3	101.0 ±3.8	96.9 ±2.5	102.8 ±3.3	97.1 ±2.7	104.0 ±4.2	95.9 ±2.7	105.0 ±3.2*
Forearm Blood Flow (ml min^{-1})	27.8 ±2.2	26.0 ±2.7	30.3 ±1.9	24.5 ±2.3	35.4 ±3.2†	24.1 ±3.0*	34.2 ±2.6†	23.6 ±3.0*
Forearm Vascular Conductance (ml min^{-1} $mmHg^{-1}$)	29.3 ±2.4	25.6 ±2.5	31.4 ±2.1	23.8 ±2.0*	36.4 ±2.9†	23.1 ±2.9*	36.1 ±3.0†	22.6 ±2.8*
α+β Block								
Heart Rate (beats min^{-1})	62 ±4	57 ±2	75 ±4†	64 ±2†	80 ±4†	66 ±2*†	80 ±4†	68 ±3*†
Mean Arterial Pressure (mmHg)	95.7 ±2.5	101.3 ±3.8	96.2 ±2.9	103.0 ±3.9	95.8 ±2.8	103.0 ±4.0	93.7 ±3.5	100.0 ±4.9
Forearm Blood Flow (ml min^{-1})	42.0 ±2.7	43.7 ±5.4	48.9 ±4.7†	45.7 ±5.9	55.1 ±5.4†	45.6 ±6.6	62.3 ±6.2†	47.8 ±7.1
Forearm Vascular Conductance (ml min^{-1} $mmHg^{-1}$)	44.2 ±3.0	42.8 ±4.9	51.0 ±4.7†	43.9 ±5.4	57.7 ±5.4†	44.3 ±6.3	67.5 ±6.9†	47.7 ±6.8*

Table 3. Blood gases and arterial catecholamine concentrations (young n=10, older n=9) during all hypoxia trials. *P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition. P_aCO_2 = arterial CO_2, SaO_2 = arterial oxyhemoglobin saturation, $CtaO_2$ = arterial oxygen content.

	Baseline		90% SaO_2		85% SaO_2		80% SaO_2	
	Young	Older	Young	Older	Young	Older	Young	Older
Control								
pH_a	7.41±0.01	7.43±0.01	7.42±0.01	7.43±0.01	7.43±0.01	7.44±0.01	7.42±0.01	7.45±0.01
P_aCO_2 (mmHg)	38.5±1.2	36.8±1.5	36.6±1.0	36.4±1.3	36.9±1.2	36.2±1.2	37.3±1.2	35.1±1.0
S_aO_2 (%)	95.5±0.3	94.2±0.4 *	88.1±0.6	88.2±0.5	82.5±0.7	83.5±0.4	78.9±0.7	80.3±1.0
P_aO_2 (mmHg)	82.3±1.8	73.3±2.0	54.4±1.3	54.3±1.0	46.1±0.9	46.4±0.8	42.1±1.0	43.1±1.3
Ct_aO_2 (ml dl^{-1})	25.3±0.8	20.7±0.9	18.4±0.5	18.6±0.4	24.9±0.7	17.6±0.4	24.9±0.7	16.6±0.4
Epinephrine (pg ml^{-1})	56.8±8.2	56.1±9.7	69.0±11.1	59.3±10.3	80.7±9.5†	73.9±9.4†	134.3±27.9†	68.8±11.6
Norepinephrine (pg ml^{-1})	240.9±19.5	294.7±24.7	231.5±13.8	268.3±24.5†	240.6±24.2	270.3±20.3	244.2±27.7	265.1±22.1 †
β-Block								
pH_a	7.43±0.01	7.44±0.01	7.42±0.01	7.43±0.01	7.43±0.01	7.44±0.01	7.43±0.01	7.44±0.01
P_aCO_2 (mmHg)	35.5±1.4	35.5±1.3	36.6±1.3	35.6±1.5	35.7±1.5	35.4±1.4	37.1±1.2	33.9±1.3
S_aO_2 (%)	95.7±0.3	94.5±0.4 *	87.8±0.4	88.4±0.5	83.5±0.5	83.6±0.5	80.0±0.4	79.5±0.3
P_aO_2 (mmHg)	83.2±1.8	74.5±2.3 *	54.1±1.0	54.9±1.5	47.5±0.9	47.0±0.8	43.6±0.5	42.4±0.5
Ct_aO_2 (ml dl^{-1})	23.8±0.7	20.0±0.6	18.6±0.5	18.6±0.4	23.9 ±0.9	17.4±0.4	24.9±0.7	16.9±0.5
Epinephrine (pg ml^{-1})	70.7±11.2	55.0±8.7	79.3±13.7	68.9±10.5	130.0±27.7†	73.8±15.3	173.2±45.0†	88.9±19.0†
Norepinephrine (pg ml^{-1})	255.7±17.6	243.3±25.9	216.4±22.6	236.5±24.9	192.3±14.5	257.1±20.0*	185.0±18.5	282.8±24.5 *†
α+β-Block								
pH_a	7.43±0.01	7.44±0.02	7.43±0.01	7.43±0.01	7.43±0.01	7.44±0.01	7.43±0.01	7.44±0.01
P_aCO_2 (mmHg)	34.9±1.1	34.0±1.6	35.5±1.3	34.9±0.9	35.1±1.3	34.4±1.0	34.5±1.2	33.8±1.1
S_aO_2 (%)	95.7±0.4	94.6±0.5 *	87.2±0.6	87.6±0.7	82.6±0.7	83.1±0.7	80.6±0.7	79.2±0.9
P_aO_2 (mmHg)	84.6±2.0	75.8±2.1 *	54±1.5	53.7±1.5	46.9±1.0	46.5±0.9	44.7±1.1	42.7±1.2
Ct_aO_2 (ml dl^{-1})	23.7±0.7	20.1±0.5	18.1±0.4	18.2±0.4	23.8±0.7	17.2±0.4	23.4±0.6	16.2±0.4
Epinephrine (pg ml^{-1})	96.5±17.6	78.5±16.0	148.7±36.2†	67.2±18.8†	235.2±58.9†	86.6±27.2	338.7±91.6†	127.7±52.3 †
Norepinephrine (pg ml^{-1})	272.1±35.8	324.1±48.4	221.9±22.3†	364.8±50.4*	218.6±18.6†	392.2±44.5*	205.3±21.1†	461.0±57.7 *†

Table 4. Ventilation during hypoxia trials. *P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition. SpO_2 = oxyhemoglobin saturation via pulse oximetry.

	Baseline		**90% SaO_2**		**85% SaO_2**		**80% SaO_2**	
	Young	**Older**	**Young**	**Older**	**Young**	**Older**	**Young**	**Older**
Control								
Minute Vent. (l min^{-1} BTPS)	7.6 ±0.5	7.5 ±0.8	14.8 ±1.5†	10.5 ±1.2*†	16.4 ±1.7†	11.5 ±1.3†	19.8 ±2.8†	13.3 ±1.8*†
End Tidal CO_2 (mmHg)	39.0 ±0.9	36.9 ±1.0	38.7 ±0.9	37.6 ±1.1	38.1 ±0.9	36.9 ±0.9	38.2 ±0.9	36.4 ±0.7
SpO_2 (%)	98.3 ±0.4	97.2 ±0.6	90.0 ±0.5†	90.5 ±0.5†	84.4 ±0.4†	85.3 ±0.4†	78.7 ±0.5†	80.4 ±0.4†
β- Block								
Minute Vent. (l min^{-1} BTPS)	7.6 ±0.5	7.3 ±0.5	13.7 ±1.9†	9.6 ±1.4†	16.9 ±2.6†	11.3 ±2.1†	18.8 ±2.9†	13.5 ±2.7†
End Tidal CO_2 (mmHg)	37.3 ±0.9	36.3 ±1.2	38.4 ±0.9	36.8 ±0.9	38.3 ±0.9	37.0 ±0.9	37.9 ±0.9	36.0 ±0.9
SpO_2 (%)	98.7 ±0.3	97.4 ±0.4	90.3 ±0.4†	90.2 ±0.6†	84.3 ±0.4†	84.2 ±0.7†	78.9 ±0.6†	79.4 ±0.6†
α+β Block								
Minute Vent. (l min^{-1} BTPS)	8.5 ±0.7	6.9 ±0.5	15.3 ±2.0†	10.8 ±1.6†	18.9 ±3.0†	13.0 ±2.3†	21.3 ±3.9†	14.5 ±2.9†
End Tidal CO_2 (mmHg)	36.9 ±1.0	34.9 ±1.2	37.9 ±0.9	36.6 ±0.8	37.8 ±0.9	36.2 ±0.7	37.6 ±0.9	36.5 ±0.6
SpO_2 (%)	98.3 ±0.4	97.6 ±0.6	89.2 ±0.6†	89.2 ±0.5†	83.8 ±0.7†	84.4 ±0.7†	79.0 ±0.7†	80.1 ±0.8†

Table 5. Hemodynamic variables during α- and β- adrenergic responsiveness.
*P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition.

α-adrenergic receptor responsiveness	Baseline		Ne (20ng 100mL $FAV^{-1}min^{-1}$)		Ne (40ng 100mL $FAV^{-1}min^{-1}$)		Ne (152ng 100mL $FAV^{-1}min^{-1}$)		Post-Baseline		Challenge	
	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older
Heart Rate (beats min^{-1})	61 ±3	56 ±2	60 ±3	56 ±2	60 ±3	56 ±2	62 ±3	57 ±2	61 ± 3	56 ±2	60 ±3	56 ±2
Mean Arterial Pressure (mmHg)	94.1 ±3.2	101.6 ±3.5	95.3 ±3.2	101.1 ±3.6	94.1 ±3.0	102.8 ±3.6	95.2 ±2.6	103.2 ±3.9	97.2 ±2.8	101.9 ±4.5	98.0 ±2.8	104.3 ±4.8
Forearm Blood Flow (ml min^{-1})	30.3 ±3.0	28.5 ±4.0	24.3 ±2.8†	24.2 ±3.4†	22.5 ±2.9†	25.3 ±3.1	18.9 ±2.6†	20.8 ±2.4†	46.5 ±5.2	40.6 ±5.3	42.8 ±5.7	39.2 ±5.3
Forearm Vascular Conductance (ml min^{-1} $mmHg^{-1}$)	32.0 ±2.5	27.3 ±3.2	25.3 ±2.4†	23.4 ±2.7†	23.6 ±2.6†	24.1 ±2.4	19.5 ±2.3†	19.9 ±1.9†	48.1 ±5.2	38.6 ±3.9	43.8 ±5.6	39.8 ±3.6
β-adrenergic receptor responsiveness	Baseline		ISO (5ng 100mL $FAV^{-1}min^{-1}$)		ISO (15ng 100mL $FAV^{-1}min^{-1}$)		ISO (50ng 100mL $FAV^{-1}min^{-1}$)		Post-Baseline		Challenge	
	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older
Heart Rate (beats min^{-1})	60 ±3	57 ±2	62 ±4	56 ±2	61 ±1	56 ±2	63 ±3	58 ±2	61 ±3	54 ±2	60 ±3	54 ±2
Mean Arterial Pressure (mmHg)	95.8 ±2.7	101.1 ±3.7	94.4 ±2.9	102.2 ±3.6	94.3 ±2.9	101.6 ±3.6	94.4 ±2.6	100.6 ±3.3	95.9 ±2.9	101.0 ±4.0	97.3 ±3.0	105.1 ±4.5
Forearm Blood Flow (ml min^{-1})	31.1 ±4.0	26.9 ±3.3	68.7 ±10.9†	56.4 ±13.5†	76.7 ±1.0†	45.8 ±8.7†	125.9 ±20.6†	74.3 ±15.2*†	47.1 ±6.6	39.9 ±5.5	48.3 ±7.6	42.8 ±5.6
Forearm Vascular Conductance (ml min^{-1} $mmHg^{-1}$)	32.2 ±3.6	26.1 ±2.6	74.3 ±13.0†	54.1 ±12.7†	76.7 ±0.1†	44.1 ±7.9*†	131.4 ±19.7†	71.5 ±3.8*†	49.2 ±6.4	38.5 ±3.9	49.8 ±7.5	39.8 ±3.6

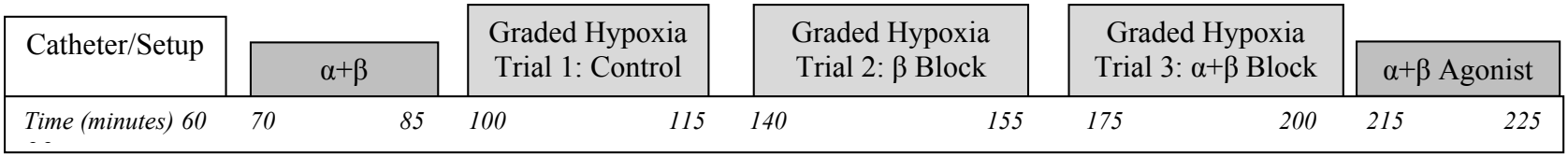
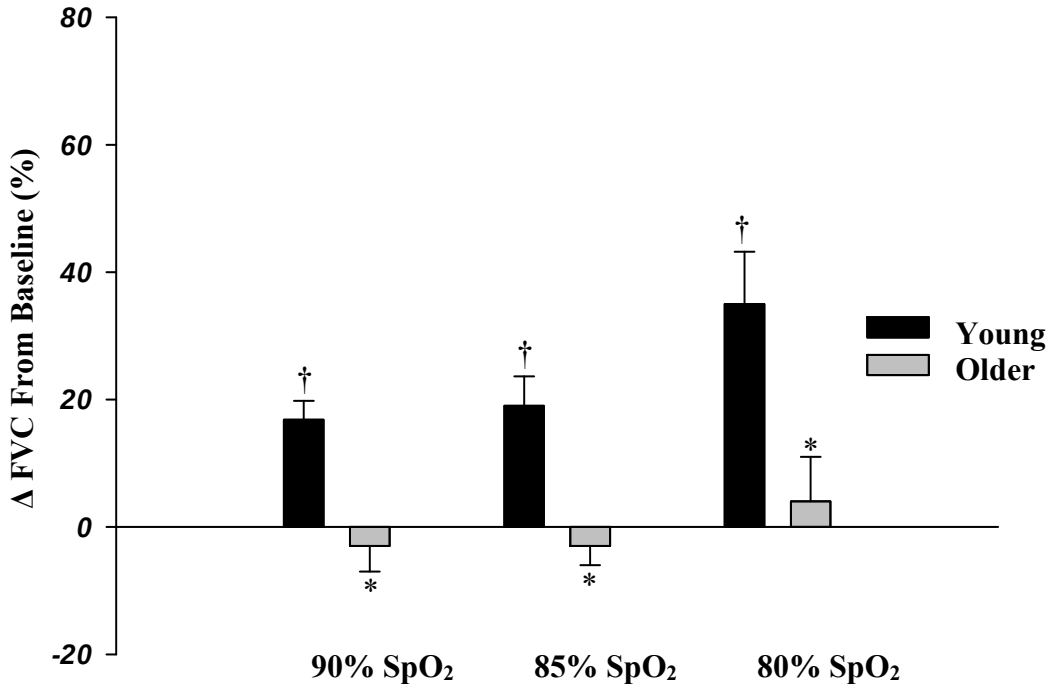
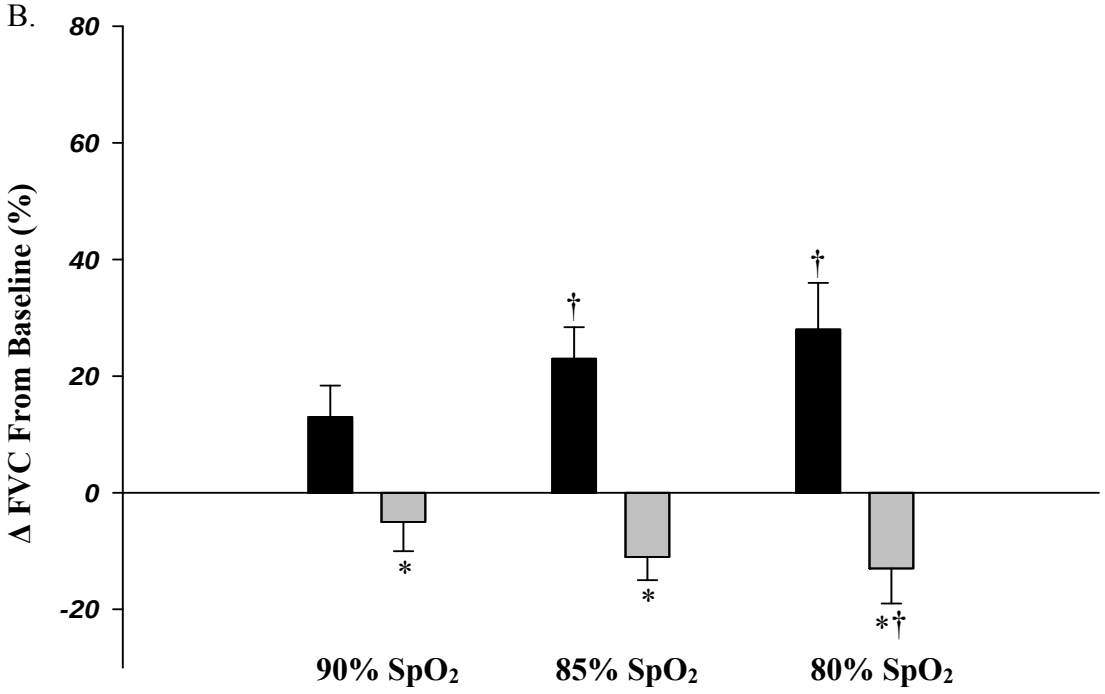


Figure 1. Study Timeline. Following brachial artery catheter insertion and rest, α- and β- adrenergic receptor responsiveness was determined. Each agonist (α-agonist: norepinephrine (NE) and β-agonist: isoproterenol (ISO)) was administered in three incremental doses for two minutes each. Hypoxia trials consisted of 2 minutes of baseline followed by isocapnic systemic hypoxia (90, 85, 80% SpO2). Each level of hypoxia was maintained at minimum for 1 minute before continuing to next level. Following the third bout of hypoxia, a single dose (medium or high) of each agonist was administered for two minutes to confirm effective α- and β- adrenergic receptor blockade.

A.


80
60
40
20
0
-20
Δ FVC From Baseline (%)
Young
Older
†
*
90% SpO₂
85% SpO₂
80% SpO₂

B.


80
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Δ FVC From Baseline (%)
†
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90% SpO₂
85% SpO₂
80% SpO₂

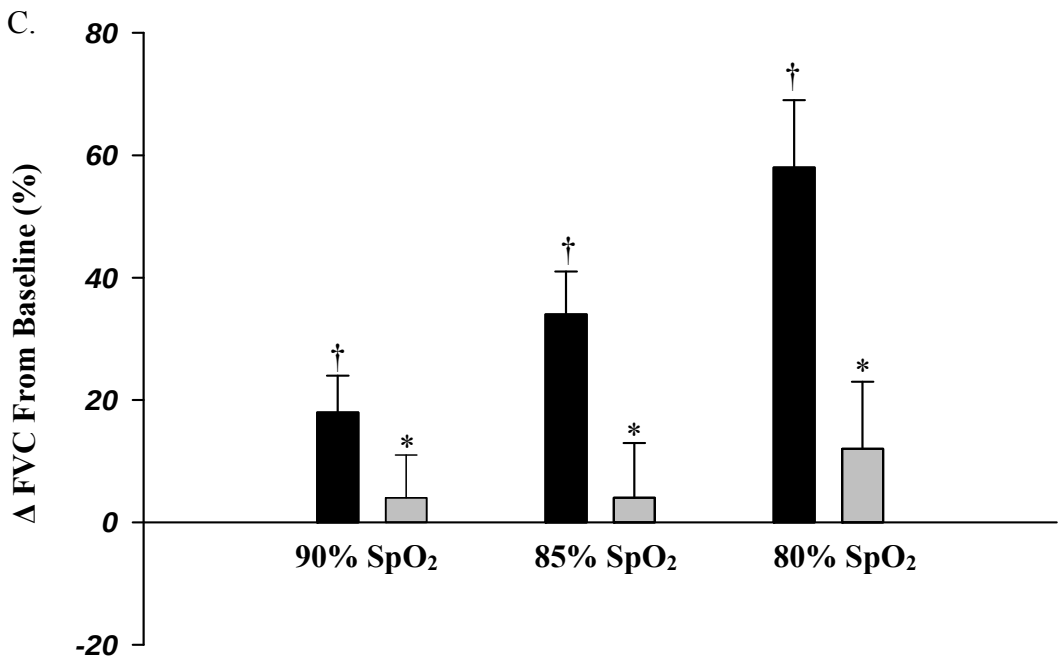

Figure 2. Hypoxic vasodilation (Δ FVC (%)) from baseline in (A) Control (B) during local β-adrenergic receptor blockade and (C) during local α+β- adrenergic receptor blockade.*P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition. In control conditions (A), young adults progressively vasodilated with increasing hypoxia whereas older adults failed to dilate at any level of hypoxia. Following local β-adrenergic receptor blockade (B), young adults progressively dilated to hypoxia similar to that observed in control conditions whereas older adults failed to dilate and appear to actively constrict to increasing levels of hypoxia. Following local inhibition of both α+β adrenergic receptors, young adults continued to exhibit progressive vasodilation, however, the age-associated impairment in hypoxic vasodilation persisted.

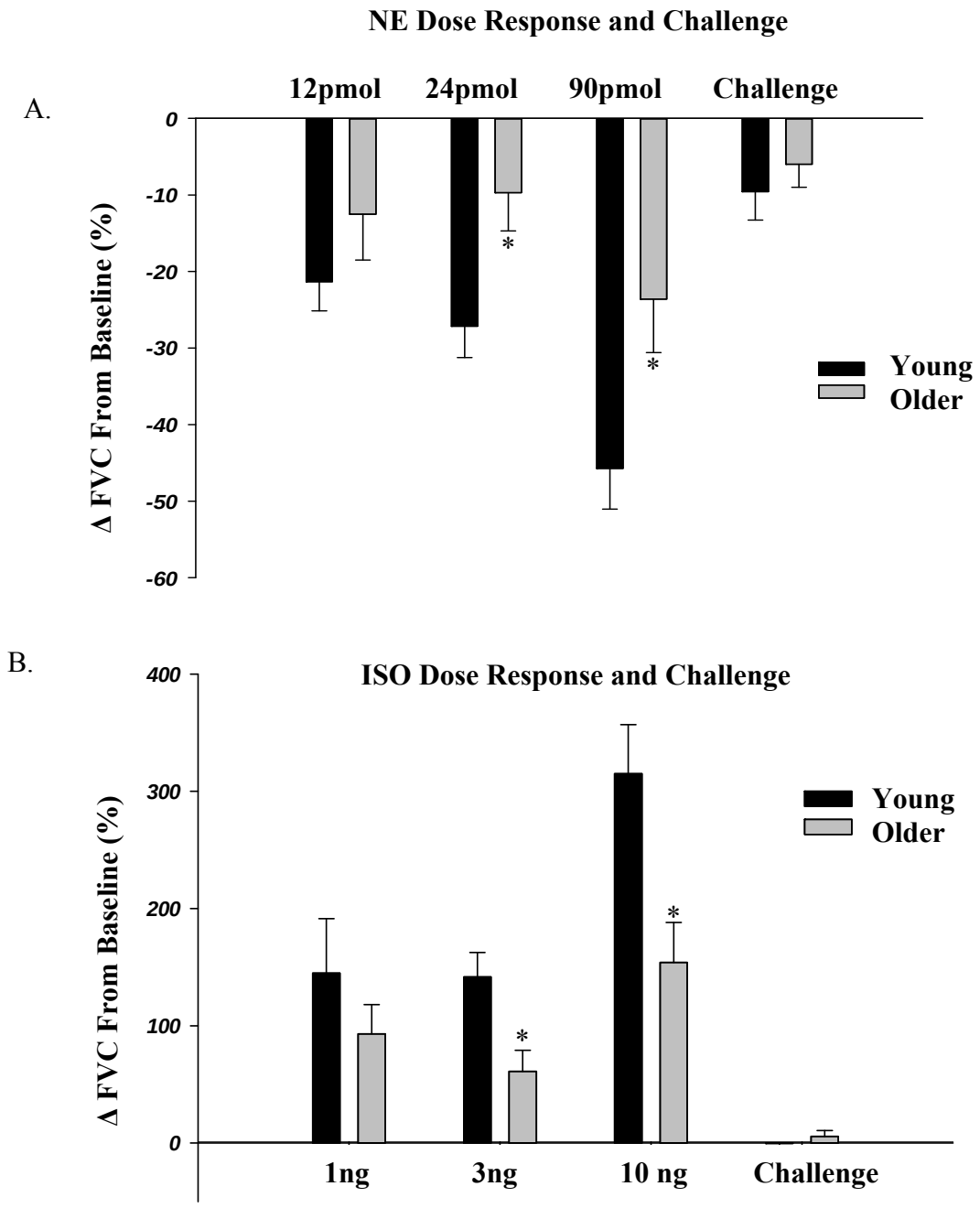

Figure 3. A) α-adrenergic and B) β-adrenergic receptor responsiveness. * P<0.05 vs. Young**.** Compared to young, older adults exhibited attenuated α-adrenergic receptor responsiveness (A) during three incremental doses of norepinephrine (NE) administered for two minutes each. Additionally, effective α-adrenergic blockade was confirmed at the end of the study by re-administering NE for two minutes at the medium dose in young and high dose in older adults. Both groups failed to significantly vasoconstrict to NE following local sympatho-adrenal blockade. Compared to young, older adults exhibited attenuated β-adrenergic receptor responsiveness (B) during incremental infusion of three doses of isoproterenol (ISO) at two minutes of each dose. Additionally, effective β-adrenergic blockade was later confirmed by re-administering a medium-high dose of ISO following local sympatho-adrenal blockade.

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CHAPTER III – FOLLOW UP STUDY

Acute ascorbic acid infusion improves peripheral hypoxic vasodilation in older adults

Summary

We tested the hypothesis that local (brachial artery catheter) infusion of ascorbic acid (AA) would reverse the age-associated impairment in peripheral hypoxic vasodilation in older adults. Forearm blood flow was measured (Doppler Ultrasound) and forearm vascular conductance (FVC) was calculated in 5 young (21±1 years) and 5 older adults (64±4 years) at rest and during systemic isocapnic hypoxia. Prior to the hypoxia trial, local sympatho-adrenal blockade was performed in each subject via combined infusion of phentolamine and propranolol (α- and β-adrenergic receptor antagonists, respectively) to isolate local vascular control mechanisms. Subjects were exposed to 15 minutes of systemic isocapnic hypoxia (85% SpO_2); the first 5 min with infusion of saline (control) and the subsequent 10 min with AA infusion (8 mg dl FAV^{-1} min^{-1}). After 5 minutes of control hypoxia, older individuals exhibited a lower forearm vasodilatory response compared with young (Δ FVC = 14±3% vs. 29±6%, $P<0.05$). AA infusion significantly improved the dilatory response in older individuals (Δ FVC = 43±9% from steady-state hypoxia levels) whereas there was no significant change in the younger group (Δ FVC = 7±2%). These findings suggest that in the absence of sympatho-adrenal influences on vascular tone, acute AA administration improves peripheral vasodilation during systemic hypoxia in older individuals such that the net response is no longer impaired compared to young healthy humans. Although the mechanisms by which AA improves local dilatory signaling in older adults during hypoxia remain to be determined, prior work from our laboratory and others suggest this may be via improved nitric oxide bioavailability.

Introduction

Systemic hypoxia activates the sympathetic nervous system (SNS) in a graded fashion and despite the greater vasoconstrictor stimulus, the peripheral vasculature in young healthy adult's exhibit vasodilation [1-3]. Peripheral hypoxic vasodilation is attributed to stimulation of β-adrenergic receptors and local endothelium-dependent vasodilation (nitric oxide and vasodilating prostaglandins) [1, 2]. Our lab has observed attenuated peripheral vasodilation to systemic hypoxia in older adults, both in the presence and absence of sympatho-adrenal activation. The lack of significant improvement in the hypoxic vasodilation following sympatho-adrenal blockade suggests that sympathetically mediated vasoconstriction is not limiting vasodilation and therefore, impaired local vasodilation may be a significant contributor to the age-associated impairment. Endothelium-dependent vasodilation declines with age and is attributed to decreased nitric oxide bioavailability. We have previously demonstrated that acute AA infusion can improve nitric oxide availability [4] and reverse the age-associated impairment in endothelium-dependent vasodilation [5, 6].

Based on our previous findings indicating that hypoxic vasodilation was impaired with age, and that local inhibition of the sympatho-adrenal system failed to reverse the age-associated impairment, we sought to determine whether improving nitric oxide availability through acute infusion of AA would improve hypoxic vasodilation in older adults.

Methods

Subjects

With Institutional Review Board approval and following written informed consent, a total of 5 young (1 female, 4 male) and 5 older (2 female, 3 male) healthy subjects participated in the

present study. All participants were non-smokers, non-obese, normotensive, and not taking any medications including over the counter supplements. Young females were studied during the placebo phase of birth control or during the early follicular phase of their menstrual cycle to minimize any potential vascular effects of sex hormones. Older females were post-menopausal (2 + years) and not taking hormonal supplements. Subject characteristics are presented in Table 1. All Studies were performed in the Human Cardiovascular Physiology Laboratory located at Colorado State University (~1500 m above sea level) following a 12-hour fast with the subjects in the supine position. All studies were performed according to the Declaration of Helsinki.

Arterial Catheterization

The non-dominant arm was chosen to be the experimental arm and after local application of anesthesia (2% lidocaine), a 20-guage, 7.6 cm catheter was inserted into the brachial artery utilizing aseptic technique. The catheter was connected to a pressure transducer for continuous monitoring of mean arterial pressure (MAP) as well as a 3-port connector to allow for drug infusions and blood sampling. Throughout the duration of the study, heparinized saline was continuously infused at a rate of 3 ml minute^{-1}.

Body Composition and Forearm Volume

Dual- energy X-ray absorptiometry (DEXA: Hologic: Bedford, MA, USA) was used to determine body composition. A regional analysis of the experimental forearm area (proximal to distal radio-ulnar joint) from the whole body DEXA scan was performed to determine forearm volume. Drug dosage was normalized according to forearm volume [4, 7]. Body mass index was calculated as body weight (kg) divided by height (meters) squared.

Systemic Isocapnic Hypoxia (85% SpO_2)

To elicit systemic hypoxia we utilized a self-regulating partial re-breathe system which allows for constant alveolar fresh air ventilation independent of changes in minute ventilation and enables end-tidal CO_2 ($EtCO_2$) to be clamped [1, 8]. Oxygen (O_2) levels were titrated down by mixing nitrogen with air in a medical gas blender to attain steady arterial O_2 saturations (SaO_2) of 85% as assessed by pulse oximetry (SpO_2) of the earlobe. Nasal breathing was prevented through the use of a nose clip while subjects breathed through a scuba mouthpiece. An anesthesia monitor was used to monitor gas concentrations at the level of the mouthpiece (Cardiocap, Datex-Ohmeda, Louisville, CO, USA) as well as to monitor heart rate (HR; 3 lead ECG). Additionally, ventilation was measured with a pneumotachograph (model 17125 UVM,Vacu-Med, Ventura, CA, USA).

Forearm Blood Flow (FBF) and Vascular Conductance (FVC)

Brachial artery mean blood velocity (MBV) and diameter was determined using a 12 MHz linear-array ultrasound probe (Vivid 7, General Electric, Milwaukee, WI, USA). The probe placed proximal to the catheter site as previously described [7]. During blood velocity measurements, the probe insonation angle was maintained at less than 60 deg and the frequency used was 5 MHz. A multigon 500M TCD spectral analyzer (Multigon Industries, Mt. Vernon, NY, USA) was used to analyze the Doppler shift frequency and subsequently determine MBV from the weighted mean of the spectrum of Doppler shift frequencies. Brachial artery diameter measurements were made in duplex mode at end-diastole in triplicate during steady state conditions. FBF was calculated as FBF=MBV x $\times \pi$ (brachial artery diameter/2)2 $\times$ 60, where the FBF is in ml min^{-1}, the MBV is in cm s^{-1}, the brachial diameter is in centimeters, and 60 was

used to convert from ml s^{-1} to ml min^{-1}. Forearm vascular conductance (FVC) was calculated as (FBF/MAP) ×100, and expressed as ml min^{-1} $100mmHg^{-1}$ [9, 10]. All studies were performed in a cool temperature-controlled environment with a fan directed toward the forearm to minimize the contribution of skin blood flow to forearm hemodynamics.

Regional α-adrenergic and β-adrenergic receptor blockade

To limit the contribution of the sympatho-adrenal system and examine local vasodilatory signaling, we locally infused phentolamine mesylate (Bedford Laboratories, Bedford, OH, USA), a non-selective α-adrenergic receptor antagonist (load 200 μg min^{-1} for 5 min, maintenance 50 μg min^{-1}*)* and propranolol hydrochloride (Baxter, Deerfield, IL,USA), a non-selective β-adrenergic receptor antagonist (load 200 μg min^{-1} for 5 min, maintenance 50 μg min^{-1}) prior to and during the trial [1].

Regional Ascorbic Acid infusion

Once steady state hypoxia was achieved for 5 minutes, AA was locally infused (8 mg 100ml FAV^{-1} min^{-1}) for an additional 10 minutes of systemic hypoxia [5].

Blood Gas Sampling

Arterial blood gas samples were collected at the end of baseline, 5 minutes into hypoxia alone and at the end of hypoxia + AA infusion. Blood gas samples were analyzed with a clinical blood gas analyzer (Siemens Rapid Point 400 series, Los Angeles, CA USA).

Experimental Protocol

The overall study timeline is presented in Figure 4. All participants arrived in the morning after an overnight fast. All measurements were performed with the subjects in the supine position within a temperature controlled room with a fan directed towards the arm and a wrist cuff inflated to limit the contribution of skin blood flow measures to the forearm. All study drugs were administered via brachial artery catheter and infused via Harvard infusion syringe pump.

Following placement of the catheter, subjects rested quietly for at minimum 30 minutes. To begin, α- and β- adrenergic receptor antagonists (phentolamine and propranolol) were locally infused for 5 minutes while participants breathed room air through the mouthpiece. Systemic hypoxia began and once 85% SpO_2 was attained, subjects remained at this level of hypoxia for a total of 15 minutes. Following 5 minutes of steady state hypoxia, AA was infused for an additional 10 minutes of hypoxia. The last 30 seconds of baseline, steady state hypoxia, and steady state hypoxia + AA was used to calculate steady state FBF and FVC (Table 7).

Data Acquisition/Analysis

Data were collected and stored on a computer at 250Hz and later analyzed off-line with signal-processing software (Windaq DATAQ Instruments, Akron, OH, USA). MAP was determined from the brachial artery pressure waveform. FBF, FVC, HR, MAP, and oxygen saturations represent an average of the last 30 seconds of each phase of interest (baseline, steady-state hypoxia, and hypoxia + AA). Minute ventilation and end-tidal CO_2 were determined from an average of the data over a minute time period. Blood gas values were obtained during the last minute of each condition (Table 7).

In the present study, FVC was chosen as the primary variable of interest because it reflects net vascular tone of the resistance vasculature. Systemic hypoxia as a stimulus does not significantly alter the arterial-venous pressure gradient and therefore changes in O_2 delivery are reflected by changes in vascular conductance. Additionally, baseline blood flows increase following local sympatho-adrenal blockade and therefore to more accurately reflect dilation or constriction we chose to present the data as a percent change in FVC from baseline (Δ FVC (%)) [9].

Utilizing SPSS statistical software (IBM, Armonk, New York) a three way repeated measure ANOVA was used to examine the impact of age, $\%SpO_2$, as well as any effect of AA. When appropriate, post-hoc comparisons were made using Tukeys HSD and significance was set at $P<0.05$. All values are presented as means ± standard error of the mean (SEM).

Results

Subject Characteristics

Table 6 presents the subject characteristics. In addition to age, total cholesterol was significantly greater in the older group (186±3 vs. 155±6 mg dl^{-1}), however the levels are still considered to be within a normal healthy range (< 200 mg dl^{-1}).

FBF and FVC response to systemic hypoxia and hypoxia + AA infusion

In the presence of local sympatho-adrenal blockade, young adults had a significantly greater resting blood flow (88±23 vs. 43±6 ml min^{-1} $P<0.05$) (Table 7). Following 5 minutes of systemic hypoxia (85% SpO_2), older adults exhibited significantly lower vasodilation from baseline (Δ FVC 14±4% vs. 30±6% $P<0.05$). AA infusion began after 5 minutes of systemic

hypoxia and continued for an additional 10 minutes. At the end of the AA infusion there was no change in the amount of vasodilation observed in young adults from steady-state hypoxia, however, older adults dilated further (~Δ FVC 40%) during AA infusion (Figure 5).

Effects of systemic hypoxia and hypoxia + AA on blood gases and ventilation

There were no significant differences between young and old in minute ventilation, PaO_2 or $\%SpO_2$ at rest or during hypoxia and hypoxia + AA (Table 7). Resting $EtCO_2$ (%) was significantly lower in the older group. Compared to rest, there were no changes in $EtCO_2$ during hypoxia or hypoxia +AA within each group (Table 7).

Arterial oxygen content ($CtaO_2$) was not different between young and old at rest (20±1 vs. 20±1 ml dl^{-1}) or during hypoxia (17±1 vs 17±1 ml dl^{-1}) and was not affected by infusion of AA. Peripheral oxygen delivery (FBF x $CtaO_2$) was lower at rest (18±5 vs. 9±2 ml min^{-1} P = 0.08) and during steady state hypoxia (9±2 vs. 21±5 ml min^{-1}, P = 0.06) in older adults. Peripheral oxygen delivery increased following AA infusion in older adults (12±3 vs. 9±2 l ml min^{-1}) and was unchanged in young (22±5 vs 21±5 ml min^{-1}).

Discussion

Hypoxia trial

The primary findings of the present study are that in the presence of sympatho-adrenal blockade, older adult's exhibit impaired peripheral vasodilation to acute systemic hypoxia and this age-associated impairment is significantly attenuated following acute infusion of AA. To date, there are a few studies examining whether older adults exhibit impaired peripheral vasodilation to a single level of acute systemic hypoxia. In 1972, a study reported that with

increasing age, the increase in forearm blood flow to a hypoxic stimulus was attenuated [11]. However, in this study, 6 of the 18 participants had chronic obstructive pulmonary disease and end-tidal CO_2 concentrations were not controlled, resulting in hypocapnea in all participants, possibly confounding ventilation and vascular tone. More recently, when young and older adults were subjected to acute isocapnic hypoxia (80% SpO_2), Casey [12] did not observe an age-associated impairment (both young and old vasodilated ~20%) whereas Kirby et al. reported a significant age impairment (0% vs . 48% vasodilation) [13], which is consistent with the findings reported in the control hypoxia condition (trial 1) of study 1(Chapter II) in this dissertation. In the present study, under sympatho-adrenal blockade and during 85% SpO_2, young adults vasodilated ~27% and older adults only vasodilated ~13%, also consistent with our findings in the third hypoxia (α+β blockade) trial of experiment 1(Chapter II) in this dissertation. Based on the cumulative findings from the present study and that presented in experiment 1 (Chapter II) of this dissertation, we conclude that there is a significant age-associated impairment in the hypoxic vasodilatory response that persists following local inhibition of sympatho-adrenal influence.

Hypoxia and AA infusion

Acute AA infusion began after 5 minutes of steady-state hypoxia and continued for an additional 10 minutes. At the end of the AA infusion (minute 15 of hypoxia), there was no significant change in the level of vasodilation from that observed prior to AA infusion (minute 5 of hypoxia) in the young subjects (~7% increase in ΔFVC from steady-state hypoxia). Conversely, older adults further dilated ~43% from steady-state hypoxia levels during AA infusion. Not only was the vasodilation from steady-state hypoxia to hypoxia + AA significant

in older adults (Δ FVC 43% vs. 7%), the absolute vasodilation from baseline to hypoxia + AA was greater in older adults compared to young (Δ FVC 62% vs. 38% $P<0.05$).

AA and Local Vasodilation

Previous work from our laboratory determined that in the absence of sympatho-adrenal influence, combined blockade of both nitric oxide and prostaglandins eliminated the hypoxic vasodilatory response in young adults [1]. Given that older adults exhibit impaired endothelial function with increasing age [6], their ability to facilitate hypoxic vasodilation through these pathways is limited. Our laboratory and others have also reported that in older adults, acute intra-arterial infusion of AA can improve endothelium-dependent vasodilation [5, 6], and that this improvement is likely due to increased nitric oxide availability [4]. Therefore, in the present study we chose to use AA as a pharmacological tool to improve local vasodilatory signaling. In agreement with this, the younger subjects in this study did not exhibit any change in their vascular response to hypoxia following AA infusion as healthy young adults typically don't exhibit impaired endothelial function or attenuated nitric oxide availability. The specific mechanism by which AA improves NO signaling in humans is unknown, but AA may favorably affect redox balance in older adults which could reduce scavenging of nitric oxide or enhance the enzymatic conversion of L-arginine to nitric oxide. Conversely, AA could indirectly limit production of local vasoconstricting substances, such as endothelin by altering the local redox balance. Endothelin is a peptide released from endothelial cells that is observed to be elevated with aging [14], and can limit endothelium-dependent vasodilation [15] because of its ability to attenuate nitric oxide signaling [16].

Experimental Considerations

In the present study, AA was acutely infused at a concentrated dose that would not be able to be attained through oral ingestion. To our knowledge, no studies have examined whether an oral dose of AA would facilitate similar improvements in hypoxic vasodilation in older adults. However, there is some evidence that within older diseased populations (type II diabetics, hypertensives and individuals with coronary artery disease) there is a positive vascular effect of oral AA supplementation [6, 17-19].

Experimental Limitations and Perspectives- See Chapter VI

Conclusion

In conclusion, there is a significant impairment in the regulation of peripheral vascular tone with age during exposure to acute systemic hypoxia. Further, this age-associated impairment is not attributed to elevated α-adrenergic vasoconstrictor tone (Experiment 1-Chapter II) and is significantly improved following acute intra-arterial infusion of AA, suggesting that attenuated local vasodilation is the major contributor to the age-associated impairment. AA acid improved local vasodilatory signaling in older adults likely through increasing nitric oxide availability. In agreement with this, we failed to observe any significant change in young adults following AA infusion, a population that does not exhibit attenuated nitric oxide availability or endothelial dysfunction.

Table 6. Subject Characteristics *P<0.05 vs. Young. In addition to age, total cholesterol was significantly elevated in older adults, although the levels were still within a normal range.

Variable	Young	Older
Male:Female	4:1	3:2
Age (years)	21±1	64±5*
Body mass index (kg m^{-2})	24.4±2.4	24.2±1.4
Body fat (%)	21.7±4.2	27.3±2.4
Forearm volume (ml)	950.4 ±114.5	923.2±155.1
Total cholesterol (mg dl^{-1})	155.8±5.7	186.4±3.4*
Triglycerides (mg dl^{-1})	90.2±27.8	91.4±14.6

Table 7. Hemodynamic, ventilatory, and arterial blood gas data at rest, during steady-state hypoxia (minute 5) and during steady-state hypoxia with AA (minute 15). *P<0.05 vs. Young. †P<0.05 vs. Baseline. ‡ P<0.05 vs. steady-state hypoxia (minute 5). PaO_2 = partial pressure arterial O_2, SaO_2 = arterial oxyhemoglobin saturation, $CtaO_2$ = arterial O_2 content.

	Baseline		**85% SpO_2 (Minute 5)**		**85% SpO_2+AA (Minute 15)**	
	Young	**Older**	**Young**	**Older**	**Young**	**Older**
Heart Rate (beats min^{-1})	60 ±4	52 ±3	74 ±4	64 ±2*	77 ±5	65 ±4*
Mean Arterial Pressure (mmHg)	92.2 ±1.8	100.3 ±4.3	95.2 ±2.4	103.1 ±4.7	96.7 ±2.8	101.6 ±4.6
Forearm Blood Flow (ml min^{-1})	88.3 ±23.3	43.4 ±5.7*	114.0 ±28.5†	51.1 ±6.9*†	121.8 ±29.1*†	72.7 ±12.6*†‡
Forearm Vascular Conductance (ml min^{-1} $mmHg^{-1}$)	94.5 ±25.1	43.1 ±5.5*	119.5 ±29.8†	49.3 ±6.2*†	127.9 ±32.1*†	70.6 ±10.3*†‡
Minute Vent. (l min^{-1} BTPS)	8.4 ±0.8	8.3 ±1.5	12.0 ±1.2	9.08 ±1.9	11.9 ±1.4	9.5 ±1.8
End Tidal CO_2 (%)	6.9 ±0.1	5.8 ±0.3*	6.7 ±0.1	5.7 ±0.2*	6.7 ± 0.1	6.0 ±0.2
P_aO_2 (mmHg)	79.0 ±2.7	85.6 ±4.0	52.4 ±0.8	47.3 ±1.4*	53.3 ±0.8	45.3 ±2.2*
S_aO_2 (%)	95.1 ±0.6	96.5 ±0.7	85.8 ±0.4	83.6 ±1.2	85.7 ±0.6	81.0 ±1.7
Ct_aO_2 (ml dl^{-1})	20.2 ±0.5	20.0 ±1.3	17.7 ±0.7	17.8 ±1.3	17.8 ±0.5	16.2 ±1.0
Oxygen Delivery (ml min^{-1})	18.1 ±4.8	9.0 ±2.0	20.7 ±5.4	9.3 ±1.9	22.0 ±5.4	12.6 ±3.4

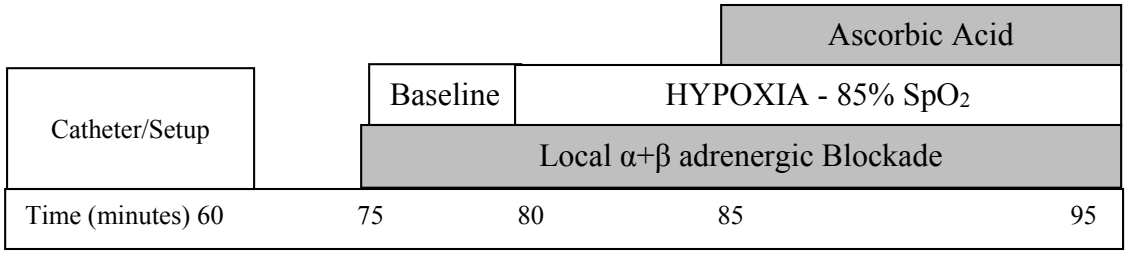

Figure 4. Study Timeline. Following brachial artery catheter insertion and rest, α+β adrenergic receptor antagonists (phentolamine and propranolol, respectively) were infused and maintained throughout the study. During the baseline period subjects breathed room air through the mouthpiece for 4-5 minutes while baseline hemodynamics and ventilation were recorded. Systemic hypoxia began (85% SpO_2) and following 5 minutes of steady-state hypoxia, ascorbic acid (AA) was infused intra-arterially for an additional 10 minutes while steady-state hypoxia (85%SpO_2) was maintained.

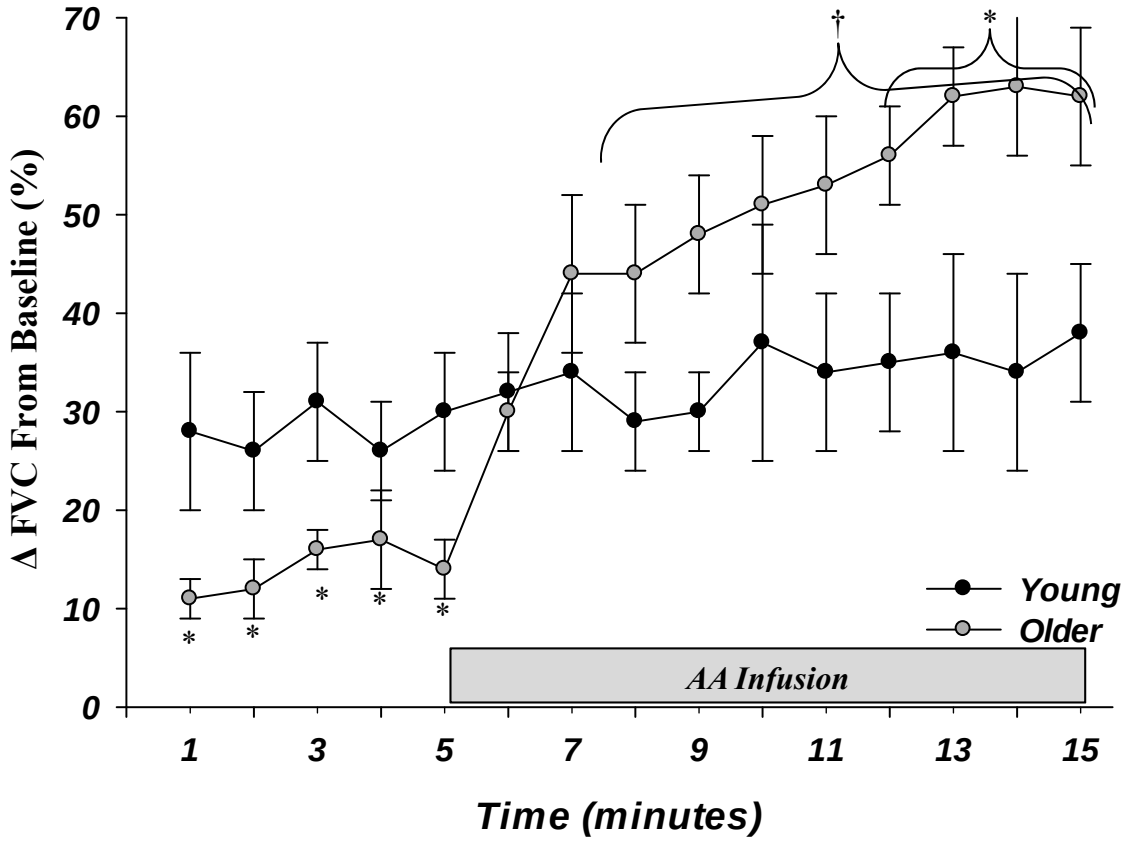

Figure 5. Vasodilatory response during hypoxia and hypoxia + AA in young and older subjects. * $P < 0.05$ vs. young. † $P < 0.05$ vs. without AA within age group. During the first five minutes of systemic hypoxia (85% SpO_2), older adults exhibited significantly lower vasodilation compared to that observed in young. AA infusion began at minute 5 of hypoxia and continued for an additional 10 minutes. AA infusion had no effect on the level of vasodilation observed in young, however, older adults further vasodilated after AA was infused.

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CHAPTER IV – MANUSCRIPT II

Impaired regulation of peripheral vascular tone during graded handgrip exercise in aging humans: role of the sympatho-adrenal system

Summary

In healthy humans, aging is associated with attenuated vasodilation and muscle blood flow during exercise. With increasing age, there is a marked increase in resting sympathetic nervous system (SNS) activity, yet whether augmented SNS mediated α-adrenergic vasoconstriction contributes to the age-associated impairment in exercising blood flow in humans is unknown. We tested the hypothesis that SNS restraint of muscle (forearm) blood flow (FBF) is greater in older compared with young adults during graded handgrip exercise (5, 15, 25% maximal voluntary contraction (MVC)). FBF was measured (Doppler ultrasound) and forearm vascular conductance (FVC) was calculated in 12 young (21±1 years) and 12 older (62±2 years) adults in control conditions and following local α+β-adrenergic receptor blockade via intra-arterial infusions of phentolamine and propranolol, respectively. Older adults exhibited significantly lower FBF and FVC at 15% MVC (177±15 vs. 216±12 ml min^{-1} and 172±16 vs. 250±18 ml min^{-1} $mmHg^{-1}$ $P<0.05$) and 25% MVC (277±17 vs. 344±24 ml min^{-1} and 253±17 vs. 365±31 ml min^{-1} $mmHg^{-1}$ $P<0.05$). Following local sympatho-adrenal blockade, the increase in FBF and FVC from rest to steady-state exercise was similar in young and older adults across exercise intensities, and the age-associated impairment persisted. Our data indicate that, during graded mild-to-moderate intensity handgrip exercise, the attenuated vasodilation (FVC) and subsequently lower skeletal muscle blood flow in older healthy adults is not due to augmented sympathetic vasoconstriction, but rather due to impairments in local vasodilatory signaling with age.

Introduction

With increasing age and despite regular exercise, there is a notable decline in resting and exercising blood flow [1-4]. This attenuated ability to deliver oxygen (O_2) to both resting and exercising skeletal muscle may be a primary contributor to lower aerobic capacity and increased risk of cardiovascular disease [5-7]. Generally, net skeletal muscle blood flow is determined by the balance between vasodilating and vasoconstricting stimuli. With age there is a decline in endothelial cell function, termed "endothelial dysfunction" [8-10] which is characterized by a loss of some inherent vasoprotective function, lower production of vasodilator signaling molecules and increased production of vasoconstrictor signaling molecules [3, 9, 11, 12]. Much of the focus of present research has attempted to improve production of local vasodilatory signals, and it appears that when local dilatory pathways are augmented, presumably through reduction in oxidative stress, exercising blood flow may increase in older populations [11, 13-15].

In addition, other vascular age-associated changes occur with age that affect the net balance between vasodilatory and vasoconstrictor stimuli. The SNS exerts tonic influence over the vasculature whereby norepinephrine (NE) is released from sympathetic nerve endings following a nerve impulse. NE binds to both α_1- and α_2- adrenergic receptors located on the vascular smooth muscle and signals vasoconstriction. As exercise ensues, the level of activity emitted from the SNS increases linearly with exercise intensity [16] as a means to maintain mean arterial pressure (MAP) [17, 18]. With respect to aging and the SNS, it is well documented that there is a significant increase in resting SNS activity [19]. Despite elevated SNS signaling at rest in older adults, this signaling does not translate into lower resting blood flow in the forearm. Additionally, following the local administration of an α-adrenergic receptor antagonist, there is

not a greater increase in resting forearm blood flow in older adults [3, 20], indicating that older adults do not have a greater degree of sympathetic restraint of forearm blood flow at rest. This phenomenon of elevated SNS vasoconstrictor signaling without a concomitant decrease in blood flow is attributed to decreased sensitivity of the α-adrenergic receptors with aging [20].

Although aging humans don't exhibit elevated sympathetic restraint in the forearm, recent animal data indicates that despite no difference in resting vessel diameter and blood flow, older mice still exhibit significantly lower skeletal muscle blood flow following a single contraction and during steady-state exercise, which was reversed following administration of an α-adrenergic receptor antagonist [21]. Further, the younger mice exhibited impairments in skeletal muscle blood flow similar to that observed in the older mice after their blood vessels were exposed to norepinephrine, an α-adrenergic receptor agonist [21]. These findings indicate that despite the lack of a role of SNS mediated restraint of skeletal muscle blood flow at rest, the SNS is able to limit skeletal muscle blood flow during exercise in older mice due to elevated α-adrenergic vasoconstrictor tone.

To date, it remains unknown whether the SNS limits peripheral vasodilation leading to attenuated skeletal muscle blood flow in older adults during exercise. We hypothesized that compared to young, older adults would have significantly lower forearm blood flow during graded handgrip exercise and this age-associated impairment would be attributed to elevated α-adrenergic vasoconstrictor tone.

Methods

Subjects

With Institutional Review Board approval and following written informed consent, a total of 12 (8 male, 4 female) young and 12 (8 male, 4 female) older healthy subjects participated in

the present study. All participants were non-smokers, non-obese, normotensive, and not taking any medications including over the counter supplements (Table 8). Females were studied during the placebo phase of birth control or during the early follicular phase of their menstrual cycle to minimize any potential vascular effects of sex hormones and all older females were post-menopausal not taking hormone replacement. All Studies were performed in the Human Cardiovascular Physiology Laboratory located at Colorado State University (~1500 m above sea level) following a 12-hour fast with the subjects in the supine position. The experimental arm of the subject was slightly elevated above heart level to minimize any potential influence of the muscle pump on forearm hemodynamics, and thus isolate local vasodilation. All studies were performed according to the Declaration of Helsinki.

Arterial Catheterization

The non-dominant arm was chosen to be the experimental arm and after local application of anesthesia (2% lidocaine), a 20-guage, 7.6cm catheter was inserted into the brachial artery utilizing aseptic technique. The catheter was connected to a pressure transducer for continuous monitoring of mean arterial pressure (MAP) as well as a 3-port connector to allow for drug infusions and blood sampling. Throughout the duration of the study, heparinized saline was continuously infused at a rate of 3 ml minute^{-1}.

Body Composition and Forearm Volume

Dual-energy X-ray absorptiometry (DEXA: Hologic: Bedford, MA, USA) was used to determine body composition. A regional analysis of the experimental forearm area (proximal to distal radio-ulnar joint) from the whole body DEXA scan was performed to determine forearm

volume (FAV). Drug dosage was normalized according to forearm volume [22]. Body mass index was calculated as body weight (kg) divided by height (meters) squared.

Graded Handgrip Exercise

Maximum voluntary contraction (MVC) was determined from the experimental arm for each subject as the average of at least three maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL, USA) that were within 3 percent of each other. Using a pulley system with weights attached, graded handgrip exercise was performed whereby individuals contracted at 5%, 15%, and 25% of their MVC for 4 minutes at each workload. Handgrip exercise was performed for a total of 12 minutes with a duty cycle of 1 second contraction and 2 second relaxation using both audio and visual cues to ensure correct timing of contraction and relaxation [23, 24].

Forearm Blood Flow (FBF) and Forearm Vascular Conductance (FVC)

Brachial artery mean blood velocity (MBV) was determined using a 4-MHz pulsed Doppler ultrasound probe (model 500M Multigon Industries, Mt. Vernon, NY) which was securely fixed to the skin and the probe insonation angle was 45 degrees relative to the skin. Brachial artery diameter was determined using a 7 MHz ultrasound probe (Sonos 4500: Hewlett Packard, Andover, MA) USA) and measurements were made in duplex mode at end-diastole in triplicate during steady state conditions. Forearm blood flow (FBF) was calculated as FBF=MBV x $\times \pi$ (brachial artery diameter/2)2 $\times$ 60, where the FBF is in ml min^{-1}, the MBV is in cm s^{-1}, the brachial diameter is in centimeters, and 60 was used to convert from ml s^{-1} to ml min^{-1}. As previously mentioned, skeletal muscle blood flow is primarily determined by changes in

the pressure gradient or driving pressure across a vessel ($\Delta P = P_{arterial} - P_{venous}$) and the caliper of the resistance vessels (r^4). During exercise, both the ΔP and r^4 are susceptible to change as the pressure gradient within the skeletal muscle bed changes during exercise as well as the caliper of the resistance vessels. In the present study, we were interested in what net blood flow (O_2 delivery) was as well as how changes in peripheral vascular tone (vasodilation and vasoconstriction) contribute to the net change in flow. Therefore, we calculated forearm vascular conductance (FVC) as (FBF/MAP) ×100, and expressed as ml min^{-1} $100mmHg^{-1}$ as an index of peripheral vascular tone. All studies were performed in a cool temperature-controlled environment with a fan directed toward the forearm to minimize the contribution of skin blood flow to forearm hemodynamics.

Brachial Artery Endothelium-Dependent and Endothelium-Independent Dilation

To characterize the endothelial function of participants and to determine if α-adrenergic vasoconstrictor signaling limited endothelium-dependent or endothelium-independent vasodilation, Acetylcholine (Ach; Miochol-E Novartis Inc.) was locally administered (16 µg 100 ml FAV^{-1} min^{-1}) for three minutes to determine endothelium-dependent vasodilation and sodium nitroprusside (SNP; Nitropress Hospira Inc.) was administered (4 µg 100 ml FAV^{-1} min^{-1}) for three minutes to assess endothelium independent vasodilation [11]. Drug order was randomized and a minimum of 10 minutes occurred between drug administrations.

Regional Sympatho-adrenal Blockade

To eliminate α-adrenergic mediated vasoconstriction at rest and during graded handgrip exercise, we locally infused phentolamine mesylate (Bedford Laboratories, Bedford, OH, USA),

a non-selective α-adrenergic receptor antagonist for 10 minutes prior to exercise (12 μg dl^{-1} FAV min^{-1}) and maintained the infusion throughout the exercise trial (5 μg dl^{-1} FAVmin^{-1}) [22, 25]. Administering an α-adrenergic antagonist can block α_2-adrenergic receptors on sympathetic nerve endings and stimulate norepinephrine release, which is able to bind β-adrenergic receptors located on the endothelium and vascular smooth muscle and elicit vasodilation [26]. Therefore, to limit any contribution of β-adrenergic receptor mediated vasodilation we locally infused propranolol hydrochloride (Baxter, Deerfield, IL,USA), a non-selective β-adrenergic receptor antagonist for ten minutes prior to exercise (10 μg dl FAV^{-1} min^{-1}) and continued the infusion at a maintenance rate (5 μg dl FAV^{-1} min^{-1}) throughout the exercise trial [22, 25].

Experimental Protocol

The overall study timeline is presented in Figure 6. All participants arrived in the morning after an overnight fast. All measurements were performed with the subjects in the supine position within a temperature controlled room with a fan directed towards the arm to reduce skin blood flow. All study drugs were administered via brachial artery catheter and were dosed according to forearm volume and infused via Harvard infusion syringe pump. Briefly, endothelium-dependent and endothelium-independent vasodilation were assessed prior to the graded exercise protocol. After sympatho-adrenal blockade the graded exercise protocol was repeated and endothelium-dependent and -independent vasodilation were re-assessed.

Data Acquisition/Analysis

Data were collected and stored on a computer at 250Hz and later analyzed off-line with signal-processing software (Windaq DATAQ Instruments, Akron, OH, USA). MAP was

determined from the brachial artery pressure waveform. FBF, FVC, HR and MAP represent an average of the last 30 seconds of each time period. A repeated measures ANOVA was used to examine differences within and between groups. When appropriate, post-hoc comparisons were made using Tukey's HSD and significance was set at $P<0.05$. All values are presented as means ± standard error of the mean (SEM).

In the present study we utilized handgrip exercise as a physiological stress that evokes a mismatch between O_2 delivery and metabolic demand, which provided us the opportunity to determine how young and older adults respond (increase O_2 delivery) to maintain homeostasis. The primary variables of interest in this study were forearm blood flow and forearm vascular conductance.

Results

Forearm Blood Flow and Forearm Vascular Conductance at Rest and During Graded Handgrip Exercise in Control Conditions

In the control trial, there was no difference in resting forearm blood flow between young and older adults (30±3 vs. 35±4 ml min^{-1}) (Table 9). In response to exercise, older adults had a significantly lower forearm blood flow during both 15% (177±15 vs. 216±12 ml min^{-1} $P<0.05$) and 25% MVC (277±18 vs. 344±24 ml min^{-1} $P<0.05$) handgrip exercise, whereas there was no age-associated impairment at 5% MVC (80±7 vs. 80±8 ml min^{-1}) (Figure 7A). On average, older adults exercising blood flows were 18% lower than young adults at both 15% and 25% MVC. While MAP was not significantly different between young and old at rest (86±3 vs. 94±2 mmHg $P=0.06$), older individuals had significantly elevated MAP across all exercise intensities (Table 9 $P<0.05$).

There were no differences in resting forearm vascular conductance between young and older adults in control conditions (36±5 vs. 37±4 ml min^{-1} $mmHg^{-1}$). Older adults exhibited significantly lower forearm vascular conductance at both 15% (172±16 vs. 250±18 ml min^{-1} $mmHg^{-1}$ $P<0.05$) and 25% MVC (253±17 vs. 365±31 ml min^{-1} $mmHg^{-1}$ $P<0.05$) (Figure 9A), on average, the older adults exhibited lower vasodilation (~30%) compared to young during both 15% and 25% MVC (Table 9).

Forearm Blood Flow and Forearm Vascular Conductance at Rest and During Graded Handgrip Exercise Following Local Sympatho-adrenal Blockade

Following local sympatho-adrenal blockade, there were no significant differences in resting forearm blood flow between young and old adults (80±13 vs. 63±6 ml min^{-1}) (Figure 7B). During graded handgrip exercise, the age-associated impairment in exercising blood flow persisted at both 15% (221±21 vs. 278±19 ml min^{-1} $P<0.05$) and 25% MVC (327±34 vs. 413±31 ml min^{-1} $P<0.05$) (Figure 7B). Additionally, MAP remained significantly elevated in the older group at rest and across all exercise intensities (Table 9).

Following sympatho-adrenal blockade, resting forearm vascular conductance was lower in older adults (65±6 vs. 82± 9 ml min^{-1} $mmHg^{-1}$) although not significantly. During handgrip exercise, the age-associated impairment persisted and peripheral vasodilation remained approximately 30% lower in older adults compared to young (Figure 8B) during both 15% MVC and 25% MVC.

Sympathetic Restraint of Forearm Blood Flow

In both the control condition and following local sympatho-adrenal blockade, there was an age-associated impairment in forearm blood flow (18% lower in older adults) and forearm vascular conductance (30% lower in older adults). This age-associated impairment was not attenuated following inhibition of α-adrenergic vasoconstrictor tone. One measure that demonstrates the degree of α-adrenergic restraint of exercising blood flow and peripheral vasodilation is to examine the increase in steady-state forearm blood flow and vascular conductance from rest to each exercise intensity, in both control and blockade conditions (Figure 9). In control conditions, older adults had a significantly smaller increase in forearm blood flow at both 15% and 25% MVC (Figure 9A). Following local sympatho-adrenal inhibition, both young and older adults increased resting blood flow to a similar extent. During exercise, older adults still exhibited significantly smaller increases in blood flow at both 15% and 25% MVC. Additionally, compared to control conditions, neither young nor old exhibited any degree of α-adrenergic restraint of blood flow as the changes observed in control are similar to the changes observed in blockade condition, despite a doubling of resting forearm blood flow in each group (Figure 9A).

Additionally, forearm vascular conductance did not improve in older adults following local sympatho-adrenal blockade. The increase in forearm vascular conductance from rest to each exercise intensity (Figure 9B) was not different from control condition in either group, indicating that α-adrenergic vasoconstrictor tone did not limiting peripheral vasodilation.

Endothelium-Dependent and Endothelium-Independent Vasodilation

Compared to young, older adults exhibited a smaller increase in forearm blood flow to Ach (Δ FBF 80±18 vs. 206±65 ml min^{-1} $P<0.05$) whereas the increase in forearm blood flow to SNP was similar in older and young adults (Δ FBF 133±20 vs.176±2 ml min^{-1}) (Figure 8). Following local sympatho-adrenal blockade, despite having a greater increase in flow to each vasodilator, the age-associated impairment to Ach persisted (Δ FBF 177±27 vs. 292±52 ml min^{-1} $mmHg^{-1}$ $P<0.05$) and the increase to SNP remained similar between older and young adults (Δ FBF 206±26 vs. 279±77 ml min^{-1}).

Discussion

The primary findings of the present study are that compared to young, older adults exhibit impaired forearm blood flow during moderate intensity handgrip exercise. Further, following local sympatho-adrenal blockade the age-associated impairment in exercising blood flow persisted. Additionally, older subjects exhibited significantly lower endothelium-dependent vasodilation that persisted following local sympatho-adrenal blockade.

Handgrip Exercise

To our knowledge, this is the first study to examine the role of the SNS in limiting the blood flow response during graded handgrip exercise in both young and older adults. Previously, Joyner and colleagues [27] implemented stellate ganglionic blockade to young adults (males) and examined forearm blood flow response to incremental handgrip exercise to exhaustion. The authors concluded that sympathetic restraint did indeed limit the forearm blood flow response at the higher exercise intensities and the augmentation of blood flow observed following ganglionic

blockade also led to an increase in tissue oxygen consumption. The method of assessing blood flow in the aforementioned study was venous occlusion plethysmography which requires the subject to stop exercising in order for blood flow to be measured. Through the use of Doppler ultrasound, forearm blood flow can be continuously measured during exercise and allows for the concomitant measure of arterial diameter to more accurately reflect steady-state blood flow. In the present study, following local sympatho-adrenal blockade, we did not observe an exercise intensity dependent degree of sympathetic restraint in either young or older adults during handgrip exercise. In fact, the increase in forearm blood flow from rest to steady-state exercise did not significantly differ between control and blockade conditions in either age group. Contrary to our hypothesis, we did not observe elevated SNS mediated restraint of forearm blood flow during handgrip exercise.

Exercise is a physiological stressor that elevates peripheral O_2 demand, resulting in increased O_2 delivery for homeostasis to be maintained and peripheral metabolic demand to be met. In the present study, older adults exhibited lower blood flow and elevated mean arterial pressure during handgrip exercise (15% and 25% MVC). Despite the elevated "driving pressure" of blood flow, net skeletal muscle blood flow remained lower and therefore the impairment is likely due to altered regulation of peripheral vascular tone, or attenuated peripheral vasodilation. Indeed, forearm vascular conductance, our index of peripheral vascular tone, was significantly lower (~30%) in older adults during handgrip exercise in both the control and sympatho-adrenal blockade conditions.

In general, factors that increase skeletal muscle blood flow include mechanical factors associated with skeletal muscle contraction as well as local released endothelium and metabolically derived vasodilating substances. The contribution of mechanical factors to the

steady-state blood flow response during steady-state exercise is relatively small compared to locally released factors and therefore it is more likely that attenuated production or availability of local vasodilating substances play a role in the age-associated impairment. In agreement with this, intra-arterial infusion of ascorbic acid can increase the bioavailability of nitric oxide, an important vasodilator whose production declines with age. Our lab previously determined that intra-arterial infusion of ascorbic acid increased forearm blood flow during mild (10% MVC) handgrip exercise in older adults without affecting forearm blood flow in young subjects [11, 13]. Another vasodilator that plays a role during exercise is adenosine tri-phosphate (ATP). ATP can be released from erythrocytes and endothelial cells in the presence of a low oxygen environment and plasma ATP concentrations are observed to increase during handgrip exercise and during systemic hypoxia [3]. Although ATP can elicit significant vasodilation, older adults have significantly lower plasma ATP concentrations during both exercise and systemic hypoxia [3] and therefore, augmenting the release of ATP from either the erythrocyte and/or endothelium may improve blood flow during exercise in older adults [28].

In addition to attenuated production of local vasodilatory signaling agents, elevated production of local vasoconstrictors may limit the ability of vasodilatory signaling. With aging, there is an increase in the production of vasoconstricting prostaglandins [10] and endothelin [8]. This shift in the balance between production of vasodilatory and vasoconstrictor agents at the local level may limit the ability of older adults to adequately increase blood flow during exercise [12].

Endothelium-Dependent and Endothelium-Independent Vasodilation

Older subjects exhibited significant attenuated vasodilation to the endothelium-dependent vasodilator (Ach). Endothelial function declines with age and numerous studies observe a

significant decline in vasodilation to Ach with aging. Following local sympatho-adrenal blockade we re-administered the same dose of Ach and SNP to determine whether sympathetically mediated vasoconstrictor signaling limited the degree of vasodilation of either Ach or SNP. Both young and older adults had greater increases in forearm blood flow to each agonist following local sympatho-adrenal blockade, however, the age impairment to Ach persisted. The question whether α-adrenergic vasoconstrictor tone can limit endothelium-dependent vasodilation has been examined previously in hypertensive patients utilizing venous occlusion plethysmography to measure flow. In the aforementioned study, phentolamine (non-selective α-adrenergic antagonist) was locally infused prior to Ach and SNP. The authors concluded that there was no improvement in Ach or SNP mediated vasodilation following α-adrenergic blockade [29]. In the present study, we utilized Doppler Ultrasound to measure changes in blood flow and in addition, implemented both α- and β-adrenergic blockade to limit any vasodilation elicited by α_2-adrenergic receptor antagonism on sympathetic nerve endings. The findings of the present study are in agreement with Panza and colleagues as we did not observe the sympatho-adrenal system to be a contributing factor to the age impairment in Ach mediated vasodilation.

Experimental Limitation and Perspectives- See Chapter VI

Conclusion

Contrary to our hypothesis, elevated α-adrenergic vasoconstrictor tone did not contribute to the age-associated impairment in the blood flow response to handgrip exercise. Following sympatho-adrenal blockade, there was no evidence of elevated α-adrenergic restraint of blood flow in young and older adults at any exercise intensity and the age-associated impairment in exercising

blood flow persisted. The lower blood flow and elevated mean arterial pressure in older adults during exercise suggests that the impairment lies in the inability of the resistance vasculature to appropriately vasodilate to the stimulus, likely due to lower production and/or availability of locally produced and released vasodilators.

Table 8. Subject Characteristics *P<0.05 vs. young. HDL = high-density lipoprotein, LDL= low-density lipoprotein. Older adults had significantly greater total cholesterol and LDL cholesterol than young adults, although the levels were still within normal range (<200 mg dl^{-1} for total cholesterol and <100 mg dl^{-1} for LDL cholesterol).

Variable	Young	Older
Male:Female	8:4	8:4
Age	21±1	62±2*
Height (cm)	174.2±3.5	173.0±2.8
Weight (kg)	80.4±4.4	77.6±4.7
% Body Fat	27.3±3.8	31.1±2.4
Forearm Volume (ml)	1034.1±68.6	907.8±60.4
Maximal Voluntary Contraction (Kg)	42.1±4.4	36.8±3.7
Triglycerides (mg dl^{-1})	78.2±13.8	90.9±12.4
Cholesterol (mg dl^{-1})	131.7±5.3	160.7±9.2*
HDL (mg dl^{-1})	44.1±2.8	47.4±4.9
LDL (mg dl^{-1})	71.1±5.0	95.3± 6.4*

Table 9. Hemodynamics at rest and during graded handgrip exercise in control and Sympatho-adrenal blockade condition. *P<0.05 vs. Young.

	Baseline		5% MVC		15% MVC		25% MVC	
	Young	**Older**	**Young**	**Older**	**Young**	**Older**	**Young**	**Older**
Control								
Heart Rate (beats min^{-1})	58±3	57±3	61±3	64±3	66±4	65±3	70±5	71±3
Mean Arterial Pressure (mmHg)	86.3 ±3.3	94.0 ±2.1	85.9 ±2.9	100.1 ±2.4*	88.4 ±3.0	103.9 ±3.4*	95.0 ±3.1	110.4 ±3.0*
Forearm Blood Flow (ml min^{-1})	30.2 ±3.3	34.8 ±4.0	80.4 ±6.7	80.2 ±8.5	215.8 ±11.6	177.4 ±14.6*	343.5 ±23.8	277.3 ±17.6*
Forearm Vascular Conductance (ml min^{-1}mmHg^{-1})	35.6 ±4.5	37.2 ±4.7	94.9 ±8.6	80.8 ±9.3	249.5 ±18.4	172.5 ±15.6*	365.3 ±31.3	252.7 ±17.6*
α + β Block								
Heart Rate (beats min^{-1})	56±3	56±3	57±3	58±3	62±3	62±3	67±3	67±4
Mean Arterial Pressure (mmHg)	87.9 ±2.7	96.8 ±4.0*	88.5 ±2.7	100.6 ±3.5*	91.9 ±3.0	102.4 ±3.0*	95.9 ±2.6	104.0 ±3.6*
Forearm Blood Flow (ml min^{-1})	69.9 ±9.4	62.9 ±6.0	141.2 ±16.5	117.3 ±12.4	276.5 ±18.6	220.6 ±20.5*	413.3 ±30.9	327.0 ±33.8*
Forearm Vascular Conductance (ml min^{-1}mmHg^{-1})	82.3 ±8.9	65.2 ±6.0	159.6 ±18.6	116.2 ±11.1*	304.2 ±22.5	215.3 ±18.6*	433.4 ±34.3	315.4 ±32.1*

Table 10. Hemodynamics during infusion of acetylcholine (Ach) and sodium nitroprusside (SNP). * P<0.05 vs. Young. Young n=9, Old n=11.

	Baseline		Ach		Baseline		SNP	
	Young	Older	Young	Older	Young	Older	Young	Older
Control								
Heart Rate (beats min^{-1})	54±2	59±3	56±2	60±3	54±2	61±3	66±2	66±4
Mean Arterial Pressure (mmHg)	84.3 ±3.3	94.0 ±2.3*	82.6 ±3.4	93.6 ±2.6*	84.9 ±3.0	94.2 ±4.2*	73.6 ±2.8	81.8 ±3.2
Forearm Blood Flow (ml min^{-1})	33.8 ±4.8	39.1 ±4.2	239.6 ±64.4	119.0 ±20.1*	31.6 ±3.6	33.9 ±3.6	207.6 ±26	167.0 ±21.6
Forearm Vascular Conductance (ml min^{-1}mmHg^{-1})	41.2 ±6.9	41.2 ±4.2	290.0 ±78.8	125.9 ±20.4*	38.0 ±5.33	36.4 ±3.4	285.9 ±39.4	205.8 ±27.5
α + β Block								
Heart Rate (beats min^{-1})	53±2	60±3	56±2	64±3	53±2	60±3	64±3	68±3
Mean Arterial Pressure (mmHg)	87.9 ±3.7	98.9 ±2.7*	85.9 ±2.9	99.7 ±3.1*	90.1 ±3.3	99.1 ±3.4*	76.2 ±2.6	86.2 ±2.8*
Forearm Blood Flow (ml min^{-1})	92.1 ±15.4	75.2 ±7.5	384.1 ±57.4	252.7 ±29.4*	81.8 ±7.1	74.0 ±7.1	361.6 ±40.0	279.8 ±30.2
Forearm Vascular Conductance (ml min^{-1}mmHg^{-1})	103.1 ±14.5	75.7 ±7.5	439.6 ±62.8	259.7 ±34.4*	90.6 ±3.3	74.4 ±6.4	480.3 ±60.5	324.0 ±34.5*

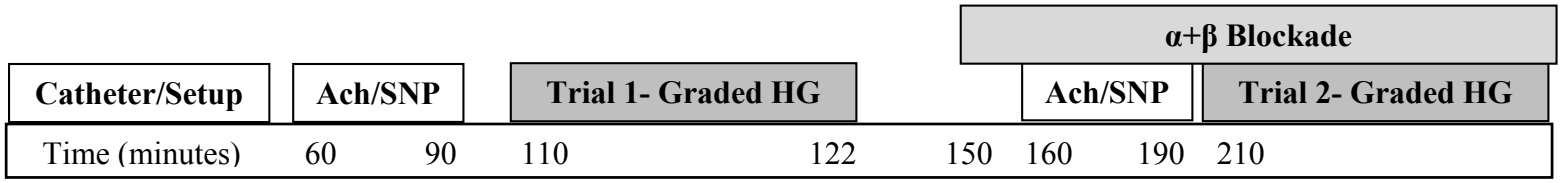


Figure 6. Study Timeline. Following insertion of the brachial artery catheter, subjects rested quietly for 30-45 minutes and then either exercise or vasodilator infusion began (randomized). Endothelium-dependent and endothelium-independent vasodilation was determined in random order utilizing acetylcholine (Ach) and sodium nitroprusside (SNP) respectively. During the graded handgrip trials, participants exercised for four minutes each at 5%, 15%, and 25% of their maximal voluntary contraction (MVC). The last 30 seconds or baseline of each workload was used to determine steady-state levels for each variable. Following the completion of control trials, phentolamine and propranolol were loaded and continually infused to limit sympatho-adrenal influence and the trials were repeated.

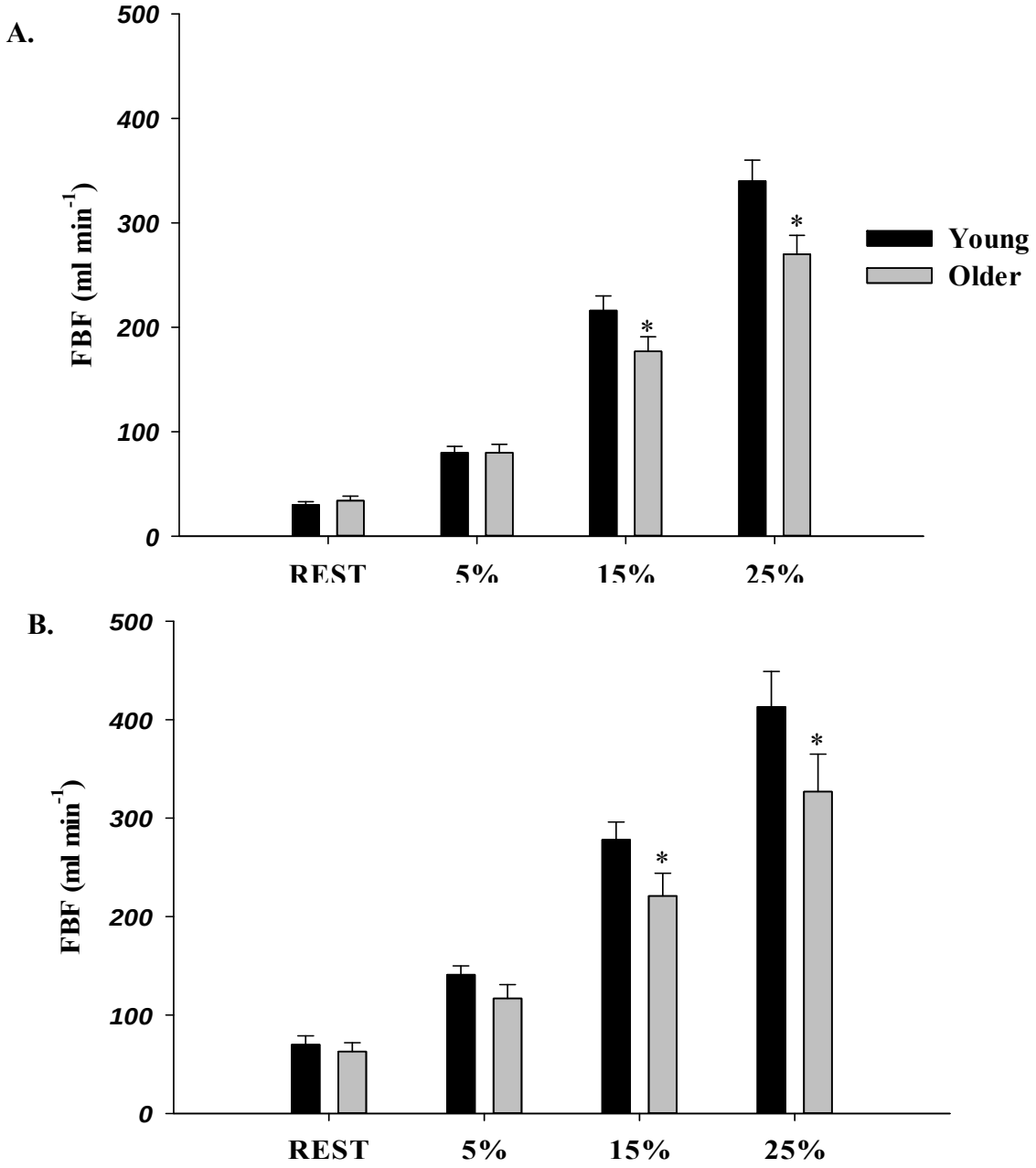


Figure 7. FBF at rest and during exercise in control (A) and following local sympatho-adrenal blockade (B) conditions. *P<0.05 vs. Young. In control conditions (A) older adults had significantly lower FBF at 15% and % 25 MVC workloads. (~18% lower FBF in older adults). Following local sympatho-adrenal blockade (B) resting and exercising blood flows were greater in both groups compared to control conditions, however, the age-associated impairment in exercising blood flow persisted at both 15% and 25% MVC (~20% lower FBF in older adults at both 15% and 25% MVC).

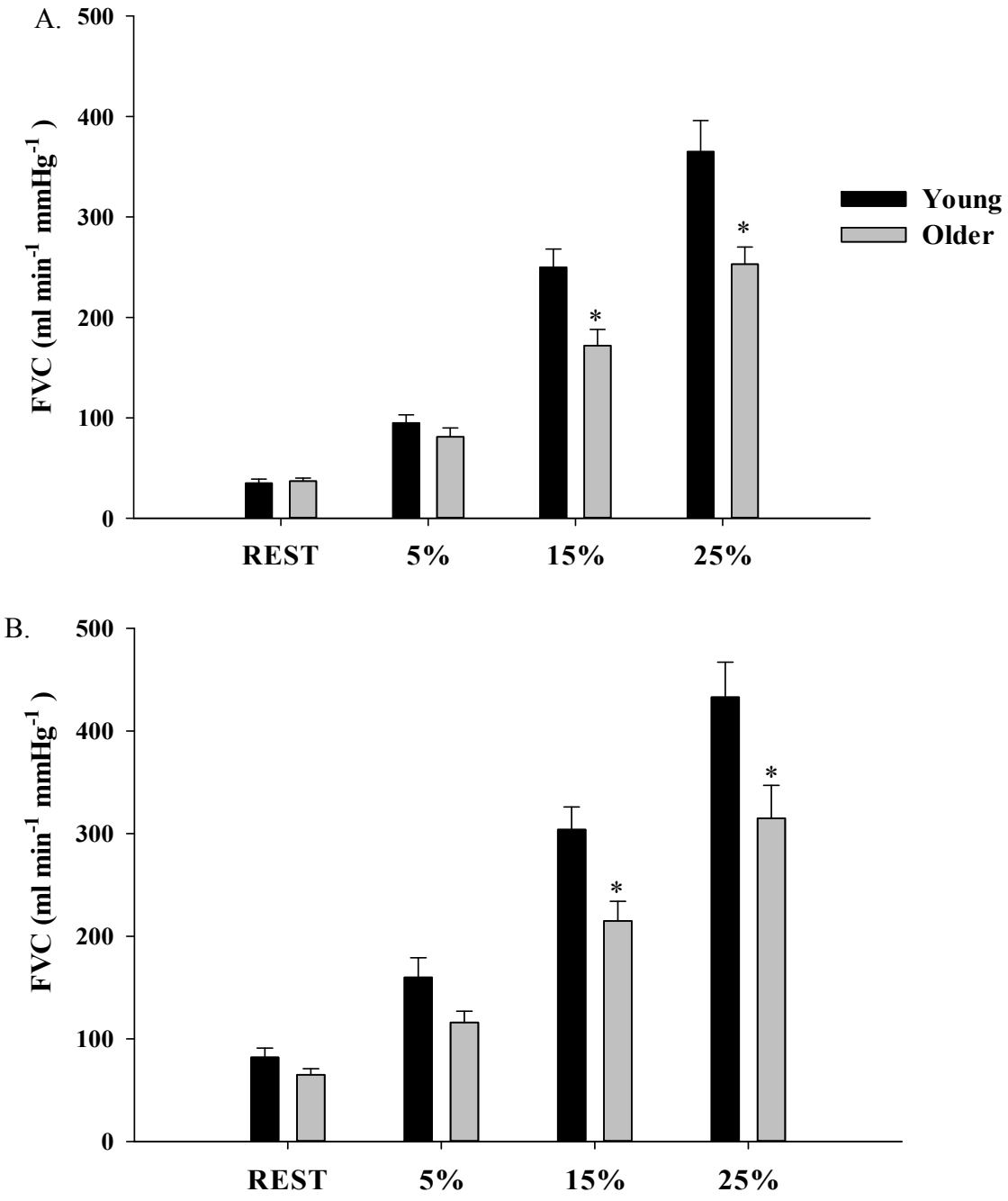
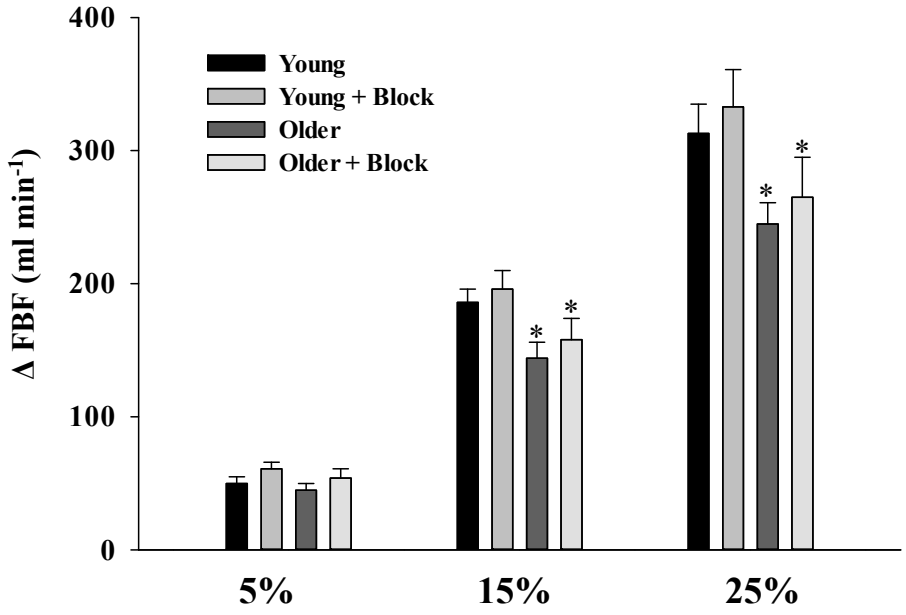


Figure 8. FVC at rest and during graded handgrip exercise in control (A) and following sympatho-adrenal blockade (B) condition *P<0.05 vs. Young. In control conditions (A) older adults had significantly lower FVC at both 15% and 25% MVC workloads (~30% lower FVC in old vs. young). Following local sympatho-adrenal blockade (B), the age-associated impairment persisted at both 15% and 25% MVC workloads (~28% lower FVC in old vs. young).

A.



B.

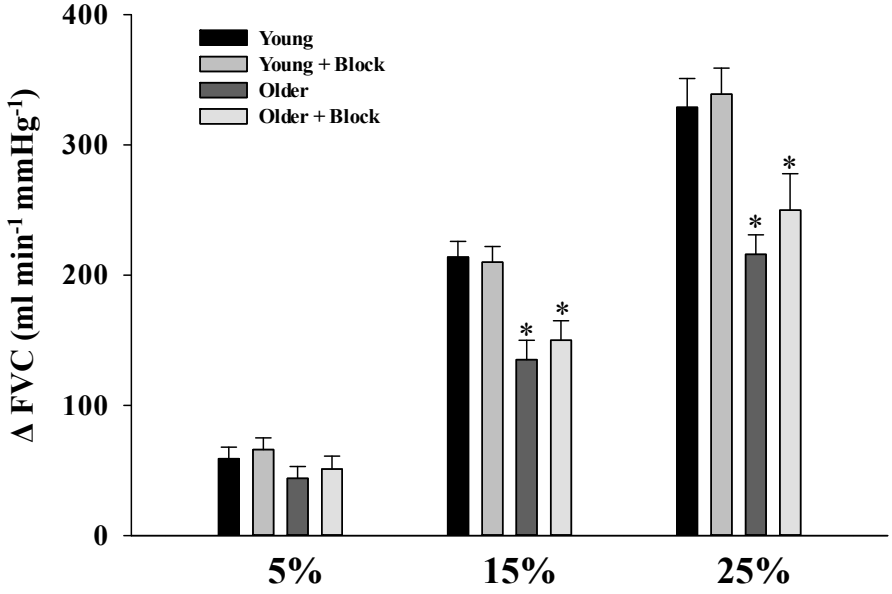


Figure 9. Increase in FBF (A) and FVC (B) from rest (Δ FBF and ΔFVC) in both young and older adults in control condition and following local sympatho-adrenal blockade. In control conditions, older subjects had a significantly smaller increase in FBF (A) and FVC (B) from rest compared to young at both 15% and 25% MVC workloads. Following sympatho-adrenal blockade, older adults still had a significantly smaller increase in FBF (A) and FVC (B) from rest at both 15% and 25% MVC workloads. Resting FBF and FVC were not significantly different between groups in either condition. The ΔFBF and ΔFVC from rest to exercise was not different between conditions in both groups despite greater resting blood flow during sympatho-adrenal blockade.

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CHAPTER V- FOLLOW UP STUDY

Acute ingestion of ascorbic acid improves forearm vasodilation during moderate intensity handgrip exercise in older adults

Summary

Intra-arterial infusion of ascorbic acid (AA) is observed to improve forearm blood flow (FBF) during handgrip exercise in older adults. We tested the hypothesis that acute oral AA ingestion would improve vasodilation and increase FBF and oxygen consumption (VO_2) during graded rhythmic handgrip exercise among older men (n=8; 65 ± 3 years). Subjects performed two bouts (12 minutes each) of graded rhythmic handgrip exercise (5, 15, 25% of maximum voluntary contraction (MVC)). Following trial 1 (control), participants ingested 2 g of AA and rested comfortably for 2 hours and repeated the exercise bout (trial 2). At rest and during exercise, we measured FBF (Doppler ultrasound) and calculated forearm vascular conductance (FVC). Using deep venous blood samples, VO_2 of the forearm muscles was calculated. Compared to control, there was no influence of AA at rest or during 5% MVC exercise. During both 15% and 25% MVC exercise, AA significantly increased FBF (248±16 vs. 199±13 ml min^{-1} and 403±33 vs. 343±24 ml min^{-1} $P< 0.05$) and FVC (244±19 vs. 198±16 ml min^{-1} $mmHg^{-1}$ and 386±38 vs 324±7 ml min^{-1} $mmHg^{-1}$ $P< 0.05$). Further, increased O_2 delivery following AA during 15% and 25% MVC was associated with significantly greater VO_2 (33.7±2.8 vs. 26.2±2.0 ml min^{-1} and 50.4±5.8 vs. 43.3±4.2 ml min^{-1} $P< 0.05$). On a separate study day, participants repeated the protocol with a placebo pill (n = 4); there were no differences in any variable between trials 1 and 2 at rest or during exercise. We conclude that acute oral AA is capable of increasing FBF via local vasodilation and VO_2 during moderate intensity handgrip exercise in older healthy humans.

Introduction

During exercise, an increase in metabolic demand drives an elevation in blood flow to working muscle as a means to adequately deliver oxygen (O_2) and nutrients to tissue [1-4]. The net blood flow response to a given exercise intensity is determined by (1) mechanical factors associated with skeletal muscle contraction; (2) locally released metabolic and endothelium derived dilators and; (3) the sympathetic nervous system (SNS) [5]. Mechanical compression of blood vessels following a contraction leads to local elevations in extra-vascular pressure and an acute increase in blood flow [6-8]. Although the augmentation in blood flow in response to a mechanical stimulus is significant, the overall contribution of mechanical factors to hyperemia during sustained steady-state exercise is small in comparison to the contribution of local and endothelial derived factors [9]. A number of local and endothelial derived factors have been implicated in governing increases in blood flow including nitric oxide and prostaglandins (PG), K^+, adenosine, adenosine triphosphate (ATP), and lactate [9]. However, to date, no single factor is considered wholly responsible for augmenting flow, rather there appears to be redundancy such that flow is maintained following the elimination of one dilator [9]. The third contributor to the net blood flow response, engagement of the SNS, occurs as exercise intensity increases and acts to elevate peripheral resistance as a means to maintain mean arterial pressure (MAP) [5, 10]. Further, as exercise intensity increases, unidentified local factors are capable of blunting sympathetically mediated vasoconstriction (functional sympatholysis), enabling adequate blood flow and O_2 delivery to exercising muscles [11-13]. In summary, net blood flow during sustained steady state exercise is predominately influenced by the presence of local dilators and the degree of sympathetically mediated vasoconstriction.

Human aging is associated with attenuated exercising blood flow to skeletal muscle [14-18]. This reduction is attributed, in part, to impaired endothelial mediated dilation [19, 20]. The age associated reduction in endothelium dependent dilation (EDD) is often linked with a global reduction in NO bioavailability [21], which can be attributed to attenuated NO production or sequestering of NO to yield it biologically unavailable as a vascular signaling molecule [22-25]. Impairments in EDD are observed to be ameliorated following endurance exercise training [26] and this positive adaptation has been associated with reductions in plasma markers of oxidative stress [27] and increased production of nitric oxide [28].

Our laboratory has previously demonstrated an age associated impairment in FBF (20-30% lower *vs*. young) during mild (10% maximum voluntary contraction (MVC)) and moderate (15% and 25% MVC) rhythmic handgrip exercise [18]. Following intra-arterial infusion of AA the age-associated blood flow impairment during 10% MVC handgrip exercise was abolished, with AA having no effect on younger individuals [18]. Furthermore, infusion of AA also reversed the impaired vasodilatory response to acetylcholine in older individuals, a classic test of endothelial function. To gain insight into the vasodilatory pathway responsible for the AA induced augmentation of flow, a follow up study co-infused the nitric oxide synthase (NOS) inhibitor (NG-monomethyl-l-arginine (l-NMMA)) with AA during rhythmic handgrip exercise in older individuals [29]. The augmented flow response associated with AA infusion (~20%) was abolished following co-infusion L-NMMA [29]. The result of these two studies suggest that intra-arterial infusion of AA can reverse the age-associated blood flow impairment and that the blood flow augmentation following AA infusion is likely working through the NO vasodilatory pathway.

Other labs have also documented improvements in resting leg blood flow [30, 31] and flow mediated dilation [32] following intra-arterial and intra-venous infusion of AA in healthy older individuals. These improvements are attributed to AA's ability to act as a potent anti-oxidant, potentially increasing NO bioavailability. This hypothesis is supported by the fact that infusion of AA has no effect on younger individuals [18] [31] or on older trained individuals [32]. Although the improvements in vascular function following intra-arterial and intra-venous infusions are well described, whether an oral dose of AA is capable of exerting positive vascular effects is less clear. Thus far, studies examining the influence of oral AA generally report positive findings when examining vascular outcomes within diseased populations such as type II diabetics [33], hypertensive [34, 35], and individuals with coronary artery disease [36]. Results examining the influence of AA on resting vascular function in otherwise healthy populations are equivocal. More recently, Wray and colleagues demonstrated that an oral antioxidant cocktail (1000 mg AA, vitamin e and alpha-lipoic acid) increased post-exercise (plantar flexion) blood flow compared to placebo in older individuals [37] and post exercise blood flow recovery has previously been demonstrated to be significantly dependent upon NO availability [38].

To date, it is unknown whether an acute oral dose of AA is capable of improving forearm blood flow in otherwise healthy older adults *during* exercise. Furthermore, whether improvements in O_2 delivery lead to O_2 greater utilization by the working tissue remains unknown. Accordingly, the primary purpose of this study was to examine FBF and oxygen consumption during mild and moderate intensity rhythmic forearm exercise following an acute AA dose (2g) administered orally. We hypothesized that oral AA consumption would improve exercising FBF and the elevated FBF following AA consumption would be associated with greater VO_2.

Methods

Subjects

With Institutional Review Board approval and following written informed consent, a total of 8 older male healthy subjects participated in the present study (Table 1). All participants were non-smokers, non-obese, normotensive, and not taking any medications including over the counter supplements. The week of the study date, participants kept a 3-day food log (two week days and one weekend day). Studies were performed after a 12-hour fast with the subjects in the supine position. The experimental arm of the subject was slightly elevated above heart level to minimize any potential influence of the muscle pump on forearm hemodynamics. All studies were performed according to the Declaration of Helsinki.

On a separate day, 4 subjects returned to the lab and performed two bouts of handgrip exercise separated by 2 hours and placebo consumption. This group served as a control to account for the influence of time on measured variables. During both visits, subjects were blinded to whether they were receiving AA or placebo.

Venous Catheterization and Blood Gas Measurements

An 18 gauge catheter (3.8 cm) was inserted in retrograde fashion into an antecubital vein of the experimental arm for deep venous blood samples. Saline was continuously infused through this catheter at a rate of approximately 3 ml min^{-1} for the duration of the study to keep it patent [39]. Venous blood samples were immediately analyzed with a clinical blood gas analyzer (Siemens Rapid Point 405 Automatic Blood Gas System, Los Angeles, CA, USA) for partial pressures of venous oxygen and carbon dioxide (PO_2 and PCO_2), venous oxygen content (ctO_2), pH, and oxygen saturation (SO_2).

Forearm Blood Flow, Vascular Conductance and Oxygen Consumption

A 12 MHz linear-array ultrasound probe (Vivid 7, General Electric, Milwaukee, WI, USA) was used to determine brachial artery mean blood velocity (MBV) and brachial artery diameter. For blood velocity measurements, the probe insonation angle was maintained at <60 degrees and the frequency used was 5 MHz. The Doppler shift frequency spectrum was analyzed via a Multigon 500M TCD (Multigon Industries, Mt Vernon NY, USA) spectral analyzer from which mean velocity was determined as a weighted mean of the spectrum of Doppler shift frequencies. Brachial artery diameter measurements were made in duplex mode at end-diastole at rest and between contractions (in triplicate) during steady-state conditions. Forearm blood flow (FBF) was calculated as:

$FBF = MBV \times \pi(\text{brachial artery diameter}/2)^2 \times 60$, where the FBF is in ml min^{-1}, the MBV is in cm s^{-1}, the brachial diameter is in cm, and 60 was used to convert from ml s^{-1} to ml min^{-1}. A fan was directed toward the experimental arm to minimize the potential contribution of skin blood flow to forearm hemodynamics. As an index of vascular tone, forearm vascular conductance (FVC) was calculated as: (FBF/Mean Arterial Pressure)*100 and expressed as (ml min^{-1} $100mmHg^{-1}$) [29]. Forearm VO_2 was calculated as: FBF x (arterial – venous O_2 content). Arterial oxygen content was assumed to be 20 ml dl^{-1} [40, 41]. Importantly, several studies have shown that arterial oxygen content does not change during mild-to-moderate forearm (handgrip) exercise in humans [39, 42].

Heart Rate and Mean Arterial Pressure

Heart rate (HR) was monitored with a 3-lead ECG. Mean arterial pressure (MAP) was measured by placing a finger pressure cuff around the middle phalanx of the middle finger on the

non-experimental arm (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). Resting arterial blood pressure was measured over the brachial artery following 30 minutes of supine rest, and just prior to each exercise trial (Cardiocap 5, Datex Ohmeda, Louisville,CO), and resting Finometer MAP was corrected for differences between the two readings [43].

Handgrip Exercise

Maximum voluntary contraction (MVC) was determined for each subject as the average of at least three maximal squeezes of a handgrip dynamometer (Stoelting, Chicago, IL, USA) that were within 3 percent of each other. Subjects lifted at weight corresponding to their % MVC, 4-5 cm over a pulley using both audio and visual cues to ensure correct timing of contraction (1 second) and relaxation (2 seconds) [44, 45].

Plasma Measures

Following catheterization and prior to exercise, plasma samples were collected for subject characterization and analyzed for cholesterol, ascorbate concentration and oxidized LDL concentration. AA and oxidized LDL samples were collected 2 hours post ingestion of AA.

Experimental Protocols

Figure 10 is a timeline for the specific trials. Each exercise trial consisted of 2 minutes of resting baseline followed by continuous incremental handgrip exercise: 4 minutes each at 5%, 15%, and 25% MVC. Following the first bout of exercise (trial 1), participants consumed 2g of AA (n= 8) (C-1000, NOW Foods, Bloomingdale, IL) and rested quietly for 2 hours before

repeating the exercise bout (trial 2). Two hours was chosen as the optimal time to increase plasma concentrations of ascorbate following oral consumption [46, 47]. Trial 1 served as the control condition to which trial 2 (AA) was compared to. On a separate study day, 4 participants repeated the two exercise trials with a placebo. On each visit, participants were blinded to which pill they were taking.

Statistics

Data are presented as mean ± S.E.M. Within each protocol, differences between trials were determined via two way repeated measures analysis of variance (ANOVA). Post hoc comparisons were made with the Holm-Sidak test. Significance was set at $P < 0.05$.

Results

Subject Characteristics

Subject characteristics for our 8 male participants are presented in Table 11. Half of these subjects repeated the study on a separate day with a placebo.

Resting Hemodynamics

There were no significant differences among resting hemodynamics between the two trials prior to and following AA or placebo consumption (Table 12).

Exercise Hemodynamics

Compared to control condition (trial 1), there were no significant differences in any hemodynamic variable at the lowest workload (5% MVC) following AA consumption. Oral AA

consumption (trial 2) significantly increased FBF at 15% MVC (248±16 vs. 198±13 ml min^{-1} $P<0.05$) and 25% MVC (403±33 vs. 343±24 ml min^{-1} $P<0.05$) (Figure 11A). On average, oral AA increased FBF 20% at both 15% and 25% MVC. Forearm vascular conductance, an index of peripheral vascular tone was also significantly greater (~20%) following oral AA consumption at both 15% and 25% MVC. VO_2 was significantly greater at both 15% MVC (33±3 vs 26±2 ml min^{-1} $P<0.05$) and 25% MVC (50±6 vs. 43±4 ml min^{-1} $P<0.05$) (Figure 11B).

Although FBF, FVC, and VO_2, increased following AA at 15% and 25% exercise, AA had no effect on HR, MAP or brachial artery diameter (Table 12). Within the time control visit, there were no significant differences between trial 1(control) and trial 2 (placebo) at rest or during exercise (Table 12 and Figure 12).

Plasma measures

Following consumption of 2g of AA, plasma ascorbate concentrations at 2 h were significantly greater than baseline (2.8±0.3 vs. 1.5±0.1 mg dl^{-1})($P< .001$) (Figure 13A) whereas there was no change in oxidized LDL (76.6±8.2 vs. 76.5± .4) (Figure 13B).

Venous blood gases

There were no significant differences between trial 1 (control) or trial 2 (AA or placebo) at rest or during exercise (Table 13).

Discussion

The primary findings of the present study are that an acute oral dose of AA in older individuals enhanced peripheral vasodilation and augmented exercising blood flow and VO_2

during moderate intensity handgrip exercise (15% and 25% MVC). There was no effect of AA at rest or during 5% MVC exercise. Additionally, increasing O_2 delivery during handgrip exercise resulted in greater O_2 consumption in older adults. Furthermore, when 4 subjects repeated the study protocol with a placebo pill, there was no significant difference in any variable at rest or during exercise, suggesting that the increase in FBF and VO_2 is likely attributed to AA consumption.

AA and Handgrip Exercise

We have previously demonstrated that the age associated impairment in skeletal muscle blood flow observed during handgrip exercise is ameliorated following intra-arterial infusion of AA [18]. Furthermore, this improvement in FBF following AA is primarily mediated through the NO vasodilatory pathway [29], thus ameliorating the age associated decline in EDD. Although not directly measured in the present study, it is likely that oral AA could facilitate increases in exercising blood flow through a similar mechanism to which it does when delivered intra-arterially; improving endothelium dependent dilation via increased nitric oxide bioavailability.

One mechanism by which AA may have improved nitric oxide bioavailability in the present study, could be via improved redox balance. Despite a significant increase in plasma ascorbate concentrations, there was no change in oxidized LDL, a surrogate marker of oxidative stress. The lack of a decline in oxidized LDL in agreement with other studies [48] and does not preclude the ability of AA to act as an antioxidant. It is plausible that oral AA limited the exercise induced increase in oxidative stress during trial 2 [49], or that any acute vascular antioxidant effects were not reflected in plasma oxidized LDL.

Previous studies examining the influence of oral AA typically report positive vascular/hemodynamic findings within patient populations such as type II diabetics, hypertension, and coronary artery disease; however the findings regarding older healthy individuals generally report no vascular improvements at rest. Indeed the lack of an effect of oral AA consumption in older healthy populations may be associated with the dose of AA provided or the method utilized to gauge improvement. Typically, primary variables of interest are resting blood flow, resting arterial blood pressure, and flow mediated dilation. Among otherwise healthy groups, the lack of additional co-morbidities may limit the detection of a positive vascular effect of an oral supplement during rest. In agreement with this, we failed to observe a change in any variable at rest and during the lowest exercise intensity following AA consumption. It is plausible that the healthy status of our study participants coupled with smaller quantity of AA circulating following oral consumption requires the vascular system to be stressed in order for any anti-oxidant effect of AA to be exerted. The likelihood of this occurrence is bolstered by the findings of Wray and colleagues [50] who failed to observe an improvement in flow mediated dilation following oral consumption of their antioxidant cocktail but did later detect improved brachial artery vasodilation during exercise in a follow up study [51].

AA and Forearm VO_2

In addition to a significant improvement in FBF at 15% and 25% exercise, we also observed a significant increase in forearm VO_2 at 15% and 25%. The increase in O_2 utilization of the tissue following AA suggests that blood flow impairments among older individuals may

be limiting oxygen utilization by the tissue. It is feasible that impaired blood flow within older populations may be a key factor contributing to reduced exercise capacity.

In the present study, we utilized the forearm to examine the acute effects of oral AA on skeletal muscle blood flow. Recently, intra-venous infusion of AA was observed to improve resting leg blood flow in older men [31] and evidence suggests that older individuals present with a greater degree of sympathetic tone in the leg [52, 53] whereby a reduction in basal nitric oxide availability could have a more pronounced effect on limb blood flow at rest and during exercise. Given this information, it is unknown how oral AA may influence resting and exercising leg blood flow among older adults, although it is plausible that AA may have a more profound effect on leg blood flow and O_2 consumption compared to the forearm. Finally, it is unknown how chronic supplementation may influence these findings. Older trained individuals fail to demonstrate any positive vascular effect to an oral antioxidant cocktail [50] or intravenous infusions of AA [32] and whether chronic supplementation with oral AA during a training program can influence any cardiovascular adaptation in unclear.

Experimental Limitation and Perspectives- See Chapter VI

Conclusion

The cumulative findings from both exercise studies (Chapter IV and V) indicate that impairments in peripheral vasodilation led to significant lower forearm blood flow during moderate intensity handgrip exercise. This attenuated vasodilation is not due to elevated α-adrenergic vasoconstrictor tone and is improved following acute ingestion of AA. Exercising blood flow during moderate handgrip exercise was augmented ~20% following AA ingestion.

Further, when O_2 delivery (blood flow) to exercising skeletal muscle was augmented following acute AA ingestion, there was an increase in O_2 utilization (~17%) by active skeletal muscle, suggesting that in healthy older individuals, enhancing O_2 delivery can enhance O_2 utilization.

Table 11. Subject Characteristics. FFM = fat free mass; LDL = low density lipoprotein; HDL = high density lipoprotein.

Variable	
Male	8
Age (years)	65±3
Body mass index (kg m^{-2})	28.6±1.4
Body fat (%)	31.4±3.0
Whole-Body FFM (kg)	59.2±3.0
Forearm volume (ml)	1148.4±53.9
Total cholesterol (mg dl^{-1})	175.8±5.4
LDL cholesterol (mg dl^{-1})	117.5±6.4
HDL cholesterol (mg dl^{-1})	43.2±3.3
Triglycerides (mg dl^{-1})	75.4±10.5

Table 12. Rest and Exercise Cardiovascular Variables. * P<0.05 vs. Respective Control Condition

Variable	Rest	5% MVC	15% MVC	25% MVC
Control (n=8)				
Heart Rate (beats min^{-1})	57±2	63±2	64±2	68±2
Mean Arterial Pressure (mmHg)	93.9±2.8	100.6±3.7	100.9±3.0	107.2±4.8
Forearm Blood Flow (ml min^{-1})	35.8±5.1	96.5±9.0	198.6±12.5	342.5±24.6
Forearm Vascular Conductance (ml min^{-1} mmHg^{-1})	37.7±5.3	96.5±9.8	198.8± 15.8	324.5± 26.9
AA (n=8)				
Heart Rate (beats min^{-1})	57±2	59±2	64±3	68±2
Mean Arterial Pressure (mmHg)	95.3±2.9	99.5±3.9	104.9±4.2	105.8±4.0
Forearm Blood Flow (ml min^{-1})	30.2±3.6	93.1±5.0	248.3±16.0*	402.8±33.1*
Forearm Vascular Conductance (ml min^{-1} mmHg^{-1})	31.2±3.1	93.4±3.0	244.5± 19.0*	386.8± 38.1*
Time Control (n=4)				
Heart Rate (beats min^{-1})	60±2	63±2	65±3	70±5
Mean Arterial Pressure (mmHg)	91.2±3.5	97.5±4.3	99.7±3.8	112.8±7.2
Forearm Blood Flow (ml min^{-1})	33.3±7.3	84.8±7.6	197.9±17.1	309.1±32.2
Forearm Vascular Conductance (ml min^{-1} mmHg^{-1})	36.5±7.4	87.8±8.9	200.5± 20.3	277.4± 26.7
Placebo (n=4)				
Heart Rate (beats min^{-1})	54±3	61±1	62±1	67±6
Mean Arterial Pressure (mmHg)	97.2±1.1	98.4±1.7	102.4±1.3	110.4±3.6
Forearm Blood Flow (ml min^{-1})	28.4±5.0	85.8±5.3	201.2±9.2	297.2±13.6
Forearm Vascular Conductance (ml min^{-1} mmH^{-1}g)	29.4±4.9	87.4±6.0	198.2± 11.2	216.4± 44.1

Table 13. Rest and Exercise Blood Gas Data. * P<0.05 vs. Respective Control Condition. PCO_2 = partial pressure of CO_2, PO_2 = partial pressure of O_2, SO_2% = percent oxyhemoglobin saturation, CtO_2 = venous O_2 content.

	REST					*5% MVC*					*15% MVC*					*25% MVC*				
	pH	**PCO_2**	**PO_2**	**SO_2%**	**CtO_2**	**pH**	**PCO_2**	**PO_2**	**SO_2%**	**CtO_2**	**pH**	**PCO_2**	**PO_2**	**SO_2%**	**CtO_2**	**pH**	**PCO_2**	**PO_2**	**SO_2%**	**CtO_2**
Ascorbic Acid Trial (n=8)																				
Control (n=8)	7.36± 0.01	46.8± 1.8	30.3± 2.0	54.2± 4.7	11.4± 1.0	7.34± 0.01	51.0± 1.5	20.8 ± 1.4	30.9 ± 3.6	6.3± 0.8	7.31± 0.01	53.0± 5.4	22.3± 1.0	33.2± 2.2	6.7± 0.5	7.28± 0.01	59.0± 2.9	24.8± 0.9	36.7± 2.5	7.7± 0.6
Ascorbic Acid (n=8)	7.36± 0.01	46.5± 1.5	28.5± 2.3	49.0± 5.0	10.2± 1.1	7.34± 0.01	52.9± 1.6	21.3 ± 1.1	31.5 ± 2.8	6.5± 0.6	7.31± 0.01	55.7± 0.9	21.9± 1.0	31.7± 2.4	6.6± 0.5	7.30± 0.01	55.2± 1.7	23.9± 1.2	36.0± 3.3	7.6± 0.7
Time Control Trial (n=4)																				
Time Control (n=4)	7.37± 0.00	49.7± 1.6	26.6± 4.5	45.2± 8.5	9.4± 1.9	7.35± 0.00	55.6± 2.1	19.7 ± 2.4	28.8 ± 6.6	5.9± 1.4	7.29± 0.02	62.9± 3.3	21.3± 1.4	31.6± 4.3	6.5± 0.9	7.25± 0.02	68.8± 4.5	25.0± 1.1	35.0± 3.5	7.4± 0.7
Placebo (n=4)	7.36± 0.00	51.5± 1.9	24.7± 3.1	39.9± 7.6	8.3± 1.6	7.34± 0.01	52.5± 1.8	19.9± 1.9	28.3± 3.9	5.8± 0.8	7.29± 0.01	59.7± 2.3	21.9± 1.4	30.9± 3.4	6.4± 0.8	7.27± 0.02	60.6± 3.3	26.6± 0.6	39.8± 1.8	8.4± 0.3

Figure 10. General Experimental Timeline: Following instrumentation and quiet rest, blood samples were collected to detemrine cholesterol, oxidized LDL and plasma ascorbate concentrations. At rest and at the end of each exercise intensity, deep venous samples were collected for blood gas analysis and VO_2 determination. Plasma oxidized LDL and ascorbate samples were collected 2h post ingestion of AA (2g).

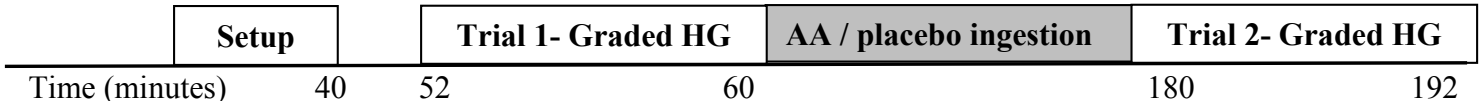


Figure 11. FBF (A) and VO_2 (B) in control conditions and 2 hours following ingestion of AA in older adults. N=8. *P<0.05 vs. Control. Following AA ingestion, older adults exhibited significantly greater FBF at both 15% and 25% MVC. In addition to an elevation in flow within these two exercise intensities, VO_2 of the forearm was also significantly greater.

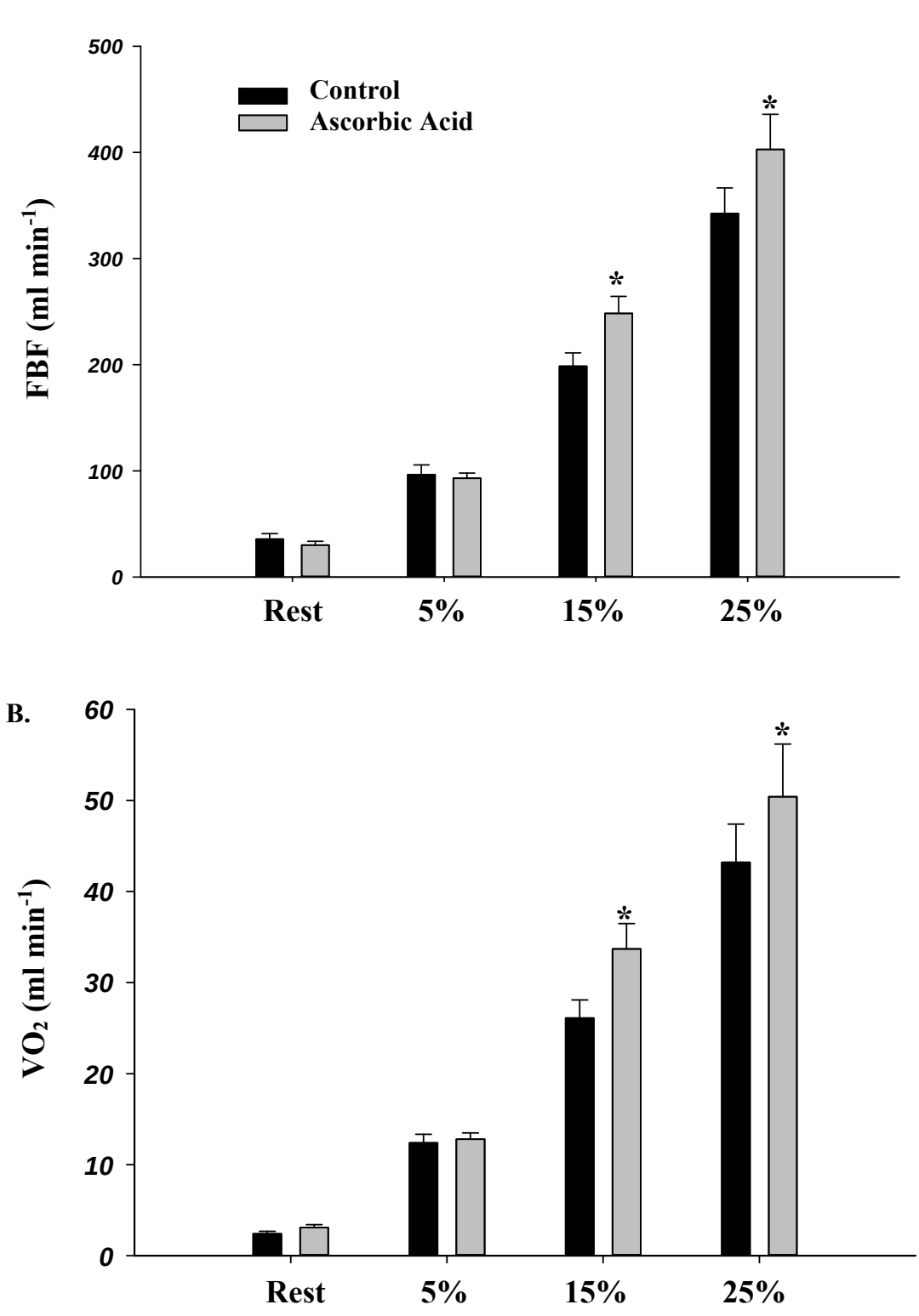
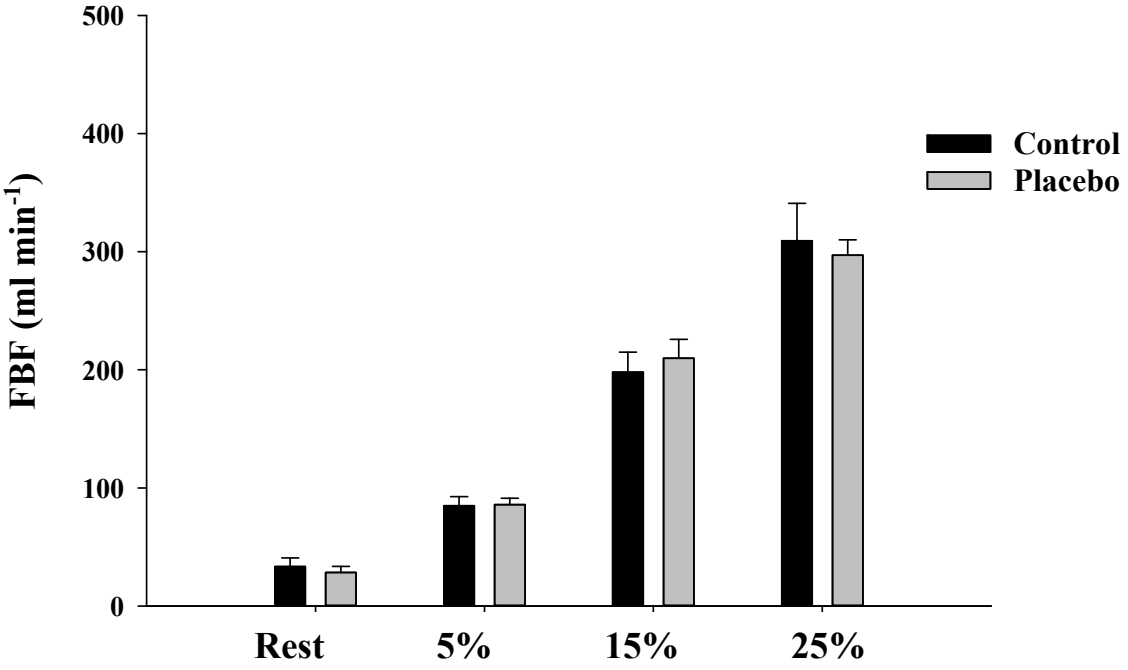


Figure 12. FBF (A) and VO_2 (B) in control conditions and 2 hours post ingestion of a placebo. N=4. There were no significant differences between control and placebo at any exercise intensity.

A.



B.

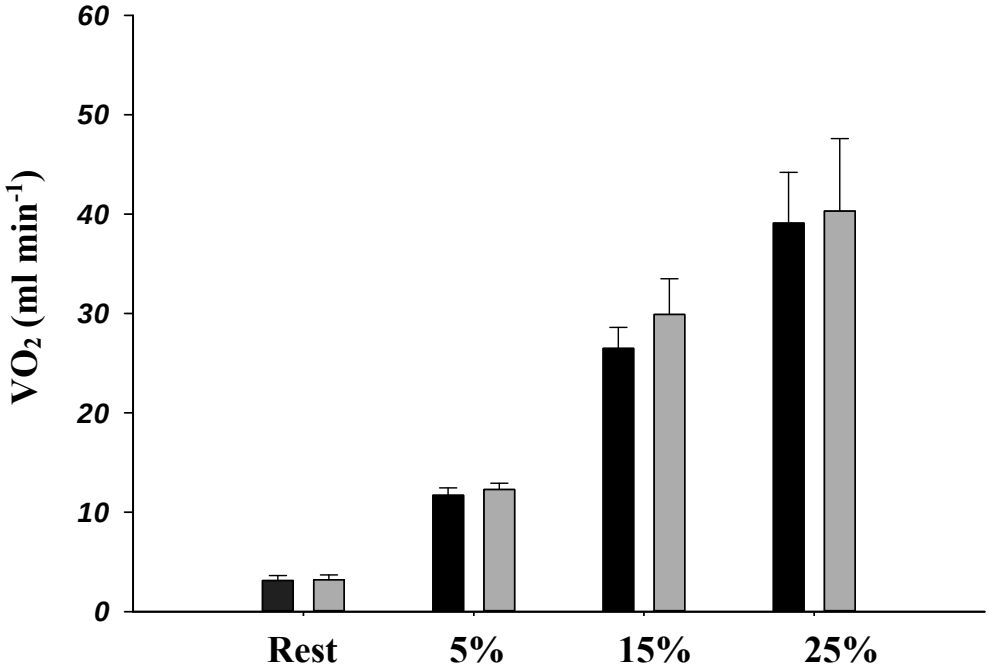
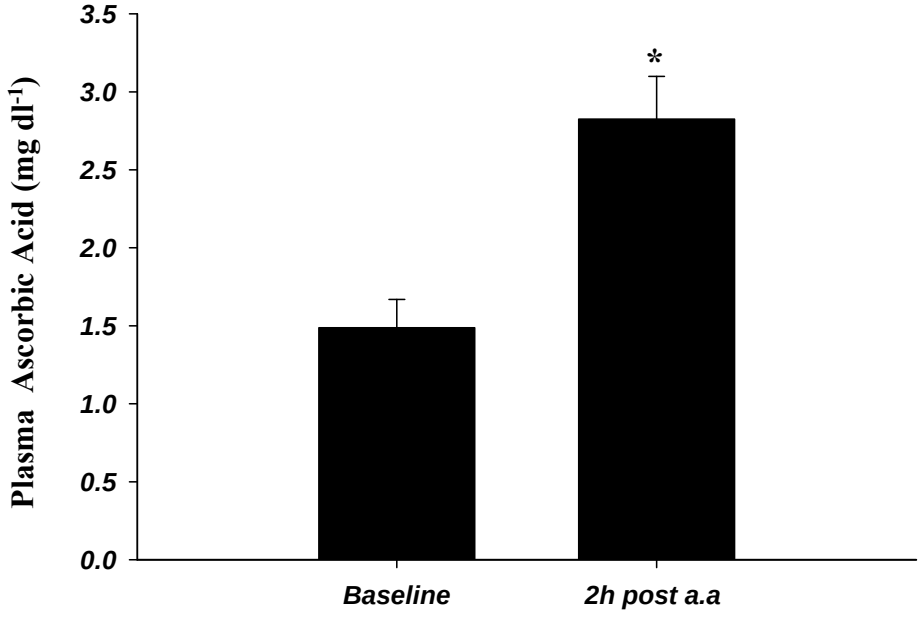
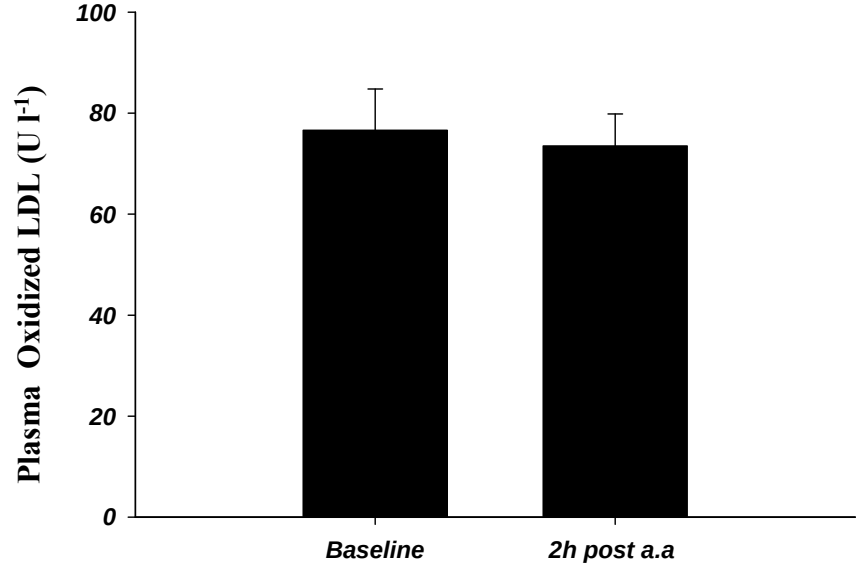


Figure 13. Plasma AA levels (A) and oxidized LDL (B) at baseline and 2 hours post ingestion of AA. Plasma AA significantly elevated from baseline at 2 hours, however, there was no change in oxidized LDL, the surrogate marker of oxidative stress following AA ingestion.
* P<0.05vs baseline.

A.



B.



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CHAPTER VI – LIMITATIONS AND PERSPECTIVES

General Experimental Limitations

Throughout this dissertation, we chose to utilize the forearm model to investigate the role the sympatho-adrenal system plays in the regulation of peripheral vascular tone. The limitation of this application is that our conclusion that age-associated impairments in the regulation of peripheral vascular tone are not due to elevated sympathetic vasoconstrictor tone, may not be applicable to the lower limb. As mentioned previously, despite elevated resting levels of sympathetic nervous system signaling, the forearm of older adults does not exhibit elevated sympathetic restraint of blood flow at rest. The lack of increased sympathetic restraint of forearm blood flow in the face of elevated sympathetic vasoconstrictor signaling is attributed to decreased α-adrenergic receptor responsiveness in the forearm. Conversely, the legs of older adults do exhibit a greater degree of sympathetic restraint of blood flow, which occurs despite decreased α-adrenergic receptor responsiveness in this limb as well. Therefore, the elevated sympathetic restraint of leg blood flow at rest in older adults may translate into greater sympathetic restraint of exercising blood flow in older adults, something we failed to observe in the forearm in the present studies.

Despite the finding that there is not a greater degree of sympathetic restraint of resting blood flow in the forearm of older adults, animal data indicates that increased sympathetic activity actively limits vasodilation in older adults, despite no differences in resting skeletal muscle blood flow or diameter of skeletal muscle arterioles. These animal studies propose that the increased α-adrenergic vasoconstrictor signaling in older animals prevents local vasodilation and importantly, limits the ability of that vasodilation to ascend up the arteriolar tree. Further, the impairment in arteriolar vasodilation was reversed following local application of an α-

adrenergic receptor antagonist. We felt that it was important to determine whether older adult humans had elevated sympathetic restraint of skeletal muscle blood flow during exercise, and the forearm model similarly reflects some of the findings reported on this topic in animal models.

Additionally, we feel that the forearm is an appropriate model to examine the regulation of peripheral vascular tone for a number of reasons. First, performing rhythmic dynamic forearm handgrip exercise recruits a small muscle mass and exercise can be easily performed for an extended period of time. Exercise utilizing small a muscle mass limits the engagement of systemic reflexes that may confound the interpretation of the primary variables of interest. With respect to pharmacology, dosing the drugs to a smaller volume limits the possibility that local arterial infusions spill-over into the systemic circulation and activate systemic reflexes. Young and older adults tend to have similar maximal voluntary contractions of the forearm muscles and therefore despite our implementation of relative workloads, the absolute load lifted is not different between young and older adults. Lastly and most importantly, endothelial function of the brachial artery is correlated with coronary endothelial function in both healthy and diseased populations and coronary endothelial dysfunction is a significant risk factor for cardiovascular disease. Given that age is an independent risk factor for cardiovascular disease, we feel that the forearm is an appropriate model to investigate regulation of vascular tone.

In this dissertation, we tested the efficacy of our sympatho-adrenal blockade in our first hypoxia study (chapter II) but did not test it again during the second hypoxia study (chapter III) or exercise study (chapter IV). We did not feel that it was necessary to test the blockade again because we utilized similar doses of adrenergic antagonists as used in experiment 1 (Chapter II) as well as other studies that demonstrate effective blockade.

Additionally, in this dissertation, ascorbic acid was both infused and ingested as a pharmacological tool to improve local vasodilator signaling. In the present study, we did not directly test the mechanism by which ascorbic acid increased hypoxic vasodilation or exercising blood flow in older adults. However, previous studies indicate that any positive vascular response intra-arterial ascorbic acid had on exercising blood flow in older adults was abolished following inhibition of the enzyme responsible for catalyzing the reaction which produces nitric oxide (eNOS), indicating ascorbic acid somehow increases local nitric oxide signaling. We don't know if acute ingestion of ascorbic acid improves exercising skeletal muscle blood flow through the same mechanism as it does when infused in higher concentrations intra-arterially, however the % increase in exercising blood flow is similar (~20%), despite how it was administered, which may indicate that oral ingestion can similarly increase nitric oxide signaling as that observed following acute intra-arterial infusion. Furthermore, exercising at a higher intensity or recruiting a large muscle mass can increase sympathetic nervous system activity, resulting in greater α-adrenergic vasoconstrictor signaling. It is unclear what the net vascular tone might be under these competing signals and therefore we don't know if the elevation in exercising blood flow in older adults following ascorbic acid would persist in these different conditions.

Perspectives

The key findings of this dissertation are that older adults exhibit significant impairment in the regulation of peripheral vascular tone during physiological stressors. Furthermore, the observed age-associated impairment is not due to altered sympatho-adrenal regulation, because following local sympatho-adrenal blockade, the impairments persisted and remained similarly

blunted when compared to younger subjects. In the absence of sympatho-adrenal influence we were able to restore hypoxic vasodilation in older adults by enhancing local vasodilatory signaling with the infusion of ascorbic acid. Additionally, oral ingestion of ascorbic acid enhanced forearm blood flow in older adults during handgrip exercise with intact sympatho-adrenal signaling. Together, these findings suggest that the age-impairment in both hypoxic vasodilation and skeletal muscle blood flow during handgrip exercise are likely due to attenuated local vasodilatory signaling.

The data in this dissertation confirm the presence of age impairment in the regulation of peripheral vascular tone to two different physiological stressors. Prior to these studies, there was no information on whether older adults had impaired vasodilation to a graded hypoxic stimulus. Further, it was unknown what role the sympatho-adrenal system played in the vascular response to hypoxia in older adults. In addition, whether the sympatho-adrenal system restrained blood flow during handgrip exercise in young or older adults was also unknown. These studies provide novel insight into the regulation of peripheral vascular tone during healthy aging. Further, these studies have mechanistically isolated the contributions of the sympatho-adrenal system and local vasodilatory signaling to the net regulation of peripheral vascular tone in both young and older adults.

With aging, there is a distinct impairment in the ability to regulate peripheral vascular tone and this is primarily attributed to impairments in local vasodilatory signaling, which leads to lower blood flow and attenuated O_2 delivery. Lower O_2 delivery may limit the ability of skeletal muscle to perform work, potentially leading to the significant reduction in aerobic capacity with age and subsequent loss of quality of life and functional independence. Many diseases are also characterized by a reduction in peripheral O_2 delivery, including peripheral artery disease,

congestive heart failure and obstructive sleep apnea. Furthermore, the observed vascular changes that occur with aging such as impaired endothelial function and elevated resting sympathetic nervous system are significantly exacerbated in disease states including heart failure, hypertension and diabetes. Given that small adjustments in peripheral vascular tone have a profound effect on O_2 delivery, understanding all the contributing factors to this regulation can have significant clinical implications.

APPENDIX A – HUMAN SUBJECTS APPROVAL

Colorado State University
Knowledge to Go Places

Research Integrity & Compliance Review Office
Office of the Vice President for Research
321 General Services Building - Campus Delivery 2011 Fort Collins, CO
TEL: (970) 491-1553
FAX: (970) 491-2293

NOTICE OF APPROVAL FOR HUMAN RESEARCH

DATE:	December 04, 2013
TO:	Dinenno, Frank, Health & Exercise Science Israel, Richard, Health & Exercise Science, Richards, Jennifer, 1582 Dept Hlth & Exer Sci, Scott, Hannah, 1570 Human Dev & Fam Stds
FROM:	Barker, Janell, Coordinator, CSU IRB 1
PROTOCOL TITLE:	Aging and Sympathetic Vasoconstriction: Rest vs. Exercise
FUNDING SOURCE:	Funding - Grants/Contracts , Other Funding
PROTOCOL NUMBER:	09-1186H
APPROVAL PERIOD:	Approval Date: November 18, 2013 Expiration Date: November 17, 2014

The CSU Institutional Review Board (IRB) for the protection of human subjects has reviewed the protocol entitled: Aging and Sympathetic Vasoconstriction: Rest vs. Exercise. The project has been approved for the procedures and subjects described in the protocol. This protocol must be reviewed for renewal on a yearly basis for as long as the research remains active. Should the protocol not be renewed before expiration, all activities must cease until the protocol has been re-reviewed.

If approval did not accompany a proposal when it was submitted to a sponsor, it is the PI's responsibility to provide the sponsor with the approval notice.

This approval is issued under Colorado State University's Federal Wide Assurance 00000647 with the Office for Human Research Protections (OHRP). If you have any questions regarding your obligations under CSU's Assurance, please do not hesitate to contact us.

Please direct any questions about the IRB's actions on this project to:

Janell Barker, Senior IRB Coordinator - (970) 491-1655 Janell.Barker@Colostate.edu
Evelyn Swiss, IRB Coordinator - (970) 491-1381 Evelyn.Swiss@Colostate.edu

Barker, Janell

Barker, Janell

Approval is to continue to recruit the remaining 201 participants with the approved recruitment and consent material. This also reflects an approved minor amendment to increase the total approved to recruit from 940 to 1,040. With a total of 839 already recruited, the balance remaining to recruit is 201. The above-referenced project was approved by the Institutional Review Board with the condition that the approved consent form is signed by the subjects and each subject is given a copy of the form. NO changes may be made to this document without first obtaining the approval of the IRB.

Approval Period:	November 18, 2013 through November 17, 2014
Review Type:	FULLBOARD
IRB Number:	00000202
Funding:	National Institute on Aging, National Institute of Health , Monfort Professor
	DEXA Scan, ECGs, Ultrasound, 2-MHz pulsed flat transcranial probe, 7.5 MHz linear transducer, 7.5 MHz linear echo probe, automated oscillometric technique, anesthesia monitor, Teflon catheters, biopsy needle, peroxidase diaminobenzidine reaction kit, coated tubes

APPENDIX B – CONSENT FORM

Consent to Participate in a Research Study
Colorado State University

TITLE OF STUDY: Regional Blood Flow Control and Vascular Function: Effects of Aging and Regular Physical Activity

PRINCIPAL INVESTIGATOR: *Frank A. Dinenno, Ph.D. 491-3203*

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH? *You are a man or woman between the ages of 18-35 or 55-90 years. You are either 1) not exercising vigorously and regularly, or 2) have exercised vigorously and regularly for a number of years. Our research is looking at the effect of aging and exercise on regional blood flow control and how your blood vessels work.*

WHO IS DOING THE STUDY? *This research is being performed by Frank Dinenno, Ph.D of the Health and Exercise Science Department, and also by Dennis Larson, M.D. and Gary Luckasen, M.D., of the Heart Center of the Rockies. Trained graduate students, undergraduate students, research assistants, or research associates are assisting with the research. These studies are paid for by the National Institute on Aging, a part of the US Government.*

WHAT IS THE PURPOSE OF THIS STUDY? *The way in which blood flow (and oxygen delivery) and blood vessels are regulated by local factors and nerves during exercise and during changes in the composition of air you breathe is being studied. Importantly, cardiovascular regulation under these conditions might change in older people, it might be different between men and women, and it might be affected by regular physical exercise. The purpose of the research is to understand differences in how blood vessels work in various groups of adults, in different muscle groups (forearm, thigh, calf), as well as in the neck. The makeup of muscle fibers is also being studied.*

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST? *This whole research project will take place over a period of approximately five years. However, your part of this study will be either:*

__X___ 1) one or two visits over a several day period, or _____ ***(your initials)***

_____ 2) several visits over a few to several weeks. _____ ***(your initials)***

WHAT WILL I BE ASKED TO DO? *This consent form applies to a large research project. You are only being asked to participate in one part of the total project. Depending on the part of the research project that you are involved in, you will be asked to participate in some of the following procedures. Many potential procedures are described in the section below. However, the procedures that you will be asked to do for this part of the study have a check mark next to them. The check marks were put there by one of the researchers. The time associated with each procedure reflects the amount of time you will spend performing or undergoing the procedure, not the total time of the study. A member of the research team will fully explain each checked procedure that applies to your participation and specifically how long each session (total time) in the laboratory will be.*

_**X**____ ***Health and Physical Activity Questionnaire***. *You will be asked to answer some questions about your health and exercise habits to determine if you can participate in the study. (~20 minutes)*

_____ ***(your initials)***

_____ ***Pregnancy Test.*** *If you are female you will be required to have a sample of your urine tested for the presence of human chronic gonadotropin (HCG), a hormone which indicates whether you may be pregnant. This will require approximately 1 cup of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study. (~10 minutes)*

_____ ***(your initials)***

__X___ ***Heart Rate and Blood Pressure.*** *Heart rate will be measured by placing three sticky electrodes on your chest and reading the electrocardiogram (ECG) signal. Blood pressure will be measured with an automated machine that requires the placement of a cuff around your upper arm (bicep), or a small cuff on your finger. (continuous monitoring throughout study)*

_____ **(*your initials)***

___x__ ***Graded Exercise Test.*** *If you are in the 55-90 yr-old age group, you will be asked to perform a maximal exercise test on a treadmill under the supervision of a physician. This test will occur in the Human Performance Clinical/Research Laboratory in the Department of Health and Exercise Science on the CSU campus. Sticky electrodes will be placed on your chest, and you will walk briskly or jog while the steepness of the treadmill is increased. Your blood pressure and heart beat will be closely measured during and immediately after the test. (~1 hour)*

_____ ***(your initials)***

_____ ***Maximal Oxygen Consumption.*** *VO_{2max} testing will be performed on a treadmill while you are walking or running and the steepness of the treadmill is increased until you can't exercise any more. You will be asked to put your mouth around a scuba-like mouthpiece and wear a nose clip to prevent breathing through your nose. The amount of oxygen your body uses for energy will be determined from the oxygen and carbon dioxide you breathe in and out during the exercise. Your heart rate will be measured using a heart rate monitor. Body mass and height will be measured on a medical beam scale. (~30 - 45 min)*

_____ ***(your initials)***

__X__ ***Body Composition.*** *The fat, muscle, and bone in your body will be measured using an x-ray device (dual-energy x-ray absorptiometer) that will scan you from head to toe while you lie quietly on a special table for approximately 20 minutes. The amount of x-ray radiation you will receive is extremely low. (~20 minutes)*

_____ ***(your initials)***

_____ ***Forearm Volume.*** *The volume of your forearm will be measured via water displacement. You will place your forearm in a large water-filled cylinder, and the spillover of this water is collected in a large graduated cylinder to determine the volume. (~5 minutes)*

_____ ***(your initials***

__X___ ***Forearm Exercise.*** *You will lay flat on a bed and squeeze your hand and forearm muscles using a handgrip device while your hand and arm are comfortably secured. The intensity of the exercise will range from very easy to moderately difficult, and you will be asked*

to perform this exercise for ~10 minutes several different times throughout the study with plenty of rest in between exercise trials. (1 – 2 hours)

_____ ***(your initials)***

_____ ***Calf Exercise.*** *You will sit in a special chair and squeeze your calf muscles (similar to standing on your toes) while your arms, hips, and shoulders are comfortably secured. The intensity of the exercise will range from very easy to moderately difficult, and you will be asked to perform this exercise for ~10 minutes several different times throughout the study with plenty of rest in between exercise trials.*
(1 – 2 hours)

_____ ***(your initials)***

_____ ***Knee Extensor Exercise.*** *You will sit in a special chair and squeeze your thigh muscles while your hips and shoulders are comfortably secured. Your feet will be secured in specially designed boots and you will be asked to extend your leg against resistance until your ankle is about at the height of your knee, relax back to a regular seated position, and then repeat. The intensity of the exercise will range from very easy to moderately difficult, and you will be asked to perform this exercise for ~10 minutes several different times throughout the study with plenty of rest in between exercise trials. (1 – 2 hours)*

_____ ***(your initials)***

__X___ ***Maximum Voluntary Contraction.*** *This will consist of 3-4 trials where you will squeeze your muscles (either forearm, calf, or thigh) and generate as much force as you can. You will be asked to generate as much force over the course of ~3 seconds and hold this force another 5 seconds. After a 2-3 minute rest period, you will be asked to do this again. This is typically used to determine how heavy of exercise you perform so everybody is exercising at similar percentages of their maximum. (~ 20 minutes)*

_____ ***(your initials)***

_____ ***Exercise Training.***

Forearm: You will be instructed to exercise five times per week, for a total of four weeks. You will be given a special exercise device and will be instructed to exercise with your non-dominant forearm squeezing your muscles 12 times per minute at 30-35% of your maximum until you can't exercise any more. When you are able to exercise at this initial workload for 30 minutes, the workload will be increased. You will need to visit the laboratory once per week to adjust the training workload as your performance improves. _____ ***(your initials)***

Calf: You will be instructed to exercise five times per week, for a total of four weeks. You will be instructed to exercise with your calf muscles and squeeze this muscle 12 times per minute at 30-35% of your maximum until you can't exercise any more. You will be instructed to perform calf extension exercise in the upright position with added weight (if necessary) to achieve the pre-determined workload. When you are able to exercise at this initial workload for 30 minutes, the workload will be increased. You will need to visit the laboratory once per week to adjust the training workload as their performance improves. _____ ***(your initials)***

Knee extensor: You will be instructed to exercise 3 times per week, for a total of eight weeks. You will be required to perform the training studies in the laboratory under supervision.

Each training session will be 60 minutes. The first two weeks will consist of short (5-10 min) high intensity exercise bouts, whereas the second two weeks will consist of long (15-45 min) low intensity exercise bout. This pattern of training will be repeated to attain a total training period of eight weeks. As your exercise performance improves, the training workload will be adjusted accordingly. _____ ***(your initials)***

<u>Whole-body</u>: You will be instructed to exercise 5 times per week, 40-50 minutes per exercise session at 60-85% of your maximum heart rate, for a total of twelve weeks. You will be asked to cycle, walk, jog, or run during this training period. You will be taught how to use heart rate monitors (provided by the lab) in order to train at the proper intensity as well as to record your exercise sessions. _____ ***(your initials)***

____ ***Ischemic Exercise.*** *You will exercise your calf or forearm with a blood pressure cuff on your thigh or upper arm that is inflated very tightly to temporarily block the blood flowing to your muscle. You will be asked to perform this exercise for ~10 minutes several different times throughout the study with plenty of rest in between exercise trials. (20 – 30 minutes)*
_____ ***(your initials)***

____ ***Cold Pressor Test.*** *You will place your hand or foot in ice water for 2-3 minutes on several occasions. (~10 minutes)*
_____ ***(your initials)***

__x__ ***Lower Body Negative Pressure.*** *You will be placed in a sealed wooden chamber while you are lying flat on a bed. The chamber is sealed at your waist. Using a standard vacuum that is attached to the chamber, suction will be applied to mimic what happens when you go from laying to standing up. This will occur several times throughout the study for about 15 minutes at a time. (~ 1 hour)* _____ ***(your initials)***

____ ***Up-right or head-down tilting.*** *You will be laying on a bed that is specially designed to be tilted ~60 degrees upright, or tilted downward ~10 degrees. This mimics what happens when you go from laying to standing up, and vice versa. (~ 1 hour)*
_____ ***(your initials)***

____ ***Forearm Negative/Positive Pressure.*** *You will place your forearm in a sealed chamber up to your elbow. Application of suction (like a vacuum) increases blood flow to your arm, whereas the opposite pressure reduces blood flow to your arm. (1-2 hours)*
_____ ***(your initials)***

____ ***Brachial Artery Compression.*** *A special device that is mounted to a frame above your forearm will be placed over your brachial artery at the elbow. When this device presses down on your arm, it will temporarily reduce the amount of blood to your forearm. This will be performed for approximately 5 minutes at a time, and will occur several times throughout the study. (1-2 hours)* _____ ***(your initials)***

__x__ ***Breathing a low Oxygen or high Carbon Dioxide Gas Mixture.*** *The purpose of this test is to mimic what happens when you go up to altitude. You will be asked to place your mouth around a scuba mouthpiece while wearing a nose clip to prevent breathing through your nose. The amount of oxygen or carbon dioxide you are breathing will be changed carefully with a specially designed system, and you will breathe this for a maximum of 20 minutes at a time.*

You will be asked to do this several times throughout the study, with plenty of time in-between each trial. The amount of oxygen that is in your blood will be measured with a light sensor on your fingertip or earlobe. (1-1.5 hours)

_____ ***(your initials)***

____ ***Venous Occlusion Plethysmography.*** *The blood flow in your forearm or calf will be measured by the use of blood pressure cuffs around your upper arm or thigh, and around your wrist or ankle. These cuffs will be inflated and deflated periodically. A sensitive gauge (similar to a rubber band) will also be placed around the maximum circumference of your forearm or calf. (2-3 hours)*

_____ ***(your initials)***

__X__ ***Doppler Ultrasound.*** *The blood flow in your arm, leg, neck, or brain will be measured using an ultrasound machine which produces sound waves to measure your blood vessel size and the speed of your blood. This also provides information about how elastic or stiff your blood vessels are. (2-3 hours)*

_____ ***(your initials)***

____ ***Reactive Hyperemia.*** *A blood pressure cuff will be placed on your upper arm or thigh and inflated really tight to temporarily block the blood to your forearm or calf. After 5, 10, or 15 minutes, the cuff will be released and the blood flow in your forearm or calf will be measured. This test is a measure of how much your blood vessels can relax and will be repeated several times throughout the study. (1- 1.5 hours)*

_____ ***(your initials)***

____ ***Flow-Mediated Vasodilation.*** *A blood pressure cuff will be placed on your forearm or your calf and inflated really tight to temporarily block the blood to your hand or foot. After 5, 10, or 15 minutes, the cuff will be released and the diameter changes of the blood vessels in your arm or leg will be measured using Doppler ultrasound. In some cases, your hand or foot will be warmed up for 15 minutes and the changes in blood vessel diameter will be measured. This will be repeated several times throughout the study. (1-1.5 hours)*

_____ ***(your initials)***

____ ***Sympathetic Nervous System Activity.*** *The measurement of sympathetic nervous system activity involves measuring the activity of one of your nerves on the side of your knee. Two small microelectrodes (small needles) will be placed through your skin. The position of one of the electrodes will be moved back and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the electrode. This search procedure will continue until the electrode being moved causes your foot to twitch. This procedure will take between 5-60 minutes. When a foot or hand twitch is observed, measurement of the activity of the sympathetic nervous system will begin. (2-3 hours)* _____ ***(your initials)***

_X___ ***Blood Sample.*** *Up to 100 ml (approximately 7 tablespoons) of your blood will be drawn from a vein on the front of your elbow or artery in a standard fashion using a sterilized hypodermic needle. (~15 minutes)*

_____ ***(your initials)***

***** For Arterial Catheterization, Venous Catheterization, or Muscle Biopsy:*** *<u>If you are allergic to lidocaine or novacaine, or have had a negative reaction to medicines injected while at the dentist, you should notify us immediately and not have any of these procedures done.</u>*

__***X***__ ***Venous Catheterization.*** *Your skin will be cleaned and a catheter (plastic needle) will then be inserted on the front side of your elbow and secured to the skin. In some cases, a local anesthetic might be used to reduce any discomfort. (~2-4 hours)*

_____ ***(your initials)***

__X__ ***Brachial Artery Catheterization.*** *Your skin will be cleaned and a local anesthetic will be given with a small needle to numb the area where the catheter will be placed (front side of your elbow). The catheter (plastic needle) will then be inserted and secured to the skin. (~2-4 hours)*

_____ ***(your initials)***

____ ***Femoral Artery Catheterization.*** *Your skin will be cleaned and a local anesthetic will be given with a small needle to numb the area where the catheter will be placed (about half way between your hip bone and groin on the front side of your leg). The catheter (plastic needle) will then be inserted and secured to the skin. (~2-4 hours)*

_____ ***(your initials)***

_***X***___ ***Drug Administration (~ 2 - 4 hours).*** *The administration of one of more of the following drugs might occur several times throughout the study.*

Vasoconstrictors – cause temporary narrowing of the blood vessels (minutes)
___ *Tyramine*
__X_ *Norepinephrine*
_X__ *Phenylephrine*
___ *Clonidine*
___ *Dexmedetomidine*
___ *L-NAME*
___ *L-NMMA*
___ *Aspirin*
___ *Ketorolac*
___ *Barium Chloride*
___ *Oubain*

Vasodilators – temporarily relax the blood vessels (minutes)
_X__ *Acetylcholine*
___ *Adenosine*
_X__ *Sodium Nitroprusside*
___ *L-Arginine*
_X__ *Phentolamine*
___ *Adenosine Triphosphate (ATP)*
___ *Potassium Chloride (K^+)*
_X__ *Isoproterenol*

No major effects
___ *Ascorbic Acid (Vitamin C)*
_X__ *Propranolol*
___ *Aminophylline*
___ *Pyridoxine* _____ ***(your initials)***

Oral Supplement Administration
__***X***__ ***Ascorbic Acid (Vitamin C)***

_____ ***(your initials)***

____ ***Muscle Biopsy.*** *A sample of muscle will be taken from a muscle on the outside of your thigh. This will take place under the supervision of a medical doctor in the Hartshorn Health Center on the CSU campus. Your skin will be temporarily numb using lidocaine, a medicine similar to novacaine. After deadening the skin, a ¼ inch incision, or cut, is made in the skin over the muscle using a sterilized scalpel. The sample is obtained using a sterilized sampling needle. The muscle sample obtained is usually about ½ the size of the eraser on the end of a pencil. You will not have to reduce your activity afterwards, but should not perform any unusual or extremely vigorous activity for a few days. You will receive written instructions regarding care of the incision, and a telephone number to contact if you have any questions. (30 - 45 minutes)*

_____ ***(your initials)***

FUTURE USE OF BLOOD OR MUSCLE SAMPLES

It is possible that we may want to use any extra blood or muscle tissue for future research not described in this consent form. For example, this may include determination of certain gene expressions that relate to various measures of cardiovascular function measured as part of this study. This information will remain private as will all of the data collected from the study. Only choose one of the following:

_____ *I give permission for the use of my blood or muscle tissue collected as part of the current study only.* _____ ***(your initials)***

_____ *I give permission for the use of my blood or muscle tissue for the current study as well as for future studies.* _____ ***(your initials)***

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY?

If you are not 18-35 or 55-90 years of age, are pregnant, are a regular smoker, or have any diseases that would affect our measurements or significantly increase the risks associated with this study, we will not be able to include you in the research.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

(The procedures that apply to your proposed participation are checked)

- _X____ *Health and Physical Activity Questionnaire – there are no known risks associated with answering health questions. All information is kept strictly confidential.*

  _____ ***(your initials)***

- __X___ *Graded Exercise Test – there is a risk of fatigue (temporary muscle tiredness), muscle strain, heart beat abnormalities (arrhythmias), a 0.01% chance of death (in people who have heart problems), a 0.02% risk of cardiac arrhythmias that would require you to go to a hospital (in people who have heart problems), and a risk of an increase or decrease in blood pressure.*

  _____ ***(your initials)***

- _____ *Maximal Oxygen Consumption – There is the possibility of fatigue, muscle strains, heart rhythm abnormality, and change in blood pressure. There is the possibility of falling off of the treadmill. Incidence of myocardial infarction (MI) is also a risk. 1 in 10,000 individuals*

with cardiovascular disease may die and 4 in 10,000 may have abnormal heart rhythms or chest pain.

_____ ***(your initials)***

- *_X____ Body composition (DEXA) scan – the risks associated with the DEXA are very low. The radiation you will receive is less than 1/3000th of the Food and Drug Administration (FDA) limit for annual exposure. The FDA is a government organization responsible for medical safety. In other words, you could receive 3000 DEXA scans in a single year and still not meet the FDA limit for radiation exposure. In this study you will receive one scan. The more radiation you receive over the course of your life, the greater the risk of having cancerous tumors or of inducing changes in genes. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is not known. Women who are pregnant or could be pregnant should receive no unnecessary radiation and should not participate in this study.* _____ ***(your initials)***

- *_X____ Muscle contractions (Exercise) – There is a slight risk of muscle strain and muscle soreness resulting from brief strong muscle contractions. Soreness should not last more than two days or affect your normal function.* _____ ***(your initials)***

- *_____ Exercise training – There is a slight risk of muscle strain and muscle soreness resulting from brief strong muscle contractions. Soreness should not last more than two days or affect your normal function and should get progressively less as training continues.* _____ ***(your initials)***

- *_____ Ischemic Exercise – There is a risk of temporary discomfort and possible cramping in the forearm or calf during and after the exercise. These symptoms will be relieved when the exercise stops.* _____ ***(your initials)***

- *_____ Cold Pressor Test – There is a risk of temporary discomfort of the hand or foot. In rare cases, subjects might feel light-headed or nauseous. These symptoms will be relieved when the hand or foot is removed from the ice water and wrapped in a blanket.* _____ ***(your initials)***

- *___X__ Lower Body Negative Pressure - There is a small risk of feeling nauseous or fainting. These symptoms will be relieved when the vacuum is turned off.* _____ ***(your initials)*** _____ ***(your initials)***

  _____ Up-right or head-down tilting – Small risk of feeling nausea or fainting during up-right tilt. These symptoms will be relieved when the table is tilted back and the subject is lying supine. There are no known risks for head-down tilt. _____ ***(your initials)***

- *_____ Forearm Positive/Negative Pressure – There is a small risk of slight discomfort or cramping if performing forearm exercise at the same time.* _____ ***(your initials)***

- *_____ Brachial Artery Compression – There is a risk of slight discomfort at the site of compression (elbow). There is also a risk of slight discomfort or cramping if performing forearm exercise at the same time.* _____ ***(your initials)***

- __X___ *Breathing a low oxygen or high carbon dioxide content gas mixture- The risks associated with this include light-headedness, headache and fainting. However, we will be monitoring all of your vital signals and will stop the procedure if this occurs. Symptoms will end momentarily after breathing normal room air.* _____ ***(your initials)***

- _____ *Venous Occlusion Plethysmography- There is a risk of temporary discomfort of the hand or foot when the blood pressure cuffs are inflated.*
  _____ ***(your initials)***

- _____ *Reactive Hyperemia/Flow-Mediated Vasodilation- There is a risk of temporary discomfort of the upper arm or thigh when the blood pressure cuffs are inflated. The discomfort might be greater the longer the cuffs are inflated.*
  _____ ***(your initials)***

- _____ *Sympathetic Nervous System Activity – Some subjects experience a temporary (seconds) pain and discomfort while the microelectrodes are being inserted. After the procedure there is a small risk of numbness, pins and needles type sensations, or pain which lasts 1-3 days. In very rare cases, numbness, pins and needles type sensations, or pain in the leg or arm has lasted several weeks or months (1-3 in 1000). These problems can be minimized by only having experienced individuals perform this technique. In addition, by minimizing the time to find the nerve to less than 60 minutes, the risk of unpleasant after-effects is reduced even more.*
  _____ ***(your initials)***

- ___X__ *Blood sample – The risks associated with blood drawing include bruising, slight risk of infection, soreness, and fainting. These are minor risks which usually do not last more than one day if they occur.* _____ ***(your initials)***

- _X____ *Venous Catheterization- The risk of allergic reaction to lidocaine is extremely low. There is a risk of bruising, slight risk of infection, local soreness, and fainting.*
  _____ ***(your initials)***

- __X___ *Arterial Catheterization – The risk of allergic reaction to lidocaine is extremely low. There is a risk that pain or discomfort may be experienced when the catheter is inserted in the artery, and local soreness after the study. In about 1 in 10 cases a small amount of bleeding under the skin will cause a bruise. There is about a 1 in 1,000 risk of infection or significant blood loss. In about 1 in 4,000 damage may occur to the artery requiring surgery.*
  _____ ***(your initials)***

- __X___ *Drug/Supplement Administration - The risks associated with drug administration include temporary increases or decreases in blood pressure and heart rate. In the case of clonidine and dexmedetomidine, you might experience mild drowsiness. These symptoms should resolve when the drug stops. With any of the vasoconstrictor drugs, there is a slight risk that ischemia (lack of blood to the tissues) could occur. Risks of these effects are minimized by calculating the amount of drug given relative to the size of your forearm or leg, and not the entire body. Finally, there is a potential risk of an allergic reaction to vasoactive drug administration. If you are allergic to aspirin, you should not participate.*
  _____ ***(your initials)***

- _____ *Muscle Biopsy – The risks associated with the muscle sample procedure include discomfort, soreness in that muscle, bruising, infection, and minor scarring. The discomfort and localized soreness are likely, but generally last only 24-48 hours. Temporary scarring is also expected. How wounds heal over time is different between people. The scar will only be about ¼ inch long, and is usually difficult to distinguish 8-12 months after the procedure. The risk of bruising is low, and infections are extremely rare.*

  _____ ***(your initials)***

- It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

WILL I BENEFIT FROM TAKING PART IN THIS STUDY? *There are no direct benefits to you for participating in this study beyond receiving information on your body composition and cardiovascular risk factors.*

DO I HAVE TO TAKE PART IN THE STUDY? *Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.*

WHAT WILL IT COST ME TO PARTICIPATE? *There is no cost to you for participating except that associated with your transportation to our facilities.*

WHO WILL SEE THE INFORMATION THAT I GIVE? *We will keep private all research records that identify you, to the extent allowed by law. Your information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private.*

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key. You should know, however, that there are some circumstances in which we may have to show your information to other people. For example, the law may require us to show your information to a court or to the Human Research Committee at CSU.

CAN MY TAKING PART IN THE STUDY END EARLY? *Your participation in the study could end in the rare event of muscle strain, if you become pregnant, or if you miss an excessive number of appointments.*

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY? *For experiments that involve the blood sample, muscle sample, fine wire electrodes, and arterial or venous catheterization, you will be paid $15/hour.*
Your identity/record of receiving compensation (NOT your data) may be made available to CSU officials for financial audits.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH? *Please be aware that for this study the University has made special arrangements to provide initial medical coverage for any injuries that are **directly related** to your participation in this research project.*

The research project will provide for the coverage of reasonable expenses for emergency medical care related to the treatment of research-related injuries, if necessary.

LIABILITY:
Because Colorado State University is a publicly-funded, state institution, it may have only limited legal responsibility for injuries incurred as a result of participation in this study under a Colorado law known as the Colorado Governmental Immunity Act (Colorado Revised Statutes, Section 24-10-101, et seq.). In addition, under Colorado law, you must file any claims against the University within 180 days after the date of the injury.

In light of these laws, you are encouraged to evaluate your own health and disability insurance to determine whether you are covered for any physical injuries or emotional distresses you might sustain by participating in this research, since it may be necessary for you to rely on your individual coverage for any such injuries. Some health care coverage's will not cover research-related expenses. If you sustain injuries, which you believe were caused by Colorado State University or its employees, we advise you to consult an attorney.

WHAT IF I HAVE QUESTIONS? *Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the principal investigator, Frank Dinenno, Ph.D., at (970)491-3203, or via email at fdinenno@cahs.colostate.edu. If you would like to ask a medical doctor about your participation in the study, you may contact one of the physicians listed below at the corresponding phone number. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, Human Research Administrator, at 970-491-1655. We will give you a copy of this consent form to take with you.*

This consent form was approved by the CSU Institutional Review Board for the protection of human subjects in research on November 18, 2010.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 9 pages.

_____ _____
Signature of person agreeing to take part in the study Date

_____
Printed name of person agreeing to take part in the study

_____ _____
Name of person providing information to participant Date

_____
Signature of Research Staff

**** List of Contact Numbers in Case of Medical Emergency**

Gary Luckasen, M.D.	Work: 970-221-1000 (24 hours a day)
Dennis Larson, M.D.	Work: 970-221-1000 (24 hours a day)
Poudre Valley Hospital Emergency	970-297-6250
Frank A. Dinenno, Ph.D.	Work: 970-491-3203
	Home: 970-266-1719