EFFECTS OF AGING AND EXERCISE TRAINING ON THE MECHANISMS OF ANGIOTENSIN II-INDUCED VASOCONSTRICTION IN RAT SKELETAL MUSCLE ARTERIOLES

A Dissertation

by

YOONJUNG PARK

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2006

Major Subject: Kinesiology

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Approved by:

Chair of Committee, Robert B. Armstrong
Committee Members, Michael D. Delp
Christopher R. Woodman
James D. Fluckey
Emily Wilson
Head of Department, Robert B. Armstrong

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ABSTRACT

Effects of Aging and Exercise Training on the Mechanisms of Angiotensin II-Induced Vasoconstriction in Rat Skeletal Muscle Arterioles. (December 2006)

Yoonjung Park, B.A., Seoul National University;

M.S., Seoul National University;

M.A., The University of Texas at Austin

Chair of Advisory Committee: Dr. Robert B. Armstrong

Aging is associated with increases in regional and systemic vascular resistance and impaired ability to increase blood flow to active muscles during exercise. Aging enhances vasoconstrictor responsiveness in both humans and animals, and an increase in Angiotensin II-induced vasoconstriction is one possible mechanism for old age-associated increase in muscle vascular resistance. The purpose of this study was to determine 1) whether aging alters Ang II-induced vasoconstriction, 2) whether exercise training attenuates the age-associated alteration in Ang II-mediated vasoconstriction, and 3) the mechanism(s) through which aging and exercise training alter Ang II-induced vasoconstriction in rat skeletal muscle arterioles. Male Fischer 344 rats were assigned to 4 groups: Young sedentary (YS; 4 months), old sedentary (OS; 24 months), young trained (YT) and old trained (OT). Exercise-trained groups performed treadmill exercises for 60 min/day at 15 m/min, on a 15º incline for 5 days/week for 10-12 weeks. First-order (1A) arterioles were isolated from soleus and gastrocnemius muscles for *in*

vitro experimentation. Intraluminal diameter changes were determined in response to the cumulative addition of Ang II (3×10^{-11} - 3×10^{-5} M). Ang II dose responses were then determined following the removal of endothelium and treatment with N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-5} M), a nitric oxide synthase (NOS) inhibitor. Ang II-induced vasoconstriction was augmented in the aged skeletal muscle arterioles, both in soleus and gastrocnemius muscles, and age-associated increases in Ang II-induced vasoconstriction were abolished with the removal of endothelium and with L-NAME. Exercise training ameliorated the age-induced increase in Ang II-vasoconstriction, and this alteration was eliminated by the removal of endothelium and with NOS inhibition. These findings suggest that aging enhances Ang II-induced vasoconstrictor responses in the arterioles from both soleus, high oxidative, and white portion of gastrocnemius, low oxidative glycolytic muscles, and this age-associated change occurs through an endothelium-dependent NOS signaling pathway. These results also demonstrated that exercise training can ameliorate the age-associated increase in Ang II vasoconstriction in the arterioles from both high oxidative and low oxidative glycolytic muscles through an endothelium-mediated NOS mechanism.

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CHAPTER I

INTRODUCTION

1.1 Aging, exercise training, cardiovascular system, and control of muscle flood flow

By the year 2020, the average life expectancy will be 82.0 years for women and 74.2 years for men, and by the year 2040, it will increase to 83.1 years for women and 75.0 years for men (94). The number of the population in the United States over 65 years old will rise to 52 million by the year 2020 and to 68 million by the year 2040. This rapid growth of the elderly will have an enormous impact on future health care costs (94). A characteristic of this population is the high prevalence of cardiovascular diseases, which negatively affect both life expectancy and quality of life, and are associated with an impaired ability for adaptation to environmental change (91). Epidemiological studies have reported that advancing age, genetic factors, diabetes, lipid levels, and sedentary lifestyle are all risk factors for cardiovascular disease, such as coronary artery disease, hypertension, congestive heart failure, and stroke (39, 57). For example, advancing aging is highly correlated with the prevalence of hypertension, incidence of atherothrombotic stroke, and incidence of coronary heart disease (57).

This dissertation follows the style and format of *American Journal of Physiology-Heart and Circulatory Physiology*.

The old age-related impairment of the cardiovascular system is associated with various detrimental changes of cardiovascular structure and function. In terms of cardiovascular structure, aging results in increased vascular intimal thickness, vascular stiffness, left ventricular wall thickness and left atrial size (37, 55, 57, 76). Functional changes of the cardiovascular system with aging include decreased end diastolic filling pressure at rest (68), although systolic function is not significantly altered with aging, which preserves ejection fraction and stroke volume (90). Also, cardiac responsiveness to β-adrenergic stimuli is decreased with aging, confirming that increases in heart rate and myocardial contractility induced by catecholamines or exercise are diminished in elderly people and peak cardiac output at maximal exercise is consequently reduced with aging (32, 56).

One of the key interventions that is able to prevent and reduce the risk of cardiovascular disease is long term regular physical activity. The benefits of chronic physical activity have been reported both in healthy subjects and old patients (12). However, aging itself limits elderly individuals to continue a physically active life style due to a significantly reduced exercise capacity and maximal aerobic capacity (41, 78). This decline in exercise capacity is in part due to an attenuated ability to increase blood flow to working muscle during exercise, as well as a diminished ability to elevate cardiac output during exercise (63). It has been reported that skeletal muscle perfusion during muscle stimulation and exercise is lower in old animals (50, 75) and humans (5, 60, 64, 82, 84, 86, 106), although skeletal muscle blood flow is not altered with aging during rest (18, 41, 111). Irion et al. (50) reported that blood flow is significantly lower

in aged rats undergoing intermittent tetanic contractions compared to younger animals. Likewise, Musch et al. (75) reported that muscle blood flow in conscious exercising rats was reduced with advanced age to highly oxidative muscle, but was increased with aging to low oxidative muscle although blood flow to total hindlimb muscles was not different between young and old rats during exercise. Human data also indicate that leg muscle blood flow is lower with advanced age during submaximal exercise in sedentary men (5, 82) and women (84) and endurance-trained men (86). For example, Proctor et al. (86) demonstrated during whole body exercise that leg blood flow and vascular conductance were lower in aged individuals compared to younger counterparts. Several potential problems with the experimental approach of using whole body exercise to determine whether local vascular factors limit skeletal muscle perfusion with aging is that the lower old age-associated exercise hyperemia could result from limited cardiac output (32, 56) or a smaller muscle mass (35) among elderly subjects. To overcome these potential limitations, Lawrenson el al. (60) measured muscle blood flow and vascular resistance during small muscle mass knee extensor exercise which would not elicit maximal cardiac output and found that knee extensor blood flow was lower and vascular resistance higher across a range of work rates in aged men relative to young subjects. These findings indicate that local factors, such as decreased responsiveness to vasodilator stimuli and/or increased responsiveness to vasoconstrictor stimuli could underlie the old age-associated reduction in muscle blood flow during exercise (60).

One of the potential local mechanisms that could mediate an impaired ability to increase skeletal muscle blood flow with aging is a diminished endothelium-dependent

vasodilator function. Furchgott and Zawadzki (34) firstly found the important role of the endothelium in the vasodilation of the vascular system caused by the endothelium-dependent vasodilator ACh. They demonstrated the role of endothelial cells to release a factor that causes vasodilation in the vessel, termed endothelium-derived relaxing factor (EDRF), which modulates the vascular response (34). This factor has been identified as nitric oxide (NO) (81). NO synthesis is catalyzed by the constitutive enzyme, nitric oxide synthase (NOS), from the terminal guanidine of L-arginine, which is calcium (Ca^{++})/calmodulin-dependent (80). Since NO is rapidly diffusible, once it is synthesized, it can easily move into the smooth muscle cell through the cell membrane where it plays an important role in regulating the constriction of smooth muscle in the vascular system (33).

Although the effect of aging on endothelium-dependent vasoreactivity varies with species and vascular beds (48, 112), aging-induced impairment of endothelial function in various vascular beds has been well documented (16, 21, 22, 30, 36, 46, 71, 98, 118). Desouza et al. (22) reported that vascular responsiveness to the endothelium-dependent vasodilator, ACh, is decreased, but vasodilatation to an endothelium-independent vasodilator, nitroprusside, is unchanged in the forearm of aged humans. Woodman et al. (118) reported similar finding with isolated feed artery from the rat skeletal muscles. They found that endothelium-dependent dilation to ACh was lower in old soleus muscle feed arteries, whereas endothelium-independent dilation to sodium nitroprusside (SNP) was not different in skeletal muscle feed arteries from young and old rats. Moreover, our laboratory has shown similar results in the arterioles isolated from

skeletal muscle in aged rats. Specifically, it was reported that endothelium-dependent vasodilator responses to ACh are diminished with aging in first-order (1A) arterioles from the soleus muscle, a highly oxidative muscle, but not from the superficial portion of gastrocnemius muscle, a low-oxidative glycolytic muscle (71, 98). Furthermore, age-related differences in ACh-induced vasodilatation of soleus muscle arterioles between young and old rats are abolished with N^{G}-nitro-L-arginine methyl ester (L-NAME), a NOS antagonist (71, 98). These findings indicate that aging impairs endothelium-dependent and NO-mediated vasodilatation in 1A soleus muscle arterioles.

Long-term aerobic exercise training has been reported to ameliorate the age-associated dysfunction of both central and peripheral cardiovascular function (78). For example, 16 weeks of endurance training results in a significant increase in leg blood flow and vasodilator capacity in both men and women (64). Also, cross-sectional studies report that physically active individuals and aged endurance athletes have greater endothelium-dependent vasodilation through the NOS mechanism (22, 106). Moreover, several animal studies have supported the finding that exercise training results in enhanced endothelium-dependent vasodilation in conduit arteries (19, 20) and skeletal muscle arterioles (71, 98). Spier et al. (98) reported that the exercise training-induced reversal of the age-related endothelial dysfunction in soleus muscle arterioles is mediated through the NO signaling pathway.

1.2 Aging, vasoconstrictor mechanisms of blood flow and exercise training

In addition to impaired endothelium-dependent vasodilator function with aging, the age-associated reduction in skeletal muscle blood flow capacity could be due to an enhancement of resistance vessel vasoconstrictor responsiveness. Three major vasoconstrictor mechanisms have been described: noradrergic-, endothelin-1 (ET-1)- and Ang II- mediated mechanisms. Since these are potentially important factors that control muscle blood flow at rest and during exercise, understanding the mechanism of effects of aging and exercise training on these factors is important.

Aging causes progressive increases in the sympathetic vasoconstrictor outflow to skeletal muscle in resting humans, and it is evidenced by elevations in muscle sympathetic nerve activity (MSNA) and basal norepinephrine (NE) spillover rates (97). However, age-associated responsiveness or sensitivity to α-adrenergic receptors is controversial. For example, no changes in age-associated responsiveness or sensitivity to α-adrenergic stimulation (26, 96), age-related attenuated vasoconstrictor responsiveness to sympathetic stimulation (14, 104), or elevated adrenergic sensitivity of the leg vasculature in older men (25, 54) have been reported. Although some studies have reported that leg blood flow is not changed with aging in isolated contracting muscles (51), in exercising rats (75), and in humans performing leg exercise (85), studies have supported an age-associated reduction in the ability to increase muscle blood flow during exercise as previously mentioned (50, 52, 60, 62, 86). Reductions in basal limb blood flow and vascular conductance with aging have been reported to be related to enhanced sympathetic α-adrenergic vasoconstriction (25). Koch et al. (54) reported greater

reduction in leg vascular conductance to sympathetic stimulation during exercise in older men whereas increase in sympathetic outflow to local cold stimulation during the cycle ergometer exercise was not different. Also, impaired functional sympatholysis in the forearm vascular beds during rhythmic handgrip exercise was found in older men indicating the impaired ability to blunt sympathetic α-adrenergic vasoconstriction with advancing age causing the reduction in blood flow to exercising muscle (24).

Although the mechanisms through which aging and exercise training alter endothelium-dependent vasodilation have been previously investigated (21, 71, 98, 106), the effects of aging and exercise training on the modulation of vasoconstrictor responses in the peripheral resistance vasculature have not been clearly delineated. Alterations in resistance vessel sensitivity to vasoconstrictors may contribute to the age-related reduction in exercise tolerance and skeletal muscle blood flow capacity. The mechanisms through which aging and exercise training affect noradrenergic- and ET-1-mediated vasoconstriction have been recently investigated in our laboratory (27, 28). Donato el al. (27) found that aging is associated with an augmented α-adrenergic vasoconstriction in soleus muscle arterioles and exercise training attenuated this augmentation in old rats. The aging- and training-associated alterations in α-adrenergic vasoconstriction are mediated through an endothelium-dependent mechanism, since the aging and exercise training effects are abolished with the removal of the endothelium.

Donato et al. (28) also reported that aging is associated with an enhancement of ET-1 sensitivity in 1A arterioles from the white portion of the gastrocnemius muscle, but not in 1A arterioles from the soleus muscle. This enhanced vasoconstrictor response to

ET-1 in gastrocnemius muscle arterioles from old rats is due to augmented vasoconstrictor response mediated through ETa receptors on the smooth muscle, but not through ETb receptors. Thus, aging has been shown to enhance vasoconstrictor responses of skeletal muscle arterioles through α-adrenergic and ET receptors. However, despite α-adrenergic and ET receptors both being present on vascular endothelial and smooth muscle cells, the aging and exercise training effects of α-receptor-mediated vasoconstriction occurred through the endothelial cells, whereas the aging effect mediated through the ETa-receptor occurred through the smooth muscle cells. No study has been reported that investigated the mechanisms for the effects of aging and training on Ang II-induced vasoconstriction, which is one of the possible vasoconstriction mechanisms in the skeletal muscle arterioles.

1.3 Ang II

Ang II is the main biologically active peptide of the renin-angiotensin system (RAS), which plays a major physiological role in regulation of the cardiovascular system. Disorders of the RAS are associated with the pathophysiology of renal diseases, hypertension, and chronic heart failure (61). Ang II is generated by the angiotensin-converting enzyme (ACE), which is an ectoenzyme that catalyzes the extracellular conversion of Ang I to Ang II. Also, another enzyme, renin, is involved in regulation of the Ang II synthesis in the RAS. Renin is released from the juxtaglomerular cells of the kidney into the circulation where it converts angiotensinogen to Ang I. Ang II also plays an important physiological role in the regulation of blood pressure, plasma volume, and sympathetic nervous activity (7); it is in this capacity that Ang II, as a potent

vasoconstrictor, can serve to regulate blood flow through alteration in vascular tone and conductance, and ultimately serve to regulate mean arterial blood pressure (102, 105).

The vascular effects of Ang II are mediated through direct action of Ang II on Ang II receptors. Final cardiovascular responses to Ang II are determined by the result of combined action of Ang II receptors. Two major subtypes of Ang II receptors have been mainly defined on the basis of their different pharmacological and biochemical properties: Ang II type 1 receptor (AT_1R) and Ang II type 2 receptor (AT_2R) (53, 69, 74).

The locations of AT_1R and AT_2R vary depending on species of animal and tissue type. AT_1R are located in the cardiovascular, renal, endocrine, and nervous systems in humans (3). In the vasculature, AT_1R are primarily concentrated on smooth muscle cells with relatively low levels in the adventitia (2, 121) and endothelial cells (88). In contrast to AT_1R, AT_2R are highly present in fetal tissues, and its expression is rapidly decreased after birth, but, in adults, AT_2R expression is detectable in various tissues including the vasculature (1). AT_2R are expressed both in endothelial and smooth muscle cells in rat mesenteric arteries and skeletal muscle arterioles (65, 77).

AT_1Rs are involved in most of the well-known physiological effects of Ang II, and this subtype is the major effector mechanism of Ang II-mediated vascular functions (92). Activation of AT_1R results in stimulation of vasoconstriction, vascular cell hypertrophy and hyperplasia, and sodium retention (7). Also, physiological effects of this receptor have also been reported to include stimulation of reactive oxygen species (ROS) (89) and induction of inflammatory (72), thrombotic (113), and fibrotic (107) processes. In terms of vasoconstriction, binding of Ang II to AT_1R stimulates G protein-

coupled activation of phospholipase C (PLC) and results in phosphatidylinositol (PIP_3) hydrolysis and formation of inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 activates the movement of Ca^{2+} from sarcoplasmic reticulum (SR), and DAG leads protein kinase C (PKC) to activate the sodium/hydrogen (Na^+/H^+) exchanger (110). Consequently, it results in increased intracellular free Ca^{2+} concentration and actin-myosin interaction in vascular smooth muscle cells and, subsequently, vascular smooth muscle contraction. Thus the overall signaling pathway through AT_1R binding results in vasoconstriction in the vasculature (110).

AT_2R has a less well-defined role in the cardiovascular system, but there is growing evidence that the AT_2R plays an important role in cardiovascular physiology. Overall, AT_2R appears to counter-regulate the excitatory effect of AT_1R, including vasodilation, antigrowth, antihypertrophic effects, and depressor regulation of blood pressure (11, 47, 66). There are evidences showing AT_2R has a significant role to control vascular tone by mediating vasodilation and counterbalancing the AT_1R-mediated vasoconstriction of Ang II. AT_2R stimulates endothelium-dependent vasodilation by release of NO (11, 61), which counteracts with the direct smooth muscle contraction that is mainly mediated by the AT_1R. Moreover, Ichiki et al. (49) found that AT_2R knock-out mice have higher blood pressure compared to the wild-type control mice, and Munzenmaier et al. (73) reported that pharmacological blockade of AT_2R augments the pressor effect of Ang II in the rat. AT_2R–mediated signaling works through a G protein coupling mechanism to mediate cardiovascular actions (47). Binding to AT_2R stimulates several cascades, including activation of protein phosphatases and

protein dephosphorylation, stimulation of phospholipase A (PLA_2) and release of arachidonic acid (AA), sphingolipid-derived ceramide, and increase in cyclic guanosine 3',5'-monophosphate (cGMP) level through a NO mechanism, which cause the vasodilation (47).

It has been well-documented that activation of AT_1R mediates vasoconstriction of conduit coronary arteries (103), but several studies relating to pharmacological properties suggest that AT_2R may mediate vasodilation of large conduit vessels (95, 117) and the coronary microcirculation (119) through the endothelium. In addition, studies have reported that, in endothelial cells, AT_1R plays a role of vasodilation through an eNOS mechanism (8), whereas activation of AT_2R on the smooth muscle cells exert a functional vasodilation via neuronal NOS (nNOS) and a soluble guanylate cyclase (sGC) pathway (15). Since Ang II exerts direct effects on both endothelial and smooth muscle cells in the vasculature, Ang II-induced vasoconstriction is determined by the net interactions between smooth muscle and endothelial cells. In order to explain the mechanisms of Ang II-induced vasoconstriction to control blood flow, Ang II-induced vasodilation through the endothelium is also critical (40), although the majority of studies in the literature have focused on the Ang II-induced vasoconstriction role through smooth muscle. Moreover, Gruetter et al. (38) reported that Ang II-induced vasoconstriction is attenuated by the release of a substance from the endothelium and enhanced by removal of endothelium. Specifically, Ang II activates NO production by vascular endothelial cells through AT_1R activation (10) and/or through AT_2R activation (93, 116). Therefore, identifying the interaction between the smooth muscle and

endothelial cell response is important for understanding the mechanism(s) of effects of aging and exercise training on Ang II-induced vasoconstriction in resistance arterioles.

Ang II has been shown to play a role in the vasomotor activity in various microvascular beds (44), but few studies investigating the direct effects of Ang II on the microcirculation have been performed. The degree of Ang II-induced vasoconstriction gradually diminishes with increasing Ang II concentration above a certain point of concentration. Zhang et al. (119) recently reported that the more potent vasoconstriction occurs at a lower concentration of Ang II (1nmol/L), where there is less vasoconstriction at higher concentrations (10nmol/L) in coronary arterioles. This is consistent with the findings in the perfused heart showing that the magnitude of coronary vasodilation is increased with increasing Ang II concentrations (83). This lower constrictor response at higher concentrations of Ang II may depend on the interaction of the smooth muscle cell and endothelial cell response to Ang II.

1.4 Aging, exercise, and Ang II

Limited information is available regarding changes in Ang II vasoreactivity with aging in the microcirculation. Although aging is associated with lower plasma renin activity in normal individuals (115), evidence suggests no aging effect on plasma Ang II concentration (29). Moreover, few studies have investigated possible changes of Ang II receptor density with aging, and what has been reported provides no clear pattern. Daubert et al. (13) reported that the density of Ang II receptor binding sites is lower in old mouse brains, whereas Heymes et al. (45) found that mRNA levels of both AT_1R and AT_2R subtypes are markedly up-regulated in the myocardium of aged rats. More

significantly, the effect of aging on Ang II-induced vasoconstriction in skeletal muscle arterioles has not been shown, although understanding the effect of aging on Ang II-induced vasoconstriction in skeletal muscle arterioles may be potentially important since Ang II-induced vasoconstriction may be one of the important contributing factors to the age-associated reduction in blood flow to skeletal muscles during exercise.

During dynamic exercise, the plasma Ang II levels are increased in an intensity-dependent manner (101, 108), and as a result, Ang II may be more involved in determining the blood flow response during exercise. Ang II is a potent vasoconstrictor in the cardiovascular system during exercise (102, 105) in that it increases the pressor response and may be involved in redirecting blood flow from the splanchnic and renal regions toward active muscles (102). However, no study has been reported regarding the effect of long-term exercise training on Ang II-induced changes in blood flow and vascular conductance. Moreover, the effect of exercise training on Ang II-induced vasoconstriction in the skeletal muscle resistance vessels also has not been investigated.

Overall, the role of Ang II has been investigated in the control of blood flow and vascular conductance during acute exercise. However, studies on the effect of aging and endurance exercise training on Ang II-mediated vascular responses are lacking.

1.5 Purpose and hypotheses

The central hypothesis for this study is that aging diminishes vascular conductance due to a shift of interaction between vascular smooth muscle cells and endothelial cells, and that exercise training serves to attenuate this effect. One possible mechanism for old age-related reductions in vascular conductance is an enhanced

vasoconstrictor responsiveness to Ang II, and that exercise training may serve to diminish Ang II-mediated vasoconstriction.

The overall purpose of this dissertation research is to determine whether aging alters Ang II vasoreactivity in skeletal muscle resistance arterioles and whether exercise training can ameliorate the putative old age-associated alteration in Ang II-induced vasoconstriction. A secondary purpose is to elucidate the mechanism(s) of the aging and exercise training effects on Ang II-induced vasoconstrictor response of skeletal muscle resistance arterioles from old rats. Therefore, six hypotheses were tested:

1) There will be higher Ang II-induced vasoconstriction of arterioles from soleus and gastrocnemius muscles in old rats compared to that in young animals.
2) Removal of the endothelium will abolish the age-associated difference of Ang II-mediated vasoconstriction in arterioles from soleus and gastrocnemius muscles.
3) Inhibition of NOS activity with L-NAME will abolish the age-associated difference in Ang II-mediated vasoconstriction in soleus and gastrocnemius muscle arterioles.
4) Exercise training will decrease Ang II-mediated vasoconstriction in aged arterioles from soleus and gastrocnemius muscles to levels at or near that in young sedentary animals.
5) Removal of endothelium will abolish the exercise training-associated reduction in Ang II-mediated vasoconstriction in aged arterioles from soleus and gastrocnemius muscles.

6) Inhibition of NOS activity with L-NAME will abolish the exercise training-associated reduction of Ang II-mediated vasoconstriction in aged arterioles from both soleus and gastrocnemius muscles.

CHAPTER II

EFFECTS OF AGING AND EXERCISE TRAINING ON THE MECHANISMS OF ANGIOTENSIN II-INDUCED VASOCONSTRICTION IN RAT SKELETAL MUSCLE ARTERIOLES

2.1 Introduction

It is well documented that aging has a detrimental effect on the cardiovascular system and that aging is an independent risk factor for cardiovascular diseases, such as atherosclerosis, hypertension, and coronary artery disease (31). In addition to increased risk of cardiovascular disease with aging, aerobic exercise capacity declines with advancing age. This decline in exercise capacity is partly due to an attenuated ability to increase blood flow to working muscle during exercise, through both declines in maximal cardiac output (63) and skeletal muscle blood flow during exercise (60).

One of the potential local mechanisms that could mediate an impaired ability to increase skeletal muscle blood flow with aging is an impaired endothelium-dependent and nitric oxide (NO)-mediated vasodilator function (16, 21, 22, 30, 36, 46, 71, 98, 118). Long-term aerobic exercise training has been also reported to ameliorate the age-associated dysfunction through the endothelium-dependent vasodilation and the NOS signaling pathway mechanism in humans and animals (22, 98, 106).

Although the mechanisms through which aging and exercise training alter endothelium-dependent vasodilation have been previously investigated, the effects of

aging and exercise training on the modulation of vasoconstrictor responses in the peripheral resistance vasculature have not been clearly delineated. Recently, however, Donato el al. (27) reported that aging is associated with an augmented α-adrenergic vasoconstriction in soleus muscle arterioles and exercise training attenuates this augmentation in old rats through an endothelium-dependent mechanism. Donato et al. (28) also found that aging is associated with an enhancement of ET-1 sensitivity in 1A arterioles from the white portion of the gastrocnemius muscle through augmented vasoconstrictor response mediated through ETa receptors on the smooth muscle and not through the endothelium.

Ang II is the main biologically active peptide of the renin-angiotensin system (RAS), which exerts both hemodynamic and renal effects. Ang II also plays an important physiological role in the regulation of blood pressure, plasma volume, and sympathetic nervous activity in the cardiovascular system (7). In regard to the regulation of blood pressure, Ang II is a potent substance capable of constricting arterioles. Two subtypes of Ang II receptors have been mainly defined on the basis of their different pharmacological and biochemical properties. Ang II type 1 receptor (AT_1R), which is involved in most of the well-known physiological effects of Ang II and exerts potent vasoconstriction in the blood vessels, and Ang II type 2 receptor (AT_2R), which has a less well-defined role but appears capable of counterbalancing some of the effects of AT_1R stimulation (53, 69, 74). However, studies have reported that, in endothelial cells, AT_1R also plays a role of vasodilation through an eNOS mechanism (8,

87), whereas activation of AT_2R in the smooth muscle cell still exert a functional vasodilation via nNOS and sGC pathway (15).

Since Ang II exerts direct effects on both endothelial and smooth muscle cells in the vasculature, Ang II-induced vasoconstriction is determined by the interaction of vasoconstrictor and vasodilator influences mediated by smooth muscle and endothelial cells. Therefore, understanding the role of the smooth muscle and endothelial cells is important to elucidate the mechanism(s) of the effect of aging and exercise training on Ang II-induced vasoconstriction in the resistance arteriole.

Limited information is available regarding changes in Ang II vasoreactivity with aging in the microcirculation. Moreover, no direct studies have been conducted regarding the effects of aging or exercise training on Ang II-induced vasoconstriction in skeletal muscle arterioles. Therefore, the purpose of this study is to determine whether and through what mechanism(s) aging and exercise training affect Ang II-mediated vasoconstriction in rat skeletal muscle resistance arterioles. Based on results showing age enhances α-adrenoceptor-mediated vasoconstriction via diminished endothelium dependent NOS mechanism (27), we hypothesized that aging would enhance Ang II-induced vasoconstriction through an impaired endothelium-dependent NOS signaling pathway, and that exercise training would attenuate the increased Ang II-mediated vasoconstriction through the endothelium NOS mechanism.

2.2 Methods

2.2.1 Animals

Male Fischer 344 young (3-6 months) and old (22-24 months) rats were obtained from the National Institutes for Aging (NIA/Harlan) and housed in a temperature-controlled (23±2°C) room with a 12:12 light-dark cycle. Water and rat chow were provided ad libitum. All animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee and complied with the guidelines of the National Research Council *Guide for the Care and Use of Laboratory Animals*.

2.2.2 Exercise Training

Training consisted of the following 10-12 weeks running program, executed on a motor-driven treadmill. During habituation, the rats walked on the treadmill at 10 m/min (0° incline) and then speed was increased to 15 m/min, 5 min/day for 3 days. After habituation on the treadmill, young and old rats were assigned to one of four groups, young sedentary (YS), young exercise-trained (YT), old sedentary (OS), and old exercise-trained (OT). Exercise-trained rats performed treadmill running at 15 m/min on a 15° incline, 60 min/day, 5 days a week for 10 to12 weeks as previously described (20, 98). A minimum of 48 hours was allowed between the execution of experiments and the final bout of exercise.

2.2.3 Microvessel Preparation

The rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and the gastrocnemius-plantaris-soleus muscle group from the hindlimb was carefully dissected free and placed in cold (4°C) physiological saline solution (PSS) that contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 1 g/100 ml BSA at pH 7.4. With a dissecting microscope (Olympus SVH10), 1A arterioles from the soleus muscle and the white portion of the gastrocnemius muscle were isolated and removed from the surrounding muscle tissue as previously described (67, 70). The arterioles were transferred to a Lucite chamber that contained PSS equilibrated with room temperature. Each end of the arteriole was cannulated with a micropipette and secured with nylon suture. After cannulation of the arterioles, the microvessel chamber was transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310), video caliper (Microcirculation Research Institute), and data acquisition system (MacLab/Macintosh) for on-line recording of intraluminal diameter. The arterioles were initially pressurized to 70 cmH_2O with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then closing the valves to the reservoirs and verifying that intraluminal pressure remained constant. The arterioles that did not hold pressure were discarded. The arterioles that were free from leaks were warmed to 37°C and allowed to develop initial spontaneous tone during a 30- to 60-min equilibration period.

2.3.4 Experimental Design

Protocol I. Concentration-response relations to the cumulative addition of Ang II [3×10^{-11} to 3×10^{-5} M] were determined in arterioles from the soleus and gastrocnemius muscles from YS, OS, YT, and OT groups. Diameter was recorded for 3 minutes following each addition of Ang II. The arterioles were allowed to develop at least 15% spontaneous tone prior to addition of Ang II.

Protocol II. To determine whether alterations induced by aging and exercise training from protocol I were mediated through the vascular endothelium, the endothelium was denuded from the gastrocnemius and soleus muscle arterioles from the YS, YT, OS, and OT by passing 5 ml of air through the lumen of the vessel. In order to insure full removal of the endothelium, the arterioles were exposed to ACh [3×10^{-5} M]. Vessels that exhibited vasodilation of more than 5% were excluded from further study. Following the ACh test, the vessels were washed several times with PSS and allowed to develop spontaneous tone prior to the Ang II dose response. The diameters of denuded 1A arterioles from gastrocnemius and soleus muscles were measured in response to increasing concentrations of Ang II [3×10^{-11} to 3×10^{-5} M].

Protocol III. Since results from protocol II indicated that the effects of aging and exercise training were endothelium-dependent, another series of studies was performed to determine whether the alteration of the endothelium by aging and exercise training was mediated through the NOS signaling pathway. After the arterioles were allowed to develop spontaneous tone, they were incubated for 20 minutes with L-NAME [10^{-5} M] and the Ang II dose response [3×10^{-11} to 3×10^{-5} M] was performed.

2.2.5 Muscle Citrate Synthase Activity

Sections of the soleus and white gastrocnemius muscles from each animal were stored at -80°C for determination of citrate synthase activity (100), a measures of muscle oxidative capacity, to determine the efficacy of the training regimen. Likewise, the heart was removed to determine whether exercise training elevated heart-to body mass ration, an indicator of an exercise trained state.

2.2.6 Data Analysis

Actual diameter was measure in response to Ang II and was expressed as a percentage of constrictor response according to the following formula:

$$\text{Vasoconstriction (\% Maximal Response)} = [(D_b - D_s)/(D_b) \times 100]$$

where D_b is the initial baseline diameter recorded immediately before the addition of the Ang II and D_s is the steady-state diameter measured after each dose of Ang II. Dose response curves were analyzed by two-way ANOVA with repeated measure on one factor (Ang II dose). Pairwise comparisons between specific levels were made through post-analysis (LSD). A one-way ANOVA was performed to determine significance of differences among groups in citrate synthase activity, body weight, and muscle weight. All values were presented as mean ± SEM. Significant differences were indicated by $P \leq 0.05$.

2.3 Results

2.3.1 Animal Characteristics

The animals' age at the time of study was approximately 6 months (range, 4-7 month) for the young rats and 25 months (range, 24-25 month). Body mass was greater in old sedentary than in young sedentary rats (YS: 342 ± 6 g; OS: 431 ± 4 g), and exercise training reduced body mass in old rats (OS: 431 ± 4 g; OT: 396 ± 6 g), but not in young rats (YS: 342 ± 6 g; YT: 331 ± 7 g) (Table 2.1). Although only soleus muscle mass was increased with age, both soleus and gastrocnemius muscle mass-to-body mass ratio were decreased with aging and exercise training in young and old rats. Also, only soleus muscle mass-to-body mass ratios was increased with exercise training in young and old rats (Table 2.1).

Heart mass and left ventricle mass-to-body mass ratio were higher in the exercise trained groups and citrate synthase activity was higher in soleus muscle from both young and old trained rats (Soleus; YS 20.0 ± 0.6, OS: 17.5 ± 0.9, YT: 25.9 ± 0.9, OT: 23.4 ± 1.3) indicating the efficacy of the exercise training regimen ($P<0.05$). However, citrate synthase activity in the white portion of gastrocnemius was not altered by exercise training in either young or old rats (Table 2.1).

2.3.2 Isolated Vessel Characteristics

Maximal intraluminal diameters of soleus muscle arterioles were not different among groups, but maximal intraluminal diameters of gastrocnemius muscle arterioles were increased with age. Exercise training tended to increase maximal diameter of gastrocnemius muscle arterioles in old rats ($P=0.057$) (YS: 151 ± 4, OS: 172 ± 5, YT:

156 ± 7, OT: 188 ± 7) (Table 2.2). Initial spontaneous tone developed was not different among groups in arterioles from soleus and gastrocnemius muscles.

2.3.3 Ang II Vasoconstrictor Studies

Vascular sensitivity (EC_{50}). There were no aging and exercise training effect on vascular sensitivity (EC_{50}) in the rat skeletal muscle arterioles (Table 2.3).

Effect of aging. Aging enhanced Ang II-mediated vasoconstrictor response in the 1A arterioles from both soleus (Figure 2.1 A) and gastrocnemius (Figure 2.1 B) muscles.

Vasoconstrictor response to single dose of Ang II. Ang II-induced vasoconstriction with a single dose of Ang II at the concentration of 1×10^{-8} M also resulted in a higher vasoconstrictor response in aged arterioles from soleus (A) and gastrocnemius (B) muscles (Figure 2.2). This difference was similar to the concentration with the cumulative addition of Ang II (Figure 2.1). These results indicate that the difference in Ang II-induced vasoconstriction between young and old skeletal muscle arterioles is concentration-dependent, not time-dependent.

Effect of removal of endothelium. The biphasic responses of Ang II-mediated vasoconstriction, which is a lower vasoconstrictor response at higher concentration of Ang II (higher than 3×10^{-7} M), were abolished with the removal of the endothelium in both young and old rats (Figure 2.3). More importantly, the removal of the endothelium resulted in an elimination of the age-associated difference in Ang II-mediated vasoconstriction of soleus (Figure 2.3 A) and gastrocnemius (Figure 2.3 B) muscle arterioles from young and old rats.

Table 2.1 Animal characteristics of YS, OS, YT, and OT groups.

	Sedentary		Exercise-Trained	
	Young (YS)	Old (OS)	Young (YT)	Old (OT)
N	29	29	23	20
Body Mass (g)	342 ± 6	431 ± 4*	331 ± 7	396 ± 6‡
Soleus Muscle Mass (mg)	150 ± 4	170 ± 4*	158 ± 5	174 ± 6
Gastrocnemius Muscle Mass (mg)	1,756 ± 33	1,804 ± 37	1,713 ± 30	1,702 ± 47
Soleus Muscle Mass/Body Mass Ratio (mg/kg)	441 ± 10	395 ± 10*	480 ± 13†	438 ± 12‡
Gastrocnemius Muscle Mass/Body Mass Ratio (mg/kg)	5,160 ± 98	4,182 ± 85*	5,208 ± 119	4,295 ± 94
Heart Mass (mg)	909 ± 33	1,168 ± 37*	949 ± 36	1,176 ± 37
Heart Mass/Body Mass Ratio (mg/kg)	2,657 ± 124	2,709 ± 94	2,867 ± 90†	2,969 ± 117‡
LV Mass/Body Mass Ratio (mg/kg)	1,822 ± 71	1,887 ± 35	2,252 ± 59†	2,215 ± 66‡
Soleus muscle citrate synthase activity (μmol/min/g wet Wt)	20.0 ± 0.6	17.5 ± 0.9*	25.9 ± 0.9†	23.4 ± 1.3‡
White portion of gastrocnemius muscle citrate synthase activity (μmol/min/g wet Wt)	12.0 ± 0.3	12.1 ± 0.6	12.1 ± 0.6	13.0 ± 1.0

Wt is weight; LV is left ventricle. * indicates significant difference between young sedentary and old sedentary, † indicates significant difference between young sedentary and young trained, ‡ indicates difference between old sedentary and old trained, $P<0.05$. Values are means ± SEM

Table 2.2 Characteristics of 1A arterioles from soleus and the superficial portion of gastrocnemius muscles from YS, OS, YT, and OT groups.

	Sedentary		Exercise-Trained	
	Young (YS)	Old (OS)	Young (YT)	Old (OT)
N	36	32	21	15
Soleus Muscle Arteriole Lumen Diameter (μm)	120 ± 3	125 ± 4	112 ± 4	118 ± 4
Gastrocnemius Muscle Arteriole Lumen Diameter (μm)	151 ± 4	172 ± 5*	156 ± 7	188 ± 7
Soleus Muscle Arteriole Spontaneous Tone (%)	51 ± 3	45 ± 3	50 ± 4	40 ± 4
Gastrocnemius Muscle Arteriole Spontaneous Tone (%)	45 ± 3	40 ± 3	50. ± 4	47 ± 4

* indicates significant difference between young sedentary and old sedentary, † indicates significant difference between young sedentary and young trained, ‡ indicates difference between old sedentary and old trained, $P<0.05$. Values are means ± SEM

Table 2.3 Vascular sensitivity (EC_{50}) to Ang II in arterioles from soleus and the superficial portion of gastrocnemius muscles.

			Soleus Muscle Arterioles EC_{50} (M)	Gastrocnemius Muscle Arterioles EC_{50} (M)
Sedentary	Young (YS)	YS	$2.3\ E^{-10} \pm 0.6\ E^{-10}$	$1.5\ E^{-10} \pm 0.3\ E^{-10}$
		YS-E	$2.4\ E^{-9} \pm 1.0\ E^{-9}$	$1.7\ E^{-8} \pm 0.3\ E^{-8}$ #
		YS+L-NAME	$4.0\ E^{-9} \pm 2.2\ E^{-9}$	$1.9\ E^{-8} \pm 0.9\ E^{-8}$
	Old (OS)	OS	$3.0\ E^{-10} \pm 1.5\ E^{-10}$	$1.6\ E^{-10} \pm 0.5\ E^{-10}$
		OS-E	$8.6\ E^{-9} \pm 6.6\ E^{-9}$ #	$7.4\ E^{-8} \pm 3.4\ E^{-8}$ #
		OS+L-NAME	$4.0\ E^{-9} \pm 2.2\ E^{-9}$	$2.6\ E^{-8} \pm 1.1\ E^{-8}$ Ψ
Exercise-Trained	Young (YT)	YT	$1.2\ E^{-10} \pm 0.4\ E^{-10}$	$1.3\ E^{-10} \pm 0.6\ E^{-10}$
		YT-E	$1.0\ E^{-8} \pm 0.4\ E^{-8}$	$1.3\ E^{-8} \pm 0.7\ E^{-8}$
		YT+L-NAME	$1.6\ E^{-8} \pm 0.6\ E^{-8}$ §	$7.0\ E^{-9} \pm 3.2\ E^{-9}$
	Old (OT)	OT	$1.7\ E^{-10} \pm 0.6\ E^{-10}$	$1.5\ E^{-10} \pm 0.5\ E^{-10}$
		OT-E	$1.8\ E^{-8} \pm 1.0\ E^{-8}$ #	$6.4\ E^{-8} \pm 1.4\ E^{-8}$ #
		OT+L-NAME	$6.7\ E^{-9} \pm 3.4\ E^{-9}$	$1.4\ E^{-9} \pm 0.5\ E^{-9}$ Ψ

-E is endothelium removed. # indicates significant effect of removal of endothelium, § indicates significant effect of treatment of L-NAME, Ψ indicates significant different between endothelium removed and L-NAME, $P<0.05$. Values are means ± SEM

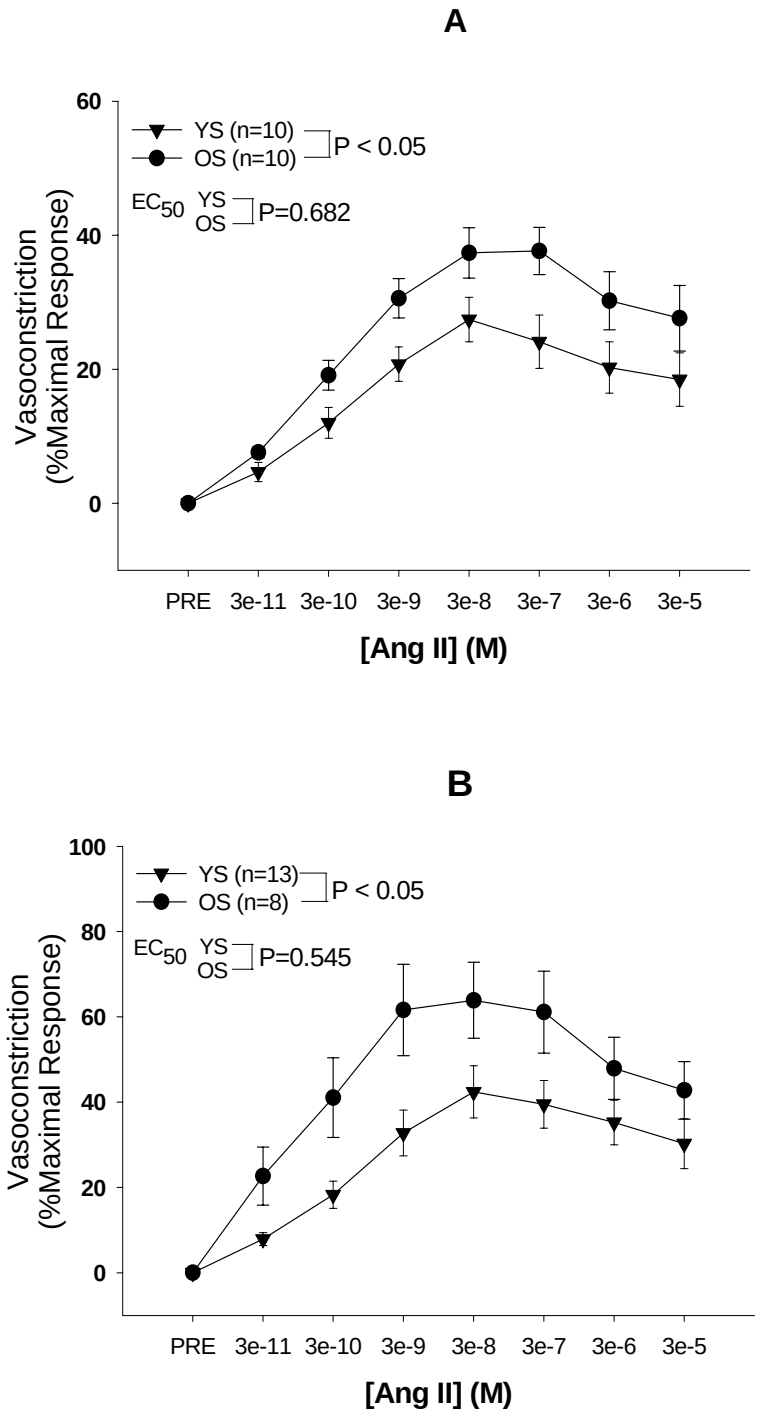

Figure 2.1 Comparison of vasoconstrictor response to the cumulative addition of Angiotensin II between young sedentary (YS) and old sedentary (OS) in soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

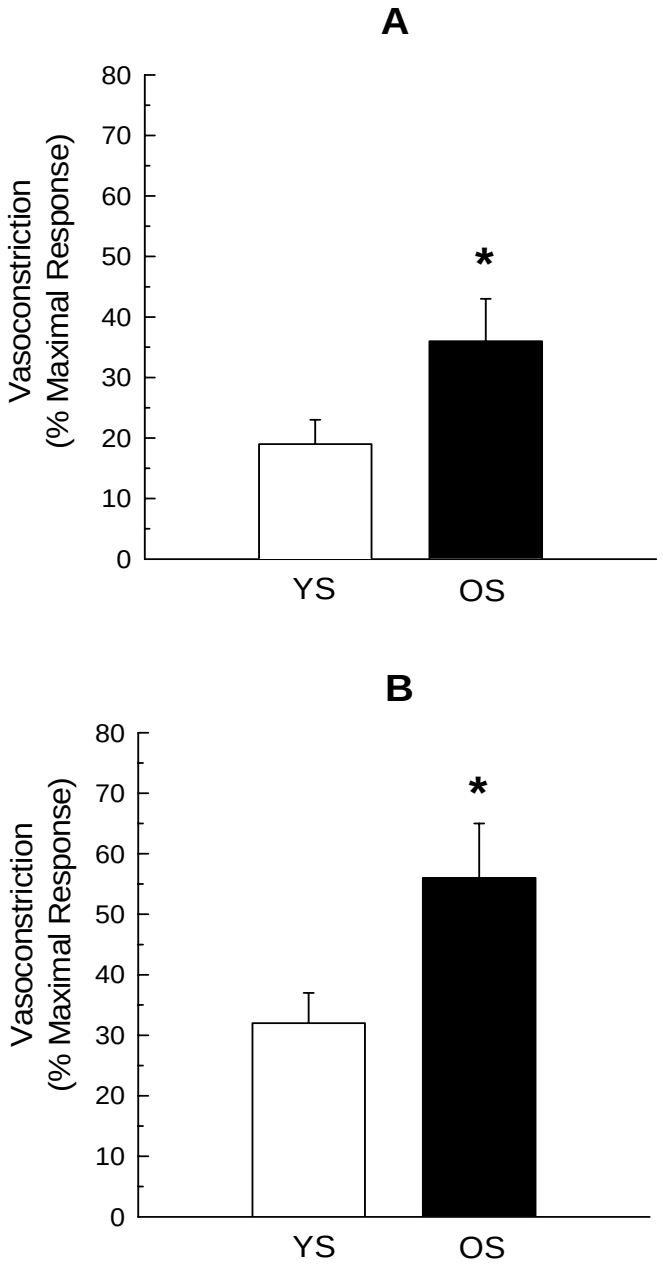

Figure 2.2 Comparison of vasoconstrictor response to the single dose of Angiotensin II [10^{-8} M] between young sedentary (YS) and old sedentary (OS) in soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM. * indicates significantly different between YS and OS. ($P<0.05$)

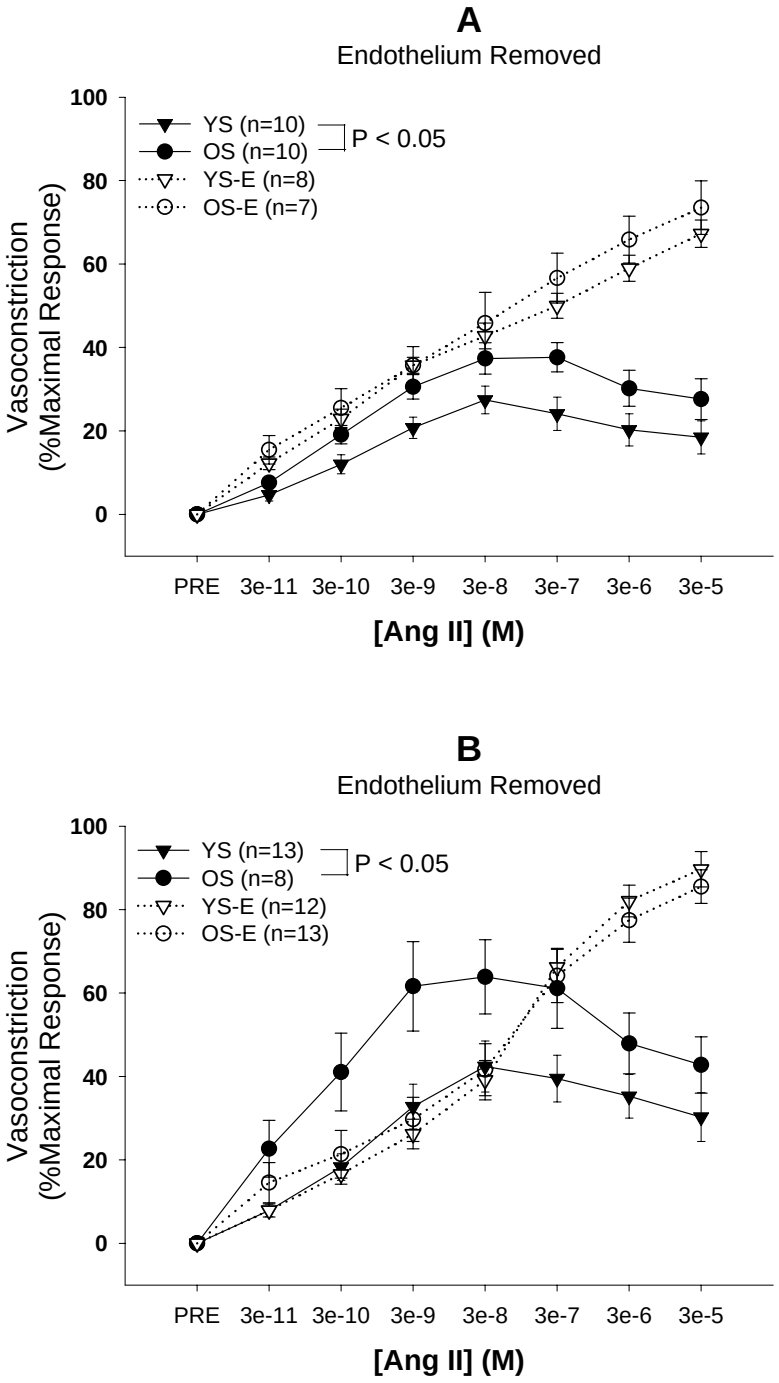

Figure 2.3 Effect of removal of endothelium on vasoconstrictor response to the cumulative addition of Angiotensin II among young sedentary (YS), endothelium removed young sedentary (YS-E), old sedentary (YS) and endothelium removed old sedentary (OS-E) in soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

Effect of NOS inhibition. Treatment with L-NAME resulted in a linear increase in the vasoconstrictor response to Ang II rather than the biphasic response occurring at the higher concentrations of Ang II in soleus and gastrocnemius muscle arterioles. (Figure 2.4). More importantly, age-related difference in Ang II-mediated vasoconstriction of soleus (Figure 2.4 A) and gastrocnemius (Figure 2.4 B) muscle arterioles were abolished by the removal of the endothelium.

Effect of exercise training. Exercise training decreased Ang II-mediated vasoconstrictor responses in arterioles from both soleus ($P<0.05$) (Figure 2.5 A) and gastrocnemius ($P<0.05$) (Figure 2.5 B) muscles of old rats, but training had no effect on arterioles from young rats from the soleus ($P=0.607$) and gastrocnemius ($P=0.965$) muscles.

Effect of removal of endothelium in old exercise trained arterioles. Following removal of vascular endothelium, the above-mentioned exercise training-mediated alterations in Ang II-induced vasoconstriction of soleus (Figure 2.6 A) and gastrocnemius (Figure 2.6 B) muscle arterioles from old rats were abolished.

Effect of NOS inhibition in old exercise trained arterioles. In the presence of L-NAME, the training-related decrease in Ang II-mediated vasoconstriction of old rat soleus (Figure 2.7 A) and gastrocnemius (Figure 2.7 B) muscle arterioles were likewise abolished.

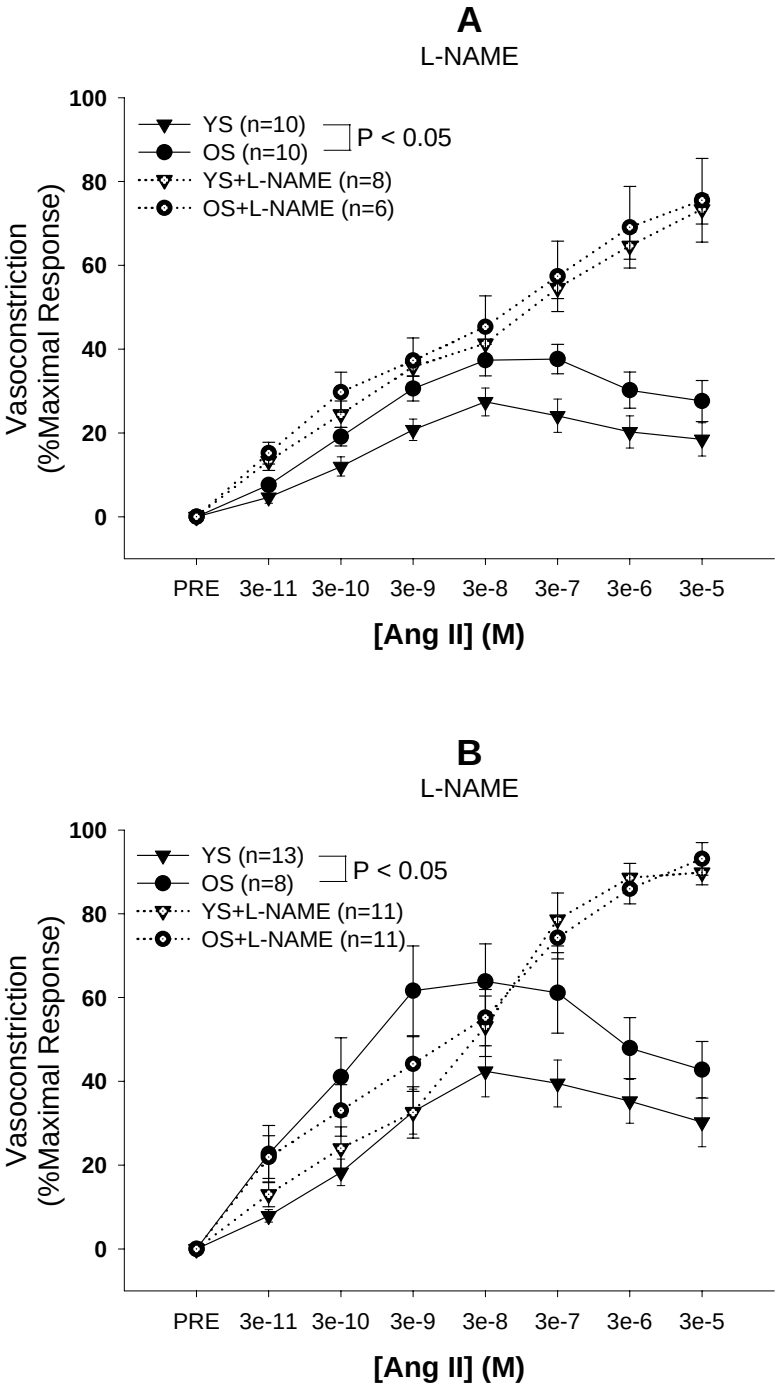

Figure 2.4 Effect of L-NAME on vasoconstrictor response to the cumulative addition of Angiotensin II among young sedentary (YS) young sedentary with L-NAME (YS+L-NAME), old sedentary (OS) and old sedentary with L-NAME (OS+L-NAME) in soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

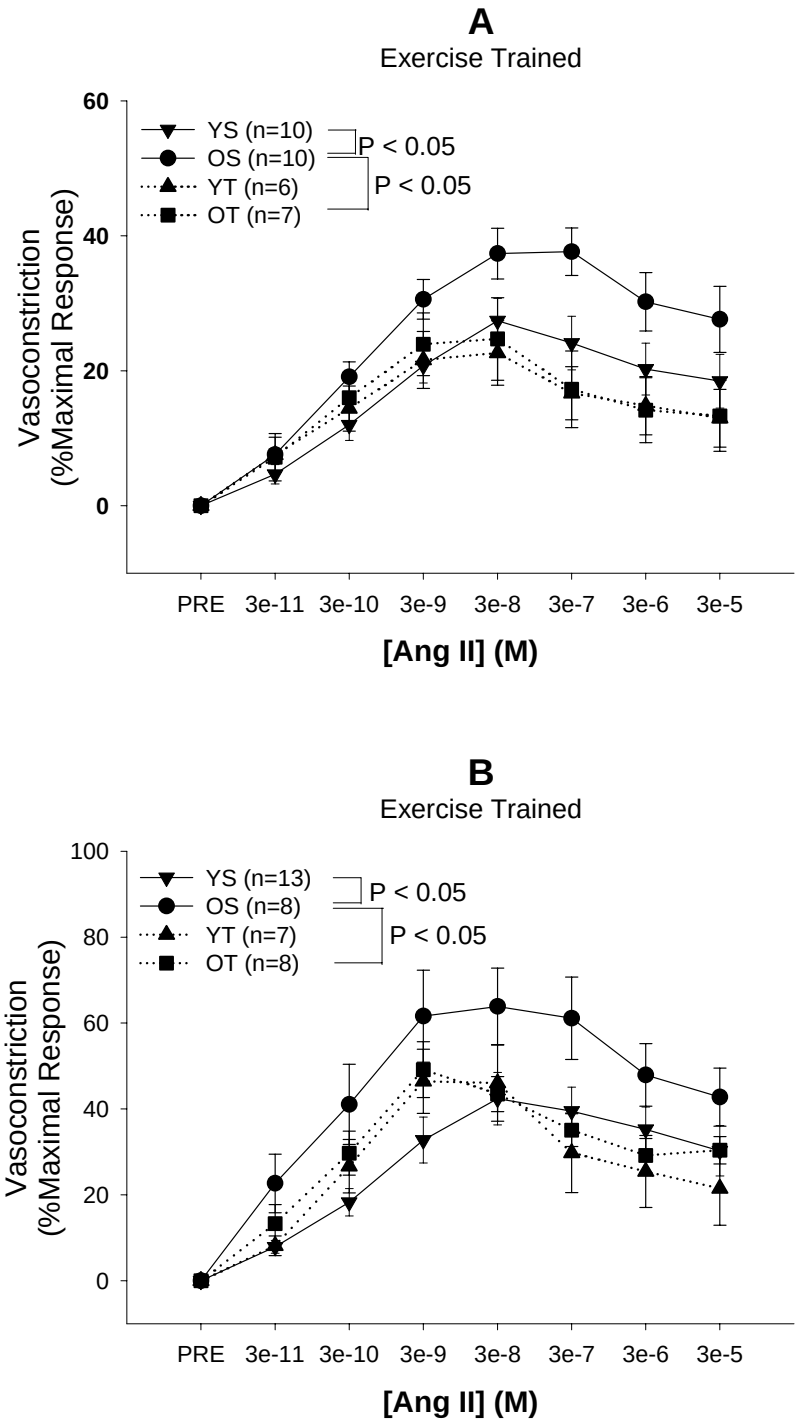

Figure 2.5 Effect of exercise training on vasoconstrictor response to the cumulative addition of Angiotensin II among young sedentary (YS), young exercise trained (YT), old sedentary (OS) and old exercise trained (OT) in soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

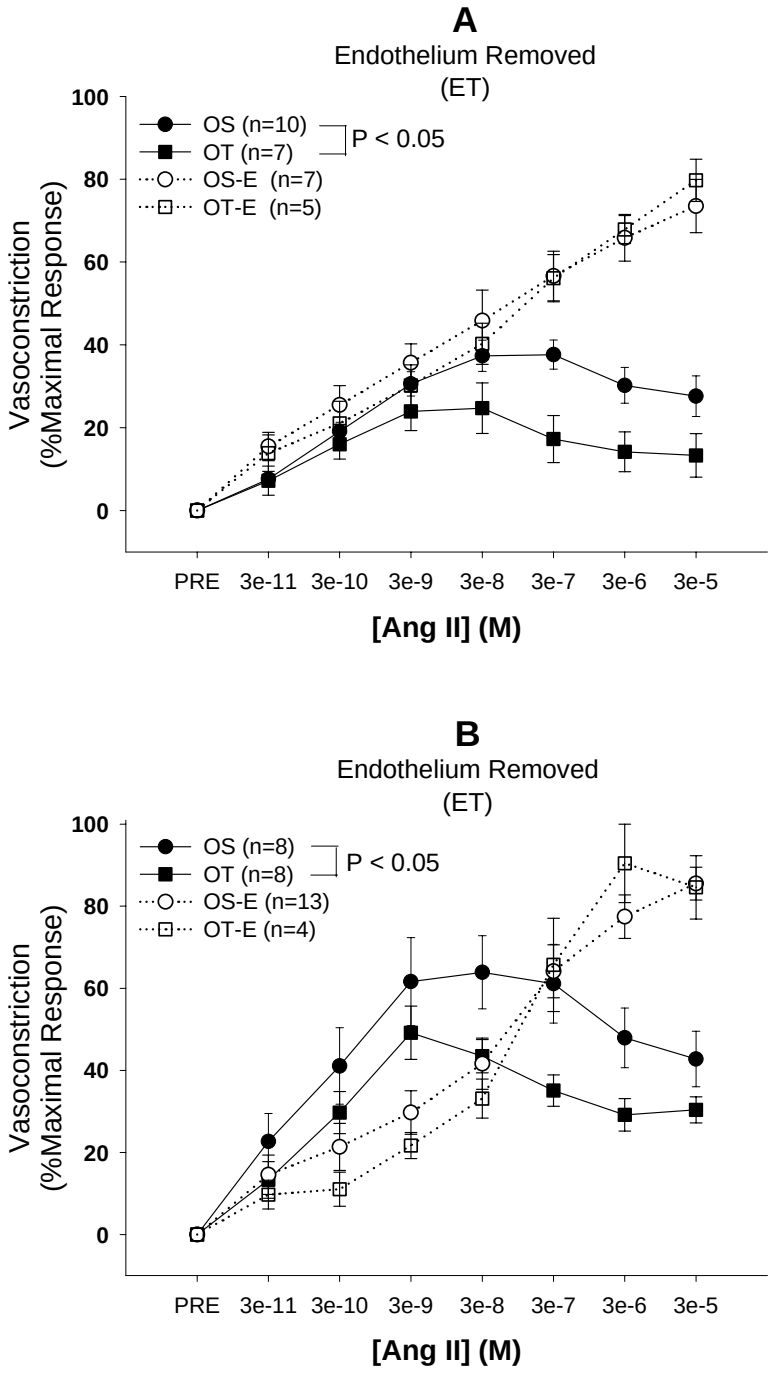

Figure 2.6 Effect of removal of endothelium on vasoconstrictor response to the cumulative addition of Angiotensin II among old sedentary (OS), old exercise trained (OT), endothelium removed old sedentary (OS-E), and endothelium removed old exercise trained (OT-E) in old soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

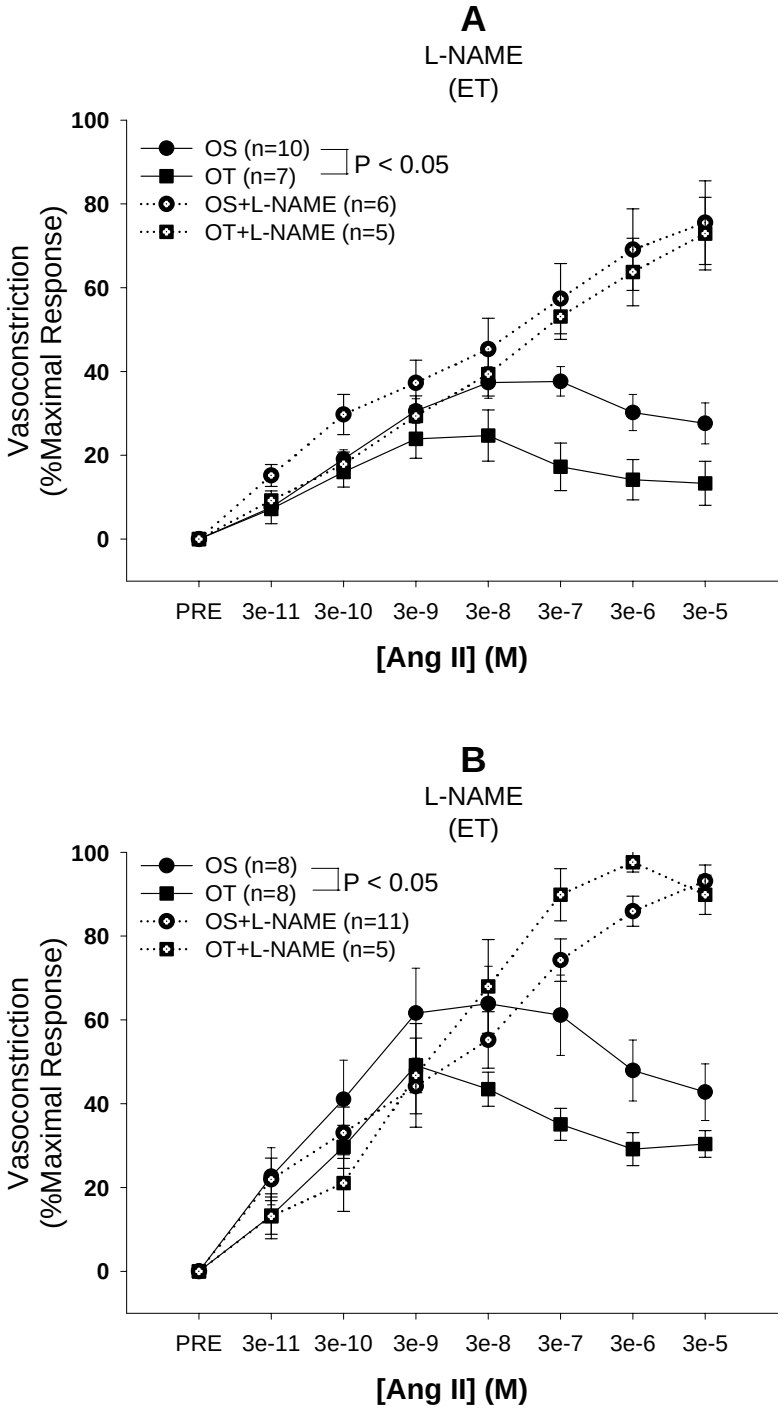

Figure 2.7 Effect of L-NAME on vasoconstrictor response to the cumulative addition of Angiotensin II among old sedentary (OS), old exercise trained (OT), old sedentary with L-NAME (OS+L-NAME), and old exercise trained with L-NAME (OT+L-NAME) in old soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

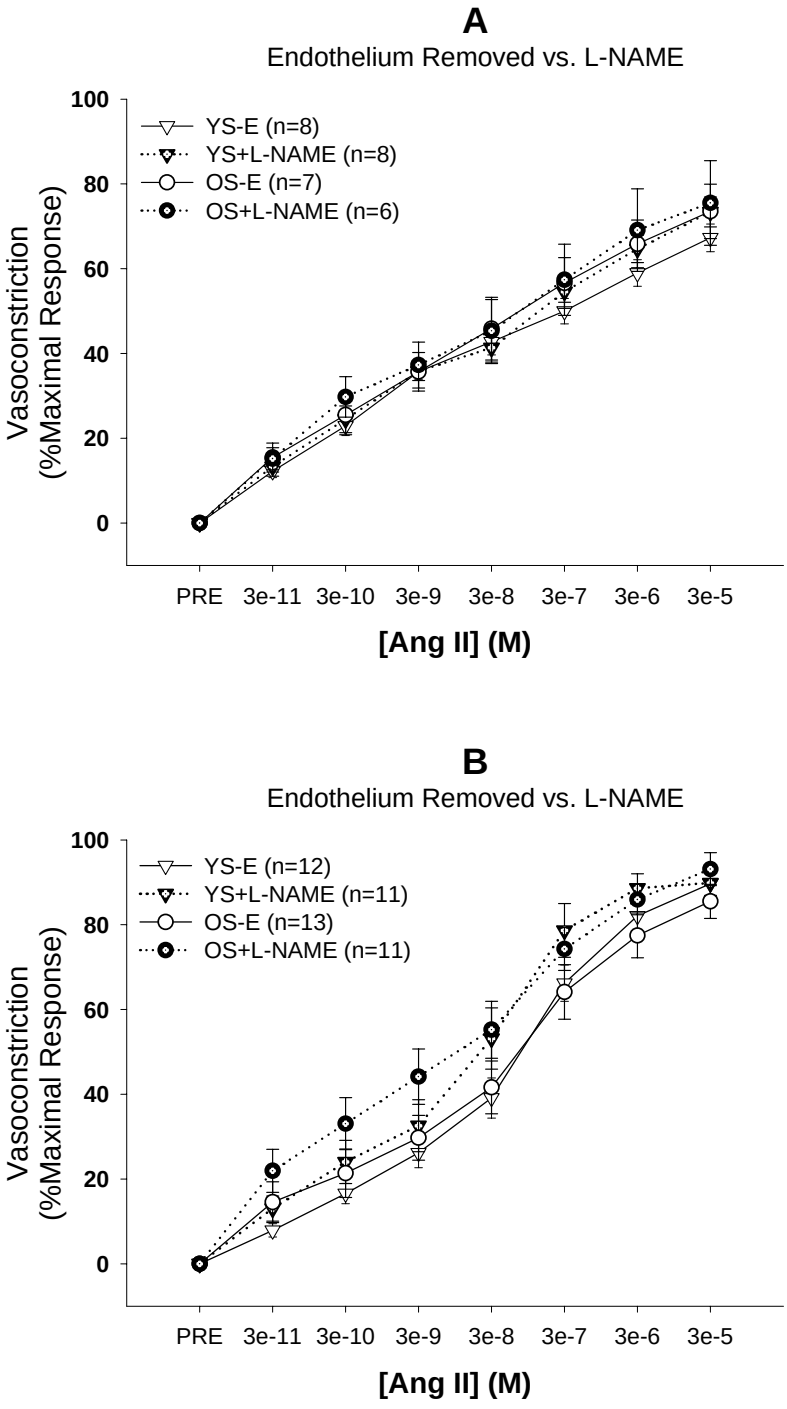

Figure 2.8 Comparison of effect of removal of endothelium and L-NAME on vasoconstrictor response to the cumulative addition of Angiotensin II in young and old sedentary soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

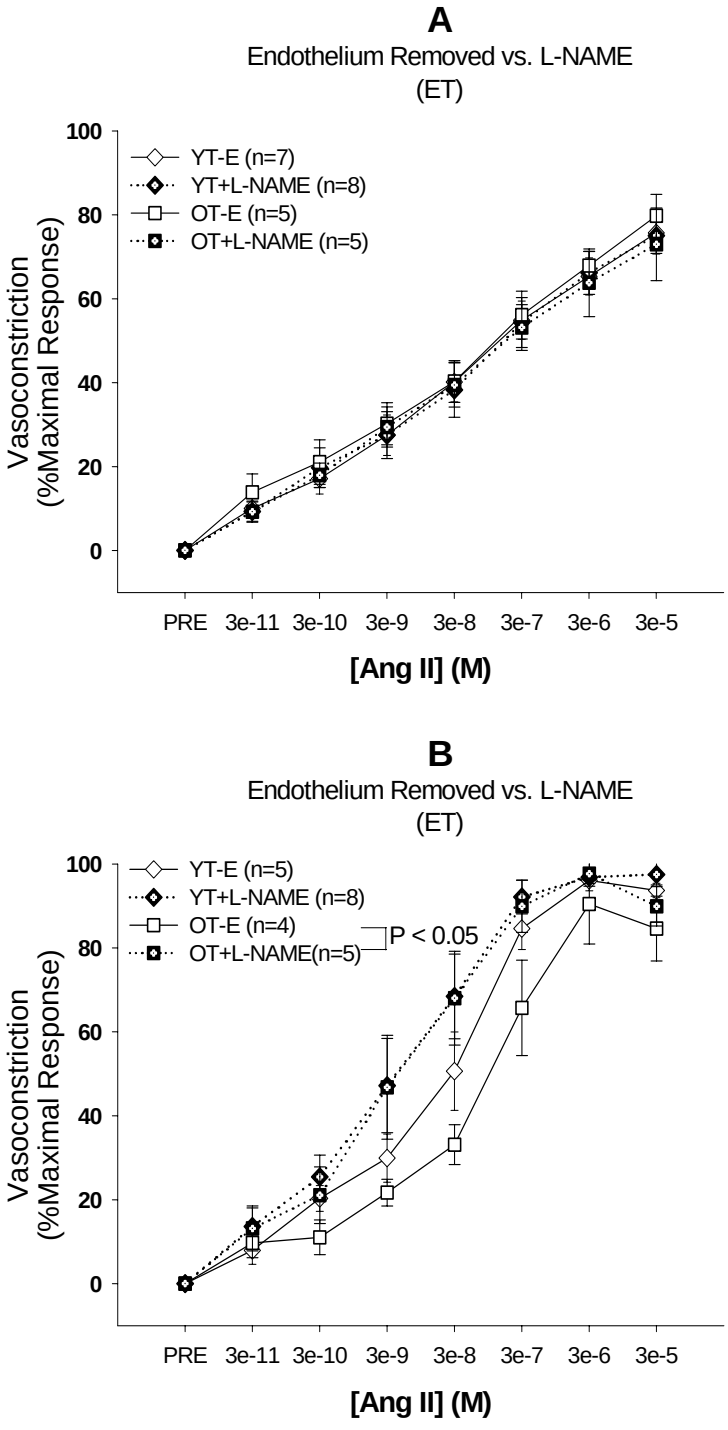

Figure 2.9 Comparison of effect of removal of endothelium and L-NAME on vasoconstrictor response to the cumulative addition of Angiotensin II in young and old exercise trained soleus (A) and gastrocnemius (B) muscle arterioles. Values are means ± SEM.

Comparison between removal of endothelium and L-NAME. Ang II-mediated vasoconstriction appeared to be identical between arterioles with the endothelium removed and those treated with L-NAME from young and old soleus muscle (Figure 2.8). In both young and old exercise trained arterioles from soleus muscle, the effects of removal of the endothelium on Ang II-mediated vasoconstriction were also not different from treatment with of L-NAME (Figure 2.9 A). However, in arterioles from exercise trained gastrocnemius muscle, Ang II-mediated vasoconstriction in L-NAME treated arterioles was higher compared to endothelium removed arterioles in old trained rats; responses in young trained rat gastrocnemius muscle arterioles were not different (Figure 2.8 B).

2.4 Discussion

The purpose of this study was to determine 1) whether aging alters Ang II-induced vasoconstriction of skeletal muscle arterioles, 2) whether exercise training modulates Ang II-induced vasoconstriction in young and old animals, and 3) mechanisms of putative aging- and exercise training-induced differences in Ang II-induced vasoconstriction in skeletal muscle arterioles. The present study provides several unique findings. First, aging enhances Ang II-induced vasoconstrictor responses in arterioles from both the highly oxidative soleus muscle and low-oxidative superficial portion of gastrocnemius muscle. Second, the age-associated enhancement of Ang II-induced vasoconstriction occurs through an endothelium-dependent NOS signaling mechanism. And third, exercise training ameliorates the age-associated increase in Ang

II-mediated vasoconstriction in both soleus and gastrocnemius muscles, and this too occurs through an endothelium-dependent NOS signaling pathway.

2.4.1 Determination of Ang II-induced vasoconstriction

Unlike other vasoconstrictor responses in the vasculature, Ang II-induced vasoconstriction is a biphasic response, where the most potent vasoconstriction occurs at the lower concentrations of Ang II (119). Our results (Figure 2.1) are consistent with those findings showing lower vasoconstrictor responsiveness with the highest concentrations of Ang II. Many studies have reported that activation of AT_1R results in vasoconstriction through increased Ca^{++} availability in the smooth muscle cell and binding to AT_2R causes vasodilation through increased NO synthesis (7, 11, 42, 65, 110). Thus, it appears that the net Ang II-induced vasoconstriction is the result of an interaction between vasoconstrictor influence via AT_1R and vasodilator influence through AT_2R. However, this view may be somewhat misleading for gaining understanding of the mechanisms of Ang II-induced vasoconstriction because several studies have reported the presence of vascular endothelial cell AT_1R which ,when stimulated, result in vasodilation (8, 87). What is clear is that the endothelium plays a very important role in modulating Ang II-induced contraction of blood vessels through an endothelium dependent vasodilator mechanism (8, 38, 40, 109, 120). Therefore, Ang II-induced vasoconstriction may be determined by the interaction of vasoconstrictor signals mediated through AT_1R and the vasodilator influence mediated through endothelial cells AT_1R and AT_2R. Although AT_2R are also located on smooth muscle cells in various vascular beds which mediate vasodilation (15), vasoconstriction

mediated through AT_1R is the predominant smooth muscle cell response. Our findings in skeletal muscle arterioles support the idea that the endothelium plays an important role in determining the biphasic Ang II-induced vasoconstrictor response (Figure 2.3).

2.4.2 Age-associated enhancement in Ang II-induced vasoconstriction

In humans, it has been shown that aging results in lower leg blood flow at rest and during exercise (5, 23, 60, 82, 84, 86). Although an impairment of endothelium-dependent vasodilator function has been reported as a mechanism for this age-associated decrease in muscle blood flow in human (21, 71, 98, 118) and rats (21, 71, 98, 118), the role of vasoconstrictor mechanisms to explain the reduced blood flow capacity to the skeletal muscle with advancing aging has not been as thoroughly explored. Recently, our laboratory found that old age-related reductions in the ability to increase muscle blood flow during exercise may be due to alterations in vasoconstrictor responsiveness. Donato et al. (27, 28) found that an augmented α-adrenergic vasoconstriction and ET 1-mediated vasoconstriction may play an important role in determining age-associated reduction in skeletal muscle blood flow. One other possible vasoconstrictor mechanism may be an enhanced Ang II-mediated vasoconstriction, since it is well established that Ang II acts as a potent vasoconstrictor (102). Studies concerning the effect of aging on Ang II-induced vasoconstriction are limited and equivocal. Mixed findings have been reported regarding the effect of aging on Ang II-related vascular responses; decreased vascular responsiveness to Ang II in aorta in rats with advancing age (9) and no effect of aging on constrictor response to Ang II in rat mesenteric resistance arteries (58). The discrepancy in these results may be due to differences in vessels studied. The present study

demonstrates that there is greater Ang II-induced vasoconstriction in arterioles from skeletal muscles of old rats (Figure 2.1).

Musch et al. (75) reported that although total hindlimb muscle blood flow is not altered with advanced age during submaximal treadmill exercise in rats, blood flow to specific muscles or muscle parts are altered based on the oxidative potential of the muscle. For example, perfusion of highly oxidative muscles (e.g., the soleus muscle) is lower during exercise in old rats, whereas blood flow to low oxidative muscles (e.g., the white portion of gastrocnemius muscle) is greater. Results from the present study showing enhanced Ang II-mediated vasoconstriction with old age in soleus muscle arterioles are consistent with the lower exercise hyperemia in the soleus muscle of aged rats. However, data from gastrocnemius muscle arterioles showing enhanced Ang II-mediated vasoconstriction appear to be at odds with the observation by Musch et al. (75) of a greater muscle perrfusion during exercise in this low oxidative muscle. Two factors could serve to explain this apparent discrepancy. First, several previous studies (6, 70, 71, 98) as well as the present study (Table 2.2) report that the luminal diameter of feed arteries and arterioles are enlarged in the white portion of gastrocnemius muscle from old rats. The increased size of the resistance vasculature in low oxidative muscles such as the white gastrocnemius muscle could contribute to the old age-related elevation in muscle blood flow during exercise despite a greater Ang II-induced vasoconstriction. A second potential factor contributing to the higher perfusion of low oxidative muscle is a change in the muscle recruitment pattern during exercise with aging. During moderate to high intensity treadmill running (20 m/min), the intensity of exercise used by Musch et

al. (75) to measure muscle blood flow in young and old rats, the muscles recruited in the young animals would predominantly be the high oxidative muscles, such as the soleus muscle, with little recruitment of low oxidative muscle (17, 59). However, perfusion of the highly oxidative hindlimb muscles in the old rats is lower than that in the young animals (75). The compromised delivery of oxygen to high oxidative muscle would likely cause premature fatigue of these muscles and necessitate the recruitment of other low oxidative muscles in order to maintain the exercise intensity imposed on the animals. Thus, the higher blood flow to low oxidative muscles in the older rats may more closely reflect a change in the motor unit recruitment pattern with age and consequently greater muscle activity and metabolism in the low oxidative muscle rather than a change in the vasomotor properties of the arterioles.

2.4.3 Mechanisms for age-related increase in Ang II-induced vasoconstriction

The present study indicates that the mechanisms for the age-associated alteration in Ang II-induced vasoconstriction is an endothelium-dependent mechanism, since aging differences were abolished with the removal of the endothelial cell layer (Figure 2.3). Previous studies have shown that aging-induced alterations in NE-mediated vasoconstrictor responses occur through an endothelium-dependent mechanism (27), whereas aging-induced enhancement of ET 1-mediated vasoconstriction is a smooth muscle cell-dependent mechanism through the ETa receptors (28). Results from the present study, as well as these of NE-mediated vasoconstriction (27), are consistent with previous studies reporting that endothelial vasodilator function declines with age (21, 71, 118). Moreover, previous studies demonstrating Ang II-induced vasoconstriction has an

endothelium-dependent component supports the finding of this study. For example, Gruetter et al. (38) reported that malfunction of the endothelium significantly increases Ang II-induced contraction in rat aorta and bovine coronary artery, and Haberl et al. (40) suggested that Ang II produces a vasodilator factor from the endothelium in the rat cerebral arterioles. Therefore, dysfunction of endothelial cell signaling with aging can be an important mechanism to determine an age-associated enhancement of Ang II-mediated vasoconstriction.

As previously described, endothelium-dependent vasodilation is n aimportant factor in determining Ang II-induced vasoconstrictor responsiveness. Three pathways responsible for endothelium-dependent vasodilation are currently known: NO, which is released by action of NOS, prostacyclin (PGI_2), which is formed through cyclooxygenase (COX), and endothelium-derived hyperpolarizing factor (EDHF). The present results indicate that the NOS signaling pathway plays the major role in determining the age-associated alteration in Ang II-induced vasoconstriction, since this age-associated difference is eliminated with treatment of L-NAME in the arterioles from soleus (Figure 2.4 A) and gastrocnemius (Figure 2.4 B) muscles. Moreover, as illustrated in Figure 2.8, the effect of NOS blockade and effect of the removal of endothelium on Ang II-induced vasoconstrictions are comparable in arterioles from soleus (Figure 2.8 A) and gastrocnemius (Figure 2.8 B) muscles. These results suggest that NO is the major mechanism responsible for the endothelium-dependent vasodilator component of Ang II-induced vasoconstriction in rat skeletal muscle arterioles. Although some studies have reported that Ang-II induced vasodilation through the endothelium is dependent on the

COX mechanism in rat cerebral arterioles (40) or, neither NOS nor COX (4), the preponderance of evidence demonstrates that Ang II-induced constriction is modulated by the release of endothelium-derived NO through endothelial AT_1R in rat carotid artery (8) and cultured endothelial cell from rat aorta (87), and endothelial AT_2R (65) in rat mesenteric resistance arteries and in rat aorta (116). Pueyo et al. (87) found that NO production in the endothelial cell is augmented by Ang II infusion and this increased NO production is abolished by losartan, an AT_1R-specific antagonist. They also found that cyclic GMP levels are increased with Ang II infusion, but it is reduced with losartan. These data suggest that Ang II can elicit endothelium-dependent vasodilation by Ang II-induced NO release and it may modulate the direct vasoconstrictor effects of Ang II on smooth muscle cells through increased cGMP production. Moreover, Ang II infusion with NG-monomethyl-L-arginine (L-NMMA), another NOS antagonist, results in less NO production and the treatment of L-NAME with Ang II decreases blood flow and vascular conductance (105).

2.4.4 The effect of exercise training on Ang II-induced vasoconstriction

Although several studies have been performed to investigate the effects of chronic exercise training on vasoconstrictor responsiveness to NE and ET-1 (20, 27, 99), no direct study elucidating the effect of endurance training on Ang II-induced vasoconstriction has been reported. It is believed that this present study is the first to examine the long-term exercise training effect on vasoconstrictor responsiveness to Ang II in rat skeletal muscle arterioles in young and old rats. Our results indicate that exercise training reduces Ang II-mediated vasoconstriction in arterioles from soleus and

gastrocnemius muscles of old rats, but has no effect on arterioles from young rats (Figure 2.5). This present study is consistent with the study of Donato et al. (27) showing that the effect of exercise training on the NE-mediated vasomotor response occurs only in aged skeletal arterioles, whereas previous studies also reported that long-term exercise training reduces NE-mediated vasoconstrictor responsiveness in abdominal aortas from young animals as well (20, 99). This discrepancy related to the effects of exercise training on NE-mediated vasoconstriction in young animals may be due to different adaptations to exercise in different vascular beds or differences in the type of artery studied, i.e., conduit arteries vs. resistance arterioles. It has shown that endurance training can increase leg blood flow in aged humans (22, 64, 106) and that the mechanism for this exercise training-induced increase in blood flow in the elderly is through endothelium-dependent vasodilation (19, 20, 22, 98, 106). Our results suggest that endurance exercise training can also ameliorate an age-associated enhancement of Ang II- induced vasoconstriction through an endothelium-dependent pathway, and that this may be one contributing mechanism to enhance blood flow in trained elderly individuals.

2.4.5 The mechanism for exercise training-associated reduction in Ang II-induced vasoconstriction

In addition, the present results suggest that the mechanism of this reduced Ang II-induced vasoconstriction in exercise trained arterioles from old skeletal muscle is the endothelium-dependent NOS signaling pathway (Figure 2.6 & Figure 2.7). It has been shown that activation of endothelial AT_1R and AT_2R results in the release of NO (8, 65,

87, 116) and PGI_2 (40), which promote vasodilation in the blood vessel. The present study indicates that NO is fully responsible for the exercise training-associated reduction in Ang II-induced vasoconstriction in the rat skeletal muscle arterioles (Figure 2.9). Although no direct studies were performed to investigate the possible PGI_2 and EDHF mechanisms on Ang II-induced vasoconstrictor responses, the notion that training primarily affects the endothelial NOS signaling pathway is supported by the finding of Spier et al. (98), who found that exercise training enhances the endothelium-mediated vasodilation via the NOS pathway in rat skeletal muscle arterioles from old rats.

One of the mechanisms to improve vasodilator function by exercise training is increased eNOS mRNA and protein expression in the vascular endothelium through a vascular shear stress stimulus (98). Exposure of the endothelium to exercise-induced increases in Ang II concentration is another possible mechanism to increase eNOS expression in the endothelium. The plasma concentration of Ang II is increased during dynamic exercise, and there is a reported doubling during exercise at 80% of maximal heart rate (114). Also, it has been reported that Ang II increases eNOS mRNA and protein expression (43, 79). Hennington et al. (43) found that acute Ang II-infusion (8 ng/kg/min) for 110 minutes resulted in increased renal eNOS mRNA, but no change in renal eNOS protein concentration, whereas chronic Ang II-infusion (5 ng/kg/min) for 10 days increased renal eNOS protein content, but no change in renal eNOS mRNA (43). Olson et al. (79) also found that in the bovine pulmonary artery endothelium that eNOS mRNA expression was increased 2.4-fold with 4 hours of 1μM Ang II infusion and eNOS protein content was elevated 3.3-fold with Ang II infusion for 8 hours. These data

suggest that chronic elevations in plasma Ang II concentration with dynamic exercise training could serve as a stimulus to increase levels of eNOS mRNA and protein in the endothelium. Consequently, there could be a greater vasodilator component to Ang II vasomotor responses in arterioles from exercise trained animals, resulting in a diminished net vasoconstrictor response.

2.4.6 Conclusion and significance

In conclusion, Ang II-induced vasoconstriction is determined by the net effects of a potent smooth muscle cell vasoconstrictor response and less potent endothelium-dependent vasodilator influence via a NOS signaling pathway. With aging, Ang II-mediated vasoconstriction is enhanced due to an age-associated dysfunction of the endothelium-dependent NOS vasodilator mechanism. Exercise training can ameliorate this age-associated enhancement of Ang II-induced vasoconstriction through an enhancement in the endothelium-dependent NOS signaling pathway.

Although the majority of studies in the literature have focused on the role of Ang II in the regulation of renal and splanchnic blood flow, Ang II is also important for the regulation of skeletal muscle blood flow during exercise. Since aging can elevate the Ang II-induced vasoconstrictor responsiveness of skeletal muscle arterioles and exercise training can ameliorate this enhanced vasoconstriction to Ang II, these data in the present study suggest that the effects of aging and exercise training on skeletal muscles vasomotor responsiveness to Ang II may play a role in old age-associated reductions in skeletal muscle blood flow and the training-induced restoration of muscle perfusion during exercise.

CHAPTER III

SUMMARY AND CONCLUSION

The overall purpose of this dissertation research was to determine whether aging alters Ang II vasoreactivity of skeletal muscle resistance arterioles and whether exercise training can ameliorate possible alterations in Ang II-induced vasoconstriction. A secondary purpose was to elucidate the mechanism(s) of possible aging and exercise training effects on Ang II-induced vasoconstrictor responses of skeletal muscle resistance arterioles from old rats. We tested six hypotheses and found that:

1) there was higher Ang II-induced vasoconstriction in arterioles from soleus and gastrocnemius muscles of old vs. young rats (Figure 2.1),
2) removal of the endothelium abolished the age-associated difference of Ang II-mediated vasoconstriction in soleus and gastrocnemius muscle arterioles (Figure 2.3),
3) inhibition of NOS activity with L-NAME abolished the age-associated difference in Ang II-mediated vasoconstriction in soleus and gastrocnemius muscle arterioles (Figure 2.4),
4) exercise training decreased Ang II-mediated vasoconstriction in aged arterioles from soleus and gastrocnemius muscles (Figure 2.5),

5) removal of the endothelium eliminated the exercise training-associated reduction in Ang II-mediated vasoconstriction in arterioles from soleus and gastrocnemius muscles of old rats (Figure 2.6), and
6) inhibition of NOS activity with L-NAME eliminated the exercise training-associated reduction of Ang II-mediated vasoconstriction in arterioles from both soleus and gastrocnemius muscles of aged rats (Figure 2.7).

In conclusion, aging results in enhanced Ang II-mediated vasoconstriction in the arterioles from the rat skeletal muscle due to age-associated dysfunction of endothelium-dependent NOS signaling pathway. However, exercise training could ameliorate this age-associated increase in Ang II-induced vasoconstriction in the arterioles from the rat skeletal muscle through an enhanced endothelium-dependent NOS signaling mechanism.

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VITA

Yoonjung Park

ADDRESS (Office)
Department of Veterinary Physiology & Pharmacology
College of Veterinary Medicine and Biomedical Sciences
Texas A&M University
College Station, TX 77843-4466
(979) 845-0976

EDUCATION

INSTITUTION	DEGREE	YEAR	FIELD OF STUDY
Texas A&M University	Ph.D.	2003-2006	Exercise Physiology
The University of Texas at Austin	M.A.	2000-2003	Exercise Physiology
Seoul National University	M.S.	1996-1998	Exercise Physiology
Seoul National University	B.A.	1992-1996	Physical Education

PROFESSIONAL EXPERIENCE

2003-2006	Graduate Research/Teaching Assistant, Department of Health and Kinesiology, Texas A&M University, College Station, TX
2001-2003	Graduate Teaching Assistant, Department of Kinesiology & Biological Science, The University of Texas, Austin, TX
1998-2000	Exercise Specialist, Sports and Health Medical Center, Asan Medical Center, Seoul, Korea
1997-1998	Graduate Research Assistant, Department of Physical Education Seoul National University, Seoul, Korea

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