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Impaired peripheral vasodilation during graded systemic hypoxia in healthy older adults: role of the sympathoadrenal system.

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Abstract:

Systemic hypoxia is a physiological and pathophysiological stress that activates the sympathoadrenal system and, in young adults, leads to peripheral vasodilation. We tested the hypothesis that peripheral vasodilation to graded systemic hypoxia is impaired in older healthy adults and that this age-associated impairment is due to attenuated β-adrenergic mediated vasodilation and elevated α-adrenergic vasoconstriction. Forearm blood flow was measured (Doppler ultrasound) and vascular conductance (FVC) was calculated in 12 young (24±1 yrs) and 10 older (63±2 yrs) adults to determine the local dilatory responses to graded hypoxia (90, 85, and 80% O_2 saturations) in control conditions, following local intra-arterial blockade of β-receptors (propranolol), and combined blockade of α+β receptors (phentolamine + propranolol). Under control conditions, older adults exhibited impaired vasodilation to hypoxia compared with young at all levels of hypoxia (peak ΔFVC at 80% SpO_2 = 4±6 vs. 35±8%; $P<0.01$). During β-blockade, older adults actively constricted at 85 and 80% SpO_2 (peak ΔFVC at 80% SpO_2= -13±6%; $P<0.05$ vs. control) whereas the response in the young was not significantly impacted (peak ΔFVC = 28±8%). Combined α+β blockade increased the dilatory response to hypoxia in young adults, however older adults failed to significantly vasodilate (peak ΔFVC at 80% SpO_2= 12±11% vs. 58±11%; $P<0.05$). Our findings indicate that peripheral vasodilation to graded systemic hypoxia is significantly impaired in older adults which cannot be fully explained by altered sympathoadrenal control of vascular tone. Thus, the impairment in hypoxic vasodilation is likely due to attenuated local vasodilatory and/or augmented vasoconstrictor signaling with age.

New and Noteworthy:

We found that the lack of peripheral vasodilation during graded systemic hypoxia with aging is not mediated by the sympathoadrenal system, strongly implicating local vascular control mechanisms in this impairment. Understanding these mechanisms may lead to therapeutic advances for improving tissue blood flow and oxygen delivery in aging and disease.

Introduction

In humans and experimental animals, acute systemic hypoxia evokes autonomic reflex responses and alterations in the synthesis of a variety of vasoactive substances within the circulation, blood vessels, and local tissue, all of which contribute to the control of vascular tone (35, 36). In many vascular beds including cerebral (3), coronary (37), and skeletal muscle (21, 52), the net effect of these changes in response to systemic hypoxia is vasodilation. In the skeletal muscle vasculature of humans, this vasodilatory response is graded with the degree of hypoxia (21, 25), despite concurrent sympathetic activation as evidenced by increases in muscle sympathetic nerve activity (MSNA) (14, 45) and norepinephrine spillover (32). Although this elevation in sympathetic outflow does not translate to increases in circulating norepinephrine due to elevations in neurotransmitter clearance (32), skeletal muscle resistance vessel α-adrenergic receptors are stimulated and limit or restrain hypoxic vasodilation (52). Studies also indicate that sympathetic activation elevates circulating epinephrine (11) leading to subsequent β-adrenergic stimulation of resistance vessels which may evoke peripheral vasodilation in humans (5, 52). Additionally, our laboratory has recently shown that local endothelium-derived nitric oxide (NO) and prostaglandins are involved in hypoxic vasodilation (34) and further, that erythrocyte (red blood cell) release of adenosine triphosphate (ATP) during progressive hemoglobin deoxygenation and may participate in the response (27). Taken together, there is a complex interaction between the sympathoadrenal system and locally-derived substances that ultimately determine the net peripheral vasodilatory response to systemic hypoxia in humans.

Many changes in both autonomic circulatory control and peripheral vascular function occur with advancing age, predisposing older adult humans to both acute (e.g. myocardial

103 infarction, stroke) and chronic (e.g. hypertension, atherosclerosis, ischemic vascular disease)
104 cardiovascular complications (51). Systemic hypoxia is not only a physiological stressor, but
105 may be a significant pathophysiological stressor observed in disease states such as obstructive
106 sleep apnea and congestive heart failure, both of which increase in prevalence with advancing
107 age (4, 7). One of the most pronounced and repeatable findings with human aging is the
108 progressive increase in basal (resting) MSNA that is typically 2-3 fold higher in older compared
109 with young healthy adults (15, 19, 20, 39). We have previously demonstrated that this elevated
110 sympathetic activity with age does not translate to greater basal forearm vasoconstriction due to
111 reduced α-receptor responsiveness at rest (18, 42). Regarding sympathetic responses to systemic
112 hypoxia, the acute increase in MSNA is not different with age (14, 26), however, our
113 understanding of how post-junctional α-receptor signaling interacts with circulating epinephrine
114 or other local vasodilatory factors to regulate vascular tone in older adults during a hypoxic
115 stimulus is currently unknown. In this context, circulating epinephrine appears to increase to a
116 similar extent in young and older adults (11), yet given potential changes in β-receptor
117 responsiveness with age (16, 40, 49), the net effect of sympathoadrenal activation on the
118 regulation of vascular tone during hypoxia is also unknown.
119 Human aging is also characterized by vascular endothelial dysfunction, which results in
120 reduced NO bioavailability (47, 48) , a potential shift from predominantly vasodilator (e.g.
121 prostacyclin) to vasoconstrictor (e.g. thromboxane) prostanoid production (48), and increased
122 endothelin-1 (ET-1) mediated vasoconstriction(53). Additionally, we have recently reported
123 impaired ATP release from erythrocytes of older healthy adults in response to hypoxia, and this
124 was related to a lack of increase in plasma ATP and impaired forearm vasodilation during a
125 single level of systemic hypoxia (80% SpO_2) (27). In theory, any change in autonomic

126 circulatory control or in the bioavailability of these local substances could alter the net vascular
127 response to systemic hypoxia in older adults and contribute to the observed impairment in
128 hypoxic vasodilation with age.

129 To date, little is known regarding peripheral vasodilator responses during *graded*
130 systemic hypoxia in aging humans, a stimulus that leads to progressive increases in both
131 sympathoadrenal activity and local vasodilator signaling in young adults. Further, there is no
132 information regarding how the sympathoadrenal system modulates vascular tone under these
133 conditions in older adults. Accordingly, the purpose of the present study was to test the
134 hypothesis that aging is associated with impaired hypoxic vasodilation during graded systemic
135 hypoxia, and that this impairment is due to attenuated β-adrenergic vasodilation and increased α-
136 adrenergic vasoconstrictor signaling with age.

137

138 **Methods**

139 *Subjects*

140 With Institutional Review Board approval and following written informed consent, a total
141 of 12 young (4 female, 8 male) and 10 older (4 female, 6 male) healthy subjects participated in
142 the present study. All subjects were free from overt cardiovascular disease as assessed from a
143 medical history, were sedentary to moderately active, non-smokers, non-obese, normotensive,
144 and not taking any medications including over the counter supplements (Table 1). Older subjects
145 were further evaluated for clinical evidence of cardiopulmonary disease with a physical
146 examination and resting and maximal exercise electrocardiograms. Females were studied during
147 the placebo phase of birth control or during the early follicular phase of their menstrual cycle to
148 minimize any potential vascular effects of sex hormones and all older females were post-

menopausal and 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, and 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 (18, 34). Throughout the duration of the study, heparinized saline (2 U/mL) was continuously infused at a rate of 3 ml/minute. Heart rate (HR) was monitored via 3-lead ECG.

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 for normalization of drug doses (18). 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 (2, 21, 34) 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 (2). 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 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 securely fixed to the skin over the brachial artery proximal to the catheter insertion site as previously described (13). 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 (34). 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, the MBV is in cm/s, the brachial diameter is in centimeters, and 60 was used to convert from ml/s to ml/min. Forearm vascular conductance (FVC) was calculated as (FBF/MAP) ×100, and expressed as ml/min/100mmHg (6, 31).

Regional α- and β- adrenergic Receptor Responsiveness

To determine the effect of age on α- and β-adrenergic receptor responsiveness at rest, norepinephrine and isoproterenol were locally infused via brachial artery catheter, respectively. Norepinephrine (Levophed, Hospira Inc., Lake Forest, IL, USA) was infused at 20, 40, 152 ng/dL/forearm volume (FAV)/minute (28), and isoproterenol (Isuprel, Hospira Inc., Lake Forest, IL, USA) was infused at 1, 3, 10 ng/dL/FAV/min (23). Saline and these agonists were infused at a rate of 2 ml/min via Harvard infusion syringe pump.

Regional Blockade of α- and β-adrenergic Receptors

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/min) and maintained the infusion during graded systemic hypoxia (5 μg/dL/FAV/min). To eliminate 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 5 minutes prior to hypoxia (10 μg/dL/FAV/ min) and continued the infusion at a maintenance rate (5 μg/dL/FAV/min) throughout the hypoxia trial. Loading doses of the drugs were given at 2 ml/min via Harvard infusion syringe pump, and

maintenance doses were given at 1 ml/min. These doses were chosen based on previous studies in our laboratory demonstrating effective adrenergic blockade (18, 22, 34).

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% SpO_2) 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 via HPLC with 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 cool temperature controlled room (21^{o} C). A fan was directed toward the forearm to limit skin blood flow, and a wrist cuff was inflated to exclude the hand circulation from our forearm hemodynamic measures (12).

Following placement of the brachial catheter, subjects rested quietly for a minimum 30 minutes. To begin, α- and β-adrenergic receptor responsiveness was randomly assessed using norepinephrine and isoproterenol, respectively. To do so, following 2 minutes of baseline measures with saline, three incremental doses of each agonist were locally infused for 2 minutes at each dose. The last 30 seconds of rest and each dose was used to calculate FBF and FVC. At a minimum, a 10 minute break was given between administrations of the α- or β- adrenergic

receptor agonist to allow drug washout and forearm hemodynamics to return to baseline, then the infusion of the second agonist was given in an identical fashion. Following the determination of both α- and β-adrenergic receptor responsiveness, all subjects underwent three trials of graded systemic hypoxia. Each hypoxia trial consisted of 4 minutes of baseline where subjects breathed room air through the mouthpiece, followed by 4 minutes of hypoxia at 90, 85, and 80% O_2 saturations (12 minutes total) and 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 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. In prior studies, the contribution of β-adrenergic receptors to the overall hypoxic vasodilatory response was assessed following local block of α-adrenergic receptors (8, 52). However, administering a non-selective α-adrenergic antagonist can inhibit α_2-adrenergic receptors on sympathetic nerve endings and facilitate norepinephrine release, which is able to bind β-adrenergic receptors located on the endothelium and vascular smooth muscle and elicit vasodilation (46), potentially resulting in an overestimation of the contribution of β-adrenergic mediated vasodilation (17, 46). Therefore, we sought to isolate the contribution of β-adrenergic mediated vasodilation prior to local inhibition of α-adrenergic receptors. 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 sympathoadrenal influences on vascular tone. Our laboratory and others have shown that the local vascular response to systemic hypoxia is repeatable over time (34, 52), indicating that any changes we observed during pharmacological blockade were not attributed to any residual effects

from the previous bout of hypoxia. Ten minutes following the last hypoxia trial, we challenged the efficacy of our local sympathoadrenal blockade with a single dose of each agonist for 2 minutes each. In anticipation that older adults would have attenuated adrenergic responsiveness (18), we elected to use the high dose of norepinephrine (152 ng/dL/FAV/minute) and isoproterenol (10 ng/dL/FAV/ minute) and the medium dose in young adults (40 and 3 ng dL/FAV/min, respectively) for this challenge.

Data Acquisition and 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 and HR from the ECG. FBF, HR, MAP, 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. Arterial blood gas values and catecholamines were obtained during the last minute of rest and each level of hypoxia. Our primary interest was in the peripheral vasodilator (or vasoconstrictor) responses to hypoxia, and thus to account for individual differences in resting vascular tone as well as alterations in vascular tone due to antagonist infusions, we quantified this as a percentage change in FVC from baseline within a given conditions (34, 52). Similar quantification was made for vasoconstrictor and vasodilator responses to norepinephrine and isoproterenol, respectively.

Utilizing SPSS statistical software (IBM, Armonk, New York) a 3-way repeated measure ANOVA was used to examine the impact of age, $\%SpO_2$, as well as any drug/condition 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

The mean age difference between young and older subjects was 39 years. There were no significant age-group differences in any measure of whole-body anthropometrics or regional tissue composition. Triglycerides and HDL-cholesterol were also not different between groups. Although within a normal range, older adults had significantly greater total and LDL-cholesterol (Table 1).

FBF and FVC Responses to Graded Systemic Hypoxia

There were no significant differences in resting FBF or FVC between young and older adults (Table 2). During the control hypoxia trial, young individuals exhibited progressive vasodilation in response to graded hypoxia (peak ΔFVC at 80% SpO_2 = 35±8%; $P<0.05$ vs. zero), whereas older adults failed to vasodilate significantly at any level of SpO_2 (peak ΔFVC = 4±6%; $P<0.05$ vs. young; Figure 2A) and the response was blunted compared to young adults at all levels of hypoxia ($P<0.05$).

β-adrenergic receptor blockade did not impact FBF or FVC at rest in either group (Table 2). Following local β-adrenergic receptor blockade, young adults continued to exhibit net vasodilation during hypoxia, the magnitude of which was only slightly less than that observed in control conditions (peak ΔFVC at 80% SpO_2 = 28±8 vs. 35±8%; $P = 0.29$; Figure 2B). Conversely, older adults actively constricted in response to graded systemic hypoxia during β-

adrenergic blockade which was significant at 85 and 80% SpO_2 (peak ΔFVC at 80% SpO_2 = -13±6%; P<0.05 vs. zero; Figure 2B), and again, demonstrated impaired responses compared to young adults at all levels of hypoxia (P<0.05).

As expected, α-adrenergic receptor blockade significantly increased FBF and FVC at rest in both young and older adults (Table 2). During the third hypoxia trial, when both α-adrenergic vasoconstriction and β-adrenergic mediated vasodilation were inhibited, young adults still exhibited significant forearm vasodilation, the magnitude of which was augmented compared with control and β-blockade conditions at 85 and 80% SpO_2 (peak ΔFVC at 80% SpO_2 = 57±11%; P <0.05 vs. control). In contrast, the older adults failed to significantly vasodilate from rest at any level of hypoxia (peak ΔFVC = 12±11%; P = 0.32 vs. zero) and the age-associated impairment in peripheral vasodilation persisted across all levels of hypoxia (Figure 2C).

Effects of Graded Systemic Hypoxia on Ventilation, Blood Gases, and Arterial Catecholamine Concentrations

At rest, there were no significant differences between young and older adults with respect to ventilation (Table 3) and resting blood gases (Table 4). Further, there was no effect of time (hypoxic bout) or age on ventilatory or blood gas responses to hypoxia. There were no significant differences in resting arterial catecholamine concentrations between young and older adults in any condition (control, β-blockade, and α+β-blockade; Table 4). Arterial epinephrine concentrations increased with the level of hypoxia in both young and older adults in the control trial, and the increase was less in older adults at 80% SpO_2 (P<0.05). Similar patterns of response were observed in the subsequent hypoxia trials, with epinephrine concentrations being elevated at rest and during hypoxia in the third trial in both groups (α+β-blockade trial; P<0.05).

Arterial norepinephrine was not different in young and older adults at rest in the control trial, and did not significantly increase during graded systemic hypoxia in either group. Similar data was obtained in the second hypoxia trial (β-block trial). In the third trial (combined α+β-blockade), both age groups demonstrated significant increases in norepinephrine during hypoxia, and this was greater in older compared with young adults ($P<0.05$).

FBF and FVC Responses to α- and β- adrenergic Receptor Agonists

Resting FBF and FVC were not different between young and older adults prior to infusion of the adrenergic agonists (Table 5). Compared to young, older individuals exhibited lower α-mediated vasoconstrictor responses at the medium and high doses of norepinephrine (Figure 3A). Similarly, older adults demonstrated impaired β-mediated vasodilation at the medium and high doses of isoproterenol compared with young (Figure 3B).

Propranolol and Phentolamine Efficacy

After the third hypoxia trial, the efficacy of the combined local α+β-adrenergic blockade was challenged with a single dose of either norepinephrine or isoproterenol (see methods for doses used). There was no significant change in FBF or FVC in response to the agonist challenge in either group, indicating effective local α- and β-adrenergic receptor blockade in young and older adults (Figure 3A and 3B).

Discussion

The primary novel findings of the present study are as follows. First, compared to young, healthy older adults demonstrate impaired forearm vasodilator responses to graded systemic hypoxia. Second, local inhibition of β-adrenergic receptors slightly reduces hypoxic vasodilation in young adults ~10%, however a robust vasodilation is still observed. In stark contrast, local β-blockade results in active forearm vasoconstriction in older adults. Third, local inhibition of α-adrenergic mediated vasoconstriction augments forearm vasodilation during hypoxia in young subjects, however older adults continued to fail to vasodilate and thus the age-associated impairment in hypoxic vasodilation persists at all levels of hypoxia. As such, the collective data indicate that the age-related impairments in forearm vasodilation during graded systemic hypoxia are primarily independent of the sympathoadrenal system in humans.

Age and Peripheral Vasodilation During Systemic Hypoxia

To our knowledge, this is the first study to determine the peripheral vascular response to graded systemic hypoxia in older adults, and further, to determine what role the age-associated changes in the sympathoadrenal system may play in the net response. In the control hypoxia trial, at the onset of hypoxia (90% SpO_2) young individuals vasodilated ~17% (ΔFVC) and progressively dilated as the level of saturation declined (ΔFVC ~35% at 80% SpO_2). Conversely, older adults failed to vasodilate at any level of hypoxia during control conditions (Figure 2A). Previous studies on this topic in older adults have utilized only a single level of systemic hypoxia, however the majority of data support our findings of an age-associated impairment in hypoxic vasodilation (11, 27, 29). Although the net vascular response during systemic hypoxia can be influenced by several factors, we next determined the role of the

sympathoadrenal system in regulating vascular tone given that this system is engaged during systemic hypoxia and that aging is associated with chronic elevations in sympathetic nervous system activity and alterations in adrenergic receptor responsiveness.

Effects of local β-adrenergic Receptor Blockade on Hypoxic Vascular Control

Systemic hypoxia elicits a significant increase in sympathoadrenal activity as evidenced by elevations in muscle sympathetic nerve activity (14) and circulating epinephrine (52). In the second hypoxia trial, we locally infused propranolol to inhibit β-adrenergic mediated vasodilation to determine the contribution of this pathway to the overall net hypoxic vasodilatory response. Previous studies in young healthy adults determining the role of β-receptor stimulation in peripheral hypoxic vascular control have yielded equivocal results. Original studies on this topic in the 1960's indicate that local blockade of β-receptors had a very modest (<10%) effect on hypoxic vasodilation (43). In contrast, more recent studies have suggested that ~50% of hypoxic vasodilation is mediated via β-receptors (52), however some caution is warranted when interpreting these latter findings. Specifically, the role of β-mediated vasodilation was assessed when α-adrenergic receptors were inhibited. Although this approach is useful for evaluating vasodilating mechanisms independent of sympathetic vasoconstriction, local non-selective α-blockade can increase norepinephrine release from sympathetic nerve endings via inhibition of pre-junctional α_2-adrenergic receptors leading to stimulation of β-receptors *independent* of circulating epinephrine (46). Importantly, this effect could be enhanced during systemic hypoxia when sympathetic nerve discharge is elevated, leading to a potential overestimation of the contribution of β-adrenergic receptors to the net dilatory responses (17, 46). Findings from the present investigation indicate that despite a significant increase in plasma epinephrine (Table 4),

401 hypoxic vasodilation is only modestly blunted (~10%) during blockade of β-receptors in young
402 adults (without concomitant α-receptor blockade). Taken together, the collective data indicate
403 that while some evidence suggests that β-receptor activation can participate in hypoxic
404 vasodilation in young adults, this may not be obligatory to observe the normal dilatory response.
405 To date, no studies have determined the contribution of peripheral β-receptors to vascular
406 control during graded hypoxia in aging humans. Given evidence that β-adrenergic receptor
407 responsiveness may be reduced with age (40), we hypothesized that inhibition of this pathway
408 would have a minimal impact on the hypoxic vasodilator response in older adults. Interestingly,
409 we observed that local β-blockade resulted in a net *vasoconstriction* in older adults during graded
410 hypoxia, a response that reached statistical significance at 85 and 80% SpO_2 levels of systemic
411 hypoxia (Figure 2B). These findings suggest that despite a lack of vasodilation in the control
412 hypoxia trial, β-mediated vasodilatory signaling may play an important role in buffering
413 vasoconstrictor signaling in older adults. This active vasoconstriction observed in older adults
414 during the second hypoxia trial appears to be due to augmented sympathetic vasoconstrictor
415 signaling, as inhibiting α-adrenergic receptors in trial 3 reversed this response (see below).
416
417 *Effects of Local α-adrenergic Receptor Blockade on Hypoxic Vascular Control*
418 In young adults, local inhibition of α-adrenergic mediated vasoconstriction augments
419 peripheral vasodilation during systemic hypoxia (9, 52). As such, the elevated sympathetic
420 outflow (14) and norepinephrine release (32) act to restrain or limit the amount of vasodilation.
421 The data from the present investigation support these previous observations. Specifically, we
422 observed that local α-adrenergic receptor blockade resulted in augmented forearm vasodilation in

423 young subjects at 85 and 80% SpO_2 levels of systemic hypoxia, and is consistent with the
424 observed graded increase in MSNA with progressive hypoxia in humans (44).
425 To date, no studies have determined whether augmented α-adrenergic vasoconstrictor
426 tone is mechanistically-linked with age-associated impairments in hypoxic vasodilation. Human
427 aging is associated with an increase in basal muscle sympathetic nerve activity (14) as well as
428 reductions in α-adrenergic responsiveness at rest (18). Previous studies indicate that the
429 sympathetic response to hypoxia is similar in young and older adults (14), and although α-
430 responsiveness appears blunted with age, we hypothesized that any age-associated impairment in
431 hypoxic vasodilation would be partly attributed to elevated α-adrenergic vasoconstriction due,
432 potentially, to less "opposition" from β-receptor or NO signaling (11, 34, 40, 49). Following
433 local sympathoadrenal blockade, basal forearm hemodynamics were elevated similarly in young
434 and older adults (Table 2), consistent with the removal of basal α-adrenergic vasoconstrictor tone
435 at rest (42). However, in contrast to the augmented vasodilation observed in young adults, the
436 older adults still failed to vasodilate to graded systemic hypoxia (Figure 2C). It should be noted
437 here that the net vasoconstriction observed in Trial 2 was no longer present when α-receptors
438 were inhibited, yet older adults still did not significantly vasodilate. Thus, the collective data
439 from the present set of experiments indicate that alterations in sympathoadrenal regulation of
440 vascular tone do not explain the impaired peripheral vasodilation during graded systemic
441 hypoxia in older adults.
442
443 *Adrenergic Receptor Responsiveness and Blockade Efficacy*
444 In the present study, we determined α- and β-adrenergic receptor responsiveness in both
445 young and older adults via graded intra-arterial doses of norepinephrine and isoproterenol,

446 respectively. Compared to young, older adults demonstrated blunted α- and β-adrenergic
447 receptor responsiveness at the medium and high doses of each agonist (Figure 3), a finding
448 consistent with prior studies from our laboratory (18) and others (49). Importantly, in the
449 present study we challenged the efficacy of our adrenergic blocking drugs using the medium and
450 high doses of each agonist in young and older adults, respectively. We chose to use a higher
451 dose for the older subjects based on our anticipated response of reduced adrenergic
452 responsiveness with age. Our data indicate that infusion of norepinephrine or isoproterenol after
453 combined infusion of propranolol and phentolamine did not significantly change forearm
454 vascular tone (Figure 3). However, in young adults, compared to control conditions (~40%
455 constriction) a small amount of vasoconstriction (~9%) persisted during the α-adrenergic
456 receptor challenge with norepinephrine, suggesting that there may have been incomplete α-
457 adrenergic blockade in some subjects. If this were the case, we may have underestimated the
458 role of the sympathetic nervous system in restraining vasodilation during hypoxia in young
459 adults. Importantly, this does not impact the primary conclusions from the present investigation
460 that the age-related impairments in forearm vasodilation during graded systemic hypoxia are
461 primarily independent of the sympathoadrenal system in humans.
462
463 *Potential Mechanisms*
464 The major key finding from the present study is that the age-associated impairment in
465 peripheral hypoxic vasodilation persists after local inhibition of sympathoadrenal control of
466 vascular tone. Although we found some age-related differences in circulating epinephrine in
467 response to hypoxia (see Table 4) and β-adrenergic receptor responsiveness (Figure 3B), these
468 observations most likely do not explain our findings related to hypoxic vascular control as β-

469 blockade in the young did not significantly attenuate hypoxic vasodilation. Thus, any age-
470 related changes in β-receptor stimulation or receptor responsiveness appear to have a minimal
471 impact on the net vascular response under control conditions. Similarly, despite some age-group
472 differences in plasma norepinephrine across the hypoxia trials, we did not find that inhibition of
473 α-adrenergic receptors (Trial 3) "normalized" hypoxic vasodilation in older adults. In fact, older
474 adults still failed to vasodilate significantly at any level of hypoxia (Figure 3C). This may be
475 related, in part, to reductions in α-adrenergic responsiveness with age; however our collective
476 observations clearly indicate that mechanisms beyond sympathoadrenal influences on vascular
477 tone underlie the impairment in hypoxic vasodilation in older adults.

478 The lack of a robust increase in hypoxic vasodilation in the older group during local
479 sympathoadrenal blockade suggests that the age-associated impairment is primarily due to local
480 vascular control mechanisms. In this context, our laboratory has previously determined that
481 during α- and β-adrenergic blockade (as in Trial 3 of the present study), the peripheral hypoxic
482 vasodilatory response is abolished in young individuals following combined inhibition of NO
483 and vasodilating prostaglandins (34). It is well known that aging is associated with a reduction
484 in endothelial-derived NO bioavailability (48) and potentially a reduction in vasodilating
485 prostaglandins (47), and thus it is plausible to speculate that endothelial dysfunction and less
486 vascular relaxation via these pathways may explain the impaired hypoxic vasodilation in older
487 adults.

488 Additionally, the erythrocyte has been proposed to be a sensor of hypoxic conditions,
489 whereby reductions in hemoglobin oxygenation stimulates release of ATP, which then binds to
490 purinergic receptors on the vascular endothelium eliciting vasodilation (24). We have recently
491 demonstrated that, in contrast to young adults, venous plasma ATP does not increase during

492 systemic hypoxia in older adults, and that isolated erythrocytes from older adults fail to release
493 ATP when deoxygenated (27). Interestingly, we and others have shown that ATP-mediated
494 dilation is dependent, in part, on endothelial-derived NO and prostaglandins (12), and therefore
495 we speculate that impaired red blood cell ATP release during hypoxia coupled with endothelial
496 dysfunction could underlie the lack of hypoxic vasodilation with age. Finally, it is also possible
497 that local vasoconstrictor signaling via ET-1, which is elevated with advancing age (50), could
498 act to restrain hypoxic dilation in older adults. Future studies will be needed to determine these
499 exact mechanisms in humans.
500
501 *Experimental Considerations*
502 There are a few experimental considerations worthy of discussion. First, despite waiting
503 20 minutes between hypoxia trials, there was a general trend for an increase in plasma
504 catecholamine concentrations with repeated hypoxia bouts (Table 4). For example, compared to
505 the control trial, both young and older adults demonstrated a significant increase in arterial
506 epinephrine concentrations at rest and during hypoxia in the third hypoxia trial (α+β blockade).
507 Additionally, norepinephrine was also significantly elevated in both age groups during the third
508 hypoxic bout. However, it is important to note that any significant increase in epinephrine or
509 norepinephrine with repeated hypoxia exposure in either age group does not impact the
510 interpretation of the peripheral vascular response data, as both α+β-adrenergic receptors (Trial 3)
511 were effectively inhibited in the trial where increases were observed. Further, our arterial
512 catecholamine concentrations are similar to previously reported date in young and older adults at
513 rest and during hypoxia (11, 52).

Second, older adults failed to increase heart rate to the same extent as young adults and this was significant across all hypoxia bouts. This is consistent with previous studies on this topic (33) and most likely reflects age-related reductions in cardiac β-adrenergic responsiveness (30, 43). Despite older adults having a significantly smaller increase in heart rate to hypoxia, it is unlikely that this is contributing to the overall age-associated impairment in hypoxic vasodilation, as there is ample heart rate reserve to elevate cardiac output in both age groups at all levels of systemic hypoxia.

Finally, although we were not statistically powered to do so, we did examine whether there was any trend for sex differences in the degree of vasodilation to hypoxia within the young and older adult groups (10). In the present study, we did not observe any sex differences in the vasodilatory response to hypoxia nor the impact of the adrenergic blockers on the response, however, given our small sample size, it is unlikely we would be able to detect a significant sex difference.

Potential Significance

In the present study we determined the effects of healthy aging on the peripheral vasomotor responses to graded systemic hypoxia within the forearm vasculature. The forearm was chosen not only to isolate the local effects of our pharmacological agents, but also due to the significant correlation between endothelial function assessed in the forearm and coronary vasculature (1). Thus, impairments in vasodilation observed in the forearm vasculature could have implications for other regions such as the coronary and possibly cerebral circulations. Further, accumulating evidence indicates that hypoxic vasodilation is impaired in patients with heart failure (38) and obstructive sleep apnea (41), populations clearly at risk for ischemic

coronary and cerebrovascular disease. Thus, improving vascular control during hypoxic stress may be a potential therapeutic target for improving tissue blood flow and oxygen delivery in aging and disease.

Conclusions

Human aging is associated with a significant impairment in the peripheral vasodilatory response to graded systemic hypoxia. This impairment is independent of age-associated alterations in sympathoadrenal control of vascular tone, and thus it is likely that reductions in the stimulus for local vasodilation (e.g. red blood cell derived ATP) and/or alterations in the local production or bioavailability of endothelium-derived substances (e.g. NO, ET-1), underlie the lack of hypoxic vasodilation in older healthy adults. Peripheral hypoxic vasodilation is also impaired in patient populations that increase in prevalence with advancing age (e.g. heart failure, obstructive sleep apnea), and as such, identifying mechanisms to improve hypoxic vascular control could prove clinically beneficial for older healthy and diseased humans.

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Figure Legends

Figure 1. Study Timeline. Following brachial artery catheter insertion and rest, α+β adrenergic receptor responsiveness was determined. Each agonist (norepinephrine and isoproterenol) was administered in three incremental doses for 2 minutes each. Hypoxia trials consisted of 4 minutes of baseline followed by 4 minutes of isocapnic systemic hypoxia at 3 different levels (90, 85, 80% SpO_2). The vascular response to graded hypoxia was assessed in control conditions, during local β-adrenergic receptor blockade, and during combined α- and β-adrenergic receptor blockade. In each condition, prior to the start of hypoxia and during the last minute of each level of hypoxia, an arterial catecholamine and blood gas sample and was collected. Following the third bout of hypoxia and local administration of both propranolol and phentolamine (adrenergic blockade), a single dose (medium or high; see methods) of each agonist (Norepinephrine (NE) and Isoproterenol (ISO) was administered for 2 minutes to confirm effective α+β receptor blockade.

Figure 2. Hypoxic Vasodilation in Young and Older Adults. A) Control trial hypoxic vasodilation (ΔFVC(%)) from baseline in young and old. B) Hypoxic vasodilation during local β-adrenergic blockade via intra-arterial propranolol (ΔFVC %) from baseline in young and old. C) Hypoxic vasodilation during local α + β-adrenergic blockade (phentolamine and propranolol) (ΔFVC %) from baseline in young and old. * $P<0.05$ vs. Young. † $P<0.05$ vs. zero within each age group. # $P<0.05$ vs. control condition. ∧ $P<0.05$ vs. β-adrenergic blockade. + $P<0.05$ vs. α + β-adrenergic blockade.

Figure 3. Adrenergic Responsiveness in Young and Older Adults. A) α-adrenergic (norepinephrine: NE) and B) β-adrenergic (isoproterenol: ISO) receptor responsiveness. Each agonist (NE and ISO) was administered in three incremental doses for 2 minutes each. There was a significant interaction between age and ΔFVC during infusion of both α- and β-adrenergic agonists. * $P<0.05$ vs. Young. Note: in both young and old, all vascular responses to each agonist were significantly different from zero.

Table 1. Subject Characteristics. *P<0.05 vs. Young. Although total and LDL cholesterol were significantly greater in older adults, they were still within a normal range.

Variable	Younger	Older
Male:Female	8:4	6:4
Age (years)	24±1	63±2*
Body mass index (kg/m^2)	24±1	25±1
Body fat (%)	25±3	30±2
Forearm volume (mL)	883±35	879±81
Total cholesterol (mg/dl)	141±7	184±13*
LDL cholesterol (mg/dl)	81±4	108±9*
HDL cholesterol (mg/dl)	46±4	53±6
Triglycerides (mg/dl)	76±9	115±22
Glucose (mg/dl)	82±5	86±9

Table 2. Systemic and Forearm Hemodynamics during all hypoxia trials. *P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition.

	Baseline		90% SaO_2		85% SaO_2		80% SaO_2	
	Young	**Old**	**Young**	**Old**	**Young**	**Old**	**Young**	**Old**
				Control				
HR (beats/min)	64 ±4	57 ±2	76 ±4†	62 ±2*†	82 ±5†	67 ±2*†	89 ±5†	70 ±2*†
MAP (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
FBF (ml/min)	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
FVC (ml/min/mmHg)	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†	29.2 ±4.0*
				β-adrenergic blockade				
HR (beats/min)	62 ±4	58 ±2	74 ±4†	62 ±2*†	80 ±4†	65 ±3*†	83 ±4†	68 ±2*†
MAP (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
FBF (ml/min)	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*
FVC (ml/min/mmHg)	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-adrenergic blockade				
HR (beats/min)	62 ±4	57 ±2	75 ±4†	64 ±2†	80 ±4†	66 ±2*†	80 ±4†	68 ±3*†
MAP (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
FBF (ml/min)	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
FVC (ml/min/mmHg)	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. Ventilation during hypoxia trials. *P<0.05 vs. Young, † 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				
Minute Vent. (l/min 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-adrenergic blockade				
Minute Vent. (l/min 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-adrenergic blockade				
Minute Vent. (l/min 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 4. Blood gases and arterial catecholamine concentrations (young n=11, older n=10) during all hypoxia trials. FHHb (fractional deoxyhemoglobin (%)) *P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition, ‡ P<0.05 vs. Control Condition.

	Baseline		90% SaO_2		85% SaO_2		80% SaO_2	
	Young	Old	Young	Old	Young	Old	Young	Old
					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†
FHHb (%)	4.2±1.1	5.1±1.5	11.9±1.6	12.6±2.4	17.5±2.1	16.5±1.2	20.6±2.2	20.5±4
Epinephrine (pg/ml)	55±8	56±10	61±9	59±10	80±10†	74±9†	134±19†	74±12*†
Norepinephrine (pg/ml)	244±20	295±27	233±14	268±25†	221±16	270±20	225±28	265±22†
					β-adrenergic blockade			
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†
FHHb (%)	5.3±1.3	4.2±1.1	11.6±1.5	12.2±1.3	16.3±1.4	16.3±1.6	20.6±1.3	19.7±1.1
Epinephrine (pg/ml)	63±8	55±9	69±7	69±11†	110±22†	74±15†	142±35†	89±19†
Norepinephrine (pg/ml)	261±18	243±26	220±23	237±25	194±15†	257±20*	185±19†	283±28*†
					α+β-blockade			
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†
FHHb (%)	5.1±1.3	4.3±1.2	11.9±2.1	12.4±2.1	17.1±2.2	16.5±1.9	20.8±2.5	19.5±2.3
Epinephrine (pg/ml)	88±16‡	79±12‡	121±25†‡	67±13†‡	209±55†	87±20*	240±53†‡	128±40*†‡
Norepinephrine (pg/ml)	279±36	324±35	225±23†	365±37*†‡	221±19†	392±32*‡	206±21†	461±44*†‡

Table 5. Hemodynamic variables during α+β adrenergic responsiveness. *P<0.05 vs. Young, † P<0.05 vs. Baseline in respective condition. Post-Baseline reflects resting values following local α+β adrenergic blockade.

α-adrenergic receptor responsiveness	Baseline		NE (20ng/100ml FAV/min)		NE (40ng/100ml FAV/min)		NE (152ng/100ml FAV/min)		Post-Baseline		Challenge	
	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older
HR (beats/min)	61 ±3	56 ±2	60 ±3	56 ±2	60 ±3	56 ±2	32 ±3	57 ±2	61 ± 3	56 ±2	60 ±3	56 ±2
MAP (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
FBF (ml/min)	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
FVC (ml/min/ mmHg)	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/min)**		**ISO (15ng/100ml FAV/min)**		**ISO (50ng/100ml FAV/min)**		**Post-Baseline**		**Challenge**	
	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older	Young	Older
HR (beats/min)	60 ± 3	57 ±2	62 ±4	56 ±2	61 ±1	56 ±2	63 ±3	58 ±2	61 ±3	54 ±2	60 ±3	54 ±2
MAP (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
FBF (ml/min)	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
FVC (ml/min/ mmHg)	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.

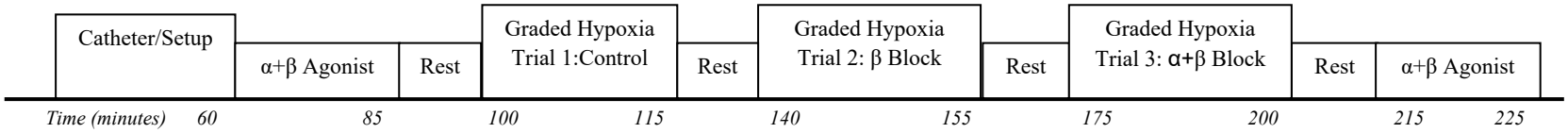

Catheter/Setup
α+β Agonist
Rest
Graded Hypoxia
Trial 1:Control
Rest
Graded Hypoxia
Trial 2: β Block
Rest
Graded Hypoxia
Trial 3: α+β Block
Rest
α+β Agonist
Time (minutes)
60
85
100
115
140
155
175
200
215
225

Figure 2.

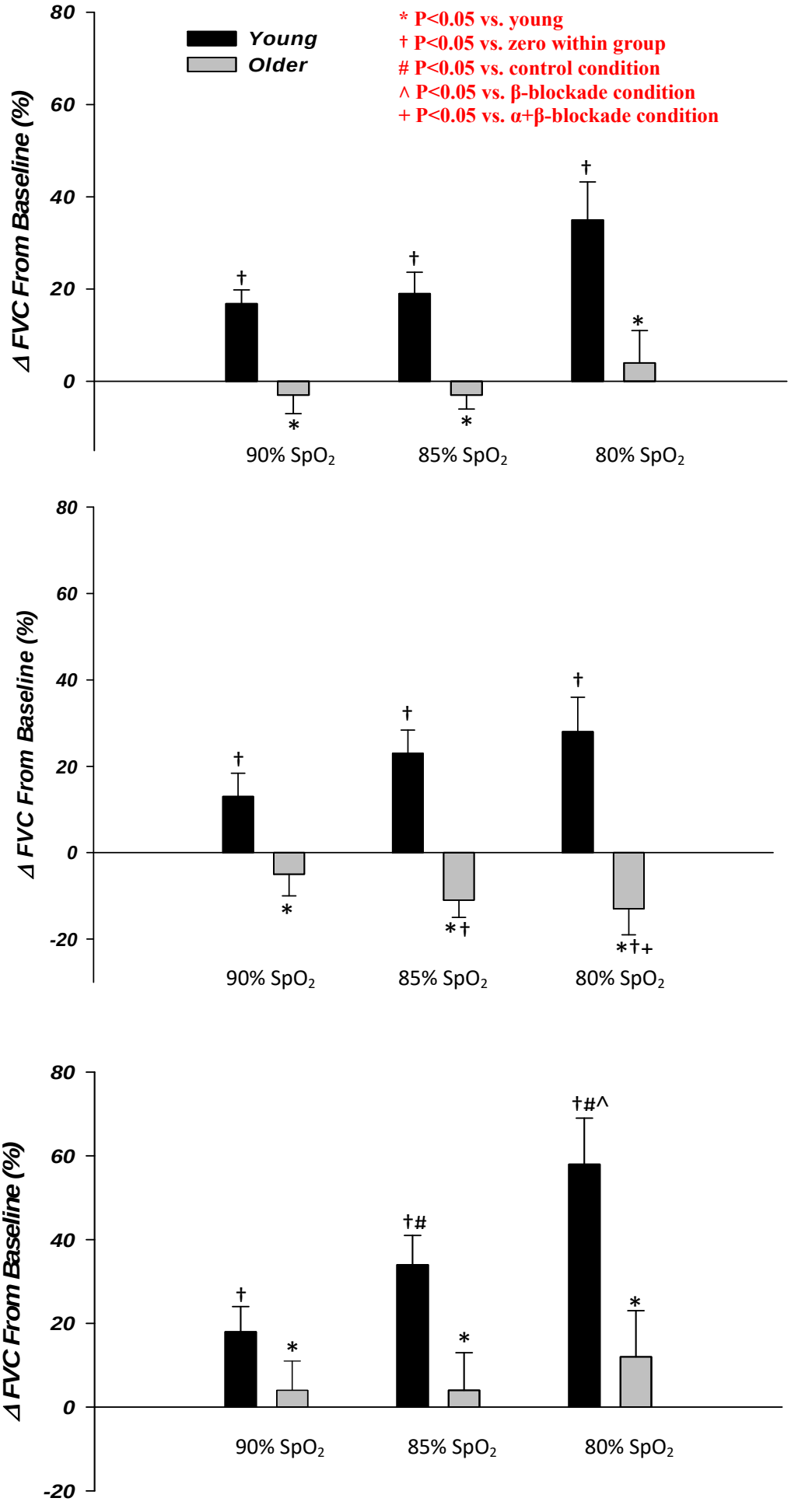
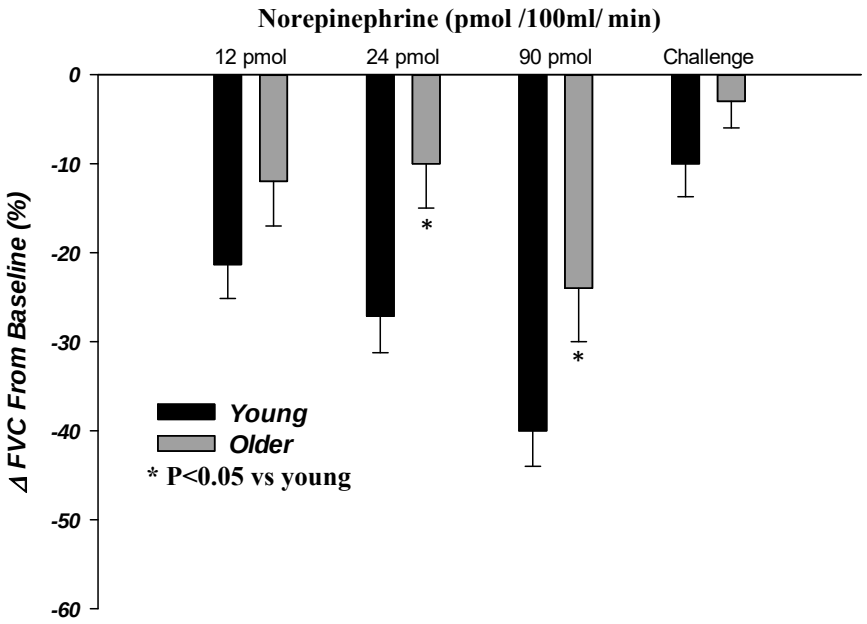

A.
Young
Older
* P<0.05 vs. young
† P<0.05 vs. zero within group
# P<0.05 vs. control condition
∧ P<0.05 vs. β-blockade condition
+ P<0.05 vs. α+β-blockade condition
Δ FVC From Baseline (%)
80
60
40
20
0
90% SpO2
85% SpO2
80% SpO2
B.
-20
C.

Figure 3.

A.


Norepinephrine (pmol /100ml/ min)
12 pmol
24 pmol
90 pmol
Challenge
0
-10
-20
-30
-40
-50
-60
Δ FVC From Baseline (%)
Young
Older
* P<0.05 vs young
*
*

B.

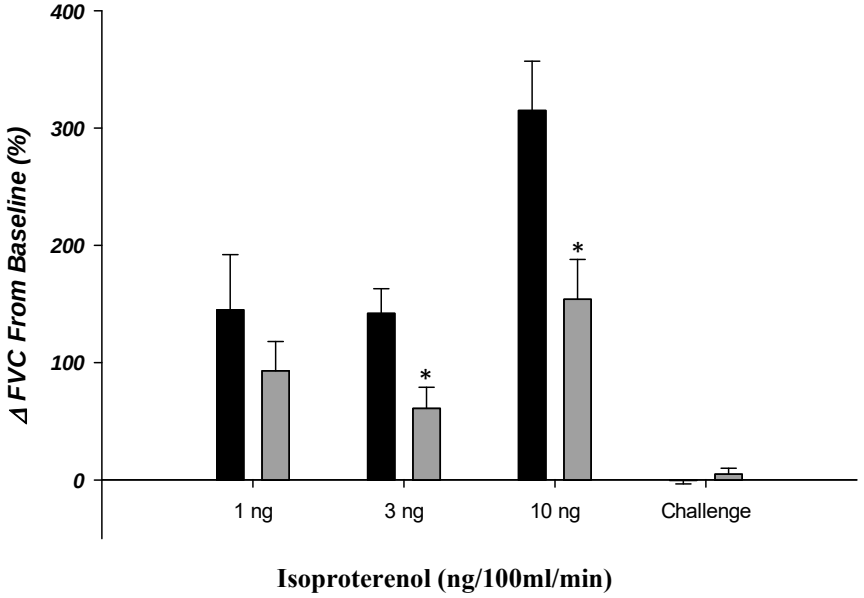
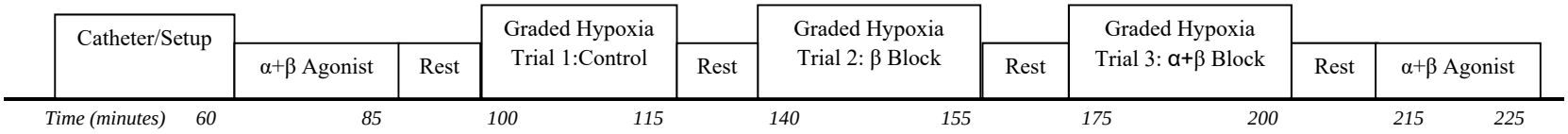
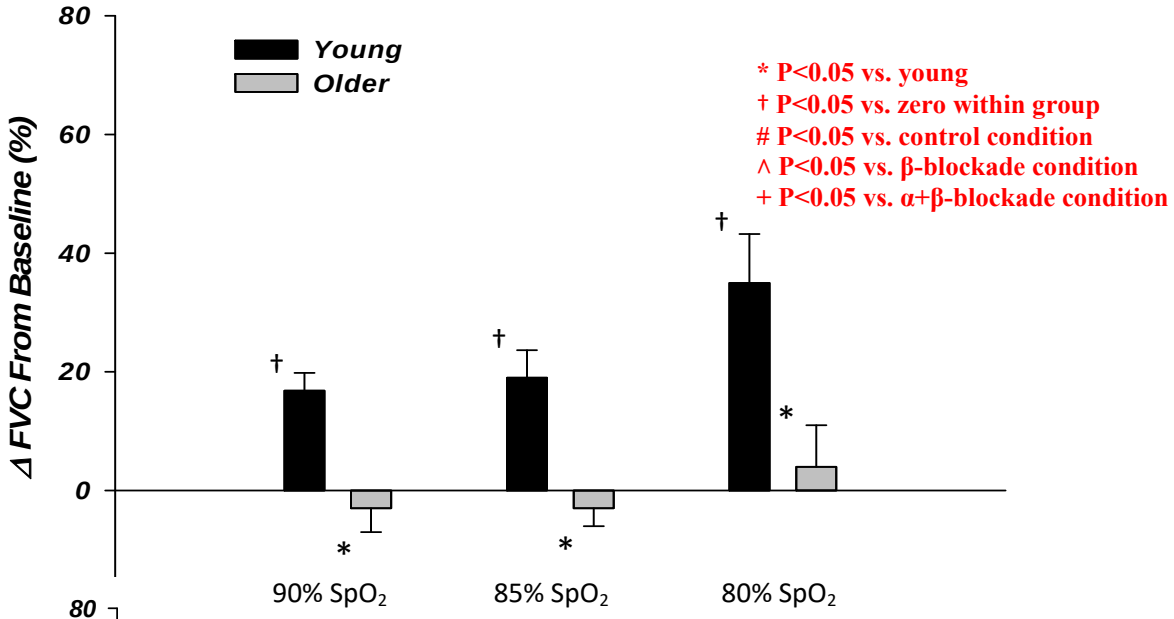

400
300
200
100
0
Δ FVC From Baseline (%)
1 ng
3 ng
10 ng
Challenge
*
*
Isoproterenol (ng/100ml/min)

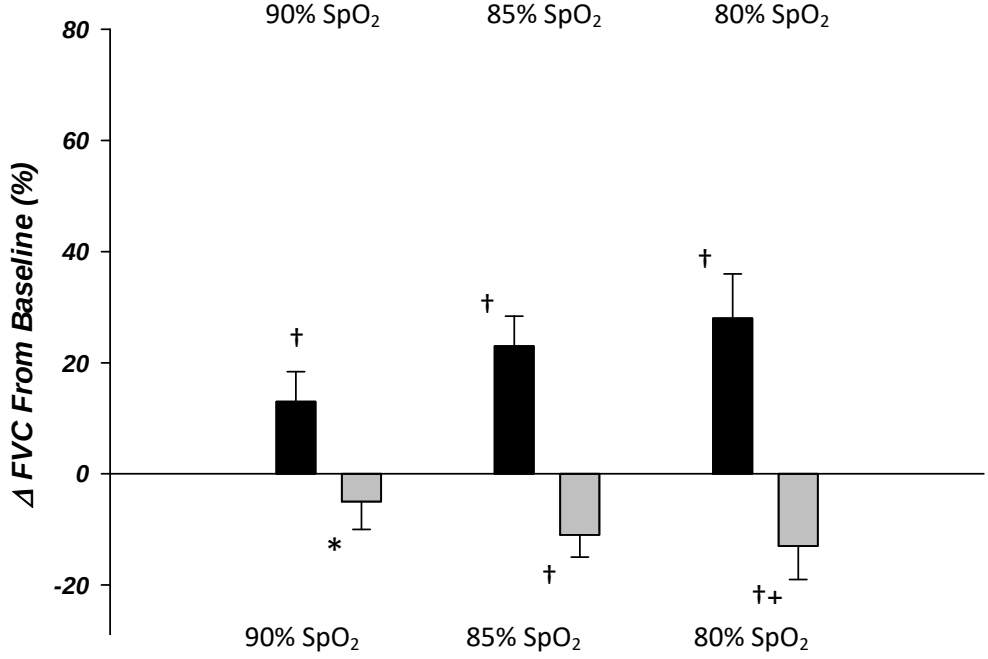
Figure 1.


Catheter/Setup
α+β Agonist
Rest
Graded Hypoxia Trial 1:Control
Rest
Graded Hypoxia Trial 2: β Block
Rest
Graded Hypoxia Trial 3: α+β Block
Rest
α+β Agonist
Time (minutes)
60
85
100
115
140
155
175
200
215
225

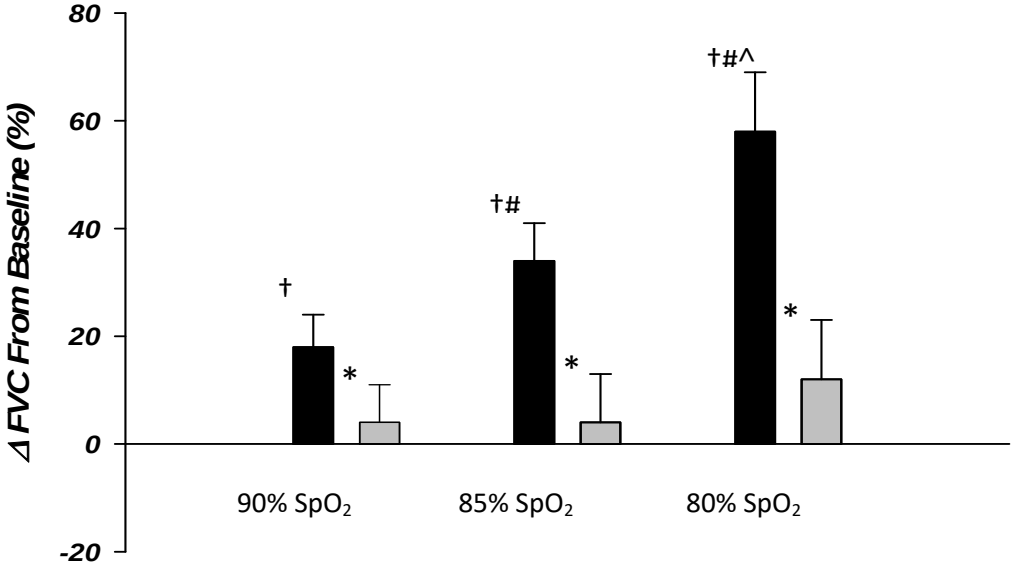
A.


Young
Older
* P<0.05 vs. young
† P<0.05 vs. zero within group
# P<0.05 vs. control condition
^ P<0.05 vs. β-blockade condition
+ P<0.05 vs. α+β-blockade condition
Δ FVC From Baseline (%)
80
60
40
20
0
90% SpO2
85% SpO2
80% SpO2

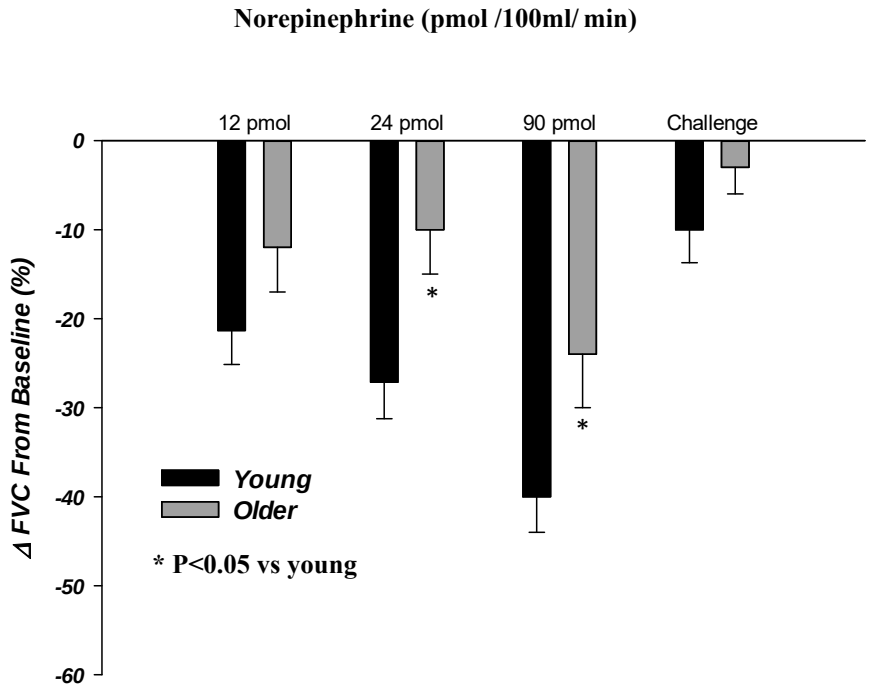
B.


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

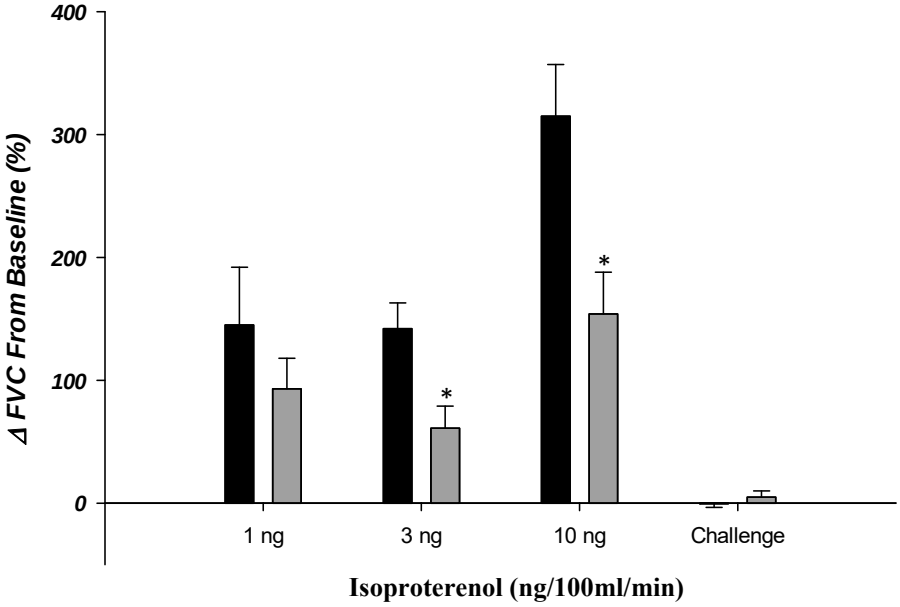
C.


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

A.


Norepinephrine (pmol /100ml/ min)
12 pmol
24 pmol
90 pmol
Challenge
0
-10
-20
-30
-40
-50
-60
Δ FVC From Baseline (%)
Young
Older
* P<0.05 vs young
*
*

B.


400
300
200
100
0
Δ FVC From Baseline (%)
1 ng
3 ng
10 ng
Challenge
Isoproterenol (ng/100ml/min)
*
*