EFFECTS OF AGING AND EXERCISE TRAINING ON STRUCTURAL AND VASOCONSTRICTOR PROPERTIES OF SKELETAL MUSCLE ARTERIOLES

A Dissertation

by

ANTHONY JOHN DONATO

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

August 2004

Major Subject: Kinesiology

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ABSTRACT

Effects of Aging and Exercise Training on Structural and Vasoconstrictor Properties of Skeletal Muscle Arterioles. (August 2004)

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Aging is associated with increases in regional and systemic vascular resistance and arterial blood pressure. One possible mechanism through which these age-associated alterations occur is enhanced vasoconstrictor responsiveness, or alterations in the structural properties of the resistance vasculature. We hypothesized that stiffness and vasoconstriction would be greater in skeletal muscle arterioles from old rats, and that endurance exercise training would ameliorate the associated with aging alterations. METHODS: Young sedentary (YS; 4 months), old sedentary (OS; 24 months), young trained (YT) and old trained (OT) male Fischer 344 rats were used. Training modality was treadmill exercise at 15 m/min up a 15o incline, 5 days/wk for 12wks. Skeletal muscle first-order arterioles were isolated for in vitro experimentation. Intraluminal diameter was measured in response to the cumulative addition of endothelin-1, norepinephrine, KCl, and isoproterenol. Stiffness was measure by examining the arterioles' stress and strain

relation to increased luminal pressure in Ca^{++} free solution. RESULTS: Skeletal muscle arterioles had augmented vasoconstriction to endothelin-1 and norepinephrine. Adrenergic vasodilation was diminished in aged rat arterioles. Stiffness increased with age. Exercise training ameliorated the age-associated changes in stiffness and norepinephrine vasoconstriction. Exercise training did not alter endothelin-1 vasoconstriction or adrenergic vasodilation. CONCLUSIONS: These findings suggest that enhanced vascular sensitivity to vasoconstrictors and increased arteriole stiffness may play a role in the increase in skeletal muscle and systemic vascular resistance and, thus, contribute to the elevated blood pressure which occurs in aging humans. These results also demonstrate some of the cardioprotective effects of exercise training.

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INTRODUCTION

1.1 The cardiovascular system and control of muscle blood flow

Appreciation of the cardiovascular system in some form or another most likely has been around since man has hunted or participated in war with neighboring tribes. The idea that "blood" was a key link to survival was probably well comprehended by very early man. Actually there is data to support the fact that sight or smell of "blood," ones own or another's, initiates very strong instinctual drives in animals and man. Thus, there may be an innate understanding of the cardiovascular system and its importance to the maintenance of life.

Unfortunately, since *Homo erectus* did not document any of these important findings we must continue our historical journey forward to the time of the ancient Egyptians. The Egyptians (1700 B.C.) were most likely the greatest forward thinkers in all of history. They were able to establish a written language, form the basics of mathematics and engineering, create division of work and laws. In addition to these monumental discoveries, they were the first to document in the *Papyrus Ebers* the relation of the pulse in the limbs to the heart. In addition, they were able to describe a vessel network to carry the pulse from the heart to the periphery. They also described the fact that the "breath" travels from the lungs to the heart. Still, they did not manage to associate the blood with the vasculature and heart (77).

In ancient Greece, the greatest philosophers of all time still had a limited

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This dissertation follows the style of the *American Journal of Physiology.*

understanding of the circulatory system. The first work in biology, which was undertaken by Plato, was eventually expanded into comparative anatomy and physiology by Aristotle. Aristotle was one of the first to make a classification system of animals and start to relate anatomical structures with physiological function (77). Still, function of the cardiovascular system with regards to cardiac function and circulating blood remained unexplored. Hippocrates (460-375 B.C.) developed the concepts of the four humors, which included the blood, although it was not thought to circulate (77). Since the mystery of the cardiovascular system remained unsolved by the ancient Greeks, the burden of discovery was shifted to the next great society, the Roman Empire.

Interestingly, it was the very successful Greek physician of Marcus Aurelius who forwarded the thought of the functions of the cardiovascular and nervous systems. Galen (131-201 A.D.) proposed that spirits, animal, vital and nutritive, flowed in the body via the nerves, arteries, and veins. He proposed that the animal spirits flowed from the central nervous system through hollow nerves in a coordinated manner to produce movement that was termed “sympathy” (104). Air flowed from lung to heart and became the vital spirit which was distributed by the boiling over of the heart, which then was distributed in the blood via the arteries. Nutritive spirits were absorbed from the intestine and proceeded to the liver where they were distributed by the veins. This schema was widely accepted due to its comprehensive explanation of the cardiovascular and nervous system. It also allowed for the inclusion of the traditional view of humors and its relation to health developed by Hippocrates (77). Thus, this view published in the book *De Usu*

Partium, became the dogma for many centuries until the true physiological function of the cardiovascular system began to become discovered in the 15th and 16th centuries.

I believe it must have been very difficult for scientists of the era to come forward with data or hypotheses to refute Galen's theory. This theory had been taught and was believed to explain most systems of the human body for approximately 1,400 years! Modern physiologists cannot even fathom a dogma so powerful and ingrained in life as Galen's theory. For example, arguably one of the most brilliant men in history, Leonardo da Vinci (1425-1519) created detailed diagrams of the cardiovascular system including the valves in the heart, veins, as well as detailed diagrams of hemodynamic flow through tubes including eddy formation. All this anatomical detail distinctly shows the possibility for an alternate hypothesis to that purported by Galen. However, da Vinci remained a devout believer in the Galen theory (77). Therefore, it is not surprising that the Galen theory was eroded slowly by the scientists of the time and not replaced impetuously.

A typical anti-Galen action was made by the Parisian military physician, Ambroise Pare (1510-1590), who reintroduced ligatures for injured soldiers anddiscouraged bloodletting as a treatment for disease (77). The final blow to the Galen theory was delivered by famed researcher Sir William Harvey (1578-1657), who when studying chick development with famous Italian anatomist Fabrizzi, was puzzled by the structure of the heart, arteries and veins. After detailed experiments measuring blood outflow with beakers, Harvey proposed in 1616 that blood must

circulate (47). Still, his famed book, *De Motu Codis,* was not published until 12 years later. The *De Motu Codis* gave the basis of the scientific method and also included a detailed explanation of the heart's pumping mechanism and the outflow of blood via the arteries and its inflow via the veins. Harvey hypothesized that arteries and veins must form anastamoses, but this could not be proven until the advent of the microscope (104). Though major errors can be discovered in Harvey's book, the principles forwarded had finally put an end to Galen's theory.

The orphaned Reverend Stephen Hales (1677-1761) picked up where Harvey's *De motu codis* left off. Hales, a true renaissance man, was for many years vicar in Teddington, England, and interested in plant physiology and human physiology. The Reverend was the first to measure both systolic and diastolic blood pressure in an assortment of animals (47). He also made the first accurate measurements of cardiac output or "circulation rate" by casting ventricles of animals and measuring heart rate. These measurements allowed Hales to estimate the resistance of the vasculature and postulate that this resistance is modified by the constriction or relaxation of vessels. He also estimated transit time of blood through capillaries (47). For these achievements, many have designated Stephen Hales as the father of hemodynamics.

Building on the principles that Stephan Hales had put forth, many mathematicians and physicists started to put together the laws of hemodynamics. Bernoulli (1700-1782) defined the laws of fluid hydraulics, Laplace (1749-1827) supplied a description of wall tension and it's relation to transmural pressure and diameter, Poiseuille (1799-1869) described the flow of Newtonian fluids through

tubes, and Starling (1860-1927) explained fluid exchange at the capillaries as extensions of Hale's initial discoveries (77). These hemodynamic equations and relations became the building blocks of modern cardiovascular research which are still used today.

Further explanation of how arteries constrict came in 1727 when Francois Petit (1664- 1741) showed that cutting sympathetic nerves causes arteries to vasodilate (104). Still, a true appreciation of the role of the sympathetic nervous system in the control of arterial tone did not come until much later. In 1853, Claude Bernard (1813-1876) showed that by lesioning and stimulating the sympathetic nerves, he could tonically restrict blood flow (47). Gaskell (1843-1912) added to these observations by demonstrating that nearly every peripheral tissue was influenced by the autonomic nervous system input via different chemical changes which he coined "parasympathetic" and "sympathetic" transmitters (104). In 1878, Gaskell further expanded the understanding of vascular tone by demonstrating its inhibition by metabolic by-products This was the first indication that local tissues could modulate tone, and therefore, potentially modulate local blood flow (104). William Bayliss (1860-1924) discovered in 1902 that vascular tone could be modulated by changes in wall tension created by changes in transmural pressure (62). This indicated for the first time that there was an inherent vascular tone due to the properties of smooth muscle and not just the influence of the sympathetic nervous system. Walter Cannon hypothesized in 1912 that the adrenal medulla released epinephrine which could alter blood flow to active muscle (117). This would be proven by Barcroft in 1956 (9). These important discoveries

demonstrated that vascular resistance and blood flow regulation was due to a combination of factors: myogenic, local, humoral, and neural.

With the present knowledge of cardiovascular hemodynamics, 20th century physiologists were able to explore the areas of cardiovascular regulation at rest and during cardiovascular stress. One of the most potent stressors of the cardiovascular system is aerobic exercise. Aerobic exercise allows the physiologist to test the maximal capacity of the cardiovascular system. Aerobic exercise also allows for a better understanding of how the distribution of tissue blood flow is regulated.

1.2 The dawn of exercise physiology

The following section will give a brief review of the dawn of exercise physiology as a science, the pioneering exercise physiologists, and their seminal findings. Where possible this review will focus on cardiovascular regulation of blood flow and the studies which led to a better understanding of why the regulation of blood flow is important in exercise physiology. The basic foundations of exercise physiology have enabled today's scientists to understand how chronic physical activity benefits the cardiovascular system in natural aging and pathophysiological conditions (e.g., coronary heart disease, hypertension, and diabetes).

Exercise physiology has been a discipline for centuries, yet scientific research yielding a better understanding of the integration of systems physiology has only recently started. The first integrative scientific experiments related to exercise where carried out by Lavoisier in 1777. He related O_2 consumption and CO_2 production to the respiratory demand during exercise (84). In 1912, Ernest H.

Starling demonstrated the length-tension relation, describing increases in left ventricular contractile force in response to increases in diastolic filling pressure (66). Starling postulated the importance of venous return during exercise on the increase in stroke volume. He stated, "… as man starts to run, his muscular movements pump more blood into the heart so increasing venous filling (and stroke volume due to length tension relation), while the central nervous system, by contracting the arteries, raises blood pressure and forces all the available blood through the active muscles" (66). Interestingly, in this statement he seems to suggest a redistribution of blood flow away from non-muscular tissues during exercise. This is truly an advanced hypothesis, since experiments to examine this possibility would not be done for another 20 years. The first studies to relate blood pressure, cardiac output, and oxygen "intake" during exercise were performed by Stenstrom and Lilistrand from 1915-1920. These studies provided the first truly integrative measurements of muscular work, respiration, and cardiovascular function during exercise (84). In addition, these studies were done in a variety of exercise modalities including swimming, running, and cross-country skiing, of which cross-country skiing produced the highest oxygen "intake." Stenstrom and Lilistrand established the Scandinavian expertise in exercise physiology, which still holds true today because many of the best exercise physiologists originate in Scandinavia. The true father of exercise physiology in the English speaking world was the Nobel laureate, A.V. Hill, who first showed that muscle metabolism measured by heat production was independent of oxygen consumption, indicating separate aerobic and anaerobic energy pathways (12). Still, not even the greatest of

scientists are without erroneous findings. Hill's theory of muscle shortening, which he hypothesized was due to polar charge attraction, was replaced by Huxley and Huxley's sliding filament theory (12). Hill, after winning the Nobel Prize, undertook human experiments in applied exercise physiology to the dismay of many of his colleagues who viewed exercise physiology as a waste of energy on a trivial topic. A.V. Hill was the first to define the term maximal oxygen uptake (VO_2 max) which since has become a bench mark of aerobic fitness (12). In addition, Hill defined its determinants (1924): 1) arterial oxygen saturation, 2) mixed venous saturation, 3) the oxygen capacity of blood, and 4) the circulation rate, thus showing he had a strong appreciation of integrative physiology. Hill also equated maximal cardiac output as an important determinant of VO_2 max. He also related VO_2 max as a critical determinant of athletic performance (12). Since these hypotheses have been made exercise physiologists have become obsessed with measures of VO_2 max, many times mistaking VO_2 max as the only important variable with respect to performance or function when many other variables may be more suitable. Still, A.V. Hill's contributions to the field allowed many more scientists to undertake experiments in applied exercise physiology and established exercise physiology as a "true science."

Joseph Barcroft (1872-1947) was a true pioneer in distribution of blood flow. He was accomplished in both animal and human models of measuring blood flow and was one of the first to measure exercising blood flow in human skeletal muscle (8). Shortly after A.V. Hill's contributions in exercise physiology, Barcroft designed several experiments in dogs to determine how blood flow distribution

affected the peripheral circulation at rest and during exercise. In 1929, he showed that blood volume was shunted away from the spleen during exercise in dogs (77). Later experiments in 1935 showed that manually constricting the aorta above the mesentery caused increases in venous return and cardiac output, while constricting flow to the legs decreased venous return and subsequently reduced cardiac output (7). These studies were the first to support popular theories of the time. First, during exercise blood was shunted away from the splanchnic region to the muscle. These data supported Starling's aforementioned ideas of the redistribution of blood flow during exercise. Second, his data supported Nobel Laureate August Krogh's basic model of the circulation (1912) that included one pump which emptied into both a compliant circulation (splanchnic) and a non-compliant circulation (muscle). These data documented how dynamic the body's blood flow distribution could be from rest to exercise. Unfortunately, the mechanisms by which the body was able to redistribute blood flow remained undetermined.

Arguably the most important homeostatic reflex regulator in the cardiovascular system was discovered in 1923 by Heinrich Herring. Herring demonstrated that increased pressure on the outside of the carotid artery resulted in a reflex by which bradycardia and hypotension ensued (117). However, the arterial baroreflex's exact reflex arc was still not elucidated until the1950s when the efferent arm of the baroreflex was shown to be the vagus and sympathetic nerves (41). It was nearly 90 years after Gaskell's initial discovery that sympathetic neurons tonically restricted blood flow before the α and β post-junctional adrenergic receptors were identified by Raymond Ahlquist (2) and norepinephrine

was discovered by von Euler (122). These discoveries allowed for exercise physiologists to put together the different physiological systems that are necessary for a majority of the redistribution of blood flow during the transition from rest to exercise.

The effects of exercise on circulating catecholamines began to be widely researched shortly after the description of adrenergic receptors. In 1957, researchers recorded plasma norepinephrine levels that were double those of baseline values after a long bout of endurance running (51). Shortly afterward, Vendsalu documented that plasma norepinephrine levels increased in proportion with exercise intensity, but that plasma epinephrine levels did not rise until heavy exercise (121). During the early 1980s, Murray Esler's research group in Australia showed that regional distribution of norepinephrine spillover was different in the gut, heart, muscle, and kidneys. Until this time, researchers assumed that activation of the sympathetic nervous system was systemic with increasing exercise intensity. Esler's studies demonstrated that the sympathetic nervous system was able to modulate its release of norepinephrine into various regions depending on the stimulus (exercise, rest, supine or upright posture, healthy or disease states) (43, 44, 54).

The arterial baroreflex's effects on heart rate (vagus and sympathetic nerves) and blood flow distribution (sympathetic nerves) during exercise were being extensively researched in the early 1960s. Due to the complex network of affectors, effectors and integration of central command, research was fraught with erroneous findings. This was primarily due to the lack of appreciation of the

complexity of the arterial baroreflex system including the cardiopulmonary baroreflex and the fact that different exercise modalities produce tremendously different effects (i.e., resistance exercise vs. aerobic; supine vs. upright exercise; quadrupeds vs. bipeds). The common conception of the baroreflex during this time was that if arterial pressure increased at rest that the heart rate would diminish and sympathetic outflow would be reduced (117). Thus, many researchers concluded that the baroreflex would work in opposition to the elevated pressure by trying to reduce blood pressure and heart rate. During exercise, however, it was known that heart rate increased concurrent with an increase in arterial blood pressure and increases in plasma epinephrine and norepinehrine. Thus, it appeared that this increased sympathetic outflow during exercise contradicted the dogma of arterial baroreflex function. Findings in the ‘60s and ‘70s consistently showed after removal of sympathetic nerves by blockade, ablation or denervation of the arterial baroreceptors, that animals and humans had impaired heart rate responses to exercise and increased splanchnic blood flows which resulted in lower blood pressures (117). These results suggested that the exercise response was due to an altered baroreceptor function causing increased heart rate and sympathetic outflow during exercise. While data accumulated for decades supporting the fact that baroreceptors “reset” to defend a higher pressure during exercise it was not until the mid 1980s that this theory was fully embraced by the scientific community (117).

The baroreceptor resetting during exercise and the differential distribution of sympathetic nervous system activity paved the way for exercise physiologists to find

that with increases in exercise overall sympathetic nervous system activity is increased, acting to vasoconstrict the non-active tissues during exercise and allowing for greater blood flow to the active tissues. Thus, during large muscle mass exercise, the sympathetic nervous system, via the arterial baroreceptors, must restrict blood flow even to the active tissue in order to preserve adequate brain perfusion.

Up to this point there was some controversy as to how much blood would perfuse an active muscle during exercise. This debate started when the factors contributing to maximal oxygen consumption where being disputed among exercise physiologists. Many of the early studies utilized in situ preparations in which the animals were anesthetized (6). Muscle was exposed and electrically stimulated while blood flow was measured with flow probes, and this generally resulted in peak muscle blood flows of approximately 100 ml/min/100 g. These results vastly underestimated active muscle blood flow in vivo. Professors Bob Armstrong and Maurice Harold Laughlin used radioactive labeled microspheres, which lodge in the terminal arterioles or capillaries, to accurately measure the regional distribution of blood flow in rats during treadmill running. The results from those studies suggested that active muscle blood flow during exercise had been vastly underestimated in the previous in situ experiments (6), and could approach 500 ml/min/100 g in some muscles of the rat. In 1985, Bengt Saltin measured active quadriceps muscle blood flow via thermodilution in humans (4). These investigators recorded flows per volume of active muscle that supported those found by Armstrong and Laughlin in the rat. Follow up studies done by the Armstrong and Laughlin laboratories showed that the addition of an α-adrenergic antagonist (phentolamine) would augment skeletal muscle blood flow at

rest and during exercise (75). Interestingly, for the purpose of my study, it appears that the white muscle blood flow was elevated significantly more (when measured as a percent increase) than in the soleus muscle after the addition of phentolamine. This suggests that white muscle resistance vessels are under greater tonic sympathetic nervous system constriction than are soleus resistance vessels. In addition, since muscle sympathetic nerve activity is assumed to be uniform within a muscle, it indicates that post-junctional adrenergic receptor density differences may account for the aforementioned results.

Great strides have been made up to this point in exercise physiology to identify regulators of arterial blood flow and arterial tone. Still, in the 1970s a new field was discovered in cardiovascular physiology and quickly caught the interest of exercise physiologists. The field was "vascular biology." This field evaluated the biology of the cells that constituted the vascular wall in normal and disease states, with early studies in the 1970s primarily evaluating arterial remodeling in response to atherosclerosis. Still, an important turn of events took place in the mid to late 70s when several discoveries determined that the endothelium possessed both endocrine and paracrine roles in regulating vascular tone locally and systemically. Early indications of the importance of the endothelial cells showed that conversion of angiotensin I to angiotensin II occurs via angiotensin converting enzyme located at the endothelial surface (3). A paper in 1975 showed that prostaglandins were released by endothelial cells (50) and prostacyclin release from the endothelium was discovered shortly afterward (87). Arguably the most important discovery in the field of vascular biology was made by Robert Furchgott, who described the endothelium dependent

vasorelaxation phenomenon (48). In his studies, he showed with intact endothelium a vasorelaxation in response to a muscarinic agonist, and without an intact endothelium there was no vascular smooth muscle relaxation. Furchgott called the vasorelaxative substance released from the endothelial cells endothelial derived relaxing factor (EDRF) (48), and would later share the Nobel Prize for this discovery. This discovery magnified the complexity of the regulation of vascular tone, because it appeared that endothelial cells could self-regulate tone via the release of both dilators and constrictors from the endothelium. Several years later, it was shown in a seminal study that arteries with vascular disease dilated less to muscarinic receptor agonists than arteries with no progression of vascular disease. These studies suggested that a lack of EDRF may be contributing to the progression of vascular disease or at least to augmented vasoactive tone in arteries with vascular disease. In the late 1980s nitric oxide was discovered to be released by the endothelium and to induce vascular relaxation (95). Interestingly, overshadowed by the excitement of nitric oxide research was another incredible finding. The endothelium released a very potent vasoconstrictor substance in addition to the EDRFs. This substance was denoted endothelin in 1988 (127). These vascular biology studies provided new insight into local control mechanisms of vascular tone, further highlighting the need to understand and integrate myogenic, local, humoral, and neural control mechanisms of vascular tone. All of these components are of immense importance in regulating blood flow and blood pressure at rest and during exercise.

1.3 Aging, exercise training, and the cardiovascular system

While the fountain of youth remains elusive, advances in medical techniques, pharmaceutical drugs, nutrition, and immunization have allowed the human life expectancy to increase. Predictions estimate that by 2030 the fastest growing segment of the population will be people 85 and older (1). While many older individuals have been able to "successfully age," advancing age is still a major independent risk factor for coronary heart disease (52) along with other vascular diseases. This begs the question: with advancing age what occurs with respect to the cardiovascular system that leads to increased prevalence of CHD? Thus, it is critical to characterize and elucidate mechanisms of the aging process, so that we can better understand the physiological states of this growing segment of the population.

One of the many systems in which function is compromised with advancing age is the cardiovascular system. Declines in cardiac function with aging have been well documented and include reduced early diastolic filling, maximal heart rate, maximal ejection fraction, maximal stroke volume, and maximal cardiac output (70).

Chronic aerobic exercise training produces increases in overall cardiac function in an older population. This includes greater maximal ejection fraction, early diastolic filling, maximal stroke volume, and maximal cardiac output versus sedentary age matched controls (57). Thus, these studies provide evidence that the decline in cardiac performance is largely a consequence of physical inactivity rather than the aging process.

Aging is associated with increases in systemic vascular resistance (SVR) (57). These increases in vascular resistance most likely contribute to the age-associated

increases in blood pressure, which also is an independent risk factor of CHD (52). One possible mechanism underlying age-associated alterations in vascular resistance and systemic pressures is the autonomic nervous system, which tightly regulates any changes in systemic blood pressure via sympathetic and parasympathetic branches. Activity of the autonomic nervous system, which supports both systolic blood pressure (SBP) and diastolic blood pressure (DBP), is altered in older individuals (63). Other autonomic changes with aging include decreased cardiac vagal modulation, increased plasma and total norepinephrine spillover, and increased sympathetic nervous system activity (SNA) to heart, gut and skeletal muscle tissue (108). These age-associated changes in the autonomic sympathetic nervous system (SNS) are also seen in Fischer 344 rats, including elevations in SNA to tail arteries (14), blood pressure, and plasma catecholamines (128).

Whether chronic aerobic exercise training reduces overall SVR remains controversial though several studies have shown reductions (13, 107) or the lack of reductions (80) in older individuals at rest. At any given exercise intensity older trained individuals show lower SVR relative to older sedentary individuals (107). It appears that exercise training has minimal effects on reductions in resting blood pressure in healthy older adults, though even a small reduction could be epidemiologically significant (126). During a bout of exercise, blood pressure is significantly reduced after exercise training (126). In a cross-sectional study it appears that exercise training has no effect on autonomic support of blood pressure (97) or muscle SNA in young or old adults at rest (108). After aerobic exercise training muscle SNA is lower in the contralateral leg for any given absolute workload (101).

Likewise, it appears that aerobic exercise training has no effect on resting renal SNA in rabbits (31), but for a given workload during acute exercise the renal SNA is lower after exercise training (32). Although conclusive evidence is lacking, the current data indicate that if exercise training does indeed reduce SVR at rest it is not due to altered autonomic nervous system outflow or changes in muscle SNA, but may be related to adaptive alterations in the resistance vasculature.

Skeletal muscle tissue makes up ~35-40% of a healthy person's body mass. Of particular interest to exercise physiologists is how locomotor muscle is affected by aging. Leg vascular resistance contributes significantly to SVR at rest and a majority of this vasculature is located in the skeletal muscle. A series of experiments conducted by Dinenno and Seals have consistently shown a decrease in resting leg blood flow independent of skeletal muscle mass in older adult males. This decreased leg blood flow (25-30% lower in aged) is accompanied by reduced vascular conductance and increased leg vascular resistance (~50% higher in aged) (33, 35). These findings are similar to lower resting calf blood flow with aging (81), as well as reduced leg blood flow in older women (90). Chronically augmented alpha adrenergic constriction in older adult males accounted for a most of the reduction in blood flow and increased vascular resistance (35). This increased α-adrenergic constriction in healthy older men could be due to several different factors including increased muscle SNA, increased α-adrenergic receptor sensitivity of resistance vessels, greater amount of vascular smooth muscle in resistance and conduit arteries, or a combination of factors. Aged rodent data suggest that muscle blood flows are unchanged at rest compared to young rats (27, 60). However, these species differences

could be due to differences in the definition of rest. Rest in the aforementioned human studies is supine and with postural muscles unloaded, while resting measurements in rodents are with the animals in a standing position with postural muscles loaded (27). Furthermore, blood flow measurements reported by Irion et al. (60) were taken with the rat under anesthesia so determination of resting flows could be confounded by an impaired autonomic nervous system. Presently, the effects of aging on resting skeletal muscle blood flow are unclear (60).

While resting blood flow to skeletal muscle is unclear, the effects of aging on muscle perfusion during acute bouts of exercise are more apparent. Aging significantly reduces the amount of blood flow to a given locomotor skeletal muscle at higher submaximal and maximal workloads in rodents and humans (60, 76, 99, 123). This appears to be independent of training status in cross-sectional studies (100). This reduction also appears to be due to increased vascular resistance in the older individuals at a given workload. Interestingly, an intervention study has shown that chronic aerobic exercise training in older sedentary individuals restores their maximal blood flow response similar to that of young individuals (13). This increase in leg blood flow was suggested to be due to a decrease leg vascular resistance, because stroke volume and cardiac output were unchanged with exercise training. These data provide significant insight as to the mechanisms of reductions in physiological functional capacity and aerobic capacity with aging. It appears that old age-associated reductions in exercise blood flow to locomotor skeletal muscle are due to increased leg vascular resistance and may be limiting to exercise capacity in older sedentary individuals.

The major regulator of both blood flow and vascular resistance are the small resistance arteries and arterioles, where vascular tone of the resistance vasculature is regulated by the balance of vasodilator and vasoconstrictor stimuli. Thus, shifts in vasoconstrictor or vasodilator stimuli or alterations in the vascular response to vasoactive stimuli could affect the overall vascular resistance of a region. For example, in the skeletal muscle circulation with aging there is a significant reduction in flow-mediated vasodilation (17, 91) and agonist-induced vasodilation (29, 91, 113). It appears that these reductions are due to attenuated endothelial mediated vasodilation, resulting from reductions in nitric oxide bioavailability (111). One of the non-endothelial dependent vasodilators which also is reduced with aging is α_2-mediated vasodilation (119). Unfortunately, these measurements were made in the forearm vasculature not in skeletal muscle involved in locomotion. Taken together these changes could shift the resistance vessel phenotype from one with a balanced vascular tone to one with a more pro-vasoconstrictor vascular tone.

Aerobic exercise training restores endothelial mediated vasodilation in skeletal muscle vasculature of older adults (29, 111). This restoration occurs through an enhancement of the nitroxidergic signaling pathway in locomotor skeletal muscle vasculature (111) and non-locomotor skeletal muscle vasculature like the forearm (29). Cross sectional studies also showed that older exercise trained individuals have no decrement in endothelial mediated vasodilation in the human forearm vasculature (113). There are no studies that have reported the β_2-

adrenergic vasodilator system in skeletal muscle resistance vessels with respect to aerobic exercise training in young or older subjects.

Aging appears to affect the vasoconstrictor responsiveness in a variety of ways. It appears that in the systemic vasculaturevasoconstriction to α_1-adrenergic agonists is decreased with aging (63). In the forearm vasculature overall responsiveness to NE is reduced (56), along with α_1-adrenergic receptor sensitivity with human aging (39). In a study using anesthetized young and old rats, NE vascular responsiveness was not different in the perfused skeletal muscle hindlimb (53). In isolated locomotor skeletal muscle arterioles there was no significant difference in NE vascular responsiveness with age (92). Similar age-related findings were reported in rat cremaster muscle (19). Another very potent vasoconstrictor, which could play an important role in the local regulation of vascular resistance, is endothelin. Unfortunately, very little is known about how the vascular reactivity of endothelin is affected by aging, and even less is known about ET-1 effects on skeletal muscle resistance vasculature. The seminal study on aging and ET-1 showed that in Wistar rat aorta there is an enhanced vasoconstrictor reactivity with aging, but no differences were seen in femoral arteries with aging (10). In summary, there is some evidence, although inconclusive, that with aging there is no change in skeletal muscle vascular reactivity to NE or ET-1 in rats. Still, the human literature on forearm skeletal muscle vasculature suggests reductions in α-adrenergic vasoconstriction.

Aerobic exercise training reduces α-adrenergic responsiveness in isolated rat aorta from young rats (25, 110). The only study to look at the effects in a resistance vasculature showed no difference in NE maximal responsiveness after swim training

in young rat cremaster muscle (125). An acute bout of exercise appears to produces reduced α-adrenergic responsiveness in young dogs and in the human forearm (15, 102). To date, there are no published studies investigating the effects of habitual exercise training on skeletal muscle α-adrenergic responsiveness or endothelin responsiveness in older animals.

1.4 Purpose and hypotheses

The purpose of this dissertation is to determine whether alterations in the intrinsic vasomotor properties of skeletal muscle resistance arterioles or structural properties of the arterial vasculature contribute to the elevation in peripheral vascular resistance and reduction in skeletal muscle blood flow with aging. A related objective was to determine whether aerobic exercise training functions to modify old age-associated alterations in the vasomotor properties and structural properties of resistance vasculature. The specific aims of this proposal are to test the hypotheses that:

I. Aging will not change vasoconstrictor responsiveness of skeletal muscle resistance arterioles, while exercise training will attenuate the vasoconstrictor responsiveness of skeletal muscle resistance arterioles.

II. Aging will attenuate skeletal muscle arteriolar responsiveness to adrenergic vasodilation, and exercise training will not reverse this effect.

III. Aging will increase the stiffness of arteries, and exercise training will not affect arterial stiffness.

CHAPTER II

ENDOTHELIN-1 VASOREACTIVITY IN RAT SKELETAL MUSCLE ARTERIOLES: IMPLICATIONS OF AGING AND EXERCISE TRAINING

2.1 Introduction

Aging is an independent risk factor for hypertension, atherosclerosis, and coronary heart disease. It has been hypothesized that vascular dysfunction precedes these pathological disease states and could contribute to the progression of these diseases (106). For example, aging is characterized by compromised endothelium-mediated vasodilator function, and this may explain why aging is an independent risk factor for these vascular diseases. Alteration in resistance vessel sensitivity to endothelial derived constricting factors may also contribute to the age-associated increase in the incidence of vascular disease. Although the age-related decline in endothelial vasodilator function has been well documented (30, 91, 113), the effects of aging on endothelium-dependent vasoconstrictors in the peripheral resistance vasculature has not been clearly delineated.

Endothelin-1 (ET-1) is a potent substance produced by endothelial cells that acts on vascular smooth muscle cell endothelin A (ETa) and endothelin B (ETb) receptors to cause vasoconstriction (127). As with other vasoconstrictor agents, there is negative feedback regulation of ET-1 vasoconstriction via the ETb receptors located on the endothelial cells. Activation of this receptor by ET-1

produces endothelial derived relaxing factors that act to oppose the ET-1 vasoconstriction.

Current research has documented augmented ET-1 release and sensitivity in patients with hypertension (79), heart failure (45), atherosclerosis and obesity (11). However a paucity of data exist documenting the effects of aging on the skeletal muscle vascular responsiveness to ET-1.

The primary purpose of this study was to determine the effects of aging on ET-1 vasoconstrictor responsiveness in arterioles. Arterioles from two different muscles were investigated; the soleus muscle, a predominantly slow-twitch highly oxidative muscle and the white gastrocnemius muscle, a fast twitch low oxidative muscle. Since aging is often associated with physical inactivity, a secondary purpose was to determine whether exercise training would attenuate possible aging-induced alterations in ET-1 responsiveness. We hypothesized that aging would enhance ET-1 mediated vasoconstriction through an attenuation of the endothelial cell ETb receptor mechanism, and that exercise training would attenuate the exaggerated ET-1 response through an endothelium-dependent mechanism

2.2 Material and methods

Animal characteristics

Male Fischer 344 young (4-6 months) and old (24-26 months) rats were obtained (Harlan Inc) and were housed in a temperature controlled (23 ± 2 ºC) room with a 12:12 light-dark cycle. The selected ages represent young adulthood and senescence (~50% mortality rate), respectively. The Fischer 344 rat model is supported by the National Institute on Aging to study the effects of old age on the

cardiovascular system in the absence of overt cardiovascular disease. All animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee and complied by the guidelines of the National Research Council *Guide for the Care and Use of Laboratory Animals* (Washington DC: National Academy Press, Revised 1996).

Exercise training

All rats were habituated to treadmill exercise, during which time each rat walked on a motor-driven treadmill at 15 m/min (0° incline), 5 min/day for 3 days. At the end of the habituation period young and old rats were assigned to either a young or old sedentary control group (YS and OS; respectively) or a young or old exercise-trained group (YT and OT; respectively). Exercise-trained rats performed treadmill running at 15 m/min (15° incline), 1hr/day, 5 days/wk, for 10-12 weeks as previously described (25, 111). Vascular responses were determined 48 hours after the last exercise bout in trained rats.

Microvessel preparation

Animals were anesthetized with an ip injection of sodium pentabarbatol and euthanized via exsanguination. The gastrocnemius-plantaris-soleus muscle complex was carefully excised from each leg. Following excision, the muscle complex was placed in cold (4º 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, pH 7.4 for isolation of gastrocnemius and soleus muscle first-order (1A) arterioles with the aid of a dissecting microscope (Olympus SVH10) as previously

described (78, 92). In the soleus and gastrocnemius muscles, 1A arterioles were defined as the first arterial branch after the feed artery entered the muscle. The arterioles (length, 0.5 - 1.0 mm; maximal inner diameter: soleus muscle, 60-159 μm; gastrocnemius muscle, 84-220 μm) were cleared of surrounding muscle fibers, removed from the muscle and placed in Lucite chambers containing MOPS buffered PSS equilibrated to room air. The arterioles were cannulated on both ends with micropipettes and secured with 11-0 surgical nylon suture. After cannulation, the chambers were 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) for recording of luminal diameter. Arterioles were pressurized to the in vivo pressure of 44 mmHg with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then closing the reservoirs, verifying that diameter remained constant. Arterioles that exhibited leaks were discarded. Arterioles free of leaks were warmed to 37º C and allowed to develop initial spontaneous tone during a 30-60 min equilibration period.

Vasoreactivity to pharmacological agonists

In a series of three studies, concentration-response relations to the cumulative addition of ET-1 (1×10^{-11} to 3×10^{-8}) were determined in endothelium intact and denuded arterioles. The arterioles were allowed to equilibrate between successive dose responses and were discarded unless at least 20% baseline tone was achieved prior to addition of agents.

Study 1

To determine whether ET-1 vasoreactivity of the skeletal muscle arterioles is altered with aging or exercise training, the diameter of 1A arterioles was measured in response to ET-1 (1×10^{-11} to 3×10^{-8} M). Arteriolar sensitivity to ET-1 was assessed by calculating the dose eliciting 50% of the maximal vasoconstrictor response (EC_{50}).

Study 2

When differences in the reactivity to ET-1 were found, a second series of studies was performed to determine whether the difference was mediated through the vascular endothelium. For these studies, the endothelium was denuded from gastrocnemius muscle arterioles of young and old rats by passing 3-5 ml of air through the lumen of the vessel. To ensure full removal of the endothelium, arterioles were exposed to the endothelium-dependent vasodilator, acetylcholine (3×10^{-5} M). Any vessel that exhibited vasodilation > 5% was excluded. Following the acetylcholine test, the vessels were washed several times with PSS and allowed to establish spontaneous tone prior to the ET-1 dose response.

Study 3

In order to determine whether the vascular smooth muscle ETa or ETb receptors were responsible for the age-associated augmentation of gastrocnemius muscle arteriolar ET-1 sensitivity, the endothelial layer was first removed as described above. One arteriole was incubated with the ETa receptor antagonist BQ-123 (10^{-6} M) for 30 minutes prior to undergoing an ET-1 dose response. A second arteriole was incubated with the ETb receptor antagonist BQ-788 (10^{-8} M) for 30

minutes prior to undergoing an ET-1 dose response. These doses of ET receptor antagonists have been previously shown to inhibit ETa and ETb receptor vasoconstriction respectively (59, 82, 83).

Muscle oxidative enzyme activity

Sections of the soleus and white gastrocnemius muscles from each animal were stored at -80°C for determination of citrate synthase activity (112), a measure of muscle oxidative capacity, to determine the efficacy of the training regimen.

Solutions and stocks

Stock solutions of drugs were prepared in PSS and frozen. Fresh dilutions of these stocks were prepared daily. All drugs were obtained from Sigma Chemical (St. Louis, MO).

Data presentation and statistical analysis

Vasoconstrictor responses were recorded as actual diameters and expressed as a percentage of possible constriction according to the following formula for analysis:

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

Where D_s is the steady-state inner diameter recorded after each dose and D_b is the initial baseline inner diameter before the first addition of drug. Spontaneous tone is expressed as a percentage of maximal intraluminal diameter. Repeated measures analysis of variance (ANOVA) was used to determine differences among young and old, sedentary and exercise-trained groups. Fisher protected least significant difference post hoc was used where appropriate. A one-way ANOVA was used to determine significance of differences among groups in animal mass, muscle mass

and muscle citrate synthase activity. All data are presented as mean ± SEM. Significance was set at $P \leq 0.05$.

2.3 Results

Animal characteristics

Body mass increased with age (YS, 335 ± 5 g; OS, 430 ± 5 g), and exercise training resulted in decreased body mass in old rats but not in young rats (YT, 337 ± 5 g; OT, 391 ± 6 g). Left ventricle to body mass ratio was significantly higher in the trained rats compared to age matched controls (Table 2.1). Gastrocnemius and soleus muscle mass to body mass ratio was reduced with aging and increased by exercise training (Table 2.1). Citrate synthase activity was significantly higher in both muscles of young and old trained rats relative to the sedentary control groups (Soleus: YS 15 ± 1, OS 11 ± 1, YT 19 ± 1, OT 14 ± 1; Gastrocnemius: YS 16 ± 1, OS 14 ± 1, YT 19 ± 1, OT 22 ± 1), confirming the efficacy of the exercise training regimen ($P<0.05$).

Vasoconstrictor studies

Aging increased ET-1 (EC_{50}) sensitivity but did not alter maximal vasoconstrictor capacity in white gastrocnemius skeletal muscle arterioles (Figure 2.1 B). Similar age-related differences were not present in soleus skeletal muscle arterioles (Figure 2.1 A). Exercise training had no effect on ET-1 vasoconstriction in either young or old trained rat gastrocnemius or soleus muscle arterioles (Figure 2.1 A&B).

In white gastrocnemius muscle arterioles denuded of the endothelium, the age-related increase in ET-1 sensitivity persisted (Figure 2.2 A), indicating that the increase in sensitivity was not due to endothelial ETb receptors or altered endothelial vasodilator function in the aged arterioles.

In the presence of the ETb receptor antagonist BQ-788, the age related increase in ET-1 sensitivity remained (Figure 2.3 B). In contrast, ETa receptor antagonist BQ-123 eliminated the age-associated increase in ET-1 sensitivity. Furthermore arteriolar vasoconstriction to ET-1 was greater in young rats with ET_a receptor inhibition (Figure 2.3 C). ET-1 sensitivity and maximal vasoconstriction was greater when mediated through the vascular smooth muscle ETa receptor compared to the ETb receptor in arterioles regardless of age.

Table 2.1 Characteristics of animals and vessel lumen diameters and tone.

	Sedentary		Trained	
	Young	Old	Young	Old
N	26	25	24	21
Body Wt (g)	335 ± 5	430 ± 5*	337 ± 5	391 ± 6‡
Heart Wt/ Body Wt Ratio (g/g*100)	0.276 ± 0.016	0.276 ± 0.002	0.309 ± 0.015†0.08	0.303 ± 0.013
LV Wt/ Body Wt Ratio (g/g*100)	0.188 ± 0.004	0.190 ± 0.002	0.200 ± 0.002†	0.208 ± 0.004‡
Soleus Muscle Wt/Body Wt Ratio (g/g*100)	0.044 ± 0.001	0.036 ± 0.001*(0.08)explain	0.048 ± 0.001†	0.042 ± 0.001‡
Gastrocnemius Muscle Wt/ Body Wt Ratio (g/g*100)	0.490 ± 0.006	0.380 ± 0.005*	0.510 ± 0.010†	0.410 ± 0.007
Soleus Muscle Arteriole Lumen Diameter (μm)	116 ± 3	118 ± 3	101 ± 4†	113 ± 4
Gastrocnemius Muscle Arteriole Lumen Diameter (μm)	151 ± 3	153 ± 4	171 ± 5†	186 ± 4‡
Soleus Muscle Arteriole Spontaneous Tone (%)	45.3 ± 3.4	45.7 ± 3.5	40.5 ± 4.3	32.0 ± 3.9‡
Gastrocnemius Muscle Arteriole Spontaneous Tone (%)	33.0 ± 1.9	30.5 ± 2.6	31.4 ± 2.5	25.5 ± 2.3

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 significant difference between old sedentary and old trained, $P < 0.05$. Values are means ± SEM.

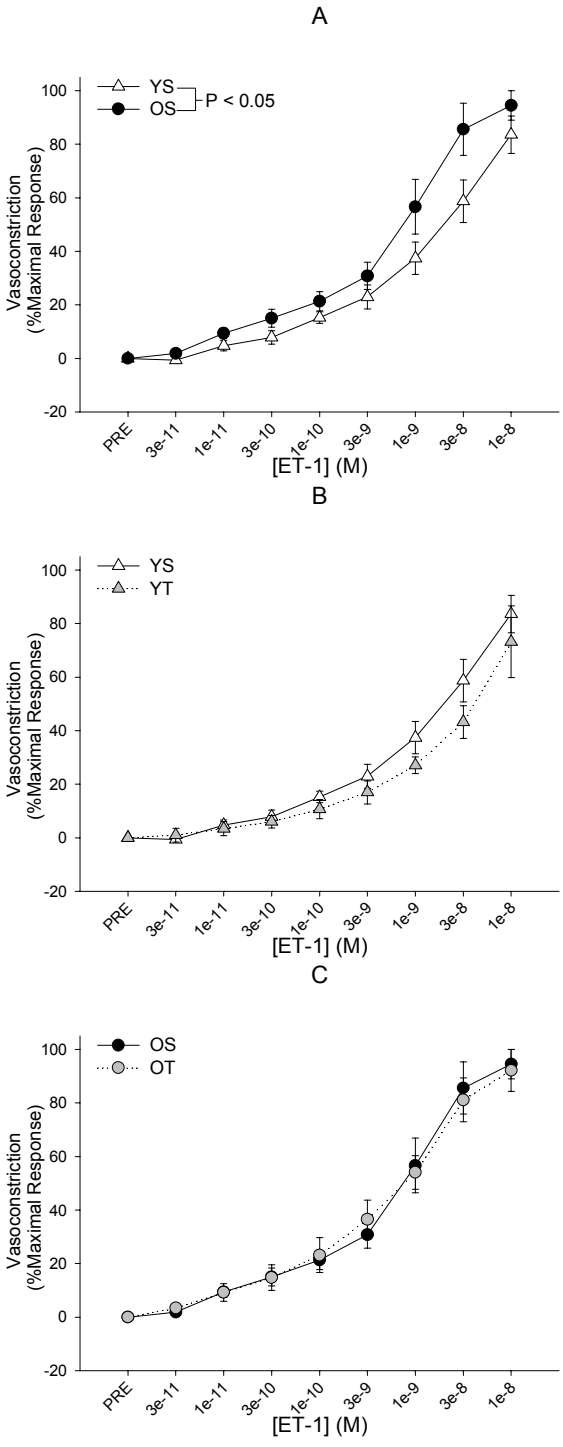

Figure 2.1. Comparisons of dose-response relations to the cumulative addition of endothelin-1 in gastrocnemius muscle arterioles from young and aged sedentary rats (A) and sedentary and trained young (B) and aged (C) rats with an intact endothelium. Values are means ± SEM.

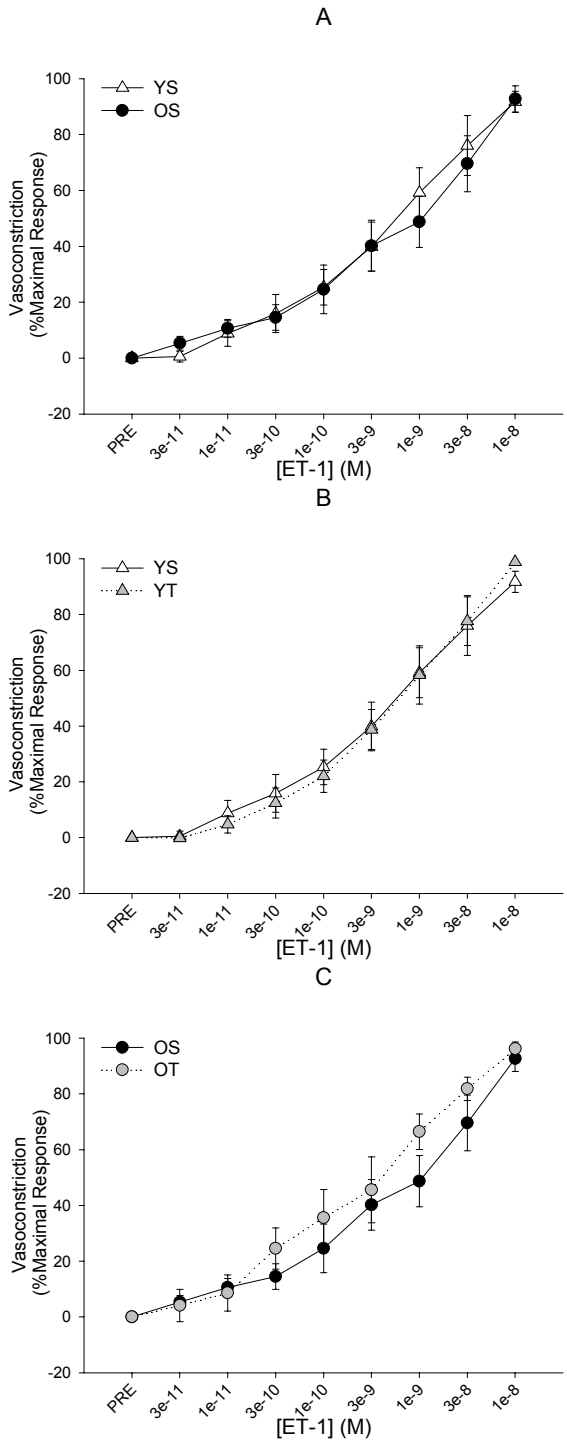

Figure 2.2. Comparisons of dose-response relations to the cumulative addition of endothelin-1 in soleus muscle arterioles from young and aged sedentary (A) rats and sedentary and trained young (B) and aged (C) rats with an intact endothelium. Values are means ± SEM.

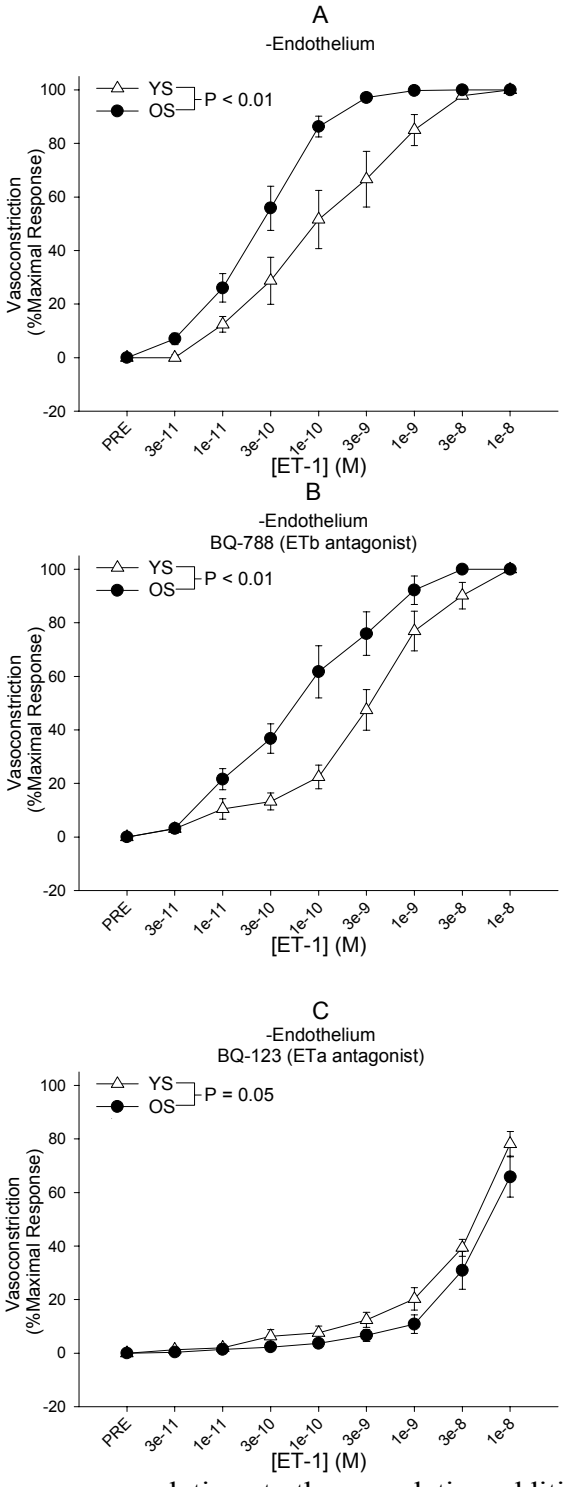

Figure 2.3 Dose-response relations to the cumulative addition of endothelin-1 in endothelium denuded gastrocnemius muscle arterioles (A) and in the presence of the endothelin B receptor antagonist, BQ-788, (B) or the endothelin A receptor antagonist, BQ-123 (C). Values are means ± SEM.

2.4 Discussion

From the present study come several novel findings. First, there is an age-associated increase in ET-1 sensitivity in white gastrocnemius muscle arterioles, but not in soleus muscle arterioles. Second, the augmented vasoconstriction to ET-1 in gastrocnemius muscle arterioles from old rats was not due to age-related changes in endothelial function. Third, the age-associated increase in ET-1 sensitivity was due to augmented smooth muscle ETa receptor mediated vasoconstriction. Fourth, vascular smooth muscle ETb receptor function was slightly diminished by aging. Finally, exercise training had no effect on ET-1 vasoconstriction in young or old rat skeletal muscle arterioles.

Age associated increases in ET-1 sensitivity: effects of muscle type

This study demonstrated that there was a greater ET-1 sensitivity in arterioles from the superficial gastrocnemius muscle, composed primarily of fast-twitch (type IIB) glycolytic fibers (26), in old rats. In contrast, aging had no effect on ET-1 sensitivity or maximal vasoconstriction in arterioles from the slow twitch (type I) highly oxidative soleus muscle. The heterogeneity of the vascular responses within muscle is similar to that among other tissues during healthy aging. For example, the seminal studies by Luscher et al. have shown augmented ET-1 vasoconstriction with aging in coronary arteries (118), and diminished or unaltered ET-1 sensitivity in small mesenteric (40)and large conduit arteries (10), respectively, with old age.

Physiologically, it is interesting that soleus muscle, which is a postural muscle that maintains chronically high blood flow at rest and exercise, shows no

change in arteriolar ET-1 sensitivity. These data beg the question, what factor(s) differs between white gastrocnemius and soleus muscles to cause one arteriole to have an age-associated increase in ET-1 sensitivity and the other to have no difference? Although no definitive answer currently exists, previous work has demonstrated that these two vessels can regulate vascular tone through distinct mechanisms. Early studies by Armstrong and Laughlin indicated that the fast-twitch glycolytic muscle was tonically vasoconstricted via adrenergic stimulation to a greater degree than the highly oxidative muscle (75). In addition, differences in the mechanisms of vasodilation have also been described. Soleus muscle arterioles principally use the nitric oxide synthase mechanism to mediate endothelium dependent vasodilation, while the gastrocnemius muscle arterioles use vasodilator prostanoids and what appears to be an endothelium-derived hyperpolarizing factor (111). Since nitric oxide attenuates the local ET-1 system, we speculated that a diminished NO bioavailability that is associated with old age in the soleus muscle might make arterioles from this muscle type susceptible to old age-related increases in ET-1 sensitivity. Contrary to our hypothesis, it appears that neither old age nor exercise training induced alterations in endothelium-dependent vasodilation or eNOS protein expression greatly influence ET-1 sensitivity in soleus muscle arterioles (111). An alternative hypothesis is that the greater NE-induced vasoconstriction with aging may lead to an increase in ET-1 sensitivity in gastrocnemius muscle arterioles, since chronically high sympathetic outflow has been shown to augment the ET-1 vasoconstriction (109). This hypothesis will require further investigation.

Mechanisms of age-related augmented ET-1 sensitivity

Arteriolar studies with and without an intact endothelial cell layer demonstrate that the old age-associated increase in ET-1 sensitivity is not dependent on or related to the vasodilator function of ET receptors on the endothelium. This confirms that augmented sensitivity of vascular smooth muscle ET receptors mediates the increased ET-1 vascular sensitivity seen with advanced age. Using specific inhibitors of ET receptors on the vascular smooth muscle, the results demonstrate that ETa receptors mediate the enhanced sensitivity to ET-1 with aging, whereas ETb receptors mediate a slightly greater vasoconstrictor response in young rats. Because the predominant mechanism through which ET-1 mediated vasoconstriction occurs in skeletal muscle arterioles is through the ETa receptor , the net effect of a greater ETa receptor mediated vasoconstriction and a lower ETb receptor vasoconstriction in arterioles from old animals is an enhanced ET-1 vasoconstrictor response. These findings are similar to those reported in hypertension, which is associated with augmented ETa receptor mediated vasoconstriction in the resistance vasculature (16, 79).

Exercise training effects on ET-1 sensitivity

A ten to twelve week aerobic exercise training program had no effect on ET-1 sensitivity or maximal responsiveness in skeletal muscle arterioles. Isolated endothelial cells exposed to shear stress have been shown to increase expression of endothelial ETb receptors (88). These data, as well as others demonstrating improved endothelial function after chronic aerobic exercise training in young and old subjects (29, 68, 111), lead us to hypothesize that increased expression of

endothelial ETb receptors via augmented muscle blood flow during exercise would result in an attenuated ET-1 vasoconstriction in the exercise trained groups. Although shear stress during the in vivo bouts of exercise were not assessed, the vascular remodeling of the gastrocnemius arterioles from trained rats (i.e., the larger diameter) is indicative of a chronic elevation in shear stress (64, 74). Despite evidence of elevated shear stress, the in vitro data do not support that endothelial ETb receptor function was augmented with exercise training (Figure 2.1& 2.2). Previous data in our laboratory indicate that exercise training augments acetylcholine-induced endothelium-dependent vasodilation in both soleus and gastrocnemius arterioles (111). Taken together, these results suggest that endothelial function may be augmented via certain endothelial pathways, but the endothelial ETb receptor pathway appears unaltered by aerobic exercise training.

Implications

The overall implications of the increased arteriolar ET-1 sensitivity in glycolytic muscle is that it may serve to elevate systemic vascular resistance. Since endothelial ET-1 release has been shown to be augmented with aging (10), a corresponding increase in arteriolar ET-1 sensitivity in humans could elevate systemic vascular resistance through chronically elevated muscle vasoconstrictor tone. Such changes in systemic vascular resistance with healthy aging could predispose older individuals to hypertension (71, 105).

CHAPTER III

EFFECTS OF AGING AND EXERCISE TRAINING ON ADRENERGIC VASOREACTIVITY IN SKELETAL MUSCLE ARTERIOLES

3.1 Introduction

While the fountain of youth remains elusive, advances in medical techniques, pharmaceutical drugs, nutrition, and immunization have allowed the human life expectancy to increase. Estimates are that by 2030 the fastest growing segment of the population will be people 85 and older (1). While many older individuals have been able to "successfully age," advancing age is still a major independent risk factor for coronary heart disease (CHD) (52) other vascular diseases (73). Aging is likewise associated with increases in systemic vascular resistance (SVR) (57). These increases in vascular resistance most likely contribute to the age-associated increases in blood pressure, which also is an independent risk factor of CHD (52). The mechanisms through which advancing age leads to increased prevalence of cardiovascular disease are not completely understood.

One possible cause of underlying age-associated alterations in vascular resistance is phenotypic changes in the skeletal muscle resistance vasculature. Skeletal muscle makes up ~35-40% of a healthy person's body mass and receives approximately 10% of cardiac output at rest (103). Leg vascular resistance contributes significantly to systemic vascular resistance at rest and is largely determined by the leg skeletal muscle. Experiments have consistently shown a decrease in resting leg blood flow independent of skeletal muscle mass in older males

and females (33, 37). This decreased leg blood flow with aging (25-30% lower in aged) is accompanied by reduced vascular conductance and increased leg vascular resistance (~50% higher in aged) (33, 35, 90). Chronically augmented alpha adrenergic constriction in older adult males accounts for a majority of the reduction in blood flow and increased vascular resistance (35). This increased α-adrenergic vasoconstriction in healthy older men could be due to several factors, including increased muscle sympathetic nerve activity, increased α-adrenergic receptor sensitivity of resistance vessels, or greater amount of vascular smooth muscle in resistance and conduit arteries.

Regular aerobic exercise has been shown to have cardioprotective effects on the coronary and peripheral vasculature and is recommended by both the American Heart Association and American College of Sports Medicine. Still, little is known about the mechanisms of the cardioprotective effects of exercise on the peripheral vasculature. Thus, the primary purpose of this study was to determine whether age-associated differences in blood pressure or blood flow could be due to alterations in adrenergic vascular sensitivity or responsiveness in skeletal muscle arterioles. The secondary purpose was to determine if the cardioprotective effects of regular endurance exercise training could be due to alterations in adrenergic vascular sensitivity or responsiveness in skeletal muscle arterioles. We hypothesized that aging would augment the vasoconstrictor responses to norepinephrine, and exercise training would attenuate or reverse these aging effects.

3.2 Material and methods

Animal characteristics

Male Fischer 344 young (4-6 months) and old (24-26 months) rats were obtained (Harlan Inc) and housed in a temperature controlled (23 ± 2 ºC) room with a 12:12 light-dark cycle. These selected time points represent young adulthood and senescence (~50% mortality rate). The Fischer 344 rat model is supported by the National Institute on Aging because these rats can be used to study the effects of old age on the cardiovascular system in the absence of overt cardiovascular disease. All animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee and complied by the guidelines of the National Research Council *Guide for the Care and Use of Laboratory Animals* (Washington DC: National Academy Press, Revised 1996).

Exercise training

All rats were habituated to treadmill exercise, during which time each rat walked on a motor-driven treadmill at 15 m/min (0° incline), 5 min/day for 3 days. At the end of the habituation period young and old rats were assigned to either a young or old sedentary control group (YS and OS; respectively) or a young or old exercise-trained group (YT and OT; respectively). Exercise-trained rats performed treadmill running at 15 m/min (15° incline), 1 hr/day, 5 days/wk, for 10-12 weeks as previously described (25, 111). Vascular responses were determined 48 hours after the last exercise bout in trained rats.

Microvessel preparation

Animals were anesthetized with an I.P. injection of sodium pentobarbital and euthanized via exsanguinations. The gastrocnemius-plantaris-soleus muscle complex was carefully excised from each leg. Following excision, the gastrocnemius-plantaris-soleus muscle complex was placed in cold (4º 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, pH 7.4 for isolation of gastrocnemius and soleus muscle first-order (1A) arterioles with the aid of a dissecting microscope (Olympus SVH10) as previously described (78, 92). In the soleus and gastrocnemius muscles, 1A arterioles were defined as the first arterial branch after the feed artery entered the muscle. The arterioles (length, 0.5 - 1.0 mm; maximal inner diameter: soleus muscle, 60-159 μm; gastrocnemius muscle, 84-220 μm) were cleared of surrounding muscle fibers, removed from the muscle and placed in lucite chambers containing MOPS buffered PSS equilibrated to room air. The arterioles were cannulated on both ends to micropipettes and secured with nylon suture. After cannulation, the chambers were 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) for recording of luminal diameter. Arterioles were pressurized to the in vivo pressure of 44 mmHg with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then

closing the reservoirs verifying that diameter remained constant. Arterioles that exhibited leaks were discarded. Arterioles free of leaks were warmed to 37º C and allowed to develop initial spontaneous tone during a 30-60 min equilibration period.

Study 1

To determine whether adrenergic vasomotor function of the skeletal muscle arterioles is altered with aging or exercise training, responses of 1A arterioles were measured to the cumulative addition of norepinephrine (NE 10^{-9} - 10^{-4} M) (with a propranolol -blockade, 10^{-5} M), and isoproteronol (ISO 10^{-9} -10^{-5} M) or increasing concentrations of isotonic potassium chloride (KCl 10 - 100 mM). Sensitivity of the arterioles to these pharmacological agonists were assessed by calculating the doses eliciting 50% of the maximal vasoconstrictor or vasodilator response (EC_{50} or IC_{50}; respectively). The arterioles were allowed to equilibrate between successive dose responses and were discarded unless at least 20% baseline tone was achieved prior to addition of vasoactive agents. Following active responses, vessels were incubated in calcium-free PSS to determine maximal lumen diameter.

Study 2

When differences in the reactivity to the pharmacological agonists were found, a second series of studies was performed to determine whether the differences were mediated through the vascular endothelium. For these studies, the endothelium was denuded from arterioles by passing 3-5 ml of air through the lumen of the vessel. To ensure full removal of the endothelium, arterioles were exposed to the endothelium-dependent vasodilator, acetylcholine (3 x10^{-5} M). Any

vessel that exhibited vasodilation > 5% was excluded. Following the acetylcholine test, the vessels were washed several times with PSS and allowed to establish spontaneous tone.

Study 3

The last series of studies was done to examine whether the age-related changes in vascular smooth muscle β_2-receptor-mediated vasodilation might be due to the downstream signaling. Arterioles were denuded of the endothelium as previously described. After stable tone was achieved, vessels underwent a dose response to forskolin (FOR 10^{-9} to 10^{-5}), which directly stimulates adenylate cyclase to increase cytosolic cAMP.

Muscle oxidative enzyme activity

Sections of the soleus and gastrocnemius muscles from each animal were stored at -80°C for determination of citrate synthase activity (112), a measure of muscle oxidative capacity, to determine the efficacy of the training regimen.

Solutions and stocks

Stock solutions of drugs were prepared in PSS and frozen. Fresh dilutions of these stocks were prepared daily. All drugs were purchased from Sigma Chemical (St. Louis, MO).

Data presentation and statistical analysis

Responses were recorded as actual diameters and expressed as a percentage of possible vasoconstriction or vasodilation according to the following formulas for analysis:

Vasoconstriction (% Maximal Response) = $[D_b - D_s)/(D_b) * 100]$

$$\text{Vasodilation (\% Maximal Response)} = [(D_s - D_b)/(D_m - D_b) * 100]$$

where D_s is the steady-state inner diameter recorded after addition of agonist and D_b is the initial baseline inner diameter before the first addition of a drug and D_m is maximal inner diameter. Spontaneous tone is expressed at a percentage of maximal intraluminal diameter. Repeated measures analysis of variance (ANOVA) was used to determine differences among young and old, sedentary and exercise-trained groups. Fisher's protected least significant difference post hoc tests were used where appropriate. A one-way ANOVA was used to determine the significance of differences among groups in animal mass, muscle mass and muscle citrate synthase activity. All data are presented as mean ± SEM. Significance was set at $P \leq 0.05$.

3.3 Results

Animals

Body mass increased with age (YS, 384 ± 7 g; OS, 404 ± 7 g), and exercise training resulted in decreased body mass in old rats but not in young rats (YT, 359 ± 4 g; OT, 367 ± 6 g). Left ventricle to body mass ratio was significantly higher in the trained rats compared to age matched controls (Table 3.1). Gastrocnemius and soleus muscle mass to body mass ratio was reduced with aging and increased by exercise training (Table 3.1).

Citrate synthase activity was significantly higher in both muscles of young and old trained rats relative to the sedentary control groups (Soleus muscle; YS 15 ± 1, OS 11 ± 1, YT 19 ± 1, OT 14 ± 1 and Gastrocnemius muscle; YS 16 ± 1, OS 14 ± 1, YT 19 ± 1; OT 22 ± 1), confirming the efficacy of the exercise training regimen.

Adrenergic vasoconstrictor studies

Aging increased maximal soleus muscle arteriolar α-adrenergic vasoconstriction and sensitivity (EC_{50}) to NE compared to young controls (Fig 3.1 A). Although chronic exercise training did not alter NE-induced vasoconstriction in the young rats, exercise training ameliorated the enhanced vasoconstrictor responses observed in the old sedentary rat soleus muscle arterioles (Fig 3.1 A). Gastrocnemius muscle arterioles exhibited no age-related differences in alpha adrenergic vasoconstriction, but exercise training attenuated alpha adrenergic vasoconstriction in old arterioles (Fig 3.1 B). Following removal of the vascular endothelium, the aforementioned aging and exercise training related changes in alpha adrenergic vasoconstriction were no longer present (Fig 3.2 A&B).

Table 3.1 Characteristics of rats, vessel lumen diameters and spontaneous tone.

	Sedentary		Trained	
	Young	Old	Young	Old
N	26	24	25	21
Body Wt (g)	335 ± 5	430 ± 5*	337 ± 5	391 ± 6‡
Heart Wt/ Body Wt Ratio (g/g*100)	0.276 ± 0.016	0.276 ± 0.002	0.309 ± 0.015†0.08	0.303 ± 0.013
LV Wt/ Body Wt Ratio (g/g*100)	0.188 ± 0.004	0.190 ± 0.002	0.200 ± 0.002†	0.208 ± 0.004‡
Soleus Muscle Wt/Body Wt Ratio (g/g*100)	0.044 ± 0.001	0.036 ± 0.001*(0.08)	0.048 ± 0.001†	0.042 ± 0.001‡
Gastrocnemius Muscle Wt/ Body Wt Ratio (g/g*100)	0.490 ± 0.006	0.380 ± 0.005*	0.510 ± 0.010†	0.410 ± 0.007
Soleus Muscle Arteriole Lumen Diameter (μm)	116 ± 3	118 ± 3	101 ± 4†	113 ± 4
Gastrocnemius Muscle Arteriole Lumen Diameter (μm)	151 ± 3	153 ± 4	171 ± 5†	186 ± 4‡
Soleus Muscle Arteriole Spontaneous Tone (%)	45.3 ± 3.4	45.7 ± 3.5	40.5 ± 4.3	32.0 ± 3.9‡
Gastrocnemius Muscle Arteriole Spontaneous Tone (%)	33.0 ± 1.9	30.5 ± 2.6	31.4 ± 2.5	25.5 ± 2.3

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 significant difference between old sedentary and old trained, $P < 0.05$. Values are means ± SEM.

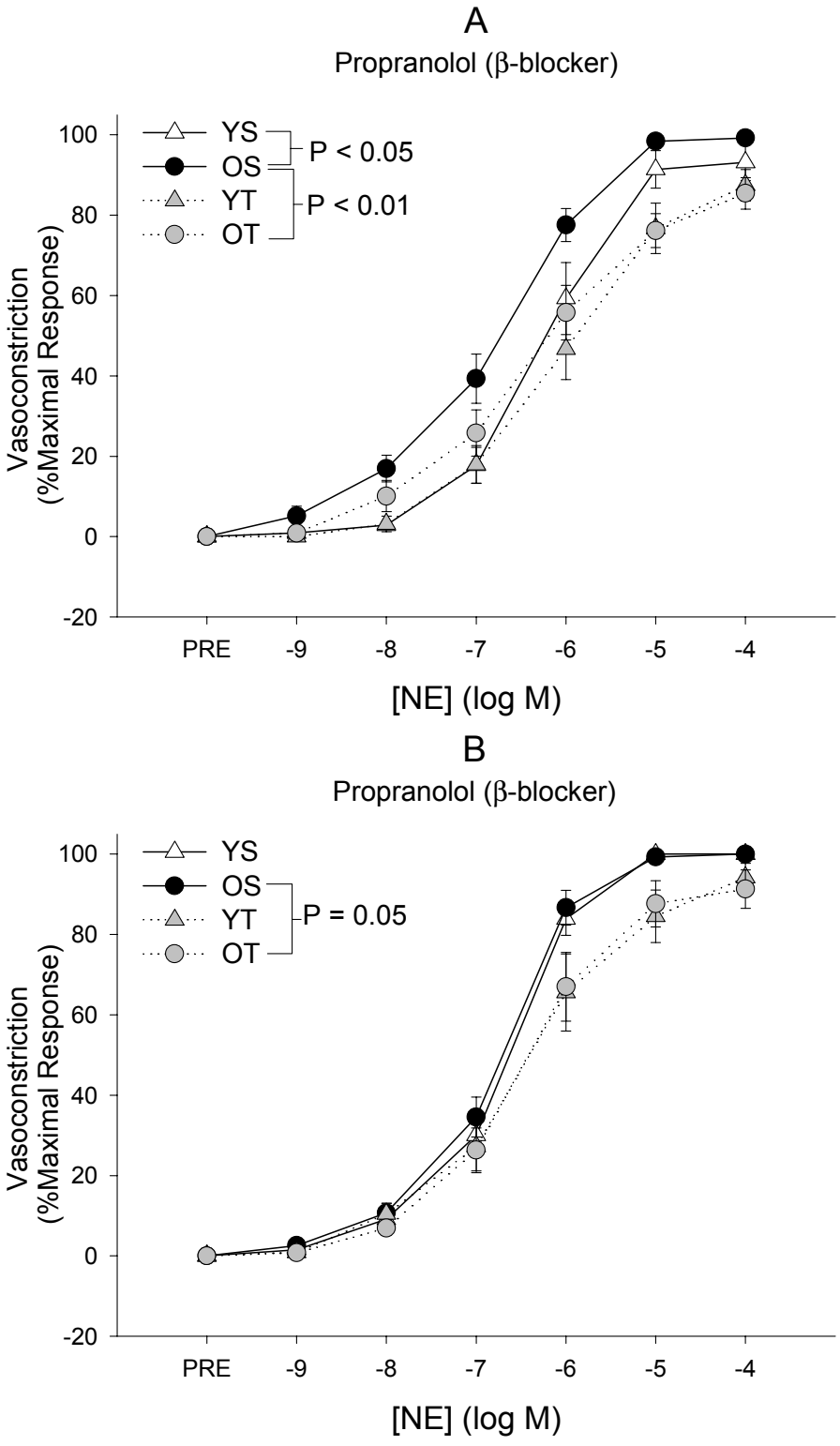

Figure 3.1 Dose-response relations to the cumulative addition of norepinephrine the presence of the beta-adrenergic receptor blocker, propranolol, in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium intact. Values are means ± SEM.

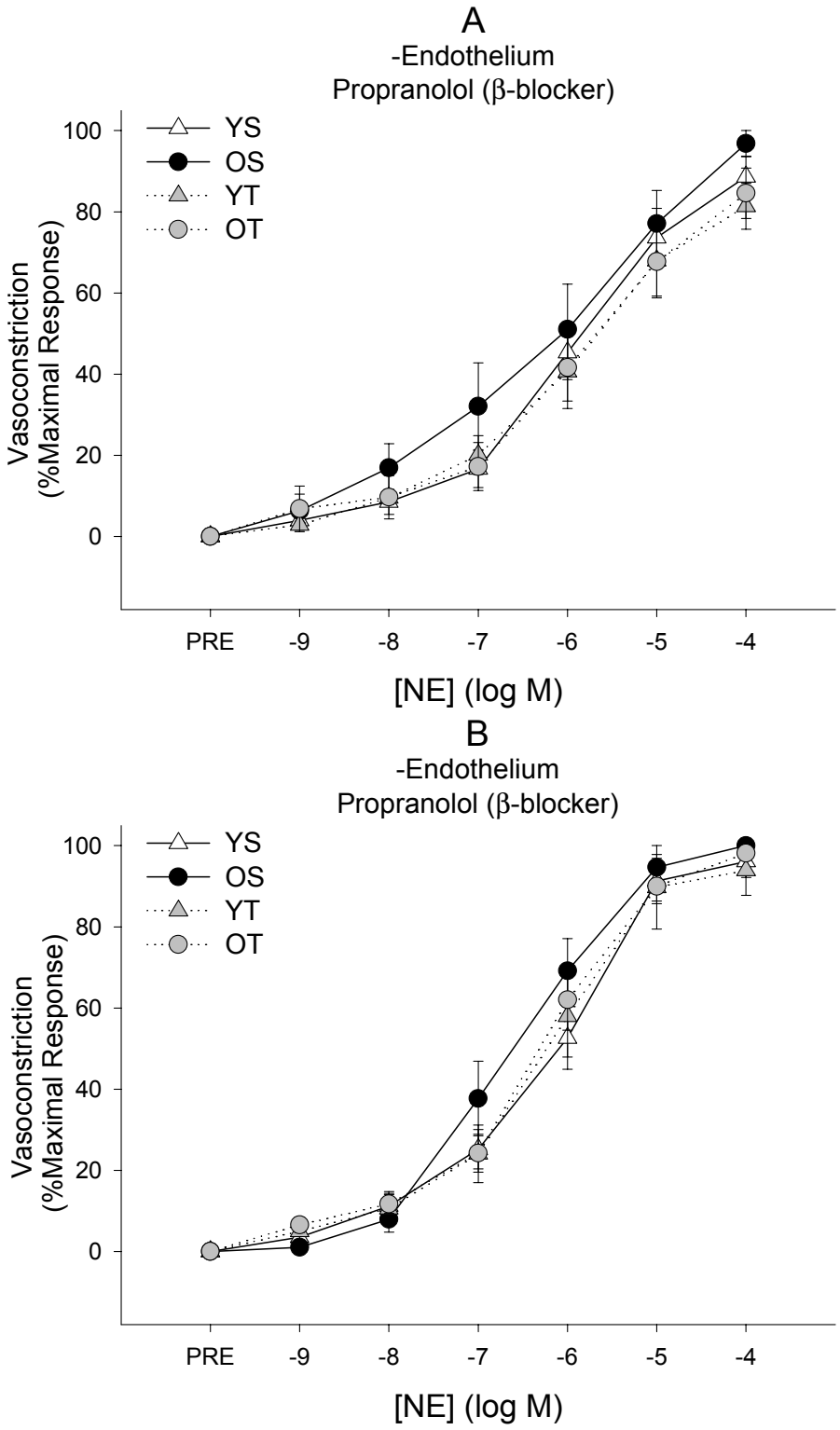

Figure 3.2 Dose-response relations to the cumulative addition of norepinephrine the presence of the beta-adrenergic receptor blocker, propranolol, in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium removed. Values are means ± SEM.

Adrenergic vasodilator studies

β-adrenergic vasodilation to ISO was reduced with aging in both soleus and gastrocnemius muscle arterioles (Fig 3.3 A&B). Exercise training did not affect the β-mediated vasodilation in the soleus muscle arterioles in either the young or old rats (Fig 3.3 A). However, gastrocnemius muscle arterioles exhibited an enhanced sensitivity to ISO in the old trained rats (Fig 3.3 B). Interestingly, the young trained gastrocnemius muscle arterioles exhibited a tendency toward a reduction in β-adrenergic vasodilation when compared to age matched control arterioles (P = .10). Age-associated reductions in vasodilation persisted following the removal of the endothelium from both soleus and gastrocnemius muscle arterioles (Fig 3.4 A&B). No differences in forskolin-mediated vasodilation occurred between young and old arterioles in either the soleus or gastrocnemius muscle arterioles (Fig 3.5 A&B).

KCl-induced vasoconstriction

KCl-induced vasoconstrictor responsiveness was reduced with aging in the gastrocnemius muscle arterioles of the sedentary rats. Aerobic exercise training reduced vasoreactivity to KCl in gastrocnemius muscle arterioles from both young and old rats (Fig 3.6 B). Removal of the endothelium ameliorated the aging and training differences (Fig 3.7 B). No alterations in KCl-mediated vasoconstriction occurred with aging or training in the soleus muscle arterioles (Fig 3.6 A).

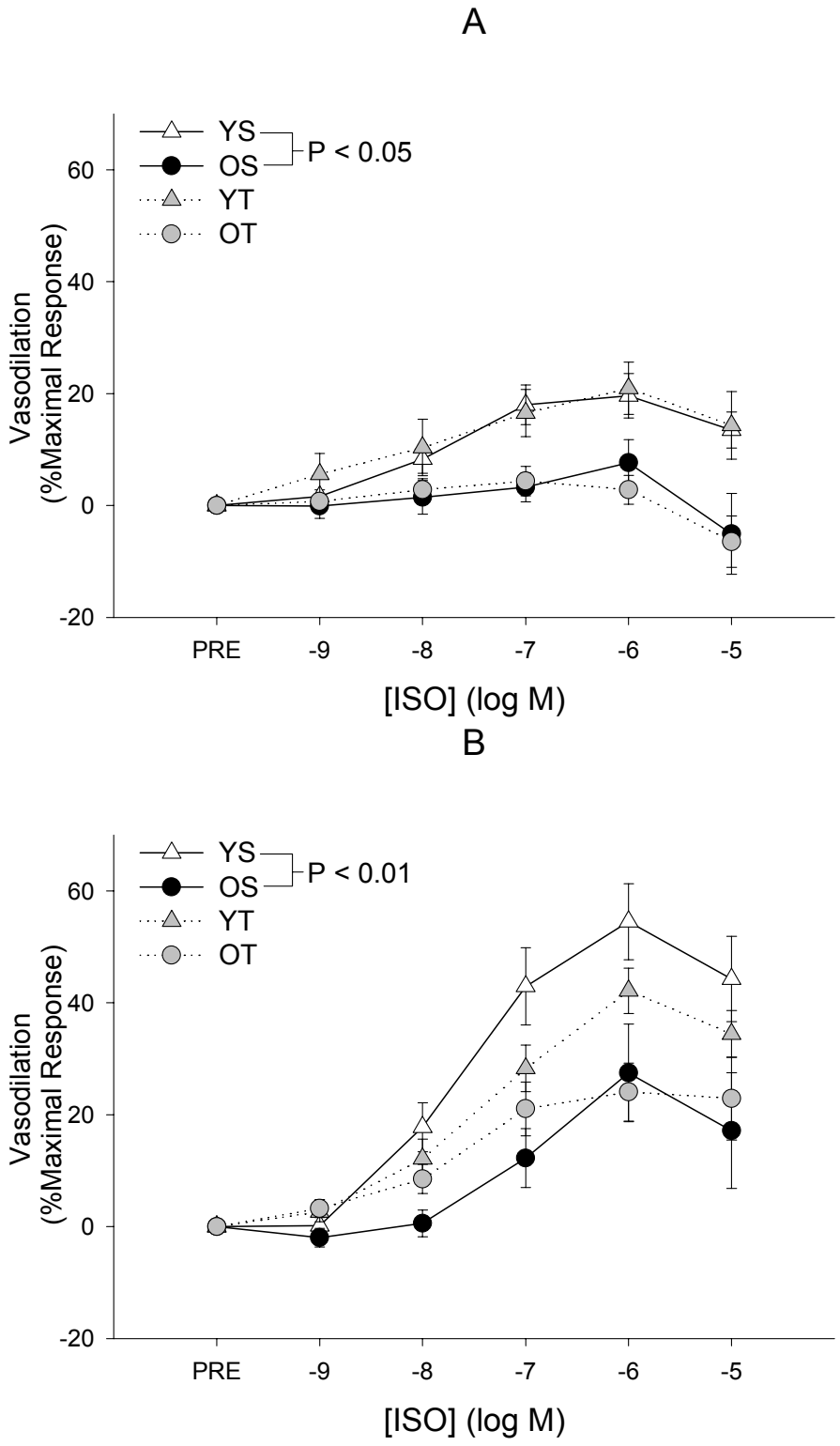

Figure 3.3 Dose-response relations to the cumulative addition of isoproterenol in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium intact. Values are means ± SEM.

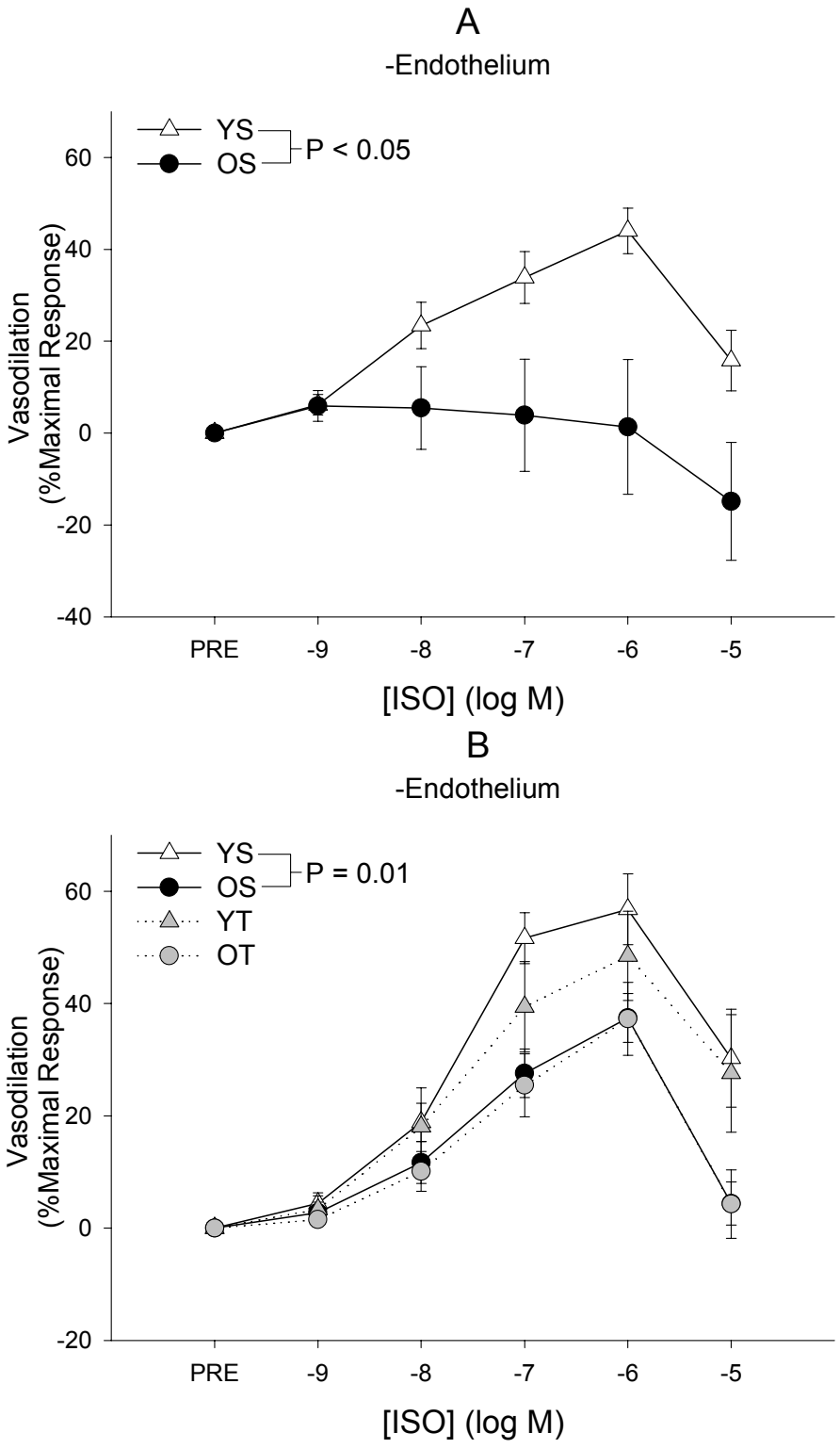

Figure 3.4 Dose-response relations to the cumulative addition of isoproterenol in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium removed. Values are means ± SEM.

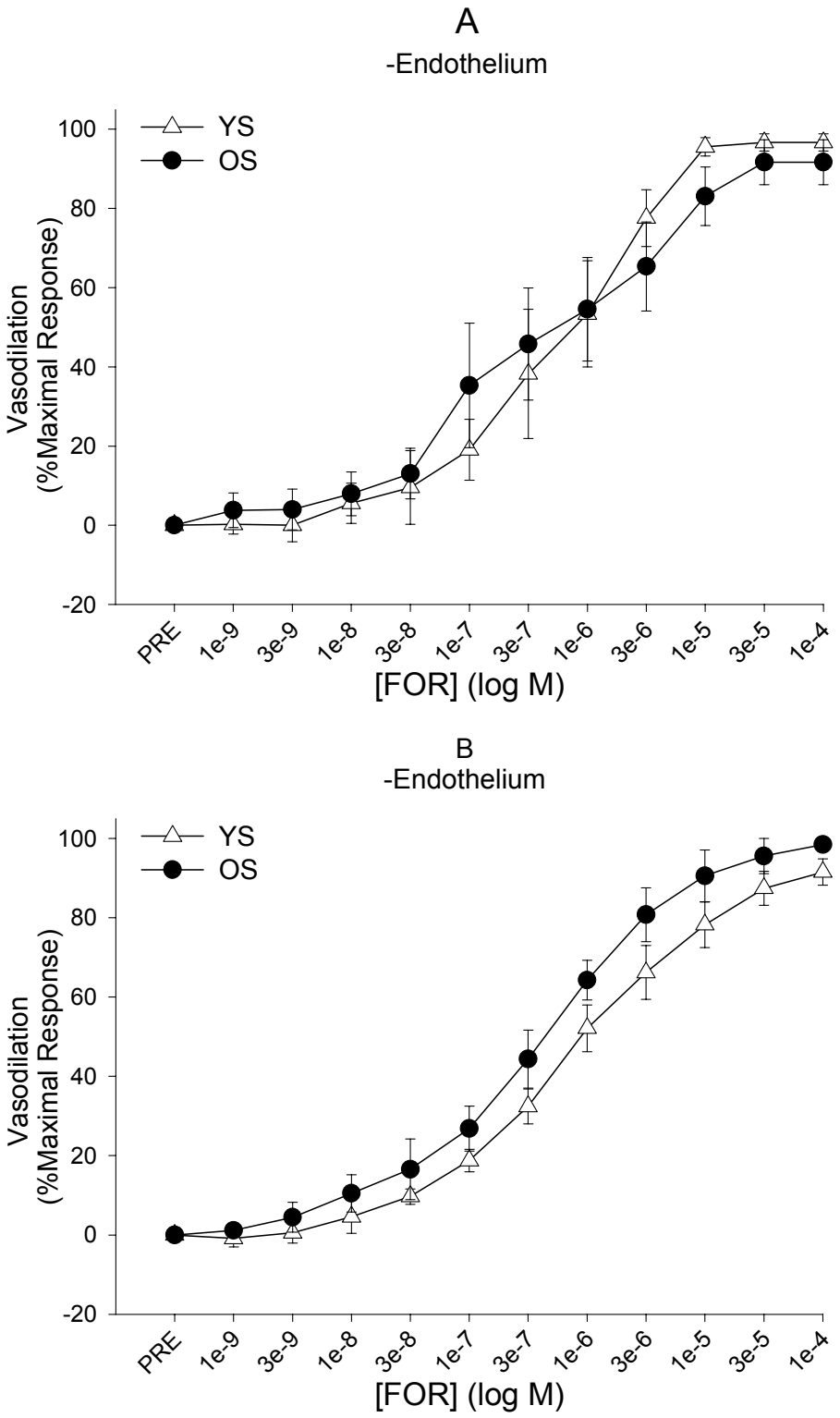

Figure 3.5 Dose-response relations to the cumulative addition of forskolin in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium removed. Values are means ± SEM.

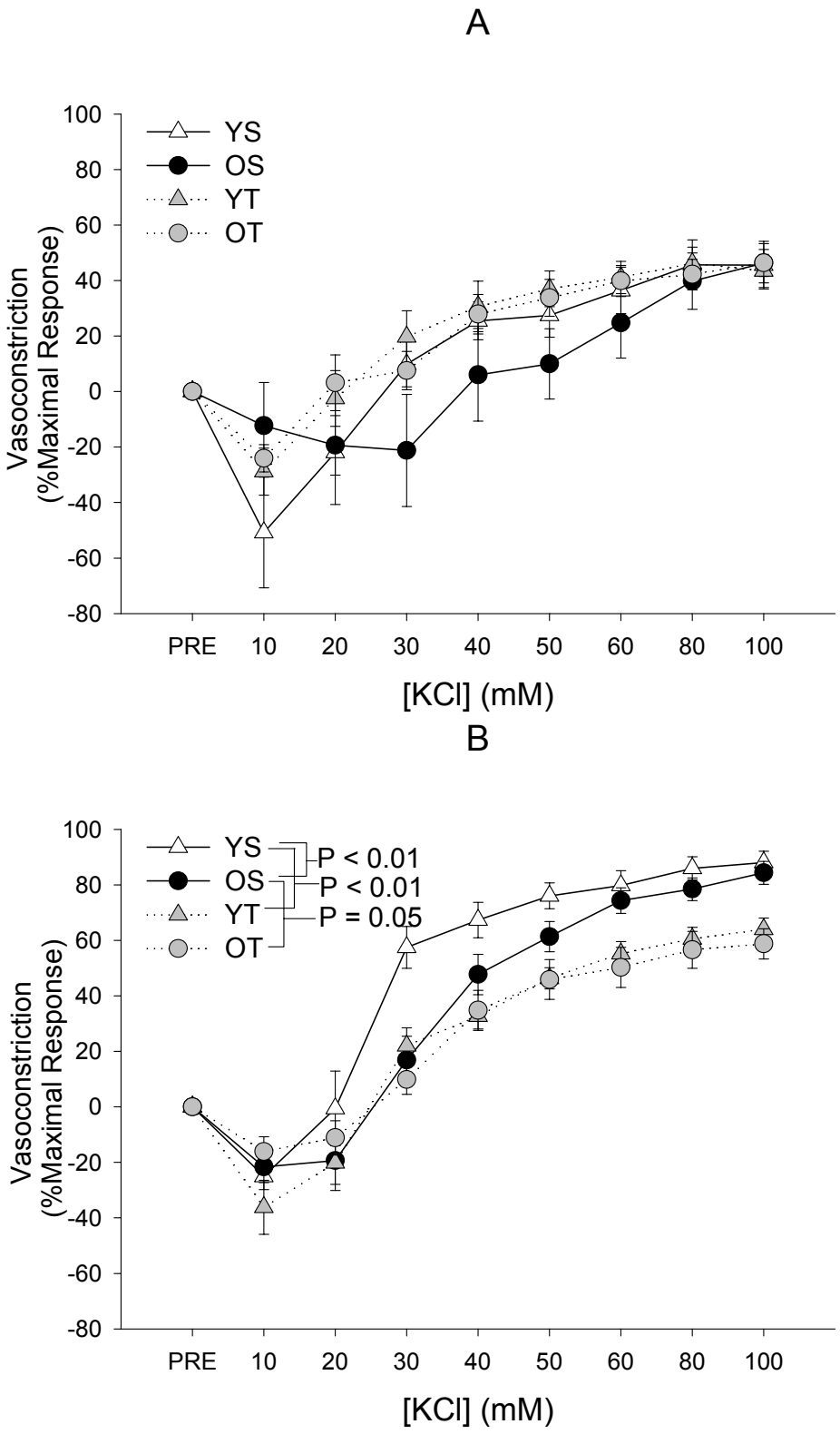

Figure 3.6 Dose-response relations increasing concentrations of isotonic KCl in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium intact. Values are means ± SEM.

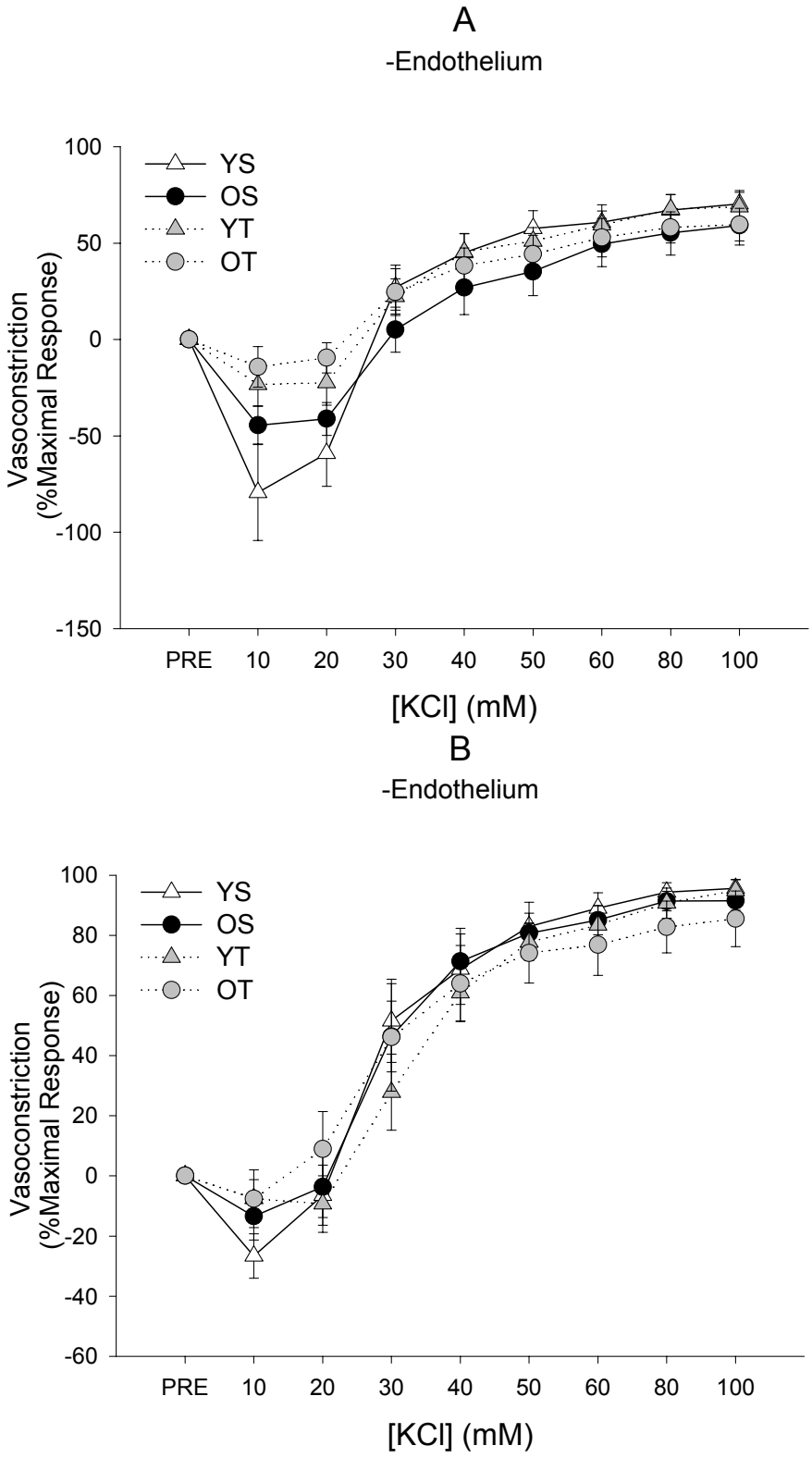

Figure 3.7 Dose-response relations to increasing concentrations of isotonic KCl in soleus (A) and gastrocnemius (B) muscle arterioles with the endothelium removed. Values are means ± SEM.

3.4 Discussion

The purpose of the present study was to determine 1) whether aging alters adrenergic receptor vasoreactivity of skeletal muscle arterioles, and 2) whether exercise training modulates adrenergic-mediated vascular responses in young and old animals. The novel findings of this study are as follows: 1) aging is associated with an enhanced α-adrenergic vasoconstriction in soleus skeletal muscle arterioles, 2) exercise training reduces α-adrenergic vasoconstriction in skeletal muscle arterioles from old rats through an endothelium-dependent mechanism, and 3) aging was associated with a reduction in β_2-adrenegic vasodilation, which does not appear to be due to an impairment of the cAMP messenger system.

In humans it has been shown that leg blood flow is lower and leg vascular resistance is higher in older individuals during both rest and exercise (33, 76, 100). One possible mechanism for this aging effect is a greater sympathetic nerve activity to the legs or an enhanced vasoconstrictor responsiveness to adrenergic stimulation. A study by Dinenno et al. demonstrated that phentolamine (α-adrenergic blocker) infusion abolishes differences in leg blood flow and vascular resistance between young and old subjects, indicating the aging effect of decreased blood flow and increased vascular resistance was the result of augmented muscle sympathetic nerve activity or elevated adrenergic sensitivity of the leg vasculature in older men (35). Indeed, previous work has shown a greater vasoconstriction to a cold pressor test during exercise in older individuals (69). Furthermore, results from the present study support the concept that aging increases adrenergic vasoconstriction in resistance arteries from highly oxidative skeletal muscle. This enhanced

vasoconstrictor responsiveness is associated with the vascular endothelium and may be the result of a diminished β-receptor mediated vasodilation that normally opposes α-receptor mediated vasoconstriction. This agrees with numerous studies demonstrating that endothelial function declines with age (29, 42, 114).

The notion that aging enhances adrenergic vasoconstriction is in contrast to a previous report that has shown forearm α-adrenergic vasoconstriction to luminal infusion of adrenergic agonists to be reduced with older age (38). Unfortunately, studies in the forearm cannot be generalized to the lower leg because of inherent differences adrenergic receptor subtype and density (98). Thus, the physiological changes in the arm may not mimic those found in the leg.

Previous work has demonstrated that chronic exercise training reduces vasoconstrictor responsiveness to NE in abdominal aortas from young animals (25, 110), while the present study only showed a tendency for a reduction in α-adrenergic responsiveness of arterioles from young trained animals. The difference in the training effects reported in previous studies and the present investigation may reflect differences in the adaptive responses in conduit versus resistance arteries in young animals. Despite the lack of a training effect in arteries from young animals, the present results demonstrate that chronic endurance exercise reduces α-adrenergic receptor sensitivity and vasoconstrictor responsiveness in skeletal muscle arterioles from older rats. In addition, the results suggest that the mechanism of this reduction in adrenoreceptor vasoconstriction is due to an α-adrenergic receptor mechanism associated with the endothelial cell layer. Previous work has demonstrated that stimulation of endothelial α_2-adrenoreceptors causes

the release of NO, prostacyclin, and EDHF, which would promote vasorelaxation (120). This interpretation in agreement with previous work from our laboratory which indicate that exercise training increases both endothelial mediated vasodilation in skeletal muscle arterioles from old rats (111), as well as by data in the human forearm demonstrating exercise training is associated with enhanced endothelium-dependent vasodilator function in older individuals (29). Since increases in vascular shear stress induced by exercise have been shown to increase endothelial nitric oxide synthase mRNA and protein expression (24, 111), it is tempting to speculate that the endothelium-dependent vasodilator mechanism through which NE vasoconstriction is blunted occurs via the nitric oxide synthase pathway.

Further evidence that aging and training-induced alterations in adrenergic vasoconstrictor responsiveness are not due to intrinsic changes in smooth muscle cell vasoconstrictor properties are supported by the KCl-mediated vasoconstrictor responses. In gastrocnemius muscle arterioles, vasoconstrictor responses to KCl were diminished by aging and exercise training (Fig 3.6 B). However, when these arterioles were denuded of the endothelial cell layer, the smooth muscle response to the depolarizing effects of KCl were no longer different among the young and old, sedentary and trained groups (Fig 3.7 B). These data indicate that smooth muscle vasoconstrictor properties remain largely unchanged with aging and training, but that endothelial function is altered by aging and physical conditioning. Although the present results do not provide insight into the mechanism through which the endothelial cells alter KCl-mediated vasoconstrictor responses, it is apparent that a

coupling mechanism(s) exists between endothelial and smooth muscle cells which is capable of influencing KCl-mediated vasoconstriction in gastrocnemius muscle arterioles. The soleus muscle arterioles did not exhibit this phenomenon, thus the reduction in vasoconstriction is likely due to alterations in adrenergic receptor number or its signalling pathway.

Interestingly, the α-adrenergic system reacts differently with aging and exercise training than does the β-adrenergic system. The β_2-adrenergic receptor mediates vasodilation via both endothelium-dependent and -independent mechanisms (120). The present results demonstrate that there is an age-associated reduction in β_2-adrenergic vasodilation and that exercise training fails to augment maximal vasodilation in skeletal muscle arterioles from either young or old rats. Still, sensitivity to isoproterenol is enhanced with training in arterioles from fast-twitch glycolytic skeletal muscle from old rats and this enhanced sensitivity is abolished by removal of the endothelium, indicating that it is due to enhanced endothelial β_2-mediated vasodilation. The reduced β_2-adrenergic vasodilation with aging agrees with previous findings in the human leg showing β_2-adrenergic vasodilation is reduced with old age (119). Our data extend these previous observations by demonstrating that the age related β_2-adrenergic vasodilator dysfunction is not due reductions in endothelial function with age, but appears to be a compromised smooth muscle cell β_2-receptor function.

In conclusion, adrenergic receptor-mediated vascular responses shift toward a more pro-constrictor phenotype in skeletal muscle arterioles during healthy aging. This is brought about by an enhanced α-adrenergic vasoconstriction in highly

oxidative slow-twitch muscle arterioles and a more generalized reduction in arteriolar α-adrenergic vasodilation. The increases in α-adrenergic vasoconstriction in skeletal muscle arterioles with aging appear to be due to a reduction in α-adrenoreceptor endothelial-mediated vasodilator function. In contrast, exercise training in old animals appears to augment endothelial α-adrenoreceptor vasodilator function, normalizing the vasoconstrictor function to levels near that of their younger counterparts. The diminished β_2-mediated vasodilation does not appear to be due to a diminished endothelial function or an impaired cAMP signaling mechanism, but may be related to a reduction in vascular smooth muscle α-receptor function. Thus, it appears that adrenergic mechanisms could explain some of the increases in systemic vascular resistance with aging. Moreover, the effect of exercise training to enhance α-adrenergic-mediated endothelium-dependent vasodilation could contribute to the beneficial effects of exercise on cardiovascular disease.

CHAPTER IV

EFFECTS OF AGING AND EXERCISE TRAINING ON ARTERIAL STIFFNESS AND STRUCTURE

4.1 Introduction

It has been well documented that senescence is associated with lower large artery compliance and increases in large artery stiffness (21, 49, 73, 89, 115). Various experimental techniques have been applied to examine arterial stiffness, including extraction of the aorta or carotid artery to determine the mechanical properties of the arterial wall (20), using ultrasonagraphy to examine the in vivo distension of a single large artery to cyclic increases in blood pressure (115), and with measurements of pulse wave velocity or augmentation index to indirectly assess arterial stiffness of long segments of artery (46, 94). Measures of arterial stiffness using the latter two approaches not only reflect the intrinsic mechanical properties of the artery, but also are strongly influenced by myogenic tone, neuronal vasoconstriction,and local release of vasodilators (61). Therefore, old age-associated elevations in muscle sympathetic nerve activity or diminished basal and stimulated NO bioavailability could independently increase arterial stiffness and not reflect a true increase in the mechanical stiffness of the vessels themselves.

In contrast to aging, it has been shown that several months of aerobic walking exercise reduces in vivo large artery stiffness in an older population (115). Such changes in arterial stiffness may be due to age-related alterations in the prevailing vasoconstrictor or vasodilator influences (111, 113) or to changes in the

intrinsic mechanical properties of the arterial wall. An acute bout of cycling exercise also reduces arterial stiffness (67). These data suggest that training-induced reductions in arterial stiffness may be due to changes in the vasoconstrictor or vasodilator influences on these large arteries. Currently, it is not clear whether changes in the intrinsic mechanical properties of the vascular wall contribute to the beneficial effects of exercise training on arterial stiffness.

Therefore, the purpose of the present study was to determine the effects of exercise training on the mechanics, stiffness and structure of large and small conduit arteries, as well as skeletal muscle resistance arterioles from young and old rats. We hypothesized that aging would result in an increase in arterial wall stiffness, and that chronic exercise training would not ameliorate the increases in stiffness seen in the old rats.

4.2 Material and methods

Animal characteristics

Male Fischer 344 young (4-6 months) and old (24-26 months) rats were obtained (Harlan Inc) and housed in a temperature controlled (23 ± 2 ºC) room with a 12:12 light-dark cycle. These selected time points represent young adulthood and senescence (~50% mortality rate). The Fischer 344 rat model is supported by the National Institute on Aging because these rats can be used to study the effects of old age on the cardiovascular system in the absence of overt cardiovascular disease. All animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee and complied by the guidelines of the National Research

Council *Guide for the Care and Use of Laboratory Animals* (Washington DC: National Academy Press, Revised 1996).

Exercise training

All rats were habituated to treadmill exercise. Each rat walked on a motor-driven treadmill at 15 m/min (0° incline), 5 min/day for 3 days. At the end of the habituation period old and young rats were assigned to either a control sedentary group (YS or OS, respectively) or an exercise-trained group (YT or OT, respectively). Exercise-trained rats performed treadmill running at 15 m/min (15° incline), 1hr/day, 5 days/wk, for 10-12 weeks as previously described (25). Vascular mechanics were determined 48 hours after the last exercise bout in trained rats. Animals were anesthetized via I.P. injection of sodium pentabarbatol and euthanized via exsanguination.

Large vessel preparation

Segments of the thoracic aorta between the intercostal artery and the diaphragm, the abdominal aorta between the diaphragm and the renal artery, and the iliac arteries were carefully exposed, excised, and placed in chilled (4^{o}C) Krebs physiological saline solution (PSS). With the aid of a stereomicroscope (Olympus SZX12), three thoracic, three abdominal, and two iliac rings were cut from each arterial segment, with axial lengths of approximately 3.0 mm. In addition, smaller rings (~1.0 mm in axial length) were cut directly adjacent to each of the larger rings for measurement of outside diameter (OD) and inside diameter (ID) with a Filar calibrated micrometer eyepiece as previously described (23, 25). The small rings were subsequently discarded after measurement, while the larger rings were used

for the mechanical studies.

The passive mechanical properties of the large arteries were first assessed by measuring passive tension in response to graded increases in stretch. All of the thoracic, abdominal, and iliac arterial rings from each experimental animal were mounted on two stainless steel wires that passed through the vessel lumen. One wire was attached to a force transducer (Model FT03c, Grass Instruments Co.) and the other to a micrometer microdrive (Stoelting/Prior Microdrive, Stoelting Co.) to permit the vessel to be stretched by known increments. The vessel rings were immersed in a 20 ml tissue bath (Harvard Apparatus) containing Krebs buffer solution equilibrated at 37^{o}C with 95% O_2-5% CO_2. Passive tension was measured and recorded using a computer and data acquisition system (MacLab Electronic Data Acquisition System). All segments started from a reference position designated as L_o. This length represents the inner diameter of the segments at their unloaded or unstretched state.. Rings from all four groups were individually stretched by increments of 20% of their initial inner diameter. The passive tension relation was assessed over a range of uniaxial displacements or stretches from 120-240% of L_o with the vessels bathed in a calcium-free Krebs solution.

The passive mechanical properties (i.e., stress-strain relations) of arterial rings were then determined by evaluating the Cauchy stress-stretch responses, calculated from the passive tension relation. In the calculation of Cauchy stress-strain, the uniaxial displacements of the passive relation are used to calculate the hoop stretch as:

$$\lambda = r/R \qquad (1)$$

where λ is the hoop stretch, a dimensionless measure of strain, r is the deformed radius at each increment of stretch, and R is the initial undeformed radius. Cauchy stresses were determined in each of the individual thoracic, abdominal, and iliac rings and averaged as one observation per vessel type per animal.

Although tension is commonly used to express resting and isometric contractile force of vessel rings, it is limited because it does not take into account potential differences in cross-sectional area or the material properties (e.g., distensibility) of vessels. Stress responses, however, account for these potential differences. The Cauchy stress is defined as the actual force or tension acting over an area in the current (deformed) configuration (58). The Cauchy stress was calculated as (20, 58)

$$t = \frac{\lambda L}{2HD} \tag{2}$$

where t is the one dimensional (1-D) Cauchy stress, λ is the circumferential stretch ratio or hoop stretch, H is the current wall thickness, D is the axial length and L is the applied load (applied tension), which is equal to passive tension. The current wall thickness (H) at each length was calculated as:

$$V = (\lambda_o + H_o)^2 - \lambda_o^2 = (\lambda + H)^2 - \lambda^2 \tag{3}$$

where V is the wall volume (a constant), $\lambda_o = 1$ (reference stretch), and H_o is the unloaded (reference) wall thickness. Equation 2 is a rearrangement of the equation determined by (22, 124).

Since equation 2 is defined in the current configuration, a 1-D strain in the current configuration can only be used to measure the deformation associated with the stress. Since the deformations of aortic rings are large, principal stretch ratios

are appropriate measures of strain for these arteries (58). Thus, the hoop stretch (λ) was calculated from measures of undeformed and deformed inner wall hoop lengths. These lengths were measured by photographing (using an Olympus SC35 camera and SZX12 stereomicroscope) the arterial rings at each increment of uniaxial displacement and measuring the inner circumference from the vessel image using a Bioquant image analysis system.

Incremental stiffness of arterial rings was then determined from the passive stress-stretch (strain) relation, using the following equation:

$$S = \frac{\Delta t_i}{\Delta \lambda_i} \tag{4}$$

where S is the incremental stiffness, Δt_i is the change in Cauchy stress (eq. 1) for each datum point, and $\Delta \lambda_i$ is the change in hoop stretch for each datum point. Incremental stiffness points were plotted as a function of their corresponding Cauchy stress points, and a linear regression analysis was used to calculate the slope of that relation (96). The slope was then used as a measure of the overall relative stiffness for each artery.

Microvessel preparation

The gastrocnemius-plantaris-soleus muscle complex was carefully excised from each leg. Following excision, the muscle complex was placed in cold (4º 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, pH 7.4. Gastrocnemius and soleus muscle first-order (1A) arterioles were isolated with the aid of a dissecting microscope (Olympus SVH10) as previously described (78, 92).

In the soleus and gastrocnemius muscle, 1A arterioles were defined as the first arterial branch after the feed artery entered the muscle. The arterioles (length, 0.5 - 1.0 mm; inner diameter, soleus muscle: 60-159 μm, gastrocnemius muscle: 84-220 μm) were cleared of surrounding skeletal muscle fibers, removed from the muscle and placed in Lucite chambers containing MOPS buffered calcium-free PSS equilibrated to room air. The arterioles were cannulated on both ends with micropipettes and secured with 11-0 surgical nylon suture. After cannulation, the chambers were 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) for recording of luminal diameter. Arterioles were pressurized to the in vivo pressure of 60 cm H_2O (44 mmHg) with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then closing the reservoirs, verifying that diameter remained constant. Arterioles that exhibited leaks were discarded.

Arterioles equilibrated at 37°C for 60 min with the vessel chamber and pressure lines filled with calcium-free PSS containing 2.0 mM EDTA. The arterioles were rinsed every fifteen minutes during the 60 min period to induce complete vasorelaxation. The intraluminal pressure was then lowered to 0 cm H_2O. A pressure/diameter relation was then established by increasing pressure in increments of 15 cm H_2O from 0 to 135 cm H_2O. After each step increase in pressure, inner diameter was continuously recorded for 3 min. Following the last pressure step, intraluminal pressure was returned to 60 cm H_2O for 5 min. The vessel was then fixed with 2% Bouins, stained with fast green, and frozen in OCT

as previously described (28, 92). Vessels were cut into 7 μm thick cross-sections on a cryotome, mounted on glass microscope slides and stained with Masson's trichrome stain.

In order to determine medial cross-sectional area (CSA), sections were examined under a light microscope with an attached video camera coupled to an image processor, optical mouse, and personal computer equipped with an image analysis system (BioQuant). Medial wall thickness (WT) at each pressure step was then calculated from CSA (measured at 60 cm H_2O) and the inner diameter (ID) as measured at each pressure step according to the following formula:

$$WT = [(4CSA/\pi + ID^2)^{1/2} - ID]/2 \quad (5)$$

The validity of estimating WT at intraluminal pressures other than 60 cm H_2O is based on the assumption that wall volume remains constant with changes in diameter. This assumption has been verified for muscle arterioles (124). Circumferential stress (σ) at each pressure step was calculated from intraluminal pressure (IP), ID, and WT using the equation:

$$\sigma = (IP \times ID)/(2WT) \quad (6)$$

Circumferential strain (ε) was calculated as the change in diameter relative to the original diameter measured at 60 cm H_2O:

$$\varepsilon = (ID_f - ID_o)/ID_o \quad (7)$$

where ID_o is the original diameter measured at 60 cm H_2O and ID_f is the measured diameter at each pressure step. Stiffness was determined from the stress/strain relation as describe for the large vessel segments.

4.3 Results

Animals

Body mass increased with age (YS, 384 ± 7 g; OS, 404 ± 7 g), and exercise training resulted in decreased body mass in old but not young rats (YT, 359 ± 4 g; OT, 367 ± 6 g). Left ventricle to body mass ratio was significantly higher in the trained rats compared to age matched controls (Table 4.1). Gastrocnemius and soleus muscle mass to body mass ratios were reduced with aging and increased by exercise training (Table 4.1). Citrate synthase activity was significantly higher in both muscles of young and old trained rats relative to the sedentary control groups (Soleus: YS 15 ± 1, OS 11 ± 1, YT 19 ± 1, OT 14 ± 1; Gastrocnemius: YS 16 ± 1, OS 14 ± 1, YT 19 ± 1, OT 22 ± 1), confirming the efficacy of the exercise training regimen ($P < .05$).

Morphology

Lumen diameters of the thoracic and abdominal aortas and the iliac arteries were larger in the aged animals compared to young controls (Fig 4.1), although no differences in arteriolar diameter were observed with aging in either of the skeletal muscles examined (Fig 4.1). In young rats, aerobic exercise training resulted in an increase in lumen diameters of the iliac artery and gastrocnemius muscle arterioles, but a smaller luminal diameters of the soleus muscle arterioles when compared to age-matched sedentary rats (Fig 4.1). In aged rats, training increased the luminal diameters of only the gastrocnemius muscle arterioles when compared to the sedentary aged rats (Fig 4.1).

Table 4.1 Fischer 344 rat characteristics.

	Sedentary		Trained	
	Young	Old	Young	Old
N	26	24	25	21
Body Wt (g)	335 ± 5	430 ± 5*	337 ± 5	391 ± 6‡
Heart Wt/ Body Wt Ratio (g/g*100)	0.276 ± 0.016	0.276 ± 0.002	0.309 ± 0.015†0.08	0.303 ± 0.013
LV Wt/ Body Wt Ratio (g/g*100)	0.188 ± 0.004	0.190 ± 0.002	0.200 ± 0.002†	0.208 ± 0.004‡
Soleus Muscle Wt/Body Wt Ratio (g/g*100)	0.044 ± 0.001	0.036 ± 0.001*(0.08)	0.048 ± 0.001†	0.042 ± 0.001‡
Gastrocnemius Muscle Wt/ Body Wt Ratio (g/g*100)	0.490 ± 0.006	0.380 ± 0.005*	0.510 ± 0.010†	0.410 ± 0.007

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 significant difference between old sedentary and old trained, $P < 0.05$. Values are means ± SE

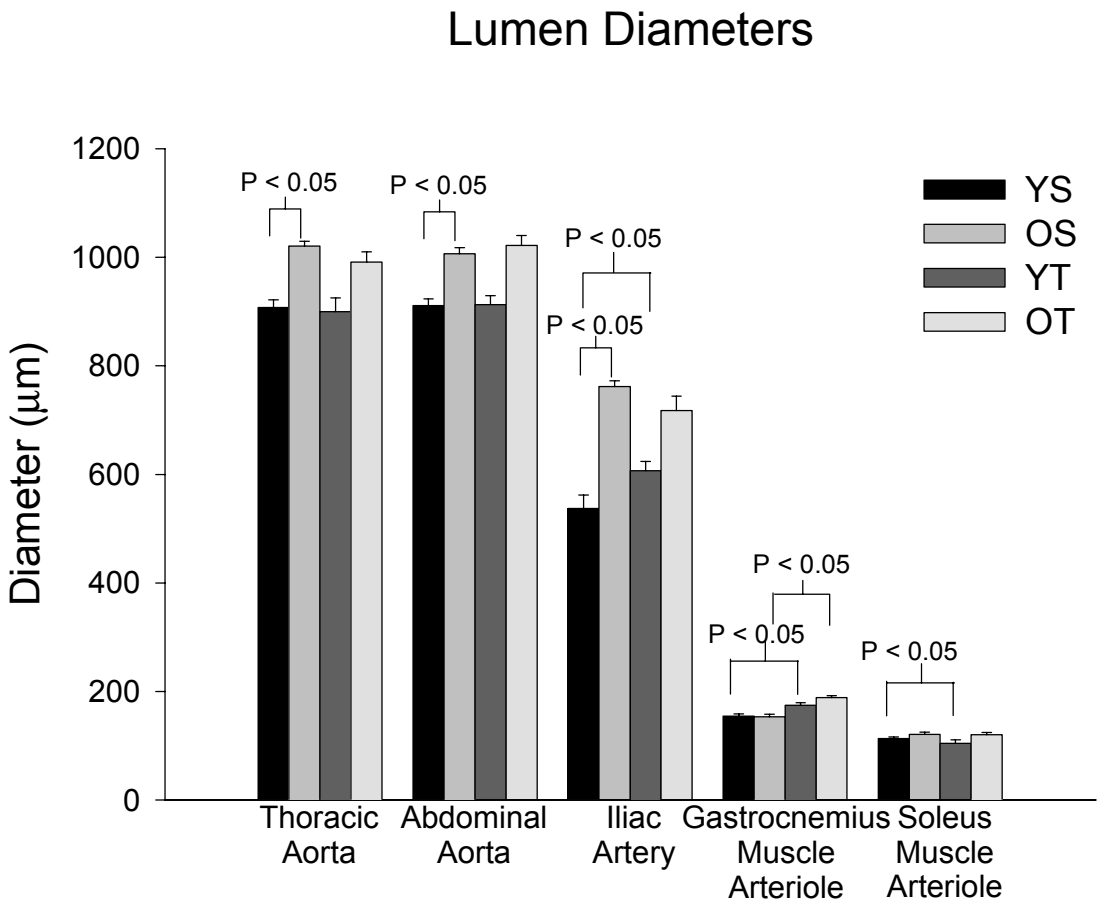

Figure 4.1 Lumen diameters of thoracic aorta, abdominal aorta, iliac artery, gastrocnemius and soleus muscle arterioles from young and old sedentary and aerobic exercise trained groups. Values are means ± SEM.

Medial wall cross sectional area was increased in the conduit arteries of the aged sedentary rats when compared to young sedentary rats (Fig 4.2). However, cross sectional area was decreased in soleus muscle arterioles of aged sedentary rats compared to young controls, with no differences observed in the gastrocnemius muscle arterioles (Fig 4.2). In aged rats, training resulted in decreased medial wall cross sectional area in the thoracic aorta and iliac arteries (Fig 4.2), and an increased medial wall cross sectional in the abdominal aorta (Fig 4.2) and soleus muscle arterioles (Fig 4.2) of aged rats. No differences in cross sectional area occurred in response to training in the young rats.

Aging resulted in an increased medial wall thickness in each of the conduit arteries (Fig 4.3), while no changes occurred in the arterioles (Fig 4.3). In old rats, there were trends toward an increase in the wall thickness in the abdominal aorta ($P = 0.07$) and a decrease in wall thickness of the iliac artery ($P = 0.081$) with training (Fig 4.3). Wall thickness was unaltered by training in any of the vessel groups from the young rats. Wall-to-lumen ratio was unaltered by aging or training in any vessel groups examined (Fig 4.4).

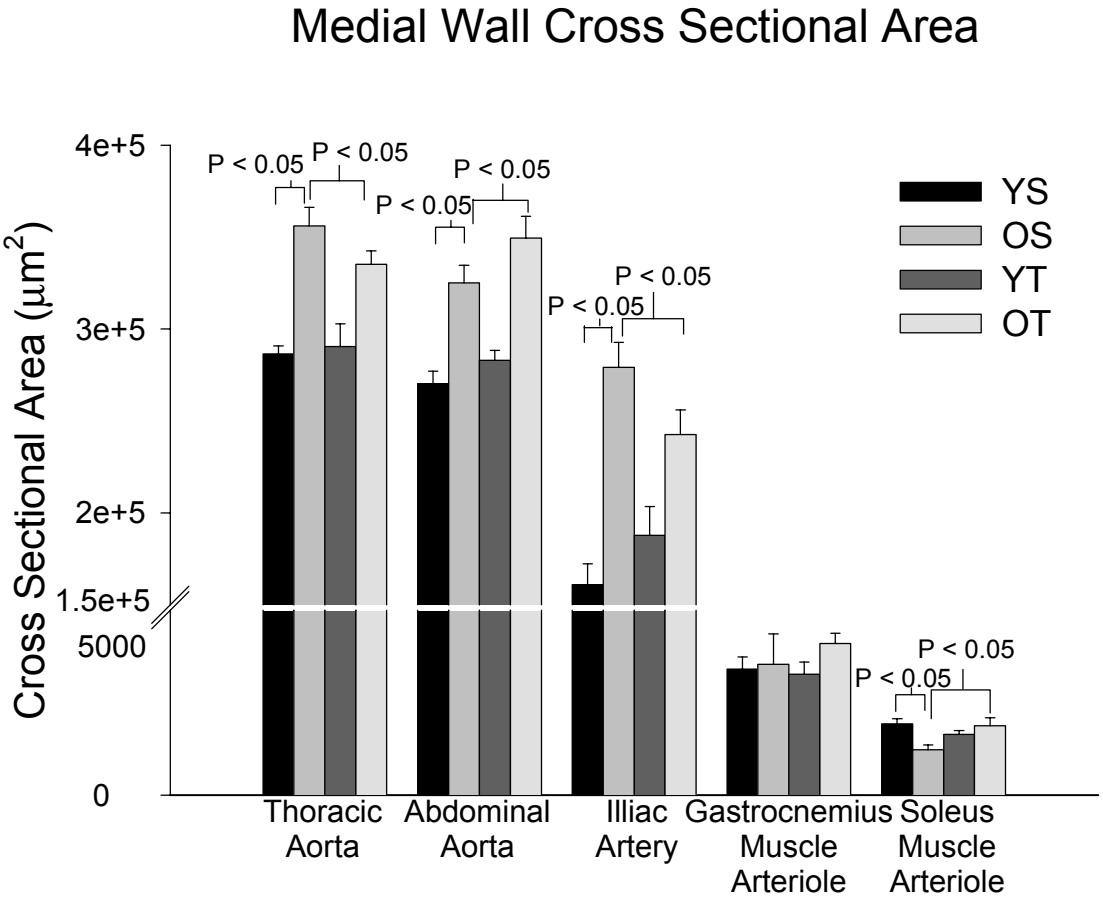

Figure 4.2 Medial wall cross sectional areas of thoracic aorta, abdominal aorta, iliac artery, gastrocnemius and soleus muscle arterioles from young and old sedentary and aerobic exercise trained groups. Values are means ± SEM.

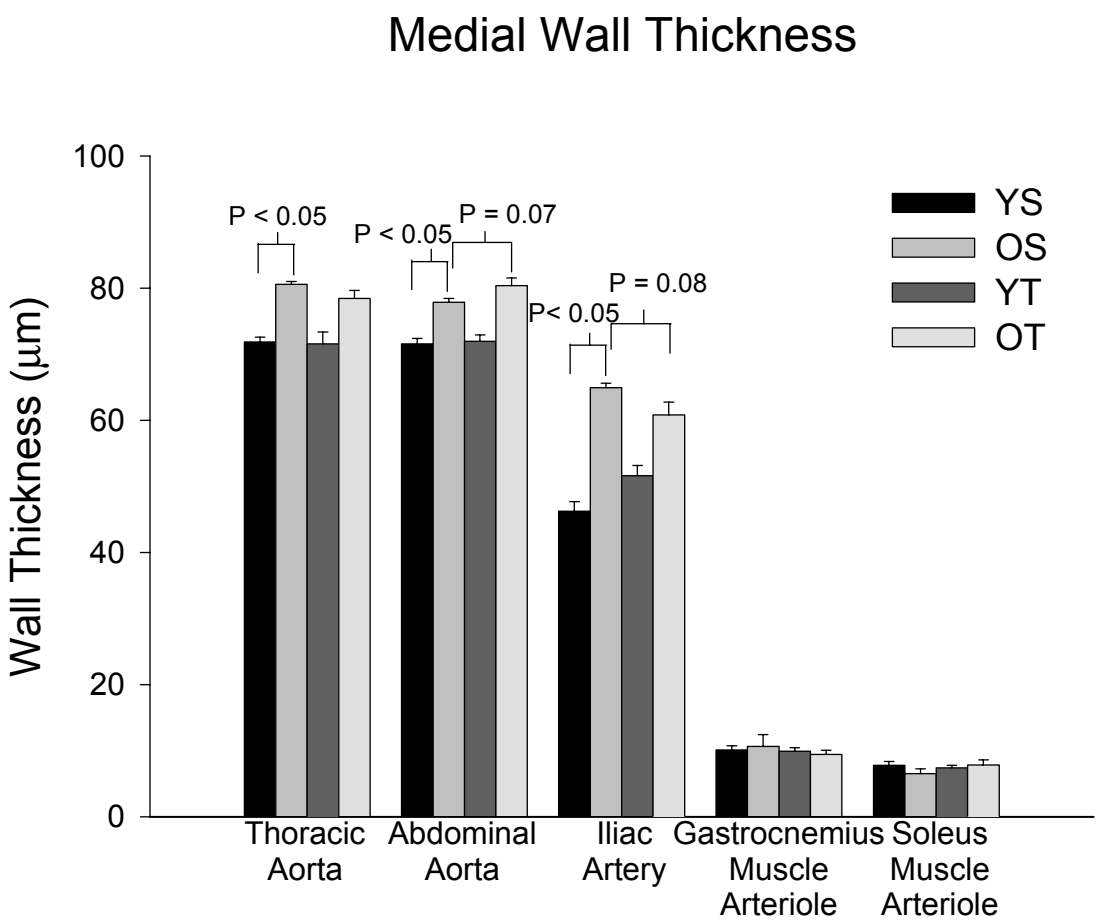

Figure 4.3 Medial wall thickness of thoracic aorta, abdominal aorta, iliac artery, gastrocnemius and soleus muscle arterioles from young and old sedentary and aerobic exercise trained groups. Values are means ± SEM.

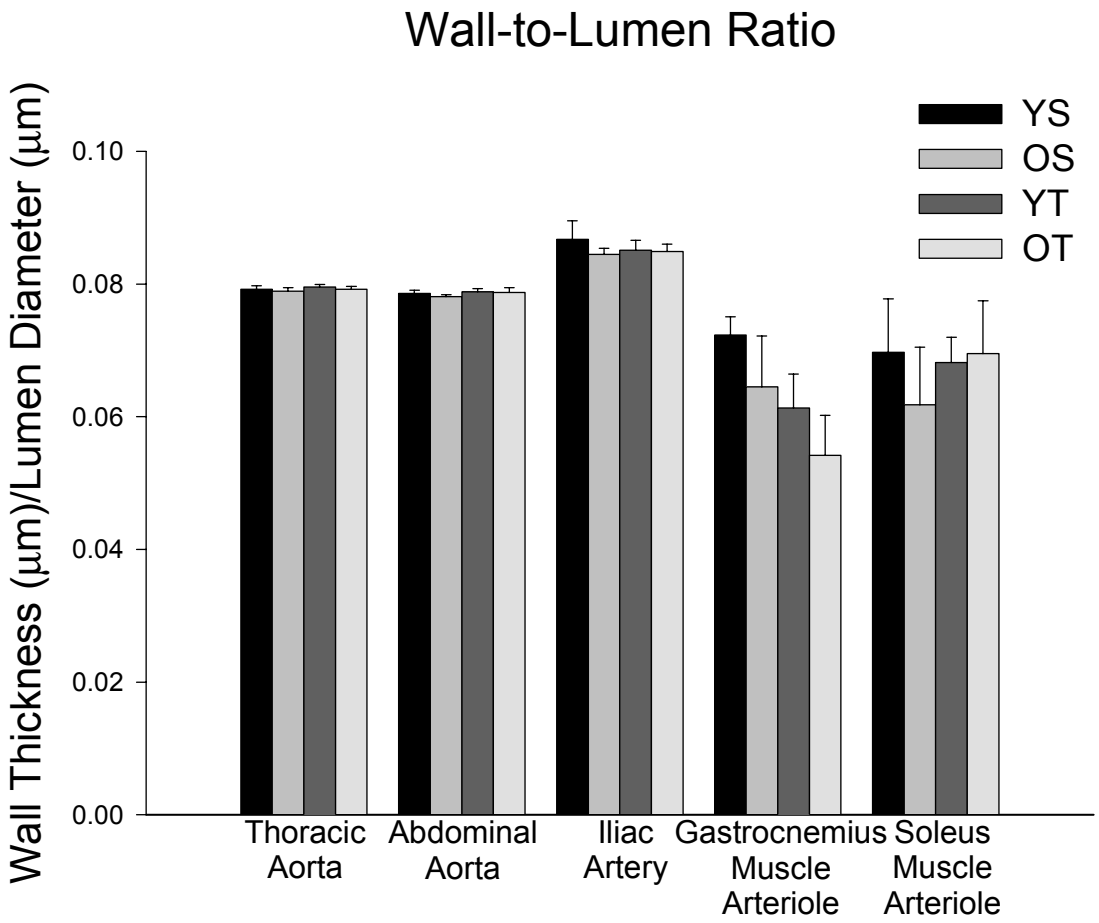

Figure 4.4 Medial wall thickness to lumen diameter ratio of thoracic aorta, abdominal aorta, iliac artery, gastrocnemius and soleus muscle arterioles from young and old sedentary and aerobic exercise trained groups. Values are means ± SEM.

Passive mechanical properties

Passive tension was increased with aging in the thoracic aorta and iliac arteries, but not in the abdominal aorta with aging (Fig 4.5). Training increased passive tension in the thoracic aorta of young rats (Fig 4.5 A). No other differences in passive tension occurred in the conduit arteries with aging or training. Passive distension of the gastrocnemius and soleus muscle arterioles was unaltered by aging or training (Fig 4.6 A&B).

There was a tendency ($P = 0.09$) for Cauchy stress to increase in the thoracic aorta (Fig 4.7 A) with aging. Training increased Cauchy stress in the young thoracic aorta and iliac artery, and tended to increase in the old iliac artery ($P = 0.07$) (Fig 4.7). There was also a higher Cauchy stress in the gastrocnemius muscle arterioles from old rats with training (Fig 4.8 B).

No differences in stretch (strain) were observed with aging in any artery group examined (Fig 4.7). Training, however, resulted in a greater strain in iliac arteries (Fig 4.7 C) and soleus muscle arterioles of young rats (Fig 4.8 A).

Stiffness was greater with aging in all conduit arteries, as well as in the gastrocnemius muscle arterioles (Fig 4.9). No differences occurred with aging in soleus muscle arterioles. Training decreased the stiffness of the abdominal aortas of both young and old rats and the iliac arteries and gastrocnemius muscle arterioles of the old rats (Fig 4.9).

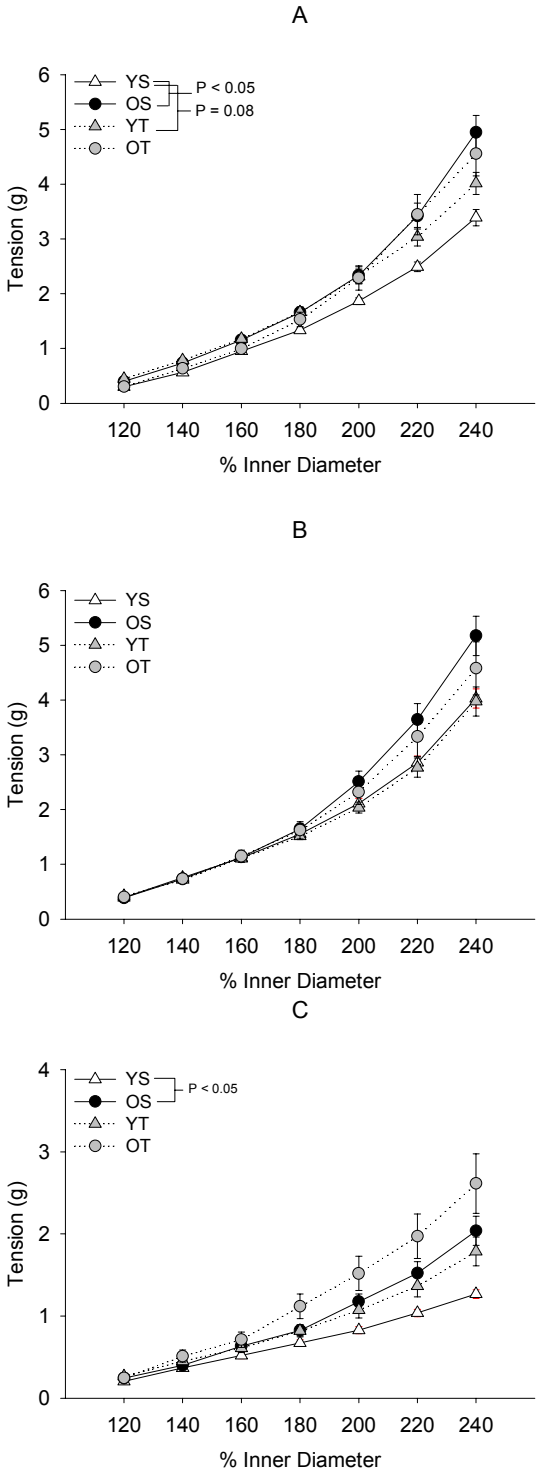

Figure 4.5 Passive stretch-tension relations in thoracic aorta (A), abdominal aorta (B), and iliac arteries (C) from young and old sedentary and exercise trained groups. Values are means ± SEM.

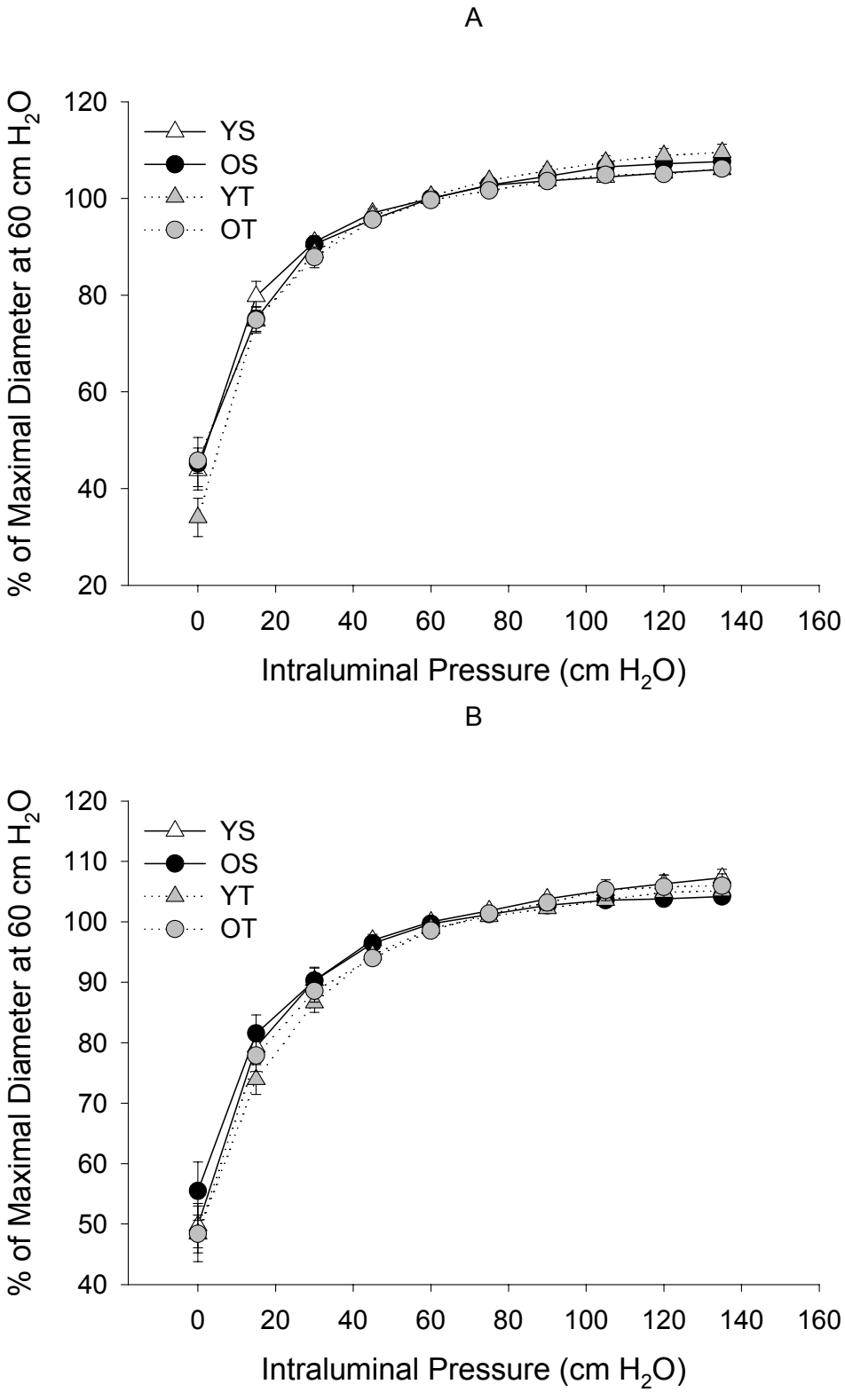

Figure 4.6 Passive pressure diameter relations in soleus (A) and gastrocnemius (B) muscle arterioles from young and old sedentary and exercise trained groups. Values are means ± SEM.

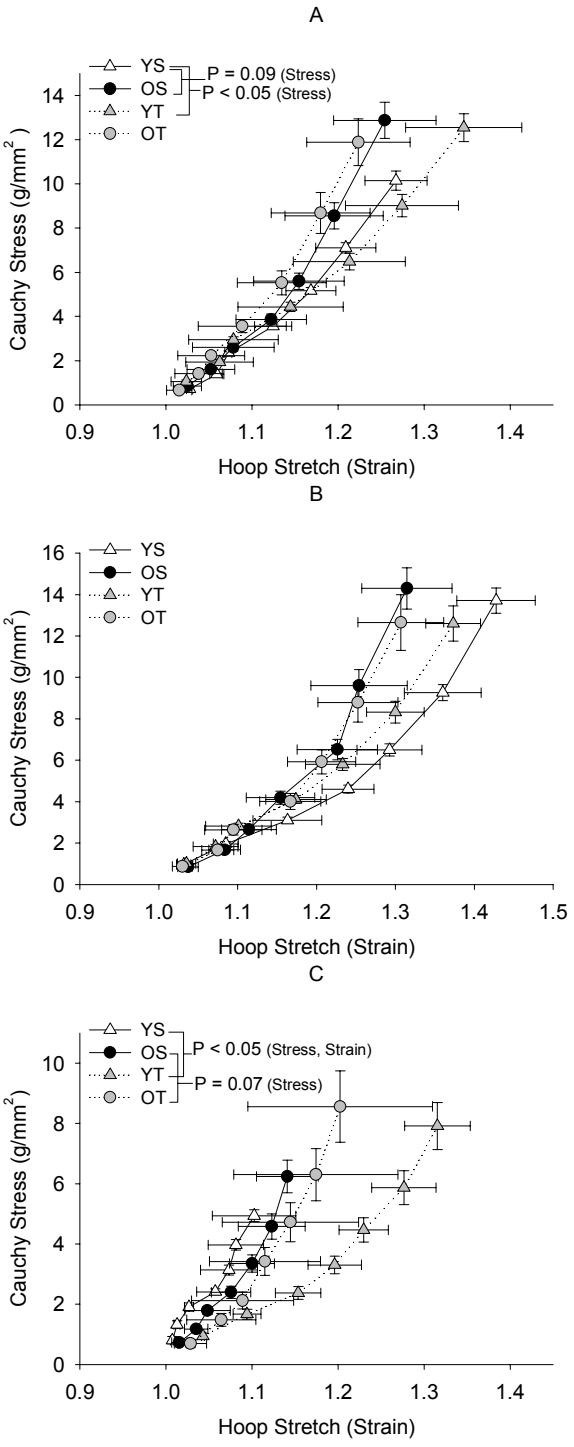

Figure 4.7 Cauchy stress/ hoop stretch relation of thoracic aorta (A), abdominal aorta (B) and iliac artery (C) from young and old sedentary and exercise trained groups. Values are means ± SEM.

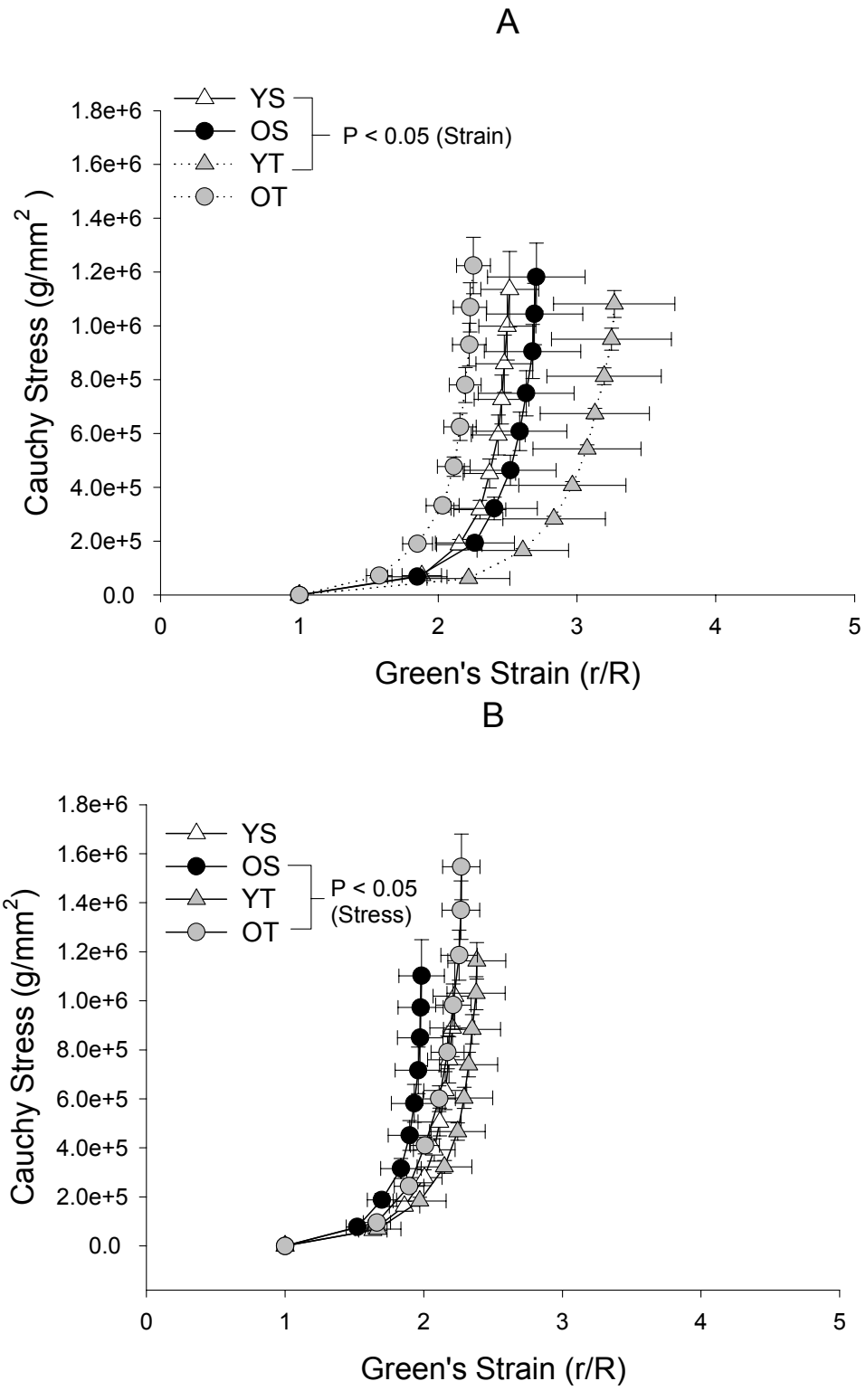

Figure 4.8 Circumferential stress and strain in isolated soleus (A) and gastrocnemius (B) muscle arterioles in response to increases in intraluminal pressure. Values are means ± SEM.

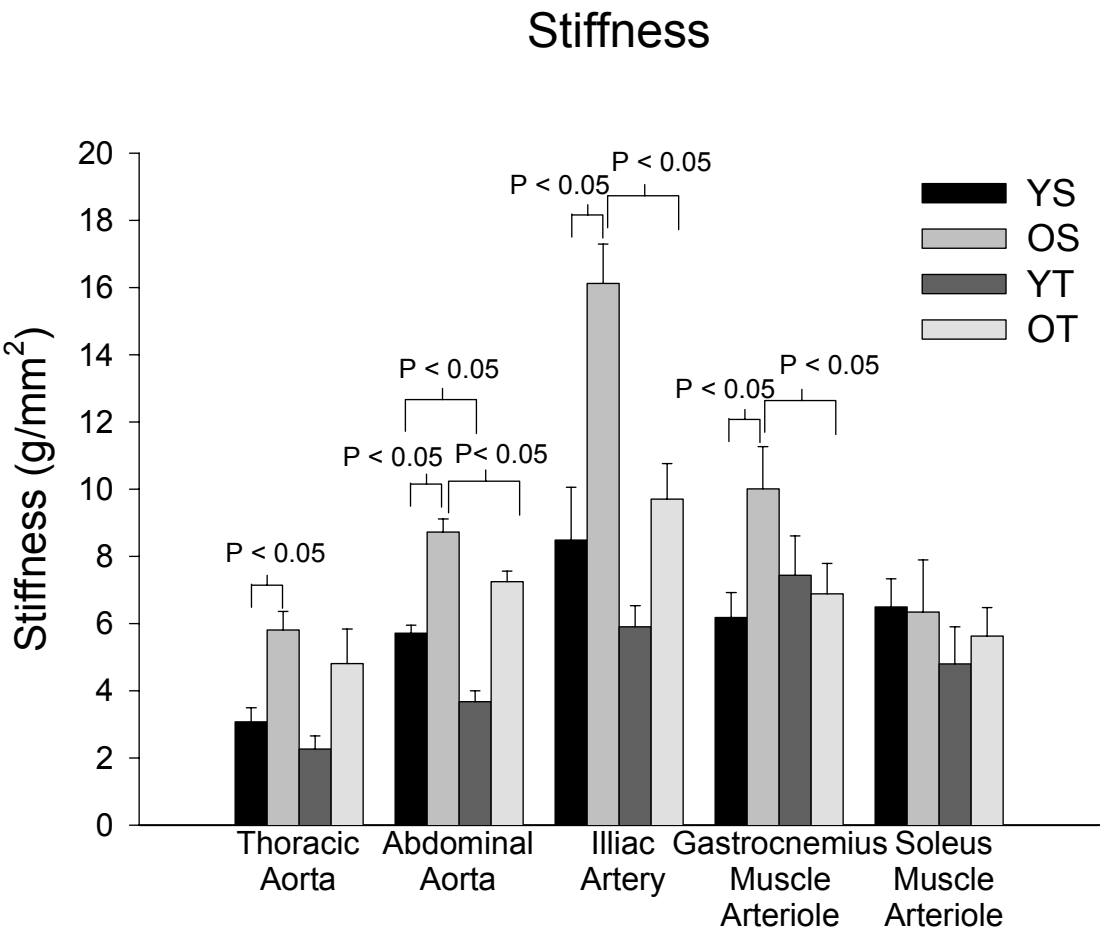

Figure 4.9 Incremental stiffness coefficient of thoracic aorta, abdominal aorta, illiac artery, gastrocnemius and soleus muscle arterioles from young and old sedentary and aerobic exercise trained groups. Values are means ± SEM.

4.4 Discussion

The primary findings in this study are as follows. First, aging increases arterial wall stiffness in conduit arteries and gastrocnemius muscle arterioles. Second, exercise training appears to ameliorate the age-associated stiffening of the arterial wall in all arteries except the thoracic aorta. Third, aging is associated with increased lumen diameter and wall thickness in conduit vessels, but not in skeletal muscle arterioles. Finally, wall-to-lumen ratio was unaffected by either aging or exercise training in any of the arterial segments investigated.

Previous work has shown that large central artery stiffness is elevated with advancing age (21, 49, 73, 89, 115). However, from these studies it is not possible to ascertain whether the increased stiffness is the result of alterations in the mechanical properties of the vessel or differences in the active tone of the smooth muscle. Work from the present study supports and extends these previous observations by demonstrating that large peripheral conduit arteries and some resistance arterioles demonstrate elevated arterial stiffness with senescence, which is the result of differences in the mechanical properties of the arterial wall. Only the arterioles from the soleus muscle did not exhibit greater arterial wall stiffness with old age, similar to a previous report (92). These data demonstrate that the intrinsic mechanical properties of the arteries change to increase stiffness independent of age-related changes in myogenic tone (92), endothelial vasodilator function (17, 29, 91, 113), and vasoconstrictor responsiveness to neural (35), humoral (55) and local (10) agonists, which could contribute to age-related differences in arterial stiffness measured in vivo. The mechanisms of arterial stiffening could be due to several

factors, including increases in the collagen to elastin ratio (21) and greater advanced glycated end-products (65), each of which has been shown to be independently related to increases arterial stiffness with aging.

Exercise training ameliorated the age-related elevation in arterial stiffness in most arterial segments. These results agree with those from old and exercise trained humans (115). They also demonstrate that peripheral conduit arteries and arterioles that exhibit age-associated increases in stiffness can be ameliorated by exercise training. Furthermore, these results indicate that exercise training in old rats modifies the structure of the arterial wall to decrease the stiffness of the arteries. The soleus muscle arterioles studied were impervious to the effects of exercise training. It appears that no such arterial wall restructuring is seen in young exercise trained rats, and this is consistent with previous results (93). Soleus muscle arterioles may be resistant to the effects of both aging and training because the soleus muscle itself is tonically active in the rat and in essence already physically 'trained' relative to the gastrocnemius muscle. Mechanisms of these changes have been largely unstudied, but researchers speculate that reductions in the amount of collagen and collagen crosslinking may lead to more elastic arteries (115).

Morphology changes indicated that smooth muscle wall thickness and arterial lumen diameters of conduit arteries were larger in old rats compared to young controls. These age-related changes are consistent with measures of carotid intima media thickness (IMT) in humans (34, 73, 116). Similar changes in vascular structure do not appear to occur in either of the small resistance arterioles. Thus, age-induced arterial remodeling appears to be restricted to large arteries not the

microvasculature. Studies from our laboratory have previously shown that aging increased gastrocnemius lumen diameter (111) and also have shown aging does not change gastrocnemius lumen diameter (92). These differences are most likely due to the particular white gastrocnemius arteriole selected for the studies. Physiologically, it appears that vascular smooth muscle wall thickness to artery lumen diameter is tightly regulated in the arterial system, since this variable remained constant in all artery and arteriolar segments regardless or aging or training state. In contrast age-associated increases in IMT-to-lumen ratio in humans have been documented (34). Although it is presently unclear what factors may contribute to this discrepancy, it is possible that differences simply reflect species differences. Alternatively, healthy human aging is characterized by sub-clinical atherosclerosis, which may be the reason IMT is greater in "healthy" older human subjects (73, 116). This confounding variable is not present in the Fischer 344 model, which does not develop atherosclerosis (18). Also, arteries measured in vivo have tone and are chronically constricted by the sympathetic nervous system to a greater extent in older individuals (108), which would underestimate the passive lumen diameter and overestimate the IMT of arteries from older individuals. The present study indicates that with atherosclerosis-free aging the medial layer thickness to artery lumen diameter ratio is a tightly regulated variable across a range of artery sizes.

Exercise training appears to primarily affect only peripheral conduit and relatively low resting blood flow gastrocnemius arteriolar lumen diameter. Similar training-associated changes do not occur in the smaller soleus muscle arterioles,

potentially due to their relatively high blood flows at rest (5). Iliac arteries and gastrocnemius muscle arterioles showed expansive arterial remodeling in response to exercise training, which is similar to previous results in our laboratory (111). In addition, these results are similar to the increase in luminal diameter that occurs in the human femoral artery after exercise training (36, 85). Wall thickness was not affected by exercise training in young or old animals, which is similar to findings reported in the carotid artery of humans (116).

In conclusion, the inherent properties of the arterial wall contribute to the age-related increase in arterial stiffening in large conduit arteries, and some small resistance arterioles. Exercise training appears to ameliorate the age-related stiffening in all arteries. Vascular smooth muscle wall thickness is increased with aging in conduit arteries, and exercise training has no effect on wall thickness. Lumen diameter is increased with aging in conduit arteries, but not in arterioles. Wall-to-lumen ratio appears to be tightly regulated with aging and exercise training in a rat model unaffected by atherosclerosis. These results definitively show that with aging a majority of the arteries become stiffer due to structural modifications in the arterial wall, and this occurs independent of alterations in vascular tone with senescence.

Large artery stiffening is associated with elvations in systolic blood pressure (72), and may contribute to the reductions in diastolic blood pressure due to inhibited recoil of the arterial wall. In addition, the increase in large artery stiffness with aging contributes to the age-associated decline in arterial baroreflex sensitivity (86). Microvascular arterial stiffening could lead to impairment in myogenic signaling pathways, which in tern may contribute to the age-related reduction in myogenic function in the microvasculature (92). Overall, exercise training appears to ameliorate the age-associated increases in arterial stiffness and, consequently, may be a contributing factor to the cardioprotective effects of chronic physical activity.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this dissertation was to determine whether alterations in the intrinsic vasomotor properties of skeletal muscle resistance arterioles or structural properties of the arterial vasculature may contribute to elevations in peripheral vascular resistance and reductions in skeletal muscle blood flow with aging. A secondary objective was to determine whether aerobic exercise training functions to modify old age-associated alterations in the vasomotor and structural properties of the arterial vasculature. The specific aims of this dissertation were to test the hypotheses that:

I. *Aging will augment vasoconstrictor responsiveness of skeletal muscle resistance arterioles, while exercise training will attenuate the vasoconstrictor responsiveness of skeletal muscle resistance arterioles.*

Taken together the findings of the present study indicate that aging is associated with augmented vasoconstrictor properties therefore, we must accept our aging hypothesis. The augmented vasoconstrictor properties were due to alterations in reactivity to receptor-specific agonists because potential mediated vasoconstriction was similar or reduced with aging. Endothelin reactivity was enhanced in arterioles from a relatively low flow highly glycolytic fast twitch skeletal muscle. This age-associated augmentation in vasoconstriction was due to an enhanced sensitivity of the ETa receptor pathway. It was not due to age-

associated differences in endothelial function. Interestingly, norepinephrine showed age-associated increases in vasoconstrictor sensitivity in the relatively high flow, highly oxidative, slow twitch skeletal muscle. These age-related changes were due to a decline in endothelium dependent adrenergic vasodilation.

Exercise training attenuated the old age-associated increases in vasoconstrictor responsiveness to adrenergic vasoconstrictors via endothelium-dependent vasodilator mechanisms. However the enhanced responsiveness to endothelin with aging was unaffected by exercise training. We must reject our hypothesis for the vasoconstrictor response to endothelin but accept it for the adrenergic vasoconstrictor response. The adrenergic vasoconstrictors appear to attenuate the vasoconstriction via a vasodilation due to endothelium-dependent mechanism.

II. *Aging will attenuate skeletal muscle arteriolar responsiveness to adrenergic vasodilation, and exercise training will partially reverse this effect.*

β-mediated adrenergic vasodilation was indeed diminished with aging in the arterioles from both skeletal muscles, and these differences were not due to changes in endothelium-mediated vasodilation or vascular smooth muscle cAMP sensitivity. Therefore, we must accept our aging hypothesis. Exercise training enhanced β-adrenergic vasodilation sensitivity in the old rat gastrocnemius arterioles but not in the soleus arterioles. Exercise training did not have significant effects on young rat arterioles. We must reject our exercise-training hypothesis except in the case of the old rat gastrocnemius arterioles.

III. Aging will increase the stiffness of arteries, and exercise training will not effect arterial stiffness.

Age-associated augmentation of vascular stiffness appeared in all segments of the arterial tree studied except the soleus muscle arterioles. Therefore, we accept our hypothesis, with the exception of soleus muscle arterioles. Exercise training ameliorated the age-associated increases in stiffness. Therefore we must reject our exercise training hypothesis.

In summary, it appears that healthy sedentary aging produces a pro-vasoconstrictor state in skeletal muscle arterioles. Adrenergic and endothelin vasoconstrictors have augmented receptor sensitivities with aging in different skeletal muscle arterioles, but an understanding of why this occurs is still unclear. Skeletal muscle fiber type and/or blood flow patterns appear to be important in determining which arterioles become altered with aging. Endothelium-independent vasodilation via adrenergic β receptors is reduced with aging. In addition, vascular stiffening with aging is relatively uniform throughout the arterial vasculature except in soleus muscle arterioles with high resting blood flows. However, arterial remodeling with aging is physiologically appropriate in that wall to lumen ratio is unchanged. Exercise training reduces adrenergic vasoconstriction in old rat skeletal muscle arterioles via an endothelium dependent mechanism, but is without effect on endothelin-mediated vasoconstriction. In addition, exercise training ameliorates the age-related increase in vascular stiffness. Although aging results in a pro-constrictor phenotype in the skeletal muscle resistance vasculature, exercise training appears to be effective in at least partially ameliorating these changes.

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Declines in physiological functional capacity with age: a longitudinal study in peak swimming performance
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