NONADRENERGIC CONTROL OF SKELETAL MUSCLE BLOOD FLOW IN THE ELDERLY

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

The objective of this thesis was to determine the role of nonadrenergic vasoactive substances in the control of blood flow in the elderly. The first study aimed to determine the individual and potentiating effects of the renin-angiotensin-aldosterone (RAAS) and alpha-adrenergic systems on the regulation of blood flow in the young and old. We observed an enhanced maximal reduction in brachial artery blood flow in response to angiotensin-II (ANG-II) in the old compared to the young, which was abolished when the alpha-adrenergic component of the response was pharmacologically eliminated with phentolamine. These data suggest that with healthy aging, the increased ANG-II-mediated vasoconstriction may be attributed, in part, to potentiation of alpha-adrenergic vasoconstriction, and indicates that the "cross-talk" between the RAAS and adrenergic systems could be an important therapeutic consideration for hypertension in the elderly. The second study of this project was focused on the role of endogenous endothelin-1 (ET-1) in the regulation of vascular tone at rest and during exercise, with a secondary emphasis on how this pathway is altered with advancing age. The first portion of the second study thus sought to characterize the role of ET-1 on blood flow, arterial blood pressure, and oxygen consumption (VO_2) during knee extensor exercise in young, healthy adults. This study documented an increase in exercising limb blood flow following ET-1 receptor subtype A (ET_A) antagonism (BQ-123), which was accompanied by a decrement in arterial blood pressure and an increase in VO_2. Together, these findings have identified

a significant role of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the ET_A receptor as an important mechanism for both restraint of blood flow in the exercising limb and support of arterial blood pressure in healthy, young adults. During the aging portion of the second study, at rest, blood flow was reduced by 30% in the elderly compared to the young. ET_A antagonism did not change resting blood flow in the young, but restored blood flow in the old to a level similar to that of the young. During exercise, BQ-123 increased blood flow and VO_2 to a similar degree in both the young and old. Likewise, the increase in arterial blood pressure during exercise was attenuated in a similar manner between groups after BQ-123 administration. Together these findings demonstrate differences in the regulatory role of the ET-1 pathway at rest and exercise with advancing age. Collectively, these studies have provided insight into the role of nonadrenergic vasoactive substances, specifically ANG-II and ET-1, in the regulation of vascular tone with age. Findings from these studies may provide new information concerning the prevention and treatment of age-associated cardiovascular diseases.

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CHAPTER 1

INTRODUCTION

In the United States, cardiovascular diseases such as atherosclerosis and hypertension, which can both lead to the development of heart failure and stroke, are the leading cause of mortality, accounting for upwards of 40% of deaths in those 65 and older (16). Since over 80% of cardiovascular deaths occur within this age-range (16), age may be considered a risk factor for cardiovascular disease (14). With aging, there are adverse alterations to the cardiovascular system, both structurally and functionally, which jointly have been thought to play a role in the progression of cardiovascular disease. One principle alteration associated with the age-related changes of the cardiovascular system is an elevation in vascular tone (8, 32), which likely contributes to the attenuated skeletal muscle blood flow observed at rest in the elderly (7, 25, 26).

Age-related changes in vascular tone may be particularly important in the cardiovascular response to exercise, when muscle perfusion must increase dramatically to meet the elevated metabolic demand of the exercising tissue. This increase in skeletal muscle blood flow during exercise is accomplished, in large part, by metabolic vasodilation within the skeletal muscle vasculature, though this response must be balanced by the concomitant need to support arterial blood pressure. There is some evidence for impaired hyperemia during leg exercise in the elderly (15, 25, 27, 36), yet very few studies to date have taken an integrative approach to examine changes in the "balancing act" between maintenance of skeletal muscle blood flow and arterial blood pressure during exercise in this cohort. Further, the potential mechanisms responsible for age-related dysregulation of vascular tone during exercise remain poorly understood.

One of the most pronounced and reproducible autonomic adaptations associated with healthy aging is a progressive increase in firing of sympathetic nerves innervating

skeletal muscle vascular beds (6, 22, 32). Accompanying this age-associated rise in muscle sympathetic nerve activity (MSNA) is a selective reduction in alpha-adrenergic responsiveness (8), presumably due to a down regulation or desensitization of alpha-adrenergic receptors. However, nonspecific alpha-adrenergic blockade does not fully restore blood flow to that of the young (8). This indicates that sympathetic-adrenergic receptor-mediated control of local skeletal muscle vascular tone is not solely responsible for the reduction in skeletal muscle blood flow in the elderly, presenting the possibility for age-related changes in nonadrenergic vasoconstrictor pathways.

Although there are an abundance of vasoactive molecules that might contribute to the vasoconstriction observed at rest and during physical activity in the elderly, the age-associated alterations in angiotensin-II (ANG-II) and endothelin-1 (ET-1) represent two potentially significant and somewhat understudied pathways that may contribute to the elevated vascular tone documented with age. ANG-II is the end-product of the renin-angiotensin-aldosterone system (RAAS), and functions in an endocrine manner as a potent endogenous vasoconstrictor through binding to the ANG-II subtype 1 (AT_1) receptors on arteriolar vascular smooth muscle (12), as well as potentiating norepinephrine (NE) release from sympathetic butons by binding to AT_1 receptors prejunctionally (4). ANG-II subtype 2 (AT_2) receptors are also present in human arteries (19, 38), though recent studies suggest that this ANG-II receptor subtype plays a minimal role in blood flow regulation in humans (10). While there are multiple places within the RAAS pathway that can potentially serve to regulate ANG-II production, one of the essential steps is the secretion of renin from the kidneys (2), which is initiated by an increase in sympathetic nerve activity (SNA) (11, 24, 28). Thus, it is plausible that the

inherent elevation in SNA with aging (6, 22, 32) expressed at the juxtaglomerular cells could increase renin production, increase the concentration of circulating ANG-II, and ultimately amplify alpha-adrenergic vasoconstriction via circulating ANG-II. The unique nature of the AT_1 receptors involvement in the regulation of the sympathetic nervous system (SNS) and RAAS make it an ideal site to investigate the interactions between these two respective systems and their collective contribution in regulating peripheral hemodynamics in the elderly.

While aging individuals have similar sympatho-adjustments to stressors which acutely increase SNA, such as static and dynamic exercise, (20, 29), the increase in peripheral vascular tone due to the interactions between the SNS and RAAS could account for a portion of the age-related attenuation in peripheral blood flow. Indeed, the documentation that AT_1 receptor density has been shown to increase with age (9, 31), and functional studies from our group identifying an age-associated increase in ANG-II-mediated vasoconstriction in the leg (36), affirm the significance of AT_1 receptors in the regulation of peripheral vascular tone. However, it is unknown whether the enhanced ANG-II-mediated vasoconstriction demonstrated in the elderly is predominately due to a hypersensitivity of the postjunctional AT_1 receptors or due to an enhanced ANG-II potentiated NE release. Thus, the first objective of this thesis was to determine the role of peripheral AT_1 receptors in the regulation of sympathetic vasoconstriction, and whether this differs with healthy aging.

Unlike ANG-II, ET-1 acts primarily in a paracrine and autocrine manner, produced by the vascular endothelial cells and binding to the ET_A receptor subtype, located on the vascular smooth muscle, and the ET_B receptor subtype, located on the

vascular endothelium (1, 37). While ANG-II production is directly linked to the SNS, stimulation of ET-1 production by the vascular endothelial cells occurs locally by vasoactive hormones, shear stress, free radicals, and hypoxia, and is inhibited by stimuli that increase concentrations of cyclic GMP such as nitric oxide and prostaglandins (13). However, despite the distinct differences in stimuli inducing the production of ANG-II and ET-1, the G-protein-coupled receptor pathway leading to ET_A receptor-mediated vasoconstriction is similar to that of the AT_1 receptor (3, 30). Thus, the examination of these two nonadrenergic receptors in conjunction with one another provides the ability to distinguish between a receptor specific versus a G-protein-linked response.

As ET-1 has been identified as one of the most potent endogenous vasoconstrictor substances (23), any age-related adaptation in the sensitivity of ET_A and ET_B receptors could profoundly alter peripheral vascular tone and ultimately skeletal muscle blood flow. Recently, it was reported that ET-1-mediated vasoconstriction of the brachial artery at rest is significantly attenuated in the elderly, while ET_A receptor blockade (BQ-123) enhanced resting blood flow in older individuals, compared to their younger counterparts who exhibited no change in blood flow (33). Also, ET_A receptor blockade has been shown to enhance vasodilation induced by acetylcholine infusion in older individuals, indicating that an age-related increase in endogenous ET-1 may be partially responsible for the reduction in endothelial-dependent vasodilation in the elderly (34). These findings support the notion that there is a concomitant age-associated increase in circulating ET-1, which may ultimately contribute to the elevated resting vasoconstrictor tone and impaired vasodilatory capacity in the elderly. However, the importance of ET-1 during exercise remains unknown.

Initial evidence suggesting a contributing role of ET-1 in the regulation of skeletal muscle blood flow during exercise has been derived, for the most part, from assays measuring circulating concentrations of plasma ET-1 during exercise. With evidence supporting (18), and refuting (5, 17), increasing concentrations of ET-1 during exercise. Previous work in animals has evaluated the tonic ET-1-mediated restraint on vascular tone via ET_A and concomitant ET_A/ET_B receptor blockade during exercise, and reported that ET_A receptor inhibition improved exercising muscle blood flow (21). Recently, our group identified an attenuated vasoconstriction in response to intra-arterial infusion of ET-1 during dynamic knee-extensor exercise in young, healthy humans. (35). However, the role of endogenous ET-1, specifically the ET_A receptor, in the regulation of exercising limb blood flow and arterial blood pressure has yet to be investigated. Thus, the second objective of this thesis was to further elucidate the role of ET-1 in the regulation of vascular tone in young healthy individuals during exercise, and second, determine the extent to which this vasoconstrictor pathway contributes to age-associated differences in vascular tone at rest and exercise.

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CHAPTER 2

ANGIOTENSIN-II POTENTIATES ALPHA-ADRENERGIC VASOCONSTRICTION IN THE ELDERLY

Abstract

Aging is characterized by a global sympathoexcitation, expressed through both the alpha-adrenergic and renin-angiotensin-aldosterone (RAAS) pathways. Overactivity of these pathways may contribute to the well-known elevation in peripheral vasoconstriction with age, as well as the sequelae of geriatric hypertension. To examine the individual and possible potentiating effects of these two pathways, 14 subjects (*n* = 8 young, 26±1 yrs; *n* = 6 old, 68±3 yrs) underwent brachial artery (BA) catheterization for local administration of angiotensin-II (ANG-II) (0.8-25.6 ng/dl/min), norepinephrine (NE) (2.5-80 ng/dl/min) and ANG-II with concomitant alpha-adrenergic antagonism (phentolamine (PHEN), 10 µg/dl/min). Ultrasound Doppler was utilized to determine BA blood flow in both the infused and contralateral (control) limb. Arterial blood pressure was measured directly and heart rate was determined via standard three-lead ECG. At maximal doses, the ANG-II-mediated reduction in BA blood flow was greater in the old (-61±6%) compared to young (-48±4%, P = 0.01), while the NE-mediated reduction in BA blood flow was similar between groups (-58±9% old, -65±7% young). In the presence of PHEN, the ANG-II-mediated reduction in BA blood flow in the old was restored to that of the young (-43±8% old, -39±6% young). Arterial blood pressure, heart rate, and control arm BA blood flow remained unchanged throughout all drug infusions. These data suggest that with healthy aging, the increased ANG-II-mediated reduction in blood flow may be attributed, in part, to potentiation of alpha-adrenergic reduction in blood flow, and indicate that "cross-talk" between the RAAS and adrenergic systems could be an important therapeutic consideration for hypertension in the elderly.

Introduction

One principle alteration associated with age-related changes of the cardiovascular system is an elevation in vascular tone (8, 31), which likely contributes to the attenuated skeletal muscle blood flow commonly observed in the elderly (7, 16, 17, 23, 25, 37). While there are many regulatory pathways that collectively contribute to the age-related decline in limb blood flow, the effects of the alpha-adrenergic and renin-angiotensin-aldosterone system (RAAS) are of particular interest. Specifically, there is evidence that alpha-adrenergic blockade in old individuals partially restores resting limb blood flow towards that of the young (8), suggesting an age-related alteration in this receptor group. Though less well studied, there is also evidence to suggest an important role of the RAAS pathway; we recently identified a significantly greater vasoconstriction in response to angiotensin-II (ANG-II) in the old compared to their young counterparts (37), implicating this pathway as an additional, potentially significant contributor to the regulation of resting skeletal muscle blood flow in the old.

While the independent contribution of these two pathways to excessive vascular tone in the elderly may be substantial, significant cross-talk exists that could produce synergistic behavior between these two pathways. Indeed, circulating ANG-II acts as a potent vasoconstrictor not only through binding to ANG-II subtype 1 (AT_1) receptors located on the vascular smooth muscle (12), but also through binding to AT_1 receptor located presynaptically on postganglionic sympathetic nerves and enhancing the release of norepinephrine (NE), attenuating the reuptake of NE, and ultimately potentiating alpha-adrenergic vasoconstriction (5, 18). Considering the evidence for an age-related increase in circulating ANG-II and AT_1 receptor density (9, 29), the direct and indirect

(alpha-adrenergic-mediated) vasoconstricting effects of ANG-II in the elderly may be profound. This age-associated increase in RAAS activity is especially important in the context of underlying changes in autonomic function with age. Indeed, one of the most pronounced and reproducible observations associated with aging is a progressive increase in muscle sympathetic nerve activity (MSNA) (6, 20, 31), which is modulated peripherally by both the alpha-adrenergic and RAAS pathways. Thus, it follows that in the presence of this elevated basal MSNA, the potentiating effect of circulating ANG-II may be even more pronounced. Considering this, presynaptic AT_1 receptors may be seen as uniquely positioned to act as a regulatory point for sympathetic nervous system control of skeletal muscle blood flow.

The degree to which postsynaptic AT_1 receptors and the potentiating effects of ANG-II on alpha-adrenergic vasoconstriction contribute to the enhanced ANG-II-mediated vasoconstriction in the elderly is currently unknown. Therefore, this study sought to evaluate the functional consequence of age-related changes in ANG-II-mediated vasoconstriction in the presence and absence of alpha-adrenergic vasoconstriction. We hypothesized that ANG-II-mediated vasoconstriction would be greater in the old compared to the young, but this age effect would be attenuated in the presence of alpha-adrenergic antagonism.

Methods

Subjects

Eight young (26 ± 1 yrs) and six old (68 ± 3 yrs) healthy subjects were enrolled in this study. All subjects were nonsmokers and were normally active. Subjects were not taking any prescription medication and were free of overt cardiovascular disease, as

indicated by a health history. Protocol approval and written informed consent were obtained according to the University of Utah and the Salt Lake City Veterans Affairs (VA) Medical Center Institutional Review Board requirements. All data collection took place at the VA Salt Lake City Geriatric, Research, Education, and Clinical Center (GRECC) Utah Vascular Research Laboratory (UVRL). Subjects remained supine in a thermoneutral environment for the duration of the study.

Protocols

Subjects reported to the UVRL fasted at 0800 on the experimental day. Catheters (18 G, 20 cm, Arrow) were placed in the brachial artery (BA) and basilic or cephalic vein using sterile technique, with the site of the BA catheter insertion approximately 10 cm distal to the axilla and both catheters advanced 6-8 cm in the proximal direction. The arterial catheter was placed in this region of the upper arm to ensure that study drugs entered the artery "upstream" (~15 cm) to the ultrasound Doppler sample volume, allowing assessment of drug effects on BA diameter and blood velocity (39). A microelectrode was placed in the peroneal nerve for direct assessment of basal muscle sympathetic nerve activity (MSNA), as described previously (34). Following catheter and microelectrode placement, the drug infusion protocol was performed, as illustrated in Figure 2.1.

Study Drugs

Lower and upper arm volumes were determined anthropometrically, and then used for the calculation of drug dosing. Total limb volume receiving infusate was calculated as:

Total volume (dl) = forearm volume + (upper arm x 0.5)

A portion of the upper arm was included in this calculation due to the proximal location of the arterial catheter.

Angiotensin-II (ANG-II) (Bachem, Germany) was diluted from 50 µg of lypholyzed powder in normal saline (0.9% NaCl) to a concentration of 0.125 µg/ml. The drug was infused intra-arterially at 0.8-1.6-3.2-6.4-12.8 ng/dl/min (3 min each dose). Norepinephrine Bitartrate (NE) (Levophed, Hospira, Lake Forest, IL) was diluted from 4 mg suspension in 5% dextrose to a concentration of 1 µg/ml. The drug was infused intra-arterially at 2.5-5-10-20-40 ng/dl/min (3min each dose). If a subject did not exhibit a plateau in BA blood flow and BA diameter after the fifth dose of ANG-II or NE, a sixth doubling dose (ANG-II, 25.6 ng/dl/min; NE 80 ng/dl/min) was administered of the respective drug. Phentolamine Mesylate (PHEN) (Regitine, Bedford Labs, Bedford, OH) was diluted from 5 mg lypholyzed powder in normal saline (0.9% NaCl) to a concentration of 83.3 µg/ml. The drug was infused at a rate of 10 µg/dl/min for 20 minutes followed by a maintenance dose 5 µg/ml/dl during the remainder of the protocol. In a subset of subjects (n = 4 young, n = 4 old), NE was also infused at 5 and 20 ng/dl/min (3 min each dose) at the end of the alpha-adrenergic blockade portion of the protocol to challenge the alpha receptor blockade (Figure 2.1).

Measurements

Ultrasound Doppler assessments

Simultaneous measurements of BA blood velocity and vessel diameter were performed in both the infused and control arms using Logiq 7 and Logiq e ultrasound Doppler systems (GE Medical Systems, Milwaukee, WI) operating in duplex mode. The Logic 7 and Logic e were equipped with linear array transducers operating at imaging frequencies of 14 and 12 MHz, respectively. Blood velocity was obtained using the same transducers with a Doppler frequency of 5 MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization.

Brachial artery diameter, blood velocity, and blood flow analyses

Mean velocity values (V_{mean}, angle-corrected and intensity-weighted area-under-the-curve) were calculated using commercially available software (Logic 7 and Logic e). End-diastolic, ECG R-wave-gated images were collected via video output from the Logic 7 and Logic e for off-line analysis of BA vasodilation using automated edge-detection software (Medical Imaging Applications, Coralville, Iowa). Using BA diameter and V_{mean} BA blood flow was calculated according to the equation: $(V_{mean} \cdot \pi\ (\text{vessel diameter}/2)^2 \cdot 60)$.

Muscle sympathetic nerve activity assessment

Basal muscle sympathetic nerve activity (MSNA) was recorded directly from the peroneal nerve, as described previously (10, 21, 32). Briefly, a tungsten microelectrode was inserted into fascicles of the peroneal nerve near the fibular head of the left leg. Neural signals were processed by a preamplifier and amplifier (Nerve traffic analyzer model 662C-3, Iowa City, IA) with a total gain of 90,000. Amplified signals were filtered (bandwidth, 700–2,000 Hz), rectified, and integrated (time constant, 0.l s) to obtain mean voltage neurograms.

Arterial blood pressure and heart rate assessment

Heart rate was monitored from a standard three-lead ECG. Arterial blood pressure was acquired continually from within the BA, with the pressure transducer placed at the level of the catheter (Transpac IV, Abbot Laboratories).

Blood chemistry

A lipid panel was performed in all subjects by standard technique. In a subset of subjects (n = 6 young, n = 5 old), resting blood samples were obtained from the brachial artery and the basilic or cephalic vein. Plasma ANG-II concentration was assessed by radioimmunoassay (Bachem, Bubendorf Switzerland), and plasma NE concentration by enzyme immunoassay (2-CAT Elisa, Labor Diagnostika Nord, Nordham Germany).

Data analysis

Ultrasound images and Doppler velocity spectra were recorded continuously. During the last 60 s of each ultrasound Doppler segment, V_{mean} was averaged across five 12 s intervals, which were matched with intima-to-intima BA diameter measurements

evaluated during diastole. Maximal reductions in BA blood flow and BA diameter during ANG-II and NE infusions were identified on an individual basis from the three highest doses of ANG II (6.4-12.8 25.6 ng/dl/min) and NE (20-40-80 ng/dl/min). Mean arterial pressure was calculated using the time integral of the arterial waveform. MSNA bursts were was identified according to appearance and timing in relation to the preceding R-wave, and expressed as burst frequency (bursts per minute).

The rate of NE spillover into plasma was determined using the following equation:

$$NE\ spillover\ (pg/min) = [(C_v - C_a) + C_a\ (E_e)] \cdot BA\ blood\ flow$$

where C_v and C_a are plasma concentrations of NE (pg/ml) in the basilic or cephalic vein and brachial artery (respectively) and E_e is the fractional extraction of epinephrine (27).

Statistical Analyses

Statistical analyses were performed with the use of commercially available software (SigmaStat 3.10, Systat Software, Point Richmond, CA). Multiple 2x6 mixed repeated-measures analysis of variance (ANOVA) were used to identify significant changes in measured variables within and between drug conditions, as well between young and old. Multiple 2x2 mixed repeated measures ANOVA were used to identify significant maximal changes in measured variable between during conditions, as well as between young and old. When a significant main effect was found ($p < 0.05$), the Holm-Sidak method was used for alpha adjustment and post hoc analysis. Student's *t*-tests were used to identify significant differences in subject characteristics between young and old.

Significance for subject characteristics was established at α=0.05. All group data are expressed as mean ± standard error.

Results

Subject Characteristics

Subject characteristics are presented in Table 2.1. Resting muscle sympathetic nerve activity (MSNA) measurements were attempted in all subjects, and an acceptable recording site was identified in 9 of the 14 subjects (*n* = 3 young, *n* = 6 old). The old exhibited significantly higher resting levels of MSNA, plasma norepinephrine (NE), and NE spillover. Plasma ANG-II was similar between young and old (Table 2.2).

Angiotensin II (ANG-II)

The overall hemodynamic response to ANG-II was a dose-dependent reduction in brachial artery (BA) blood flow (Figure 2.2A, Table 2.2) and diameter (Figure 2.3A, Table 2.2), with an apparent plateau in vasoconstriction in both groups. Due to individual variability in maximal response to the drug, not all subjects received the same number of doses. All subjects received the first 4 doses (0.8-1.6-3.2-6.4 ng/dl/min), 11 of 14 subjects (*n =6* young, *n = 5* old) received the fifth dose (12.8 ng/dl/min), and 4 of the 14 subjects (*n* = 1 young, *n* = 3 old) required a sixth dose (25.6 ng/dl/min) to reach maximal vasoconstriction. This maximal reduction in BA blood flow identified during doses 4-6 (6.4-12.8-25.6 ng/dl/min) was significantly greater in the old compared to the young (Figure 2.2B).

Throughout the dose-response protocol, heart rate (HR), mean arterial pressure (MAP), and BA blood flow in the contra-lateral arm were unchanged (Table 2.2), confirming that the drug remained localized in the vasculature of the infused arm.

Norepinephrine (NE)

During the NE dose-response, significant age-independent reductions in BA blood flow and BA diameter were observed (Table 2.3). As with ANG-II, due to individual variability in maximal response to the drug, not all subjects received the same number of doses of NE. All subjects received the first 4 doses (2.5-5-10-20 ng/dl/min), 11 of the 14 subjects (n =7 young, n = 4 old) received the fifth dose (40 ng/dl/min), and 10 of the 14 subjects (n = 7 young, n = 3 old), required a sixth dose (40 ng/dl/min). The maximal reduction BA blood flow identified during doses 4-6 (20-40-80 ng/dl/min) was similar between young and old (Figure 2.2B). As with ANG-II, HR, MAP, and BA blood flow in the contra-lateral arm were unchanged during NE infusion (Table 2.3), confirming that the drug remained localized in the vasculature of the infused arm.

ANG-II during Nonspecific Alpha-Adrenergic Antagonism

Alpha-adrenergic-receptor blockade by a continuous infusion of phentolamine (PHEN) induced a significant age-independent increase in blood flow (young, Δ149 ± 39ml/min, $P<0.05$; old, Δ182 ± 47 ml/min; $P<0.05$). NE was administered (5 and 20 ng/dl/min) at the end of the PHEN infusion (n = 8) to confirm alpha-adrenergic receptor blockade. Neither NE dose produced a significant change in BA blood flow (253 ± 92 ml/min, baseline; 241 ± 29 ml/min, NE 5 ng/dl/min; 223 ± 3 ml/min, NE 20 ng/dl/min).

Similar to ANG-II alone, the overall response to ANG-II during PHEN was a dose-dependent reduction in brachial artery (BA) blood flow in both groups, with an attenuated magnitude of change in blood flow in response to ANG-II at the higher doses in both groups (Figure 2.2A, Table 2.3). Compared to the ANG-II alone trial, the maximal reduction in BA blood flow during ANG-II + PHEN was similar in the young

group, but was attenuated in the old (Figure 2.2B, Table 2.3), effectively abolishing the age-related differences in ANG-II-mediated vasoconstriction observed during ANG-II alone (Figure 2.2B, Table 2.3).

Brachial Artery (BA) Diameter

BA diameter was reduced in the young (doses: 6.4 and 12.8 ng/dl/min) and in the old (doses: 6.4 and 12.8 ng/dl/min) (Figure 2.3A). Similar levels of vasoconstriction of the BA were induced by ANG-II alone and NE (Figure 2.3B) in both the young (ANG-II: -0.57±0.11mm; NE: -0.51 ± 0.19mm) and the old (ANG-II: -0.50 ± 0.13mm; NE: -0.68 ± 0.06mm). In the presence of alpha-adrenergic receptor antagonism, the ANG-II-mediated vasoconstriction of the BA induced by ANG-II was ablated in both the young (ANG-II+PHEN: -0.06 ± 0.03mm; $P<0.05$) and the old (ANG-II+PHEN: 0.00 ± 0.02mm; $P<0.05$) (Figure 2.3B).

Discussion

To better understand the mechanisms regulating the peripheral circulation with advancing age, this study sought to examine the interaction between the alpha-adrenergic and renin-angiotensin-aldosterone (RAAS) systems. A significant increase in sympathetic nervous system (SNS) activity was observed in the elderly, which was accompanied by an age-related increase in brachial artery (BA) vasoconstriction in response to intra-arterial angiotensin-II (ANG-II) administration. Following alpha adrenergic blockade, ANG-II-mediated vasoconstriction was restored to that of the young. These data reveal that with healthy aging, an increased ANG-II receptor sensitivity may be attributed in part to a potentiation of alpha-adrenergic vasoconstriction, and implicate the “cross-talk” between the RAAS and adrenergic systems as an important consideration in therapeutic

strategies targeting these two pathways. In the context of an age-related increase in SNS activity, these findings may also represent a potential mechanism contributing to enhanced alpha-adrenergic-mediated vascular tone associated with healthy aging.

ANG-II and Blood Flow Regulation with Age

This study has identified an enhanced dose-dependent as well as maximal reduction in BA blood flow to intra-arterial infusion of ANG-II in healthy old individuals compared to their young counterparts (Figure 2.2, Table 2.2). These findings are in agreement with previous work from our group, identifying a greater reduction in leg blood flow in old individuals compared to the young following femoral artery ANG-II infusion (37). Through the inclusion of an ANG-II dose response protocol (Figures 2.1 and 2.2), the assessment of circulating ANG-II, and control limb measurements, this study expands on this previous work, confirming the apparent increase in ANG-II mediated reduction in blood flow in healthy, old adults.

This enhanced reduction in blood flow induced by exogenous ANG-II in the old contrasts with the work of Hogikyan and Supiano (13), who observed a similar change in forearm blood flow in old and young individuals in response to brachial artery (BA) infusion of ANG-II. This discrepancy may be, in part, due to the differences in methodology and drug dosing. The aforementioned study (13) used venous occlusion plethysmography to measure forearm blood flow and administered a dose of ANG-II that elicited a systemic pressor response. In contrast, in the present study ultrasound Doppler was utilized to determine BA blood flow, there was no significant change in mean arterial pressure (MAP), and BA blood flow in the contra-lateral (noninfused) arm during intra-arterial infusion of ANG-II was documented to be unchanged (Table 2.2). Therefore,

through a controlled and comprehensive experimental design, the present findings build on previous observations, and now identifying a clear that the ANG-II-mediated reduction in blood flow is enhanced in the elderly.

ANG-II Dose-Response during Alpha-Adrenergic Antagonism and Age

ANG-II acts as a potent vasoconstrictor not only through binding to ANG-II subtype 1 (AT_1) receptors located on the vascular smooth muscle (12), but also by potentiating alpha-adrenergic vasoconstriction (5, 18). Thus, recognizing the enhanced reduction in blood flow induced by ANG-II with age (37) (Figure 2.2), we sought to evaluate the potential impact of age on AT_1 receptors located presynaptically on postganglionic sympathetic nerves, as this receptor group is known to potentiate the release and inhibit the reuptake of NE (5, 30).

To pharmacologically probe the role of these presynaptic AT_1 receptors, we repeated the ANG-II dose response protocol with concomitant infusion of the nonselective alpha-adrenergic receptor antagonist PHEN. In support of our hypothesis, the age-associated increase in the ANG-II-mediated reduction in blood flow was abolished after alpha-adrenergic blockade, such that the maximal reduction in blood flow in response to ANG-II was similar to that observed in the young group (Figure 2.2B). These data thus indicate that alpha-adrenergic vasoconstriction is responsible for nearly 20% of the maximal reduction in blood flow induced by intra-arterial ANG-II infusion in the old (Figure 2.2B), approximately double that observed in the young (10%).

The interaction between the RAAS and alpha-adrenergic pathways in the peripheral circulation of young, healthy subjects is well documented. Both Seidelin *et al*. (28) and Webb *et al.* (33) observed that BA infusions of low-doses of ANG-II (at

concentrations that do not effect forearm blood flow) enhanced the sympathetically-mediated reduction in blood flow induced by lower body negative pressure. Importantly, when NE and ANG-II were infused concomitantly in these studies, no potentiating effect of ANG-II was evident, providing evidence in support of presynaptic AT_1 receptors as the site of action. Using higher doses of ANG-II, Lyons *et al.* (18) demonstrated a marked reduction in blood flow in response to intra-arterial ANG-II in the young that was significantly reduced (~36%) in the presence of PHEN. However, the use of significantly higher doses of ANG-II and the lack of blood pressure measurements in the former study limits a direct comparison with the present findings. Specifically, the present study extends the evidence for an alpha receptor-mediated contribution to the vasoconstrictor action of ANG-II in the young, and an enhanced synergistic interaction between ANG-II and the alpha-adrenergic system which contributes to the age-associated increase in the ANG-II-mediated reduction in blood flow.

AT_1 and Alpha-Adrenergic Receptor Distribution

By design, all study drugs were infused into the BA proximal to where the vessel was imaged, allowing the unique opportunity to assess BA diameter changes in response to ANG-II, PHEN, and NE. Independent of age, ANG-II produced a dose-dependent vasoconstriction of the BA diameter (Figure 2.3A, Table 2.2), which initially was thought to provide evidence for AT_1 receptors in the BA. However, during co-infusion with PHEN, ANG-II administration did not change BA caliber (Figure 2.3), indicating that ANG-II-mediated vasoconstriction of the BA was, in fact, achieved through the alpha-adrenergic pathway. The alpha-adrenergic dependence of this response is further supported by the similar maximal reductions in BA diameter induced by ANG-II and NE

(Figure 2.3B). Thus, it seems AT_1 receptors may be distributed heterogeneously throughout the arterial tree. Specifically, AT_1 receptors located on vascular smooth muscle appear to be located distal to the BA, while those located presynapticlly on sympathetic-adrenergic nerves may be located in both the proximal and distal portions of the arm vasculature.

The ANG-II-mediated vasoconstriction of the BA documented in this study is in contrast with earlier studies from our group performed in the leg. Specifically, we previously observed minimal changes in the diameter of the common femoral artery (CFA) diameter in response to ANG-II infusion (3, 37), while the alpha-1 adrenergic agonist phenylephrine provoked a significant reduction in CFA caliber (3, 35, 36, 38). The explanation for this disparity is most likely related to limb differences. Studies that have utilized reflex increases in muscle sympathetic nerve activity (MSNA) have observed sympathetically-mediated vasoconstriction of the BA (1), while the leg does not appear to have significant sympathetic innervation at the level of the CFA (14, 15, 24), despite the presence of alpha-1-adrenergic receptors in this large conduit vessel (3, 35, 36, 38). Together, the prior and current data indicate that the age-independent ANG-II potentiation of alpha-adrenergic vasoconstriction may be limited to those alpha receptors that are sympathetically innervated, and thus support a presynaptic site of action for this potentiating effect of ANG-II and the RAAS on the alpha-adrenergic system, as reported previously (28, 33).

Perspectives

To our knowledge, this is the first study to demonstrate that a significant part of the vasoconstrictive action of ANG-II in the old is mediated through the alpha-adrenergic

system, which may have important implications for the regulation of skeletal muscle blood flow in this population. Indeed, healthy aging is associated with a concomitant rise in MSNA (6, 31) (Table 2.1) and a reduction in skeletal muscle blood flow (7, 16, 17), and it has been suggested that upwards of 80% of the age-associated reduction in skeletal muscle blood flow can be explained by the alpha-adrenergically-mediated contribution to vascular tone (8). In this light, the potentiating effects of ANG-II on NE release and alpha-adrenergic vasoconstriction with age may not only indicate an important site of ANG-II modulation of sympathetic-adrenergic vasoconstriction, but also a potential mechanism contributing to enhanced alpha-adrenergic-mediated vascular tone associated with healthy aging.

The interaction between the RAAS and sympathetic-adrenergic system may be of particular relevance in cardiovascular disease states characterized by an elevation in activity of these two respective systems, such as hypertension and heart failure (2, 11). Indeed, recognition of the current underlying contribution of alpha-adrenergic vasoconstriction to ANG-II-mediated vasoconstriction in the elderly may be an important mechanism by which hypertension develops with advancing age. This concept is predicated on the observed efficacy of the AT_1 receptor blockade in reducing resting blood pressure (4), vascular compliance (26), and vascular tone (19) in hypertensive patients. These functional vascular changes that are potentially due to the ability to block the presynaptic AT_1 receptor, limiting NE release (22). Thus, we speculate that the present data, identifying an apparent enhanced contribution of alpha-adrenergic vasoconstriction to ANG-II-mediated vasoconstriction with age, may represent one important pathway through which hypertension develops in the elderly.

Summary

This study has identified an enhanced in ANG-II-mediated reduction in limb blood flow in healthy older individuals, and demonstrated that this apparent increase in sensitivity to ANG-II is due, at least in part, to an ANG-II-mediated potentiation of alpha-adrenergic vasoconstriction. These findings suggest that cross-talk between the RAAS and adrenergic systems may be an important regulator of resting vascular tone and muscle blood flow with advancing age.

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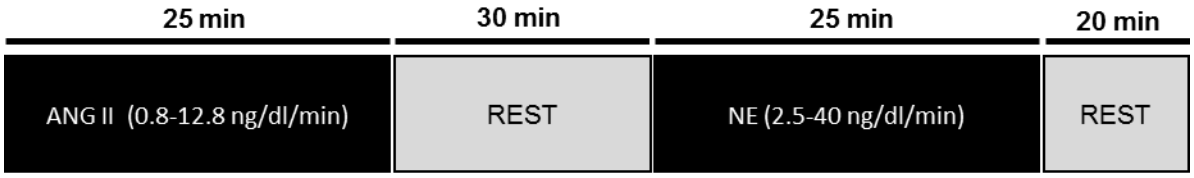
FIGURE 2.1: Protocol Timeline. 5 doubling doses of Angiotensin-II (ANG-II) and norepinephrine (NE) were administered (3 min per dose), with the goal of a "saturating dose" that produced maximal local receptor occupancy. If deemed necessary, a sixth doubling dose of ANG-II (25.6 ng/dl/min) or NE (80 ng/dl/min) was given in order to induce maximal changes in BA blood flow and BA diameter. The order of ANG-II and NE administration was balanced, but the remainder of the protocol was ordered due to lasting effects of phentolamine (PHEN) on brachial artery blood flow.


25 min
30 min
25 min
20 min
ANG II (0.8-12.8 ng/dl/min)
REST
NE (2.5-40 ng/dl/min)
REST

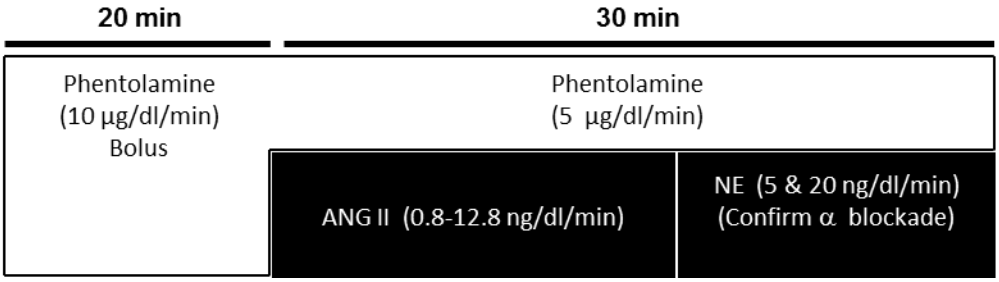
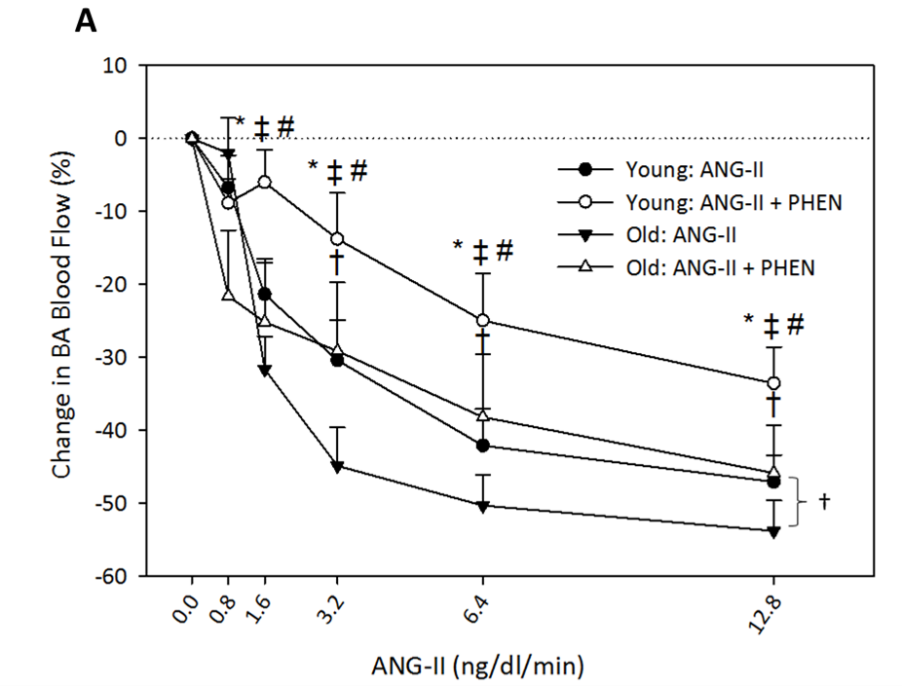

20 min
30 min
Phentolamine
(10 µg/dl/min)
Bolus
Phentolamine
(5 µg/dl/min)
ANG II (0.8-12.8 ng/dl/min)
NE (5 & 20 ng/dl/min)
(Confirm α blockade)

FIGURE 2.2 A: Changes in brachial artery (BA) blood flow in young (circles) and old (triangles) subjects during continuous infusion of angiotensin-II (ANG-II) before (filled symbols), and after (open symbols) alpha-adrenergic blockade with phentolamine (PHEN). **B:** Maximal reductions in BA blood flow induced by norepinephrine (NE), ANG-II alone, and ANG-II after nonselective alpha-adrenergic blockade with PHEN. The reduction in BA blood flow in response to NE (black bars) was similar in both groups. The ANG-II (light grey bars) - mediated reduction in BA blood flow was significantly greater in old compared to young subjects, and this age-related response to ANG-II was ablated in the presence of alpha-adrenergic blockade (dark grey bars). *Significantly different then baseline in both you and old. ‡Significantly different from PHEN in both young and old. # Significantly different from ANG-II alone in both young and old. ## Significantly different from ANG-II alone in the old. † Significantly different from young, $P<0.05$.


A
Change in BA Blood Flow (%)
10
0
-10
-20
-30
-40
-50
-60
0.0
0.8
1.6
3.2
6.4
12.8
ANG-II (ng/dl/min)
Young: ANG-II
Young: ANG-II + PHEN
Old: ANG-II
Old: ANG-II + PHEN
* ‡ #
†

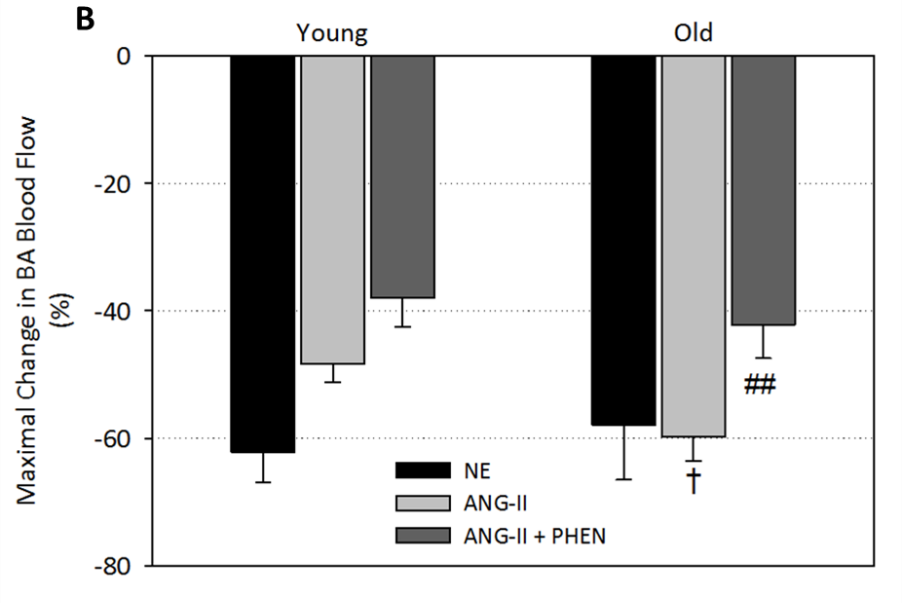
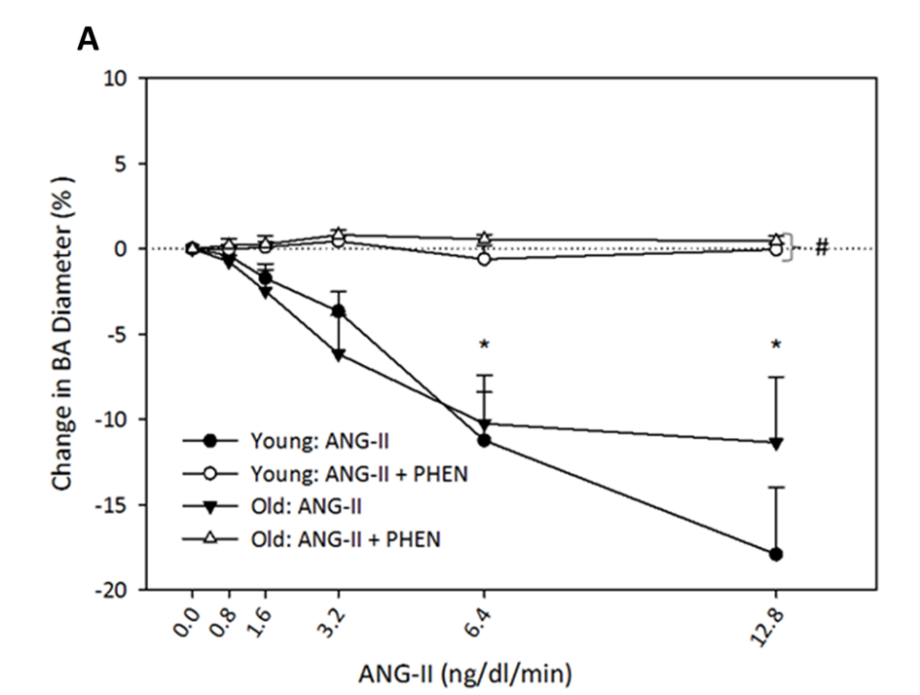

B
Young
Old
Maximal Change in BA Blood Flow (%)
0
-20
-40
-60
-80
NE
ANG-II
ANG-II + PHEN
##
†

FIGURE 2.3 A: Changes in brachial artery (BA) diameter in young (circles) and old (triangles) subjects during continuous infusion of angiotensin-II (ANG-II) alone (filled symbols), and ANG-II with concomitant alpha-adrenergic blockade with phentolamine (PHEN) (open symbols). **B:** Maximal reductions in BA diameter induced by Norepinephrine (NE), ANG-II alone, and ANG-II with concomitant alpha-adrenergic blockade via PHEN. Diameter changes in response to NE (black bars) and ANG-II (light gray bars) were similar in both groups, while BA diameter was virtually unchanged in the presence of alpha-adrenergic blockade (dark grey bars). * Significantly different from baseline in both you and old. # Significantly different from ANG II alone in young and old, $P<0.05$.


A
10
5
0
-5
-10
-15
-20
Change in BA Diameter (%)
#
*
*
Young: ANG-II
Young: ANG-II + PHEN
Old: ANG-II
Old: ANG-II + PHEN
0.0
0.8
1.6
3.2
6.4
12.8
ANG-II (ng/dl/min)

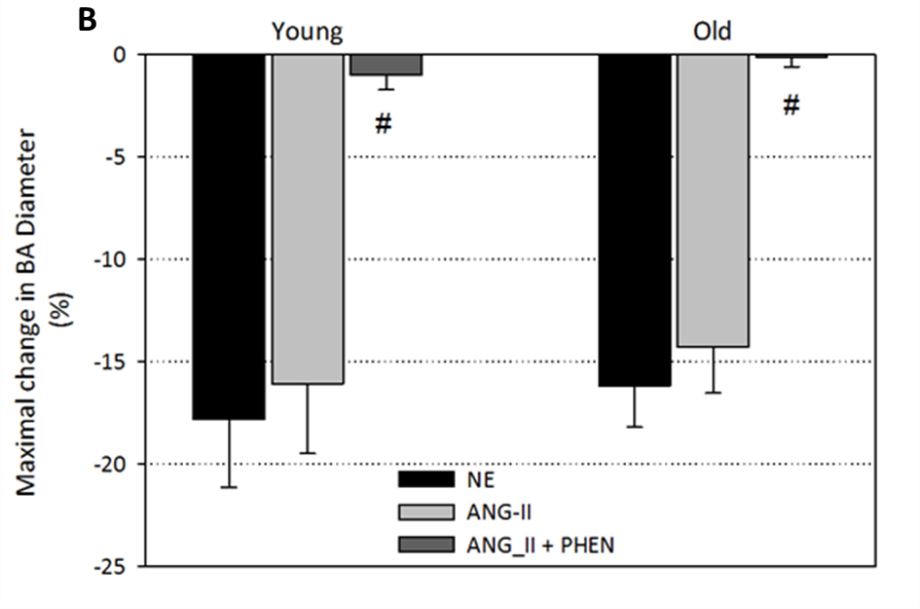

B
Young
Old
0
-5
-10
-15
-20
-25
Maximal change in BA Diameter (%)
#
#
NE
ANG-II
ANG_II + PHEN

Table 2.1. Subject characteristics

	YOUNG	**OLD**
Age, yrs	26±1	68±3†
Height, cm	176±2	173±4
Weight, kg	74±3	84±4
Body mass index, kg/m^2	24±1	28±1†
Arm volume, dl	16.7±1.4	15.7±0.8
Glucose, mg/dl	79±7	84±2
Total Cholesterol, mg/dl	134±22	146±7
Triglycerides, mg/dl	62±10	83±5†
HDL, mg/dl	47±7	48±7
LDL, mg/dl	76±12	83±3
MSNA (bursts/min) (*n = 9)*	21±3	40±6†
Plasma NE (venous, pg/ml) (*n = 11)*	371±54	877±162†
NE spillover (pg/min) (*n = 11)*	7855±1469	35592±5978†
Plasma ANG-II (arterial pg/ml) (*n = 10)*	64.2±8.7	58±12.7

ANG-II, Angiotensin-II; HDL, high density lipoprotein; LDL, low density lipoprotein; MSNA, muscle sympathetic nerve activity; NE, norepinephrine. Values are mean ± SE. † Significantly different from young, P <0.05.

Table 2.2. Hemodynamic responses to intra-arterial infusions of angiotensin II (ANG-II)

Dose (ng/dl/min)	Baseline	0.8	1.6	3.2	6.4	12.8
YOUNG						
Heart rate, beats/min	63±4	63±4	67±8	62±3	62±3	59±2
Mean arterial pressure, mmHg	99±4	102±4	100±3	99±5	100±5	107±4
Brachial artery diameter (mm)	3.69±0.20	3.67±0.19	3.62±0.19	3.55±0.19	3.30±0.25*	2.88±0.26*
Brachial artery velocity (cm/s)	6.5±0.6	6.1±0.6	5.2±0.4*	4.7±0.3*	4.6±0.4*	4.7±0.6*
Brachial artery blood flow (ml/min)	42±6	39±6	32±8	28±3*	23±3*	18±1*
Brachial artery blood flow (Δml/min)	0±0	-3±2	-9±3*	-14±4*	-19±4*	-16±3*
Brachial artery vascular conductance (ml/min/mmHg)	0.43±0.06	0.39±0.06	0.33±0.05*	0.30±0.04*	0.26±0.04*	0.20±0.03*
Non-infused brachial artery blood flow (Δml/min)	0±0	-2±2	0±1	3±3	2±2	-3±5
OLD						
Heart rate, beats/min	61±5	63±5	61±5	64±5	63±5	61±5
Mean arterial pressure, mmHg	111±3†	111±3	111±4	116±7†	118±3†	120±4†
Brachial artery diameter (mm)	4.27±0.38	4.24±0.38	4.18±0.40	4.00±0.34	3.83±0.36*†	3.76±0.45*†
Brachial artery velocity (cm/s)	8.4±1.6	8.0±1.1	5.7±0.9*	5.1±0.9*	5.0±0.9*	5.1±0.9*
Brachial artery blood flow (ml/min)	65±5†	63±5	42±4*†	35±4*	32±4*†	31±3*†
Brachial artery blood flow (Δml/min)	0±0	-3±5	-22±7*†	-30±6*†	-33±5*†	-38±7*†
Brachial artery vascular conductance (ml/min/mmHg)	0.58±0.04†	0.57±0.05	0.39±0.04*	0.31±0.03*	0.27±0.03*	0.26±0.03*
Non-infused brachial artery blood flow (Δml/min)	0±0	0±3	2±2	2±2	4±4	-5±8

Values are means ± SE. † Significantly different than young, $P < 0.05$. * Significantly different than baseline, $P < 0.05$.

Table 2.3. Hemodynamic responses to intra-arterial infusions of norepinephrine (NE)

Dose (ng/dl/min)	Baseline	2.5	5	10	20	40
YOUNG						
Heart rate, beats/min	62±3	60±3	64±4	59±3	60±3	59±3
Mean arterial pressure, mmHg	102±3	100±4	102±3	99±4	99±4	103±4
Brachial artery diameter (mm)	3.67±0.20	3.66±0.20	3.60±0.19	3.44±0.16*	3.19±0.21*	2.93±0.23*
Brachial artery velocity (cm/s)	7.4±0.5	7.0±0.8	6.6±0.5	6.0±0.5*	5.9±0.6*	5.2±0.7*
Brachial artery blood flow (ml/min)	49±7	46±7	41±5*	34±4*	28±3*	21±4*
Brachial artery vascular conductance (ml/min/mmHg)	0.48±0.07	0.47±0.80	0.41±0.06	0.35±0.04*	0.28±0.04*	0.21±0.04*
Non-infused brachial artery blood flow (Δml/min)	0±0	-2±3	2±2	8±8	2±4	6±6
OLD						
Heart rate, beats/min	61±5	57±5	60±5	60±4	59±4	59±4
Mean arterial pressure, mmHg	113±5	114±4	114±5	113±6	113±6	109±3
Brachial artery diameter (mm)	4.40±0.41	4.36±0.42	4.28±0.44	4.12±0.40*	3.81±0.48*	3.27±0.41*
Brachial artery velocity (cm/s)	7.5±1.5	7.9±1.6	7.1±1.9	6.4±1.2*	5.7±1.0*	6.1±1.5*
Brachial artery blood flow (ml/min)	63±8	65±10	54±10*	49±9*	36±5*	33±11*
Brachial artery vascular conductance (ml/min/mmHg)	0.56±0.07	0.57±0.08	0.47±0.10	0.43±0.07*	0.25±0.07*	0.21±0.10*
Non-infused brachial artery blood flow (Δml/min)	0±0	1±2	1±4	7±6	2±4	1±4

Values are means ± SE. * Significantly different than baseline, $P < 0.05$.

Table 4. Hemodynamic responses to intra-arterial infusions of angiotensin II (ANG-II) with alpha-receptor antagonism

Dose (ng/dl/min)	Baseline	PHEN	0.8	1.6	3.2	6.4	12.8
YOUNG							
Heart rate, beats/min	61±3	65±3	65±3	62±3	60±2‡	62±2	62±3
Mean arterial pressure, mmHg	102±2	102±3	100±3	100±4	102±5	110±3‡	108±3
Brachial artery diameter (mm)	3.71±0.22	3.86±0.22*	3.86±0.22	3.86±0.21	3.87±0.21	3.83±0.20‡	3.77±0.23
Brachial artery velocity (cm/s)	10.7±1.6	31.6±5.2*	28.0±4.4	29.2±4.6	26.6±4.3‡	23.6±3.4‡	22.1±2.0‡
Brachial artery blood flow (ml/min)	71±13	220±44*	192±33	201±35	180±30‡	162±29‡	150±20‡
Brachial artery blood flow (Δml/min)	0±0	149±39*	-29±11	-20±10	-40±19‡	-58±20‡	-92±26‡
Brachial artery vascular conductance (ml/min/mmHg)	0.70±0.13	2.19±0.44*	1.90±0.32	2.00±0.34	1.76±0.28‡	1.51±0.29‡	1.37±0.19‡
Non-infused brachial artery blood flow (Δml/min)	0±0	4±2	1±3	-1±2	1±4	5±4	4±4
OLD							
Heart rate, beats/min	64±6	67±2	65±7	66±6	65±7	64±6	65±8
Mean arterial pressure, mmHg	115±5†	115±5†	115±4†	115±4†	116±4†	119±5	123±6
Brachial artery diameter (mm)	4.20±0.46	4.32±0.26*	4.34±0.27	4.34±0.27	4.36±0.27‡	4.36±0.27‡	4.38±0.26‡
Brachial artery velocity (cm/s)	11.1±1.8	26.7±6.5*	21.1±6.1	21.0±6.3	19.3±5.8‡	18.2±5.4‡	14.1±5.5‡
Brachial artery blood flow (ml/min)	93±24	275±43*	231±30	228±26‡	214±31‡	198±33‡	166±40‡
Brachial artery blood flow (Δml/min)	0±0	182±47*	-44±16	-47±15‡	-61±18‡	-77±26‡	-110±60‡
Brachial artery vascular conductance (ml/min/mmHg)	0.79±0.18	2.26±0.90	2.08±0.93	1.91±0.91	1.76±0.86‡	1.59±0.76‡	1.25±0.76‡
Non-infused brachial artery blood flow (Δml/min)	0±0	-2±3	2±2	8±8	2±4	6±6	7±7

PHEN, phentoamine. PHEN hemodynamics used as baseline parameters for ANG-II dose-response. Values are means ± SE. † Significantly different than young, $P < 0.05$. *Significantly different than baseline, $P < 0.05$. ‡Significantly different than PHEN, $P < 0.05$.

CHAPTER 3

TAMING THE “SLEEPING GIANT”: THE ROLE OF ENDOTHELIN-1 IN THE REGULATION OF SKELETAL MUSCLE BLOOD FLOW AND ARTERIAL PRESSURE DURING EXERCISE

Abstract

The degree to which endogenous ET-1 participates in the regulation of skeletal muscle vascular tone and arterial blood pressure during dynamic exercise is largely unknown. Thus, in eight young (24±2 yrs), healthy volunteers, we examined changes in common femoral artery (CFA) blood flow (ultrasound Doppler), mean CFA blood pressure, heart rate, leg a-vO_2 difference, leg VO_2, pH, and net ET-1 and lactate release at rest and during knee extensor exercise (0-5-10-15-20-30W) before and after endothelin receptor subtype A (ET_A) blockade (intra-arterial BQ-123, 10 nmol/min/liter of thigh volume). At rest, BQ-123 evoked no change in CFA blood flow or blood pressure. During exercise, net ET-1 release across the exercising leg increased ~3-fold. BQ-123 increased CFA blood flow by ~20% (113 ± 76, 176 ± 83, 304 ± 108, 364 ± 130, 502 ± 117, and 570 ± 178 ml/min at 0-5-10-15-20-30W, respectively) and attenuated the exercise-induced increase in mean CFA blood pressure by ~6% (-3 ± 3, -3 ± 2, -6 ± 3, -7 ± 1, -8 ± 1, -9 ± 3 mmHg at 0-5-10-15-20-30W, respectively) in an intensity-dependent manner. The increase in CFA blood flow was accompanied by a ~9% increase in leg VO_2 with unchanged a-vO_2, suggesting a reduced efficiency of the active muscle following ET_A receptor blockade. Together, these findings have identified a significant role of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the ET_A receptor as an important mechanism for both restraint of blood flow in the exercising limb and support of arterial blood pressure in healthy, young adults.

Introduction

During dynamic exercise of increasing intensity, oxygen demand of the exercising muscle is dramatically elevated, requiring a marked increase in skeletal muscle blood flow that is accomplished through a combination of systemic sympathoexcitation and local metabolic vasodilation (22). Sympathetic activation acts both centrally and peripherally, increasing cardiac output and inducing vasoconstriction of the internal organs and less active skeletal muscle in order to redistribute blood flow to more active tissue (7, 45, 47). However, in the face of local metabolic byproducts produced by the exercising muscle, the sympathetic vasoconstrictor stimulus appears less effective, a phenomenon termed "functional sympatholysis" (41). In combination with regional vasodilatory influences, this reduction in overall vasoconstrictor "efficacy" evokes a requisite increase in limb blood flow and vascular conductance within the exercising limb to ensure adequate delivery of oxygen to the exercising tissue.

While vasodilation of active skeletal muscle is an essential component in the cardiovascular response to exercise, it must be balanced with the concomitant need to support arterial blood pressure. Indeed, leg blood flow values as high as 4 L/kg/min have been observed during small muscle mass exercise (42), suggesting that the skeletal muscle represents a "sleeping giant" that must be under tonic restraint to avoid hypotension during intense, whole-body exercise (46). Studies utilizing isolated arm and leg exercise modalities support this concept, demonstrating a reduction in arterial blood pressure when additional muscle mass is recruited at near maximal exercise intensities, even in the face of drastic increases in sympathetic nervous system activity (10, 42, 50, 55). From these observations, the concept has emerged that exercise-induced metabolic

vasodilation must be restrained to some degree in order to protect against precipitous drops in arterial blood pressure as the limit of cardiac output is reached. Though a small number of studies have documented some degree of sympathetic restraint within the exercising limb (19, 37), the preponderance of data from studies in both animals and humans have demonstrated substantial blunting of sympathetic vasoconstriction in the exercising skeletal muscle vasculature (9, 11, 44, 48, 52, 56). In the face of this intensity-dependent loss of regional sympathetic control, it is likely that nonadrenergic vasoconstrictor pathways may contribute importantly to the balance of skeletal muscle blood flow and arterial blood pressure during exercise.

One such vasoconstrictor that may be relevant in this regard is Endothelin-1 (ET-1), the most potent endogenous vasoconstrictor (61). In the periphery, ET-1 provokes a sustained vasoconstrictor response by binding to endothelin subtype A (ET_A) and B (ET_B) receptors located primarily on the vascular smooth muscle (4, 61). This endothelial-derived peptide is released in response to a variety of stimuli, including increases in pulsatile stretch (26), shear stress (31), hypoxia (21), and a reduction in pH. Since these physical and chemical stimuli are among the host of changes that take place within the skeletal muscle during exercise (25), ET-1 has been implicated as an important signaling molecule in the response to exercise. Indeed, circulating concentrations of ET-1 have been documented to increase in an intensity-dependent manner during exercise (28), suggesting a potential role of ET-1 in blood flow distribution and the support of arterial blood pressure. Previous work in animals has evaluated the tonic ET-1-mediated restraint on vascular tone via ET_A and concomitant ET_A/ET_B receptor blockade during exercise, and reported that ET_A receptor inhibition improved exercising muscle blood flow (35).

Recently, our group identified an attenuated vasoconstriction in response to intra-arterial infusion of ET-1 during dynamic knee-extensor exercise in young, healthy humans (57). However, the role of endogenous ET-1, specifically the ET_A receptor, in the regulation of exercising limb blood flow and arterial blood pressure has yet to be investigated.

Therefore, the current study sought to determine the endogenous contribution of the ET_A receptor in the regulation of blood flow and arterial blood pressure during dynamic exercise of increasing exercise intensities. We hypothesized that: 1) intra-arterial, local ET_A receptor blockade would enhance exercising skeletal muscle blood flow in an intensity-dependent manner; and 2) The reduction in vascular tone induced by ET_A receptor blockade would attenuate the increase in arterial blood pressure present during exercise of increasing intensity.

Methods

Subjects

Eight young (24 ± 2 yrs), healthy subjects were enrolled in the present study. All subjects were nonsmokers and were normally active. Subjects were not taking any prescription medication and were free of overt cardiovascular disease, as indicated by a health history. Protocol approval and written informed consent were obtained according to the University of Utah and the Salt Lake City Veterans Affairs (VA) Medical Center Institutional Review Board requirements. All data collection took place at the VA Salt Lake City Geriatric, Research, Education, and Clinical Center (GRECC) in the Utah Vascular Research Laboratory (UVRL).

Protocols

Subjects reported to the UVRL at 0800 on the experimental day. After 30 min of supine rest, two catheters (common femoral artery (CFA) and femoral vein) were placed using sterile technique, as previously reported (1, 5, 60). After catheter placement, subjects rested for ~30 min, and then underwent the protocol as outlined in Figure 3.1. Due to the duration of the study, subjects were given 1/2 cup of corn flakes and 1/2 cup of skim milk before the start of the control (saline) and BQ-123 trials to prevent hypoglycemia (Figure 3.1). All data collection took place with subjects in a semirecumbent position (60° reclined), and all studies were performed in a thermoneutral environment.

Due to the long lasting effects of BQ-123, the drug administration portion of the protocol always occurred after the saline portion of the protocol. Thus, to minimize the risk that the observed changes in CFA blood flow and arterial blood pressure were the consequence of an exercise ordering effect, rather than the effect of the drug *per se*, subjects returned to the laboratory on a separate day to undergo an exercise time control study. For this visit, catheters were not placed, and no drugs were administered. Apart from these differences, the timeline for this study was identical to the drug infusion day, with the addition of leg blood flow measurements immediately before and after a light meal to examine possible postprandial hemodynamic effects.

Drug Infusions

Thigh volumes were determined anthropometrically, and then used for the calculation of drug dosing. A selective ET_A receptor antagonist (BQ-123 Clinalfa,

Calbiochem-Novabiochem, La¨ufelfingen, Switzerland) was prepared in normal saline (0.9% NaCl) and administered intra-arterially (CFA) at 10 nmol/min/liter of thigh volume (infusion rates 0.8-1.5 ml/min). This dose has been documented to induce an apparent plateau in vasodilation in both the forearm (13, 16) and quadriceps (51), without affecting arterial blood pressure. BQ-123 has been documented to have a high affinity for the ET_A receptor (17) and effectively counteracts the vasoconstrictor effect of ET-1 infusion in the human forearm (15). During the control trial, normal saline (0.9% NaCl) was administered intra-arterially into the CFA at the same infusion rates as BQ-123.

Exercise Model

The knee extensor (KE) paradigm implemented in this study has been described previously (2, 23, 43, 59). Briefly, subjects were seated on an adjustable chair with a cycle ergometer (model 828E; Monark Exercise AB, Vansbro, Sweden) positioned behind them. Resistance was created by applying friction to the flywheel, which was turned by the subject via a metal bar that connects the crank arm of the ergometer and the subject's foot, which is placed in a metal boot. Sixty contractions per minute were maintained at each work rate. Subjects exercised for 3 min at six exercise intensities (0-5-10-15-20-30W) with 3 min recovery following two successive exercise bouts (Figure 3.1).

Measurements

Ultrasound Doppler assessments

Measurements of CFA blood velocity and vessel diameter were performed in the infused leg using a Logiq 7 ultrasound Doppler system (GE Medical Systems,

Milwaukee, WI) operating in duplex mode. The Logic 7 was equipped with a linear array transducer operating at an imaging frequency of 14 MHz. The CFA was insonated 2-3 cm proximal to the bifurcation of the CFA into the superficial and deep branches. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 5 MHz, operated in the high-pulsed repetition frequency mode (2-25 kHz). Care was taken to avoid aliasing the blood velocity spectra by using scale adjustments, especially during exercise. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less (24). The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization.

Common femoral artery diameter, blood velocity, and blood flow analyses

At all sample points, arterial diameter (cm) and angle-corrected, time-averaged, and intensity-weighted mean blood velocity (V_{mean}) values were calculated using commercially available software (Logic 7). Using measured arterial diameter and V_{mean}, CFA blood flow was calculated according to the equation:

$$\text{CFA blood flow (ml/min)} = (V_{mean} \times \pi\ (\text{vessel diameter}/2)^2 \times 60)$$

Arterial blood pressure, vascular conductance, and heart rate assessment

Arterial blood pressure measurements were collected continuously from the indwelling catheter placed in CFA with the pressure transducer placed at the level of the catheter (Transpac IV, Abbott Laboratories). Mean CFA blood pressure (mmHg) was calculated as diastolic arterial pressure + (arterial pulse pressure x 0.33). On the time control study day, mean arterial blood pressure was determined noninvasively using

finger photoplethysmography (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). CFA vascular conductance was calculated as CFA blood flow / mean CFA blood pressure. Heart rate was monitored from a standard three-lead ECG recorded in duplicate on the data acquisition device (BIOPAC U.S.A.) and the Logic 7.

Blood chemistry

A lipid panel was obtained for all subjects by standard techniques. At each exercise intensity, samples (3-4 ml) of femoral arterial and venous blood were collected. 1 ml of arterial and venous blood were presented anaerobically to a GEM 4000 blood-gas analyzer and co-oximeter (Instrumentation Laboratories, Bedford, MA) to obtain arterial and venous total hemoglobin (tHb) oxyhemoglobin saturation (SO_2), partial pressure of oxygen (PO_2), hematocrit (hct), lactate, and pH. Blood oxygen content (CO_2) (ml/dl) was calculated as:

$$\text{Blood oxygen content (ml/dl)} = 1.39\ (\text{tHb}) \times (SO_2/100) + 0.003 \times PO_2$$

Leg oxygen consumption (VO_2) (ml/min) was calculated as:

$$\text{Leg oxygen consumption (ml/min)} = (CaO_2 - CvO_2) \times \text{CFA blood flow}$$

where CaO_2 and CvO_2 represent arterial and venous oxygen content. The remaining blood was spun down for plasma samples, and stored at -80°C until analysis.

Plasma ET-1 concentrations were evaluated via sandwich radioimmunoassay (Stressgen Bioreagents). Using arterial (C_A) and venous (C_V) plasma ET-1 or lactate concentrations, with corrections for hematocrit (hct) and CFA blood flow, "net ET-1

release" and "net lactate release" were calculated (18, 53) according to the following equation:

$$\text{Net ET-1 or lactate release} = (C_V - C_A) \times (\text{CFA blood flow} \times ((101 - (\text{hct}/100))$$

Statistical Analyses

Statistics were performed with the use of commercially available software (SigmaStat 3.10; Systat Software, Point Richmond, CA). Multiple 2x7 repeated measures analysis of variance (ANOVA) were used to identify significant changes in measured variables within and between drug groups and across exercise intensities. Multiple 1x7 repeated measures ANOVA were used to identify significant changes in BQ-123-induced changes. When a significant main effect was found ($p < 0.05$), the Holm-Sidak method was used for alpha adjustment and post hoc analysis. All group data are expressed as mean ± standard error.

Results

Subject Characteristics

Subject characteristics are presented in Table 3.1.

BQ-123 at Rest and Exercise

Endothelin subtype A (ET_A) receptor inhibition BQ-123 was infused continuously (10 nmol/min/liter of thigh volume; infusion rate 0.8-1.5 ml/min) for 45 min. This intra-arterial infusion did not significantly change heart rate (Table 3.2), mean CFA blood pressure, CFA blood flow, CFA vascular conductance, leg VO_2, Leg a-vO_{2diff}, pH, net lactate release, or net ET-1 release (Figures 3.2, 3.3, and Table 3.2). During exercise,

BQ-123 induced a significant intensity-dependent increase in CFA blood flow and vascular conductance (Figures 3.2 and 3.3), accompanied by an intensity-dependent reduction in mean CFA blood pressure (Figures 3.2 and 3.3) and no change in heart rate (Table 3.2) compared to the saline trial. The vasodilation provoked by BQ-123 during exercise led to an increase in leg VO_2 (Table 3.2) without an alteration in leg a-vO_{2diff} (Table 3.2). Additionally, a significant increase in the net ET-1 release and no significant changes in net lactate release or pH were observed during BQ-123 infusion during exercise compared to the saline trial (Table 3.2).

Exercise Time Control Study

All eight subjects who participated in the drug infusion protocol returned to the lab for an exercise time control protocol. This additional protocol allowed for the assessment of CFA blood flow and mean arterial blood pressure during multiple exercise bouts and the consequence of food consumption without the influence of catheter insertion and drug infusions. Measurements were highly reproducible, with no significant difference in CFA blood flow or mean arterial blood pressure between the two exercise bouts (Figure 3.4).

Discussion

There are several key findings from the present study. During small muscle mass (knee-extensor) exercise, common femoral artery (CFA) blood flow and vascular conductance were significantly elevated in the exercising limb following ET_A receptor inhibition, demonstrating a substantial (~20%) ET_A-mediated restraint of skeletal muscle blood flow at the higher exercise intensities. ET_A blockade also blunted the intensity-

dependent rise in arterial blood pressure produced during exercise, suggesting that the ET-1 pathway may also play an important role in the support of blood pressure. The increase in CFA blood flow following BQ-123 was accompanied by an increase in leg VO_2 with no change in a-vO_2, effectively decreasing intramuscular efficiency. A significant rise in net ET-1 release across the exercising leg in an intensity-dependent manner was also observed. Collectively, these findings have identified a significant role of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the ET_A receptor as an important mechanism for both restraint of blood flow in the exercising limb and support of arterial blood pressure in healthy, young adults.

ET_A Receptor Antagonism during Exercise

With increasing exercise intensity, one of the principle ways in which skeletal muscle blood flow is elevated to meet the metabolic demands of the exercising muscle is through the reduction of vascular tone in the vascular beds perfusing the exercising muscle. Indeed, the attenuated ability of infused sympathomimetics to evoke vasoconstriction during exercise in an intensity-dependent manner provides evidence that sympathetic vasoconstrictor pathways are at least partially inhibited during exercise (8, 11, 52, 56, 57). However, this increase in skeletal muscle blood flow must be balanced by the concomitant need to support arterial blood pressure (45). With the knowledge that ET-1 provokes a sustained vasoconstriction (14, 35) and circulating levels increase during exercise (28), we sought to evaluate the putative role of the endothelin subtype A (ET_A) receptors in the regulation of skeletal muscle blood flow and maintenance of arterial blood pressure in the exercising limb vasculature during bouts of exercise of

increasing intensity. Results indicate that the ET-1 vasoconstrictor pathway plays an important role in the regulation of limb blood flow, arterial blood pressure, and intramuscular efficiency as discussed below.

In support of our first hypothesis, we observed a progressively greater BQ-123-induced increase in CFA blood flow with increasing exercise intensity (Figure 3.3). This observed response is in contrast to that of McEniery *et al.* (34), who examined the effect of ET_A receptor blockade on limb blood flow during 15 min of static intermittent handgrip exercise in hypertensive and normotensive individuals. This study reported no difference in forearm blood flow during exercise between the blocked and control trials in the normotensive group, suggesting no apparent role of the ET_A receptor in the regulation of vascular tone during handgrip exercise. This discrepancy between the current study and that of McEniery *et al.* may be partially attributable to the differences in limbs that were exercised. First, our group (58) and others (40) have identified substantial differences in the regulation of blood flow between the arms and legs and this limb specificity might explain the differing responses to ET_A receptor blockade between the former and present studies. Additionally, in this previous study (34), the vascular beds perfusing the forearm may not vasodilate to a degree in which the maintenance of arterial blood pressure is challenged. The use of the knee-extensor exercise paradigm in the present study allowed for the quadriceps muscle to be exercised, a large postural muscle-group with a vasodilatory capacity large enough to challenge the maintenance of arterial blood pressure. With this approach, we have identified for the first time a functional role of the ET-1 pathway in the regulation of exercise muscle blood flow in the large, ambulatory muscle groups of the leg.

In addition to restraint of muscle blood flow, ET_A receptor-mediated maintenance of vascular tone in the exercising skeletal muscle vasculature also appears to play a significant role in the support of arterial blood pressure. Indeed, we observed an overall reduction in the "exercise pressor" response (36) following ET_A receptor blockade (Figure 3.2). Interestingly, this hypotensive effect was intensity-dependent (Figure 3.3), indicating a proportionally greater role of ET-1 in supporting blood pressure at higher exercise intensities. In the presence of the increased perfusion induced by ET_A receptor blockade, there is the possibility for an enhanced washout of metabolic byproducts and subsequent alteration in the metabolic milieu within the exercise muscle. This could attenuate firing of group III and IV afferent nerves and attenuate the metaboreflex-induced pressor response, providing an alternate explanation for the reduction in blood pressure in the BQ-123 trial. However, pH and net lactate release, two important metabolic variables thought to stimulate group III and IV afferent nerves (20), were unchanged between trials (Table 3.2), increasing confidence that the observed changes in arterial blood pressure were directly related to ET_A receptor inhibition.

To our knowledge, this is the first study to identify a significant role of ET-1 in the regulation of arterial blood pressure during exercise. In animals, Maeda et al. (29) failed to show a change in exercising arterial blood pressure following ET_A receptor blockade trial, though the systemic administration of the ET_A receptor blocking drug preclude direct comparison with the present findings. It is tempting to speculate that this observed reduction in blood pressure demonstrates the role of the ET-1 pathway in protecting against the possibility of hypotension during exercise, as is seen when additional muscle mass is recruited at near maximal exercise intensities (10, 42, 50, 55).

The intensity-dependent contribution of the ET_A receptor in the maintenance of vascular tone also plays a role in the regulation of intramuscular efficiency. Following ET_A receptor blockade, leg oxygen consumption (VO_2) during exercise increased with no alteration in leg arterial venous oxygen difference ($a\text{-}vO_{2diff}$) (Table 3.2). Previously, it was thought that during exercise paradigms in which cardiac output does not limit blood flow to the exercising muscle, muscle was effectively overperfused (3), evident by the observations that the venous blood draining the exercising muscle remaining fairly saturated with O_2 (3, 38). However, the current study is in agreement with previous work documenting an increase in skeletal muscle VO_2 when blood flow to the exercising skeletal muscle was increased via stellate ganglion block (19). In light of this increase in leg VO_2 induced by ET_A receptor blockade, the absence of an alteration in net lactate release (Table 3.2) indicates that the restraint in oxygen delivery mediated by endogenous ET-1 does not play role in governing relative exercise intensity. Collectively, these data suggest that the ET-1 vasoconstrictor pathway appears to importantly contribute to optimizing O_2 delivery and VO_2 through restraint of skeletal muscle blood flow during exercise. Whether the documented increase in leg VO_2 induced by ET_A receptor inhibition in the current study is directly due to an alteration in metabolism of the exercising skeletal muscle or just through an enhancement of oxygen delivery requires further investigation.

In many pathophysiologies, an increase in exercising limb blood flow could be viewed as beneficial, inasmuch as O_2 delivery to the exercising tissue is improved. However, the cardiovascular response to exercise appears to be governed by a very complex combination of vasodilatory and vasoconstricting influences that optimize

exercising muscle perfusion while protecting arterial blood pressure. Data from the present study suggest that the ET-1 pathway is a significant contributor to maintaining this balance during exercise. Indeed, disrupting ET-1-mediated vasoconstriction via ET_A receptor inhibition appears to produce an overall deleterious effect in the peripheral circulation, evidenced by the fact that BQ-123 produced an increase in limb blood flow that was met with a decrease in arterial blood pressure and increased O_2 consumption (i.e. reduced efficiency) (Figures 3.2 and 3.3). When viewed in conjunction with the marked rise in net ET-1 release during exercise (Table 3.2), these observed responses indicate that ET-1 provides an important restraining influence to muscle blood flow during exercise, tempering the tendency of this "sleeping giant" to vasodilate at the expense of arterial blood pressure and intramuscular efficiency.

Mechanisms Mediating ET-1 Release during Exercise

Increases in ET-1 production has been documented to increase in response to increases pulsatile stretch (26), shear stress (31) and a reduction in pH (25), all of which occur in an intensity-dependent manner during exercise. ET-1-mediated vasoconstriction is thought to be primarily mediated by the ET_A receptor located on vascular smooth muscle (33). At rest, infusion of ET-1 induces a sustained, slow-developing, long lasting vasoconstriction (33, 57), attributable to the influx of intracellular and extracellular calcium into the cytosolic space (39). The ET-1-mediated increase in cytosolic calcium initially occurs transiently, followed by a sustained increase in cytosolic calcium (39). The rapid (30 sec), transient increase in calcium is induced via a G protein-dependent signal transduction pathway of phospholipase C activation, inositol triphosphate (IP_3) generation, and the release of calcium stored in the sarcoplasmic reticulum (32). Because

the IP_3-mediated pathway is dependent on ET-1 concentration, the exceedingly low concentrations of ET-1 at rest lead to the breakdown of IP_3 and the reuptake of intracellular calcium (33). This potentially explains the limited role of ET-1 in the regulation of vascular tone in the young at rest, a population that exhibits low levels of circulating ET-1 (Table 3.2) (27, 54). However, during exercise, where there is a significant increase in circulating concentrations ET-1 (Table 3.2), the ET-1-mediated increase in IP_3 and subsequent release of intracellular calcium might explain the rapid alterations in the ET_A receptors' contribution to vascular tone exhibited in this study (Figures 3.2 and 3.3).

Clinical Implications

The importance of the ET-1 vasoconstrictor pathway in the regulation of blood flow to the exercising skeletal muscle may be of particular relevance in populations whose phenotype is associated with high circulating levels of ET-1 and an elevated ET-1 contribution to vascular tone such as hypertension (49), heart failure (6), chronic obstructive pulmonary disease (12), and the geriatric population (30). All of these populations are associated with the same degree of exercise intolerance, which might be linked to the excessive increase in vascular tone induced by the ET-1 vasoconstrictor pathway. This raises the interesting possibility of the ET-1 pathway as being a potential peripheral therapeutic target to improve skeletal muscle blood flow and ultimately exercise tolerance in these populations, though further studies are necessary to evaluate whether the current observations can be extended to these patient groups.

Experimental Considerations

Due to the slow acting kinetics of BQ-123 binding and clearance, the study was ordered so the control (saline) trial always preceded the BQ-123 trial. However, an exercise time control trial on a separate day demonstrated highly reproducible measurements of CFA blood flow and mean arterial blood pressure during repeated bouts of KE exercise (Figure 3.4), providing confidence in the comparison of the control and the BQ-123 trials. Additionally, due to the inability to correct hematocrit for trapped plasma volume, observed changes in net ET-1 and lactate release during exercise might be due to hemoconcentration induced during exercise. However, when hematocrit was held constant by incorporating resting hematocrit levels into the equation used to calculate the net release of these two molecules, changes in net ET-1 and lactate release due to hemoconcentration accounted for less than one percent of the exercise-induced increase observed at every work rate. Finally, we cannot exclude the possibility that some of the observed changes in peripheral hemodynamics are due to ET-1 subtype B (ET_B) receptor activation, as BQ-123 is highly selective for ET_A receptors. However, a recent study utilizing combined ET_A and ET_B receptor inhibition failed to identify a significant role for ET_B receptors in the regulation of vascular tone in humans (54).

Summary

The present study has revealed a significant role of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the ET_A receptor as an important mechanism for both restraint of blood flow in the exercising limb and support of arterial blood pressure in healthy, young adults.

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FIGURE 3.1. Experimental protocol. Arrows indicate points at which leg blood flow was recorded and arterial and venous blood samples obtained.

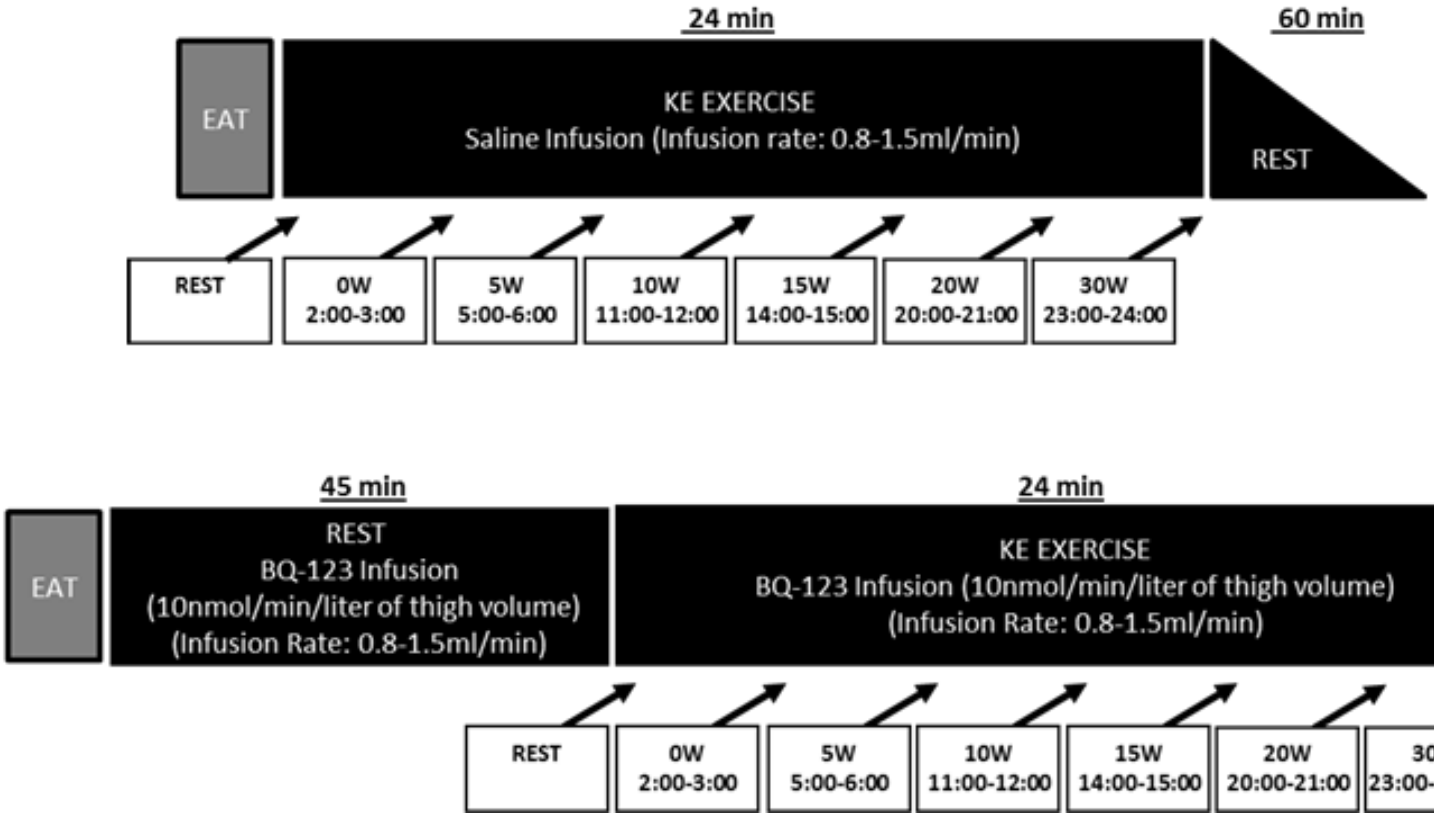
24 min
60 min
EAT
KE EXERCISE
Saline Infusion (Infusion rate: 0.8-1.5ml/min)
REST
REST
0W
2:00-3:00
5W
5:00-6:00
10W
11:00-12:00
15W
14:00-15:00
20W
20:00-21:00
30W
23:00-24:00
45 min
24 min
EAT
REST
BQ-123 Infusion
(10nmol/min/liter of thigh volume)
(Infusion Rate: 0.8-1.5ml/min)
KE EXERCISE
BQ-123 Infusion (10nmol/min/liter of thigh volume)
(Infusion Rate: 0.8-1.5ml/min)
REST
0W
2:00-3:00
5W
5:00-6:00
10W
11:00-12:00
15W
14:00-15:00
20W
20:00-21:00
30W
23:00-124:00

FIGURE 3.2. Common femoral artery (CFA) blood flow (*top*), mean CFA blood pressure (*middle*), and CFA vascular conductance (*bottom*) at rest and exercise during continuous infusion of saline (black circles) and BQ-123 (open circles). The intensity-dependent increase in CFA blood flow, mean CFA blood pressure, and CFA vascular conductance were attenuated during ETA receptor inhibition. * Significant difference from saline, $P<0.05$

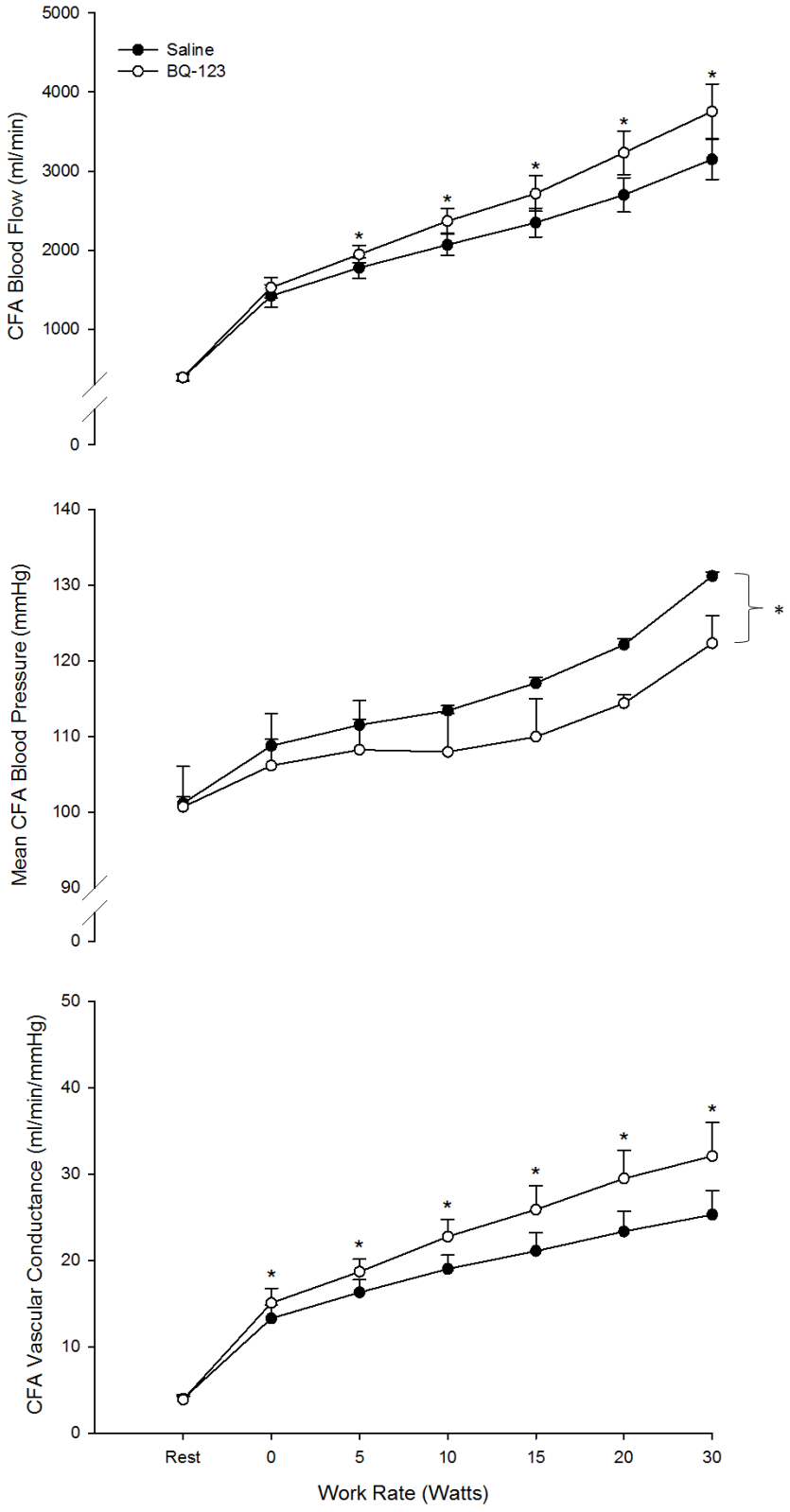
Saline
BQ-123
CFA Blood Flow (ml/min)
5000
4000
3000
2000
1000
0
Mean CFA Blood Pressure (mmHg)
140
130
120
110
100
90
0
*
CFA Vascular Conductance (ml/min/mmHg)
50
40
30
20
10
0
Rest
0
5
10
15
20
30
Work Rate (Watts)

FIGURE 3.3. BQ-123-induced changes in femoral artery (CFA) blood flow (*top*), mean CFA blood pressure (*middle*), and CFA vascular conductance (*bottom*) at rest and during exercise. The contribution of the ET_A receptor to CFA vascular tone and mean CFA blood pressure is enhanced only during exercise in an intensity-dependent manner. + Significant difference from BQ-induced changes at rest, $P<0.05$.

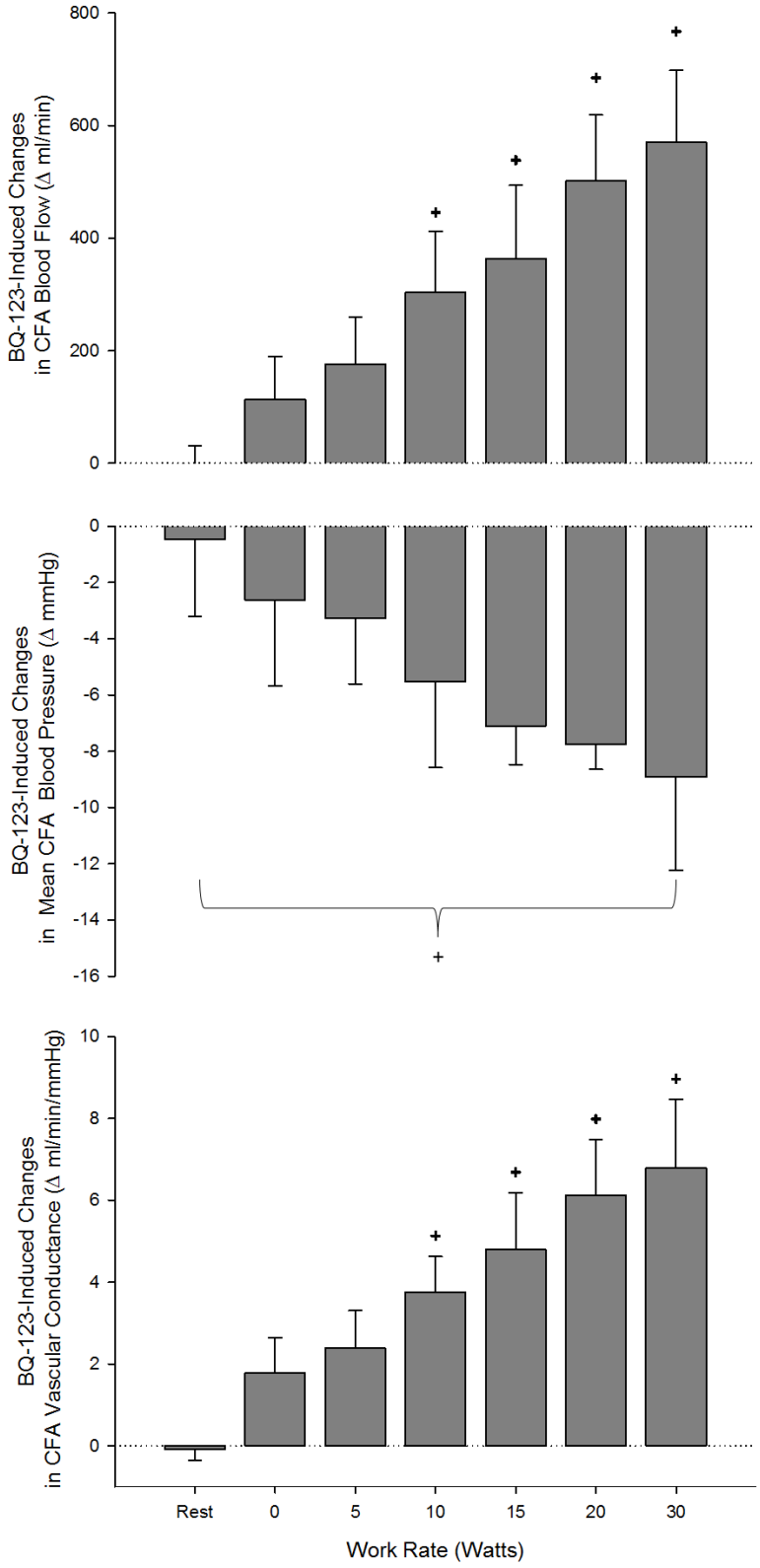
BQ-123-Induced Changes in CFA Blood Flow (Δ ml/min)
800
600
400
200
0
BQ-123-Induced Changes in Mean CFA Blood Pressure (Δ mmHg)
0
-2
-4
-6
-8
-10
-12
-14
-16
BQ-123-Induced Changes in CFA Vascular Conductance (Δ ml/min/mmHg)
10
8
6
4
2
0
Rest
0
5
10
15
20
30
Work Rate (Watts)

FIGURE 3.4: Common femoral artery (CFA) blood flow and mean arterial blood pressure during multiple time points in the exercise time control study are displayed. No significant differences were observed between measurements at rest or at equivalent exercise intensities.

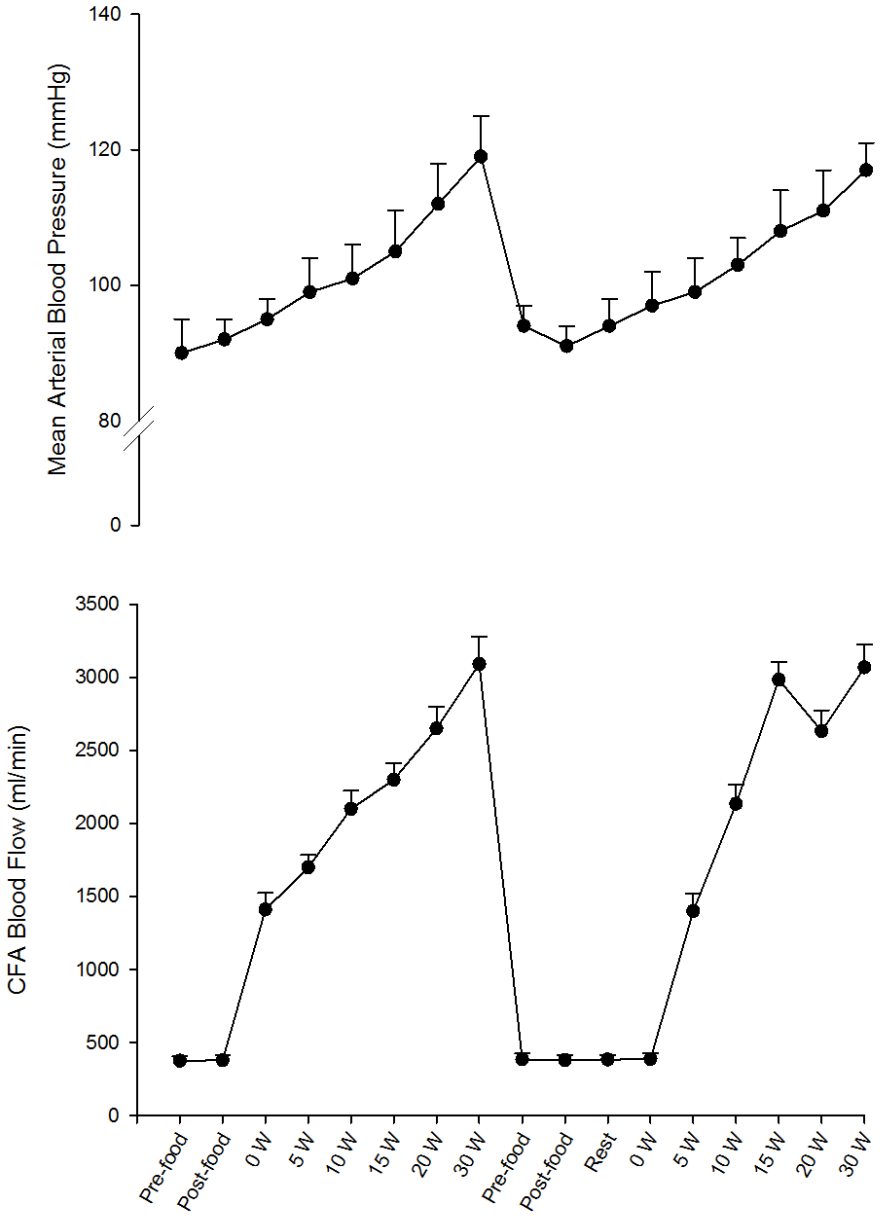
Mean Arterial Blood Pressure (mmHg)
140
120
100
80
0
CFA Blood Flow (ml/min)
3500
3000
2500
2000
1500
1000
500
0
Pre-food
Post-food
0 W
5 W
10 W
15 W
20 W
30 W
Pre-food
Post-food
Rest
0 W
5 W
10 W
15 W
20 W
30 W

Table 3.1. Subject Characteristics

Age, yr	24±2
Height, cm	172±2
Weight, kg	71±6
Body mass index, kg/m^2	24±5
Systolic blood pressure, mmHg	107±5
Diastolic blood pressure, mmHg	73±3
Quadriceps muscle mass, kg	2.0±0.1
Maximum knee-extensor, W	42±4
Glucose, mg/dl	68±6
Total Cholesterol, mg/dl	153 ±10
Triglycerides, mg/dl	69±12
HDL, mg/dl	60±9
LDL, mg/dl	78±9

HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 3.2. Impact of BQ-123 infusion on selected physiological variables at rest and during exercise

Work Rate (Watts)	Rest	0	5	10	15	20	30
Saline							
HR, beats/min	66±4	80±5$^{\$}$	87±6$^{\$}$	91±6$^{\$}$	94±7$^{\$}$	97±7$^{\$}$	101±8$^{\$}$
Leg VO_2, ml/min	20±5	117±16$^{\$}$	146±17$^{\$}$	195±22$^{\$}$	248±33$^{\$}$	307±41$^{\$}$	372±41$^{\$}$
Leg a-vO_2 difference, ml/dl	5.0±0.9	8.3±0.9$^{\$}$	8.2±0.8$^{\$}$	9.3±0.7$^{\$}$	10.2±0.7$^{\$}$	11.1±0.8$^{\$}$	11.6±0.5$^{\$}$
Net ET-1 release, ng/min	26±3	38±4$^{\$}$	46±10$^{\$}$	56±9$^{\$}$	46±10$^{\$}$	64±11$^{\$}$	60±18$^{\$}$
Net lactate release, mmol/min	4±1	19±8$^{\$}$	57±18$^{\$}$	114±5$^{\$}$	178±53$^{\$}$	223±7$^{\$}$	517±79$^{\$}$
Venous pH	7.37±0.01	7.35±0.01$^{\$}$	7.34±0.02$^{\$}$	7.31±0.01$^{\$}$	7.29±0.01$^{\$}$	7.26±0.01$^{\$}$	7.23±0.02$^{\$}$
BQ-123							
HR, beats/min	70±4	84±5$^{\$}$	88±5$^{\$}$	95±6$^{\$}$	94±7$^{\$}$	102±8$^{\$}$	110±12$^{\$}$
Leg VO_2, ml/min	19±1	122±13$^{\$}$	198±18*$^{\$}$	234±22*$^{\$}$	289±38*$^{\$}$	357±53*$^{\$}$	431±51*$^{\$}$
Leg a-vO_2 difference, ml/dl	4.8±0.7	8.0±0.5$^{\$}$	10.1±0.5$^{\$}$	9.9±0.7$^{\$}$	10.4±0.8$^{\$}$	10.7±0.8$^{\$}$	11.3±0.5$^{\$}$
Net ET-1 release, ng/min	22±3	71±11*$^{\$}$	68±14*$^{\$}$	75±12*$^{\$}$	91±13*$^{\$}$	85±21*$^{\$}$	82±16*$^{\$}$
Net lactate release, mmol/ml	8±1	28±8$^{\$}$	50±19$^{\$}$	77±21$^{\$}$	129±33$^{\$}$	346±87$^{\$}$	518±16$^{\$}$
Venous pH	7.38±0.01	7.36±0.01$^{\$}$	7.34±0.01$^{\$}$	7.33±0.01$^{\$}$	7.31±0.01$^{\$}$	7.28±0.01$^{\$}$	7.25±0.02$^{\$}$

HR, heart rate; VO_2, oxygen consumption; a-vO_2diff , arterial venous oxygen difference; ET-1, Endothelin-1. * Significant difference from saline, $P<0.05$; $^{\$}$ Significant difference from rest, $P<0.05$.

CHAPTER 4

IMPACT OF ENDOGENOUS ENDOTHELIN-1 ON THE REGULATION OF VASCULAR TONE AT REST AND DURING DYNAMIC EXERCISE IN THE ELDERLY

Abstract

While the endothelin-1 (ET-1) vasoconstrictor pathway contributes significantly to age-related elevations in resting peripheral vascular tone, the regulatory influence of ET-1 during exercise in the elderly is unknown. Thus, in eight young (24 ± 2 yrs) and eight old (70 ± 3 yrs) healthy volunteers, we examined changes in common femoral artery (CFA) blood flow (ultrasound Doppler) and blood pressure during knee extensor exercise before and after endothelin receptor subtype A (ET_A) blockade (intra-arterial BQ-123, 10 nmol/min/liter of thigh volume). Heart rate, CFA blood pressure, CFA diameter, CFA mean blood velocity, leg a-vO_{2diff} difference, leg VO_2, pH, plasma ET-1, and net lactate release were measured at rest and across a wide range of absolute and relative knee-extensor exercise intensities (0-5-10-15-20-30W and 20-40-60-80% of maximal work rate (WR_{max})). At rest, BQ-123 evoked a significant increase in CFA blood flow in the old (+31 ± 10%) compared to young (+2 ± 7%), with no change in mean CFA blood pressure. During exercise, net ET-1 release across the exercising leg increased 3-4 fold in both groups. BQ-123 increased CFA blood flow at all exercise intensities (ranging from 9 to 23%) in both young and old groups. The exercise-induced increase in mean CFA blood pressure was reduced to a similar degree in both groups (-2 to -10 mmHg) following BQ-123. The increase in CFA blood flow was accompanied by an increase in leg VO_2 in both groups, suggesting a reduced efficiency following ET_A receptor blockade. Together, these findings demonstrate distinct differences in the regulatory role of the ET-1 vasoconstrictor pathway at rest and during exercise with advancing age.

Introduction

Healthy aging is associated with an elevation in vascular tone (11, 40), contributing to the attenuated resting skeletal muscle blood flow observed in the elderly (8, 25, 26, 33, 34, 48). These age-related changes in vascular tone and the associated decline in skeletal muscle blood flow persist during exercise (4, 26, 34, 35). Because adequate muscle perfusion is vital to meet the metabolic demand of the tissue and maintain normal muscle function at rest and during exercise, a better understanding of the mechanisms which contribute to the regulation of vascular tone in the elderly is essential. The functional consequence of these adaptations are substantial; indeed, impairment in perfusion of the exercising muscle may limit the capacity for physical activity, ultimately leading to immobility and increased risks of cardiovascular disease in the elderly (6).

The underlying mechanisms contributing to this age-related increase in vascular tone, and the apparent disparity in regulation of vascular tone between resting and exercising states, has long been a topic of investigation. It has been established that resting sympathetic nervous system (SNS) activity increases with advancing age (7, 40), an adaptation that contributes significantly to basal vascular tone. There is also some evidence that this age-related increase in SNS activity persists during exercise, as an exaggerated increase in plasma norepinephrine spillover and elevated vascular resistance have been observed during supine cycling in elderly men (42). However, more recent studies have revealed an interesting dichotomy between rest and exercise with respect to age-related changes in vasoconstrictor pathways. At rest, our group (46) and others (10, 11) have demonstrated a blunted vasoconstriction to intra-arterial administration of alpha-adrenergic receptor agonists in the old compared to the young (11, 46), only to see these

responses somewhat reversed during exercise (10, 46). Contrasting responses between rest and exercise may also be seen in nonadrenergic pathways; ANG-II-mediated vasoconstriction is significantly augmented with age at rest (48), but this age-associated increase in sensitivity to ANG-II is abolished during exercise (48). Notably, during higher-intensity exercise, we have documented that both alpha-adrenergic- (46) and ANG-II-mediated vasoconstriction (48) are "lysed" to a similar degree in young and old. These findings unveil the complexities of the age-associated alterations in blood flow between rest and exercise, and also raise the question of what other vasoconstrictor pathways may be involved in blood flow regulation in this cohort.

Recently, increased consideration has been placed on endothelin-1 (ET-1) and its contributing role in the age-associated increase in vascular tone. There is evidence from both animal and human studies for increased activity of the ET-1 pathway with advancing age (15, 19), which has been shown to contribute to the age-related reduction in resting skeletal muscle blood flow (43, 45) via the endothelin subtype A (ET_A) receptor pathway (23, 45). Surprisingly, very few studies have sought to determine the role of ET-1 in the regulation of exercising skeletal muscle blood flow. Recent work in animals have evaluated the tonic restraint on blood flow induced by ET-1 with ET_A as well as concomitant ET_A and ET_B receptor blockade during exercise and reported that the vasodilatory effects of blockade were significantly less during exercise compared to control and mediated primarily by the ET_A receptor in the systemic circulation (30). In young humans, our group recently identified a substantial ET-1-mediated vasoconstriction during light knee-extensor exercise that was abolished as exercise

intensity increased (47). However, the contribution of endogenous ET-1 to the regulation of exercising limb blood flow was not evaluated in this study.

In light of the evidence for an ET-1 contribution to the elevated vascular tone in the elderly at rest and the documented rise in ET-1 during exercise (28), the ET-1-ET_A receptor interaction represents one potential mechanism for the elevated vascular tone observed during exercise in healthy, older individuals. Therefore the current study sought to determine the endogenous contribution of the ET_A receptor to the age-associated increase in vascular tone at rest and during dynamic exercise. We hypothesized that: 1) ET_A receptor blockade would enhance resting skeletal muscle blood flow in old, but not young; 2) ET_A receptor blockade during exercise would enhance skeletal muscle blood flow and reduce arterial blood pressure to a greater extent in older individuals, compared to their younger counterparts.

Methods

Subjects

Eight young (24 ± 2 yrs) and eight old (70 ± 3 yrs) healthy subjects were enrolled in the present study. All subjects were nonsmokers and were normally active. Subjects were not taking any prescription medication and were free of overt cardiovascular disease, as indicated by a health history. Protocol approval and written informed consent were obtained according to the University of Utah and the Salt Lake City Veterans Affairs (VA) Medical Center Institutional Review Board requirements. All data collection took place at the VA Salt Lake City Geriatric, Research, Education, and Clinical Center (GRECC) in the Utah Vascular Research Laboratory (UVRL).

Protocols

Subjects reported to the UVRL at 0800 on the experimental day. After 30 min of supine rest, two catheters (common femoral artery (CFA) and femoral vein) were placed using sterile technique as previously reported (1, 3, 51). After catheter placement, subjects rested for ~30 min, and then underwent the protocol as outlined in Figure 4.1. Due to the duration of the study, subjects were given 1/2 cup of corn flakes and 1/2 cup of skim milk before the start of the control (saline) trial and BQ-123 trial to prevent hypoglycemia (Figure 4.1). All data collection took place with subjects in a semirecumbent position (60° reclined), and all studies were performed in a thermoneutral environment.

Owing to potential difference in maximal knee-extensor (KE) capacity in the old individuals compared to their young counterparts, absolute as well as relative exercise intensities were incorporated into the study design (Figure 4.1). The inclusion of multiple absolute work rates offered the advantage of viewing the ET_A receptor contribution to vascular tone between groups in a condition where the exercising leg musculature performs a similar amount of mechanical work and thus a comparable metabolic cost. In contrast, inclusion of work rates based on percent of maximal effort afforded the opportunity for between-group comparisons of measurements which are influenced by relative exercise intensity, such as arterial blood pressure and lactate release (38).

Due to the long lasting effects of BQ-123, the drug administration portion of the protocol always occurred after the saline portion of the protocol. Thus, to minimize the risk that the observed changes in CFA blood flow or mean arterial blood pressure was the consequence of an exercise ordering effect, rather than the effect of the drug *per se*,

subjects returned to the laboratory on a separate day to undergo an exercise time control study. For this visit, catheters were not placed, and no drugs were administered. Apart from these differences, the timeline for this study was identical to the drug infusion day, with the addition of measurements immediately before and after a light meal to examine possible postprandial hemodynamic effects.

Drug Infusions

Thigh volumes were determined anthropometrically, and then used for the calculation of drug dosing. A selective ET_A receptor antagonist (BQ-123 Clinalfa, Calbiochem-Novabiochem, La¨ufelfingen, Switzerland) was prepared in normal saline (0.9% NaCl) and administered intra-arterially (CFA) at 10 nmol/min/liter of thigh volume (infusion rates 0.8-1.5 ml/min). This dose has been documented to induce an apparent plateau in vasodilation in both the forearm (14, 17) and quadriceps (43), without affecting arterial blood pressure. BQ-123 has been documented to have a high affinity for the ET_A receptor (18) and effectively counteracts the vasoconstrictor effect of ET-1 infusion in the human forearm (16). During the control trial, normal saline (0.9% NaCl) was administered intra-arterially into the CFA at the same infusion rates as BQ-123.

Exercise Model

The knee extensor (KE) paradigm implemented in this study has been described previously (2, 26, 36, 50). Briefly, subjects were seated on an adjustable chair with a cycle ergometer (model 828E; Monark Exercise AB, Vansbro, Sweden) positioned behind them. Resistance was created by applying friction to the flywheel, which was turned by the subject via a metal bar connected to the crank arm of the ergometer and a

metal boot in which the subject's foot was placed in. Sixty contractions per minute were maintained at each work rate, subjects exercised at for 3 min at 0, 5, 10, and 15W, as well as 20, 40, 60, and 80% of their predetermined maximal work rate (WR_{max}) with 3 min recovery following three successive exercise bouts (Figure 4.1).

Measurements

Ultrasound Doppler assessments

Measurements of CFA blood velocity and vessel diameter were performed in the infused leg using a Logiq 7 ultrasound Doppler system (GE Medical Systems, Milwaukee, WI) operating in duplex mode. The Logic 7 was equipped with a linear array transducer operating at an imaging frequency of 14 MHz. The CFA was insonated 2-3 cm proximal to the bifurcation of the CFA into the superficial and deep branches. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 5 MHz, operated in the high-pulsed repetition frequency mode (2-25 kHz). Care was taken to avoid aliasing the blood velocity spectra by using scale adjustments, especially during exercise. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less (27). The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization.

Common femoral artery diameter, blood velocity, and blood flow analyses

At all sample points, arterial diameter and angle corrected, time averaged, and intensity-weighted mean blood velocity (V_{mean}) values were calculated using

commercially available software (Logic 7). Using measured arterial diameter and V_{mean}, CFA blood flow was calculated according to the equation:

$$(V_{mean} \cdot \pi \text{ (vessel diameter/2)}^2 \cdot 60)$$

Artery blood pressure, vascular conductance, and heart rate assessment

Arterial blood pressure measurements were collected continuously from the indwelling catheter placed in CFA with the pressure transducer placed at the level of the catheter (Transpac IV, Abbott Laboratories). Mean CFA blood pressure (mmHg) was calculated as diastolic arterial pressure + (arterial pulse pressure x 0.33). On the exercise time control study day, mean arterial pressure was determined noninvasively using finger photoplethysmography (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). CFA vascular conductance (LVC; ml/min/mmHg) was calculated as CFA blood flow / mean CFA blood pressure. Heart rate was monitored from a standard three-lead ECG recorded in duplicate on the data acquisition device (BIOPAC U.S.A) and the Logic 7.

Blood chemistry

A lipid panel was obtained for all subjects by standard techniques. At each exercise intensity, samples (3-4 ml) of femoral arterial and venous blood were collected. 1 ml of arterial and venous blood were presented anerobically to a GEM 4000 blood-gas analyzer and cooximeter (Instrumentation Laboratories, Bedford, MA) to obtain arterial and venous total hemoglobin (tHb) oxyhemoglobin, saturation (SO_2), partial pressure of oxygen (PO_2), hematocrit (hct), lactate, and pH. Blood oxygen content (CO_2) (ml/dl) was calculated as:

$$1.39\ (tHb) \times (SO_2/100) + 0.003 \times PO_2$$

Leg oxygen consumption (VO_2) (ml/min) was calculated as:

$$(CaO_2 - CvO_2) * \text{CFA blood flow}$$

where CaO_2 and CvO_2 represent arterial and venous oxygen content respectively. The remaining blood was spun down for plasma samples, and stored at -80°C until analysis.

Plasma ET-1 concentrations were evaluated via sandwich radioimmunoassay (Stressgen Bioreagents). Using arterial (C_A) and venous (C_V) plasma ET-1 and lactate concentrations, with corrections for hematocrit (hct) and CFA blood flow, "net ET-1 release" and "net lactate release" were calculated (20, 44) according to the following equation:

$$\text{Net ET-1 or lactate release} = (C_V - C_A) * (\text{CFA blood flow}*((101 - (hct/100))$$

Statistical Analyses

Statistical analyses were performed with the use of commercially available software (SigmaStat 3.10, Systat Software, Point Richmond, CA). A 2x10 mixed repeated measures analysis of variance (ANOVA) was used to identify significant changes in measured variances within and between drug conditions and between young and old at rest. Multiple 2x9 mixed repeated-measures ANOVA were used to identify significant changes in measured variables within and between drug conditions, between young and old, across exercise intensities, and BQ-123-induced changes. When a significant main effect was found ($p < 0.05$), the Holm-Sidak method was used for alpha

adjustment and post hoc analysis. Student's *t*-tests were used to identify significant differences in subject characteristics between young and old. Significance for subject characteristics was established at $\alpha = 0.05$. All group data are expressed as mean ± standard error.

Results

Subject Characteristics

Subject characteristics are presented in Table 4.1.

BQ-123 at Rest

In both young and old groups, the continuous administration of the endothelin-1 (ET-1) antagonist BQ-123 (10 nmol/min/liter of thigh volume; infusion rate 0.8-1.5 ml/min) did not significantly change heart rate (Δ -2 ± 3 bpm, young; Δ -4 ± 2 bpm, old) or mean CFA blood pressure (Δ-1 ± 3 mmHg, young; -2 ± 2 mmHg, old) after 45 min of infusion. However, BQ-123 did provoke a significant increase in CFA blood flow (Figure 4.2) and CFA vascular conductance in the old (Δ 0.9 ± 0.3 ml/min/mmHg), but not in the young (Δ 0.0 ± 0.4 ml/min/mmHg). Prior to BQ-123, resting CFA blood flow was significantly reduced in the old compared to the young (Figure 4.2), and the BQ-123-mediated increase CFA blood flow effectively restored CFA blood flow in the old to that of the young (Figure 4.2).

BQ-123 during Exercise

During exercise, BQ-123 induced significant intensity-dependent increases in CFA blood flow and vascular conductance (Figures 4.3, 4.4, 4.5 and 4.6) in both the

young and the old. These hemodynamic changes were accompanied by an intensity-dependent reduction in mean CFA blood pressure (Figures 4.3, 4.4, 4.5, and 4.6) in both groups. In both the young and the old, the vasodilation provoked by BQ-123 during exercise contributed to an increase in leg VO_2 without an alteration in leg a-vO_{2diff} (Tables 4.2 and 4.3). Additionally, a significant increase in the net ET-1 release and no significant change in net lactate release or pH were observed during BQ-123 infusion compared to the saline trial (Tables 4.2 and 4.3).

Exercise Time Control Study

15 of the original 16 subjects (n= 8 young ; n = 7) who participated in the drug infusion protocol returned to the lab for an exercise time control protocol. This additional protocol allowed for the assessment of CFA blood flow and mean arterial blood pressure during multiple exercise bouts and the consequence of food consumption on hemodynamics without the influence of catheter insertion and drug infusions. Measurements were highly reproducible, with no significant differences between CFA blood flow or mean arterial blood pressure at rest or at equivalent exercise intensities (Figure 4.7).

Discussion

The present study has identified a functional role of the endothelin-1 (ET-1) vasoconstrictor pathway in the regulation of vascular tone with advancing age. At rest, endothelin receptor subtype A (ET_A) blockade reversed the age-associated reduction in common femoral artery (CFA) blood flow, confirming a significant contribution of ET-1 to the apparent elevation elevated resting vascular tone in the elderly. During exercise,

both the young and the old exhibited a robust increase in CFA blood flow following ET_A receptor inhibition, demonstrating a clear ET-1-mediated restraint of skeletal muscle blood flow. ET_A blockade also attenuated the exercise-induced increase in mean CFA blood pressure in both young and older groups. These hemodynamic changes were accompanied by a comparable increase in leg VO_2 in both groups, suggestive of decrease in intramuscular efficiency. Together, these findings have identified a clear role for the ET_A receptor in the regulation of vascular tone during exercise that is independent of age or resting responses, supporting the concept that endogenous ET-1 is an important component in the cardiovascular response to dynamic exercise in both young and old healthy humans.

ET_A Receptor Antagonism at Rest

An increasing number of studies from our group (13, 48) and others (8, 11, 32, 39) have collectively demonstrated that healthy aging is associated with a reduction in resting limb blood flow. Findings from the present study confirm these previous reports, as leg blood flow was reduced approximately 30% in the old compared to their young counterparts (Figure 4.2, bottom panel). As expected, the impact on ET_A receptor blockade in peripheral hemodynamics differed significantly between groups. Administration of BQ-123 did not affect CFA blood flow in the young group, but resulted in a progressive increase in CFA blood flow in the old that ultimately restored flow to a level that was similar to the young (Figure 4.2). Interestingly, prior to BQ-123 administration, net ET-1 release was similar between groups. However, following ET_A receptor blockade, circulating ET-1 increased almost 2-fold in the old, but remained unchanged in the young group (Tables 4.2 and 4.3). The present findings build upon

recent studies reporting a significantly greater contribution of the ET_A receptor to vascular tone in the old compared to the young (43, 45), but with the important addition of arterial and venous ET-1 levels, data which provide further insight concerning the relationship between circulating ET-1 and hemodynamic measurements. Together, these former and present findings present an aging vascular phenotype characterized by an increase in the endogenous contribution of ET-1 to vascular tone, accompanied by an overall increase in bound ET-1 and/or enhanced ET-1 bioavailability in older individuals.

ET_A Receptor Antagonism during Exercise

From rest to exercise, perfusion of the exercising skeletal muscle increases in order to match the metabolic demand of the active muscle, achieved through the vasoconstriction of less metabolically active tissue as well as the modulation of vasodilator and vasoconstrictor influences in the exercising muscle (24). Although the mechanisms governing the regulation of skeletal muscle blood flow during exercise have not been fully elucidated, there is evidence in young, healthy individuals for a reduction in the effectiveness of both adrenergic (9, 37, 46) and nonadrenergic (5) vasoconstrictor pathways from rest to exercise. Importantly, we have documented a similar inhibition of alpha-adrenergic-(49) and ANG-II-mediated vasoconstriction (48) between young and old individuals during exercise, suggesting that the role of these pathways in regulation of exercising blood flow is not altered with advancing age. Based on these previous findings and on the known role of ET_A inhibition to modulate vascular tone in the elderly at rest (Figure 4.1) (43, 45), the ET-1 pathway seems a likely contributor to blood flow regulation during exercise in this cohort.

To our knowledge, this is the first study to demonstrate a significant role of ET-1 during exercise in healthy, older individuals. Following ET_A receptor blockade, both young and old exhibited a progressive increase in CFA blood flow with increasing absolute exercise intensities (Figures 4.3 and 4.5), accompanied by an attenuation in the exercise-induced increase in mean CFA blood pressure (Figures 4.3 and 4.5). The BQ-123 induced changes in CFA blood flow were similar between young and old, and significant differences in BQ-123 induced changes in mean CFA blood pressure with age were only evident during the 10W and 15W work rates (Figure 4.5). These differences in mean CFA blood pressure were due to the greater relative intensities these absolute work rates represented in the old compared to the young, which is also indicated by the significantly higher net lactate release present in the old compared to the young (Tables 4.2 and 4.3) at these respective work rates. When comparing alterations induced by ET_A receptor blockade during relative work rates between young and old, similar results were unveiled: both groups exhibited similar BQ-123-induced increases in CFA blood flow and a reduction in the exercise-induced increase in mean CFA blood pressure (Figures 4.4 and 4.6, right panels).

The increase in CFA blood flow following ET_A receptor blockade was accompanied by a comparable decline in intramuscular efficiency in both groups, as evident by the rise in leg oxygen consumption (VO_2) (Tables 4.2 and 4.3). Similar to work by Joyner *et al.* (21), these data indicate that in the young and old, the maintenance of vascular tone is not only vital in the support of arterial blood pressure, but might also play a role in limiting the perfusion of the exercising muscle during submaximal work in order to prevent an exaggerated O_2 delivery and ultimately an unnecessary increase in

VO_2. In light of this increase in leg VO_2 induced by ET_A receptor blockade, the absence of an alteration in net lactate release in both the young and the old (Tables 4.2 and 4.3) indicates that the restraint in oxygen delivery mediated by endogenous ET-1 does not play role in governing relative exercise intensity. Whether the documented increase in leg VO_2 induced by ET_A receptor inhibition in the current study is directly due to an alteration in metabolism of the exercising skeletal muscle or just through an enhancement of oxygen delivery requires further investigation.

These observed cardiovascular responses following BQ-123 are in contrast to recent human work examining the effect of ET_A receptor blockade on limb blood flow during 15 min of static intermittent handgrip exercise in middle-aged hypertensive and normotensive individuals (29). This study reported no difference in forearm blood flow during exercise between the blocked and control trials in the normotensive group, suggesting the ET_A receptor did not contribute significantly to the modulation of vascular tone during handgrip exercise. The present study extends these findings through use of clearly delineated age groups and exercise involving an ambulatory muscle group with significantly greater vasodilatory capacity than the arm. With this approach, we have identified endogenous ET-1 as an important component in the cardiovascular response to dynamic exercise, regardless of age.

The Dichotomy of Vascular Tone Regulation with Age:

Rest versus Exercise

The current assessment of cardiovascular responses to ET_A receptor blockade at rest and during exercise have provided an interesting, yet contrasting story regarding the role of ET-1 in the elderly. At rest, BQ-123 administration revealed a marked, ET-1-

mediated restraint of CFA blood flow in the elderly, but the age-specific nature of this response was not present during exercise (Figures 4.5 and 4.6). These findings add to the growing number of studies utilizing vasoactive drug infusions to probe age-related changes in blood flow regulation, many of which have identified significant differences between resting and exercising responses. Indeed, while older individuals exhibit a reproducible reduction in resting alpha-adrenergic vasoconstriction compared to the young, there is evidence for both similar (49) and exaggerated (10) vasoconstriction in the elderly during exercise. Similarly, old individuals demonstrate an enhanced vasoconstriction in response to inter-arterial ANG-II at rest, while during exercise no age-associated differences in response to ANG-II are present (48). The mechanisms responsible for this unique dichotomy in responses between rest and exercise in these previous studies remain unclear. However, in the present study, determination of circulating ET-1 levels may provide some explanation for the observed differences between young and old following ET_A receptor blockade. Specifically, BQ-123 administration did not alter resting net ET-1 release or CFA blood flow in the young, while ET-1 release increased almost 2-fold in the old after BQ-123, which was accompanied by a ≈30% increase in CFA blood flow (Tables 4.2 and 4.3). In contrast, exercise-induced changes in net ET-1 release did not differ between young and old in either saline or BQ-123 trial. Combined with the parallel changes in CFA blood flow, blood pressure (Figures 4.3 and 4.4), and VO_2 (Tables 4.2 and 4.3) between groups, this similar rise in ET-1 release during exercise suggests an equally important role for ET-1 in the regulation of vascular tone between young and old, and highlights the diverse manner

in which endogenous vasoconstrictors such as ET-1 govern vascular tone at rest and during dynamic exercise.

Perspectives

In light of the nonsignificant differences in BQ-123-induced changes between the young and old during exercise, the ET-1 vasoconstrictor pathway still might be relevant in the regulation of vascular tone in the old during exercise. While aging and hypertension represent two independent risk factors for cardiovascular disease, aging is associated with increases in systolic blood pressure, diastolic blood pressure, and pulse pressure (22). This increase in hypertension is in part associated with increases in endothelial dysfunction. While most studies have focused on the age-associated changes in endothelial-dependent nitric oxide production (12, 31, 41), it is apparent that the ET-1 vasoconstrictor pathway plays a significant role both in the regulation of blood pressure and blood flow at rest and during exercise in patients with hypertension. At rest, in middle-aged to older patients with essential hypertension, a nonselective endothelin receptor antagonist, bosentan, significantly lowered arterial blood pressure (23). Additionally, the local infusion of BQ-123 in to the forearm of middle-aged to older patients with essential hypertension has been documented to increase forearm blood flow (29), indicating that the ET-1 vasoconstrictor pathway plays a role in blood flow restraint during exercise in this patient population.

Experimental Considerations

Due to the slow acting kinetics of BQ-123 binding and clearance, the study was ordered so the control (saline) trial always preceded the BQ-123 trial. However, a time

control trial on a separate day demonstrated highly reproducible measurements of CFA blood flow and mean arterial blood pressure during repeated bouts of KE exercise (Figure 4.5), providing confidence in the comparison of the control and the BQ-123 trials. Additionally, due to the inability to correct hematocrit for trapped plasma volume, a portion of the increase in net ET- and lactate release observed during exercise might be due to hemoconcentration induced during exercise. However, when hematocrit was held constant by incorporating resting hematocrit levels into the equation used to calculate net ET-1 and lactate release, changes in release of these two molecules due to hemoconcentration accounted for less than one percent of the exercise-induced increases observed at every work rate. Finally, we cannot exclude the possibility that some of the observed changes in peripheral hemodynamics are due to ET-1 subtype B (ET_B) receptor activation, as BQ-123 is highly selective for ET_A receptors. However, a recent study utilizing combined ET_A and ET_B receptor inhibition have failed to identify a significant role for ET_B receptors in the regulation of vascular tone in humans (45).

Conclusions

The findings from this study have unveiled a clear role for the ET_A receptor in the regulation of vascular tone during exercise that is independent of age or resting responses, supporting the concept that endogenous ET-1 is an important component in the cardiovascular response to dynamic exercise in both young and old healthy humans.

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FIGURE 4.1. Experimental protocol. Arrows indicate points at which leg blood flow was recorded and arterial and venous blood samples were obtained.

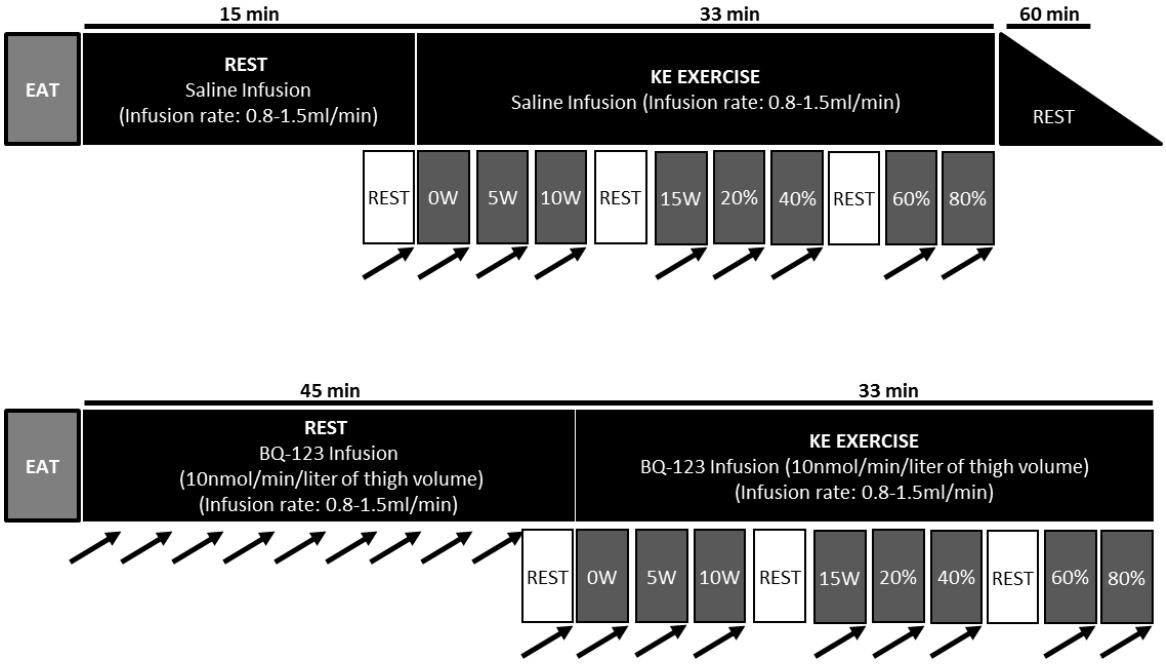
15 min
33 min
60 min
EAT
REST
Saline Infusion
(Infusion rate: 0.8-1.5ml/min)
KE EXERCISE
Saline Infusion (Infusion rate: 0.8-1.5ml/min)
REST
REST
0W
5W
10W
REST
15W
20%
40%
REST
60%
80%
45 min
33 min
EAT
REST
BQ-123 Infusion
(10nmol/min/liter of thigh volume)
(Infusion rate: 0.8-1.5ml/min)
KE EXERCISE
BQ-123 Infusion (10nmol/min/liter of thigh volume)
(Infusion rate: 0.8-1.5ml/min)
REST
0W
5W
10W
REST
15W
20%
40%
REST
60%
80%

Figure 4.2. Resting common femoral artery (CFA) blood flow expressed as percent change (top panel) and absolute values (bottom panel) during continuous infusion of BQ-123 in the young (black circles) and old (grey triangles). ‡ Significant difference from young, $P<0.05$; # Significant difference from minute 0, $P<0.05$.

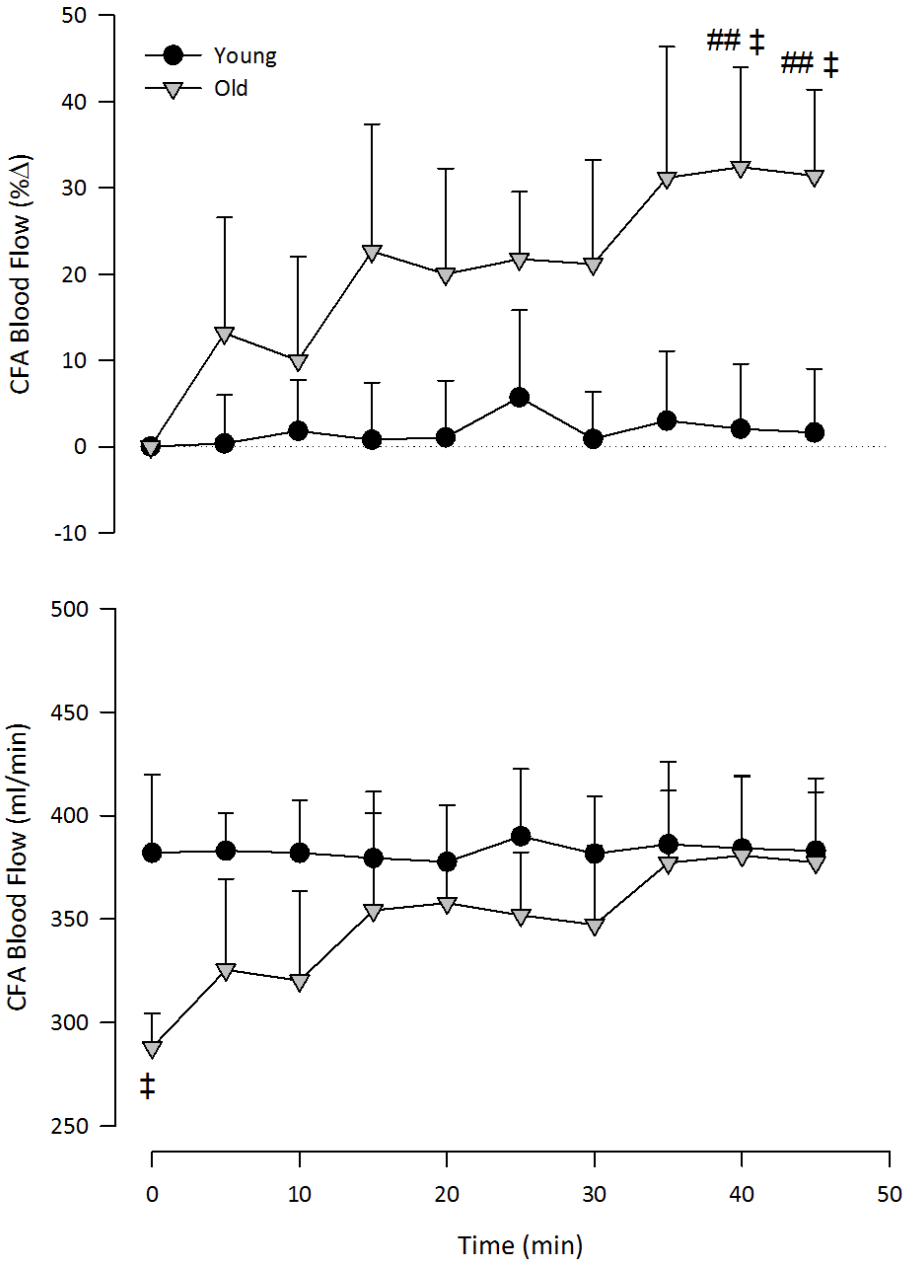
Young
Old
CFA Blood Flow (%Δ)
50
40
30
20
10
0
-10
‡
‡
CFA Blood Flow (ml/min)
500
450
400
350
300
250
‡
0
10
20
30
40
50
Time (min)

FIGURE 4.3. Common femoral artery (CFA) blood flow (top), mean CFA blood pressure (middle), and CFA vascular conductance (bottom) at rest and exercise of absolute work rates during continuous infusion of saline (filled symbols) and BQ-123 (open symbols). * Significant difference from saline in both the young and old, $P<0.05$; *** Significant difference from saline in the young, $P<0.05$; ‡ Significant difference from young, $P<0.05$.

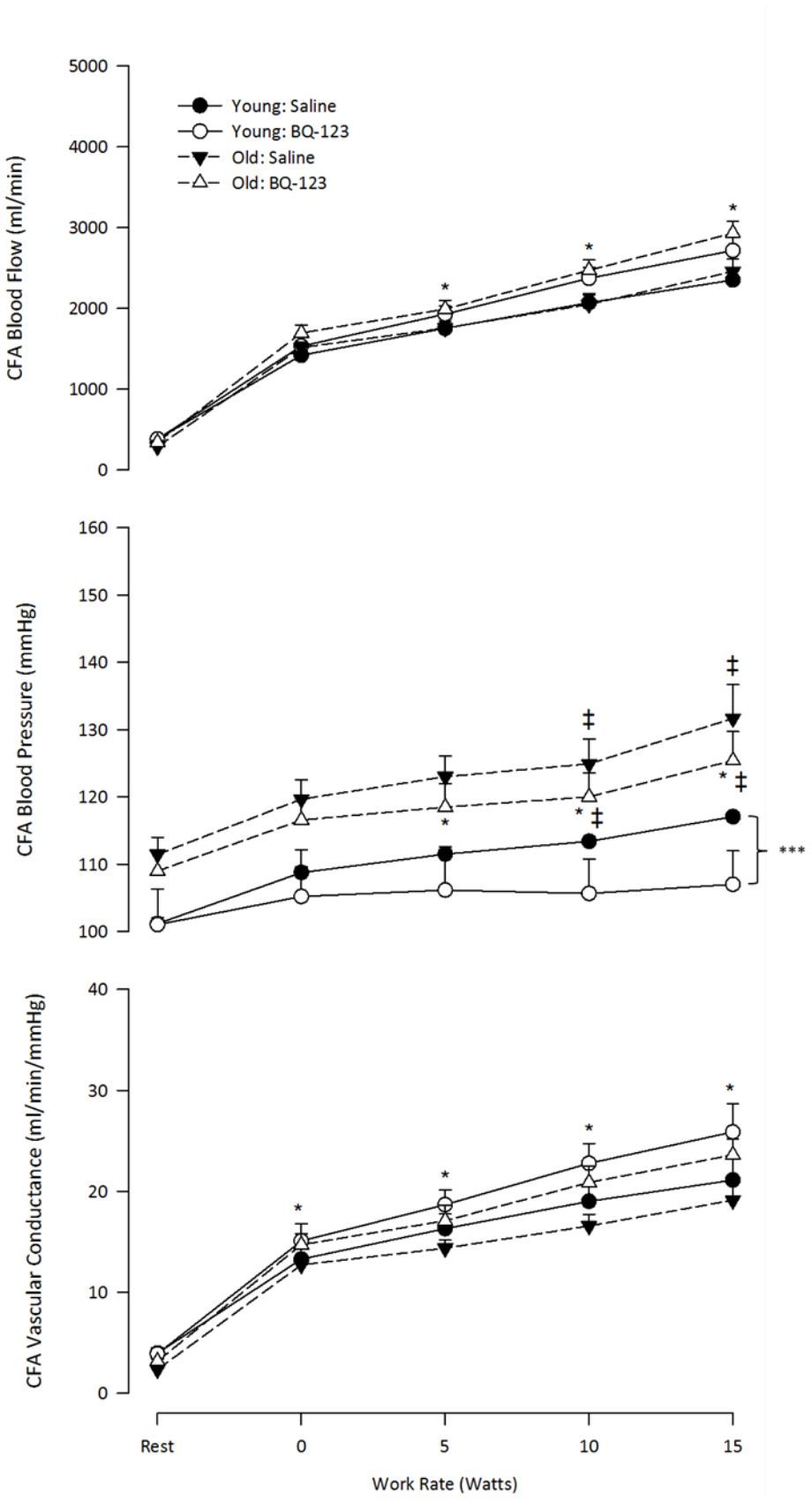
Young: Saline
Young: BQ-123
Old: Saline
Old: BQ-123
CFA Blood Flow (ml/min)
5000
4000
3000
2000
1000
0
CFA Blood Pressure (mmHg)
160
150
140
130
120
110
100
***
CFA Vascular Conductance (ml/min/mmHg)
40
30
20
10
0
Rest
0
5
10
15
Work Rate (Watts)

FIGURE 4.4. Common femoral artery (CFA) blood flow (top), mean CFA blood pressure (middle), and CFA vascular conductance (bottom) at rest and exercise of relative work rates during continuous infusion of saline (filled symbols) and BQ-123 (open symbols). * Significant difference from saline in both the young and old, $P<0.05$; **Significant difference from saline in the old, $P<0.05$; *** Significant difference from saline in the young, $P<0.05$.

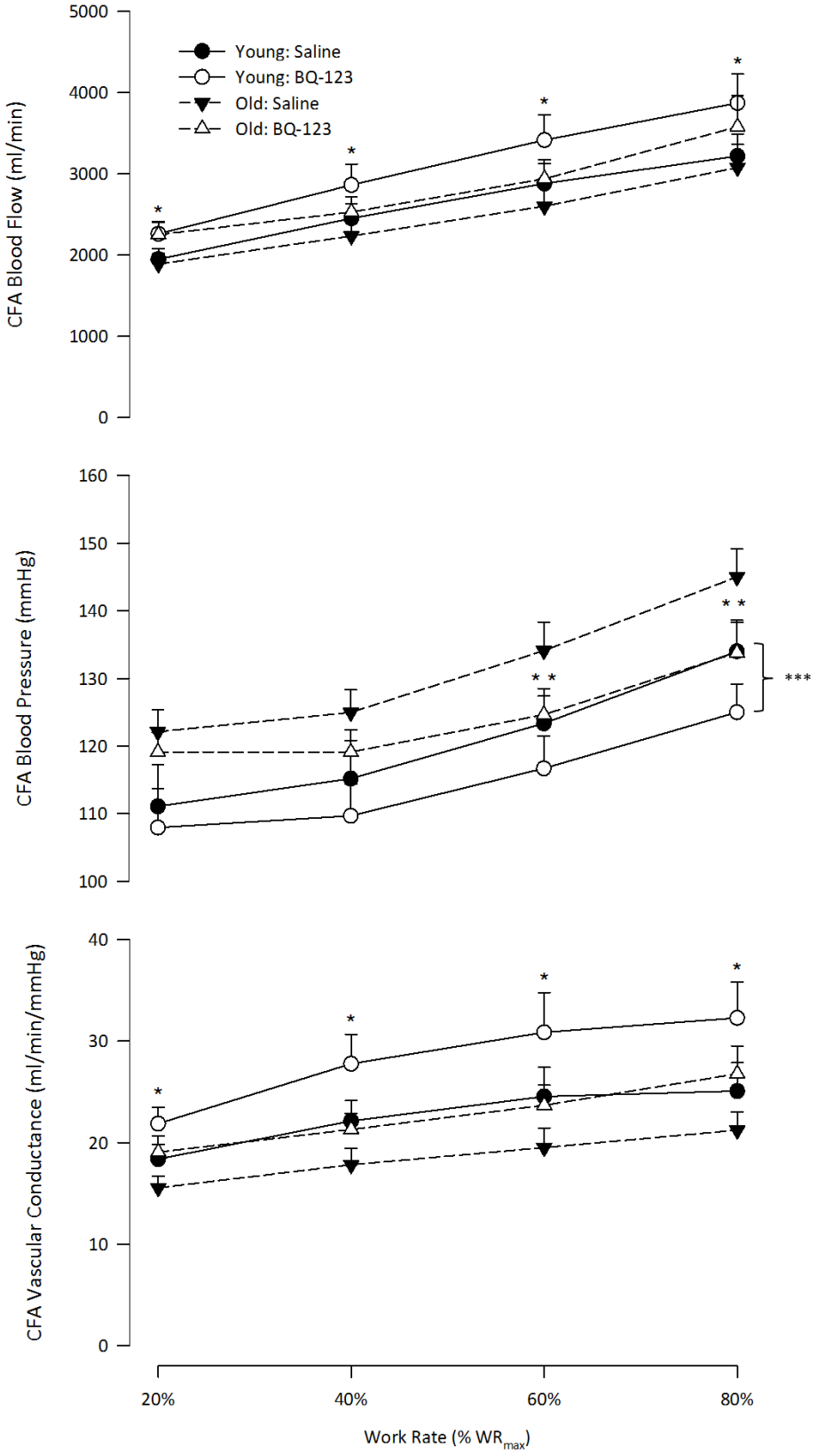
Young: Saline
Young: BQ-123
Old: Saline
Old: BQ-123
CFA Blood Flow (ml/min)
5000
4000
3000
2000
1000
0
CFA Blood Pressure (mmHg)
160
150
140
130
120
110
100
***
CFA Vascular Conductance (ml/min/mmHg)
40
30
20
10
0
20%
40%
60%
80%
Work Rate (% WRmax)

FIGURE 4.5. BQ-123-induced changes in common femoral artery (CFA) blood flow (*top*), CFA blood pressure (*middle*), and CFA vascular conductance (*bottom*) at rest and exercise of absolute work rates in young (black bars) and old (grey bars). + Significant difference from BQ-induced changes at rest in the young and old, $P<0.05$.

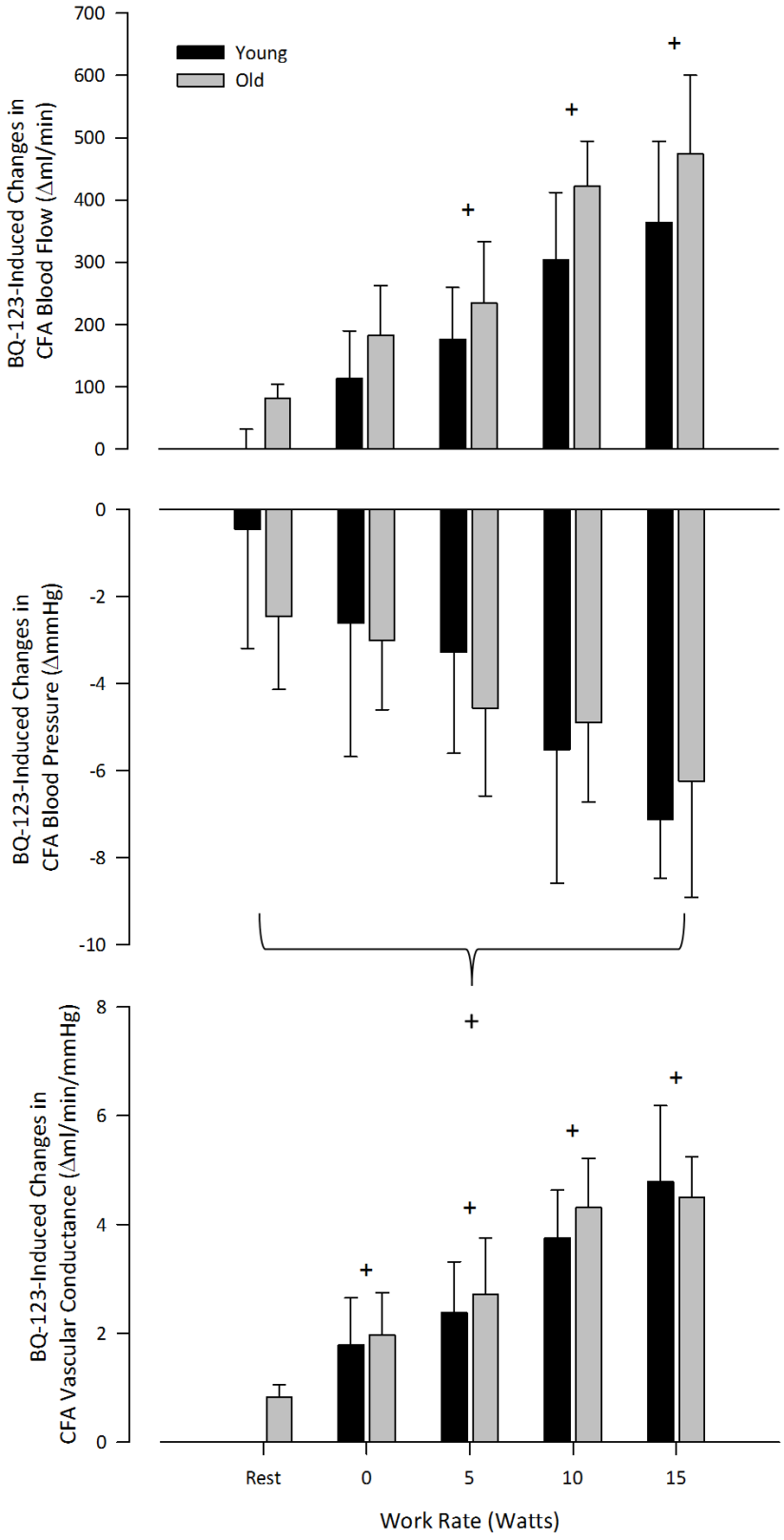
Young
Old
BQ-123-Induced Changes in CFA Blood Flow (Δml/min)
700
600
500
400
300
200
100
0
BQ-123-Induced Changes in CFA Blood Pressure (ΔmmHg)
0
-2
-4
-6
-8
-10
BQ-123-Induced Changes in CFA Vascular Conductance (Δml/min/mmHg)
8
6
4
2
0
Rest
0
5
10
15
Work Rate (Watts)

FIGURE 4.6. BQ-123-induced changes in common femoral artery (CFA) blood flow (*top*), CFA blood pressure (*middle*), and CFA vascular conductance (*bottom*) at rest and exercise of relative work rates in young (black bars) and old (grey bars). + Significant difference from BQ-induced changes at rest in the young and old, $P<0.05$; ++ Significant difference from BQ-induced changes at rest in the old, $P<0.05$; +++ Significant difference from BQ-induced changes at rest in the young, $P<0.05$.

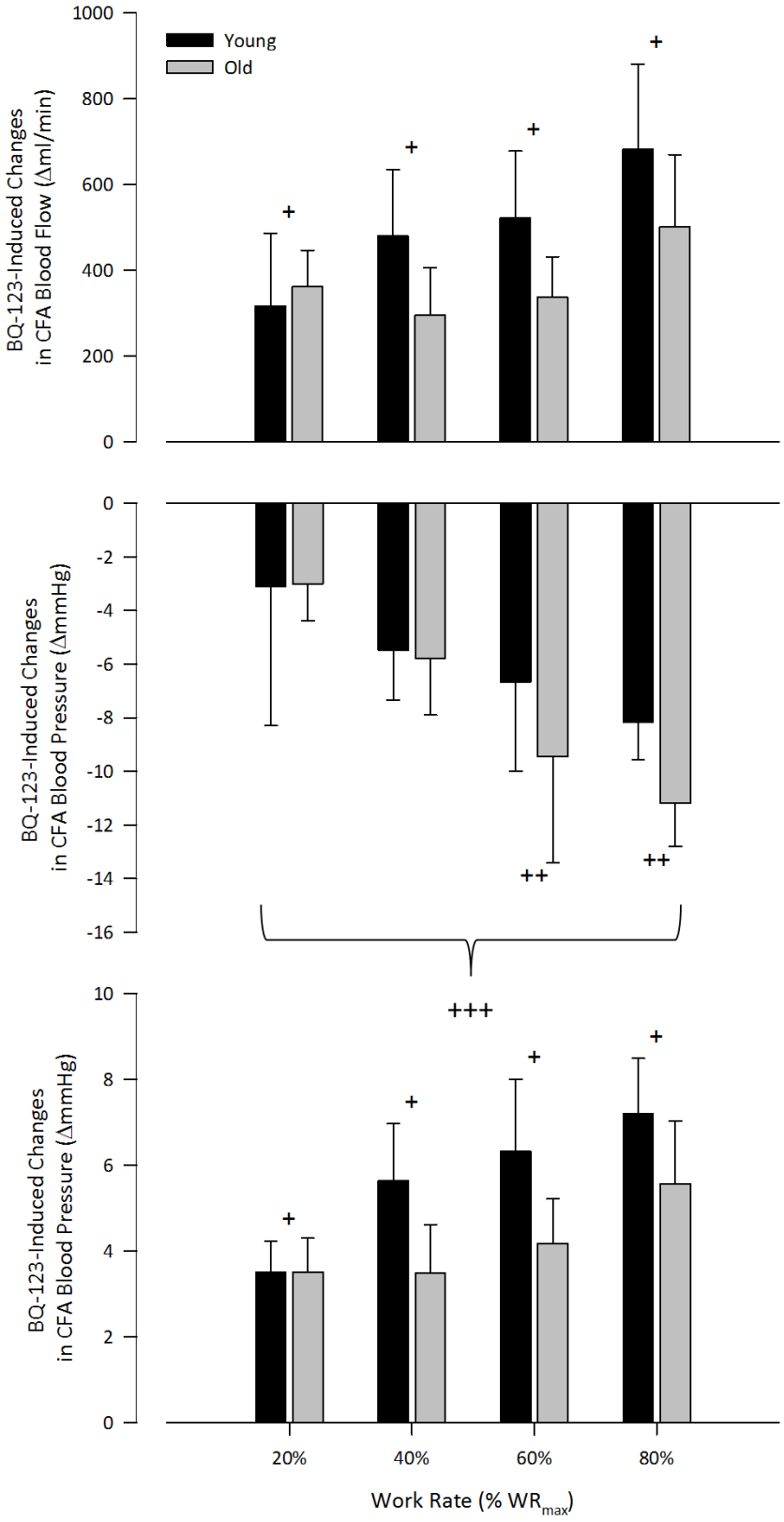
Young
Old
BQ-123-Induced Changes in CFA Blood Flow (Δml/min)
1000
800
600
400
200
0
BQ-123-Induced Changes in CFA Blood Pressure (ΔmmHg)
0
-2
-4
-6
-8
-10
-12
-14
-16
++
++
+++
BQ-123-Induced Changes in CFA Blood Pressure (ΔmmHg)
10
8
6
4
2
0
20%
40%
60%
80%
Work Rate (% WR$_{max}$)

FIGURE 4.7: Common femoral artery (CFA) blood flow and mean arterial blood pressure during the time control study in the young (black circles) and old (grey circles) are displayed. No significant differences were observed between measurements at rest or at equivalent exercise intensities

.

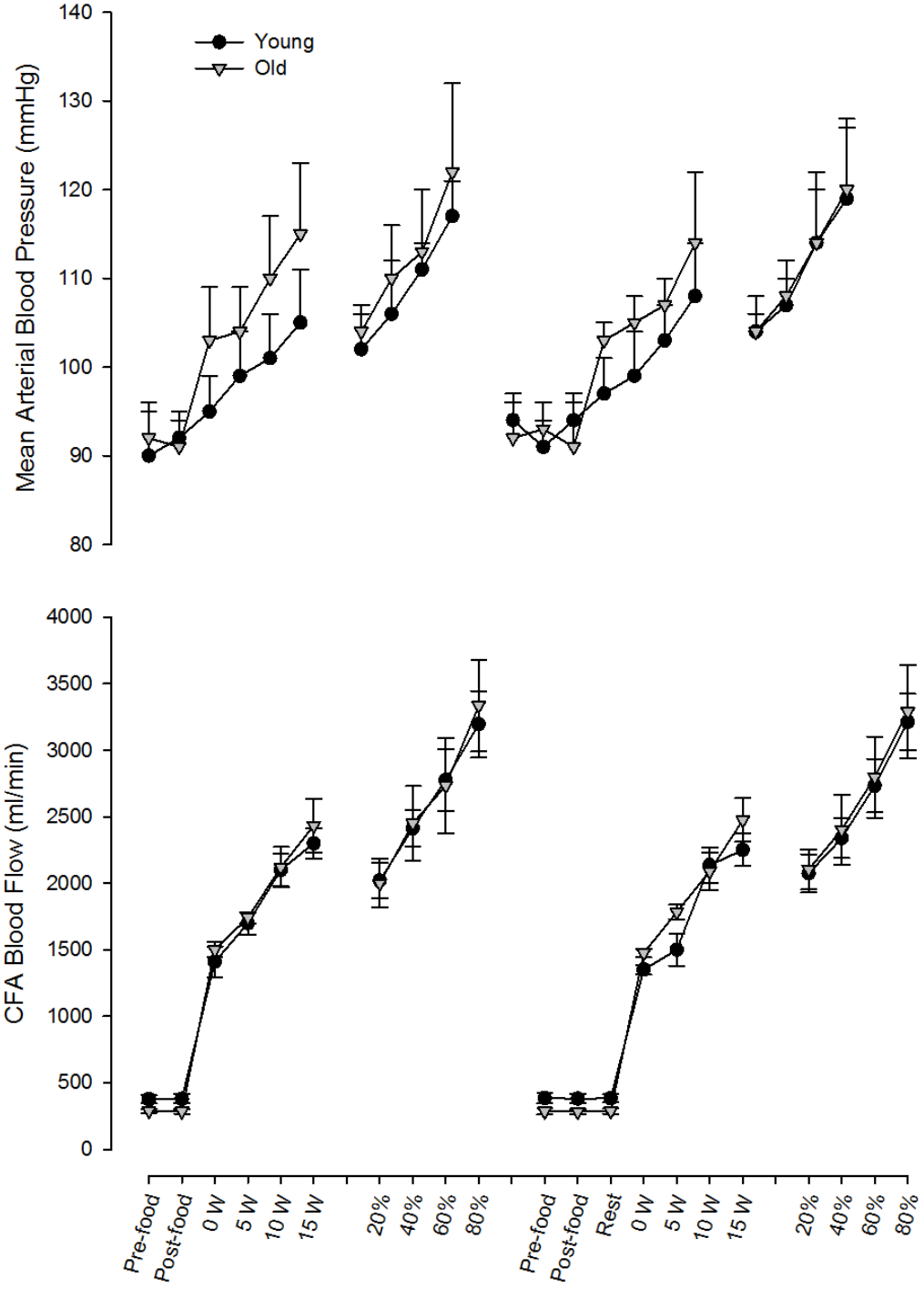
Young
Old
Mean Arterial Blood Pressure (mmHg)
140
130
120
110
100
90
80
CFA Blood Flow (ml/min)
4000
3500
3000
2500
2000
1500
1000
500
0
Pre-food
Post-food
0 W
5 W
10 W
15 W
20%
40%
60%
80%
Pre-food
Post-food
Rest
0 W
5 W
10 W
15 W
20%
40%
60%
80%

Table 4.1. Subject Characteristics

	Young	Old
Age, yr	24±2	70±3 ‡
Height, cm	172±2	172±3
Weight, kg	71±6	75±3
Body mass index, kg/m^2	24±5	25±1
Systolic blood pressure, mmHg	107±5	116±3
Diastolic blood pressure, mmHg	73±3	78±2
Quadriceps muscle mass, kg	2.0±0.1	2.1±0.1
Maximum knee extensor, W	42±4	29±5‡
Glucose, mg/dl	68±6	67±2
Total Cholesterol, mg/dl	153±10	173±8‡
Triglycerides, mg/dl	69±12	96±14‡
HDL, mg/dl	60±9	55±8
LDL, mg/dl	78±9	83±10

HDL, high density lipoprotein; LDL, low density lipoprotein ‡ Significantly different from young, $P < 0.05$.

Table 4.2. Impact of saline infusion on selected physiological variables at rest and during exercise

Work Rate	Rest	0W	5W	10W	15W	20%	40%	60%	80%
Young									
HR, beats/min	66±4	80±5$^{\$}$	87±6$^{\$}$	91±6$^{\$}$	94±7$^{\$}$	88±5$^{\$}$	94±5$^{\$}$	101±8$^{\$}$	112±6$^{\$}$
Leg VO_2, ml/min	20±5	117±16$^{\$}$	146±17$^{\$}$	195±22$^{\$}$	248±33$^{\$}$	174±17$^{\$}$	236±41$^{\$}$	328±46$^{\$}$	365±45$^{\$}$
Leg a-vO_2 difference, ml/dl	5.0±0.9	8.3±0.9$^{\$}$	8.2±0.8$^{\$}$	9.3±0.7$^{\$}$	10.2±0.7$^{\$}$	10.2±0.8$^{\$}$	10.6±0.5$^{\$}$	11.6±0.5$^{\$}$	11.4±0.4$^{\$}$
Net ET-1 release, ng/min	26±3	38±4$^{\$}$	46±10$^{\$}$	56±9$^{\$}$	46±10$^{\$}$	58±13$^{\$}$	66±17$^{\$}$	68±20$^{\$}$	63±15$^{\$}$
Net lactate release, mmol/min	4±1	19±8$^{\$}$	57±18$^{\$}$	114±5$^{\$}$	178±53$^{\$}$	74±25$^{\$}$	195±45$^{\$}$	354±54$^{\$}$	698±138$^{\$}$
Venous pH	7.37±0.01	7.35±0.01$^{\$}$	7.34±0.02$^{\$}$	7.31±0.01$^{\$}$	7.29±0.01$^{\$}$	7.31±0.01$^{\$}$	7.28±0.01$^{\$}$	7.25±0.01$^{\$}$	7.20±0.02$^{\$}$
Old									
HR, beats/min	67±3	83±9$^{\$}$	85±5$^{\$}$	79±6$^{\$}$	87±7$^{\$}$	79±5‡$^{\$}$	81±6‡$^{\$}$	89±10‡$^{\$}$	100±11‡$^{\$}$
Leg VO_2, ml/min	19±1	126±17$^{\$}$	158±19$^{\$}$	185±21$^{\$}$	253±32$^{\$}$	186±20$^{\$}$	269±39$^{\$}$	344±51$^{\$}$	377±62$^{\$}$
Leg a-vO_2 difference, ml/dl	5.3±0.4	8.7±1$^{\$}$	9.0±0.6$^{\$}$	9.1±0.6$^{\$}$	10.6±0.8$^{\$}$	10.4±0.8$^{\$}$	10.6±1.2$^{\$}$	11.5±1$^{\$}$	11.9±1.0$^{\$}$
Net ET-1 release, ng/min	21±4	50±6$^{\$}$	50±7$^{\$}$	58±4$^{\$}$	62±10$^{\$}$	51±4$^{\$}$	58±9$^{\$}$	69±4$^{\$}$	77±13$^{\$}$
Net lactate release, mmol/min	5±1	91±30‡$^{\$}$	106±16‡$^{\$}$	212±4‡$^{\$}$	302±57‡$^{\$}$	173±47$^{\$}$	216±62$^{\$}$	437±76$^{\$}$	629±136$^{\$}$
Venous pH	7.37±0.01	7.31±0.01$^{\$}$	7.33±0.01$^{\$}$	7.28±0.02$^{\$}$	7.24±0.01$^{\$}$	7.31±0.02$^{\$}$	7.22±0.01$^{\$}$	7.21±0.01$^{\$}$	7.24±0.23$^{\$}$

HR, heart rate; VO_2, oxygen consumption; a-vO_2diff, arterial venous oxygen difference; ET-1, Endothelin-1. ‡ Significant difference from young, $P<0.05$; $^{\$}$ Significant difference from rest, $P<0.05$.

Table 4.3. Impact of BQ-123 infusion on selected physiological variables at rest and during exercise

Work Rate	Rest	0W	5W	10W	15W	20%	40%	60%	80%
Young									
HR, beats/min	70±4	84±5$^{\$}$	88±5$^{\$}$	95±6$^{\$}$	94±7$^{\$}$	93±5$^{\$}$	97±5$^{\$}$	106±9$^{\$}$	116±7$^{\$}$
Leg VO_2, ml/min	19±1	122±13$^{\$}$	198±18*$^{\$}$	234±22*$^{\$}$	289±38*$^{\$}$	277±24*$^{\$}$	315±51*$^{\$}$	348±51*$^{\$}$	381±48*$^{\$}$
Leg a-vO_2 difference, ml/dl	4.8±0.7	8.0±0.5$^{\$}$	10.1±0.5$^{\$}$	9.9±0.7$^{\$}$	10.4±0.8$^{\$}$	10.1±0.6$^{\$}$	10.2±0.8$^{\$}$	11.1±0.4$^{\$}$	10.1±0.4$^{\$}$
Net ET-1 release, ng/min	22±3	71±11*$^{\$}$	68±14*$^{\$}$	75±12*$^{\$}$	91±13*$^{\$}$	72±13*$^{\$}$	88±24*$^{\$}$	92±20*$^{\$}$	92±10*$^{\$}$
Net lactate release, mmol/min	8±3	28±8$^{\$}$	50±19$^{\$}$	77±21$^{\$}$	129±33$^{\$}$	74±20$^{\$}$	136±24*$^{\$}$	406±86*$^{\$}$	707±153*$^{\$}$
Venous pH	7.38±0.01	7.36±0.01$^{\$}$	7.34±0.01$^{\$}$	7.33±0.01$^{\$}$	7.21±0.01$^{\$}$	7.32±0.01$^{\$}$	7.32±0.01$^{\$}$	7.27±0.01$^{\$}$	7.25±0.02$^{\$}$
Old									
HR, beats/min	69±3	81±5$^{\$}$	86±7$^{\$}$	83±6$^{\$}$	91±6$^{\$}$	79±7‡$^{\$}$	86±6‡$^{\$}$	90±5‡$^{\$}$	100±9‡$^{\$}$
Leg VO_2, ml/min	25±3	130±12$^{\$}$	187±19*$^{\$}$	225±15*$^{\$}$	300±45*$^{\$}$	283±27*$^{\$}$	317±27*$^{\$}$	355±55*$^{\$}$	404±73*$^{\$}$
Leg a-vO_2 difference, ml/dl	4.8±1.0	8.3±0.1$^{\$}$	9.2±0.8$^{\$}$	9.9±0.7$^{\$}$	10.5±0.8$^{\$}$	9.8±0.8$^{\$}$	10.4±0.5$^{\$}$	10.9±0.9$^{\$}$	11.0±1.1$^{\$}$
Net ET-1 release, ng/min	37±5*‡	94±11*‡$^{\$}$	95±12*‡$^{\$}$	100±12*‡$^{\$}$	110±10*$^{\$}$	99±10*‡$^{\$}$	95±8*$^{\$}$	105±11*$^{\$}$	115±14*$^{\$}$
Net lactate release, mmol/min	9±20*‡	110±31‡$^{\$}$	127±35‡$^{\$}$	264±52‡$^{\$}$	347±62‡$^{\$}$	155±47$^{\$}$	295±54$^{\$}$	502±82$^{\$}$	562±95$^{\$}$
Venous pH	7.36±0.01	7.32±0.01$^{\$}$	7.30±0.01$^{\$}$	7.26±0.02$^{\$}$	7.25±0.01$^{\$}$	7.29±0.01$^{\$}$	7.28±0.02$^{\$}$	7.24±0.02$^{\$}$	7.23±0.01$^{\$}$

HR, heart rate; VO_2, oxygen consumption; a-vO_2diff, arterial venous oxygen difference; ET-1, Endothelin-1. * Significant difference from saline, $P<0.05$; ‡ Significant difference from young, $P<0.05$; $^{\$}$ Significant difference from rest, $P<0.05$.

CHAPTER 5

CONCLUSION

Healthy aging is associated with alterations to regulatory pathways controlling the cardiovascular system. While these alterations initially might not pose a threat to overall cardiovascular health, chronic changes might expedite the progression of cardiovascular disease in the geriatric population. One principle age-related change to the cardiovascular system is an elevation in vascular tone (2, 7), which likely contributes to the attenuated skeletal muscle blood flow observed at rest in the elderly (1, 3-6). Because adequate muscle perfusion is vital to meet the metabolic demand of tissue and maintain normal muscle function at rest and during exercise, a better understanding of the mechanisms which contribute to the diminished blood flow in the elderly is essential.

While there are an abundance of vasoactive molecules that might contribute to age-associated alterations in vascular tone at rest and during physical activity, angiotensin-II (ANG-II) and endothelin-1 (ET-1) represent two potentially significant and somewhat understudied pathways. Indeed, healthy aging is associated with an increase in ANG-II vasoconstrictor activity at rest (10). However, until now it was unknown whether the age-related increase in ANG-II-mediated vasoconstriction is predominately due to a hypersensitivity of the postjunctional AT_1 receptors or due to an enhanced ANG-II potentiation of NE release and ultimate alpha-adrenergic vasoconstriction. ET-1 has also been documented to play a vital role in increasing vascular tone with age (8, 9), but the contribution of this potent endogenous vasoconstrictor during exercise had, until now, remained poorly defined. Thus, two studies were undertaken to study the role of these respective pathways on the regulation of vascular tone with advancing age.

In the first study, we aimed to determine the individual and possible potentiating effects of the alpha-adrenergic and renin-angiotensin-aldosterone (RAAS) pathways using intra-arterial infusions of ANG-II, norepinephrine (NE), and ANG-II with concomitant alpha-adrenergic receptor antagonism (phentolamine (PHEN)). At maximal doses, the ANG-II-mediated reduction in brachial (BA) blood flow was greater in the old compared to the young, while the NE-mediated reduction in BA blood flow was similar between groups. In the presence of PHEN, the ANG-II-mediated reduction in BA blood flow in the old was restored to that of the young. These findings highlight the key role the alpha-adrenergic pathway in enhancing the ANG-II-mediated reduction in skeletal muscle blood flow with advancing age.

The first objective of the second study was to better characterize the role of endogenous endothelin-1 (ET-1) in the regulation of skeletal muscle blood flow at rest and during exercise in young, healthy adults. Using ET-1 receptor subtype A (ET_A) antagonism (BQ-123), we documented no change in common femoral artery (CFA) blood flow at rest. During exercise, common femoral artery (CFA) blood flow and vascular conductance were significantly elevated in the exercising limb following ET_A receptor inhibition, demonstrating a substantial (~20%) ET_A-mediated restraint of skeletal muscle blood flow at the higher exercise intensities. ET_A blockade also blunted the intensity-dependent rise in CFA blood pressure produced during exercise, suggesting that the ET-1 pathway may also play an important role in the support of blood pressure. The increase in CFA blood flow following BQ-123 was accompanied by an increase in leg VO_2 with no change in a-vO_{2diff}, effectively decreasing intramuscular efficiency. Together, these findings have identified a significant role of the ET-1 pathway in the cardiovascular

response to exercise, implicating vasoconstriction via the ET_A receptor as an important mechanism for both restraint of blood flow in the exercising limb and support of arterial blood pressure in healthy, young adults.

The second objective of the second study was to investigate the differing role of endogenous endothelin-1 (ET-1) in the regulation of skeletal muscle blood flow during exercise in the elderly compared to their younger counterparts. Using ET-1 receptor subtype A (ET_A) antagonism (BQ-123), we documented no change in CFA blood flow in the young, and a ~30% increase in CFA blood flow in the old at rest. During exercise, net ET-1 release across the exercising leg increased ~3-fold. BQ-123 increased CFA blood flow by ~20% and attenuated the exercise-induced increase in mean CFA blood pressure by ~6% in an intensity-dependent manner. The increase in CFA blood flow was accompanied by a ~9% increase in leg VO_2 with unchanged a-vO_{2diff}, suggesting a reduced efficiency of the active muscle following ET_A receptor blockade. Collectively, these findings exhibit distinct differences in role the ET-1 pathway place in cardiovascular control at rest and during exercise with advancing age.

It is of utmost importance that we understand and study the age-associated changes in the cardiovascular system in order to investigate therapeutic avenues to improve the quality of life with advancing age. Findings from the studies contained herein may help to achieve this goal, as we have provided new insight into concerning the role the RAAS and the ET-1 pathway in the regulation of vascular tone in the elderly. We propose that the findings regarding the synergistic properties between the RAAS and adrenergic system and the ET-1 vasoconstrictor pathway not only expand our

understanding the physiology of aging, but also demonstrate the potential for targeting these pathways in the prevention and treatment of age-associated cardiovascular diseases.

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