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Dynamics of skeletal muscle blood flow and vasodilation with age

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DYNAMICS OF SKELETAL MUSCLE BLOOD FLOW AND VASODILATION WITH AGE

by

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PH.D. THESIS

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ABSTRACT

Aging is associated with attenuated blood flow and vasodilator responses during rhythmic exercise. Older adults also demonstrate attenuated blood flow and vasodilator responses following single skeletal muscle contractions (contraction-induced rapid onset vasodilation, ROV) within the forearm. These age-associated attenuations within the forearm have been demonstrated to be a result of endothelial and neural mechanisms. The objective of this research was to examine: 1) whether age-associated attenuations within the forearm are from mechanical factors; 2) whether age-associated attentions in ROV are present within the leg, as well as explore potential mechanisms for these age-associated attenuations in ROV; 3) examine whether aging is associated with a slower rate of adjustment in vasodilation (vasodilator kinetics) during rhythmic exercise preceding steady-state exercise; and 4) examine approaches to ameliorate age-related attenuations in blood flow and vasodilation within the leg across the entire exercise transient (onset to steady-state).

The novel findings of this research are that 1) age-associated attenuations in ROV within the forearm are independent of mechanical factors; 2) older adults demonstrate attenuated ROV responses within the leg; 3) age-related attenuations in ROV within the leg are not explained by enhanced sympathetic adrenergic vasoconstriction; 4) older adults exhibit prolonged vasodilator kinetics preceding steady-state exercise; and 5) when examined in a cross-sectional design chronic exercise training improves ROV, vasodilator kinetics, as well as steady-state blood flow and vasodilator responses in older adults; 6) acute supplementation with dietary nitrate fails to exert any effect on blood flow and vasodilator responses during any domain of exercise. Collectively, this work establishes that aging is associated with reductions in blood flow and vasodilation across the entire exercise transient (onset to steady-state) within the leg, which is

offset by chronic exercise training. Mechanistically, the current data suggests that mechanical and sympathetic factors do not explain age-related reductions in ROV in the arm and leg, respectively. Furthermore, acute supplementation of dietary nitrate does not impact leg blood flow and vasodilator responses in older adults during any domain of the exercise transient.

PUBLIC ABSTRACT

Advancing age is associated with reductions in blood flow and vasodilation in response to rhythmic exercise. Recent data within the forearm suggests that these reductions are apparent following a single muscle contraction, and prolong the rate of adjustment for blood flow and vasodilation preceding steady-state exercise. These age-associated maladaptations may lead to general intolerance to exercise. However, most of these findings are exclusive to the forearm, and it is unknown whether these age-associated reductions are also present within the leg. The goal of this research was to examine 1) mechanisms that contribute to the age-related reductions in blood flow and vasodilation at the onset of exercise; and 2) how blood flow and vasodilation adapt to rhythmic exercise, and whether this is altered with age. Additionally, we sought to determine whether chronic exercise training or consuming dietary nitrate improves the blood flow and vasodilator responses to single, as well as rhythmic contractions in older adults. We demonstrate mechanical factors do not play a role within age-associated reductions in ROV within the forearm. Furthermore, we demonstrate similar age-associated reductions in ROV between the forearm and leg, and this is not due to enhanced sympathetic vasoconstriction. Moreover, the rate of adjustment for vasodilation is prolonged in older adults preceding steady-state exercise. Finally, chronic exercise training, but not acute dietary nitrate, improves these responses in older adults, such that there are no differences from young adults. Together, these studies demonstrate that the mechanisms that contribute to age-associated reductions in blood flow and vasodilation are multi-factorial, but chronic exercise training acts to preserve these responses. Future studies using short-term exercise training interventions are needed to determine whether improvement in aerobic capacity are able to offset the effect of advancing age on blood flow and vasodilation in response to skeletal muscle contractions.

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CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

Overview of Blood Flow and Vasodilation during Exercise

Dynamic exercise elicits hemodynamic responses such that skeletal muscle blood flow in humans is capable of exceeding 100-fold of resting values during maximal exercise, and this blood flow response is linearly related to the metabolic demands of contracting tissue (3, 31, 94, 109, 153, 259). Regulation of skeletal muscle blood flow and perfusion during dynamic exercise necessitates a complex integration of central (e.g. cardiac output), local (e.g. vasodilators and vasoconstrictors), as well as mechanical factors (52, 68, 70, 112, 130, 190, 248, 250), such that vasodilation is the driving force for this increase in blood flow. This integration of local and central factors acts synergistically in such a way that: 1) oxygen (O_2) delivery is tightly coupled to the metabolic demand of working tissue; and 2) systemic arterial blood pressure (BP) is maintained. Movement (e.g. exercise) is produced by a coordinated recruitment of skeletal muscle motor units and subsequent contraction of skeletal muscle. Energy for skeletal muscle contraction results from adenosine triphosphate (ATP) generated from various sources of metabolism. At rest, skeletal muscle is reliant on oxidative metabolism to ensure adequate matching of O_2 supply to metabolic demand (109, 130) . With the transition into dynamic exercise, there is an immediate increase in ATP utilization from high-energy phosphate metabolism. Further generation of ATP is dependent on both the intensity and duration of skeletal muscle activity, resulting from anaerobic and aerobic sources. Collectively, the integration of central, local and mechanical factors contribute to vasodilation driving the increase in blood flow during dynamic exercise.

During the transition from rest to exercise (e.g. exercise onset), skeletal muscle O_2 consumption (VO_{2m}) increases exponentially, while microvascular partial pressure of oxygen

drops in order to meet metabolic demand, however some evidence indicates that this response is not instantaneous (127, 240). The delay in VO_{2m} is suggested to be due to intracellular factors (metabolic inertia), rather than O_2 delivery at this junction (98). However, these results may be constrained to exercise utilizing small muscle mass (145, 162, 207, 208, 240). In this context, steady-state exercise responses utilizing larger muscle mass in young healthy adults demonstrate that VO_{2m} is not constrained by O_2 delivery as demonstrated by experiments which manipulate O_2 delivery with exogenous ATP or hypoxic exposure (increasing and decreasing O_2 delivery, respectively) (47, 98). Furthermore, enhancing O_2 delivery via exogenous ATP transiently reduces leg VO_2 after 30 seconds of high intensity knee extensor exercise (37, 38, 125, 205). When taken with available human data (47), it is apparent that the magnitude and rapidity of O_2 delivery is inherently linked to VO_{2m} and thus capacity to perform and maintain exercise. Indeed, inadequate matching of O_2 delivery to demand results in slowed pulmonary and muscle VO_2 kinetics, promoting exercise tolerance (16, 17, 114, 225). From this, it can be concluded that delays in O_2 delivery may influence O_2 utilization during the rest to exercise transition. Therefore any feedback during this adaptation period is critical in establishing an adequate O_2 delivery response. Matching of O_2 delivery (that is, blood flow) to O_2 consumption (VO_{2m}) is a critical regulator of functional/exercise capacity and tolerance.

By far, the majority of data concerning local regulation of exercise hyperemia and vasodilation has been examined during steady-state exercise (e.g. after a few minutes of exercise). Previous work has demonstrated that no one single vasoactive substance or mechanical component is solely responsible for the increase in blood flow and vasodilation during steady state exercise (130). Rather, an intricate pattern of redundancy is present, whereby when one signaling pathway is inhibited or reduced, skeletal muscle blood flow preserved (52,

132, 183, 246, 248). This vasodilator redundancy effectively ensures that skeletal muscle blood flow is protected when other vasodilating substances are experimentally inhibited, or their mechanism of action blunted (130). By the nature of these studies and associated measurements examining solely steady-state responses, mechanisms of blood flow and vasodilation at the onset of exercise are largely ignored despite regulatory responses evident as soon as after a single skeletal muscle contraction. Blood flow and vasodilator responses following a single muscle contraction suggest that rapid vasodilation is integral in initiating the increase in blood flow (O_2 delivery) requisite for dynamic exercise (54, 57, 59, 68, 121, 252, 295). Subsequently, examination of exercise onset (e.g. following a single skeletal muscle contraction) and transitions between exercise intensities may be more physiologically relevant as humans rarely operate at a steady-state response, yet rather are in a constant transition between workloads of varying metabolic costs. In this context, examination of blood flow and vasodilator responses across an exercise transient (onset to steady-state) glean insight into the dynamic vascular control mechanisms that initiate exercise hyperemia as well as those that sustain hyperemia during steady-state exercise. With this in mind, no previous work has characterized blood flow and vasodilator responses across an exercise transient (onset to steady-state) within the same subjects.

Contraction-induced Rapid Vasodilation

At the onset of exercise (rest-to-exercise transition) there is an immediate increase in skeletal muscle blood flow that is a function of exercise intensity (54, 121, 139, 295, 297). This immediate increase in skeletal muscle blood flow following a single skeletal muscle contraction is termed contraction-induced rapid onset vasodilation (ROV). Of particular interest to ROV are the peak (change from baseline) and total hyperemic and vasodilator responses. Accordingly,

ROV reaches peak values approximately 5-7 cardiac cycles post-contraction (59, 61, 121, 295). (39, 59, 61, 121, 242, 252, 295). Examination of hyperemic and vasodilator responses to a single skeletal muscle contraction is important as it permits interrogation of vascular regulatory responses without the confounding influence of subsequent skeletal muscle contractions. In this way, much of the mechanical impedance due to repetitive muscle contractions is avoided. While a single skeletal muscle contraction is not entirely representative of normal physical activity as a whole, it does provide information on the underlying mechanisms that initiate exercise hyperemia.

Feed-forward to Dynamic Exercise

Rapid hyperemic and vasodilator responses following a single skeletal muscle contraction are postulated to act as a feed-forward mechanism into steady-state rhythmic exercise (39, 51, 53, 54, 139, 223). As previously mentioned, this immediate and robust increase in blood flow and vasodilation is dependent on the intensity of muscle contractions, such that O_2 delivery matches O_2 demand. The initiation of blood flow and vasodilation are in part a result of rapid vascular regulatory mechanisms, which are critical at the onset of exercise (rest-to-exercise transition) as well as during changes in exercise intensity (exercise-to-exercise transition) (54, 121, 252, 270, 295, 297). Hyperemic responses to rhythmic exercise are driven not only by exercise intensity, but also the time-course/rate of adaptation (kinetic response) to reach, and maintain steady-state blood flow. Calculation of the kinetic responses to exercise is an important indicator of one's ability adapt to constant load exercise (127, 243). Blood flow during rhythmic exercise is bi-phasic in nature, plateauing (e.g. steady-state) within a few minutes of the onset of exercise (212, 242, 251, 252, 267-269). The kinetic response of blood flow and vasodilation may be separated into three distinct phases. The rapid increase (phase I) in blood flow and

vasodilation at the onset of rhythmic contractions (5-7 seconds) is thought to feed-forward such that blood flow adequately meets metabolic demand, which may be related to contraction-induced ROV (242, 251, 252, 268, 295, 296). Mechanistically, phase I responses are postulated to be due to the repetitive mechanical compression of skeletal muscle microvasculature, however it has come to light that there is indeed a rapid vasodilation that acts synergistically with mechanically-induced vasodilation (251, 252, 270, 294-297). Phase II describes a slower response that demarcates the transition into steady-state responses, eventually plateauing (2-6 minutes). Phase II mechanisms include integration of vasodilators such that steady-state blood flow is adequate to meet the augmented metabolic demand. Phase III may emerge as a slower response, usually only apparent during heavy or high-intensity exercise (296). Collectively, these vascular regulatory responses are crucial in the ability of the vasculature to respond to changes in the frequency and magnitude of contractile activity (e.g. exercise intensity). Historically, this plateau during phase II has been used to characterize steady-state blood flow and vasodilator responses to dynamic exercise, however utilization of this response fails to account for the time course between the onset of exercise and this plateau phase. Blood flow and vasodilator phase responses to dynamic exercise can be interrogated by approaching the exercise transient from a systems control perspective. Application of a systems control analysis models the different phases of the blood flow and vasodilator responses to dynamic exercise, both the magnitude and timing of these responses may be elucidated, giving insight into regulation of the vasculature during exercise (127, 224, 243).

Vascular Aging

A growing body of evidence indicates that the aging process enhances susceptibility to the development of chronic diseases, particularly cardiovascular related diseases (202). By the

year 2030, one in five Americans will be over the age of 65 (227). As the aging population is rapidly expanding, the mounting pervasiveness of chronic diseases grows in parallel with the prevalence of physical inactivity (28, 29, 149, 150, 192, 298). According to the Centers for Disease Control and Prevention, over 750,000 deaths per year are attributed to cardiovascular disease, with 35% of deaths occurring in adults over the age of 65 (194). A hallmark of cardiovascular aging is a dysfunctional arterial phenotype, termed endothelial dysfunction, which is the initial step in atherogenesis (100). Endothelial dysfunction is an antecedent to cardiovascular disease, and is most notably associated with reduced nitric oxide (NO) bioavailability and/or signaling, as well as enhanced sympathetic outflow (79, 134, 149, 150, 281, 283, 285). Despite reductions in mortality using modern medicine, cardiovascular disease remains one of the leading causes of death and disability within the United States (1, 4, 64).

While advanced age is one of the primary risk factors in the development of a dysfunctional arterial phenotype, sedentary time or reduced physical activity also contributes to enhanced cardiovascular disease risk (1, 4, 27-29, 64, 171, 298). Older adults report the highest rate of physical inactivity and poorer physical health (1, 4, 194). Furthermore, aging is associated with alterations in the structure and function of the cardiovascular system (90), resulting in adverse cardiovascular responses to exercise relative to young adults. These age-associated maladaptations manifest as declines in aerobic capacity (e.g. VO_{2peak}) which may be due to impaired O_2 delivery to skeletal muscle. This reduction in aerobic capacity is strongly and inversely related to all-cause mortality, and peak limb vasodilation in older adults (24, 45, 83, 118, 143). Indeed, maximal O_2 consumption declines each decade of life after the age of 30 by approximately 10% per decade (35), which is exacerbated by lifestyle and chronic diseases (28, 284).

Activities of daily living and standardized clinical physical performance tests, characterized by repeated short bursts of activity (222), rely on the capability of the vasculature to rapidly respond to situations of varying metabolic cost to maintain performance (e.g. sit-to-stand; timed up-and-go) (12, 36, 305). Many activities of daily living are improved with exercise training (20, 49, 115). Coupled with declines in aerobic capacity, exercise tolerance, and skeletal muscle blood flow, age-associated impairments in O_2 delivery may be related to poorer physical function, and reduced exercise intolerance (101, 129, 172, 179, 201).Given that blood flow and vascular conductance exhibit adaptive responses across an exercise transient (i.e. phases I, II, III), and that no information presently exists on the kinetics of these responses within the leg in the aging population, this presents an important gap within the literature concerning how local regulation of blood flow and vasodilation are altered with age across the entire exercise transient and whether chronic exercise training to preserve aerobic capacity mitigates these adverse effects. The mechanisms behind these age-related reductions are multi-factorial, with no one underlying mechanism solely responsible, but are postulated to be due to endothelial, neural, as well as mechanical components. *Chapters 5 and 6 examine whether exercise training mitigates age-related reductions in blood flow and vasodilation during exercise. A version of Chapter 5 has previously been published within the Journal of Applied Physiology (118). A version of Chapter 6 has recently been submitted for review also within the Journal of Applied Physiology.*

Limb Differences in Vascular Responsiveness

The majority of evidence regarding the local regulation of blood flow during exercise has been examined utilizing a forearm model, while a paucity of information has been gleaned using a leg model. The forearm model presents ideal experimental conditions to interrogate the vasculature of a limb with relatively small muscle mass, thus reducing contribution of central

cardiovascular mechanisms (heart rate, mean arterial pressure, cardiac output) from confounding the measurement of local regulation of blood flow during exercise (249). However, it can be argued that the lower limbs represent a more physiologically relevant vascular bed when examining exercise responses, as humans are bipedal in nature. Coupled with this, the lower limbs likely have a greater propensity to develop peripheral vascular disease (7, 167, 168). Furthermore, the lower limbs are subject to greater hydrostatic forces and are integral in helping to maintain systemic arterial pressure due to the relatively high sympathetic innervation and a greater percentage of cardiac output is directed towards the lower limbs (244).

At rest, blood flow is reduced in older adults in the lower, but not upper limbs even when normalized for muscle mass (73, 80). When exposed to exogenous vasodilators, the forearm and leg vasculature exhibit heterogenous responses, in that the forearm vasculature adjusts more rapidly than the leg for a given increase in blood flow with pharmacological manipulation (195). Accumulating evidence indicates that aging is associated with attenuated exercise hyperemia and vasodilation in the upper limbs following a single muscle contraction (39, 141) as well as during steady state exercise (80, 155, 226, 234, 300). Additionally, endothelial functional is reduced with age, even when normalized for shear rate, a stimulus for vasodilation, is taken into account in both the upper and lower limbs (198, 216, 241, 257, 283). This suggests that endothelial function may mediate some of the age-related reductions in exercise hyperemia and vasodilation in the lower limbs with age. It should be noted however, that age-associated attenuations in forearm steady-state blood flow with aging is not a universal finding (80, 141, 198, 239). However, older adults consistently exhibit reductions in blood flow and vasodilation during knee extensor exercise across exercise intensities concomitant with elevations in arterial pressure (e.g.

pressor response) relative to young adults (15, 80, 155, 226, 229). Collectively, these findings suggest that processes that alter skeletal muscle tone with aging may be limb specific.

In addition to the aforementioned information, blood flow dynamics (e.g. kinetic response) appear to be altered between limbs (296). Furthermore, aging alters the dynamics of skeletal muscle blood flow and vasodilation within the forearm across the rest to exercise transition that is consistent with a reduction in NO bioavailability (41). It remains unknown whether these responses may be extrapolated to the leg. Collectively, these data indicate that exercise hyperemia and vasodilation are severely attenuated with age; however the mechanisms and magnitude for age-related reductions appear to differ between limbs. *Chapter 2 of this dissertation addresses the gap in literature pertaining to mechanisms for the age-related reduction in contraction-induced rapid vasodilation within the forearm, and a version of this chapter has recently been published in Experimental Physiology (116). Chapter 3 of this dissertation addresses whether there are limb differences with age in respect to contraction-induced rapid vasodilation and a version of this Chapter has been previously published in Physiological Reports* (120).

Mechanisms of Blood Flow Regulation with Advancing Age

Mechanisms responsible for the regulation of blood flow and vasodilation are complex, involving mechanical, neural, and endothelial factors (52, 110, 130). Extending this concept, blood flow to any vascular bed is dependent upon vascular tone and perfusion pressure across the vascular bed (52). In a broad sense, regulation of vascular tone is dependent upon the balance between vasodilator and vasoconstrictive substances (52, 248, 261). As previously noted, signaling of vasodilation is fundamentally redundant, with no single factor being solely responsible for vasodilation (52, 112, 130, 213). Given such redundancy in the control of blood

flow and vasodilation during exercise, substances or factors that modulate blood flow and vasodilation must be rapidly acting (<5 sec), and should not desensitize over time (52, 112, 261).

Mechanical Mechanisms

Mechanical compression of blood vessels occurring during contraction of skeletal muscle elicits a rapid increase in blood flow and vasodilation (53, 139, 297). Mechanosensitive endothelial and smooth muscle cells respond to changes in intramuscular pressure, as well as changes in intra-luminal frictional forces (e.g. shear stress) (51). During a skeletal muscle contraction, compression of a vascular bed forces blood out of venous capacitance vessels, thereby lowering venous pressure, increasing the arteriovenous pressure gradient, and eliciting a greater influx of arterial flow (93, 297). The arteriovenous pressure difference across a vascular bed imparts a mechanical component to hyperemia, aptly termed the skeletal muscle pump (130). Altering perfusion pressure via limb position manipulation modifies the arteriovenous pressure gradient, due to the influence of gravity. This notion is supported by evidence demonstrating that, when a given limb is positioned below heart level, the resultant elevation in gravitational forces enhance perfusion pressure thus, creating an augmented pressure and flow response (61, 124, 294, 295, 297).

Conversely, facilitation of venous emptying by positioning a limb above heart level markedly reduces hyperemic responses (294). Due to the rapid hyperemia with extra-vascular compression and the influence of perfusion pressure, the skeletal muscle pump has been postulated to act as one of the mechanisms by which rapid vasodilation occurs after a single skeletal muscle contraction (54, 139, 297). Indeed, mechanical compression of blood vessels within the forearm elicits a rapid hyperemic and vasodilator response (139, 297). Kirby et. al. (139) showed that sustained extra-vascular compressions of the forearm evoke a greater

vasodilation than single compressions alone, such that mechanically evoked contractions share a non-linear relationship with increases in extravascular pressure and peak vasodilator responses (139). Credeur et. al. (61) extended upon these findings by showing a rapid vasodilator response was present in the legs of young adults and that extra-vascular compression (300 mmHg) elicited rapid hyperemic responses within the leg. These ROV responses were found to be greater when the leg was positioned below-heart level, thereby enhancing perfusion pressure. These results collectively indicate that blood flow and vasodilation may increase as products of perfusion pressure manipulation and mechanical distortion in the vasculature of the lower limbs.

While mechanical compression of vascular beds elicits a rapid hyperemia and vasodilation, this response is of considerably less magnitude compared to single skeletal muscle contractions, independent of limb (139, 297). Both Tschakovsky and Credeur (61, 297) showed that following a single muscle contraction a rapid vasodilation is observed, with a magnitude that is greater when the forearm was positioned below heart level (augmented perfusion pressure). Accordingly, these rapid hyperemic and vasodilator responses have been shown to be dependent upon contraction-intensity (295). Further evidence for a mechanical contribution to hyperemic and vasodilator responses stems from animal studies, wherein increasing contraction frequency and intensity elicit robust hyperemic and vasodilator responses, which have been shown to be independent of neural and endothelial factors (263-265). Interestingly, the rise in blood flow and vasodilation appear to be disassociated with the onset of locomotion (263-265). Sheriff (263) showed that with alterations in treadmill grade or speed muscle blood flow corresponded to changes in contraction frequency suggesting a direct mechanical coupling between contraction frequency and skeletal muscle blood flow within experimental animal models.

Recent evidence has suggested that properties intrinsic to the arterial wall may modulate the hyperemia and vasodilation with exercise (34, 52, 53, 124). In this context, aging is associated with increases in blood pressure, presumably as a result of reductions in the elastic properties and arterial wall remodeling (303). Increased vascular stiffness may create an environment not conducive to vasodilation during exercise. This age-related increase in vascular stiffness may create an inertia that the smooth muscle is required to overcome at a given level of vasoconstriction. These adverse alterations with age have previously been shown to propagate pulsatile penetrations of blood pressure and blood flow into the microvasculature of the brain (174) and kidney (174, 176). During exercise skeletal muscle blood flow may increase up to 100-fold of resting values, therefore it too may be considered a high-flow organ during exercise. Conversely, the microvasculature of skeletal muscle may be relatively well protected against age-related elevations in pulsatile projections due to vascular wall hypertrophy, arterial remodeling and higher resistance to flow at rest serving to offset reductions in arterial compliance (174, 175). Collectively, this evidence indicates that the skeletal muscle pump in concert with rapid vasodilation plays a role in initiating the hyperemic response at the onset of exercise (e.g. following a single contraction) as evidenced by a greater magnitude of response with the dependent limb below, as compared to at or above heart level. Whether the mechanical component of contraction-induced rapid vasodilation is changed with age is currently unknown.

Sympathetic Mechanisms

Dynamic exercise elicits a large systemic hemodynamic response which involves a balance between activation of sympathetic nervous system vasoconstriction and peripheral vasodilation of active muscle tissues. At the onset of dynamic exercise the autonomic nervous system is integral in blood flow re-distribution. In order for exercise to continue, blood flow is

re-distributed to active contracting tissue in order to match O_2 demand (147, 236, 286, 287). Parasympathetic withdrawal allows for an increase in heart rate, and enhanced sympathetic outflow increases cardiac output, and the redistribution of blood flow to active skeletal muscle. Concomitant to the increase in cardiac output, enhanced sympathetic outflow elicits increases in vascular tone. In general, vascular tone within skeletal muscle resistance vasculature is primarily controlled by α-adrenergic receptors on smooth muscle. During dynamic exercise α-adrenergic receptor responsiveness to sympathetic outflow is reduced in active skeletal muscle, facilitating an increase in blood flow (O_2 supply) to metabolically active contracting tissue (i.e. functional sympatholysis). Functional sympatholysis is graded with exercise intensity and reflects a delicate balance between maintenance of systemic arterial pressure, and adequate O_2 supply to contracting tissue. In this context, sympathetic restraint of blood flow during exercise is critical for maintenance of arterial blood pressure as the vasodilator capacity of skeletal muscle far exceeds the ability of the heart to increase cardiac output (244). However, many of these studies fail to examine the temporal responses of sympathetic vasoconstriction. Indeed, there appears to be evidence for rapid blunting of sympathetic vasoconstriction at the onset of exercise, apparent even after a single muscle contraction (forearm) (67, 293). How sympatho-excitatory stimuli influence exercise blood flow is of on-going investigation as responses may vary with age and training status.

Aging is associated with increased muscle sympathetic nerve activity (MSNA) and attenuated responsiveness to sympathetic vasoconstriction at rest relative to young adults in both the arm and leg (62, 63, 72, 73, 274). Moreover, age-related elevations in MSNA are associated with increased systemic arterial blood pressure, however this effect is not observed in young men and women (11, 131). Koch et. al. (142) demonstrated that older adults exhibit greater

sympathetic responsiveness during cycling exercise, a phenomenon that has been replicated with forearm handgrip and knee-extension exercise (75, 82, 140, 185). Conversely, Richards et. al. (237) provided evidence that elevations in sympathetic vasoconstriction may not act to restrain forearm skeletal muscle blood flow and vascular conductance during exercise in young or older adults. Interestingly, lifelong exercise training/physical activity acts to preserve this ability to attenuate sympathetic vasoconstriction during exercise (185).

Attenuation of sympathetic vasoconstriction has been observed as early as after a single skeletal muscle contraction in young healthy males (67, 293). Casey et al. (40) showed that sympathetic stimulation reduces contraction-induced peak and total forearm hyperemic and vasodilator responses in young adults, whereas older adults exhibit no such reductions. Subsequent administration of a non-selective α-adrenergic agonist (phentolamine) abolished these age-related differences in peak and total hyperemic and vasodilator responses. Collectively, these results indicate that α-adrenergic modulation may explain some of the age-related differences in the rapid hyperemic and vasodilator responses at least within the forearm at the onset of exercise. It remains unknown whether the lower limbs exhibit similar responses as they represent a larger vascular bed and are more responsive to sympathetic activation. *Whether enhanced sympathetic vasoconstriction contributes to the attenuated contraction-induced ROV response within the leg of older adults is the focus of Chapter 4 and a version of this Chapter has been previously published in the Journal of Applied Physiology (117).*

Endothelial Mechanisms

The most widely recognized vasodilator, NO, has a host of physiological regulatory functions in conjunction with other signaling molecules, converging on the modulation of vascular tone both at rest and during exercise (52, 112, 235, 257). NO is synthesized within the

endothelium via endothelial nitric oxide synthase (eNOS) through hydroxylation of L-arginine to Nω-hydroxy-L-arginine in the presence of co-factors, subsequently oxidized to L-citrulline and NO (31, 94, 200, 277). Additionally, NO is synthesized endogenously from eNOS via site specific phosphorylation in response to laminar fluid shear stress (e.g. blood flow) (89, 273, 304). Endothelial-derived NO is rapidly transported to localized vascular smooth muscle where it induces production of the secondary messenger cyclic guanosine monophosphate (cGMP) which in turn inhibits calcium re-uptake concomitant with cell hyperpolarization, lowering intra-cellular calcium, and inducing vascular smooth muscle relaxation (89, 91, 92, 97).

It is generally recognized that the reliance on NO to elicit vasodilation in the skeletal muscle vasculature declines with age, precipitating development of endothelial dysfunction (102, 186, 187, 215). It is unclear whether this is due to reductions in bioavailable NO or alterations to NO signaling (2, 69, 271). Taken together, reductions in NO bioavailability or signaling, concomitant with age-related endothelial dysfunction, may act to shift the vasodilator balance towards a more vasoconstrictive phenotype contributing to impaired local control of blood flow and vasodilation (78, 79, 96, 157, 203). Although the role of NO in modulating resting vascular tone is apparent, the role NO plays in exercise hyperemia is complex and inadequately understood in aging humans.

NO, while not solely obligatory for exercise hyperemia, plays a key role in modulating the hyperemic and vasodilator responses (81, 99, 111, 235, 253, 254). Historically, the role of NO in exercise hyperemia and vasodilation has been predominately studied by inhibiting endogenous NO production at the level of the endothelium by non-specific inhibition of NO synthase (eNOS). Within the forearm, local inhibition eNOS reduces the hyperemic and vasodilator responses to both single contractions as well as during steady-state dynamic exercise

(33, 41, 43, 104, 254, 266). Within the leg of young adults, inhibition of eNOS reduces resting blood flow, vascular conductance, O_2 delivery, and increases O_2 extraction ultimately resulting in unaltered leg VO_2 (185, 204, 235). Inhibition of eNOS during passive leg movement (PLM) reduces the hyperemic response in young adults approximately 80-90%, with no apparent increases in O_2 extraction or leg VO_2 (47, 48, 185, 204, 205, 255). However, not all studies have demonstrated reductions in blood flow in response to eNOS inhibition during PLM (235). This blunted response during PLM with eNOS inhibition indicates that both the immediate increase in blood flow at the onset of movement as well as continuous movement (beyond 10s) is dependent, at least in part, on NO. Conversely, NO does not appear to play an essential role during dynamic exercise as demonstrated by the lack of effect of eNOS inhibition on blood flow during knee-extensor exercise (30, 111, 112, 254).

When combined with non-selective cyclooxygenase inhibition (double blockade), leg blood flow is further reduced at rest and during passive leg exercise as well as during dynamic knee-extensor exercise (48, 111, 182, 183, 255). Combined blockade of NO and prostanoids reduces vascular conductance both at rest and during exercise (47, 48, 182, 183, 205), but has been reported to subsequently return to control values (255) during leg-extensor exercise. This double blockade of NO and prostanoids reduce leg O_2 delivery during PLM, with reductions persisting into dynamic exercise, while O_2 extraction is increased at rest as well as during PLM, persisting for almost a minute into dynamic leg exercise (48). These alterations in O_2 delivery and extraction may not be enough to offset a decrease in leg VO_2 during double blockade during the first two minutes of dynamic leg exercise (48). These findings demonstrate that reductions in O_2 delivery may blunt muscle O_2 uptake during the initial phases of dynamic leg exercise, and may be more apparent as exercise intensity increases (48, 183, 208). Collectively, these results

not only highlight the intricate redundancy of vasodilators in modulating vascular tone at rest and during exercise, but also the importance of examining hyperemic responses across an exercise transient as opposed to steady-state levels alone.

In older adults eNOS inhibition reduces leg blood flow and vascular conductance at rest (289). In response to PLM, eNOS inhibition does not necessarily have an effect on leg blood flow, but reduces peak leg vascular conductance, mainly due to an increase in MAP (289). Within the forearm, eNOS inhibition reduces the hyperemic and vasodilator responses at the onset of exercise at low and moderate intensities (10-20% maximal voluntary contraction; MVC) but not at higher intensities (40% MVC) (43). Importantly, although ROV responses were reduced with inhibition of eNOS in older adults, they were still significantly reduced relative to young adults. Additionally, eNOS inhibition reduces the forearm hyperemic response during steady state-exercise by approximately 12% (253), and prolongs the kinetic response (i.e. the speed at which steady-state is achieved) of blood flow and vasodilation within the forearm during the transition from rest to steady-state exercise in young adults, with no changes in older adults (41). Park, et. al. (215) showed that within cannulated skeletal muscle feed arteries of older adults, endothelial-dependent vasodilation (via acetylcholine), as well as flow-induced vasodilation are attenuated relative to young adults. These reductions were coupled with a slower vasodilator kinetic profile, in part due to an NO-mediated mechanism. Furthermore, these attenuations were concomitant to a greater fold-change in phosphorylated eNOS to total eNOS ratio in young adults, relative to older adults in response to flow-induced vasodilation, suggesting an enhanced signaling in young adults. When taken in context with previous data, vascular responsiveness is improved with exercise training, suggesting these age-related reductions may be ameliorated (15, 278).

Consequently, enhancing NO bioavailability offers a new avenue to mitigate reductions in blood flow and vasodilation in populations where NO bioavailability is reduced. In efforts to potentiate NO bioavailability, infusion of the antioxidant ascorbic acid (AA) enhances steady-state hyperemic and vasodilator response in older, but not young adults, presumably through a NO-mediated mechanism (e.g. enhanced endothelium-dependent vasodilation) (141, 292). However, AA failed to improve hyperemic and vasodilator responses to single skeletal muscle contractions suggesting that oxidative stress may play a regulatory role during steady-state exercise, but not at the onset of exercise (141). Conversely, available data from the human leg suggests that infusion of the antioxidants (N-acetylcysteine or AA) thereby potentiating NO bioavailability abolishes age-related differences in vascular conductance at rest but does not augment exercise hyperemia in older sedentary subjects. These discrepant findings have been attributed to limb differences with exercise hyperemia as well as NO playing a larger role in forearm exercise hyperemia relative to the leg. Furthermore, it should be noted however, administration of antioxidants such as AA and N-acetylcysteine may benefit vascular tone through reductions in oxidative stress, but not necessarily enhancing NO bioavailability (195, 196, 204, 206, 232, 235, 248, 253, 254). Extending this concept within the context of aging, Limberg et. al. (160) demonstrated that in the forearm of young healthy adults, potentiation of NO bioavailability via phosphodiesterase-5 (PDE-5) inhibition did not have any effect on forearm blood flow and vasodilation in response to exercise. Conversely, Nyberg (209, 210) showed that PDE-5 inhibition augmented O_2 delivery, which was related to elevations in O_2 uptake selectively in the leg of older, but not young adults.

Despite the plethora of evidence for the role of NO acting as a vascular regulatory molecule, the rapidity of vascular responses to single contractions suggests metabolic

vasodilators also hold a key role. Indeed, interstitial potassium (K^+) has been shown to play a role in the regulation of vascular tone with exercise and active hyperemia (5, 6, 46, 106, 151). Evidence from animal models indicate that altering the vascular membrane potential via ouabain (Na^+/K^+ ATP_{ase} inhibitor) delayed the timing of vasodilation to electrical stimulations, as well as attenuating the timing of perfusion pressure to reach steady state levels (46). Furthermore, Armstrong (5) demonstrated that smooth muscle cell hyperpolarization elicited by K^+ as a result of muscle contraction elicited a rapid, robust vasodilation via inwardly rectifying K^+ channels and sodium potassium ATP_{ase}. Within the human forearm, inhibition of K^+ channels and sodium potassium ATP_{ase} reduces peak vasodilation following a single skeletal muscle contraction by approximately 30-45%, with further reductions evident with combined inhibition of NO and prostaglandins (approximately 60% attenuation) (59). Additionally, inhibition of inwardly rectifying K^+ channels reduces steady-state blood flow by approximately 30% of control values (60).

Collectively, this evidence suggests that endothelial factors such as NO and K^+ play a role in exercise hyperemia across the lifespan. Namely, there is a reduction in NO-mediated vasodilation during exercise with advanced age, and this reduction in NO bioavailability or signaling acts to impair exercise hyperemia and vasodilation with age. Whether enhancement of bioavailable NO in older adults improves exercise hyperemia and vasodilation is unknown. *Chapter 7 examines whether potentiation of NO bioavailability via inorganic nitrate supplementation mitigates age-related differences in both onset and steady-state exercise hyperemia within the leg.*

Summary

Given that advancing age is associated with alterations in blood flow distribution within exercising skeletal muscle, the ratio of O_2 demand to O_2 delivery may be altered at exercise onset, promoting generalized exercise intolerance. This evidence, coupled with reduced aerobic capacity/physical function in older adults suggests that the age-associated reductions in exercise hyperemia and vasodilation may contribute to increased frailty and reduced independence of older adults. In this way, the aging vasculature may not retain the ability to adapt to rapid transitions from rest to exercise or between changes in exercise intensity. Given the uncertainties listed, key questions remain unanswered regarding how the local regulation of blood flow is altered with age. Specifically, it is currently unclear as to 1) whether mechanical influences mediate immediate exercise hyperemia in older adults; 2) whether ROV responses vary between limbs (arm vs. leg); 3) whether sympathetic vasoconstriction contributes to age-related attenuations in contraction-induced ROV responses within the leg (similar to what has been shown in the arm); 4) whether chronic exercise training preserves blood flow and vasodilator responses in response to single skeletal muscle contractions with age; 5) whether aging results in a prolonged vasodilator kinetic response within the leg and whether this is offset by chronic exercise training; and 6) whether potentiation of NO bioavailability mitigates age-associated attenuations in exercise hyperemia and vasodilation both at the onset of exercise as well as during steady state exercise. Collectively, this research will address the physiological mechanisms for reductions in blood flow and vasodilation at the onset of exercise in older adults, as well as characterize how advancing age impacts the blood flow and vasodilator responses across an entire exercise transient (onset, kinetics, and steady-state). Additionally, interventions to ameliorate these age-related reductions will be explored from both a chronic exercise training, as well as a nutraceutical stand-point.

Specific Aims

Specific Aim 1 (Chapter 2)

Specific Aim 1.1 Examine whether the relative mechanical contribution to contraction-induced ROV is altered in older adults.

Hypothesis 1.1 Due to increases in peripheral artery stiffness, extravascular compression will not elicit similar elevations in vasodilation as compared to contraction-induced ROV.

Specific Aim 1.2 Examine the influence of perfusion pressure on contraction-induced ROV within the forearm of young and older adults.

Hypothesis 1.2 Elevation of perfusion pressure will augment contraction-induced ROV within the forearm of both young and older adults.

Specific Aim 1.3 Examine the relationship between peripheral arterial stiffness and contraction-induced ROV in young and older adults.

Hypothesis 1.3 Elevations in peripheral arterial stiffness will be inversely associated with contraction-induced ROV within young and older adults.

Specific Aim 2 (Chapter 3)

Specific Aim 2.1 Characterize the influence of age on contraction-induced rapid vasodilation within the leg.

Hypothesis 2.1 Advanced age will reduce contraction induced rapid onset vasodilation within the leg of older adults.

Specific Aim 2.2 Examine whether age-related ROV responses vary between limbs.

Hypothesis 2.2 The lower limbs will exhibit a greater reduction in contraction-induced rapid vasodilation in older adults relative to the upper limbs.

Specific Aim 3 (Chapter 4)

Specific Aim 3.1 Determine whether enhanced sympathetic vasoconstriction contributes to the attenuated contraction-induced ROV response observed in the leg of older adults.

Hypothesis 3.1 Acute elevations in sympathetic nervous system activity will reduce ROV to a greater extent in young compared to older adults.

Specific Aim 4 (Chapter 5)

Specific Aim 4.1 Determine whether long term exercise training can prevent the reductions in ROV that occurs in untrained older adults.

Hypothesis 4.1 Chronic endurance exercise training in older adults will ameliorate the age-related impairments in contraction-induced rapid vasodilation.

Specific Aim 4.2 Characterize the relationship between cardiorespiratory fitness, age, and contraction-induced ROV.

Hypothesis 4.2 A higher cardiorespiratory fitness will be strongly related to contraction-induced rapid vasodilation across the age-span.

Specific Aim 5 (Chapter 6)

Specific Aim 5.1 Examine whether the kinetics of blood flow and vasodilation are impaired within the leg of sedentary older adults as a function of age, or physical training.

Hypothesis 5.1 Aging will result in attenuated vasodilator kinetics within the leg of older adults, which will be mitigated by chronic exercise training in older adults

Specific Aim 5.2 Characterize the relationship between hyperemic and vasodilator responses at the onset of exercise to the kinetics of blood flow and vasodilation during steady-state exercise in the leg.

Hypothesis 5.2 The blood flow and vasodilator responses following a single muscle contraction will be inversely related to the overall kinetic profile of blood flow and vasodilation during steady-state exercise.

Specific Aim 6 (Chapter 7)

Specific Aim 6.1 Examine whether dietary nitrate improves leg blood flow and vasodilation in older adults, both at the onset, as well as during steady-state exercise.

Hypothesis 6.1 Dietary nitrate will improve leg blood flow and vasodilation in older adults across an exercise transient in older adults.

CHAPTER 2: AGE-ASSOCIATED IMPAIRMENTS IN CONTRACTION-INDUCED RAPID VASODILATION WITHIN THE FOREARM ARE INDEPENDENT OF MECHANICAL FACTORS

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Introduction

The mechanisms involved in exercise-induced hyperemia have been examined for decades, however no one cohesive mechanism has been found to be responsible for the initial phase of exercise hyperemia (130). Following a single skeletal muscle contraction blood flow rapidly increases, peaking within ~4 – 5 seconds, termed contraction-induced rapid onset vasodilation. In young adults, contraction-induced ROV is generated through a series of factors, including endothelial (43, 59, 141), and mechanical factors (51, 54, 297). With regard to the latter, evidence from both human and animal models suggest that the mechanical compressive changes (due to muscle contraction) within the microvasculature act synergistically with vasoactive factors (e.g. nitric oxide, potassium channels etc.) to elicit a rapid vasodilation that feeds-forward into steady-state exercise in young healthy humans (51). However, contraction-induced ROV is reduced in older adults, with the mechanisms for this attenuation attributed to local (endothelial) and neural (sympathetic adrenergic) factors (40, 43, 141). Whether mechanical factors are implicated as a mechanism for age-related attenuations in contraction-induced ROV is currently unknown.

Mechanical factors profoundly influence skeletal muscle blood flow, as demonstrated from both human and animal models (51, 61, 124, 262). It has previously been shown that posture or limb position evokes gravitational (i.e. hydrostatic) fluid shifts, which subsequently

modify the arterio-venous pressure gradient and alter skeletal muscle blood flow (61, 124, 294, 297). In a similar sense, contraction-induced ROV responses may be influenced via manipulation of the forearm by placing the experimental limb at different positions relative to heart level (e.g. above vs. below-heart level). In this way, gravity-induced increases in perfusion pressure elicit greater hyperemic and vasodilator responses (and *vice versa*) following a single skeletal muscle contraction in young adults, and this has traditionally been attributed to the influence of the skeletal muscle pump (61, 297). Additionally, evidence from both animal and human models indicate that acute extra-vascular compression may closely mimic muscle contraction, but without the confounding influence of muscle activation, eliciting an ROV response (51). This response is temporally disassociated with that of a single skeletal muscle contraction such that the time course of vasodilation due to mechanical compression occurs much sooner (~2-3 cardiac cycles) than a single skeletal muscle contraction (~5 cardiac cycles) (61, 139), resulting in alterations in total blood volume moved. The majority of the evidence for mechanical factors influencing skeletal muscle blood flow during exercise has been examined in young, healthy populations, with virtually no data examining whether advancing age influences this contribution of mechanical factors.

Advancing age results in an increased arterial stiffness due to adverse structural (collagen and elastin remodeling) and functional mechanisms (reduced endothelial function) predisposing this population to elevated risk for cardiovascular disease, as well as contributing to age-associated attenuations in exercise hyperemia and vasodilation (169, 170, 192, 280, 283). Indeed, evidence from the Framingham Heart Study demonstrates that carotid-femoral pulse-wave velocity (PWV), an index of central artery stiffness, is inversely associated with reactive hyperemic flow velocity (56, 163, 177). However, aortic PWV as estimated by carotid-femoral

PWV, does not necessarily reflect the "stiffness" that the isolated forearm exercise model is exposed to. There is some evidence to suggest that peripheral artery stiffness (e.g. carotid-radial PWV) is altered with age (8, 95) however, these results are not universal (174, 175). Given that advancing age is associated with structural and functional changes within the vasculature, mechanical forces may modulate hyperemic and vasodilator responses to exercise, particularly at the onset (e.g. ROV).

Given that ROV is an important component of blood flow regulation at the onset of exercise, and due to the fact that older individuals exhibit a reduced steady state exercising blood flow, investigating whether the mechanical contribution to ROV changes with age, as well as examining the mechanical components of the hyperemic and vasodilatory responses under changes in perfusion pressure is relevant in the context of reductions in functional performance and work capacity with age. The present study sought to examine the mechanical contribution to contraction-induced ROV in both young and older adults. We tested the hypothesis that 1) the mechanical contribution to ROV within the forearm is not different between young and older adults, 2) manipulation of perfusion pressure modulates ROV responses, such that enhanced perfusion pressure augments ROV responses to a similar extent in young and older adults, and 3) upper limb conduit artery stiffness would be inversely related to contraction-induced ROV within the forearm in both young and older adults.

Methods

Ethical approval

The nature, risks, and benefits of all study procedures were explained to the subjects, and their written informed consent was obtained before participation in the study. All procedures

were reviewed and approved by the Institutional Review Board at the University of Iowa and complied with the latest version of the *Declaration of Helsinki*.

Subjects

A total of 24 healthy normotensive young (n=12) and older adults (n=12) participated in the study. All subjects completed written, informed consent and a general health history screening. Subjects were generally healthy, non-obese (body mass index: ≤ 30 kg/m^2), non-smokers, and not taking any prescription medications that would influence blood pressure or exercise hyperemia. Additionally, all young female subjects were studied during the early follicular phase of their menstrual cycle, or during the placebo phase if on an oral contraceptive. Older female subjects were post-menopausal, and not taking any hormone replacement therapies. Studies were performed in the morning after an overnight fast, with subjects refraining from exercise, alcohol, and caffeine for 24 hours before reporting to the laboratory.

Experimental Protocol

Following 15 minutes of quiet supine rest, carotid-radial PWV (c-rPWV) was performed in duplicate with the experimental arm position at-heart level (~0˚). For contraction and extra-vascular compression trials (via pneumatic cuff inflation), subjects lay in the supine position with the experimental (left) arm in three different randomized positions: above (45˚), at (~0˚), and below (45˚) heart level (Figure 1). These positions (above and below) were used to manipulate forearm perfusion pressure (124, 297) with the at-heart level position (~0°) used as a reference condition. Therefore, comparisons were made relative to the control condition (at-heart level) to interrogate the influence of hydrostatic forces by using the following arm positions: 1) above-heart level to decrease perfusion pressure and facilitate venous emptying, and 2) below-heart level to enhance perfusion pressure (124, 297). Prior to each contraction/compression trial the

experimental arm was positioned to the given position, and 5 minutes of rest was allowed for stabilization of haemodynamics.

Single Muscle Contractions and External Mechanical Compression

Subjects performed single dynamic forearm contractions (1s concentric-eccentric contraction followed by relaxation) at 20% of the subject's maximal voluntary contraction (MVC) using a custom-made, handgrip device attached to a simple pulley system (moving hanging weights ~4 - 5 cm). Each subject's MVC was determined prior to experimental trials using an isometric handgrip dynamometer (Stoelting, Chicago, IL). External mechanical compressions (200 mmHg; 1s inflation followed by rapid, complete deflation) was achieved using a rapidly inflating/deflating pneumatic cuff placed around the maximal circumference of the forearm (Hokansen E20, Bellevue, WA). This level of external compression was used to mimic intramuscular pressures observed during skeletal muscle contractions, and is based on previous evidence wherein higher levels of external compression do not elicit any further increases in hyperemia (59, 124, 139). Single forearm skeletal muscle contractions and compressions were performed in duplicate across all three arm positions. Study procedures were randomized (above vs. at vs. below-heart level) and counterbalanced (contraction vs. compression) within each subject. Following each contraction/compression trial the dependent arm was always positioned at-heart level, and 15 minutes of rest preceded the next trial.

Measurements

Heart rate was measured via continuous three-lead electrocardiogram (ECG) and systemic blood pressure (BP) was measured continuously (beat-to-beat) via finger plethysmography (Nexfin, Edwards Lifesciences, Irvine, CA) on the non-exercising (right) hand, which was kept at-heart level throughout the study. Prior to the experimental protocol, and

following a 15-minute rest period in a supine position, brachial BPs were taken in duplicate using an automated cuff (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). ECG-gated c-rPWV was then recorded at the left carotid and radial artery using a pencil type micromanometer (Millar Instruments, Houston, Texas) and SphygmoCor system (AtCor Medical, Sydney, Australia) to assess arterial stiffness of the upper-limb vessels.

Forearm Blood Flow and Vascular Conductance

Brachial artery diameter (cm) and blood velocity ($cm \cdot s^{-1}$) were determined with a 12-MHz linear array Doppler probe (model M12L; Vivid 7, General Electric, Milwaukee, WI). Blood velocity was measured with a probe insonation angle previously calibrated to 60°. Measured velocity waveforms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (113). Brachial artery diameter measurements were obtained at end-diastole during rest (baseline; before contraction). Forearm blood flow (BF) was calculated as the product of mean blood velocity ($cm \cdot s^{-1}$) and brachial artery cross-sectional area (cm^2) and expressed as millilitres per minute ($ml \cdot min^{-1}$). Vascular conductance (VC) was calculated as the quotient of BF and mean arterial pressure (MAP) x 100 (expressed as $ml \cdot min^{-1} \cdot 100\ mmHg^{-1}$).

Data Analysis and Statistics

Data were collected at 250 Hz and analysed offline with signal-processing software (WinDaq, DATAQ Instruments). Beat-to-beat arterial pressure was derived from the Nexfin pressure waveform and was recorded simultaneously with beat-to-beat blood velocity measurements. Baseline BF and MAP represents an average of the last 30-seconds of the baseline period before each single contraction or compression, and were used to quantify the hyperemic response. Of particular interest are the peak and total hyperemic and vasodilator

responses post-contraction and compression. Peak hyperemic and vasodilator responses are expressed as the absolute change (Δ) in BF and VC from baseline in each respective arm position. Total BF (ml) and VC (ml·100 $mmHg^{-1}$) were defined as the area under the curve over 30 cardiac cycles post-contraction after respective baseline values were subtracted for a given flow or conductance curve (164). The relative mechanical influence on contraction-induced ROV responses was calculated as (297):

Equation 1: Relative Mechanical Contribution to ROV

$$\frac{VC_{compression}}{VC_{contraction}} * 100\%$$

Data are expressed as means ± SE. Baseline data was compared between young and older adults using analysis of variance (ANOVA). In order to address the primary question, ROV responses (peak, total, relative mechanical contribution) were compared between young and older adults at-heart level via independent t-tests. In order to address the influence of perfusion pressure on ROV responses, two-way repeated measures ANOVA was used to compare responses relative to at-heart level (e.g. above-heart vs. at-heart, below-heart vs. at-heart) on contraction and compression-mediated vasodilation (age x position) as well as the relative mechanical influence on contraction-induced ROV. Pearson product-moment correlations were used to examine the relationship between contraction-induced ROV and peripheral arterial stiffness. All statistical analyses were completed using SigmaStat software version 12.0 (Systat Software Inc., San Jose, CA). Significance was set *a priori* at $P < 0.05$.

Results

All 24 subjects completed the experimental protocol. Subject characteristics measured prior to experimental trials are shown in Table 1. Older adults had higher BMI, brachial BP (SBP, DBP, and MAP) and carotid-radial PWV than the young adults.

Rapid Hyperemic and Vasodilator Responses to Single Skeletal Muscle Contractions and Single Extra-vascular Compressions

Resting haemodynamic (prior to each condition), brachial artery BF and VC responses are presented in Table 2. There were no differences in MAP, BF, or VC across conditions (above, at, and below) or between groups (contraction vs. compression) either in older or young adults. Figure 2 illustrates the temporal rapid vasodilator responses in young (A & C) and older adults (B & D) following single forearm contractions and single forearm mechanical compressions with changes in perfusion pressure (above, at, and below-heart level). In general, each response (contraction and compression) elicited a robust hyperemic and vasodilatory response in both young and older adults, peaking within ~2-5 cardiac cycles post-contraction, returning back to baseline within 10-15 cardiac cycles during each arm position.

Figure 3 illustrates peak (A & B) and total (C & D) hyperemic and vasodilator responses following a single skeletal muscle contraction and single mechanical compression of the forearm. At-heart level responses to single muscle contractions were attenuated in older vs. young adults for total ROV (14±2 vs. 19±3 ml·100 $mmHg^{-1}$; $P < 0.05$) but not peak ROV (75±7 vs. 93±16 $ml \cdot min^{-1} \cdot 100\ mmHg^{-1}$; $P = 0.13$). Older adults also demonstrated attenuated peak (28±4 vs. 42±6 $ml \cdot min^{-1} \cdot 100\ mmHg^{-1}$; $P < 0.05$) and total (4±1 vs. 8±2 ml·100 $mmHg^{-1}$; $P < 0.05$) vasodilator responses to single mechanical compressions relative to young adults. The relative mechanical contribution to at-heart level ROV responses were not different between young and older adults for either peak (46±4% vs. 40±5%; $P = 0.21$) or total ROV (37±6 vs. 32±5%; $P = 0.27$).

Influence of Perfusion Pressure on Hyperemic and Vasodilator Responses to Single Skeletal Muscle Contractions and Single Extra-Vascular Compressions

Reductions in perfusion pressure by placing the arm above-heart level reduced peak and total BF but not VC responses to single skeletal muscle contractions. Conversely, reductions in perfusion pressure reduced total BF, as well as peak and total VC during extra-vascular compression trials in both young and older adults (Figure 3; main effect of position $P < 0.05$). Enhancing perfusion pressure (e.g. arm below-heart level) augmented peak BF and VC responses to single skeletal muscle contractions (main effect of position $P < 0.05$) as well as peak BF and VC responses to extra-vascular compression (main effect of position $P < 0.05$) in both young and older adults. A main effect of age was observed for total VC during single skeletal muscle contractions while the arm was positioned below-heart level (main effect of age $P < 0.05$), but not above-heart level (main effect of age $P = 0.12$), however there were no significant age x position interactions ($P = 0.14$-0.77).

Mechanical Contribution to Contraction-Induced ROV in Young and Older Adults

Figure 4 illustrates the relative mechanical contribution to ROV within young and older adults with reductions and elevations in perfusion pressure relative to at-heart level. Reductions in perfusion pressure did not alter the mechanical contribution to ROV in young or older adults (main effect of position $P = 0.07$-0.45), while elevations in perfusion pressure (e.g. arm below-heart level) enhanced the mechanical contribution to peak BF, VC and total VC in both young and older adults to a similar extent (main effect of position $P < 0.05$). A main effect of age was observed for peak and total VC ($P < 0.05$), however there were no significant age x position interactions ($P = 0.23$-0.44).

Relationships between Upper Limb Arterial Stiffness and Contraction-Induced ROV

Older adults exhibited elevated c-rPWV relative to young adults at rest (Table 1; $P < 0.05$). However, there were no associations between c-rPWV and peak VC ($r = 0.02$; $P = 0.93$) or

total VC (r = -0.01; P = 0.96) at-heart level in the group as a whole, or when separated by age-groups (r = 0.24-0.39; P = 0.21-0.72). Similarly, there were no associations between c-rPWV and peak or total VC at the above-heart level position (r = 0.18 and -0.14; P = 0.41 and 0.52) or at the below-heart level position (r = -0.09 and -0.14; P = 0.67 and 0.51) when examined for the entire group. When separated into age groups (young and older), no associations were observed between c-rPWV and peak VC or total VC (r = - 0.30 – 0.49; P = 0.11- 0.77).

Discussion

This investigation sought to explore whether the mechanical contribution to ROV within the forearm is altered with age, and whether contraction-induced ROV is related to peripheral artery stiffness. Additionally, we examined whether vascular responses to single skeletal muscle contractions or compressions are modulated by changes in perfusion pressure in healthy young and older adults. Our novel findings are: 1) the mechanical contribution to contraction-induced ROV within the forearm is not altered with advancing age; 2) manipulating perfusion pressure alters the rapid hyperemic response and vasodilator response, as well as the mechanical contribution to ROV in young and older adults to a similar extent; and 3) peripheral artery stiffness (c-rPWV) is not related with ROV, despite age-related differences in c-rPWV. Collectively, our findings demonstrate that mechanical factors do not appear to play a significant role in the age-related attenuations in ROV within the forearm, and contraction-induced ROV responses are largely independent of peripheral artery stiffness. Additionally these results extend upon previous findings in young adults and highlight an important role of perfusion pressure modulating contraction-induced ROV responses within the forearm in healthy young and older adults.

Mechanical Contribution to ROV with Age

Prior work in young adults and animals has demonstrated that an acute increase in extra-vascular/intraluminal pressure elicits a rapid vasodilatory response (51, 53, 139, 263, 297). The present study corroborates these findings by demonstrating that acute external compression (200 mmHg) elicits a rapid vasodilation that peaks within ~2-3 cardiac cycles post-compression in young adults (Figure 2 C & D). Furthermore, our findings are similar to those of Tschakovsky, et. al. (297) and Kirby, et. al. (139), demonstrating external compression of the forearm contributes approximately 20-60% to peak BF and/or VC responses depending on the position of the experimental arm relative to heart level (Figure 4). Interestingly, with the arm positioned at-heart level, older adults demonstrated attenuated peak and total VC responses to single external compression (Figure 3), suggesting that the isolated skeletal muscle pump may be slightly impaired in older adults. However when considered with the age-related lower contraction-induced responses, the relative mechanical contribution to ROV within the forearm remains unaltered (Figure 4). While external compression of resistance microvasculature may not represent the actual mechanical distortion that occurs with a muscle contraction *per se*, our data, along with previous work, indicates that the mechanical contribution to rapid vasodilation when the arm is in a neutral position (at-heart level) is similar between young and older adults (124, 297). In this context, the mechanisms for the age-related attenuation in contraction-induced ROV within the forearm have previously been shown to be due to local factors (e.g. endothelial nitric oxide signaling/bioavailability) (43, 141) as well as enhanced α-adrenergic vasoconstrictor tone (40). Collectively, it appears as though age-related reductions in contraction-induced ROV are not due to mechanical influences within the forearm.

Perfusion Pressure and Contraction-Induced ROV with Age

The idea that hydrostatic forces contribute significantly to perfusion pressure are supported by both animal and human data (93, 262, 297). Mechanical contributions attributed to the skeletal muscle pump (e.g. arterio-venous pressure gradient) in concert with ROV are postulated to be important determinants responsible for the rapid increase in blood flow at the onset of contractions, at least in young adults (297). This evidence stems from experimental manipulation of arterio-venous pressure gradients through venous emptying eliciting transient elevations in BF and vasodilation both with and without skeletal muscle contractions (294, 297). Conversely, it could be argued that the immediate hyperemic and vasodilator responses within this experimental paradigm are not due to the skeletal muscle pump, but rather is a function of perfusion pressure (i.e. when arm is positioned below-heart level). In a similar sense, Jasperse et. al. (124) demonstrated, using both *in vivo* and *in vitro* protocols, that positional differences in reactive hyperemia (as opposed to active hyperemia) are predominately due to changes in perfusion pressure eliciting alterations in vasodilation, and these differences are likely attributed to changes in vascular function *per se*. Therefore, the greater hyperemic and vasodilator responses are predominately observed when perfusion pressure is enhanced (105, 124, 291).

Previous evidence within the forearm of young adults, demonstrates that manipulation of perfusion pressure via changes in arm position relative to heart level alters ROV responses following a single contraction (297). Within the current study, our findings demonstrate that when perfusion pressure is increased or decreased relative to at-heart level (i.e. below or above heart-level) rapid hyperemic and vasodilator responses to single skeletal muscle contractions and extra-vascular compression are altered to a similar extent in young and older adults. Interestingly, a main effect of age was observed for total VC responses with the arm positioned below-heart level, suggesting that age-associated differences apparent with the arm at-heart level

persist despite enhancements in perfusion pressure. Additionally, we demonstrate that the relative mechanical contribution to contraction-induced ROV is greater when perfusion pressure is enhanced (Figure 4).

Peripheral Arterial Stiffness and Contraction-Induced ROV

Advanced age is associated with increases in arterial stiffening and structural remodeling (154, 170, 192, 280). Given that reduced arterial vascular function is associated with reductions in exercise hyperemia (103), we reasoned that basal muscular artery stiffness would be inversely related to contraction induced ROV. Within the current study, age-associated differences were observed for c-r PWV, however, in contrast to our hypothesis, no significant associations were observed between non-invasively measured resting upper-limb arterial stiffness and contraction-induced ROV. Acknowledging that muscular artery stiffness (c-rPWV) does not always follow the same drastic increase with age as aortic PWV (carotid-femoral PWV) (174), these results suggest that muscular artery stiffness may not directly influence exercise hyperemia within the forearm of young and older adults.

Experimental Considerations

There are a few experimental considerations that warrant discussion. First, it cannot be determined whether forearm cuff inflations at 200 mmHg truly mimic the internal compressive forces (intramuscular pressures) elicited by voluntary skeletal muscle contractions at 20% MVC. In the present study, high external pressures (200 mmHg) were employed to mimic peak hyperemic responses observed in response to single submaximal voluntary contractions, similar to previous evidence (59, 124, 139). In this context, it should be noted that a non-linear relationship between external pressure and ROV has been previously demonstrated (139). That is, step-wise increases in external pressure did not result in similar percent increases in ROV

with responses plateauing at 100 mmHg with no further vasodilation observed until 300 mmHg. In keeping with previous evidence in young adults, 200 mmHg was chosen for the current study. Furthermore, within the leg, no differences in the ROV are apparent with graded external compression (61). In contrast, contraction-induced ROV has been shown to be linearly related to exercise intensity [e.g. MVC] (139). The high external pressures induced by cuff inflation in the present study resulted in a transient but lower hyperemic response compared to single voluntary dynamic contractions regardless of arm position (i.e. perfusion pressure) in healthy young and older adults. Evidence from various animal models indicate that the intra-muscular pressure elicited by both electrical stimulation and extra-vascular compression may widely vary, however Mohrman & Sparks showed that within canine gastrocnemius, tension developed as a result of electrical stimulation was linearly related to intra-muscular balloon pressure (178). These findings support the idea that the microvasculature is affected by the intramuscular environment when an external stimulus is applied.

Second, it is important to note that intramuscular pressure is also dependent on muscle architecture. Previous studies examining intramuscular pressure responses to applied external pressure or voluntary muscle contractions have utilized the pennate fibres of the vastus lateralis and medialis (245, 260). In the context of the current study, we did not specifically measure intramuscular pressure, and it is unknown if specific fibre types influence intramuscular pressure during skeletal muscle contractions. Finally, upper-limb conduit artery stiffness was measured via c-rPWV. This measurement uses a time delay between two pressure recordings (carotid and radial arteries) to measure the velocity of the pulse-wave. This measure of peripheral artery stiffness may not truly reflect the stiffness of the microvessels within the forearm as there are numerous branching points between the carotid and radial arteries. Nonetheless, age-associated

differences were observed for c-rPWV, however this was not associated with the vasodilator response to single skeletal muscle contractions within the forearm.

Conclusion

This study has provided novel evidence regarding the mechanical influences on contraction-induced ROV within the forearm of young and older adults. Specifically, age-related reductions in contraction-induced ROV within the forearm do not appear to be influenced by mechanical factors, and ROV responses are independent of peripheral artery stiffness. Furthermore, contraction-induced ROV responses are innately influenced by alterations in perfusion pressure to a similar extent in young and older adults. Collectively, our present data suggest that while mechanical factors may not play a role within age-related reductions in contraction induced ROV within the forearm, manipulation of perfusion pressure modulate these responses and alter the mechanical contribution to contraction-induced ROV within the forearm of both young and older adults to a similar extent.

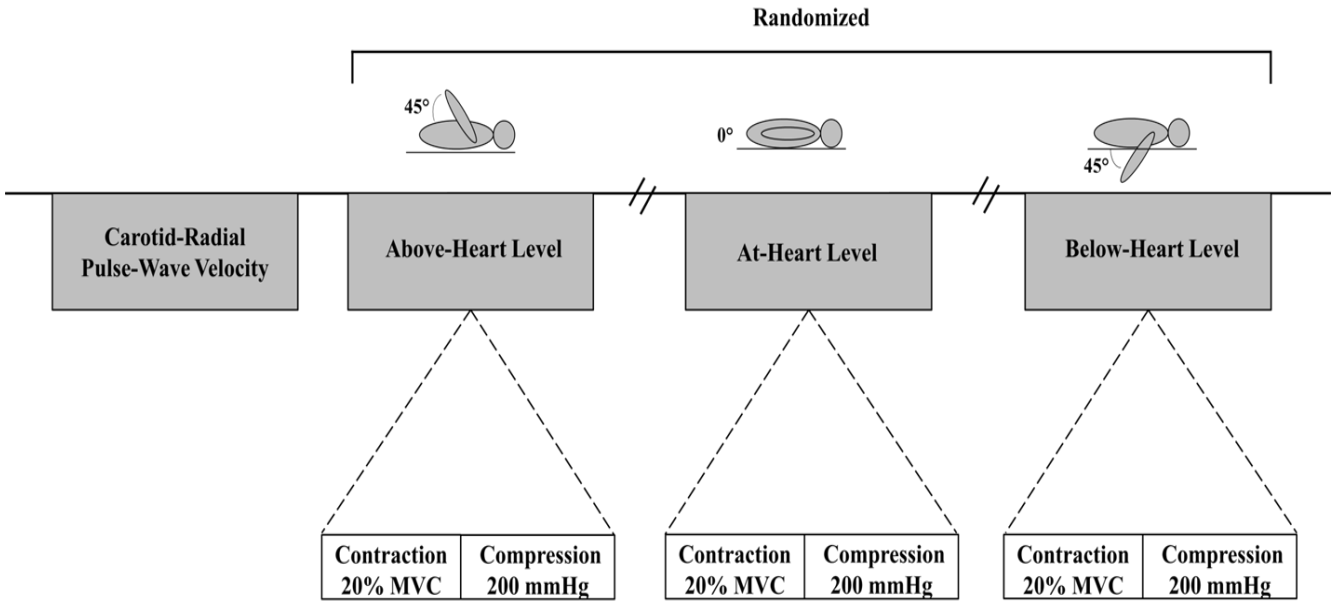

Figure 1: Experimental timeline. Study procedures were randomized (at, above, below) and counterbalanced (contraction vs. compression) within each subject prior to the study day.

Table 1: Subject Characteristics for Aim 1

Variable	Older (n=12)	Young (n=12)
Age (year)	67±1	24±1
Sex (M/F)	6/6	8/4
Height (cm)	169±2	174±4
Weight (kg)	74±3	69±5
Body Mass Index ($kg \cdot m^2$)	25.9±0.8*	22.4±1.0
MVC (kg)	34±3	37±3
Brachial SBP (mmHg)	126±4*	113±3
Brachial DBP (mmHg)	79±2*	68±2
MAP (mmHg)	95±2*	83±2
Brachial PP (mmHg)	47±3	45±2
Carotid-radial PWV ($m \cdot s^{-1}$)	10.4±0.3*	9.3±0.5

Values are mean ± SE. MVC, maximal voluntary contraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP; mean arterial pressure; PP, pulse pressure; PWV, pulse-wave velocity * $P < 0.05$ vs. young adults

Table 2: Baseline Hemodynamics Prior to Each Condition

		Contraction			Compression		
		Above	At	Below	Above	At	Below
Young	MAP (mmHg)	94±3	92±2	93±3	93±3	92±2	94±3
	BF (ml·min^{-1})	34±5	35±4	35±5	29±4	33±4	35±6
	VC (ml·min^{-1}·100 mmHg^{-1})	37±6	38±6	38±6	31±5	36±5	38±7
Older	MAP (mmHg)	106±3	103±3	106±3	106±3	105±2	102±2
	BF (ml·min^{-1})	34±6	39±6	38±5	34±7	37±4	32±5
	VC (ml·min^{-1}·100 mmHg^{-1})	33±6	39±6	37±6	34±8	37±5	32±4

Mean±SE. MAP, mean arterial pressure; BF, forearm blood flow; VC, forearm vascular conductance

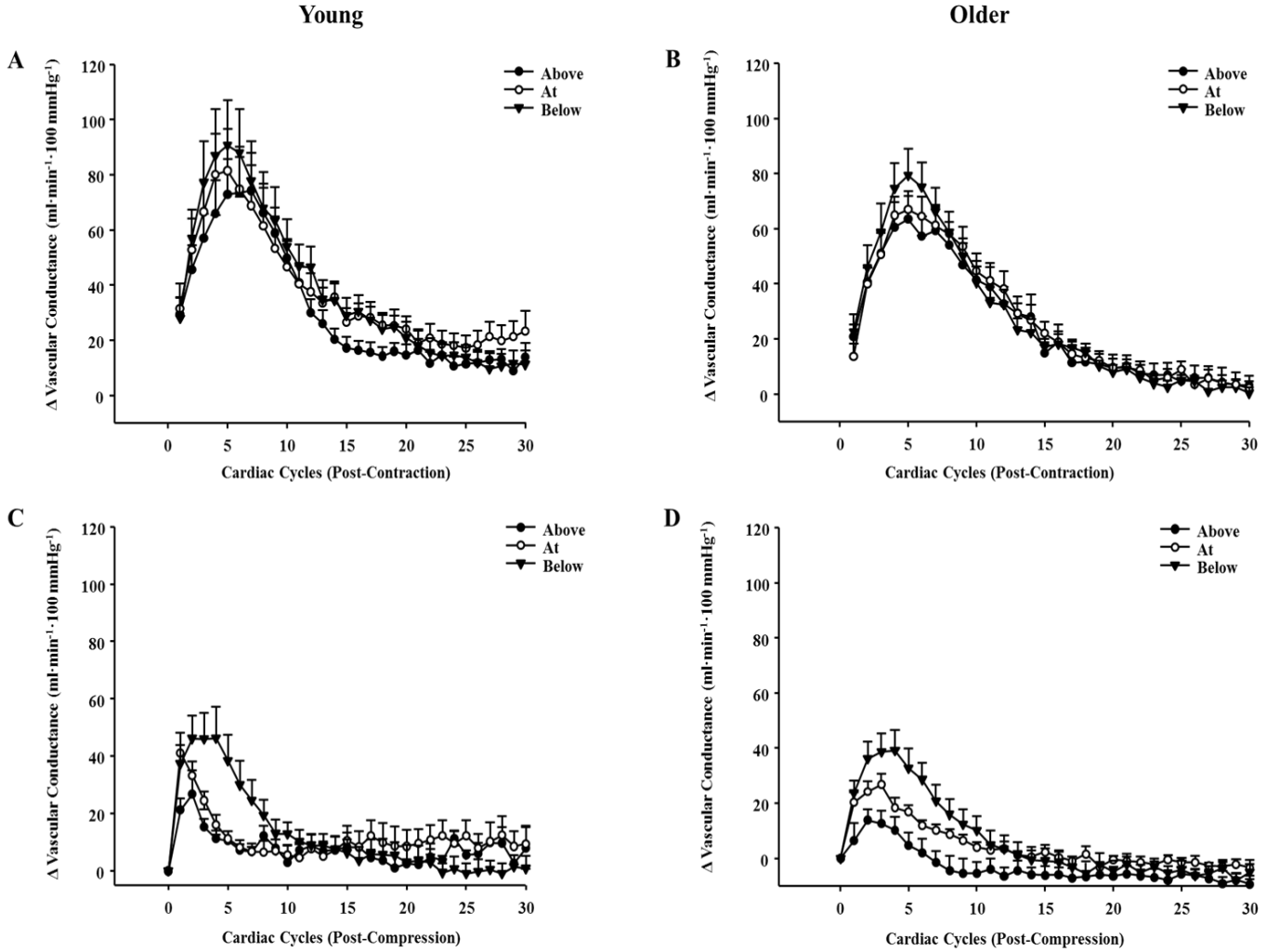

Figure 2: Change in vascular conductance (Δ vascular conductance) over 30 cardiac cycles following a single forearm contraction (20% MVC; A & B) and single mechanical compression (200 mmHg; C & D) with changes in arm position relative to heart level (above, at, and below-heart level) in young (A & C) and older adults (B & D)

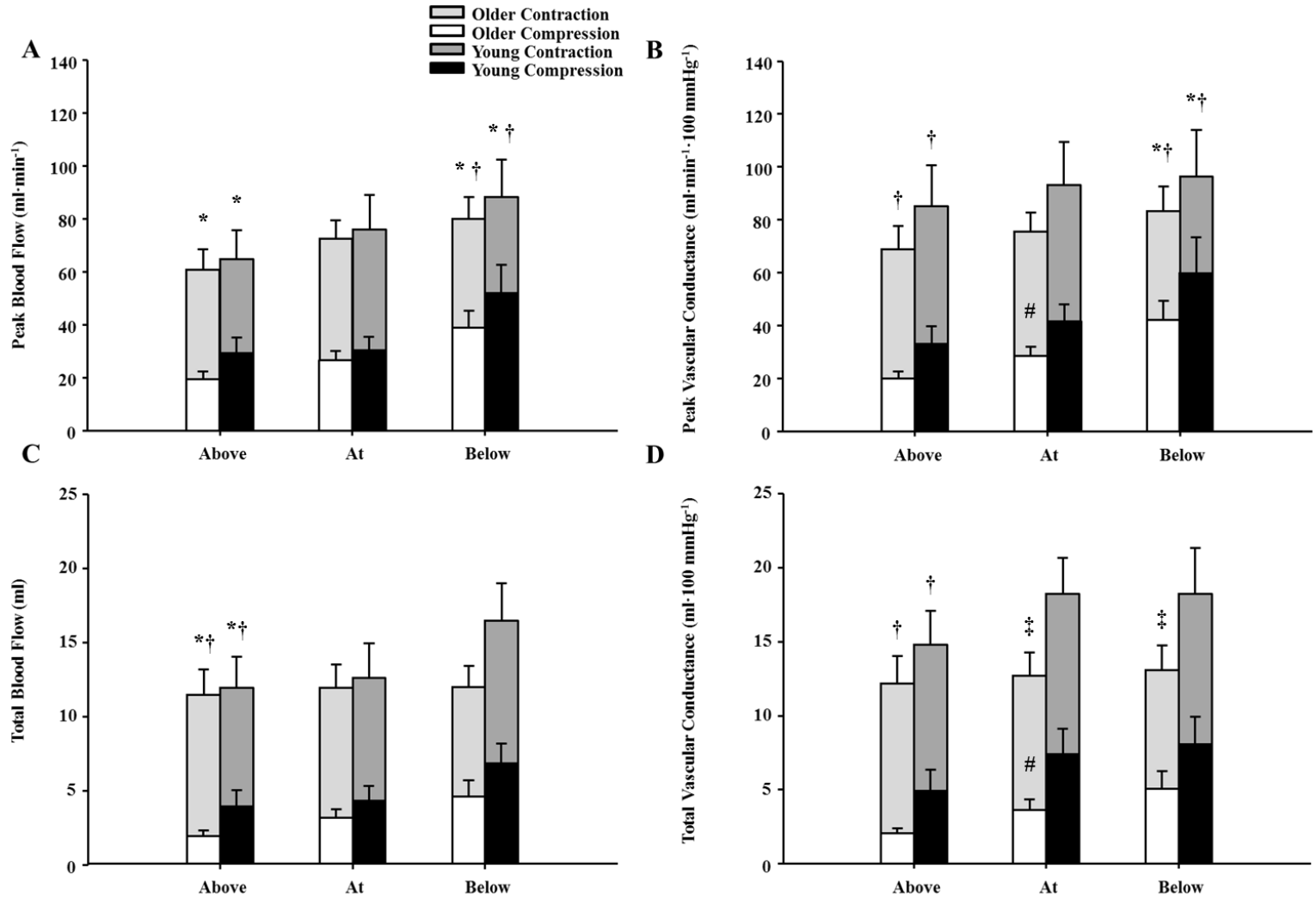

Figure 3: Peak (A & B) and total (C & D) hyperemic and vasodilator responses following a single skeletal muscle contraction and single mechanical compression of the forearm in young and older adults across experimental trials (above-, at-, and below-heart level). * $P < 0.05$ vs. at-heart level during single skeletal muscle contraction. † $P < 0.05$ vs. at-heart level during single external compression. ‡$P < 0.05$ vs. young adults during single skeletal muscle contraction. # $P < 0.05$ vs. young adults during single external compression

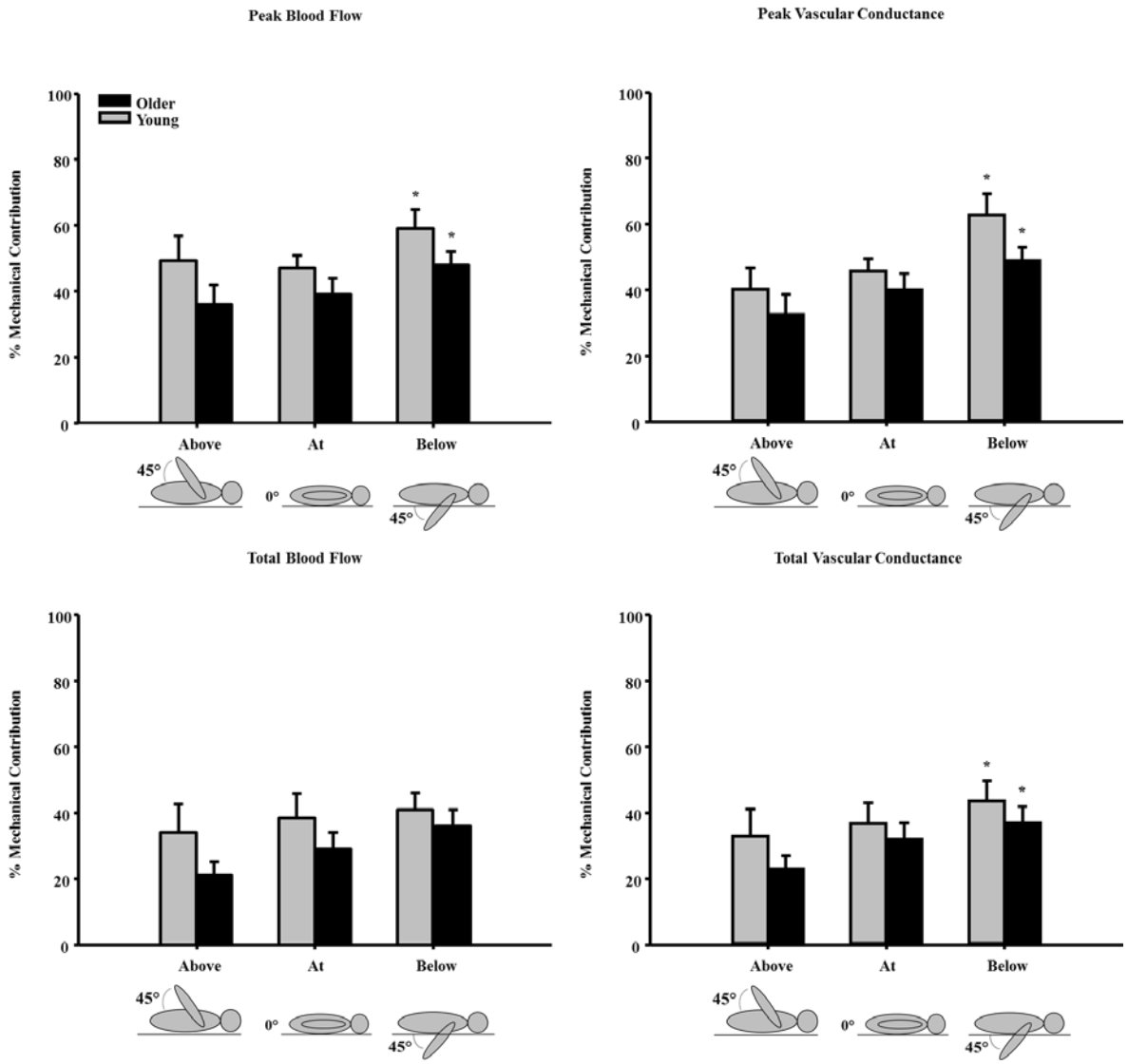

Figure 4: Relative mechanical contribution to peak (A & B) and total (C & D) hyperemic and vasodilator responses in young and older adults across experimental trials (above-, at-, and below-heart level). * $P < 0.05$ vs. at-heart level

CHAPTER 3: RAPID ONSET VASODILATION WITH SINGLE MUSCLE CONTRACTIONS IN THE LEG: INFLUENCE OF AGE

A version of Chapter 3 has previously been published: Hughes WE, Ueda K, Treichler DP, Casey DP. Rapid onset vasodilation with single muscle contractions in the leg: influence of age. *Physiol. Rep.* 2015; 8:e12516.

Introduction

At the onset of exercise there is an obligate, rapid rise in blood flow and vasodilation, ultimately contributing to the fine-tuning of exercise hyperemia and meeting the metabolic demands of contracting tissue during steady state exercise. The immediate exercise hyperemia and vasodilation following a single muscle contraction tends to peak within approximately five cardiac cycles post-contraction, is graded with exercise intensity, and has been termed ROV (51, 54, 191, 295, 297). The rapid vasodilation following a contraction is inherently linked to steady-state blood flow, acting as a feed-forward mechanism, contributing to the rapid transition between exercise workloads (252, 268). The local regulation of contraction-induced rapid vasodilation has been attributed to a complex interplay between endothelial (5, 43, 59), mechanical (139, 297), and adrenergic factors (40, 123), with no one factor having an obligatory role in this immediate hyperemic and vasodilator response.

In humans the majority of the evidence for contraction-induced rapid vasodilation has been characterized in the forearm. Collectively, this evidence has shown that older adults exhibit an attenuated rapid vasodilator response compared to young adults, which may be due to a diminished role of nitric oxide (NO) signaling, enhanced α-adrenergic vasoconstrictor tone and/or a reduced ability to blunt sympathetic vasoconstriction (40, 43). While these studies contribute to the knowledge of local regulation of blood flow and vasodilation, it is not known if

age-related impairments in contraction-induced rapid vasodilation can be generalized to the lower limbs. Recent evidence suggests that rapid vasodilation following a single isometric contraction is observed in the leg of young men, and the magnitude of this response is similar between the arm and leg (61). However, it is unclear how aging influences contraction-induced rapid vasodilation in the leg. In this context, the lower limbs are subject to greater hydrostatic forces (e.g. gravity), and locomotion presents a constant hemodynamic challenge to the leg vasculature (244). Moreover, evidence suggests that limb-specific differences in vasodilator responsiveness exist with aging. That is, the blood flow and vasodilator responses to exogenous vasodilators, limb ischemia, and steady- state exercise in the leg of older individuals are attenuated to a greater extent relative to the forearm (80, 195, 199, 216, 231, 241). Furthermore, the lower limbs have a greater propensity to develop atherosclerotic lesions, and have a higher prevalence of peripheral arterial disease (301). Collectively, these findings suggest that older adults would exhibit an impairment of contraction-induced rapid vasodilation in the leg, and this impairment is potentially greater than what has been demonstrated in the forearm (39, 40, 43, 141). Therefore, the primary purpose of this study was to characterize the effects of aging on contraction-induced rapid vasodilation in the leg. Additionally, we aimed to examine whether potential age-related impairments in rapid vasodilation following a single muscle contraction were greater in the leg compared to the forearm.

Methods

Subjects

A total of 14 young (9 men and 5 women, age: 20-26 yr) and 16 older (10 men and 6 women, age: 60-72 yr) subjects volunteered to participate in this study. Subjects completed a general health history screening and written informed consent. Subjects were generally healthy,

nonobese (body mass index: ≤ 30 kg/m^2), nonsmokers, not taking any vasoactive medications, and were self-reported as sedentary to recreationally active, with no regular physical training. Studies were performed after an overnight fast, and subjects refrained from exercise, alcohol, and caffeine for 24 hours before reporting to the laboratory. Young female subjects were studied during the early follicular phase of their menstrual cycle or the placebo phase of oral contraceptives to control for the potential influence of sex hormones on primary outcome variables (44, 173). All older female subjects were post-menopausal and were not taking any form of hormone replacement therapy. All study protocols were approved by the Institutional Review Board at the University of Iowa.

Body Composition, Forearm, and Leg Tissue Mass

Body composition was determined by dual energy X-ray absorptiometry (DEXA; Hologic software version APEX 4.0). Total mass and fat free mass of the left forearm and right leg were determined from regional analysis from the whole-body DEXA scan using bony landmarks for normalization of blood flow and vascular conductance responses for between group and between-limb comparisons. Body mass index (BMI) was calculated as body weight (kg) divided by height (meters) squared.

Heart Rate and Systemic Blood Pressure

Heart rate (HR) was recorded via continuous three-lead electrocardiogram, and systemic blood pressure was assessed (beat-to-beat) via finger plethysmography (Nexfin; Edwards Lifesciences, Irvine, CA) on the non-exercising hand. Brachial artery pressure was measured in duplicate using an automated cuff (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA) prior to beginning exercise trials while the subjects were in a supine position following 15 minutes of rest.

Pre-Study Day Measurements: Determination of Work Rate Maximum

Work rate maximum (WR_{max}) was determined from a single leg knee extensor incremental maximal exercise test completed during a familiarization session prior to the study day. Subjects were seated in a semi-recumbent position on a modified adjustable bucket seat that accommodated variable body and leg lengths allowing each subject's lower leg to move through a 90-180 degree range of motion during the knee extension exercise. Both knees were flexed at 90 degrees with a form fitting orthopedic boot attached to the right ankle. The boot was attached to a leg shaft located behind the knee that had a one way clutch bearing that allowed for no resistance as the leg returns to 90 degrees flexion (eccentric). Resistance was developed by contracting (concentric) against the leg shaft with the device electronically developing the torque. WR_{max} testing consisted of an initial workload of 5W that incrementally increased every minute by 3W and 5W in female and male subjects, respectively. Subjects kicked dynamically through a full range of motion at a cadence of 40 kicks per minute. The single leg knee extensor incremental maximal test continued until the subject could not maintain a full range of motion or 40 contractions per minute. The final workload completed was recorded as maximal kicking load from which relative workloads were calculated (158).

Study Day: Single Muscle Contractions

Subjects performed single knee extension contractions on the custom-made, computer controlled leg ergometer (described above) at 20%, 40%, and 60% WR_{max} with the order of exercise intensities randomized across subjects. Subjects were instructed to contract and relax on a verbal command from laboratory personnel. To compare rapid hyperemic and dilator responses between limbs (leg vs. arm), single forearm contractions were performed with a handgrip device at 10%, 20%, and 40% of the subject's maximal voluntary contraction (MVC),

determined (using a handgrip dynamometer) as the average of three maximal squeezes performed on the pre-study measurement day. While supine, the weight for each respective exercise intensity was lifted 4 to 5 cm over a pulley for a single, 1-second muscle contraction. WR_{max} and MVC intensities were randomized prior to the experimental protocol and each contraction intensity was performed in duplicate to calculate the average response for each subject for a given condition. Each contraction (leg and arm) was visually observed by the laboratory personnel to ensure proper timing of contraction and two minutes of relaxation were given between each contraction to allow continuous measures of limb hemodynamics post-contraction. All single muscle contractions (knee extensions and forearm contractions) were performed on the same day.

Measurement of Blood Flow

Common femoral (~2-3 cm proximal to bifurcation) and brachial artery diameter and blood velocity were determined with a 12-MHz linear-array Doppler probe (model M12L; Vivid 7, General Electric, Milwaukee, WI). Blood velocity was measured with a probe insonation angle previously calibrated to 60°. Measured velocity waveforms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (113). Artery diameter measurements were obtained at end diastole at rest (before contraction) and 1 minute post-contraction. Limb blood flow (BF) was calculated as the product of mean blood velocity ($cm \cdot s^{-1}$) and artery cross-sectional area (cm^2) and expressed as milliliters per minute ($ml \cdot min^{-1}$).

Data Analysis and Statistics

Data were collected at 250 Hz and analyzed offline with signal processing software (WinDaq; DATAQ Instruments). Mean arterial pressure (MAP) was derived from the Nexfin

pressure waveform and heart rate was determined from the electrocardiogram. Baseline BF and MAP represent an average of the last 30 s of the resting time period before each muscle contraction and were used to quantify the hyperemic response. Vascular conductance (VC) was calculated as BF/MAP (and expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$). Rapid hyperemic and vasodilator responses are expressed as the change in (Δ) BF and VC, respectively. Of particular interest are the immediate (i.e. 1st cardiac cycle post-contraction), peak, and total dilator responses post-contraction. Total BF (ml) and VC ($ml \cdot mmHg^{-1}$) were defined as the area under the curve for 30 cardiac cycles post-contraction after respective baseline values were subtracted for a given flow or conductance curve. To account for the possible influence of muscle mass, BF and VC were normalized to muscle mass (kg) (80, 217).

All values are expressed as mean ± SE. Analysis of variance (ANOVA) was used to analyze demographic variables between age groups. To address the primary question of whether contraction-induced rapid vasodilation is impaired in the leg with aging, a 2-way repeated measures ANOVA was used to evaluate leg blood flow and vasodilator differences between age groups (young vs. older) across exercise intensities (20%, 40% and 60%). To compare responses between limbs, BF and VC at 20% and 40% MVC and WR_{max} were analyzed via independent two-way ANOVA (20% and 40%). When significance was detected, Tukey's post hoc analysis was used to identify differences between groups. All statistical analyses were completed using SigmaStat software version 12.0 (Systat Software Inc., San Jose, CA). Statistical difference was set a priori at $P < 0.05$.

Results

Subject characteristics are shown in Table 3. Young and older subjects were of similar height, weight, and body mass ($P>0.05$), but older adults had a higher percent body fat ($P<0.05$).

Additionally, young and older subjects exhibited similar brachial blood pressures, forearm and leg muscle masses, and forearm MVC ($P>0.05$), but WR_{max} in the leg was lower in the older adults ($P<0.05$).

Characterizing ROV in the Leg with Aging

Baseline (i.e. resting) BF, MAP, and VC did not differ with age across each trial (Table 4). When expressed as the absolute change from baseline, the immediate, peak, and total hyperemic and vasodilator responses following single knee extension contractions were attenuated in older adults across exercise intensities (Table 5; $P < 0.05$). Similarly, the relative change from baseline in the immediate and peak hyperemic and vasodilator responses were also blunted in the older compared to young adults at each workload (Table 5; $P < 0.05$). Figure 5 illustrates the time course for the absolute rapid hyperemic and vasodilator responses normalized for muscle mass following single knee extension contractions at 20%, 40% and 60% WR_{max} in young and older adults. The immediate, peak, and total hyperemic and vasodilator responses (normalized for muscle mass) were substantially attenuated in the older adults across exercise intensities ($P < 0.05$; Figure 6 A-C). Moreover, when the relative change in immediate and peak hyperemia and vasodilation normalized for muscle mass was examined, older adults still exhibited lower responses (data not shown).

Examination of Potential Limb Differences with Aging

Table 6 shows all parameters of absolute rapid vasodilation (immediate, peak, and total) at both 20% and 40% exercise intensity. At the 20% exercise intensity all parameters of absolute rapid vasodilation (immediate, peak and total) were substantially greater in the leg compared to the forearm in both young and older adults. To account for the inherent volume and muscle mass differences between the thigh and forearm, we also compared the vasodilator responses

normalized for muscle mass in each respective limb. Utilizing this approach revealed a significant main effect of limb on the peak and total vasodilator response at 20% and 40% exercise intensity ($P < 0.05$). However, when normalized for muscle mass, greater vasodilator responses were observed in the forearm (Figure 7). Of particular interest to the current study, there was also a main effect of age but not a significant age x limb interaction. That is, despite older adults having lower vasodilator responses compared to their young counterparts, the degree of attenuation was similar between the arm and leg (Figure 7).

Discussion

Previous studies by our group, as well as others, have demonstrated that contraction-induced rapid vasodilation is blunted in the forearm of older adults (39, 40, 43, 141). In the present study we examined whether age-related impairments in the hyperemic and vasodilator response to a single muscle contraction are also present in the leg. Furthermore, we aimed to examine whether the potential age-related reduction in leg vasodilation following a single muscle contraction is greater than that seen in the forearm. Our primary novel findings are 1) older adults exhibit a substantially attenuated rapid hyperemic and vasodilator response in the leg and 2) the age-related reductions in rapid vasodilation appear to be similar between the arm and the leg.

To the best of our knowledge, only one other study has examined rapid vasodilation after a single skeletal muscle contraction in the leg. Credeur and colleagues (61) demonstrated that in young men, a single isometric contraction in the leg produced an intensity dependent increase in blood flow and vasodilation, similar to that seen in the forearm. They also found that the vasodilator response to single isometric contractions was influenced of body position (greater when leg was positioned below heart level), and largely independent of mechanical factors. The

results in young adults from our current study are in agreement with Credeur et al.(61), in that a single dynamic muscle contraction in the leg (e.g. knee extension) produced an intensity dependent increase in blood flow and vascular conductance. The novelty of our current findings is that the rapid hyperemic and vasodilator response following a dynamic muscle contraction in the leg is substantially attenuated in older adults across a range of intensities. Additionally, the blunting of rapid vasodilation in the leg with aging is evident regardless of expressing the response in absolute or relative terms (Table 6) and is independent of muscle mass (Figure 6).

Similar to previous reports (80, 306) the older adults in the present study had a lower WR_{max} compared to their young counterparts (Table 3) and consequently performed single contractions at a lower absolute workload in each trial. Therefore, it could be argued that the blunted rapid vasodilation in the older adults is simply due to a lower absolute workload performed at each relative intensity. However, close examination of our data suggest this is not likely the case. When we compare the hyperemic and vasodilator responses in the older adults at 60% WR_{max} (~17W) to that of the young adults at 40% WR_{max} (~16W), there is still a significant age-related difference in the immediate, peak and total BF and VC (expressed for both absolute and normalized for muscle mass). Additionally, if we were to match a subset of the subjects (n = 9 for each age group) for identical workloads within each relative exercise intensity and effectively eliminate any difference in WR_{max} between age groups, the attenuated hyperemic and vasodilator responses in older adults still persist. This is true for the immediate, peak, and total BF and VC and is independent on whether the response is quantified in absolute terms or normalized for muscle mass.

There is strong evidence that aging is associated with a wide number of modifications to arterial structure and function (149, 192). Moreover, the control of blood flow to dynamically

contracting skeletal muscle is altered with aging during submaximal exercise (80, 141, 155, 226, 229, 234, 253). Studies in both animals and humans suggest that the age-related impairments in the control of muscle blood flow are apparent at the very onset of exercise [i.e. as early as the first contraction] (39, 40, 43, 123, 141). To date, all evidence for age-related differences in rapid onset vasodilation in humans has been restricted to the forearm as a model (39, 40, 43, 141). Nonetheless, findings from these studies clearly demonstrate that the peak blood flow and vasodilator response to a single muscle contraction is significantly blunted in older compared to young adults.

Recent evidence from our lab has pointed to a role for reduced NO bioavailability and/or signaling as well as enhanced α-adrenergic vasoconstriction as possible mechanisms for the blunting of rapid vasodilation in the forearm with aging (40, 43). Therefore, it is conceivable that the attenuated rapid vasodilation in the leg of older adults in the present study might also be due to alterations in each of or a combination of the aforementioned mechanisms. In support of this notion, endothelium-dependent vasodilation (as assessed by flow-mediated dilation) is reduced in the leg of older adults (199, 216), with some of the evidence suggesting that the age-related impairments are greater in the leg compared to the arm (198). Moreover, the leg blood flow response to passive leg movement is severely blunted with aging and the contribution of NO to this response in older adults is significantly reduced (289). Additionally, intra-arterial infusions of an α-adrenergic receptor antagonist (phentolamine) effectively removes sympathetic restraint in the forearm and abolishes the age-related differences in hyperemic and vasodilator responses to single muscle contractions (40). Although it is unclear whether sympathetic restraint of the rapid vasodilator response in the lower limbs exists, some studies suggest the legs demonstrate an enhanced vasoconstrictor tone and augmented adrenergic responsiveness compared to the

forearm in young adults (76, 219). With respect to aging, older adults have a greater tonic vasoconstriction in the leg when compared to their young counterparts (274), however the α-adrenergic vasoconstrictor responsiveness to agonist-mediated stimulation is reduced (274, 306). Furthermore, during dynamic exercise, sympathetic vasoconstrictor responsiveness appears to be augmented in older men (142). Taken together with our current results, these previous findings suggest a possible role for NO-mediated mechanisms as well as enhanced α-adrenergic vasoconstriction in the leg of older adults as a possible explanation for the age-related differences in contraction-induced rapid vasodilation.

In the present study we also examined whether the age-related blunting of rapid vasodilation in response to a muscle contraction is more pronounced in the leg compared to the forearm. In both young and older adults the absolute change in all parameters of contraction-induced vasodilation (immediate, peak, and total) was greater in the leg as compared to the forearm at similar relative exercise intensities (20% and 40% MVC WR_{max} and MVC, respectively). The greater rapid vasodilation observed in the leg as compared to the forearm is in agreement with previous studies in young males (61). However, these limb differences observed in both age groups of the current study may simply be a product of the leg having a greater volume and muscle mass than the forearm. To try to circumvent these inherent differences between limbs we normalized the vasodilator responses for the muscle mass of either the forearm or thigh. This approach revealed a greater rapid vasodilator response in the forearm in both young and older adults (Figure 7). However, it should be noted that the muscle mass values derived via DEXA used to normalize the responses in the leg included the entire thigh. This in turn, likely led to an overestimation of the muscle mass involved in the contraction. Therefore, the significantly lower rapid vasodilator responses observed in the leg after normalizing for

muscle mass might be explained by the large denominator used in the calculation. Along these lines, when estimates of quadriceps muscle mass (via anthropometric methods) are used to normalize blood flow and vasodilator responses during steady state exercise at similar relative intensities, the response is substantially greater in the leg compared to the forearm (Donato et al. 2006). Nonetheless, our results indicate that, regardless of how vasodilator responses are expressed (absolute or normalized for muscle mass) the age-related attenuation of contraction-induced rapid vasodilation appears to be similar between limbs.

Experimental Considerations

There are a few experimental considerations for the current study that warrant discussion. First, the type of muscle contraction performed in the forearm and leg differed slightly. That is, the single leg knee extensor model employed in the present study consisted of a passive relaxation (e.g. no resistance during the eccentric portion), whereas the weight was constant during the contraction and relaxation phases of the forearm contractions (no passive relaxation). Therefore, it is conceivable that the differences in the blood flow and vasodilator responses between limbs are confounded to some degree by the type of muscle contraction performed. Second, our present findings suggest that the age-related attenuation of contraction-induced rapid vasodilation appears to be similar between limbs. However, it should be noted that the older adults demonstrated a similar handgrip strength (MVC) as their young counterparts, but had a significantly (~30%) lower exercise capacity (WR_{max}) in the leg. Since relative workloads were used to examine the rapid vasodilator response of the arm and leg, the discrepancy in strength/exercise capacity between limbs with aging may have masked a greater age-related deficit in rapid onset vasodilation in one limb vs. the other.

Lastly, in the current study we did not observe age-related differences in leg blood flow at rest (Table 4). This is in contrast to several other studies that have demonstrated that older adults have lower resting leg blood flow (73, 180, 274). However, it should be noted that these previous studies reflect supine blood flow, whereas, the resting blood flow measurements in the present study were performed with the subjects in a semi-recumbent position. Indeed, Donato et. al. (80) also reported that resting blood flow is similar in upright seated young and older adults. They speculated that the equal resting leg blood flow and vascular conductance between age groups while seated was potentially due to a pronounced increase in leg vascular tone in the young adults, while older individuals have a diminished response while in an upright position.

Conclusion

To our knowledge, this is the first study to demonstrate that aging blunts contraction-induced rapid vasodilation in the leg across exercise intensities and is largely independent of muscle mass. These findings are in agreement with previous studies demonstrating a reduced rapid vasodilator response in the forearm of older adults (39, 40, 43, 141). Our current data also suggest that age-related impairments in contraction-induced vasodilation do not appear to be different between limbs (arm vs. leg). The primary mechanism(s) and their interactions which contribute to the substantially attenuated rapid hyperemic and vasodilator response in the leg of older adults remains unknown, but due to similar age-related reductions in contraction-induced rapid vasodilation between the leg and arm, it can be hypothesized that the mechanisms might be similar between limbs.

Table 3: Subject Characteristics for Aim 2

Variable	Young Adults (n=14)	Older Adults (n=16)
Age (years)	23 ± 1	66 ± 1
Men/Women	9/5	10/6
Height (cm)	174 ± 2	172 ± 2
Weight (kg)	75 ± 3	78 ± 3
Body Mass Index ($kg \cdot m^2$)	24.7 ± 0.6	26.5 ± 0.7
% Body Fat	27.1 ± 1.9	31.9 ± 1.7 *
Forearm Muscle Mass (kg)	0.94 ± 0.08	0.90 ± 0.06
Thigh Muscle Mass (kg)	7.5 ± 0.5	6.9 ± 0.3
MVC (kg)	41 ± 3	41 ± 3
WR_{max} (Watts)	41 ± 4	29 ± 2*
Systolic BP (mmHg)	117 ± 2	123 ± 3
Diastolic BP (mmHg)	72 ± 2	75 ± 2
MAP (mmHg)	87 ± 2	91 ±2

Values are means ± SE. MVC, maximal voluntary contraction; WR_{max}, work rate maximum. * $P < 0.05$ vs. young adults

Table 4: Baseline Hemodynamics under each Condition

	Leg						Factor P-Values		
	Young			*Older*					
	20% WR_{max}	40% WR_{max}	60% WR_{max}	20% WR_{max}	40% WR_{max}	60% WR_{max}	Age	Intensity	Interaction
Diameter (cm)	0.89±0.03	0.89±0.03	0.89±0.03	0.95±0.03	0.97±0.03	0.96±0.03	<0.05	0.97	0.98
MAP (mmHg)	94±3	95±3	95±3	98±3	98±2	99±2	0.06	0.92	0.99
BF ($ml \cdot min^{-1}$)	172±13	173±14	167±13	175±20	176±16	180±18	0.64	0.99	0.94
VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	1.8±0.1	1.7±0.2	1.8±0.1	1.8±0.2	1.8±0.2	1.8±0.2	0.92	0.99	0.94
HR (bpm)	65±3	65±3	64±3	61±1	61±1	62±1	0.06	0.99	0.89
	Arm						Factor P-Values		
	Young			*Older*					
	10% MVC	20% MVC	40% MVC	10% MVC	20% MVC	40% MVC	Age	Intensity	Interaction
Diameter (cm)	0.37 ± 0.02	0.37±0.02	0.37 ± 0.02	0.41±0.02	0.41±0.02	0.41±0.02	<0.05	0.99	0.99
MAP (mmHg)	89±3	89±3	89±3	95±2	95±2	95±2	<0.05	0.98	0.99
BF ($ml \cdot min^{-1}$)	38±6	39±6	41±6	47±3	50±4	52±4	<0.05	0.66	0.98
VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	0.45 ±0.1	0.46±0.1	0.48±0.1	0.50±0.03	0.53±0.04	0.55±0.04	0.17	0.78	0.98
HR (bpm)	64±3	63±2	63±3	64±2	64±2	65±2	0.68	0.98	0.95

Mean±SE. MAP, mean arterial pressure; BF, blood flow; VC, vascular conductance; HR, heart rate; WRmax, work rate maximum; MVC, maximal voluntary contraction

Table 5: Comparison of Absolute and Relative Blood Flow and Vascular Conductance not normalized for Muscle Mass

		Young Adults			Older Adults			Factor P-values		
		20% WR_{max}	40% WR_{max}	60% WR_{max}	20% WR_{max}	40% WR_{max}	60% WR_{max}	Age	Intensity	Interaction
Absolute Responses										
Δ Blood Flow ($ml \cdot min^{-1}$)	Immediate	285±39	320±47	373±48	118±18	195±21	181±22	<0.05	<0.05	0.18
	Peak	598±57	724±68	802±83	354±27	431±34	485±29	<0.05	<0.05	0.14
	Total	108±15	127±16	137±21	47±5	68±7	72±6	<0.05	<0.05	0.47
Δ Vascular Conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Immediate	3.1±0.4	3.4±0.5	4.0±0.6	1.2±0.2	2.0±0.2	1.9±0.2	<0.05	<0.05	0.24
	Peak	6.9±0.7	8.2±0.8	9.0±1	3.7±0.3	4.6±0.4	5.0±0.3	<0.05	<0.05	0.26
	Total	1.1±0.2	1.3±0.2	1.4±0.2	0.5±0.1	0.7±0.1	0.7±0.1	<0.05	<0.05	0.46
Relative Responses										
% Δ Blood Flow ($ml \cdot min^{-1}$)	Immediate	170±21	189±26	223±20	75±14	119±12	118±19	<0.05	<0.05	0.28
	Peak	356±28	422±30	488±34	221±24	272±29	308±30	<0.05	<0.05	0.19
% Δ Vascular Conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Immediate	1.7±0.2	1.8±0.3	2.2±0.2	0.8±0.1	1.2±0.1	1.2±0.2	<0.05	<0.05	0.17
	Peak	3.8±0.3	4.5±0.3	5.1±0.4	2.2±0.2	2.8±0.3	3.2±0.3	<0.05	<0.05	0.41

Values are mean ± SE. WR_{max}, work rate maximum

Table 6: Comparison of Absolute Change in Rapid Vasodilation in the Arm and Leg of Young and Older Adults at Similar Intensities

		Young		Older		Factor P-values		
		Δ Leg VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Δ Arm VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Δ Leg VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Δ Arm VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Age	Limb	Interaction
20% Workload	Immediate	3.1±0.4*†	0.4±0.1	1.2±0.2*	0.4±0.03	<0.05	<0.05	<0.05
	Peak	6.9±0.7*†	1.2±0.1	3.7±0.3*	0.8±0.06	<0.05	<0.05	<0.05
	Total	1.1±0.2*†	0.1±0.02	0.5±0.1*	0.1±0.01	<0.05	<0.05	<0.05
40% Workload	Immediate	3.4±0.5*†	0.7±0.1	2.0±0.2*	0.5±0.1	<0.05	<0.05	<0.05
	Peak	8.2±0.8*†	1.6±17	4.6±0.4*	1.1±0.1	<0.05	<0.05	<0.05
	Total	1.3±0.2*†	0.2±0.02	0.7±0.1*	0.3±0.02	<0.05	<0.05	<0.05

Mean ± SE. VC, vascular conductance; 20% workload, WR_{max} (leg), MVC (arm); 40% workload, WR_{max} (leg), MVC (arm). * $P < 0.05$ vs. arm; † $P < 0.05$ vs. Older

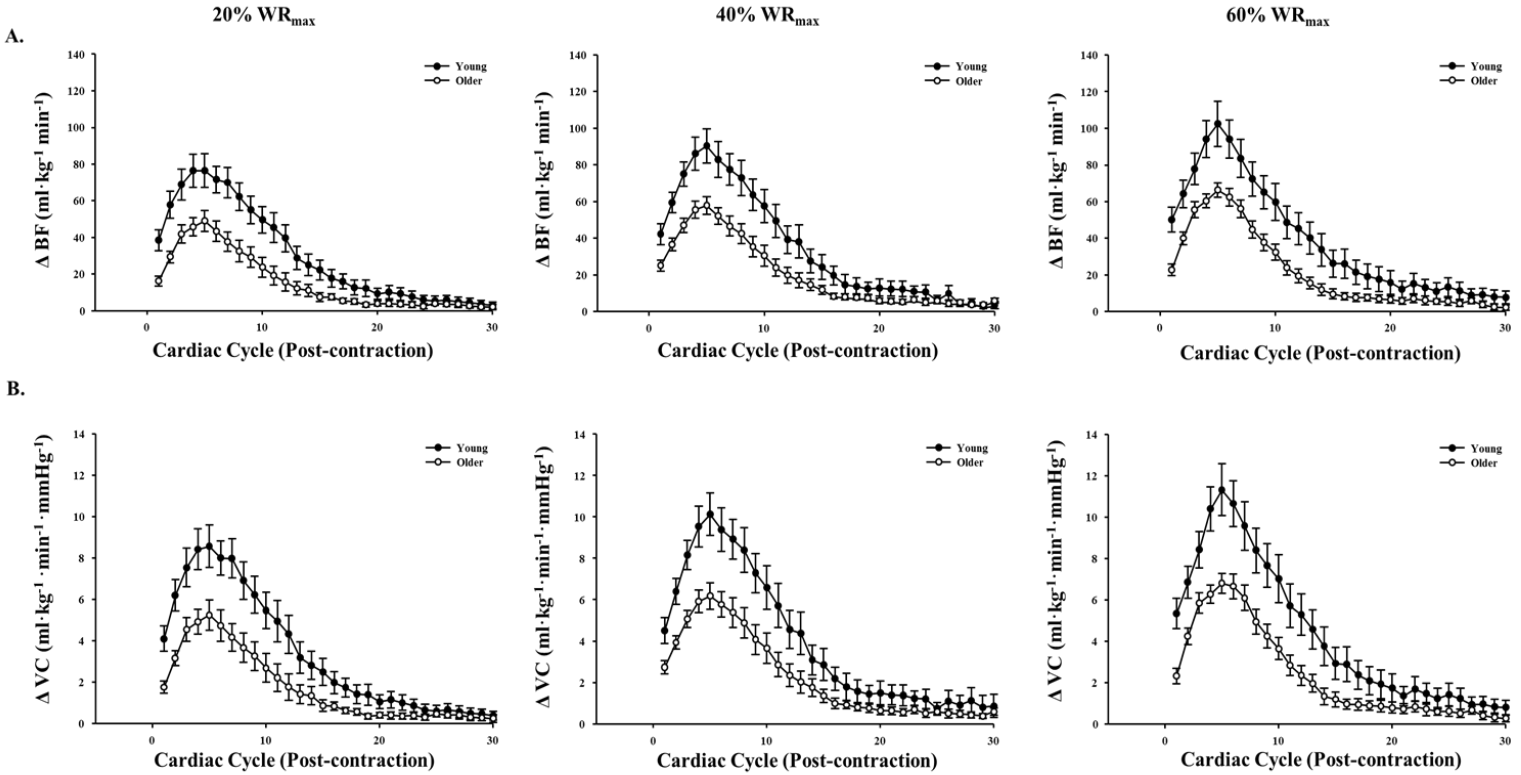

Figure 5: Hyperemic (change [Δ] in blood flow [BF] and (B) vasodilator (Δ vascular conductance [VC] responses over 30 cardiac cycles following single leg extension contractions at 20%, 40%, and 60% WR_{max}

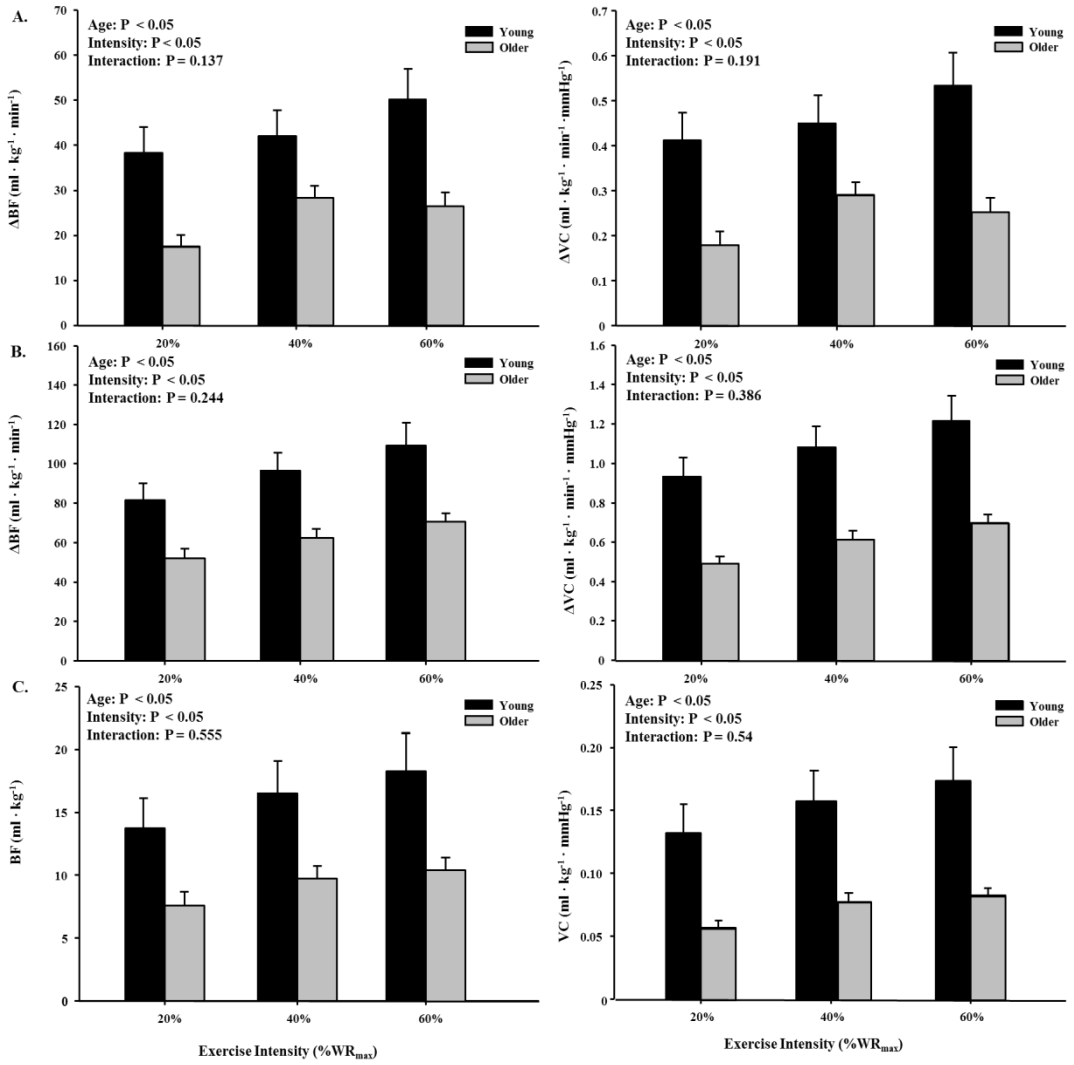

Figure 6: Immediate (A; first cardiac cycle post-contraction); peak (B), and total (C) hyperemic (ΔBF) and vasodilator (ΔVC) responses normalized for muscle mass to single leg-extension contractions between young and older adults at 20%, 40%, and 60% WR_{max}

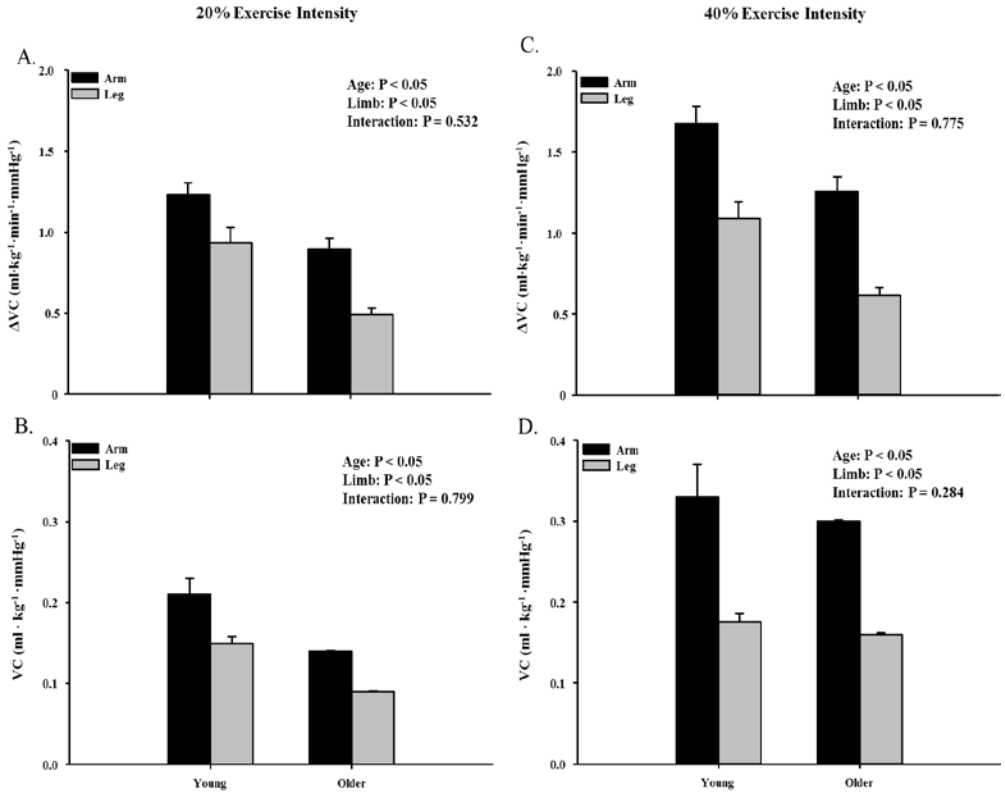

Figure 7: Peak and total ΔVC normalized for muscle mass at 20% (A & B), and 40% (C & D) exercise intensity in the arm and leg

CHAPTER 4: SYMPATHETIC NERVOUS SYSTEM ACTIVATION REDUCES CONTRACTION-INDUCED RAPID VASODILATION IN THE LEG OF HUMANS INDEPENDENT OF AGE

A version of Chapter 4 has been previously published: Hughes WE, Kruse NK, Casey DP. Sympathetic nervous system activation reduces contraction-induced rapid vasodilation in the leg of humans independent of age. *J Appl Physiol*. 2017; 123(1):106-115.

Introduction

Advancing age is associated with a decline in skeletal muscle blood flow and vasodilation at rest and during dynamic exercise (73, 76, 155, 180, 196, 226, 229, 300). However, a large majority of the studies examining age-related alterations in blood flow of contracting skeletal muscle have focused primarily on responses during steady-state submaximal exercise (130, 233). By the nature of these studies and associated measurements, regulation of blood flow and vasodilation at the onset of exercise has been largely ignored, despite evidence to suggest rapid vasodilation is integral in initiating the increase in blood flow requisite for dynamic exercise (51, 54, 296). In this context, increases in skeletal muscle blood flow and vasodilation are apparent immediately following a single skeletal muscle contraction [i.e. contraction-induced rapid onset vasodilation, ROV]. Evidence from our group as well as others suggests that older adults demonstrate a significantly lower hyperemic and vasodilator response to single muscle contractions [i.e. attenuated ROV] compared to their young counterparts (39, 40, 43, 120, 141). Although the majority of the human studies demonstrating age-related impairments in ROV have done so in the forearm (39, 40, 43, 120, 141), we have recently shown that ROV is also blunted in the leg of older adults and the magnitude of this impairment appears to be similar between limbs (120).

One mechanism potentially contributing to the impaired ROV in older adults is an enhanced sympathetic vasoconstriction. Advancing age is associated with a progressive increase in muscle sympathetic nerve activity (MSNA) and norepinephrine concentrations, acting to increase peripheral vascular resistance and thereby enhance vasoconstriction (21, 193, 274, 279). Along these lines, sympathetic stimulation via lower-body negative pressure attenuates the ROV response in the forearm of young, but not older adults (40). Additionally, subsequent non-selective blockade of α-adrenergic receptors (via phentolamine) abolishes age-related differences in ROV (40). Moreover, ROV in animals is attenuated via α-adrenergic stimulation (272) and age-related differences are abolished with α-adrenergic blockade (123). Collectively, this evidence indicates that impairments in blood flow and vasodilation at the onset of exercise with aging are due in part to an enhanced sympathetic restraint at least in the forearm.

Within the context of human locomotion, the lower limbs represent a large vascular bed exposed to greater hydrostatic forces relative to the upper limbs, and may be more representative of the physiological environment of normal activity. Interestingly, older adults exhibit reductions in resting leg blood flow and vascular conductance, which has been attributed to enhanced sympathetic vasoconstriction as well as increased oxidative stress (73, 76, 122, 180, 196). Furthermore, during dynamic exercise, leg blood flow and vasodilation are reduced with aging (185, 226, 229, 234, 300), and this attenuation does not appear to be due to a reduction in endothelial vasodilators, but rather enhanced sympathetic vasoconstriction (142, 185, 247). Specifically, the leg vasculature of older adults appears to be more responsive to acute sympathetic stimulation during dynamic exercise (142). Despite evidence supporting sympathetic vasoconstriction contributing to age-related reductions in leg blood flow during dynamic steady-state exercise, it is unclear if this is apparent at the onset of exercise. Therefore,

the aim of the present investigation was to determine whether enhanced sympathetic vasoconstriction contributes to the attenuated contraction-induced ROV response observed in the leg of older adults. We hypothesized that acute elevations in sympathetic nervous system activity would reduce ROV to a greater extent in young compared to older adults. Additionally, we hypothesized that the larger reduction in ROV with acute sympathetic stimulation in young adults would effectively abolish the age-related differences in contraction-induced rapid vasodilation in the leg of humans.

Methods

Subjects

A total of 25 healthy normotensive adults participated in the study. All subjects were healthy, non-obese (body mass index: ≤ 30 kg/m^2), nonsmokers and not taking any vasoactive medications as self-reported via a health history screening questionnaire. Additionally, subjects who reported having Raynaud's Disease were excluded from participation in this study. Studies were performed in the morning after an overnight fast and refraining from exercise, alcohol, and caffeine for 24 hours before reporting to the laboratory. Young female participants were studied during the early follicular phase of their natural menstrual cycle or the placebo/low hormone phase of oral contraceptives to control for the influence of sex hormones on exercise hyperemia (159, 173). All older female subjects were postmenopausal and were not taking any form of hormone replacement therapy. Subjects completed written informed consent and all study protocols were approved by the Institutional Review Board at the University of Iowa.

Systemic Hemodynamic Measurements

Following a 15-minute rest period in a semi-recumbent position, brachial blood pressure (BP) was measured in duplicate using an automated cuff (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). If BP values deviated by more than 5 mmHg, a third measurement was taken. Heart rate (HR) was measured via continuous three-lead electrocardiogram and systemic BP was assessed (beat-to-beat) via finger plethysmography (Nexfin, Edwards Lifesciences, Irvine, CA) over the middle phalanx of the left hand.

Leg Blood Flow and Vascular Conductance

Common femoral artery diameter (~2 cm proximal to bifurcation) and blood velocity were determined with a 12-MHz linear array Doppler probe (model M12L; Vivid 7, General Electric, Milwaukee, WI). Blood velocity was measured with a probe insonation angle previously calibrated to 60˚. Measured velocity waveforms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (113). Femoral artery diameter measurements were obtained during end-diastole at rest (before contraction) and 1-min post-contraction. Leg blood flow (BF) was calculated as the product of mean blood velocity ($cm \cdot s^{-1}$) and artery cross-sectional area (cm^2) and expressed as milliliters per minute ($ml \cdot min^{-1}$). Vascular conductance (VC) was calculated as the quotient of BF and mean arterial pressure (MAP) (expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$).

Single Muscle Contractions

Subjects performed dynamic single contractions in the right leg as previously described at 20% and 40% of their maximum work rate (WR_{max}) determined on a previous study day (118, 120). Subjects were seated in a semi-recumbent position on a modified adjustable bucket seat that accommodates variable body and leg lengths allowing the subject's lower leg to move through a full range of movement (90° flexion to ~0° extension). Both knees were flexed at 90°

with a form fitting orthopedic boot attached to the right ankle. The boot is attached to a leg shaft located behind the right knee that has a one-way clutch bearing allowing for no resistance as the leg returns to 90° flexion (eccentric). Resistance (torque) is developed by contracting (concentric) against the leg shaft with the device electronically developing the torque via an alternating current motor turning at a constant RPM transferred to the leg shaft. A dedicated computer monitors the elapsed time and the angle of the leg controlling the actual torque presented to the leg during contraction. In this way, the subject was required to develop enough power to extend the leg through a full range of motion against a given torque eliminating any inertial load. Subjects were instructed to contract and relax through a full range of movement (90° flexion to ~0° extension) within ~1s, on a verbal command from laboratory personnel. WR_{max} intensities were randomized prior to the experimental protocol, and each contraction intensity was performed in duplicate to calculate the average response for each subject for a given condition. Each contraction was visually observed by the laboratory personnel to ensure proper timing of contraction. One minute of relaxation were given between each contraction to allow continuous measures of limb hemodynamics post-contraction.

Cold-Pressor Test

The cold-pressor test (CPT) was used to examine the influence of increased sympathetic nervous system activity on ROV following single muscle contractions in the leg. Local cold stimulation has previously been shown to elicit a robust sympathetic stimulus, effectively increasing muscle sympathetic nerve activity two-fold, within 1-2 minutes (256, 299). During each CPT trial, the subject's contralateral (left) foot (at the level of approximately 5-7 cm above the malleolus) was lowered into the ice water (4˚C) for a total of four minutes (108, 142).

Experimental Protocol/Design

Following a 15-minute rest period in a semi-recumbent position and baseline brachial BP measurements subjects underwent a familiarization CPT at the start of the study. The initial (familiarization) CPT was utilized because the first exposure to cold has been shown to cause exaggerated increases in MAP when compared to subsequent CPTs (108). Therefore we aimed to avoid having the first exposure to cold and potential exaggerated pressor responses occur during one of the leg kicking trials. Following the CPT familiarization trial, subjects performed a total of four [two CPT and two non-CPT/control] leg-kicking trials (Figure 8). The order of control (no CPT) and CPT trials, as well as exercise intensity (20% vs. 40% WR_{max}) were randomized prior to the study day, and 20 minutes of quiet rest separated each trial that contained a CPT. Each trial consisted of 2 single contractions at either 20% or 40% WR_{max}. For the CPT trials, the contractions took place during the final 2 minutes of foot immersion within the ice bath. HR, systemic arterial BP, and femoral artery mean blood velocity were captured continuously throughout each trial.

Data Analysis and Statistics

Data were collected at 250Hz and analyzed offline with signal-processing software (WinDaq, DATAQ Instruments). Beat-to-beat MAP was derived from each Nexfin BP waveform and was synchronized to blood velocity (via Doppler ultrasound) measurements. Baseline BF and MAP represent an average of the last 30 seconds of the rest prior to each single contraction. To account for baseline changes in BF and VC with CPT, rapid flow and vasodilator responses after muscle contraction were adjusted (i.e., post-contraction value - baseline value) and expressed as the change in (Δ) BF and VC. Of particular interest to this study, the peak ΔVC and total VC responses post-contraction were analyzed between conditions. Total leg BF (ml) and VC ($ml \cdot mmHg^{-1}$) were defined as the area under the curve over 30 post-contraction cardiac

cycles after subtracting respective baseline values for a given flow or conductance curve. In order to further investigate age-related differences in the hyperemic and vasodilator response to sympathetic stimulation (CPT), the percent reduction (%Δ) between age-groups were compared. Given evidence for the potential confound of absolute work driving the age-related differences in exercise hyperemia and vasodilation (306) we performed additional analyses on a subset of subjects matched for an approximate absolute workload (~10W). Additionally, due to the potential influence of sex on ROV (40) we also performed additional analyses to compare responses within and between age groups based on sex.

Group data are expressed as mean±SE. Baseline characteristics between groups were analyzed via one-way analysis of variance (ANOVA). Hemodynamics prior to single leg contractions were analyzed with a two-way repeated measure ANOVA. Age and sex related differences of the primary outcome variables post-contraction (peak ΔBF and ΔVC and total BF and VC) were analyzed using two-way repeated measures ANOVA. Percent reductions in the primary outcome variables were analyzed with independent one-way ANOVA's. When significant F-ratios were detected, post-hoc comparisons were made using Tukey's post hoc test for pair-wise comparisons. Significance was set a priori at $P<0.05$. All statistical analyses were performed using SigmaStat software version 12.0 (Systat Software Inc., San Jose, CA).

Results

Subject characteristics are shown in Table 7. Older adults had a higher BMI and lower WR_{max} relative to young adults ($P<0.05$). There were no age differences for any of the BP variables ($P>0.05$).

Baseline (resting) Hemodynamic Responses across Trials

Baseline hemodynamics during control and CPT trials prior to single leg contractions are shown in Table 8. Compared to control trials, exposure to the CPT increased baseline MAP in both young and older adults ($P<0.05$). CPT also caused an increase in HR in the young adults ($P<0.05$), whereas no chronotropic effect was observed in older adults. Baseline levels of VC were reduced during the CPT in young adults across exercise intensities and at 20% WR_{max} in older adults ($P<0.05$).

Hyperemic and Vasodilator Responses to Single Muscle Contractions with Age

Figure 9 illustrates the temporal rapid hyperemic (A & B) and vasodilator (C & D) responses in young and older adults following single contractions during control and CPT trials at 20% and 40% WR_{max}. Peak hyperemic and vasodilator responses tended to occur during the 6th cardiac cycle post-contraction in both young and older adults. Under control conditions, peak (Figure 10) and total (Figure 11) hyperemic and vasodilator responses following a single skeletal muscle contraction were attenuated in older compared to young adults at 20% and 40% WR_{max} (main effect of age $P<0.05$).

Vasoconstrictor Responsiveness to Single Leg Contractions

During the CPT trials, peak hyperemic and vasodilator responses were reduced in both young and older adults at 20% and 40% WR_{max} ($P<0.05$, Figure 10). Additionally, sympathetic stimulation via CPT reduced the total hyperemic and vasodilator responses in young and older adults at 20% WR_{max} ($P<0.05$), whereas the total responses were only reduced in the young adults at 40% WR_{max} ($P<0.05$, Figure 11). When expressed as a percent reduction (% change in VC during CPT vs. control), there were no age-related differences for peak ($P=0.55$ at 20% WR_{max}; $P=0.42$ at 40%WR_{max}) or total ($P=0.34$ at 20% WR_{max}; $P=0.23$ at 40%WR_{max}) vasodilator responses (Figure 12).

Absolute Workload Sub-Analysis

The mean ± SE workloads for the subset of young (n=9) and older (n=9) subjects were 9.7±0.4 and 9.6±0.6 W, respectively. Table 9 shows BF and VC data at baseline and in response to single leg contractions during control and CPT trials. In agreement with relative workload responses, young adults demonstrated greater peak vasodilator responses compared to older adults during control and CPT trials ($P<0.05$ for both). However total vasodilator responses were not significantly different between young and older adults across conditions (P=0.08-0.1) at a comparable absolute workload. Additionally, the magnitude of vasoconstriction (% change in VC during CPT vs. control) was similar for the peak (-20±7% vs. -25±4% P=0.53) and total (-22±8% vs. -28±4%P=0.47) responses between young and older adults.

Potential Sex Differences in Rapid Hyperemic and Vasodilator Responses to Single Leg Knee-Extension with Acute Sympathetic Stimulation

Within groups: Young men demonstrated greater peak hyperemic and vasodilator responses than young women during both control and CPT at 20% WR_{max} ($P<0.05$). At 40% WR_{max}, young men demonstrated greater peak hyperemic and vasodilator responses during control ($P<0.05$), but no differences were observed for peak vasodilator responses during CPT (P=0.09) Total hyperemic and vasodilator responses were not different between young men and women during control or CPT across exercise intensities (P=0.06-0.27). Importantly, there were no sex differences evident in the percent reduction due to CPT in peak (-22±6% vs. -29±6% at 20% WR_{max}; -22±6% vs. -20±12% at 40% WR_{max}, P=0.44-0.88) or total VC (-26±7% vs. -31±8% at 20% WR_{max}; -21±10% vs. -22±13% at 40% WR_{max}, P=0.70-0.96).

There were no sex differences observed in any measure of ROV between older men and women during control or CPT (P=0.18-0.79). Additionally, the percent reduction due to CPT in

peak and total hyperemic and vasodilator responses within the leg were not different between older men and women across exercise intensities (P=0.40-0.79).

Between groups: Young males demonstrated greater peak hyperemic and vasodilator responses during both control and CPT compared to older men across exercise intensities (P<0.05 for all). There were no statistically significant differences between young and older men for total hyperemic or vasodilator responses during control or CPT across exercise intensities (P=0.07-0.14). Furthermore, the percent reduction due to CPT were not different between young and older men across exercise intensities for peak VC (-22±6% vs. -31±5% at 20% WR_{max}; -22±6 vs. -33±10% at 40% WR_{max}, P=0.39-0.90) or total VC (-26±7% vs. -31±5 at 20% WR_{max}; -21±10% vs. -35±10% at 40% WR_{max}; P=0.34-0.66).

Older women demonstrated attenuated peak hyperemic (P<0.05) but not vasodilator responses at 20% and 40% WR_{max} (P=0.13-0.18) relative to young females during control trials. There were no differences between the two groups during CPT trials (P=0.17-0.47). No age-associated differences were observed within young and older females for total hyperemic or vasodilator responses during control or CPT trials across exercise intensities (P=0.08-0.23). Furthermore, there were no age-associated differences between young and older females for the percent reduction due to CPT across exercise intensities for peak (-29±7% vs. -20±7% at 20% WR_{max}; -20±13% vs. -31±6% at 40% WR_{max}, P=0.38-0.65) or total VC (-31±8% vs. -41±10% at 20% WR_{max}; -22±13% vs. -32±6% at 40% WR_{max}, P=0.43-0.51).

Discussion

This is the first study to examine the influence of sympathetic vasoconstriction on the contraction-induced rapid hyperemic and vasodilator responses in the leg of young and older

adults. The novel finding of this study is that acute sympathetic stimulation via a CPT significantly blunts ROV in the contracting leg of both young and older adults, and the magnitude of change is similar between age groups. Additionally, age-related differences in ROV still persist in the face of acute sympathetic stimulation. Taken together, our present findings suggest that sympathetic vasoconstriction does not appear to fully explain age associated reductions in contraction-induced rapid hyperemia and vasodilation in the lower limbs of humans.

Lower Limb Vasoconstrictor Responses and Age-associated Reductions in Contraction-Induced ROV

We have previously demonstrated that contraction-induced ROV is attenuated to a similar extent in the upper and lower limbs of older adults across a range of exercise intensities (120). Furthermore, we have demonstrated that within the forearm, sympathetic stimulation reduces contraction-induced ROV in young, but not older adults (40). Moreover, non-selective α-adrenergic blockade effectively abolishes the age-related differences in ROV in the forearm (40). In the present study, contraction-induced ROV in the leg was blunted during acute sympathetic stimulation to a similar degree between age groups (Figures 10 & 11). While contraction-induced ROV is not reduced in the forearm of older adults during sympathetic stimulation (40), previous evidence has shown that older adults demonstrate greater vascular tone within the leg at rest (274) as well as reduced blood flow and vasodilation during dynamic exercise (142, 185). Taken together, this evidence might suggest that while regulation of vascular tone at the onset of exercise is altered with age, the regulatory mechanisms may differ between limbs. However, it should be noted that the contrasting ROV responses between limbs during sympathetic stimulation with aging might not truly reflect limb specific differences but rather methodological

differences. Along these lines, our previous study in the arm (40) utilized lower body negative pressure to induce acute sympathetic stimulation whereas a CPT was used in the current study. Differences in the magnitude of sympathetic stimulation and consequent hemodynamic changes between the two experimental conditions could influence ROV independent of potential inherent limb differences. In support of this notion, lower body negative pressure and CPT have been shown to elicit divergent hemodynamic responses as well as arterial blood flow patterns at rest (214).

Sympathetic Vasoconstriction, ROV and Sex Differences

Previous work from our group indicates sex may play a role in the ROV responses in the forearm during sympathetic stimulation (40). Specifically, young men exhibited greater sympathetically-mediated reductions in ROV during low-intensity contractions, but not during moderate to higher intensity contractions, suggestive of greater vasoconstrictive responsiveness that is abolished with increasing exercise intensity. In contrast to what we previously reported in the forearm, our current findings in the leg suggest that the reduction in ROV during acute sympathetic stimulation is similar between young men and women regardless of exercise intensity. Moreover, the magnitude of reduction in ROV (expressed as a relative or absolute change) during sympathetic stimulation did not differ between age groups within each sex. It is important to note that these statistical comparisons and conclusions regarding potential sex-related differences are derived from a relatively small sample size (n = 6-7 for each group), which could contribute to the lack of differences observed within and between groups.

Feedforward Mechanisms to Dynamic Exercise

The capacity to rapidly increase blood flow and vasodilation at the onset of contractions has important implications for the regulation of skeletal muscle blood flow during dynamic

exercise. Rapid vasodilation at the onset of exercise contributes to the bi-phasic nature of exercise hyperemia (295), and presumably facilitates the transition from rest to steady-state dynamic exercise. While it is well documented that vascular tone is altered with aging at rest and during repeated muscle contractions (74, 247), far less is known regarding the dynamics of vasodilation leading up to steady-state dynamic exercise. However, emerging evidence points to age-related impairments in the local control of blood flow in contracting human skeletal muscle occurring as early as the first contraction (39, 40, 43, 120, 141), which is due in part to enhanced α-adrenergic mediated vasoconstriction and reductions in nitric oxide (NO)-mediated local vasodilation (40, 43). Decreases in NO bioavailability have also been implicated in the blunted transient leg blood flow response to movement-induced hyperemia in older adults (105, 166, 181, 290). Interestingly, the age-related reductions in forearm blood flow during steady state exercise do not appear to be due to an enhanced sympathetic vasoconstriction (237). This evidence suggests that sympathetically mediated vasoconstriction possibly plays a greater role in the age-related impairments in exercise hyperemia at the very onset of exercise compared to under steady-state conditions. Furthermore, this discrepancy also highlights a knowledge gap related to the vasodilator and vasoconstrictor mechanisms involved in the regulation of muscle blood flow during the transition from rest to steady state exercise. Although, we recently demonstrated the speed (i.e. kinetics) of skeletal muscle blood flow and vasodilation during rhythmic forearm contractions are slower in otherwise healthy aging humans, possibly due to blunted NO signaling (41), the role sympathetic vasoconstriction plays in slowing vasodilator kinetics with age has not been explored.

Attenuation of sympathetic vasoconstriction during exercise is critical in the matching of O_2 supply to demand during dynamic exercise via re-distribution of blood flow to contracting

tissues (e.g. functional sympatholysis) (247). Previous evidence in the forearm of young adults indicates that attenuation of sympathetic vasoconstriction is immediate (after a single muscle contraction) (67). Conversely, sympathetic vasoconstriction appears to limit rapid vasodilation in the forearm of older adults (40) as well as contributes to the age-related reductions in muscle blood flow during steady-state dynamic exercise in the leg (74, 185, 306). In the present study, acute sympathetic stimulation reduced ROV in the leg ~20-30% of both young and older adults, with the magnitude of reduction being similar between age groups and across exercise intensities (Figure 12). Noting previous evidence from our lab of a similar attenuation in ROV between the arm and leg with age following a single muscle contraction (120), it does not appear that a greater tonic sympathetic vasoconstriction explains the drastic age-related differences in contraction-induced rapid vasodilation within the leg. Considering the idea that ROV is thought to play a critical role in modulating skeletal muscle blood flow during steady-state dynamic exercise conditions, the ability to attenuate sympathetic vasoconstriction within the leg after a single contraction may play a critical role in O_2 delivery and exercise tolerance (248).

Experimental Considerations

There are a few experimental considerations that warrant mention. First, we used a CPT to increase sympathetic activity with the goal of enhancing vasoconstriction in the contracting leg. Although we did not directly assess sympathetic outflow via direct measurement of MSNA or norepinephrine spillover, previous evidence has clearly demonstrated that a local CPT is capable of doubling MSNA in both young and older adults (142, 197, 256, 299). Along with inducing sympathetic vasoconstriction, the CPT also resulted in concomitant increases in systemic arterial pressure, which in turn could potentially confound our results. However, we do not believe that the increase in MAP during the CPT confounds our main outcome measure (i.e.

vascular conductance). In this context, the calculation of vascular conductance takes into account changes in arterial pressure. Additionally, our method for calculating ROV (Δ from respective baseline) allowed for comparison despite baseline differences between age groups as well as between trials (control vs. CPT).

The conclusion that there are no age-associated differences in the reduction of ROV in response to acute sympathetic stimulation is in contrast to our original hypothesis. Our conclusion is based off the finding that the relative reduction in ROV during CPT (compared to control conditions) is similar between young and older adults. However, it could be argued that based on the calculation of percent reduction in VC due to CPT [(CPT-control)/control x 100], the much lower VC in older adults during control conditions results in a smaller denominator and thereby possibly making the % reduction appear greater than it actually is. Although this is possible, close examination of our data reveals that this is likely not the case. When examined as an absolute difference (i.e. CPT-Control), there are still no differences in the peak and total VC between young and older adults (P=0.12-0.63). These results, taken in context with the percent reduction due to CPT suggest that sympathetic stimulation reduces ROV within the leg independent of age.

Similar to our previous studies (118, 120), the older adults demonstrated a lower WR_{max} in the leg than their young counterparts. In turn, their absolute work rates at each respective workload (5 ± 1 vs. 8 ± 1 W at 20% WR_{max} and 11 ± 1 vs. 16 ± 1 W at 40% WR_{max}) were lower, which could have influenced our current findings. Also, previous evidence suggests that any age-related difference in the ability to inhibit sympathetic vasoconstriction in the exercising leg is more closely associated with work performed rather than age *per se* (306). Therefore we addressed this potential confound by comparing ROV responses under control and CPT

conditions in a subset of young and older adults matched for a similar absolute workload (~10W). Results from this sub-analysis clearly demonstrate 1) blunted ROV responses with age still persist when matched for an absolute workload, and 2) the magnitude of reduction in the ROV response during acute sympathetic stimulation is still similar between young and older adults. Finally, using the current study design we were unable to examine whether α-adrenergic blockade abolishes the age-related differences in ROV in the leg, similar to our previous work in the forearm (40). However, removal of sympathetic α-adrenergic vasoconstriction via phentolamine abolishes age-related differences in femoral blood flow and vasodilation at rest, as well as during a CPT (76).

Conclusion

The results from the present study are the first to provide experimental evidence that contraction-induced rapid vasodilation within the leg is reduced by sympathetic stimulation, independent of age as well as sex. Furthermore, while sympathetic stimulation reduces rapid vasodilation following a single muscle contraction in the leg of young adults, it does not fully explain the blunted ROV response commonly observed in the leg of older adults.

Table 7: Subject Characteristics for Aim 3

	Young Adults (n = 13)	Older Adults (n = 12)
Age (years)	24 ± 1	67 ± 1*
Men/Women	7/6	6/6
Height (cm)	176 ± 3	169 ± 2
Weight (kg)	73 ± 3	73 ± 3
Body Mass Index ($kg \cdot m^2$)	23.4 ± 0.5	25.7 ± 1.0*
WR_{max} (Watts)	41 ± 4	26 ± 3*
Systolic Pressure (mmHg)	121 ± 3	122 ± 3
Diastolic Pressure (mmHg)	74 ± 2	75 ± 2
Mean Arterial Pressure (mmHg)	89 ± 2	91 ± 2

Values are means ± SE. WR_{max}, Work Rate Maximum. * $P < 0.05$ vs. young adults

Table 8: Baseline (resting) Hemodynamics under Each Condition

		Young		Older	
		Control	CPT	Control	CPT
Blood Flow (ml·min^{-1})	**20% WR_{max}**	184±15	171±14	135±27	108±22*
	40% WR_{max}	205±27	181±18	136±27*	114±22
MAP (mmHg)	**20% WR_{max}**	98±3	112±5†	102±4	107±4†
	40% WR_{max}	99±3	111±5†	101±3	108±3†
HR (b·min^{-1})	**20% WR_{max}**	70±4	76±4†	63±2	64±2*
	40% WR_{max}	69±4	76±4†	62±2	64±2*
VC (ml·min·mmHg^{-1})	**20% WR_{max}**	1.9±0.1	1.6±0.2†	1.3±0.3	0.9±0.2*†
	40% WR_{max}	2.1±0.3	1.7±0.2†	1.3±0.3*	1.1±.02

Mean ± SE; MAP, mean arterial pressure; HR, heart rate; VC, vascular conductance. * P<0.05 vs. young adults; † P<0.05 vs. control

Table 9: Leg Hemodynamics at Rest and Following a Single Contraction at ~10W

		Young (n=9)			Older (n=9)		
		Baseline	ΔPeak	ΔTotal	Baseline	ΔPeak	ΔTotal
Control	Blood Flow ($ml \cdot min^{-1}$)	226±36	876±113	141±17	140±29*	560±99*	122±19
	VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	2.0±0.2	9.9±2	1.6±0.2	1.4±0.3*	5.9±1.0*	1.3±0.2
CPT	Blood Flow ($ml \cdot min^{-1}$)	192±23	741±127†	136±27	128±23*	426±74*†	97±19
	VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	1.7±0.3†	8.3±2†	1.5±0.3†	1.1±0.3*	4.4±0.8*†	0.9±0.2†

Mean±SE; CPT, cold-pressor test; VC, vascular conductance. * $P < 0.05$ vs. young adults; † vs. control

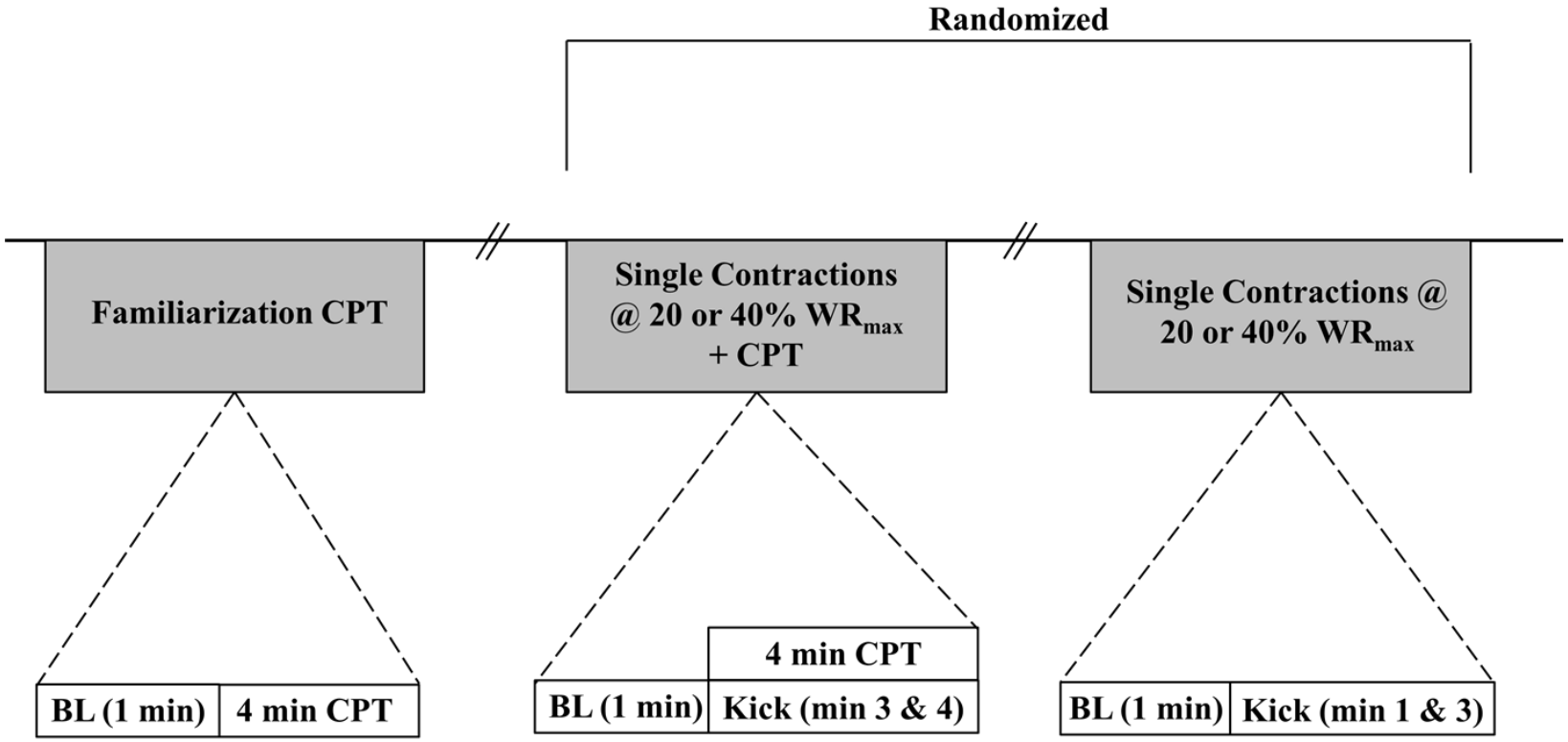

Figure 8: Experimental timeline. CPT, cold-pressor test, BL, baseline, WR_{max}, work-rate maximum

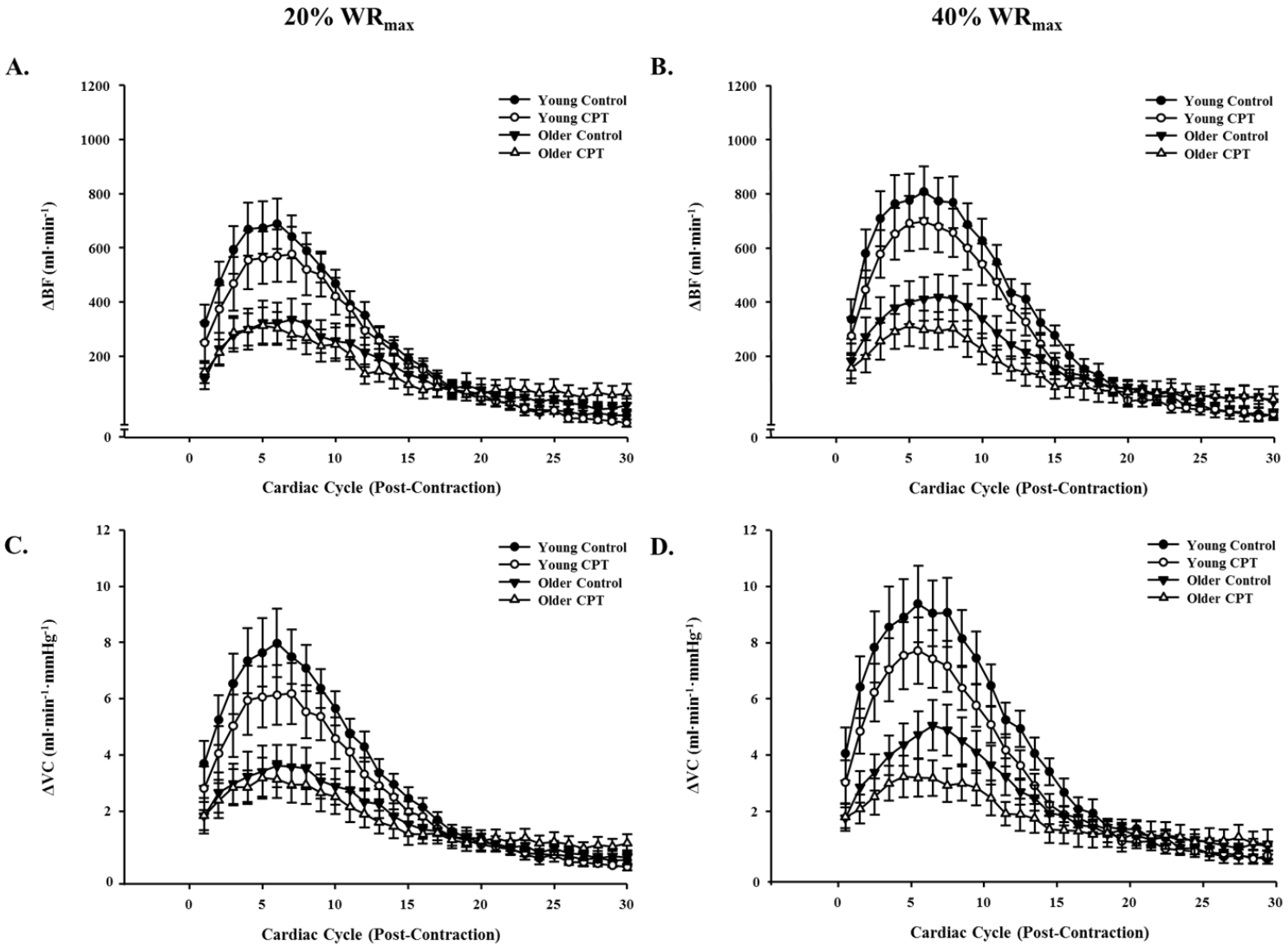

Figure 9: Hyperemic [change (Δ) in blood flow (BF); A and B] and vasodilator [Δ vascular conductance (VC); C and D] responses over 30 cardiac cycles following a single leg knee extension at 20% (A and C) and 40% WR_{max} (B and D) during control and sympathetic stimulation (cold-pressor test; CPT) conditions in young and older adults

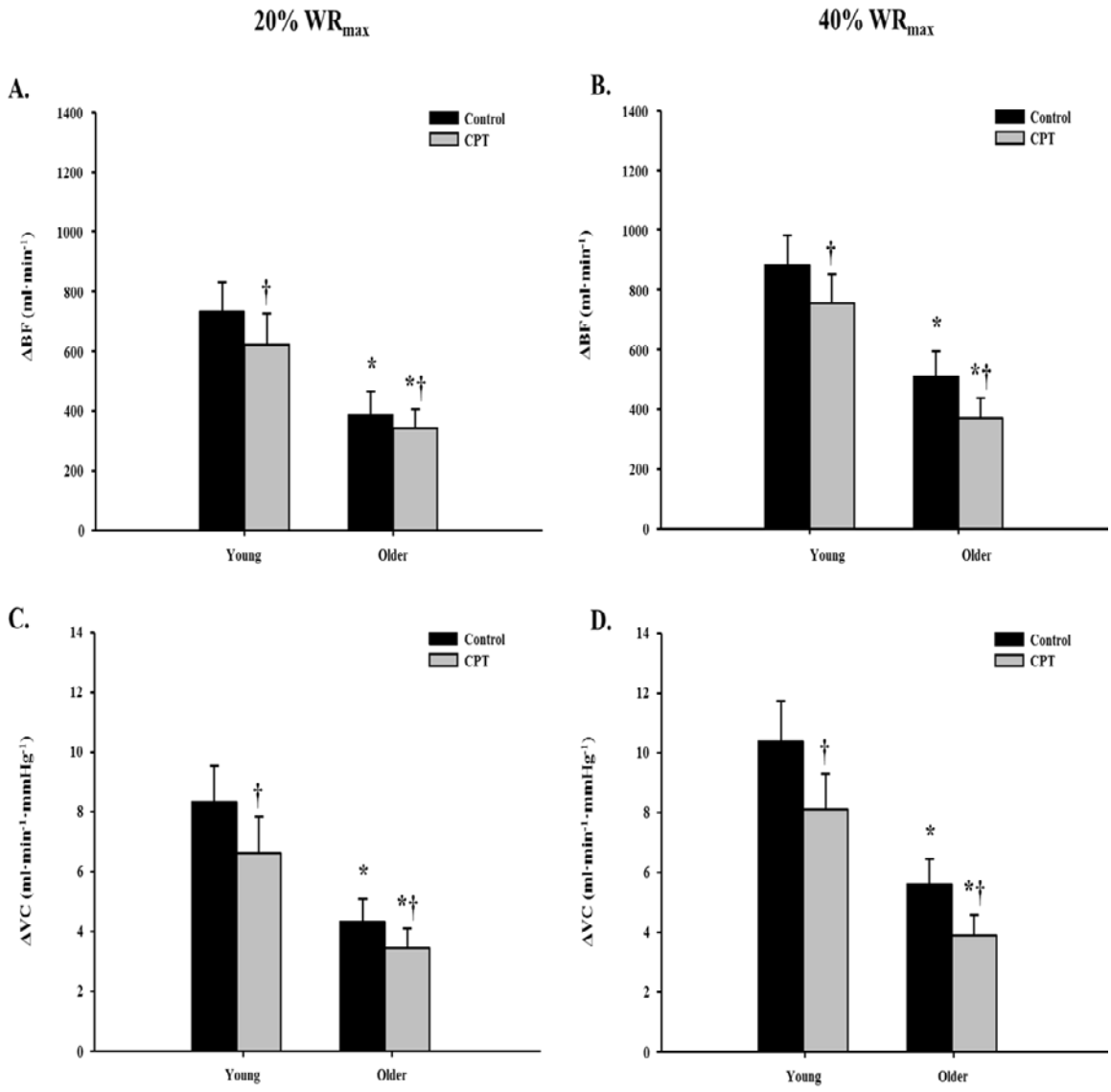

Figure 10: Peak hyperemic (A and B) and vasodilator (C and D) responses in young and older adults at 20% (A and C) and 40% WR_{max} (B and D). * P<0.05 vs. young; † P<0.05 vs control

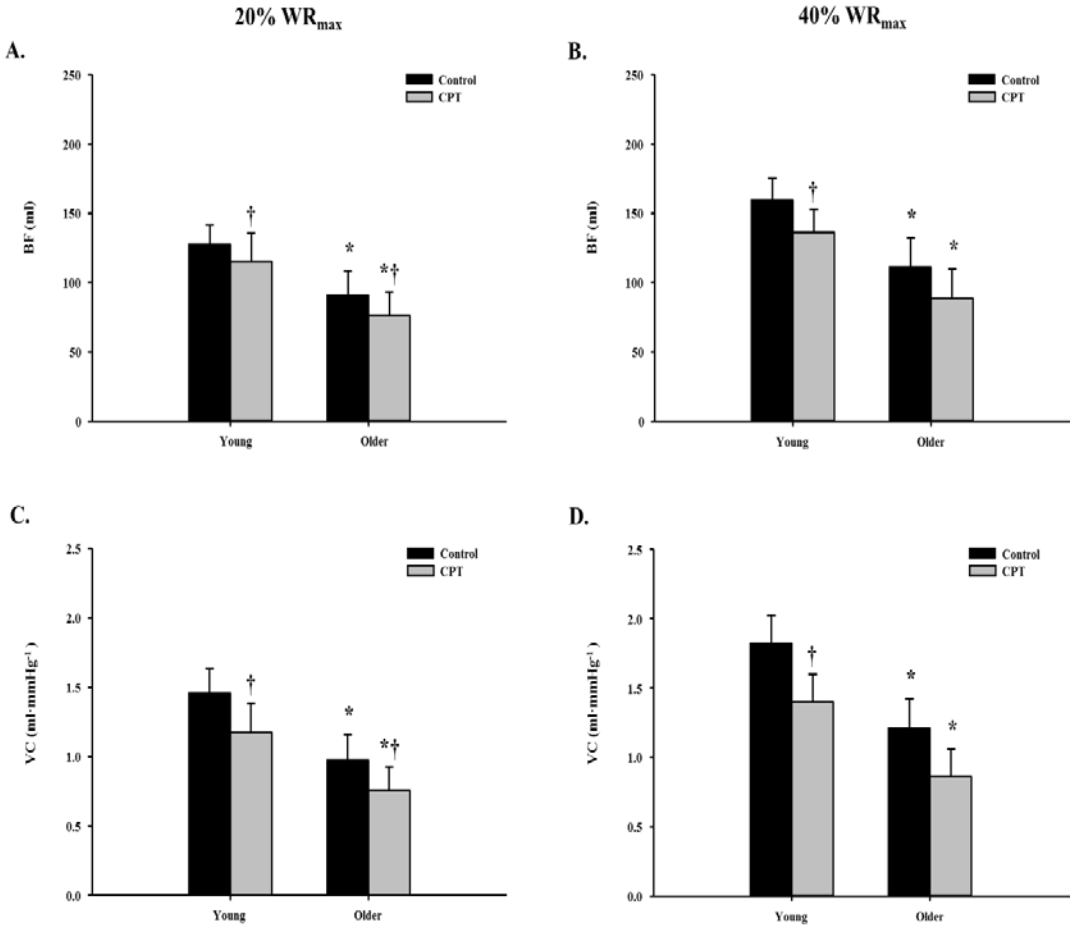

Figure 11: Total hyperemic (A and B) and vasodilator (C and D) responses in young and older adults at 20% (A and C) and 40% WR_{max} (B and D). * $P<0.05$ vs. young; $^{\dagger}P<0.05$ vs. control

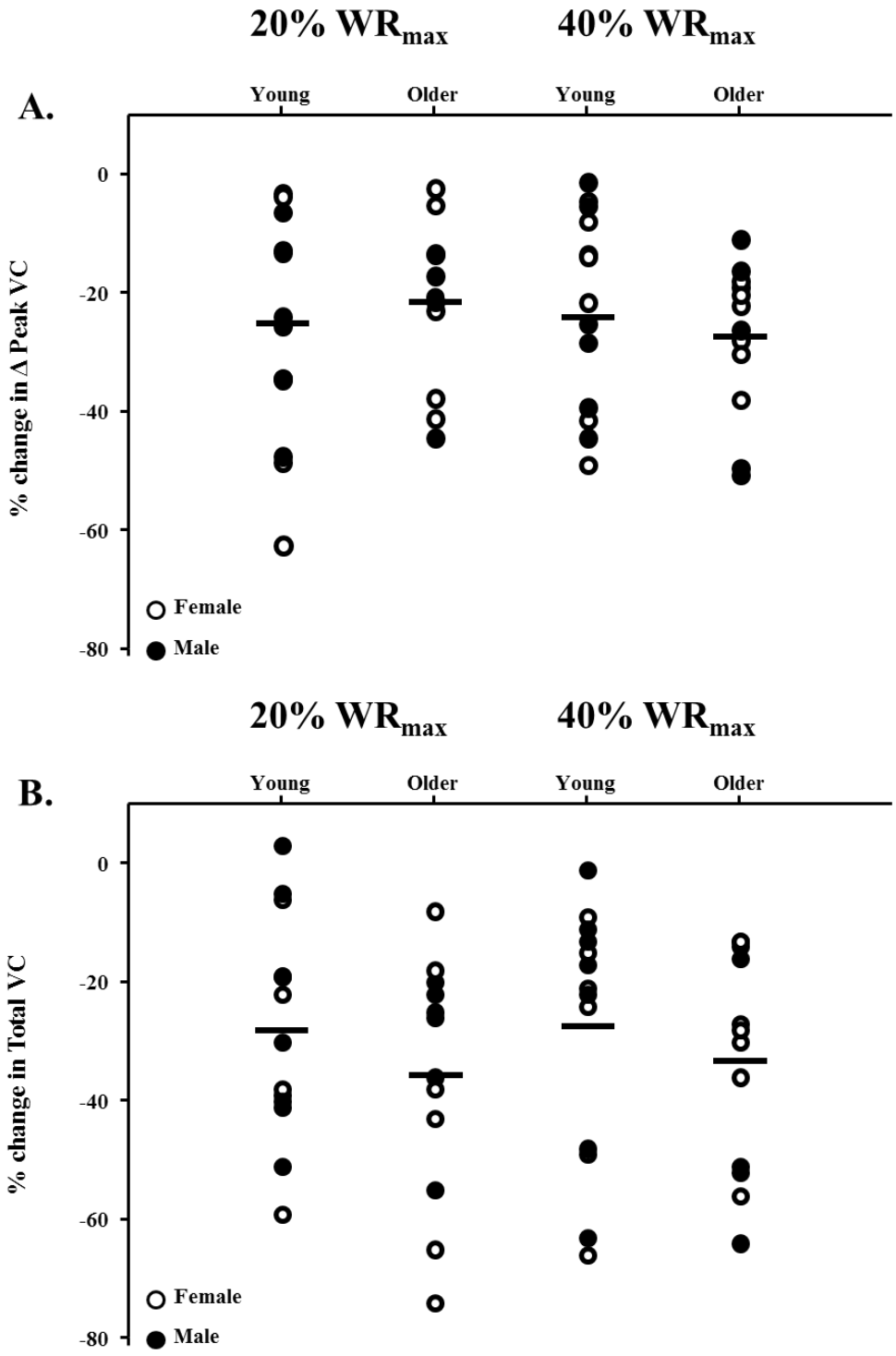

Figure 12: Percent reduction (%Δ) in peak (A) and total (B) vasodilator responses to single leg knee extension at 20% and 40% WR_{max} in response to sympathetic stimulation in young and older adults. Open circles (○) represent females and closed circles (●) represent males of each age group

CHAPTER 5: CHRONIC ENDURANCE EXERCISE TRAINING OFFSETS THE AGE-RELATED ATTENUATION IN CONTRACTION-INDUCED RAPID VASODILATION

A version of Chapter 5 has been previously published: Hughes WE, Ueda K, Casey DP. Chronic endurance exercise training offsets the age-related attenuation in contraction-induced rapid vasodilation. *J Appl Physiol*. 2016; 120:1335-1342.

Introduction

Aging is associated with impairments in vascular function and the regulation of skeletal muscle blood flow, as well as reductions in aerobic exercise capacity (15, 110, 171, 300). These age-related impairments in the regulation of skeletal muscle blood flow, particularly during exercise, are apparent after a single skeletal muscle contraction (39, 40, 43, 120, 141) and persist under steady state conditions (41, 155, 226, 229, 231). Blunted skeletal muscle blood flow responses in older adults have been attributed to alterations in local vasodilatory mechanisms, elevations in sympathetic vasoconstrictive activity or an impaired ability to modulate sympathetic vasoconstriction during exercise (75, 110, 142, 184). Collectively, these adverse changes in the vasculature and decreased limb blood flow can potentially lead to an inadequate matching of O_2 delivery to the metabolic demand of the contracting muscle (15, 155, 247).

Rapid onset vasodilation (ROV) describes the immediate increase in blood flow and vasodilation elicited with a single muscle contraction (51, 252, 295, 297) and is thought to serve a critical role in modulating the blood flow response during steady state exercise conditions (251, 252, 270). The influence of age on ROV is well characterized in the human forearm (39, 40, 43, 141), and more recently in the leg (120). Collectively, these studies indicate that older adults exhibit attenuated peak and total hyperemic and vasodilator responses following a single muscle contraction (39, 40, 43, 141), which appears to be independent of limb (120). At least in the

forearm, these age-related decrements in contraction-induced rapid vasodilation are thought to be due in part to diminished nitric oxide (NO) bioavailability or signaling and enhanced α-adrenergic vasoconstrictor tone (40, 43). However, to date all studies examining ROV in both young and older adults has exclusively examined the hyperemic and vasodilator responses in sedentary or recreationally active individuals.

Chronic exercise training has been shown to ameliorate many age-associated changes in vascular function (184, 204, 258), while physical inactivity is a risk factor for the development of chronic diseases (29, 220). Indeed, there is a strong inverse relationship between cardiorespiratory fitness (e.g. VO_{2peak}) and overall mortality (24, 83), highlighting the benefits of life-long exercise and high levels of physical activity. However, it is unclear whether the attenuated ROV in contracting muscle of older adults is due to aging per se or the reduction of physical activity and functional fitness that often accompanies the aging process (77, 171). Moreover it is unknown whether long term exercise training can prevent the blunting of ROV that occurs in untrained older adults. With this information as background, we tested the hypothesis that chronic endurance exercise training in older adults would ameliorate the age-related impairments in contraction-induced ROV. Additionally, we sought to examine whether a relationship between cardiorespiratory fitness and ROV exists in young and older adults.

Methods

Subjects

Sixteen older endurance exercise trained (9 men, 7 women, age: 59-85 yr) subjects volunteered to participate in this study. To address our primary aim, data from our previous study which included 16 older and 14 young sedentary to recreationally active adults (120) were used for comparison with the present group of older exercise trained adults (see Table 10 for

complete demographics). All subjects completed a general health history screening and written informed consent and were generally healthy, free of any diagnosed cardiovascular or metabolic complications, nonobese (body mass index: ≤ 30 kg·m^2), nonsmokers, and not taking any vasoactive medications. Exercise trained older adults self-reported chronic endurance exercise training ≥ 4 days per week, ≥ 1 hr per session per day and for at least the past year. On average subjects reported meeting these training requirements for the past 19 ± 4 years and several of them were still actively competing in races (both running and cycling). Trained status was confirmed with a graded, maximal exercise stress test. Studies were performed after an overnight fast, and subjects refrained from exercise, alcohol, and caffeine for 24 hours before reporting to the laboratory. Young female subjects were studied during the early follicular phase of their menstrual cycle or the placebo phase of oral contraceptives to control for the potential influence of sex hormones on primary outcome variables (173). All older female subjects were post-menopausal and were not taking any form of hormone replacement therapy. All study protocols were approved by the Institutional Review Board at the University of Iowa.

Pre-Study Day Measurements

Body Composition, Forearm, and Leg Tissue Mass

Body composition was determined by dual energy X-ray absorptiometry (DEXA; Hologic software version APEX 4.0). Total mass and fat free mass of the left forearm and right leg were determined from regional analysis from the whole-body DEXA scan using bony landmarks for normalization of blood flow and vascular conductance responses. Body mass index (BMI) was calculated as body weight (kg) divided by height (meters) squared.

Measurement of Exercise Capacity

Peak exercise O_2 consumption (VO_{2peak}; $ml \cdot kg^{-1} \cdot min^{-1}$) was determined in all subjects using respiratory gas analysis (Parvo Medics TrueOne 2400, Sandy, UT, USA) during incremental treadmill exercise using a Bruce protocol performed to exhaustion as previously described (88).

Determination of Work Rate Maximum

Work rate maximum (WR_{max}) was determined from a single leg knee extensor incremental maximal exercise test completed during a familiarization session prior to the study day as previously described (120). Briefly, subjects were seated in a semirecumbent position on a modified adjustable bucket seat that accommodated variable body and leg lengths allowing each subject's lower leg to move through a 90-180˚ range of motion during the knee extension exercise. Resistance was developed by a custom-made computer controlled leg ergometer. Briefly, resistance (torque) was developed by an AC motor turning at a constant RPM that was transferred to the leg shaft, against which subjects contracted. The computer monitored the elapsed time, angle of the leg and controlled the actual torque presented to the leg during contraction. In this way, the subject was required to develop enough power to extend the leg through a full range of motion. WR_{max} testing consisted of an initial workload of 5W that incrementally increased every minute by 3W and 5W in female and male subjects, respectively. Subjects kicked dynamically through a full range of motion at a cadence of 40 kicks per minute. The single leg knee extensor incremental maximal test continued until the subject could not maintain a full range of motion or a cadence of 40 contractions per minute. The final workload completed was recorded as maximal kicking load from which relative workloads were calculated.

Study Day Measurements

Heart Rate and Systemic Blood Pressure

Heart rate (HR) was recorded via continuous three-lead electrocardiogram, and systemic blood pressure was assessed (beat-to-beat) via finger plethysmography (Nexfin; Edwards Lifesciences, Irvine, CA) on the non-exercising hand. Brachial artery pressure was measured in duplicate using an automated cuff (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA) prior to beginning exercise trials while the subjects were in a supine position following 15 minutes of rest.

Single Muscle Contractions

Subjects performed dynamic single contractions in both the arm and leg as previously described (120). Briefly, single forearm contractions were performed with a handgrip device at 10%, 20%, and 40% of the subject's maximal voluntary contraction (MVC), determined (using a handgrip dynamometer) as the average of three maximal squeezes performed on the pre-study measurement day. Single knee extension contractions were performed at 20%, 40%, and 60% WR_{max}. Subjects were instructed to contract and relax on a verbal command from laboratory personnel. WR_{max} and MVC intensities were randomized prior to the experimental protocol, and each contraction intensity was performed in duplicate to calculate the average response for each subject for a given condition. Each contraction (leg and arm) was visually observed by the laboratory personnel to ensure proper timing of contraction. Two minutes of relaxation were given between each contraction to allow continuous measures of limb hemodynamics post-contraction. All single muscle contractions (knee extensions and forearm contractions) were performed on the same day.

Measurement of Blood Flow

Brachial and common femoral (~2-3 cm proximal to bifurcation) artery diameter and blood velocity were determined with a 12-MHz linear-array Doppler probe (model M12L; Vivid 7, General Electric, Milwaukee, WI). Blood velocity was measured with a probe insonation angle previously calibrated to 60°. Measured velocity waveforms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (113). Artery diameter measurements were obtained at end diastole at rest (before contraction) and 1 minute post-contraction. Limb blood flow (BF) was calculated as the product of mean blood velocity ($cm \cdot s^{-1}$) and artery cross-sectional area (cm^2) and expressed as milliliters per minute ($ml \cdot min^{-1}$).

Data Analysis and Statistics

Data were collected at 250 Hz and analyzed offline with signal processing software (WinDaq; DATAQ Instruments). Beat-to-beat mean arterial pressure (MAP) was derived from the Nexfin pressure waveform and was recorded simultaneously with beat-to-beat blood velocity measurements. Heart rate was determined from the electrocardiogram. Baseline BF and MAP represent an average of the last 30 seconds of the resting time period before each muscle contraction and were used to quantify the hyperemic response. Vascular conductance (VC) was calculated as BF/MAP (and expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$). Rapid hyperemic and vasodilator responses are expressed as the change in (Δ) BF and VC from baseline, respectively. Of particular interest are the peak (ΔVC) and total (VC) dilator responses post-contraction. Total BF (ml) and VC ($ml \cdot mmHg^{-1}$) were defined as the area under the curve over 30 post contraction cardiac cycles after respective baseline values were subtracted for a given flow or conductance curve. To account for the possible influence of muscle mass, BF and VC responses were also examined after normalizing for muscle mass (kg).

All values are expressed as mean ± SE. Analysis of variance (ANOVA) was used to analyze demographic variables between groups. To address the primary question of whether chronic exercise training offsets the age-related impairment in contraction-induced ROV, comparisons were made between the older trained adults and our previously published data on young and older untrained adults (120). Independent, one-way ANOVAs were used to compare groups across exercise intensities in both the arm (10%, 20%, and 40%) and leg (20%, 40% and 60%). When significance was detected, Tukey's post hoc analysis was used to identify differences between groups. Pearson's correlation coefficients were calculated to assess the relationship between exercise capacity (VO_{2peak}) and ROV (peak and total VC). All statistical analyses were completed using SigmaPlot software version 11.0 (Systat Software Inc., San Jose, CA). Statistical difference was set a priori at $P < 0.05$.

Results

Subject characteristics are shown in Table 10. Young, untrained and trained older subjects were of similar height, weight, body mass, and percent body fat ($P > 0.05$). Brachial blood pressures, forearm and leg muscle masses, and forearm MVC were also similar between groups ($P > 0.05$ for all). There were no differences in WR_{max} between trained and untrained older adults. Compared to young adults, untrained older adults had a lower WR_{max} ($P < 0.05$), but there were no differences between trained older adults and young adults. Lastly, and important to the current study, exercise trained older adults had higher VO_{2peak} compared to their untrained counterparts ($P < 0.05$).

Rapid Hyperemic and Vasodilator Responses to Single Muscle Contractions in Trained Older, Untrained Older, and Young Adults

Figure 13 illustrates the temporal rapid hyperemic and vasodilator responses at the highest arm (A & C; 40% MVC) and leg (B & D; 60% WR_{max}) exercise intensities in the three groups of subjects (exercise trained older adults, untrained, and young adults). The responses in the young and untrained older adults have been previously published (120) and are included to highlight the effect of chronic exercise training on the temporal vasodilator response following a single muscle contraction in the arm and leg. The peak responses for the arm and leg tended to occur in the first 3-5 cardiac cycles, and this timing of peak responses were similar between groups. The peak hyperemic and vasodilator responses in the arm were greater in trained older adults at moderate exercise intensities (20%-40% MVC) compared to their older untrained counterparts ($P < 0.05$; Figure 14A & 14B). There were no differences between trained and untrained older adults for total BF at any exercise intensity (Figure 14C & 14D). Additionally, there were no differences across contraction intensities for the peak and total hyperemic and vasodilator responses in the arm between trained older adults and young adults ($P > 0.05$). Peak and total hyperemic (Figure 15A & 15C) and vasodilator (Figure 15B & 15D) responses were greater in the older trained adults across leg exercise intensities compared to older untrained adults ($P < 0.05$) while there were no differences between trained older and young adults ($P > 0.05$). Normalizing responses to muscle mass elicited similar results in that peak arm hyperemic and vasodilator responses were greater in trained older adults at moderate exercise intensities compared to untrained counterparts (data not shown $P < 0.05$). Additionally, peak and total hyperemic and vasodilator responses were greater in trained older adults across leg exercise intensities compared to untrained older adults, with no differences between trained older and young adults (data not shown, $P > 0.05$).

Relationship between Exercise Capacity and ROV within the Arm and Leg

Figure 16 illustrates the relationship between VO_{2peak} and peak vasodilator responses at the highest exercise intensities in the arm (16A) and leg (16B). VO_{2peak} was moderately associated with arm peak and total VC across exercise intensities ($r = 0.31$-0.61; $P < 0.05$ for all) in the group as a whole. When separated by age, VO_{2peak} was associated with peak ($r = 0.59$-0.64; $P < 0.05$) and total ($r = 0.39$-0.52 $P < 0.05$) arm VC across exercise intensities in older adults, but only at 40% in young adults ($r = 0.53$; $P < 0.05$). Within the leg, VO_{2peak} was associated with peak ($r = 0.55 – 0.65$; $P < 0.05$) and total ($r = 0.39$-0.50, $P < 0.05$) VC across exercise intensities in the entire group. When separated by age, VO_{2peak} was moderately associated with peak leg vasodilator responses across exercise intensities in older adults ($r = 0.55$-0.68 $P < 0.01$ for all), and at 40-60% WR_{max} in young adults ($r = 0.60$ and 0.65 respectively, $P < 0.05$ for both). When separated by training status (trained older vs. untrained adults) there were no associations between VO_{2peak} peak or total VC in the arm or leg of untrained older adults ($P > 0.05$). Conversely, trained older adults exhibited associations between VO_{2peak} and peak ΔVC across arm exercise intensities ($r = 0.68$-0.70; $P < 0.05$ for all) and at 40% and 60% WR_{max} in the leg ($r = 0.62$-0.70; $P < 0.05$).

ROV, Age, and Physical Fitness: Influence of Workload

As untrained older adults had a lower leg WR_{max} it could be argued that because of the tight relationship between blood flow and metabolic rate, any age or physical fitness related differences may be due to differences the absolute work performed between groups. In order to address this issue, we compared the peak and total responses normalized for work rate (e.g. flow and/or conductance per Watt) between groups. Normalizing the data in this way revealed similar results as presented above for the absolute hyperemic and vasodilator responses. Trained older adults still demonstrated higher peak hyperemic (98 ± 8 vs. 68 ± 8, 56 ± 4 vs. 41 ± 4 and 44 ± 3

vs. 32 ± 3 ml· min^{-1}·W^{-1}) and vasodilator responses (1.1 ± 0.1 vs. 0.7 ± 0.1, 0.6 ± 0.1 vs. 0.4 ± 0.1, and 0.5 ± 0.04 vs. 0.3 ± 0.03 ml·min^{-1}·$mmHg^{-1}$·W^{-1}) across exercise intensities (20-60% WR_{max}) compared to untrained older adults ($P < 0.05$ for all). Additionally, the total hyperemic response normalized for work rate was higher at 20% (15 ± 2 vs. 9 ± 1) and 60% (7 ± 1 vs. 4 ± 0.4) WR_{max} in trained vs. untrained older adults ($P < 0.05$ for both).

To further address the potential confound of work rate between groups, we compared the peak and total hyperemic and vasodilator responses in a subset of subjects from each of the three groups that performed similar absolute workloads (mean ~10-11 W for each group). The mean ± SE workloads for the subset of trained older (n=10), untrained older (n=11) and young (n=11) adults were, 10.6 ± 0.4, 10.5 ± 0.3, and 10.3 ± 0.3 W, respectively. As illustrated in Figure 17, peak hyperemic (17A) and vasodilator responses (17B) were greater in trained older adults compared to untrained older adults ($P < 0.05$ for both) when matched for workload, while peak ΔVC was lower in untrained older adults compared to young adults ($P < 0.05$). Total hyperemic (17C) and vasodilator (17D) responses were not different between groups ($P = 0.07$ for both). When the responses were normalized for muscle mass in the subset of subjects matched for workload, there were no differences between groups ($P = 0.09$-0.17) for peak and total ΔBF and ΔVC. In the same subset of subjects, VO_{2peak} was associated with peak ΔVC in the group as a whole ($r = 0.48$, $P < 0.01$), but not when separated by training status ($r = -0.08$-0.44, $P > 0.05$ for all). Total VC and VO_{2peak} were not associated in any group, regardless of how responses were expressed ($r = -0.54$-0.39, $P > 0.05$ for all). There were no significant associations between VO_{2peak} and peak ΔVC (normalized for muscle mass), in the group as a whole ($r = 0.15$, $P > 0.05$), or when separated by training status (trained, untrained older adults, and young; $r = -0.43$-0.48, $P > 0.05$ for all).

Discussion

We and others have previously shown that contraction-induced rapid hyperemic and vasodilator responses are attenuated in untrained or sedentary older adults (39, 40, 43, 120, 141) and this attenuation appears to be similar between the arm and leg (120). In the present cross-sectional study we demonstrate for the first time that 1) older adults with a history of chronic endurance exercise training demonstrate greater contraction-induced rapid hyperemic and vasodilator responses within both the arm and leg compared to their age matched counterparts, and 2) vasodilator responsiveness to single muscle contractions is moderately associated with exercise capacity (VO_{2peak}). These conclusions are supported by the fact that chronically trained older adults demonstrated a substantially greater peak ROV in the arm (Figure 14) as well as an approximately 2 fold greater peak and total ROV response in the leg (Figure 15) compared to untrained older adults across a range of relative (%) workloads. Moreover, the group differences between the older trained and untrained adults still persist when 1) the ROV responses are normalized to the absolute work rate performed; and 2) the groups are matched for a similar absolute workload (Figure 17). Finally, our findings also demonstrate that exercise capacity is positively related to the peak ROV response, regardless whether the peak ROV responses are expressed at the same relative exercise intensity or matched for workload. Taken together, these results suggest that chronic endurance exercise offsets the age-related attenuation in ROV in both the arm and leg of older adults.

Benefits of Exercise Training on Blood Flow in Older Adults

During sub-maximal dynamic exercise there is a general decline in steady state blood flow and vasodilation with age (155, 226, 229-231, 233, 234, 241), which may be offset with exercise training (15, 218). The decline in exercise blood flow with aging is presumably due to

an imbalance between local vasodilator and vasoconstrictor signaling, elevated sympathetic vasoconstrictive tone, as well as an impaired ability to blunt sympathetic vasoconstriction within the contracting muscle, (73, 140-142, 253). Conversely, lifelong physical activity (185) as well as exercise interventions (184) appears to preserve the ability to blunt sympathetic vasoconstrictive tone (functional sympatholysis) in the leg of older adults and maintain sufficient O_2 delivery in older adults (228, 300). However, it should be noted that the documented benefits of lifelong physical activity or chronic exercise training on the regulation of muscle blood flow during exercise may be sex specific. That is, exercise trained older women do not appear to exhibit the same preservation of exercise hyperemia and vasodilation that trained older men do (218, 229, 230). In the present study, chronic exercise training appears to offset the age-related attenuation of peak and total hyperemic and vasodilator responses following a single muscle contraction in the leg of both older men and women. However, this study was not adequately powered to detect sex by exercise training interactions and therefore we cannot make any conclusions on whether chronic exercise training is less effective in improving ROV in older women compared to men.

Training Benefits Beyond "Active" Tissue

Exercise training adaptations to vascular structure and function are not solely restricted to the active limb (e.g. metabolically active limb) and may be conferred to the vasculature perfusing non-exercising tissue [e.g. inactive skeletal muscle] (22, 213). In the context of the present study, the majority of exercise trained older adults reported participation in primarily activities involving the lower limbs, and as illustrated in Figures 14 and 15, this chronic exercise training offsets age-related impairment of ROV responses in both limbs. Along these lines, lower body exercise training often results in an increase in femoral artery diameter, but also preserves

endothelial function in the brachial artery (e.g. “inactive” limb), such that age-related differences in both conduit and resistance arteries are abolished (71, 307). It should be noted that these training adaptations are apparent in men, as these effects have not been observed in post-menopausal women (221). These favorable vascular adaptations in conduit arteries to exercise training in “inactive” skeletal muscle are hypothesized to be mainly due to repeated bouts of elevated shear stress, with endothelial adaptations evident even after a single bout of acute exercise (288). Taken together, these data confirm and highlight the systemic benefits of chronic exercise training on the vasculature in older adults.

Possible Mechanisms

This study provides insight into how chronic endurance exercise training offsets age-related attenuations in contraction-induced ROV, however, it is important to emphasize that discerning the underlying mechanisms modulating this response was not the purpose of the present investigation. Instead, this study was designed to highlight and characterize the relationship between chronic exercise training, exercise capacity and age-related attenuations in contraction-induced ROV. Therefore, we will only briefly highlight plausible mechanisms by which exercise training may offset age-related attenuation of the ROV response to single muscle contractions. Previous evidence from our laboratory demonstrate that age related impairments in contraction-induced ROV in the arm are in part, due to a reduction in NO bioavailability or signaling, as well as an increase in sympathetic vasoconstriction (40, 43). In this context, evidence from habitually exercising older adults indicates that lifelong exercise training preserves both NO signaling (204, 282) as well as functional sympatholysis within the leg vasculature in older individuals (185). Therefore, it is plausible that the mechanisms by which

chronic exercise training offsets age-related attenuation of ROV in older adults are similar to those observed under steady state conditions during submaximal dynamic exercise.

Experimental Considerations

It should be noted that our current study design does not allow us to examine whether endurance exercise trained older adults exhibit similar ROV responses relative to trained young adults. Evidence suggests that young, highly-trained adults exhibit augmented exercise blood flow responses at submaximal and maximal workloads relative to young sedentary counterparts in both the arm and leg (302). Therefore it can be assumed that young trained adults might exhibit augmented ROV responses, however this has yet to be confirmed. Given that the aim of the study was to examine how chronic exercise training influences characteristics of ROV in a cross sectional manner, we could not control which specific type of chronic exercise subjects participated in. Furthermore, as we present a large range of chronic exercise trained older adults, we were unable to control the number of years the group as a whole participated in. Along these lines, improvements to vascular function are apparent even with short-term exercising training interventions, particularly in populations with established vascular dysfunction (71, 184, 211, 275, 307). Additionally, while the older trained and untrained groups were well matched for many characteristics besides exercise training and VO_2, there was a trend ($P = 0.11$) for a higher %body fat in the untrained compared to exercise trained older adults. Since contraction-induced rapid onset vasodilatation has been shown to be blunted with obesity (23), differences in body composition could contribute in part to the augmented ROV responses observed in the exercise trained group. However, we believe these limitations should not detract from the present study; instead, it should be viewed in a larger picture, in that older adults with a history of performing

habitual endurance exercise demonstrate an augmented contraction-induced rapid vasodilation compared to their sedentary older peers.

Conclusion

To our knowledge, this is the first study to demonstrate that chronic exercise training or physical activity offsets the age-related attenuation in contraction-induced ROV in both the arm and leg. Additionally, exercise capacity is associated with contraction-induced rapid hyperemic and vasodilator responses in older adults. These findings extend upon previous studies demonstrating the ability of chronic aerobic exercise to offset age-related impairments of endothelial function as well as steady state exercise hyperemia and vasodilation, further highlighting the benefits of lifelong exercise training.

Table 10: Subject Characteristics for Aim 4

Variable	Young Adults (n=14)	Untrained Older Adults (n=16)	Trained Older Adults (n=16)
Age (years)	23 ± 1	66 ± 1*	66 ± 2*
Men/Women	9/5	10/6	9/7
Height (cm)	174 ± 2	172 ± 2	170 ± 2
Weight (kg)	75 ± 3	78 ± 3	72 ± 2
Body Mass Index ($kg \cdot m^2$)	24.7 ± 0.6	26.5 ± 0.7	24.7 ± 0.7
% Body Fat	27.1 ± 1.9	31.9 ± 1.7	28.0 ± 2.0
Forearm Muscle Mass (kg)	0.94 ± 0.08	0.90 ± 0.06	0.85 ± 0.07
Thigh Muscle Mass (kg)	7.5 ± 0.5	6.9 ± 0.3	7.0 ± 0.3
VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	44.3 ± 2.6	30.6 ± 1.5*	37.5 ± 1.9†
MVC (kg)	41 ± 3	41 ± 3	40 ± 3
WR_{max} (Watts)	41 ± 4	29 ± 2*	37 ± 3
Systolic Pressure (mmHg)	117 ± 2	123 ± 3	123 ± 3
Diastolic Pressure (mmHg)	72 ± 2	75 ± 2	77 ± 2
Mean Arterial Pressure (mmHg)	87 ± 2	91 ± 2	92 ± 2

Values are means ± SE. MVC, maximal voluntary contraction; WR_{max}, Work Rate Maximum. * $P < 0.05$ vs. young adults, † vs. Untrained Older Adults

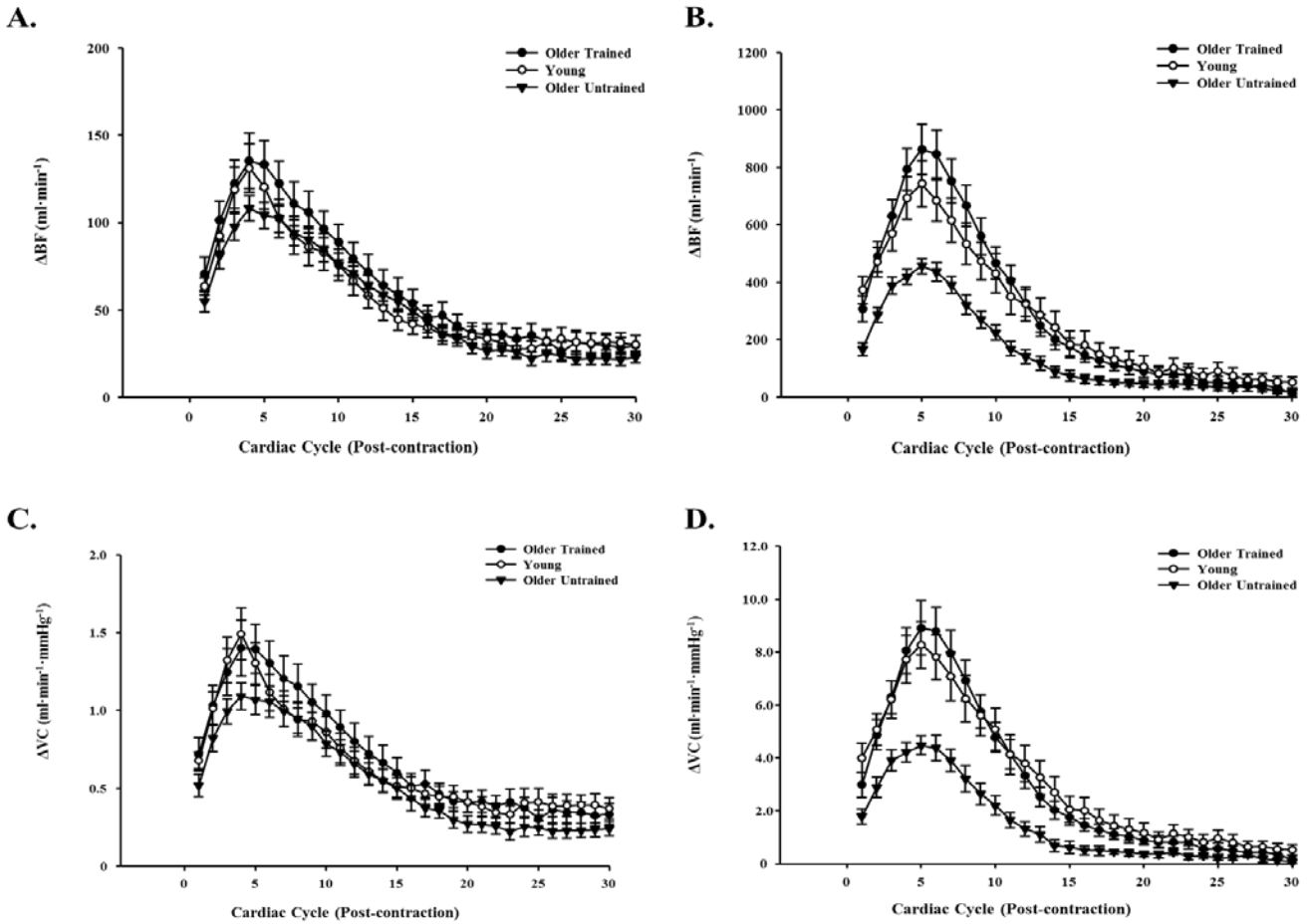

Figure 13: Hyperemic [change (Δ) in blood flow (BF); A & B] vasodilator [Δ vascular conductance (VC); B & D] responses over 30 cardiac cycles following single muscle contractions at the highest arm (A & C; 40% MVC) and leg exercise intensities (B & D; 60% WR_{max})

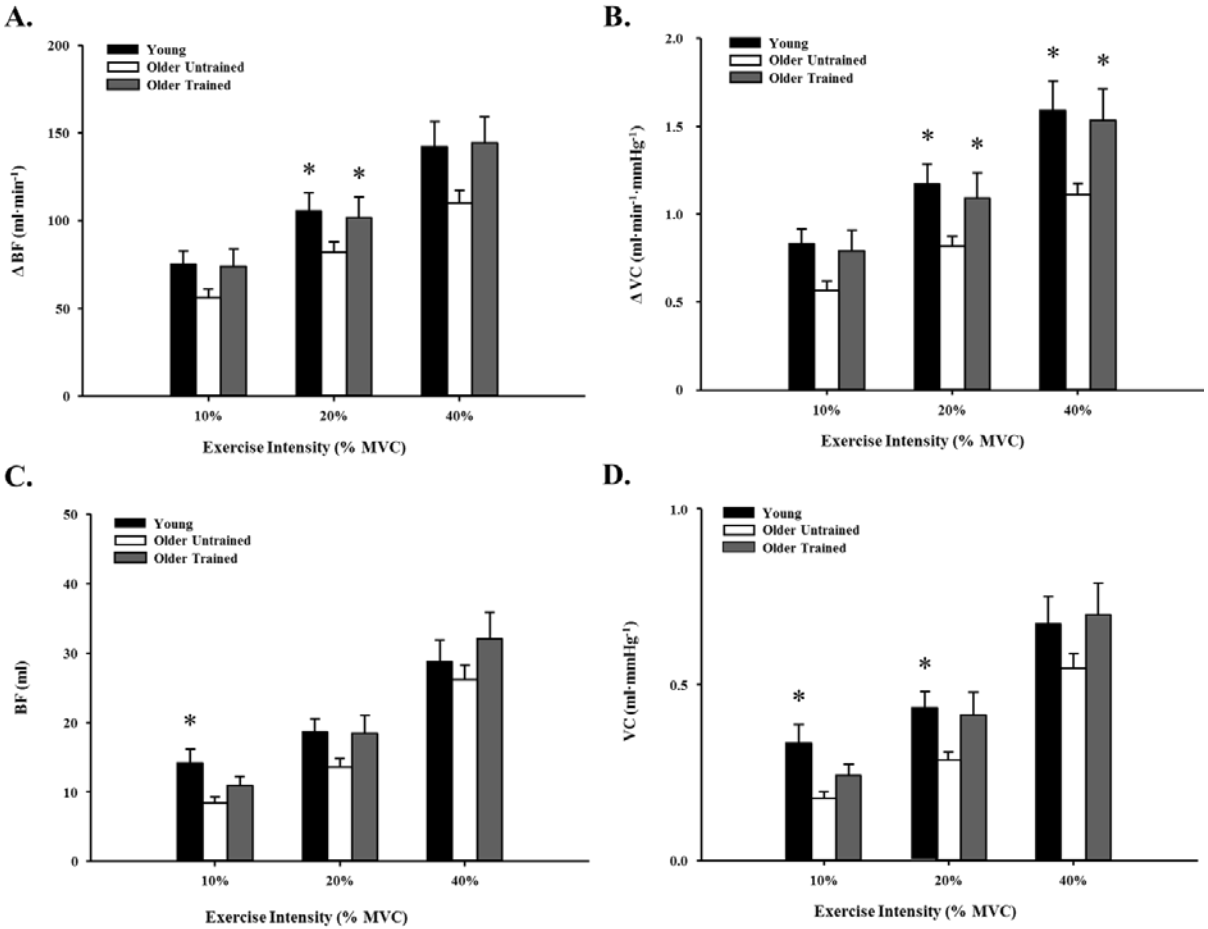

Figure 14: Peak (A & B) and total (C & D) hyperemic and vasodilator responses in the arm for young, untrained and trained older adults. * $P < 0.05$ vs. untrained older adults

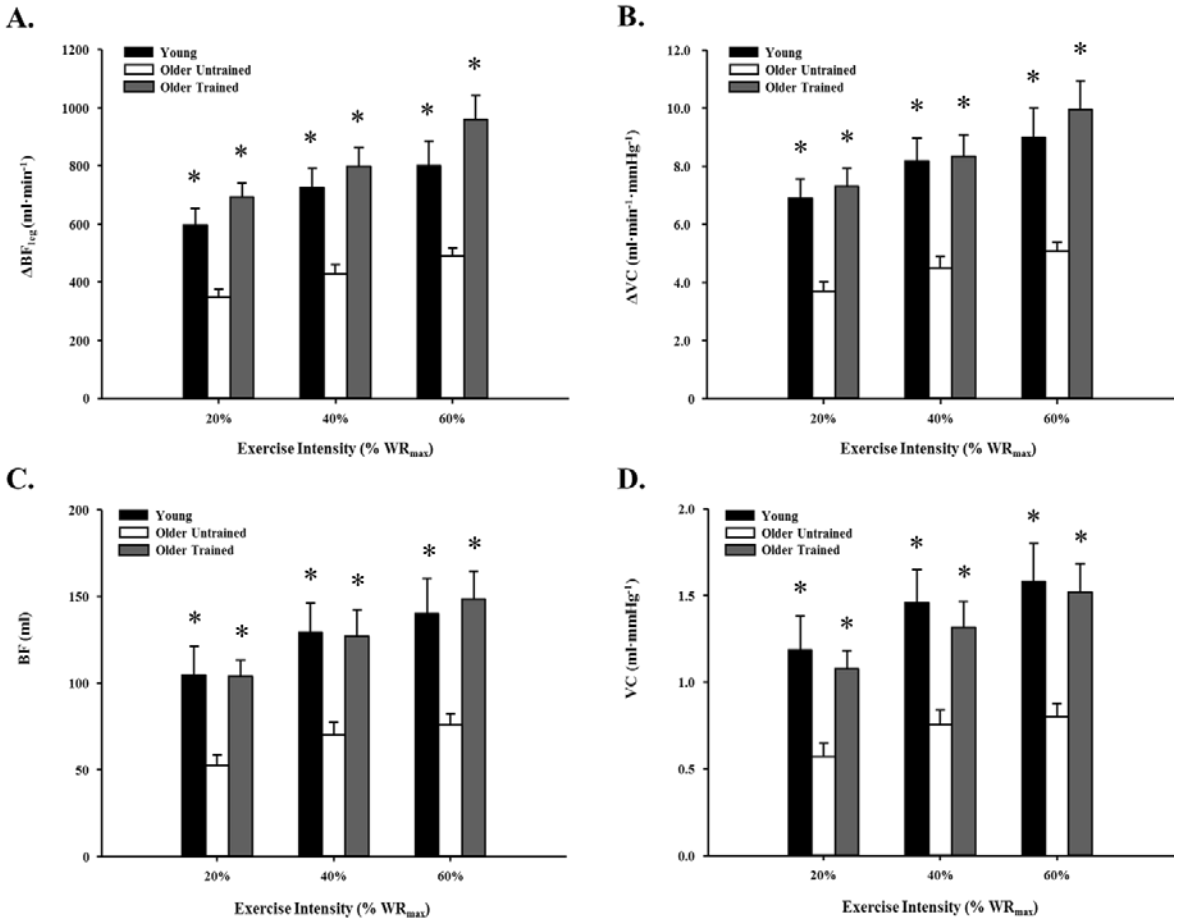

Figure 15: Peak (A & B) and total (C & D) hyperemic and vasodilator responses in the leg for young and untrained and trained older adults. * $P < 0.05$ vs. untrained older adults

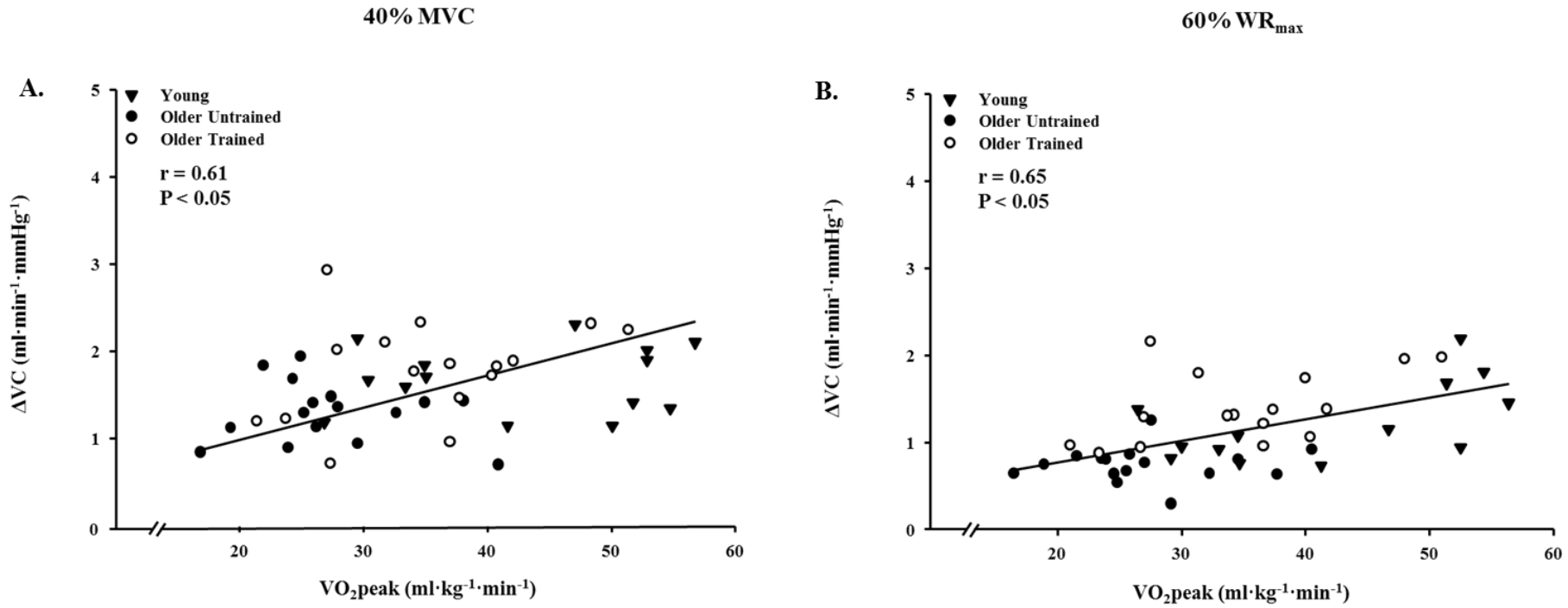

Figure 16: Relationship between exercise capacity (VO_{2peak}) and peak vasodilator responses (ΔVC peak) in the arm (A) and leg (B) of young and older adults

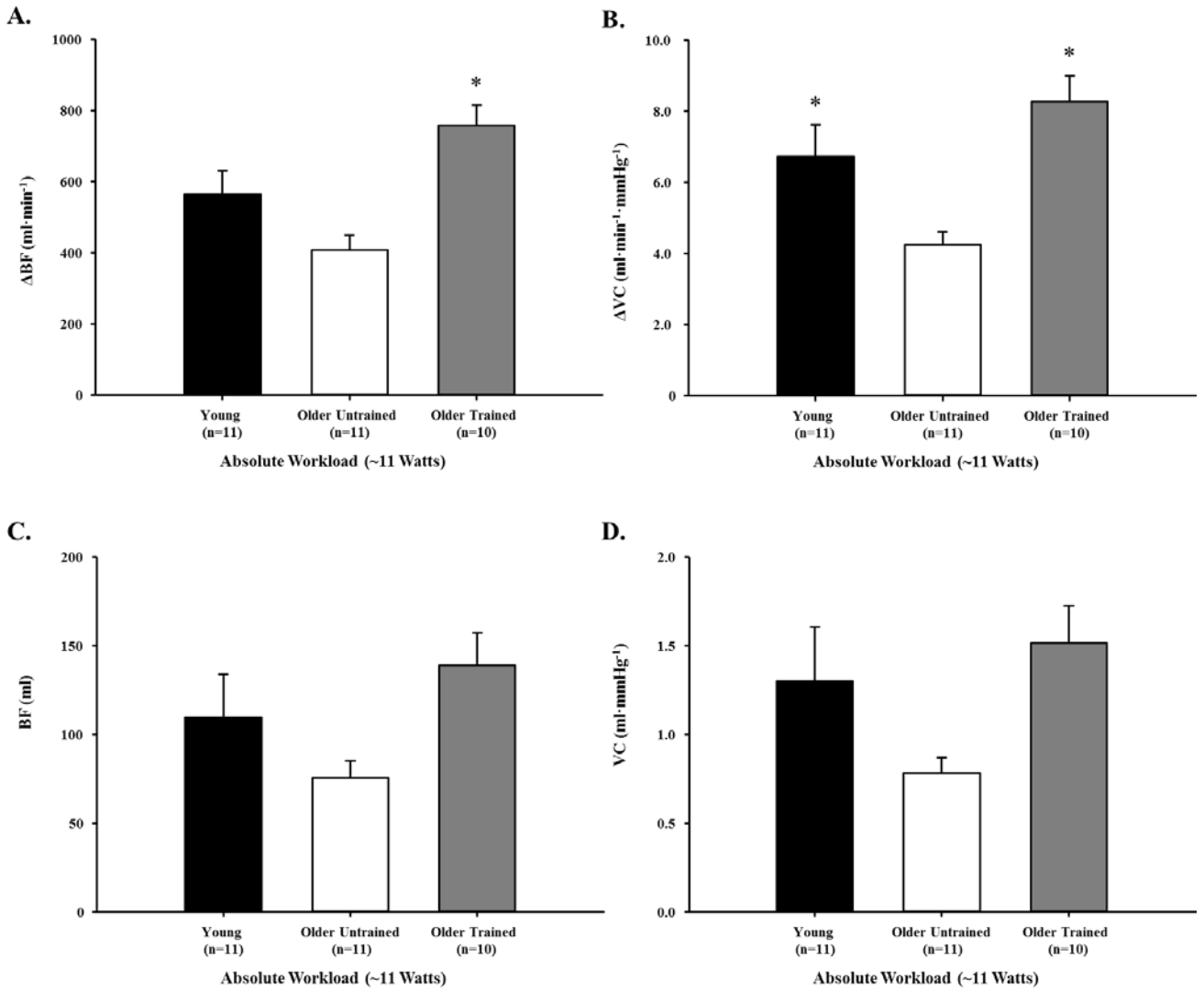

Figure 17: Peak (A & B) and total (C & D) hyperemic and vasodilator responses in the leg at an absolute workload of ~11 W for young adults and untrained and trained older adults. * $P < 0.05$ vs. untrained older adults

CHAPTER 6: HABITUAL EXERCISE TRAINING IN OLDER ADULTS OFFSETS THE AGE-RELATED PROLONGATION OF VASODILATION WITHIN THE LEG DURING SINGLE LIMB LOWER BODY EXERCISE

A version of this chapter is currently under review within the Journal of Applied Physiology

Introduction

Aging is associated with attenuated exercise hyperemic and vasodilator responses at the onset of exercise, as well as during steady-state exercise (80, 110, 120). Both short-term exercise training interventions and habitual exercise training appears to offset these age-related attenuations (15, 118, 204). These impairments in vasodilation with advancing age may act to compromise skeletal muscle blood flow (BF) and potentially reduce oxygen (O_2) delivery to contracting tissue, thereby facilitating premature exercise intolerance (16-18). Importantly, it is believed that sedentary behavior, and by extension, the impaired ability to match skeletal muscle BF to metabolic demand of contracting tissue with age increases cardiovascular disease risk (28, 29). The hyperemic and vasodilator responses at exercise onset and during steady-state have been studied independently (39, 80, 110, 120, 229), however, there is a paucity of evidence regarding how these two events are related. In this context, the mechanisms that initiate the hyperemic and vasodilator response at the onset of exercise may be distinct from those that sustain exercise hyperemia and vasodilation during steady-state exercise.

The onset of exercise illustrates an ongoing challenge to the cardiovascular system in order to meet the metabolic demands within the contracting skeletal muscle. In the transition from rest to exercise BF and vasodilation increase immediately in a biphasic manner, composed of an immediate rapid increase, followed by a slower sustained increase until reaching a plateau (e.g. steady-state) (162, 268, 269). This exercise transient (onset to steady-state) is graded with

exercise intensity and reflects the delicate ratio of oxygen O_2 delivery to O_2 demand within contracting skeletal muscle (16, 17). Examining the rate of adjustment (e.g. kinetic response) of BF and vasodilation provides insight into how regulatory responses at the onset of exercise influence steady-state responses. Along these lines, contraction-induced rapid onset vasodilation (ROV) responses demonstrate that there are key regulatory responses at the initiation of exercise without the influence of subsequent skeletal muscle contractions (268). Data from our laboratory demonstrate that contraction-induced ROV is impaired in leg of older adults relative to young adults, an effect which is offset by habitual aerobic exercise training in older adults (118, 120). Given that BF and vasodilator responses both at the onset of exercise (e.g. following a single skeletal muscle contraction) (120) as well as during steady-state exercise are attenuated in older adults (80, 110, 233), it remains unclear whether advancing age also impairs the kinetic response preceding steady-state exercise.

Data from animal models suggest that the time course (i.e. vasodilatory kinetics) of endothelium-dependent vasodilation is impaired in isolated arterioles of older rats (14, 16). Extending upon these findings in animals, Park et al. (215) demonstrated that the time course of responses to intraluminal flow and endothelium-dependent vasodilation (response to acetylcholine)in isolated human skeletal muscle feed arteries of older adults is slowed (greater tau) compared to young adults. Furthermore, Casey, et. al. (41) demonstrated that during rhythmic forearm hand-grip exercise, older untrained adults demonstrate slower forearm vasodilator kinetics (greater tau) at both relative and absolute workloads, mediated in part by nitric oxide (NO). However, no information to date has examined vasodilator kinetics in the legs of older adults. Given that the upper and lower extremities may express different hyperemic and vasodilator responses to exercise (239, 296), the purpose of this study was to examine 1) whether

advancing age prolongs leg vasodilator kinetics preceding steady-state exercise; 2) whether older adults who are self-reported as habitually aerobic exercise trained demonstrate the same age-related impairments to vasodilator kinetics within the leg; and 3) whether vasodilator kinetics are associated with contraction-induced ROV within the leg. We hypothesized that compared to young adults, advancing age would prolong vasodilator kinetics within the leg, and this effect would be offset by habitual exercise training in older adults. Additionally, we hypothesized that contraction-induced ROV would be inversely associated with the vasodilator kinetic profile in that greater ROV responses would be associated with accelerated vasodilator kinetics.

Methods

A total of 37 subjects were recruited (young adults, older untrained and older trained adults). All subjects completed a general health history screening and written informed consent, and were generally healthy, free of any diagnosed cardiovascular or metabolic complications, non-obese (body mass index: ≤ 30 kg·m^2), nonsmokers, and not taking any vasoactive medications. Peak aerobic capacity/training status was quantified in all subjects using a maximal graded exercise test. Studies were conducted after an overnight fast, with subjects refraining from exercise, alcohol, and caffeine for 24 hours before reporting to the laboratory for vascular testing. Young female subjects were studied during the early follicular phase of their menstrual cycle, or the placebo phase of oral contraceptives to control for the potential influence of sex hormones on primary outcome variables (159). All older female subjects were post-menopausal and not taking any form of hormone replacement therapy. All study protocols were approved by the Institutional Review Board at the University of Iowa

Experimental Protocol

All subjects completed three separate study days separated by at least 48 hours. Study Day 1 consisted of work-rate maximum testing (WR_{max}) and familiarization with study protocol. Study Day 2 consisted of an incremental treadmill test until exhaustion with gas analysis. Study Day 3 was the experimental testing session and consisted of a resting brachial blood pressure (BP) taken with the subjects in a supine position following 20 minutes of quiet rest. Subjects were then placed in a semi-recumbent position on the custom-made leg-ergometer and equipped with electrocardiogram electrodes and a blood pressure finger cuff. Participants then completed dynamic single contractions, followed by three minutes of rhythmic knee-extension exercise at two randomized contraction intensities (20% and 40% WR_{max}) with simultaneous measurement of femoral artery diameter and mean blood velocity for calculation of BF and vascular conductance (VC). Single contractions always preceded rhythmic exercise, and all exercise intensities were randomized prior to Study Day 3. Single contractions were performed in duplicate to calculate the average response for each subject for a given condition.

Pre-Study Day Measurements

Determination of Work Rate Maximum

WR_{max} was determined from a single leg knee-extensor incremental maximal exercise test completed during a familiarization session prior to the experimental testing day (Study Day 3) as previously described (117, 118). Briefly, subjects were seated in a semi-recumbent position on a modified adjustable bucket seat that accommodates variable body and leg lengths, allowing each subject's lower leg to move through a 90-180 degree range of motion during the knee-extension exercise. Resistance was developed by a custom-made computer controlled leg ergometer. Resistance (torque) was developed by an AC motor turning at a constant RPM that is transferred to the leg shaft, against which subjects contracted. The computer monitored the

elapsed time and the angle of the leg controlling the actual torque presented to the leg during contraction. In this way, the subjects were required to develop enough power to extend the leg through a full range of motion. WR_{max} testing started with an initial workload of 5W that incrementally increases every minute by 3W and 5W in female and male subjects, respectively. Subjects kicked dynamically through a full range of motion at a cadence of 40 contractions per minute. The single leg knee extensor incremental maximal was terminated when the subjects could not maintain a full range of motion or a cadence of 40 contractions per minute. The final workload completed was recorded as maximal kicking load from which relative workloads were calculated.

Measurement of Peak Aerobic Capacity

Peak exercise O_2 consumption (VO_{2peak}; $ml \cdot kg^{-1} \cdot min^{-1}$) was determined in all subjects using respiratory gas exchange analysis (Parvo Medics TrueOne 2400, Sandy, UT, USA) during incremental treadmill exercise using a Bruce protocol performed to exhaustion, as previously described (88).

Study Day Measurements

Heart Rate and Systemic Blood Pressure

Heart rate (HR) was recorded via continuous three-lead electrocardiogram, and systemic BP were assessed (beat-to-beat) via finger plethysmography (Nexfin; Edwards Lifesciences, Irvine, CA) on the non-exercising hand. Resting brachial artery pressure was measured in duplicate using an automated cuff (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA) prior to beginning exercise trials while the subjects were in a supine position following 20 minutes of rest.

Single Muscle Contractions

Subjects performed dynamic single leg knee-extension contractions as previously described (117, 118, 120). Briefly, contractions were performed at both 20% and 40% WR_{max} which were randomized within each subject. Subjects were instructed to contract and relax on a verbal command from laboratory personnel. Each contraction was visually observed by the laboratory personnel to ensure proper timing of contraction. Two minutes of relaxation was given between each contraction to allow continuous measures of limb hemodynamics post-contraction and allow for limb hemodynamics to return to baseline. It should be noted that some of the single contraction data has been previously reported elsewhere (118, 120) and was included for comparative purposes to address our specific hypotheses.

Steady-State Exercise

Rhythmic single leg knee-extensions were performed at 20% and 40% WR_{max} for three minutes. Subjects contracted to the sound of a metronome (40 contractions per minute) to ensure correct timing. The order of exercise intensity within each subject was randomized prior to each BF study day.

Measurement of Blood Flow

Common femoral (~2-3 cm proximal to bifurcation) artery diameter and blood velocity was determined with a 12-MHz linear-array Doppler probe (model M12L; Vivid 7, General Electric, Milwaukee, WI) with a probe insonation angle previously calibrated to 60°. Measured velocity waveforms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (113). Artery diameter measurements were obtained at end diastole at rest (before contraction) and immediately upon cessation of

rhythmic contractions. Leg BF was calculated as the product of mean blood velocity ($cm \cdot s^{-1}$) and artery cross-sectional area (cm^2) and expressed as milliliters per minute ($ml \cdot min^{-1}$).

Data Analysis and Statistics

Data was collected at 250 Hz and analyzed offline with signal processing software (WinDaq; DATAQ Instruments). Beat-to-beat mean arterial pressure (MAP) was derived from the Nexfin pressure waveform and was recorded simultaneously with beat-to-beat blood velocity measurements. Heart rate was determined from the electrocardiogram. Baseline BF and MAP values represents an average of the last 30 seconds of the resting time period prior to each muscle contraction (single and rhythmic). VC was calculated as the quotient of BF and MAP (expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$). Rapid hyperemic and vasodilator responses were expressed as the change in (Δ) BF and VC from baseline, respectively. Of particular interest are the peak and total vasodilator responses post-contraction. Total BF (ml) and VC ($ml \cdot mmHg^{-1}$) are defined as the area under the curve over 30 post-contraction cardiac cycles after respective baseline values are subtracted for a given flow or conductance curve.

BF and VC kinetic responses were modeled using a systems control analysis where responses were interpolated according to the duty cycle (40 contractions per minute) yielding 120 data points for the entire 3 minute exercise bout. The on-transient kinetics of exercise hyperemia and vasodilation during exercise was analyzed using a non-linear least-square curve-fitting procedure containing one, or two component exponential model (analogous to phases of blood flow) based on individual responses and defined as (107, 267, 269):

Equation 2: Exponential Model for Blood Flow and Vasodilator Kinetics

$$VC \text{ or } BF = BF/VC_{baseline} + Amp\left[1 - e^{\frac{t-TD_1}{\tau_1}}\right] \cdot Y_n + Amp_n\left[1 - e^{\frac{t-TD_n}{\tau_n}}\right]$$

Where *BF/VC* is the dependent variable at any time point, and $BF/VC_{baseline}$ is the resting value prior to the onset of muscle contractions. *Amp* represents the amplitude of the dependent variable response (BF, VC), *TD* represents the time delay of response, and τ represent the number of duty cycles required to achieve 63% of the phase amplitude for the dependent variable, defined as:

Equation 3: Mean Response Time for Comparison between Multiple Components

$$MRT = \frac{Amp_1}{Amp_1 + Amp_n}(\tau_1 + TD_1) + \frac{Amp_n}{Amp_1 + Amp_n}(\tau_n + TD_n)$$

Where MRT represent the time to reach 63% of total steady-state amplitude, and *n* represents the number of model components (e.g. 1 phase vs. 2 phase model). Time to reach 63% of steady-state amplitude provides insight into how quickly blood flow and vasodilator responses respond to constant-load dynamic exercise. Individual responses were inspected and fit with the appropriate component model. MRT allows for comparisons for subjects that express different component models (e.g. 1 component vs. 2 compartments) (107, 267, 269). Goodness of fit for each model was rationalized inspecting individual and group residual data plots.

All values are expressed as mean ± SE. Analysis of variance (ANOVA) was used to analyze demographic variables between groups. To determine whether vasodilator kinetics are prolonged with age in the leg, and examine the effect of habitual aerobic exercise training in older adults, independent, one-way ANOVAs were used to compare groups across exercise intensities. When significance was detected, Tukey's *post hoc* analysis was used to identify differences between groups. Pearson product moment correlation coefficients were used to assess the relationship between contraction-induced ROV and vasodilator kinetics preceding steady-state exercise. All statistical analyses and non-linear curve fitting were completed using

SigmaPlot software version 11.0 (Systat Software Inc., San Jose, CA). Statistical difference was set a priori at $P < 0.05$.

Results

Subject characteristics are shown in Table 11. Older untrained adults demonstrated lower WR_{max} and VO_{2peak} relative to young and older trained adults ($P < 0.05$), while there were no differences between older trained and young adults ($P = 0.22$-0.81). Young adults were taller than older untrained and older trained adults ($P < 0.05$) with no differences between older untrained and older trained ($P = 0.86$). Young, older untrained, and older trained adults were of similar, weight and body mass index ($P = 0.35$-0.99) and resting brachial blood pressures were similar between groups ($P = 0.26$-0.36).

Rapid Hyperemic and Vasodilator Responses to Single Skeletal Muscle Contractions

The rapid hyperemic and vasodilator responses to single muscle contractions in the leg have been reported previously by our group (118, 120). Additional subjects were collected and added to existing data to investigate the relationship between ROV and kinetic responses, and did not alter the previously reported group differences in ROV. In brief, older untrained adults exhibited attenuated peak VC responses to single leg knee-extension contractions at 20% and 40% WR_{max} relative to both young and older trained adults ($P < 0.05$). There were no differences in ROV between young and older trained adults ($P = 0.41$-0.99).

Blood Flow and Vasodilator Responses to Rhythmic Leg Contractions

Baseline (resting) and exercise hemodynamics prior to each exercise bout are shown in Table 2. Systemic MAP assessed via finger plethysmography was higher in trained older adults prior to contractions at 20% WR_{max}, while BF and VC were not different between groups prior to

either 20% (P = 0.19-0.30) or 40% WR_{max} rhythmic contractions (P = 0.06-0.08). Resting HR was lower in older trained adults relative to young adults only (P < 0.05).

In response to rhythmic knee-extension exercise BF and VC significantly increased in all groups across intensities (P < 0.05). At 40% WR_{max} there were age/training differences observed for steady-state VC (one-way ANOVA P < 0.05) with older untrained adults exhibiting attenuated responses relative to both young and older trained adults (P < 0.05 for both). Steady-state BF responses was lower in older untrained relative to older trained only (P < 0.05), while there were no differences between older trained and young (P = 0.06) or older untrained and young (P = 0.18).

Figure 19 illustrates the overall mean fit kinetic responses for leg BF and VC in all groups at 20% and 40% WR_{max}, while Figure 20 illustrates MRT values across age groups and exercise intensities for BF (A) and VC (B). At 20% WR_{max}, there were no differences in the MRT between young, older untrained and older trained adults for BF or VC (P = 0.84 and 0.98 respectively). Conversely, at 40% WR_{max} older untrained adults demonstrated a prolonged MRT for VC relative to both young adults and older trained adults (P < 0.05). There were no differences between groups for BF MRT at 40% WR_{max} (P = 0.43).

Relationship between ROV and Vasodilator Kinetics

There were no associations between peak ROV responses and VC MRT at 20% WR_{max} (r = -0.08; P = 0.67) or 40% WR_{max} (r = - 0.22; P = 0.20) in the group as a whole (young, older untrained, older trained adults). Additionally when examined as individual groups there were no significant associations for peak ROV and MRT at 20% WR_{max} (r = -0.21-0.48; P = 0.17-0.52) or 40% WR_{max} (r = -0.21 –0.23; P = 0.52-0.90). Total VC was not associated with VC MRT in the

group as a whole (r = -0.18 – -0.02; P = 0.31 – 0.90) or when examined within each group (r = -0.20 – 0.62; P = 0.06 – 0.89) at 20% or 40% $WR_{max.}$

Discussion

This is the first study to examine the influence of advancing age and habitual aerobic exercise training on the hyperemic and vasodilator responses across an entire exercise transient (onset, kinetics, and steady-state) with specific focus on the vasodilator kinetic response in a group of young, older untrained and older trained adults. The novel findings of this study demonstrate that: 1) in addition to reductions in vasodilation at the onset and during steady-state exercise, advancing age prolongs the kinetics of vasodilation within the leg preceding steady state exercise at higher workloads; 2) habitual aerobic exercise training in older adults offsets this age-related impairment in leg vasodilator kinetics at higher workloads; and 3) peak contraction-induced ROV is not associated with vasodilator kinetics in a group of young, older untrained and older trained adults. Taken together, these findings suggest that chronic/habitual aerobic exercise training exerts beneficial effects across an exercise transient (onset to steady-state). Furthermore, it does not appear as though peak vasodilator responses following a single skeletal muscle contraction within the leg are associated with accelerated vasodilator kinetics.

Aging and Vasodilator Kinetics

Previous work from both animal and human models demonstrate that aging impairs hyperemic and vasodilator responses both at the onset of exercise (14, 40, 41, 43, 120, 123), as well as during steady-state exercise (15, 110). However, these findings fail to demonstrate whether the rate of adaptation during exercise is altered with age. Evidence from NIRS-derived skeletal muscle deoxygenation suggests that older adults demonstrate prolonged responses relative to young adults during cycling exercise (66, 188). Extending upon this concept, Casey et.

al. (41) demonstrated that within the exercising forearm, older adults exhibit prolonged vasodilator kinetics (greater tau) at relative and absolute exercise intensities compared to young adults. These age-associated impairments in vasodilator kinetics were attributed in part, to NO bioavailability and/or signaling as evidenced by slowing of tau in young, but not older adults during inhibition of NO synthase. Similarly, Park et. al. (215) showed that in isolated human skeletal muscle feed arteries, older adults exhibited prolonged vasodilator responses to simulated flow, as well as in response to acetylcholine, suggesting that the age-related decline in endothelial function mediates some of the slowing of vasodilation in response to exercise. When taken in context with available animal data (16) this suggests that aging results in a mismatch between O_2 delivery and consumption within exercising aged skeletal muscle, an effect that is related to endothelium-dependent vasodilator function. Within the current study, older untrained adults exhibited prolonged vasodilator kinetics during the highest exercise intensity (40% WR_{max}) relative to young adults and older trained adults (Figure 20). Additionally, when a subset (n=30) was matched for an absolute workload ranging from 8-12W (9W, ~26%-36% WR_{max}), older untrained adults still demonstrated a prolonged VC MRT compared to young and habitually aerobic exercise older trained adults ($P < 0.05$). These results suggest that advancing age affects the dynamics of vasodilation across the entire exercise transient (onset to steady-state) in response to both relative vs. absolute exercise intensities.

Exercise Training and Vasodilator Kinetics

Habitual exercise training programs or short-term exercise interventions have been shown to exert beneficial vascular benefits in response to both single contractions, as well as rhythmic exercise in older adults (15, 118). Within the present study, habitually aerobic exercise trained older adults exhibited greater contraction-induced ROV responses (118), accelerated vasodilator

kinetics, as well as greater steady-state BF and VC responses relative to older untrained adults at the highest exercise intensity (40% WR_{max}) but no different than young adults (Table 12 and Figure 20). Importantly, chronic exercise trained older adults also exhibited accelerated vasodilator kinetics at the highest exercise intensity (40% WR_{max}) relative to untrained older adults, but no different than young adults (Figure 20). This is in agreement with our hypothesis that habitually aerobic exercise older trained adults would demonstrate accelerated vasodilator kinetics relative to older untrained adults. Previous data from Shoemaker et. al. (269) demonstrated that in previously untrained young healthy males, 10 days of endurance exercise training (cycle ergometry at 65% VO_{2peak}) accelerated femoral artery mean blood velocity and VC kinetics, however no training-induced differences were observed at rest or once steady-state was achieved. Additionally, older adults who undergo short term exercise training interventions (cycling or single leg extension exercise) have exhibited either no change in mean blood velocity (19) or NIRS-derived deoxygenated hemoglobin concentration ([HHB]), yet demonstrated an improved ratio of the change (Δ) in deoxygenated hemoglobin to change in pulmonary oxygen uptake (an estimate for matching of microvascular BF to muscle oxygen utilization) (188). Mechanistically, this improvement in vasodilator kinetics may be attributed to changes in improved O_2 utilization in skeletal muscle (19), as well as improvements in vascular function such as enhanced endothelium-dependent vasodilation (18, 165). Given these results from both human and animal data, it appears that habitual aerobic exercise training in older adults preserves vasodilator responses at the onset of exercise, as well as during steady-state conditions. The novel findings of the present study illustrate that the age-related slowing of leg vasodilator kinetics is offset with habitual aerobic exercise training.

Relationship between Vasodilator Kinetics and Contraction-Induced ROV

Historically, hemodynamic responses to rhythmic exercise have typically been reported after steady-state exercise has been achieved, as this demarcates matching between O_2 demand and O_2 utilization (130). While these studies give insight into steady-state hyperemia and vasodilation during rhythmic exercise, they overlook the kinetic response, or rate of adaptation, during the transition to steady-state exercise at a constant workload. In this context, humans rarely operate at constant steady-state but rather undergo transitions between exercise intensities (e.g. rest to exercise and/or stepwise transition from a lower intensity to a high intensity). Examining the kinetic response to constant-load exercise provides some insight into the capacity of the vasculature to respond or adapt to a metabolic pertubation. In doing so, the gap between the local regulation of BF and vasodilation at the onset of exercise and steady-state is diminished. Within the current study, no relationship was observed between peak contraction-induced ROV and VC MRT in the group as a whole (young, older untrained, older trained), or when examined as individual groups at either exercise intensity.

Experimental Considerations

There are a few experimental considerations that warrant discussion. First, the current data only provide a cross-sectional representation on the effects of habitual exercise training with advancing age, and we did not make comparisons to an exercise-trained young adult population. Previous evidence from our lab, as well as others has shown that exercise training elicits beneficial vascular effects both at the onset of exercise (118) as well as during rhythmic exercise (15). Second, there are a number of methods that have been utilized to model BF and vasodilator kinetics in response to simulated flow and rhythmic exercise. As such, the extent and application of these respective findings can only be taken in the context of which they were examined. In the current study, BF and vasodilator responses were examined in the common femoral artery while

the subject kicked at a cadence of 40 times per minute. Previous investigations into the BF and vasodilator kinetic responses to exercise have used a variety of cadences (20-60 kicks per minute), examined responses bi-laterally (162, 267), or have utilized near-infrared spectroscopy to interrogate microvascular oxygen/de-oxygenated dynamics (32, 144, 146). In this regard, the present study provides data on the kinetics of vasodilation in a large conduit artery that feeds into a larger skeletal muscle (quadriceps). There is evidence to suggest that vasodilator kinetics within capillaries differs from that of conduit arteries (107). Furthermore, Koga, et. al. demonstrated that during upright cycling, skeletal muscle deoxygenation and microvascular hemodynamics differ between specific muscles within the quadriceps (144). It is important to note, the purpose of this study was not to examine microvascular oxygen dynamics, but rather interrogate how advancing age alters bulk BF to contracting skeletal muscle across an exercise transient.

Conclusion

To our knowledge, this is the first study to quantify and characterize the relationship between the dynamics of blood flow and vasodilation across the entire exercise transient (onset, kinetics, and steady-state) in the same subjects, and whether aging and habitual aerobic exercise training alters these responses. Our current data, along with previous data (41) collectively suggest that the vasodilator kinetics (i.e. VC MRT) are attenuated in older untrained adults relative to young adults. Furthermore, this age-related prolongation in vasodilator kinetics is offset by chronic aerobic exercise training in older adults. Finally, no relationship between peak contraction-induced vasodilation and vasodilator kinetics was observed. Taken together, these results demonstrate the age-related reductions in the hyperemic and vasodilator responses to exercise are offset with chronic exercise training.

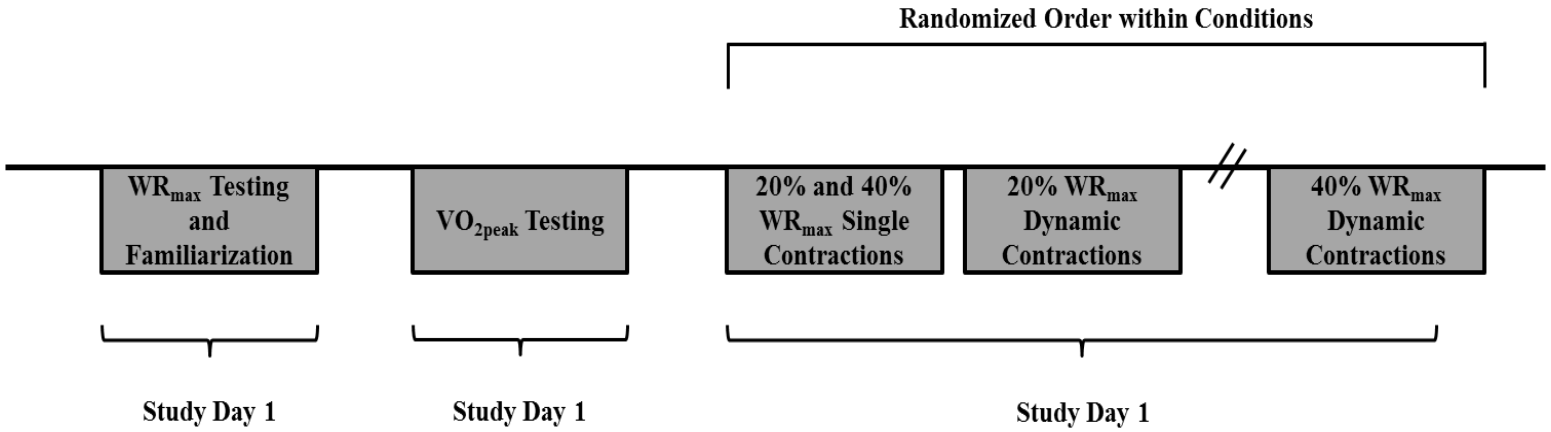

Figure 18: Expected experimental timeline. WR_{max}, work rate maximum; VO_{2peak}, peak oxygen consumption. Diagonal hash marks indicate a period of 20 minutes of quiet rest

Table 11: Subject Characteristics for Aim 5

Variable	**Young** (n=10)	**Older Untrained** (n=13)	**Older Trained** (n=14)
Age (yr)	24 ± 1*†	66 ± 1	63 ± 2
Men/Women	7/3	8/5	8/6
Height (cm)	179 ± 3	171 ± 2	171 ± 2
Weight (kg)	79 ± 4	72 ± 3	72 ± 3
Body Mass Index ($kg \cdot m^2$)	24.8 ± 0.7	24.6 ± 0.9	24.5 ± 0.8
VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	49.7 ± 3.1*	32.4 ± 1.9	40.4 ± 2.5*
WR_{max} (Watts)	39 ± 4*	28 ± 2	39 ± 3*
Systolic Pressure (mmHg)	117 ± 2	123 ± 3	123 ± 4
Diastolic Pressure (mmHg)	71 ± 1	74 ± 2	76 ± 2
Mean Arterial Pressure (mmHg)	87 ± 1	90 ± 2	92 ± 2

Values are means ± SE. WR_{max}, Work-rate Maximum. * $P < 0.05$ vs. untrained older adults; † vs. trained older adults

Table 12: Systemic and Leg Hemodynamics at Rest and during Exercise

		Young		Older Untrained		Older Trained	
		20% WR_{max}	40% WR_{max}	20% WR_{max}	40% WR_{max}	20% WR_{max}	40% WR_{max}
HR ($b \cdot min^{-1}$)	Baseline	66±3	66±3	62±2	63±2	59±2	59±2
	Exercise (Δ from BL)	26±3	32±2	24±2	29±2	21±2	29±3
MAP (mmHg)	Baseline	98±3	99±3	105±3	106±5	111±3*	110±3
	Exercise (Δ from BL)	19±3	25±3	34±4*	37±4*	30±3	40±3*
BF ($ml \cdot min^{-1}$)	Baseline	222±26	236±29	173±30	165±19	239±23	213±20
	Exercise (Δ from BL)	1101±135	1254±147	910±109	946±79†	1310±147	1650±172
VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	Baseline	2.3±0.2	2.4±0.3	1.7±0.3	1.6±0.2	2.2±0.2	2.0±0.2
	Exercise (Δ from BL)	9.1±1.3	10.4±1.3	6.2±0.8	6.3±0.7*†	9.4±1.4	10.9±1.4

Values are mean±SE. BL, baseline; WR_{max}, work-rate maximum; BF, leg blood flow; VC, leg vascular conductance; HR, heart rate. * $P < 0.05$ vs. Young; † $P < 0.05$ vs. older trained

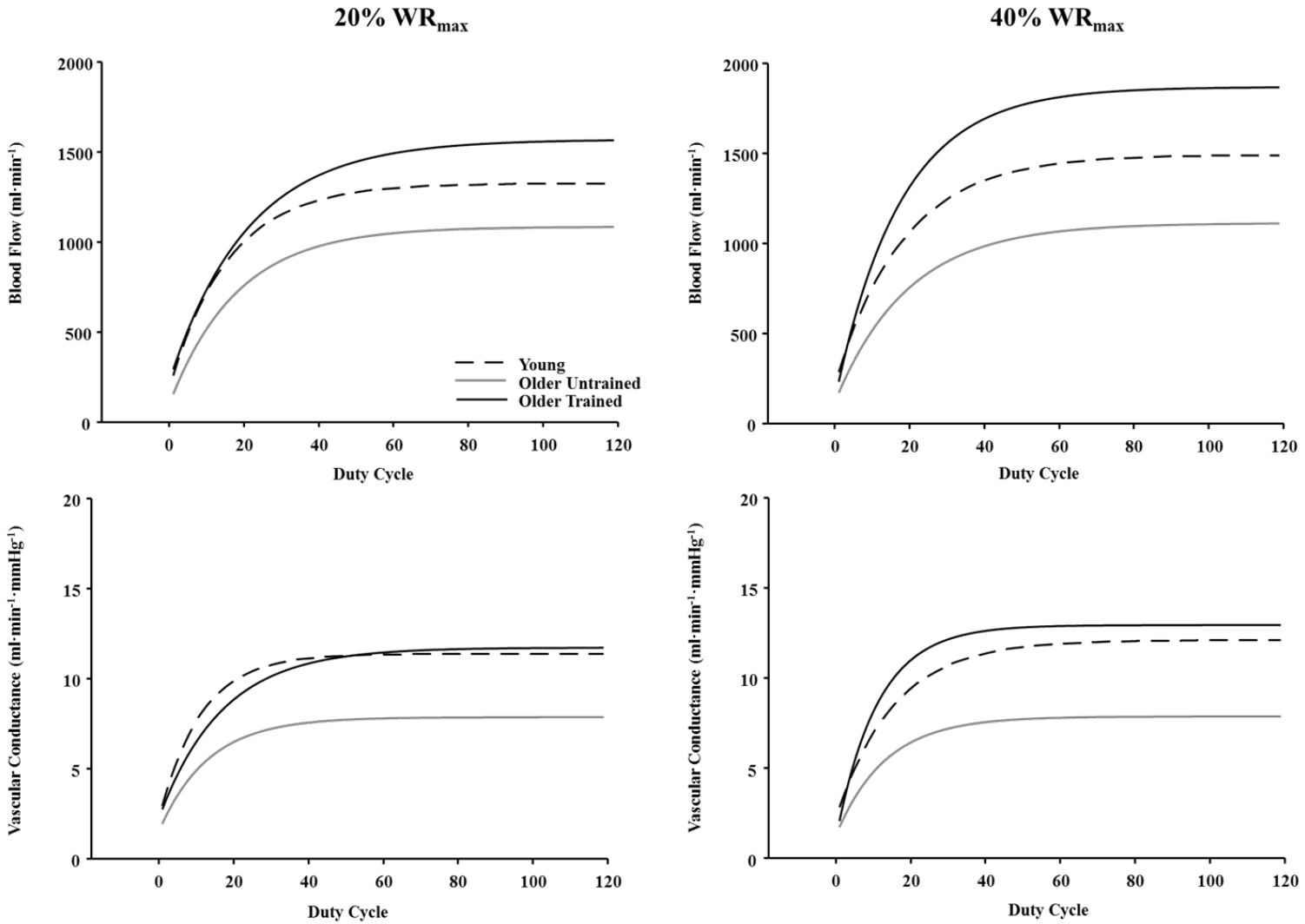

Figure 19: Group mean fit for the on-transient blood flow and vasodilator kinetics at 20% and 40% WR_{max}

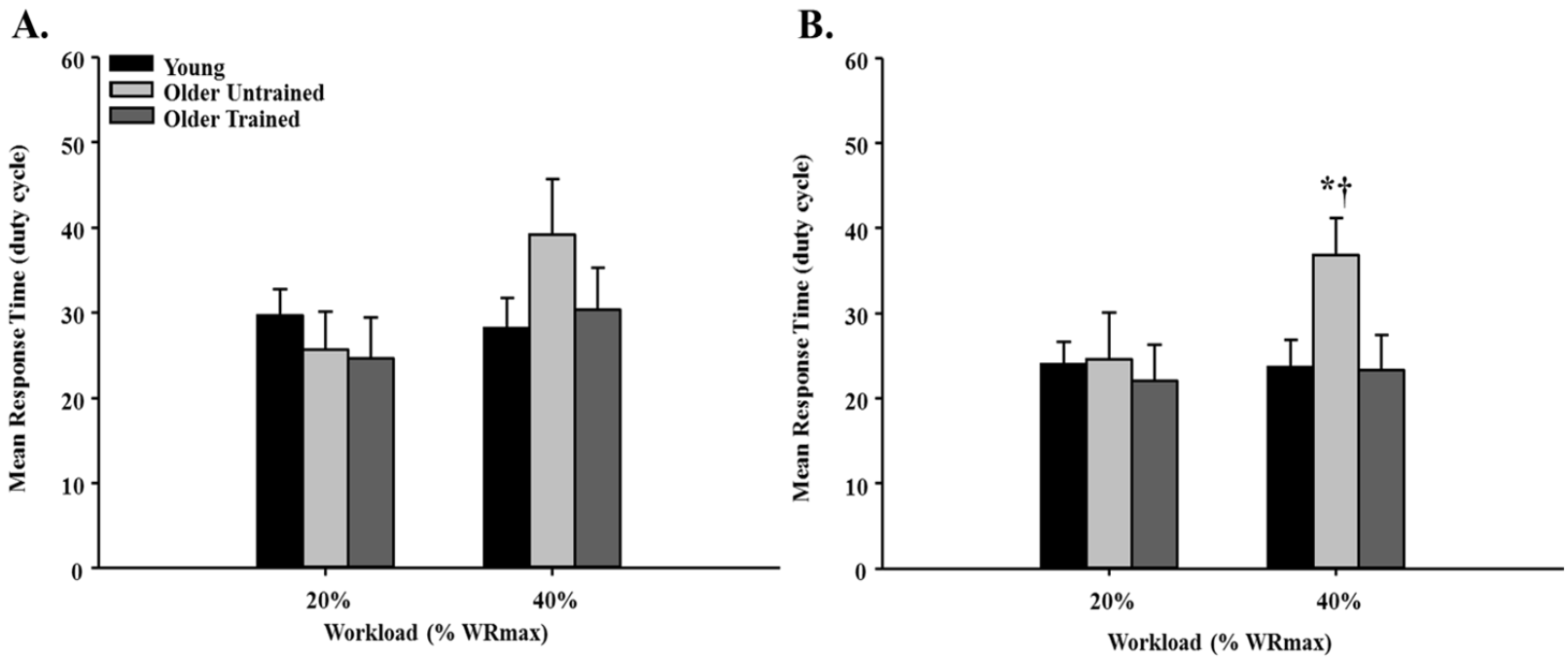

Figure 20: Mean response time for blood flow (A) and vasodilator (B) kinetics in young, older untrained and older trained adults during dynamic knee-extension exercise at 20% and 40% WR_{max}. * $P < 0.05$ vs. young adults, † vs. older trained adults

CHAPTER 7: IMPACT OF ENHANCING NITRIC OXIDE BIOAVAILABILITY ON LEG BLOOD FLOW AND VASODILATOR KINETICS WITH AGE

Introduction

Nitric oxide (NO) plays a key role in modulating the hyperemic and vasodilator responses to exercise (112, 210, 253). NO-mediated vasodilation within the skeletal muscle vasculature declines with age (283), and it is unclear whether this is due to reductions in bioavailable NO or alterations in NO signaling (187, 210, 283). Previous evidence within the forearm demonstrate that local inhibition of endothelial NO synthase (eNOS) reduces the hyperemic and vasodilator responses both at the onset of skeletal muscle contractions, as well as the transition period(kinetics) preceding steady-state exercise (41, 43, 135, 160). Importantly, previous evidence indicates this NO-mediated reduction in O_2 delivery is apparent during the initial phase of exercise (~180 seconds), but compensated with a marked elevation in O_2 extraction (via higher arterio-venous O_2 difference) (48). Taken together, these results highlight the role of NO within the transition from rest to dynamic exercise, however, the impact of age-related reductions NO bioavailability on exercise hyperemia and vasodilation across exercise transient remains inadequately understood.

Over the past decade, inorganic, or dietary nitrate has been increasingly recognized as a novel mechanism to enhance NO bioavailability by augmenting the nitrate–nitrite–NO pathway independent of endogenously produced NO (50, 126, 161). Subsequently, this increase in NO bioavailability offers new avenues for the improvement of a variety of age-related health outcomes (i.e. blood pressure and exercise capacity) (65, 126, 156, 161). In particular, evidence from animal models demonstrate that dietary nitrate increases the O_2 driving pressure at the capillary-myocyte interface during rest to exercise transitions, a critical period of time for

matching of O_2 delivery to O_2 demand (85). However, the available data on enhancing NO bioavailability and blood flow in humans is conflicting (138, 238), and the role of NO in modulating blood flow and vasodilator responses across an entire exercise transient (onset to steady-state) remains unclear. Early efforts involved interventions to reduce oxidative stress, thereby theoretically enhancing NO bioavailability; however the effects of these approaches may not be solely due to enhancement of NO bioavailability. For example, infusion of the antioxidant N-acetylcysteine abolishes age-related differences in vascular conductance at rest, but does not augment exercise hyperemia in older sedentary subjects within the leg, (204). Additionally, Kirby et. al. (141) demonstrated that infusion of ascorbic acid does not augment contraction-induced rapid onset vasodilation, but does enhance steady-state blood flow in the forearm of older adults, such that there were no differences relative to young adults. In an effort to potentiate NO signaling, Limberg et. al. (160) demonstrated that in the forearm of young healthy adults, phosphodiesterase-5 (PDE-5) inhibition had no effect on blood flow and vasodilation in response to handgrip exercise. Conversely, Nyberg et. al. (210) showed that PDE-5 inhibition augmented O_2 delivery in the leg of older, but not young adults indicating that some of the age-related reductions in leg blood flow during exercise are due to altered NO signaling. Therefore, the purpose of this study was to examine whether potentiating NO bioavailability independent of enzymatic synthesis of NO via acute dietary nitrate supplementation in older adults improves blood flow and vasodilation in the leg across the exercise transient (i.e. onset, kinetics, and steady-state).

Methods

A total of 10 sedentary older subjects participated in a randomized, double-blind crossover study. All subjects completed a general health history screening and written informed

consent, and in general were healthy, free of any diagnosed cardiovascular or metabolic complications, nonobese (body mass index: ≤ 30 kg·m^2), and nonsmokers. Studies were conducted in the morning after an overnight fast, with subjects refraining from exercise, alcohol, and caffeine for 24 hours before reporting to the laboratory. Older female subjects were post-menopausal (> 1 year after last menstrual cycle) and not taking any form of hormone replacement therapy. This study was approved by the Institutional Review Board at the University of Iowa. This study is a registered clinical trial (ClinicalTrials.gov Identifier NCT03459027).

Experimental Protocol

All subjects completed 3 study days (Figure 21). Study Day 1 consisted of work-rate maximum testing (WR_{max}), familiarization with study protocol, and two clinical functional measurements (timed up and go, 30-second sit to stand). Study Day 2 and Study Day 3 were the experimental testing sessions (nitrate or placebo), and consisted of subjects placed in a resting supine position for 20 minutes, followed by resting measurements of brachial blood pressure. A venous catheter for venous blood samples was placed in the antecubital vein under aseptic conditions. Participants were then placed in a semi-recumbent position on a custom made leg-ergometer and completed single and rhythmic leg extension exercise with their right leg at two different contraction intensities (20% and 40% WR_{max}). Measurements of femoral artery diameter and mean blood velocity were recorded for calculation of blood flow and vascular conductance At least 5 days were allotted between Study Day 2 and Study Day 3, which served as a “washout” period. All exercise intensities and supplementation order were randomized prior to the experimental protocol.

Pre-Study Day Measurements:

Determination of Work Rate Maximum

WR_{max} was determined from a single leg knee-extensor incremental maximal exercise test completed during a familiarization session prior to the experimental testing day (Study Day 3) as previously described (120) . Briefly, subjects were seated in a semi-recumbent position on a modified adjustable bucket seat that accommodates variable body and leg lengths, allowing each subject's lower leg to move through a 90-180 degree range of motion during single leg knee-extension exercise. Resistance was developed by a custom-made computer controlled leg ergometer. Resistance (torque) was developed by an AC motor turning at a constant RPM that is transferred to the leg shaft against which subjects contracted. The computer monitored the elapsed time and the angle of the leg controlling the actual torque presented to the leg during contraction. In this way, the subjects were required to develop enough power to extend the leg through a full range of motion. WR_{max} testing started with an initial workload of 5W that incrementally increases every minute by 3W and 5W in female and male subjects, respectively. Subjects kicked dynamically through a full range of motion at a cadence of 40 kicks per minute. The single leg knee extensor incremental maximal was terminated when the subjects could not maintain a full range of motion or a cadence of 40 contractions per minute. The final workload completed was recorded as WR_{max} from which relative workloads were calculated.

Additionally, two standardized clinical functional measurements were performed by subjects: 1) a timed "up and go" test, designed to examine mobility (222), and 2) a 30 second sit-to-stand test as a measure of lower body strength (128). These clinical function tests were used to characterize the physical health of older adults.

Study Day Measurements

Heart Rate and Systemic Blood Pressure

Heart rate (HR) was recorded via continuous three-lead electrocardiogram, and systemic BP was assessed (beat-to-beat) via finger plethysmography (Nexfin; Edwards Lifesciences, Irvine, CA) on the non-exercising hand. Resting brachial artery pressure was measured in duplicate using an automated cuff (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA) prior to beginning exercise trials while the subjects were in a supine position following 20 minutes of rest.

Venous Blood Sampling

Venous blood samples were collected from the antecubital vein for determination of NO metabolites, $[NO_3^-]$ and $[NO_2^-]$. Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and immediately centrifuged at 3,000 rpm for 15 minutes. Plasma samples were then aliquoted into Eppendorf tubes at frozen at -80C for future analysis. Measurements of plasma $[NO_3^-]$ and $[NO_2^-]$ were performed within 30 minutes of thawing using a Sievers chemiluminescence NO analyzer (NOA 280i; Sievers Instruments, Boulder, CO). Plasma samples were de-proteinated using an ice cold ethanol precipitation. Briefly, plasma was added in a 1:2 ratio to ice-cold (0˚C) ethanol and placed on ice for 30 minutes. Samples were then centrifuged at 14,000 rpm for 5 minutes. The supernatant was used for determination of $[NO_3^-]$ and $[NO_2^-]$ (13, 58). Plasma $[NO_3^-]$ and $[NO_2^-]$ were determined by their addition to vanadium III chloride in hydrochloric acid at 90C and to potassium iodide in acetic acid at room temperature, respectively.

Single Muscle Contractions

Subjects performed dynamic single leg knee-extensions as previously described (120). Briefly, contractions were performed at both 20% and 40% WR_{max} which were randomized

within each subject. Subjects were instructed to contract and relax on a verbal command from laboratory personnel. Each contraction was visually observed by the laboratory personnel to ensure proper timing of contraction. Two minutes of relaxation was given between each contraction to allow continuous measures of limb hemodynamics post-contraction and allow for limb hemodynamics to return to baseline.

Steady-State Exercise

Rhythmic single leg knee-extensions were performed at 20% and 40% WR_{max} (randomized) for three minutes. Subjects contracted to the sound of a metronome (40 contractions per minute). Contraction and relaxation cycles were visualized by lab personnel to ensure correct timing.

Measurement of Blood Flow

Common femoral (~2-3 cm proximal to bifurcation) artery diameter and blood velocity was determined with a 12-MHz linear-array Doppler probe (model M12L; Vivid 7, General Electric, Milwaukee, WI) with a probe insonation angle previously calibrated to 60°. Measured velocity waveforms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (113). Artery diameter measurements were obtained at end diastole at rest (before contraction) and immediately upon cessation of rhythmic contractions. Leg blood flow was calculated as the product of mean blood velocity ($cm \cdot s^{-1}$) and artery cross-sectional area (cm^2) and expressed as milliliters per minute ($ml \cdot min^{-1}$).

Nitrate Supplementation

Subjects consumed a beverage containing either active beetroot powder (Human[n]; 250 mg NO_3^- and 20 mg NO_2^-) or placebo (non-active beetroot powder with same color, taste and

consistency). Blood plasma sampling and exercise trials were repeated 120 minutes following consumption of either beverage. Analysis of plasma [NO_3^-], [NO_2^-] are similar to previous work from our lab (25) and others utilizing dietary nitrate supplementation (50, 126), and demonstrate that plasma [NO_2^-] is elevated above baseline at 120 minutes following acute consumption (119).

Data Analysis and Statistics

Data was collected at 250 Hz and analyzed offline with signal processing software (WinDaq; DATAQ Instruments). Beat-to-beat mean arterial pressure (MAP) was derived from the Nexfin pressure waveform and was recorded simultaneously with beat-to-beat blood velocity measurements. Heart rate was determined from the electrocardiogram. Baseline BF and MAP represented an average of the last 30 seconds of the resting time period prior to each muscle contraction (single and rhythmic) and are used to quantify the hyperemic response. Vascular conductance (VC) was calculated as the quotient of BF and MAP (and expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$). Rapid hyperemic and vasodilator responses were expressed as the change in (Δ) BF and VC from baseline, respectively. Of particular interest are the peak and total vasodilator responses post-contraction. Total BF (ml) and VC ($ml \cdot mmHg^{-1}$) were defined as the area under the curve over 30 post contraction cardiac cycles after respective baseline values are subtracted for a given flow or conductance curve.

Kinetic Analysis

BF and VC kinetic responses were modeled using a systems control analysis where responses were interpolated according to the duty cycle (40 contractions per minute) yielding 120 data points for the entire 3 minute exercise bout. The on-transient kinetics of exercise hyperemia and vasodilation during exercise was analyzed using a non-linear least-square curve-

fitting procedure containing one, or two component exponential model (analogous to phases of blood flow) based on individual responses and defined as (107, 267, 269):

Equation 4: Exponential Model for Blood Flow and Vasodilator Kinetics

$$BF\ or\ VC = BF/VC_{baseline} + Amp\left[1 - e^{\frac{t-TD_1}{\tau_1}}\right] \cdot Y_n + Amp_n\left[1 - e^{\frac{t-TD_n}{\tau_n}}\right]$$

Where *BF/VC* is the dependent variable at any time point, and $BF/VC_{baseline}$ is the resting value prior to the onset of muscle contractions. *Amp* represents the amplitude of the dependent variable response (BF, VC), *TD* represents the time delay of response, and τ represents the number of duty cycles required to achieve 63% of the steady-state amplitude for the dependent variable, where:

Equation 5: Mean Response Time for Comparison between Multiple Compartments

$$MRT = \frac{Amp_1}{Amp_1 + Amp_n}(\tau_1 + TD_1) + \frac{Amp_n}{Amp_1 + Amp_n}(\tau_n + TD_n)$$

Where MRT represent the time to reach 63% of total steady-state amplitude, and *n* represents the number of model components (e.g. 1 phase vs. 2 phase model). Time to reach 63% of steady-state amplitude provides insight into how quickly blood flow and vasodilator responses respond to constant-load dynamic exercise. Individual responses were inspected and fit with the appropriate component model. MRT allows for comparisons for subjects that express different component models (e.g. 1 component vs. 2 compartments)

All values were expressed as mean ± SE. The primary question was whether supplementation with dietary nitrate improves exercise hyperemia and vasodilation across an exercise transient above that of a placebo in older adults. In order to address this aim, main comparisons between the active and placebo group over time were made using a two-way ANOVA. Relationships between functional capacity (sit-to-stand and timed up-and-go) and WR_{max} were analyzed via Pearson product-moment correlation. All curve-fitting and statistical

analyses were completed using SigmaPlot software version 11.0 (Systat Software Inc., San Jose, CA). Statistical difference was set *a priori* at $P < 0.05$.

Results

Subject characteristics are presented in Table 13. All ten subjects completed the randomized crossover study consuming both the dietary nitrate and placebo beverages. Resting BP measurements during the dietary nitrate and placebo days are listed in Table 14. There were no group x time interactions for measures of BP in response to either dietary nitrate or placebo supplementation. The average count of completed sit-to-stand cycles was 19±1 (over 30 seconds), and subjects completed the timed-up and go over the course of 4.9±0.2s. There were no associations between WR_{max} and clinical measures of functional capacity (timed up-and-go $r = -0.30$, $P = 0.41$; sit-to-stand $r = 0.23$, $P = 0.53$).

Plasma Measures of $[NO_3^-]$ and $[NO_2^-]$

Acute ingestion of dietary nitrate rich beetroot juice increased plasma $[NO_3^-]$ 221% (48.3±5.0 μM to 143.2±7.3 μM; $P < 0.05$) and $[NO_2^-]$ 22% (783.6±24.2 to 950.1±32.8 nM; $P < 0.05$). In response to placebo supplementation plasma $[NO_3^-]$ increased 30% (39.7±4.9 μM to 82.8±4.7 μM; $P < 0.05$) while $[NO_2^-]$ remained unchanged after placebo (792.1±31 to 811.1±34.7 nM, $P = 0.45$). The increase in plasma $[NO_3^-]$ and $[NO_2^-]$ in response to acute dietary nitrate supplementation were greater than that of placebo ($P < 0.05$).

Contraction-Induced Rapid Onset Vasodilation

Figure 22 illustrates peak (A & B) and total (C & D) VC responses pre- and post-nitrate/placebo supplementation at 20% (A & C) and 40% WR_{max} (B & D). Acute supplementation with dietary nitrate or placebo had no effect on peak or total vasodilator responses following a single skeletal muscle contraction in older adults (interaction P = 0.37-

0.81). Similarly, acute dietary nitrate supplementation had no effect on the rapid hyperemic responses (pre vs. post) compared to placebo supplementation at 20% for peak (617±79 to 564±100 ml·min^{-1} vs. 655±79 to 636±69 ml·min^{-1}; interaction P = 0.74) and total (121±20 to 111±25 ml vs. 127±24 to 108±16 ml; interaction P = 0.70), or 40% peak (719±89 to 654±108 ml·min^{-1} vs. 740±69 to 636±69 ml·min^{-1}; interaction P = 0.78) and total (138±21 to 136±29 ml vs. 139±23 to 132±20 ml; interaction P = 0.81).

Vasodilator Kinetics

Figure 23 depicts the mean kinetic responses in the dietary nitrate and placebo groups at 20% (A) and 40% WR_{max} (B), while Figure 24 illustrates the group means for VC MRT pre- and post- supplementation at 20% (A) and 40% WR_{max} (B). There were no differences in MRT between dietary nitrate and placebo groups pre vs. post supplementation for MRT in BF (interaction P = 0.46 and 0.93 for 20% and 40% WR_{max}, respectively) or VC (interaction P = 0.89 and 0.59 for 20% and 40% WR_{max}, respectively). A main effect of time was observed for BF and VC at 40% WR_{max} ($P < 0.05$).

Blood Flow and Vasodilator Responses to Rhythmic Leg Contractions

Systemic and leg hemodynamics for all subjects in response to dietary nitrate and placebo conditions at rest and during steady-state exercise are presented in Table 15. There was no effect of dietary nitrate or placebo on hyperemic or vasodilator steady-state responses during rhythmic exercise at 20% (interaction P = 0.68 and 0.57, respectively) or 40% WR_{max} (interaction P = 0.48 and 0.70, respectively). Heart rate responses (Δ from BL) during steady-state exercise were not different pre vs. post supplementation in either the dietary nitrate or placebo group (interaction P = 0.93 and 0.23 for 20% and 40% WR_{max}, respectively). Similarly, MAP responses were not

different (pre vs. post supplementation) or between groups (dietary nitrate vs. placebo) at 20% (interaction P = 0.21) or 40% WR_{max} (interaction P = 0.87).

Discussion

The key findings from the present study are: despite increases in plasma [NO_3^-] and [NO_2^-] via acute dietary nitrate, there were no improvements in 1) rapid hyperemic or vasodilator responses at the onset of exercise (contraction-induced ROV); 2) kinetics of vasodilation preceding steady-state exercise; or 3) steady-state hyperemic and vasodilator responses at two different exercise intensities (20% and 40% WR_{max}). Collectively, these finding suggest that acute dietary nitrate supplementation does not improve hyperemic and vasodilator responses in the leg across an exercise transient in older adults

Effects of Acute Dietary Nitrate on Rapid Hyperemic and Vasodilator Responses

Previous evidence has illustrated a role for NO in age-associated reductions in contraction-induced ROV within the forearm (43). Specifically, acute inhibition of eNOS blunts contraction-induced ROV responses in young, but not older adults, in response to forearm handgrip exercise. These results suggest that endogenously produced NO exerts a vasodilator effect at the onset of exercise and this vasodilator effect is reduced with age (43). Over the past decade, several studies have demonstrated a beneficial effect of acute and chronic nitrate supplementation on various cardiovascular and performance parameters at rest as well as during exercise. Such benefits include reductions in blood pressure (50, 65, 119, 133), increased maximal oxygen uptake, time trial performance, critical power, as well as reaction time in both healthy and diseased populations (9, 10, 50, 136, 137). However, the mechanisms that potentially link nitrate supplementation to exercise performance remain poorly understood. Given that older adults exhibit an attenuated contraction induced ROV compared to their

younger counterparts (120), we hypothesized that enhancing NO bioavailability via dietary nitrate supplementation would enhance contraction-induced BF and vasodilation in older adults within the present study. In contrast to our hypothesis, acute supplementation with dietary nitrate did not enhance contraction-induced ROV (peak or total) within the leg of older adults (Figure 22). While these results do not detract from the role of NO as an important vasodilator within the leg, they do add to the body of evidence for the lack of effect that dietary nitrate has on hyperemic and vasodilator responses to exercise, particularly in normoxic conditions (42, 138).

Effects of Acute Dietary Nitrate on Vasodilator Kinetics

In addition to contributing to age-associated reductions in hyperemia and vasodilation at the onset of exercise, lack of NO has been implicated in mediating the age-associated prolongation of vasodilator kinetics preceding steady-state exercise. Casey et. al. (41) demonstrated that during forearm handgrip exercise older adults exhibit a slower blood flow and vasodilator response preceding steady-state exercise relative to young adults, which was attributed to a decreased NO bioavailability and/or signaling. Park, et. al. (215) as well as Behnke and Delp (16) have demonstrated that the kinetics of vasodilation in response to acetylcholine and simulated flow are slower in isolated arterioles from older humans and rats, suggesting an endothelium-dependent mechanism is responsible for the slower vasodilator responses with age. Taken together, these results suggest that advancing age prolongs the kinetics of vasodilation preceding steady-state exercise in an endothelium-dependent manner.

As demonstrated in Chapter 6, the kinetics of vasodilation are slower in the leg of untrained older adults relative to both young, as well as chronically exercise trained older adults. Given that aging is associated with reduced endothelium-dependent vasodilation we hypothesized that elevating plasma [NO_3^-] and [NO_2^-] via dietary nitrate would mitigate age-

associated prolongation of vasodilator kinetics within the leg of older adults. However, our results do not support this hypothesis in that acute supplementation with dietary nitrate had no effect on the kinetics of vasodilation compared to placebo at two relative exercise intensities (20% and 40% WR_{max}) within the leg of older adults (Figure 23 & 24). Kellawan, et. al. (135) demonstrated within the forearm of young adults that eNOS inhibition prolongs MRT of vasodilation to rhythmic handgrip exercise, while PDE-5 inhibition had no effect on vasodilator kinetics. When combined (eNOS + PDE-5 inhibition), there were no differences from control trials, essentially demonstrating a preserved vasodilator kinetic response in the face of reduced NO bioavailability/signaling. Adjacent to these findings, Christensen et. al. (48) reported that at least in young adults eNOS inhibition reduces O_2 delivery during the initial phase of dynamic leg exercise, however responses normalize as exercise continued (~180 seconds). While the current study failed to demonstrate an effect of dietary nitrate on vasodilator kinetics within the leg of older adults, enhancement of NO signaling via the cGMP pathway may provide alternative insight into the role for NO on exercise hyperemia and vasodilation.

Effects of Acute Dietary Nitrate on Blood Flow and Vasodilator Responses to Rhythmic Contractions

To date, the effect of acutely elevating plasma [NO_3^-] and [NO_2^-] via dietary nitrate on steady-state conduit blood flow and vasodilation in humans is conflicting. Kim, et. al. demonstrated in young healthy men that acute supplementation with dietary nitrate (12.9 mmol) did not influence hyperemic responses during low-intensity forearm handgrip exercise, despite elevations in plasma [NO_3^-] and [NO_2^-] and reductions in aortic pulse-wave velocity (138). Conversely, Richards, et. al. (238) reported that acute supplementation with dietary nitrate increases forearm blood flow via local vasodilation, despite elevations in forearm VO_2. The lack

of effect within the current study may potentially be explained by three factors. First, the dosage used may have been too little. In support of this, within the current study, the concentration of nitrate and nitrite were ~4.03 mmol and ~0.29 mmol respectively. This concept is discussed further in depth within experimental considerations. Second, it has previously been established that the effectiveness of the nitrate-nitrite-NO pathway is enhanced during hypoxia. This is supported by data from both our laboratory (42), as well as others (238). Specifically, data from our lab indicate that acute ingestion of dietary nitrate in older adults enhances exercise-induced forearm vasodilator responses during hypoxic exposure (42) and 8 weeks of sodium nitrate supplementation improves forearm blood flow in patients with peripheral artery disease, a population known to have decreased endothelial function (148). Additionally, the study by Richards et. al.(238) also noted enhanced vasodilation during hypoxic exposure with acute dietary nitrate supplementation. Finally, limb differences in vascular responses may explain the lack of effect of dietary nitrate on the hyperemic and vasodilator responses within the current study (239, 296). In this context, this is the first study to examine the effect of dietary nitrate on blood flow and vasodilator responses to exercise within the leg. Taken together, our current data does not support a role for acute dietary nitrate supplementation in enhancing skeletal muscle blood flow and vasodilation during steady-state exercise within a population of otherwise healthy older adults. Along these lines, it may be that some form of overt endothelial dysfunction or exposure to cardiovascular disease risk factors (e.g. hypertension, obesity, etc) is needed in order to elicit benefits from supplementation with dietary nitrate

Experimental Considerations

There are several experimental considerations that warrant addressing. First, it is possible that the dose used within this study was too low (~4.03 mmol NO_3^- and ~0.29 mmol NO_2^-).

Indeed, there was a lack of effect of dietary nitrate on cardiovascular parameters at rest (no change in blood pressure), which is in contrast to previous studies (55, 119, 133, 152). Human and animal studies have previously used higher doses of dietary nitrate both acutely and chronically (26, 133, 238, 276, 308) demonstrating a dose-response effect. In this regard, Kapil et. al.(133) reported step-wise increases (1.3 and 2 fold increase) in plasma nitrite with ingestion of 4 and 12 mmol of potassium nitrate respectively. Furthermore, animal models (young rats) demonstrate that higher doses of dietary nitrate (1 $mmol \cdot kg^{-1} \cdot day^{-1}$) slows the fall in microvascular partial pressure of oxygen at the onset of electrically stimulated contractions (signifying a higher oxygen driving pressure at capillary-myocyte interface), and this effect is both specific to the dose as well as muscle fiber type (84-87). Furthermore, it is possible that the lack of effect on blood flow and vasodilator kinetics within the leg is perhaps due to an increase in O_2 consumption/utilization, rather than an increase in bulk skeletal muscle blood flow. In this context, Richards, et. al. demonstrated that acute dietary nitrate during moderate to high intensity forearm handgrip exercise improves blood flow via local vasodilation, an effect that is not associated with reduced skeletal muscle VO_2. One key discrepancy between this and the current study besides the population studied, is that the current study used lower body leg kicking exercise which utilizes a larger muscle mass relative to forearm handgrip exercise. It is interesting to note however, that previous evidence supports a role for dietary nitrate in enhancing knee-extensor contractile efficiency in young adults (9). It could be argued that no significance was found in any variable due to low effect sizes observed within this study. Given the low effect sizes observed (Cohen's d-ROV: 0.30-0.47; kinetics: 0.25-0.36; steady-state: 0.20-0.34), exploratory analysis indicated that even if the sample population was doubled around the mean, an acute dose of dietary nitrate supplementation still would have no effect on blood flow

and vasodilator responses across an exercise transient in older adults. Finally, we cannot rule out a possible effect of prior exercise/"priming" exercise influencing vasodilator kinetics (main effect of time at 40% WR_{max}). In this regard, previous evidence using pulmonary VO_2 kinetics as well as NIRS-derived muscle deoxygenation kinetics has demonstrated a "priming" effect on VO_2 kinetics that is associated with improved oxygen delivery to contracting muscle in response to repeated bouts of exercise (189). Within the experimental design we attempted to minimize this effect as post-measurements were performed 2 hours after consumption of the nitrate/placebo beverage and exercise intensities were randomized within each subject.

Conclusion

This is the first study to examine the impact of dietary nitrate on blood flow and vasodilator responses across an exercise transient in older adults during lower body exercise. Acute dietary nitrate supplementation with beetroot juice did not improve hyperemic and vasodilator responses at any point across an exercise transient (ROV, kinetics, or steady-state) in older adults. These results suggest that dietary nitrate may not be effective in modulating the hyperemic and vasodilator responses during lower body exercise in older adults, whether it is at the very onset, preceding steady-state exercise, or once steady-state exercise has been achieved. Whether dietary nitrate consumption improves these responses in populations with risk factors for cardiovascular disease, or with known cardiovascular disease warrants further study.

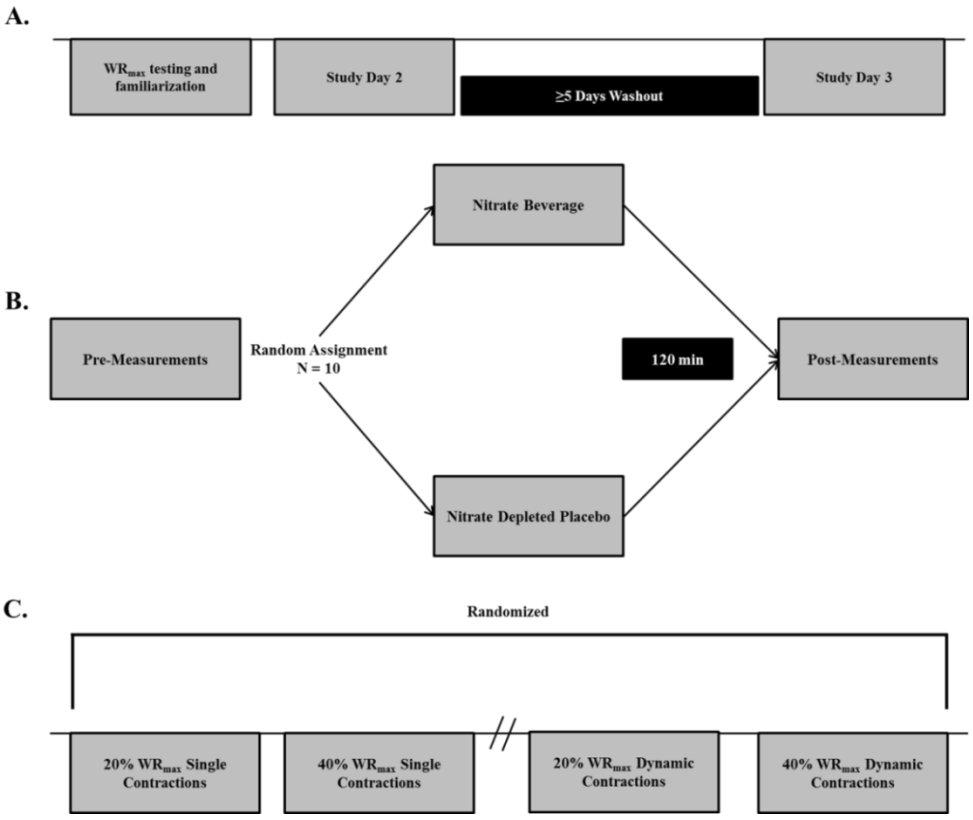

Figure 21: A. Experimental timeline overview. **B**: Pre and post measurements within each study day. **C**: Within study day time line

Table 13: Subject Characteristics for Aim 6

Variable	
Age (years)	68±1
Men/Women	7/3
Height (cm)	171±3
Weight (kg)	76±4
Body Mass Index ($kg \cdot m^2$)	25.8±1
WR_{max} (Watts)	28±2

Values are means ± SE. WR_{max}, Work Rate Maximum

Table 14: Resting Hemodynamics

Variable	Nitrate		Placebo		ANOVA Terms		
	Pre	Post	Pre	Post	Group	Time	Interaction
Systolic Pressure (mmHg)	125±3	129±3	123±3	129±3	0.64	<0.001	0.40
Diastolic Pressure (mmHg)	78±2	76±2	76±2	77±2	0.78	0.75	0.11
Mean Arterial Pressure (mmHg)	92±2	92±2	90±2	93±2	0.72	0.01	0.13
Brachial Pulse Pressure (mmHg)	48±2	53±2	48±2	52±2	0.73	0.01	0.83

Values are means ± SE

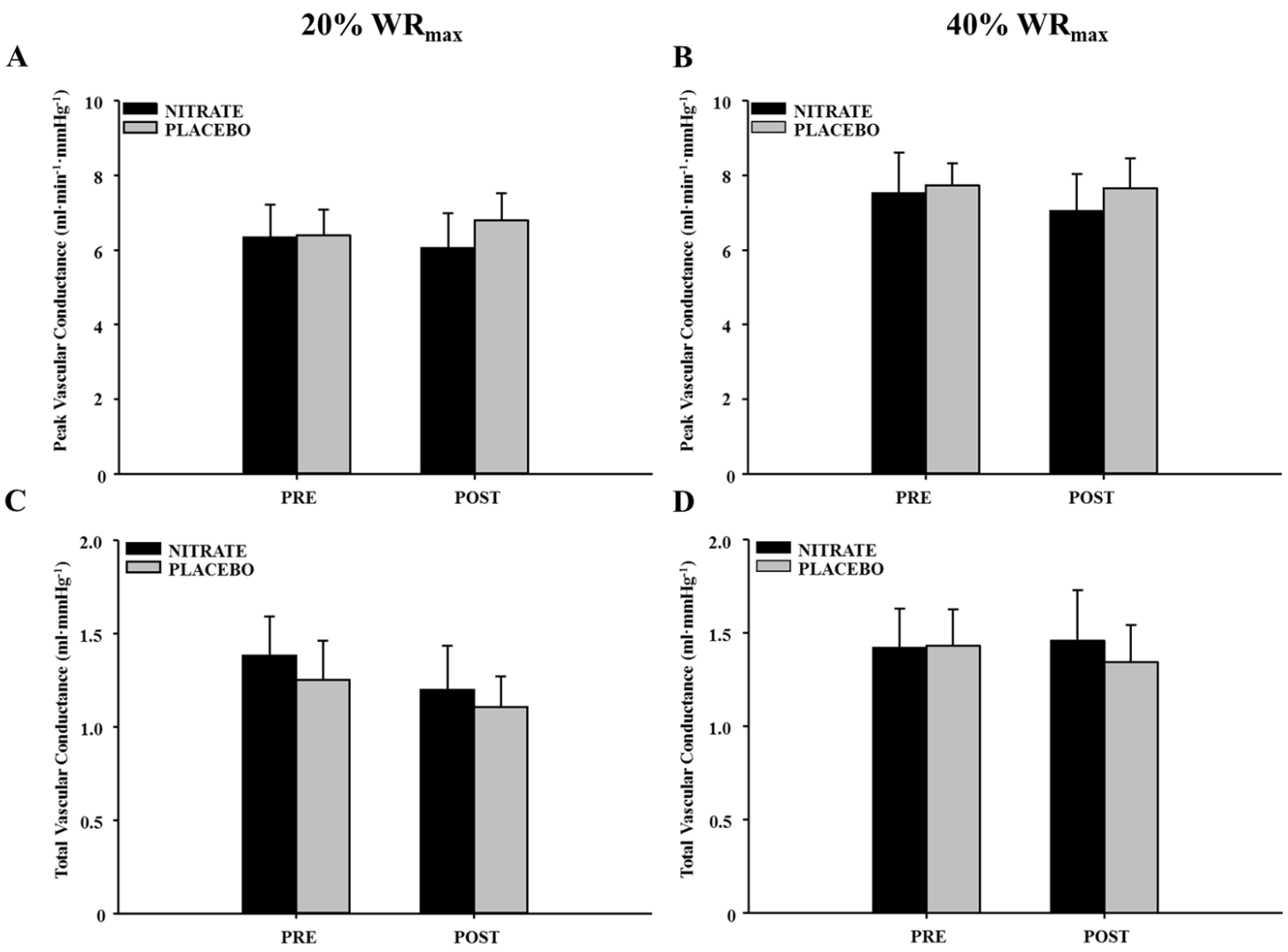

Figure 22: Rapid vasodilator responses to single skeletal muscle contractions in response to acute supplementation with dietary nitrate and placebo

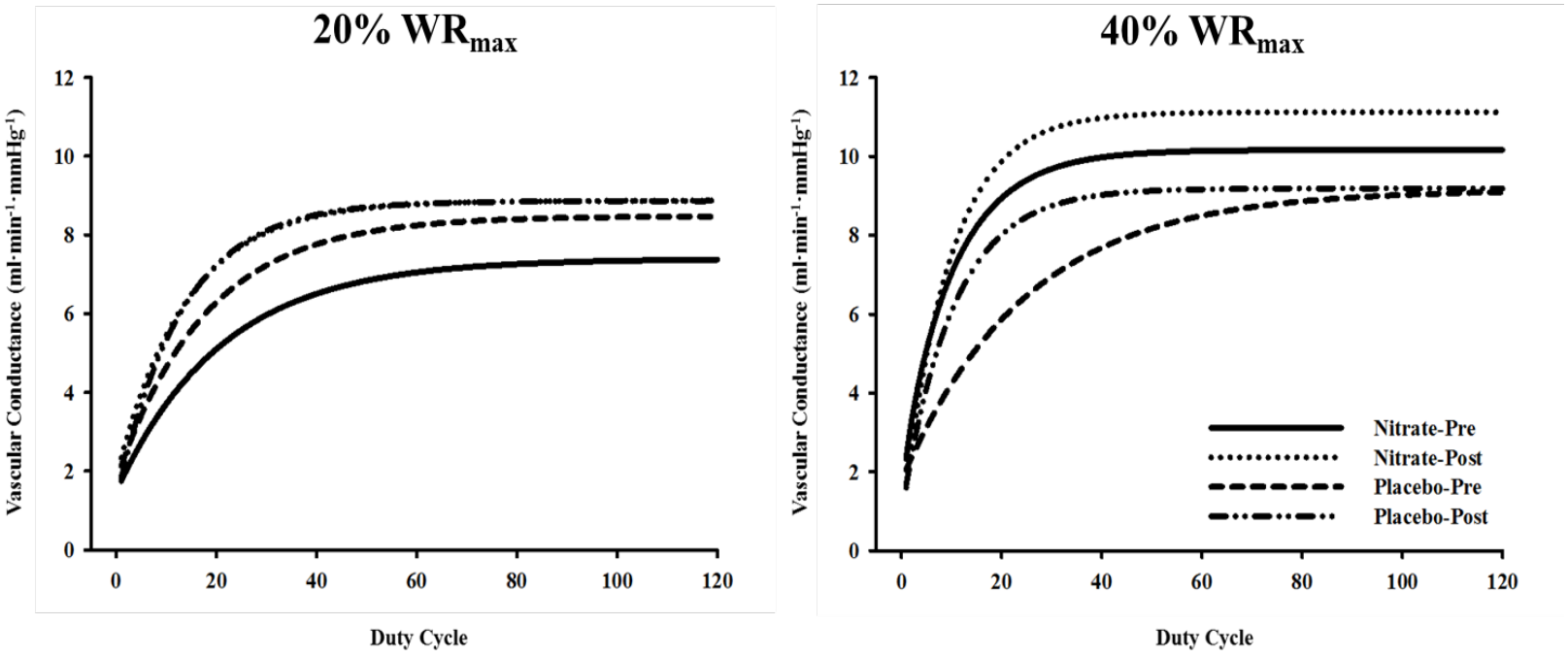

Figure 23: Group kinetic responses at 20% and 40% work-rate maximum (WR_{max}) prior to and post-supplementation with dietary nitrate and placebo

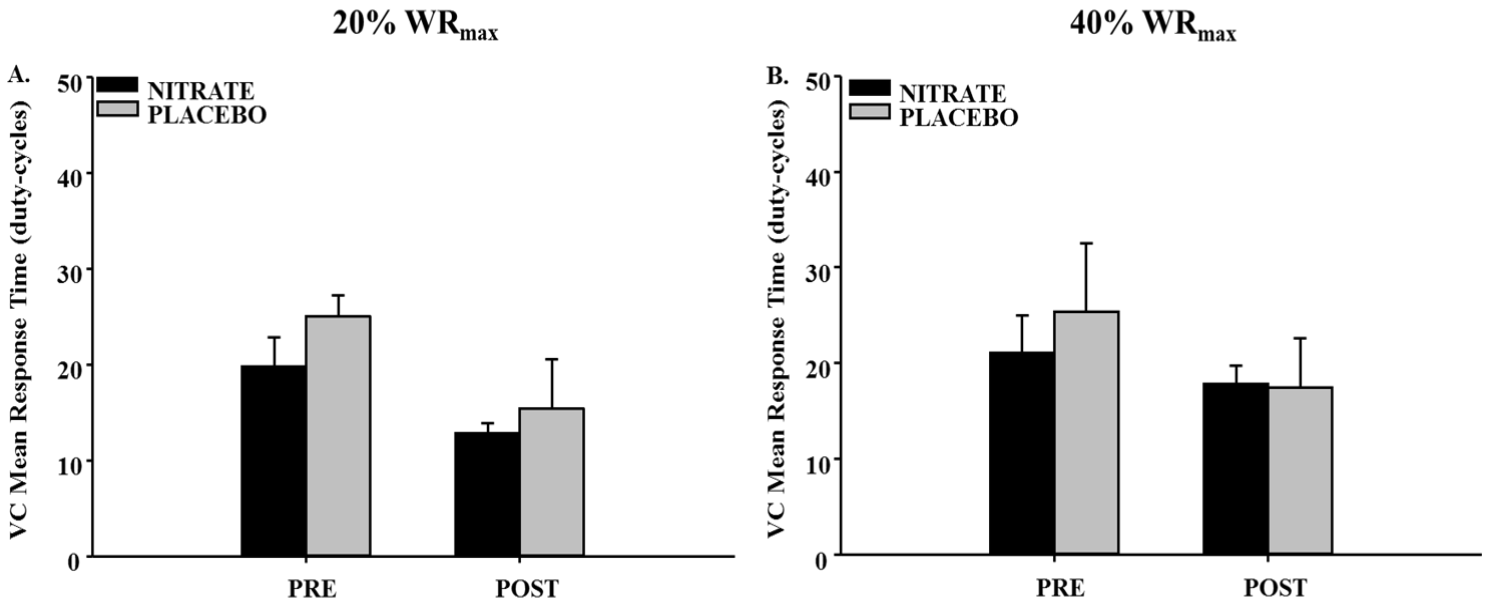

Figure 24. Mean response time pre and post supplementation with dietary nitrate and placebo at 20% and 40% work-rate maximum (WR_{max})

Table 15: Systemic and Leg Hemodynamics

		NITRATE				PLACEBO			
		BL	20% (Δ from BL)	BL	40% (Δ from BL)	BL	20% (Δ from BL)	BL	40% (Δ from BL)
PRE	HR ($b \cdot min^{-1}$)	61±2	25±3	62±2	31±2	63±2	24±3	60±2	24±2
	MAP (mmHg)	109±4	33±5	113±6	35±5	105±4	26±5	109±4	39±4
	BF ($ml \cdot min^{-1}$)	180±36	873±95	207±35	1080±126	222±37	928±93	210±55	1127±134
	VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	1.6±0.3	5.9±0.8	1.8±0.3	7.1±1.1	2.1±0.3	6.8±0.7	1.9±0.5	7.3±1.0
POST	HR ($b \cdot min^{-1}$)	59±2	25±3	60±2	30±3	63±2	30±3	60±2	32±3
	MAP (mmHg)	106±6	24±5	112±4	34±6	114±5	26±5	112±4	39±6
	BF ($ml \cdot min^{-1}$)	242±47	895±108	294±65	1306±136	210±31	993±152	184±32	1138±176
	VC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	2.3±0.4	6.6±0.1	2.6±0.6	8.5±1.2	1.8±0.3	7.0±1.2	1.6±0.3	7.6±1.5

Mean±SE. HR, Heart rate; MAP, mean arterial pressure; BF, blood flow; VC, vascular conductance; BL, baseline

Chapter 8: CONCLUSIONS

Skeletal muscle blood flow and vasodilator responses to exercise are attenuated with advancing age both at the onset of exercise, as well as during steady-state exercise. Mechanisms for these age-associated attenuations have been examined within the forearm, and are attributed to endothelial and neural components. Through a series of experiments we have attempted to comprehensively address age-related changes in skeletal muscle blood flow within the leg across an entire exercise transient in older adults, as well as explore interventional approaches aimed at improving skeletal muscle blood flow during leg exercise in older adults. The purpose of this research was to 1) determine whether mechanical factors are involved in the age-related attenuation of contraction-induced rapid onset vasodilation within the forearm; 2) examine whether contraction-induced ROV is attenuated in the leg of older adults, and whether these attenuations are similar to those seen in the arm; 3) examine whether sympathetic vasoconstriction contributes to the age-related attenuation of contraction-induced ROV within the leg; 4) determine whether chronic aerobic exercise training in older adults offsets age-related attenuations in contraction-induced ROV; 5) examine whether advancing age prolongs the rate of adaptation (e.g. vasodilator kinetic response) of blood flow and vasodilation to rhythmic leg kicking exercise, as well as determine whether attenuations in ROV at the onset of exercise are related to vasodilator kinetics; and 6) determine whether chronic exercise training or acute supplementation of dietary nitrate augment blood flow and vasodilator responses to exercise in older adults across and exercise transient (onset to steady-state). Outlined below are the specific aims addressed throughout this dissertation with original hypotheses and a brief descript of results as to whether they support these original hypotheses.

Specific Aims

Specific Aim 1 (Chapter 2)

Specific Aim 1.1 Examine whether the relative mechanical contribution to contraction-induced ROV is altered in older adults.

Hypothesis 1.1 Due to increases in vessel stiffness, extravascular compression will not elicit similar elevations in vasodilation as compared to contraction-induced ROV.

Not Supported: The mechanical contribution to contraction-induced ROV within the forearm was not altered with advancing age. Older adults demonstrated reductions in total ROV in response to single skeletal muscle contractions and to single extra-vascular compression; however the relative mechanical contribution to at-heart level ROV responses were not different between young and older adults.

Specific Aim 1.2 Examine the influence of perfusion pressure on contraction-induced ROV within the forearm of older adults.

Hypothesis 1.2 Elevation of perfusion pressure will augment contraction-induced ROV within the forearm of older adults.

Supported: Elevation and reductions in perfusion pressure by placing the experimental arm below and above heart level, respectively, altered both contraction-induced and compression-induced ROV responses in young and older adults. Additionally, the relative mechanical contribution to contraction-induced ROV was greater when perfusion pressure was enhanced (e.g. below-heart level).

Specific Aim 1.3 Examine the relationship between upper limb arterial stiffness and contraction-induced ROV in older adults.

Hypothesis 1.3 Elevations in upper limb arterial stiffness will be inversely associated with contraction-induced ROV within older adults.

Not Supported: Peripheral conduit artery stiffness as measured by carotid-radial pulse-wave velocity was not related to contraction-induced ROV despite age-related differences in carotid-radial PWV at rest.

Specific Aim 2 (Chapter 3)

Specific Aim 2.1 Characterize the influence of age on contraction-induced ROV within the leg.

Hypothesis 2.1 Advanced age will reduce contraction induced ROV within the leg of older adults.

Supported: Older adults demonstrated blunted contraction-induced ROV responses (44%-50%) within the leg relative to young adults.

Specific Aim 2.2 Examine whether age-related ROV responses vary between limbs.

Hypothesis 2.2 The lower limbs will exhibit a greater reduction in contraction-induced ROV in older adults relative to the upper limbs.

Not Supported: Despite differences in muscle mass between the arm and leg, age-related reductions in contraction-induced ROV were similar between the arm and leg.

Specific Aim 3 (Chapter 4)

Specific Aim 3.1 Determine whether enhanced sympathetic vasoconstriction contributes to the attenuated contraction-induced ROV response observed in the leg of older adults.

Hypothesis 3.1 Acute elevations in sympathetic nervous system activity will reduce ROV to a greater extent in young compared to older adults.

Not Supported: Acute sympathetic stimulation via a cold-pressor test significantly blunted contraction-induced ROV within the leg to a similar extent in young and older adults regardless of exercise intensity.

Specific Aim 4 (Chapter 5)

Specific Aim 4.1 Determine whether long term exercise training can prevent the blunting of ROV that occurs in untrained older adults.

Hypothesis 4.1 Chronic endurance exercise training in older adults will ameliorate the age-related impairments in contraction-induced ROV.

Supported: Older adults with an extensive history of chronic/habitual aerobic exercise training demonstrated augmented contraction-induced ROV responses within the leg relative to age-matched untrained older adults. Furthermore, these responses in trained older adults were similar to young adults.

Specific Aim 4.2 Characterize the relationship between cardiorespiratory fitness, age, and contraction-induced ROV.

Hypothesis 4.2 A higher cardiorespiratory fitness will be strongly related to contraction-induced ROV across the age-span.

Supported: Vasodilator responsiveness to single skeletal muscle contractions were positively associated with cardiorespiratory fitness whether expressed in relative or absolute exercise intensity terms.

Specific Aim 5 (Chapter 6)

Specific Aim 5.1 Examine whether the kinetics of blood flow and vasodilation are impaired within the leg of sedentary older adults, and whether chronic exercise training mitigates these reductions.

Hypothesis 5.1 Aging will result in attenuated vasodilator kinetics within the leg of older adults, and chronic exercise training will offset these age-related attenuations.

Supported: Untrained older adults demonstrated prolonged vasodilator kinetics relative to young adults, and chronically aerobic exercise trained older adults demonstrated no impairments in vasodilator kinetics.

Specific Aim 5.2 Characterize the relationship between hyperemic and vasodilator responses at the onset of exercise to the kinetics of blood flow and vasodilation during steady-state exercise in the leg.

Hypothesis 5.2 The blood flow and vasodilator responses following a single muscle contraction will be inversely related to the overall kinetic profile of blood flow and vasodilation during steady-state exercise.

Not Supported: Vasodilator responses to single skeletal muscle contractions were not related to vasodilator kinetics preceding steady-state exercise.

Specific Aim 6 (Chapter 7)

Specific Aim 6.1 Examine whether dietary nitrate improves leg blood flow and vasodilation in older adults, both at the onset, as well as during steady-state exercise.

Hypothesis 6.1 Acute ingestion of dietary nitrate will improve leg blood flow and vasodilation in older adults across an exercise transient in older adults.

Not Supported: Acute ingestion of dietary nitrate did not alter leg blood flow and vasodilation during any domain of exercise (onset, kinetics, or steady-state) above that of a placebo in older adults.

Summary and Implications

Utilizing predominately a cross-sectional design, the information gathered from these series of experiments demonstrates that normal healthy aging results in reductions in blood flow and vasodilation across an entire exercise transient. That is, from the very onset of exercise, and carrying over into steady-state exercise, older untrained adults exhibit reduced, and slower responses to rhythmic knee-extension contractions. Prospective mechanisms contributing to the age-related reduction in blood flow and vasodilation were examined, suggesting that enhanced sympathetic vasoconstriction may not fully explain these reductions. Additionally, chronic exercise training, but not acute dietary nitrate supplementation enhances the blood flow and vasodilator responses to dynamic knee-extension exercise in older adults. The implications of this data suggest that while normal healthy aging is associated with reductions in skeletal muscle blood flow and vasodilation across an exercise transient, habitual exercise training ameliorates these maladaptive responses to normal aging. Taken in a larger context, these results highlight the deleterious effects of sedentary aging, as well as the potent effects of exercise training in a

population that is prone to development of cardiovascular disease. The age-associated reductions in skeletal muscle blood flow and vasodilation to dynamic exercise could predispose this population to the development of premature exercise intolerance, facilitating a cascade of maladaptive responses, ultimately enhancing risk for cardiovascular disease. While dietary nitrate does not enhance these blood flow and vasodilator responses to exercise, this may be due to the relatively healthy population studied, and some form of overt cardiovascular disease or presence of risk factors must be present to observe beneficial effects of dietary nitrate on exercise blood flow and vasodilation. Specifically, while acute dietary nitrate does not enhance blood flow and vasodilator responses across an exercise transient, additional experiments are needed to determine if dietary nitrate improves these responses 1) over a longer duration (chronic supplementation), 2) whether potential improvements are dependent on the dose of dietary nitrate consumed, and 3) whether populations that exhibit more overt forms of cardiovascular disease (e.g. hypertension, peripheral artery disease, heart failure) demonstrate effects on skeletal muscle blood flow during exercise.

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