REGULATION OF ENDOTHELIAL PHENOTYPE IN RAT SOLEUS MUSCLE FEED ARTERIES: INFLUENCE OF AGING AND EXERCISE TRAINING

A Dissertation

by

DANIEL WAYNE TROTT

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

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Kinesiology

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Approved by:

Chair of Committee,	Christopher Woodman
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	Cristine Heaps
	Michael Massett
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ABSTRACT

Regulation of Endothelial Phenotype in Rat Soleus Muscle Feed Arteries: Influence of Aging and Exercise Training. (December 2010)

Daniel Wayne Trott, B.S. University of New Mexico

Chair of Advisory Committee: Dr. Christopher Woodman

Aging is associated impaired endothelial function in the skeletal muscle vasculature which contributes to decreased ability to increase muscle blow during exercise. This endothelial dysfunction is mediated, primarily, by impairments in the nitric oxide (NO) pathway in the skeletal muscle vasculature. The major purpose of this dissertation is to determine the mechanisms that mediate age-related endothelial dysfunction in rat soleus feed artery (SFA) and determine whether exercise training ameliorates this impairment in endothelial function. Therefore in these series of studies we sought to test three major hypotheses: 1) That exercise training reverses age-related decrements in endothelium-dependent dilation in SFA and that this improved endothelium-dependent dilation is the result of increased NO bioavailability due to increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content; 2) That age-related endothelial dysfunction in rat SFA is mediated in part, by NAD(P)H oxidase-derived reactive oxygen species (ROS); 3) and, that impaired endothelium-dependent dilation in senescent SFA is due to an impaired potential for p-eNOSser1177. To test these hypotheses, SFA from young (4

month) and old (24 month) Fischer 344 rats were isolated for either determination of endothelium-dependent and –independent dilations or biochemical analyses. Results from these investigations suggest that 1) exercise training reverses the detrimental effects of aging on endothelial function in skeletal muscle feed arteries by enhancing the capacity to scavenge superoxide, increasing the bioavailability of NO; 2) ROS contribute to impaired endothelium-dependent dilation in old SFA; whereas, ROS appear to play a role in ACh-mediated dilation in SFA from young rats; 3) and, that the PI3 kinase/protein kinase B (Akt)/eNOS pathway is preserved with age.

DEDICATION

To Nikki, an excellent wife, far more precious than jewels

“A mouthful of sea air, or a stiff walk in the wind’s face, would not give grace to the soul, but it would yield oxygen to the body, which is next best."

-Charles Spurgeon

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CHAPTER I

INTRODUCTION

1.1 Clinical relevance

Aging is a primary risk factor for cardiovascular disease (1). This is of importance as people 65 and older are the most rapidly expanding segment of the United States population and cardiovascular disease in this population is projected to result in a considerable rise in health care costs (137). In addition, maximal exercise capacity declines with age (1, 32). This decline in exercise capacity may contribute to reduced ability to perform activities and a decreased quality of life. There is experimental evidence that the ability to increase muscle blood flow during exercise is impaired with age and this may contribute to reductions in exercise capacity (4, 31, 91). As a result, determining the alterations that occur in the cardiovascular system with age has been an important research avenue. Findings from this research should result in effective cardiovascular disease prevention and treatment programs to decrease the health care cost burden and improve quality of life in the elderly.

1.2 Early discoveries in endothelial function

Since the seminal discovery of endothelium-derived relaxing factor (EDRF) by Furchgot and Zawadski in 1980 (43), the endothelium has been recognized as an

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important modulator of vascular function. Arterial smooth muscle contraction or relaxation plays a key role in regulating blood flow, and hence oxygen supply to downstream tissues. In these key experiments, Furchgot and Zawadski treated strips of rabbit vascular smooth muscle with acetylcholine (ACh) with the endothelium intact or removed. Strips with the intact endothelium relaxed while strips with the endothelium removed contracted. In addition, when a strip with the endothelium removed was incubated together with an endothelium intact strip, ACh evoked relaxation in both strips. These important experiments demonstrated the release of an endothelium derived relaxing factor (EDRF). Since then, EDRF has been identified as nitric oxide (NO) (53). NO is a rapidly diffusible gas that activates smooth muscle soluble guanlyate cyclase and facilitates cyclic GMP formation, which then activates K^+ channels resulting in smooth muscle cell hyperpolarization and relaxation. In addition, the endothelium has been shown to produce the dilator prostacylin and a number of other vasodilators referred to as endothelium-derived hyperpolarizing factors (EDHF) (9, 37).

In 1994, Celemajer et al. demonstrated that brachial artery endothelium-dependent dilation is impaired with advancing age and that this impaired endothelial function is associated with an increased risk for coronary artery disease (11, 12). More recently, it has been demonstrated in human and animal models that impaired NO bioavailability is associated with an age-related impairment in endothelial function in conduit and resistance vessels (6, 16, 19, 81, 133). Importantly, the impaired NO-mediated response appears to be endothelium-dependent as sodium-nitroprusside (a direct NO donor) induces

similar responses in young and old humans and animals (3, 20, 107, 114, 132). These findings suggest that the ability of the vascular smooth muscle to dilate in response to NO is preserved with age. These findings have resulted in considerable research activity in an attempt to determine the mechanism(s) responsible for age-related impairments in endothelium-dependent vasodilation.

1.3 Exercise and skeletal muscle blood flow

In humans exercise results in increased oxygen consumption ($\dot{V}O_2$), largely due to the increased oxygen demand of contracting skeletal muscle. In normal, healthy subjects, cardiac output increases four- to five-fold from rest to maximal oxygen consumption ($\dot{V}O_2$max), whereas $\dot{V}O_2$ increases 12- to 16-fold suggesting that increased cardiac output alone is insufficient to explain the increase in $\dot{V}O_2$. During exercise that induces increases in cardiac output, blood flow to visceral organs decreases, while muscle blood flow increases (2). This indicates that the capacity to redistribute blood flow during exercise is an important mechanism accounting for the increase in muscle blood flow, $\dot{V}O_2$ and exercise capacity. A major component of this redistribution is mediated through the relaxation of vascular smooth muscle of the resistance arteries and arterioles that perfuse skeletal muscle. The smooth muscle tone of these vessels is regulated by neurohumoral factors (e.g. catecholamines, angiotensin II), metabolic factors (e.g. K^+, CO_2, O_2, adenosine), intrinsic myogenic tone (response to intraluminal pressure), endothelial-derived dilators (NO, prostacyclin, EDHF) and constrictors (endothelin-1, prostanoid derived constrictors). Interestingly, muscle blood flow is dramatically increased during

exercise due to dilation of the resistance vessels that feed the muscle despite increases in adrenergic nerve activity and circulating catecholamines which generally act as constrictors (129). This phenomenon is referred to as “functional sympatholysis” (95). The mechanisms accounting for functional sympatholysis are not fully understood, however, a number of factors have been proposed including; neural factors, substances released from muscle itself, circulating substances and endothelial derived factors (60). Notably, NO is released by both contracting skeletal muscle fibers and the vascular endothelium and inhibition of NOS appears to blunt exercise hyperemia in some investigations (99, 102) but not in others (40, 93).

1.4 Aging, exercise and skeletal muscle blood flow

Aging results in a decline in maximal aerobic capacity that can be partially explained by reduced cardiac output (1, 32, 86); however, there is also considerable evidence that muscle blood flow is attenuated with age during exercise (49, 55, 83, 91, 92). This impairment in exercise hyperemia likely creates a mismatch between O_2 supply and O_2 demand by the contracting muscle, limiting exercise tolerance. Multiple investigators have reported blunted muscle blood flow responses to electrically stimulated muscle contractions in old rats (49, 55). Interestingly, in rats during treadmill exercise, total hindlimb blood flow is not compromised with age, however the distribution of blood flow within and between muscles is altered such that blood flow to oxidative muscles is significantly blunted (83). In older humans, exercise hyperemia is impaired in both leg and forearm (31, 62, 91, 92). Importantly, exercise hyperemia is blunted

with age in small muscle mass exercise where cardiac output is not maximal, suggesting that reduced cardiac output with age does not fully explain the observed reductions in muscle blood flow (73). The attenuated exercise hyperemia appears to be due in part to reduced vascular conductance in old subjects when compared to young (86), suggesting impaired vasodilation in the resistance vasculature. Several possible mechanisms have been proposed to explain the increased vascular resistance with age including, impairments in NO-mediated dilation (62, 98), greater sympathetic nerve activity and/or sensitivity to catecholamines (24), and greater circulating concentrations of other constrictors such as endothelin-1 (29). In particular, two lines of evidence suggest that impairments in NO bioavailability mediate greater vascular resistance during exercise. First, inhibition of NO blunts exercise hyperemia to a greater extent in young versus old subjects (98). Second, infusion of vitamin C (which improves NO bioavailability) improves exercise hyperemia in elderly subjects (62, 115).

Complimenting the observation that age results in blunted exercise hyperemia in oxidative muscle (83), impaired endothelial function has been observed in feed arteries and first order (1A) arterioles of soleus (oxidative) muscle with age (81, 107, 132, 133). Skeletal muscle feed arteries and arterioles play an important role in regulating skeletal muscle blood flow at rest and during exercise. The feed artery is a primary control point for total muscle blood flow to the soleus muscle, whereas the 1A arterioles regulate blood flow distribution within and between muscle fibers (129, 130). Both of these vessels exhibit impairment in NO-mediated dilation, supporting the concept that impaired NO

bioavailability plays an integral role in age-related impairments of exercise hyperemia.

1.5 Mechanisms of NO bioavailability regulation

The balance of NO-production and NO-degradation determines vascular NO bioavailability. Nitric oxide synthase (NOS) is the enzyme which is primarily responsible for production of NO. NOS catalyzes the reaction converting L-arginine to L-citruline and NO. In mammals NOS exists in three isoforms endothelial (eNOS), inducible (iNOS) and neuronal (nNOS). In the endothelium, eNOS is the primary enzyme responsible for NO production and the activity of this enzyme is regulated by numerous postranslational modifications (46). The major pathway of NO-degradation is through its reaction with superoxide anion ($O_2^{\cdot -}$) (48). Therefore, most research on age-related endothelial dysfunction has focused on mechanisms regulating NO production and NO degradation.

In its non-active state eNOS is bound to the cell membrane structural protein caveolin and is phosphorylated on threonine residue 495 (p-eNOS(thr495) inhibiting the ability of eNOS to bind with calmodulin (39, 79). Stimulation of endothelial cells with mechanical or chemical stimuli can lead to an increase in intracellular Ca^{2+} (65). The increased intracellular Ca^{2+} interacts with calmodulin and the Ca^{2+}/calmodulin (CaM) complex binding with eNOS results in a dissociation with caveolin-1 (44, 79). Heat shock protein 90 also acts as a stabilizer of the eNOS/CaM protein complex (44). In addition, stimulation of endothelial cells leads to dephosphorylation of eNOS(thr495) and phosphorylation of eNOS on a number of serine residues including 116 (p-

eNOSser116), 1177 (p-eNOSser1177), 633 (p-eNOSser633) and 617(p-eNOSser617). (7, 8, 22, 25, 38, 78) Phosphorylation at these sites appears to enhance electron flux through the enzyme by facilitating the dimerization of eNOS which is required for its activity (68). Dephosphorylation of p-eNOSthr495 is primarily mediated by protein phosphatase 2A (47). Phosphorylation of eNOS on serine residues 1177 and 635 are primarily mediated by Phosphoinositol-3-Kinase (PI3K) activation and subsequent downstream activation of protein kinase B (Akt), which then phosphorylates eNOS (22, 38). Protein kinase A (PKA) and AMP kinase have also been shown to play a role in eNOS phosphorylation (7, 8, 22, 25, 38). Importantly, the activity of these kinases appears to be regulated differently depending on the stimuli (i.e. ligand/receptor mediated vs. shear stress) (7, 63).

Lastly, the relationship of eNOS with its substrate (L-arginine) and cofactor (tetrahydrobiopterin, BH_4) are important in regulation of eNOS activity. In numerous cardiovascular diseases, supplementation of L-arginine has been shown to improve vascular function in an NO-dependent manner (14, 45). These observations have lead to the “arginine-paradox” where supplementation with L-argnine improves dilator function despite intracellular L-arginine concentrations in millimolar range much higher than the observed K_m for eNOS (micromolar range) (45). Recent evidence suggests that intracellular trafficking of L-arginine is altered with disease, that asymmetric-dimethyl arginine may compete as an eNOS substrate with L-arginine and that arginase-1 may catabolize L-arginine (45).

BH_4 is an eNOS cofactor which plays an important role in the dimerization of the enzyme (127). This dimerization facilitates electron flow through the enzyme and results in production of NO (127). In conditions of BH_4 deficiency endothelial function and NO production are impaired (15, 17, 103, 127). The deficiency of L-arginine and/or BH_4 results in “eNOS uncoupling” where eNOS exists in monomer form, electron flow through the enzyme is altered and molecular oxygen (rather than NO) becomes the final electron acceptor resulting in the production of $O_2^{\cdot -}$.

In addition to the rate of production, NO bioavailability is also determined by the rate of degradation of NO. NO has a half-life of less than 5 seconds under physiological conditions (52). In addition, before the discovery that EDRF was NO, $O_2^{\cdot -}$ was shown to be a mediator of EDRF degradation (48) and in conditions of excess $O_2^{\cdot -}$, $O_2^{\cdot -}$ and NO react to form the peroxynitrite ($ONOO^{\cdot -}$) (48, 126). This reduces NO bioavailability and the resulting $ONOO^{\cdot -}$ (a highly reactive molecule) can cause cellular damage, further impairing endothelial function (126). There is a considerable body of evidence that endothelial dysfunction is reversible with the acute administration of exogenous superoxide dismutase (SOD, a major scavenger of $O_2^{\cdot -}$), or SOD mimetics and that this improvement is primarily mediated by improved NO bioavailability (16, 33, 35, 62, 115, 123).

1.6 Aging and NO bioavailability

Aging is associated with increased risk of cardiovascular disease, impaired endothelial function, reduced exercise capacity, and blunted exercise hyperemia. Reduced NO bioavailability appears to play a role in each of these

changes, so much recent research has focused on alterations in the NO-vasodilator pathway that occur with age. As discussed above, a number of studies have demonstrated impaired NO-mediated, endothelium-dependent dilation with age in humans and animals (3, 6, 16, 20, 107, 132). A number of studies have examined the mechanisms of impaired age-related NO bioavailability. Aging has been shown to decrease (16, 33, 50, 132), increase (13, 50, 84, 107, 126) or not change (27, 74, 104, 105, 113, 132) eNOS protein content across a spectrum of species and vessel sizes. Similarly, age-induced alterations of Akt and eNOS phosphorylation appear to vary. In unstimulated rat aorta, p-eNOS(ser1177) and p-Akt(473) are blunted and p-eNOS(thr495) is enhanced with age (104, 106). In addition, aortas from old rats exhibit attenuated eNOS-Akt and eNOS-Hsp90 and enhanced eNOS-Cav-1 protein-protein interactions (105), all suggesting impairment of eNOS activation. In contrast, in human brachial artery, endothelial cell p-eNOS(ser1777) is greater in old subjects when compared to young (27). In rat coronary arterioles, p-eNOS(ser1177) is preserved with age and increases to a similar extent with stimulation by intraluminal flow or vascular endothelial growth factor (VEGF) (74). In contrast to the changes observed with eNOS protein content and phosphorylation, eNOS activity appears to be consistently blunted with age (5, 13, 105, 106). In total, these results suggest that there are age-related alterations in the ability to regulate eNOS activity and NO production; however, whether these changes occur in skeletal muscle vasculature is unknown.

Reactive oxygen species (ROS) have been implicated as a contributor to age-related endothelial dysfunction. It is well documented that aging results in enhanced vascular $O_2^{\cdot -}$ concentrations in numerous models (6, 16, 56, 103, 126). In addition, scavengers of $O_2^{\cdot -}$ and other antioxidants have been shown to improve age-related endothelial dysfunction by restoring NO bioavailability (6, 16, 33, 35, 62, 115).

A number of potential sources of ROS may explain the increased vascular ROS concentrations with age. NAD(P)H oxidase has been implicated as a major source of vascular $O_2^{\cdot -}$. Inhibition of NAD(P)H oxidase reduces vascular $O_2^{\cdot -}$ concentrations in senescent rat coronary arterioles and mesenteric arteries (16, 56). In mouse carotid arteries, NAD(P)H oxidase activity is greater with age and inhibition of NAD(P)H oxidase improves endothelial function in these vessels (33). In contrast, in rat soleus 1A arterioles, inhibition of NAD(P)H oxidase blunts flow-induced dilation in vessels from both young and old animals. Xanthine oxidase has been implicated as a source of vascular $O_2^{\cdot -}$ with age in rats (56, 85); however, in aged humans inhibition of xanthine oxidase does not improve endothelial function (34). Mitochondrial-derived ROS may also play a role in age-related endothelial dysfunction (125).

Interestingly, eNOS itself also appears to contribute to vascular $O_2^{\cdot -}$ production with age. There is emerging evidence that aging results in a deficiency of vascular BH_4, an essential eNOS cofactor and recent studies report lower levels of vascular BH_4 in rat skeletal muscle arterioles (17, 103). This has also been reported in mouse aorta, carotid and mesenteric arteries (6, 136). In

addition, exogenous sources of BH_4 have been shown to improve endothelial function in skeletal muscle from old rats and brachial artery of aged humans (17, 36). Inhibition of eNOS results in reduced vascular $O_2^{\cdot -}$ concentrations in vessels from aged rats and mice (56, 103, 136). These data implicate reduced levels of BH_4 as a potential mechanism for reduced NO-production by eNOS, and the potential for increased NO-degradation by $O_2^{\cdot -}$, both contributing to endothelial dysfunction. It is possible that ROS production from other sources and eNOS uncoupling are linked as $O_2^{\cdot -}$ from other sources may oxidize BH_4, resulting in eNOS uncoupling, generating more $O_2^{\cdot -}$ and creating a feed-forward cycle resulting in greater vascular oxidant stress.

The product of $O_2^{\cdot -}$ dismutation, either spontaneously or mediated by SOD, is H_2O_2. The role of H_2O_2 in vascular aging is somewhat controversial. H_2O_2 appears to act as an EDHF and mediate phosphorylation of eNOS on ser1177, suggesting that it plays a role as a vasodilator in the vasculature (80, 121). Indeed, in soleus first order (1A) and coronary arterioles from aged rats scavenging of H_2O_2 results in blunted flow-induced dilation (61, 103). In contrast, H_2O_2 contributes to dysregulation of NO production in aged rat mesenteric arteries (141) and promotes an inflammatory phenotype in aged rat conduit arteries (125).

In summary, it appears that aging induces complex changes that contribute to both a decrease in the rate NO production and an increase in the rate of NO degradation. However, the precise mechanisms that account for the decline in NO bioavailability with age appear to differ depending on the vessel

studied (3). Specifically, in the skeletal muscle vasculature, aging blunts endothelium-depedent dilation in both soleus feed arteries (SFA) and 1A arterioles; however, with age eNOS protein content decreases (132) or does not change (135) in SFA but increases in soleus 1A arterioles (107). Thus, changes that occur in one vessel may not be applicable to the whole vasculature.

1.7 Aging, exercise training and NO bioavailability

As aging results in endothelial dysfunction, a major area of study is to determine whether exercise can improve endothelial function in the senescent vasculature. This has been hypothesized as aerobic fitness is inversely related to cardiovascular events (64). In addition, increased shear stress and pressure, two physical changes that occur in the vasculature with exercise have been shown to modulate endothelial phenotype (38, 87, 122, 124, 134, 135). Of particular importance, short-term increases in shear stress or intraluminal pressure have been shown to improve endothelial function in senescent rat skeletal muscle feed arteries (134, 135).

Aerobic exercise training improves endothelial function in some vascular beds including senescent human forearm (41, 97), mouse conduit vessels (33) and rat skeletal muscle arterioles (103, 107, 108, 111, 112). Resistance training improves endothelial function in femoral arteries from old rats (50). In cross-sectional studies, physically active elderly subjects exhibit improved endothelium-dependent dilation responses compared to their sedentary counterparts (20, 114). Importantly, exercise training, appears to have disparate effects on endothelial phenotype depending on the size and location of the artery (71). This

finding suggests that alterations with exercise training in one vessel may not always translate to the entire vasculature.

Despite potential differences due to differing vessels and training programs, several possible mechanisms have emerged to explain the improvement in endothelial function with exercise. First, inhibition of NOS appears to abolish the improvement in endothelial function with training (33, 103, 107, 108, 110, 111). Using fluorescence measurements, vascular NO concentrations in response to increases in intraluminal flow were higher after exercise training in senescent rat soleus 1A arterioles (103). Exercise training also increases basal p-eNOS(ser1177) in conduit vessels from old mice (33). Together these observations suggest that exercise training improves NO bioavailability in the aged vasculature. Interestingly, in soleus 1A arterioles, exercise training increases vascular BH_4 concentrations, but also increases vascular $O_2^{\cdot -}$ concentrations (103). In addition, after exercise training scavenging of $O_2^{\cdot -}$ or H_2O_2 blunts flow-induced dilation in arterioles from both young and old rats (103). These observations suggest that with exercise training ROS may play a greater role in endothelium-dependent dilation in some vessels.

As discussed above, the feed artery is an important control point for regulating total muscle blood flow in a given muscle (129). Because of it's importance in regulation of muscle blood flow (129) and because rat soleus muscle blood flow response to exercise is blunted with age (83), our laboratory has chosen to study endothelial function in the soleus muscle feed artery (SFA).

With age, impairments in the NO-pathway leading to blunted endothelial dysfunction are well documented in SFA (132-135); however, little is known about the mechanisms that cause impairments in NO bioavailability, particularly, whether aging alters the mechanisms of NO production, NO degradation or both. In the senescent SFA, the effects of exercise on endothelial function are unknown; however, exposing senescent SFA *in vitro* to short-term increases in pressure or flow (both signals occurring during exercise) improves NO-mediated, endothelium-dependent dilation (134, 135). In addition, whether ROS contribute to (as in the soleus 1A), or impair (as in numerous other vessels) endothelium-dependent dilation in the SFA is also unknown.

1.8 Purpose and hypotheses

The major purpose of this dissertation is to determine the mechanisms that mediate age-related endothelial dysfunction in rat SFA and to determine whether exercise training ameliorates this impairment in endothelial function. Therefore in these series of studies we sought to test three major hypotheses:

1) Exercise training reverses age-related decrements in endothelium-dependent dilation in SFA and that this improved endothelium-dependent dilation is the result of increased NO bioavailability due to increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content.

2) Age-related endothelial dysfunction in rat SFA is mediated in part, by NAD(P)H oxidase-derived ROS.

3) Impaired endothelium-dependent dilation in senescent SFA is due to an age-related impairment in PI3K/Akt dependent phosphorylation of eNOS on serine residue 1177.

CHAPTER II

EXERCISE TRAINING REVERSES AGE-RELATED DECREMENTS IN ENDOTHELIUM-DEPENDENT DILATION IN SKELETAL MUSCLE FEED ARTERIES*

2. 1 Introduction

Aging is associated with a decline in endothelial function characterized, in part, by impaired endothelium-dependent vasodilator responses in central and peripheral arteries (3, 12, 20, 81, 116, 132, 133). The resulting endothelial dysfunction is believed to contribute to an increased risk of cardiovascular disease in older adults. In addition, the decline in endothelial function may contribute to impaired muscle blood flow and reduced exercise tolerance in the elderly (4, 55, 73, 83, 91, 98, 128). The mechanism(s) for the age-related decrement in endothelium-dependent dilation is not fully understood; however, previously published studies indicate that a decline in the bioavailability of nitric oxide (NO) plays an integral role (3, 14, 16, 81, 114, 133).

Endurance exercise training improves endothelium-dependent dilation in some conduit arteries in young healthy subjects (18, 70, 77, 82, 88, 110, 112) and in skeletal muscle arterioles/resistance arteries (72, 76, 107, 108). Importantly, examination of the effects of training in the arteriolar tree of skeletal

*Reprinted with permission from Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. **Trott DW, Gunduz F, Laughlin MH, and Woodman CR.** *J Appl Physiol* 106: 1925-1934, 2009 by the American Physiological Society

muscle indicates that the improvement in endothelium-dependent dilation induced by exercise training is not uniformly distributed throughout the arteriolar tree (72, 76). The beneficial effect of exercise training has also been reported to be associated with increased expression of endothelial nitric oxide synthase (eNOS) (71, 101, 131), enhanced production of NO (31), and improved NO-mediated, endothelium-dependent dilation (82). In addition, exercise training has been reported to increase expression of cytosolic and extracellular superoxide dismutases (SOD-1 and ecSOD) in the aorta of mice and pigs, which may improve endothelial function by enhancing the capacity to scavenge superoxide and prolonging the biological half-life of NO (42, 96).

Previous studies indicate that endurance exercise training is also an effective intervention for attenuating or reversing age-induced endothelial dysfunction in first order (1A) arterioles perfusing skeletal muscle (107, 108). Specifically, Spier et al. (107, 108) reported that endurance exercise training improves endothelium-dependent vasodilator responses in 1A arterioles from soleus and gastrocnemius muscles of aged rats, and that the improved endothelium-dependent dilation was mediated by enhanced NO bioavailability. In addition, exercise training appears to enhance antioxidant status and improve endothelium-dependent dilation in conduit arteries of aged human subjects (20, 35, 41).

In skeletal muscle, it has been established that a primary control point for regulating *total* muscle blood flow during exercise is the feed artery (129).

Indeed, previous research indicates that feed arteries, which lie immediately external to skeletal muscle, provide the principal site of resistance to flow through individual skeletal muscles and play an integral role in mediating increases in skeletal muscle blood flow during physical activity (69, 129, 130). Thus, an exercise training-induced improvement in vasodilator responses in skeletal muscle feed arteries could potentially work in concert with enhanced endothelial function previously reported in skeletal muscle arterioles (107, 108) by increasing the capacity to augment *total* muscle blood flow (feed arteries) and the ability to *redistribute* the augmented blood flow (arterioles) to active skeletal muscle fibers. Given that exercise in young animals has been shown to exert changes in endothelium-dependent dilation in the resistance artery network of skeletal muscles in a non-uniform manner and the importance of feed arteries to perfusion of skeletal muscle, it is important to know whether exercise training in aged animals has a beneficial impact on soleus muscle feed arteries. We know that exercise training has been shown to improve endothelium-dependent dilation in aged soleus muscle 1A arterioles (107, 108); however, the efficacy of endurance exercise training to improve endothelial function in senescent soleus muscle feed arteries is not known. Therefore, the purpose of this study was to test the hypothesis that exercise training reverses age-related decrements in endothelium-dependent dilation in soleus muscle feed arteries (SFA). We also hypothesized that endurance exercise training would improve endothelium-dependent dilation in senescent SFA by increasing NO bioavailability due to

increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content.

2.2 Methods

2.2.1 Experimental design

Endothelium-dependent dilation in response to application of acetylcholine (ACh) was examined in SFA isolated from young and old sedentary and exercise trained rats, using standard techniques. The relative contribution of NO synthesis by NOS was evaluated by examining vasodilator responses in the presence of N^{ω}-nitro-L-arginine (L-NNA*;* 300 μM) to inhibit nitric oxide synthase (NOS). The contribution of cyclooxygenase (COX) was evaluated by examining vasodilator responses in the presence of indomethacin (Indo; 5 μM) to inhibit COX and the role of non-NOS and non-COX pathways to the responses was determined by examining vasodilator responses in the presence of L-NNA + Indo to inhibit NOS and COX. Feed arteries were also harvested for examination of expression of eNOS, eNOS phosphorylation on serine residue 1177 (p-eNOS(ser1177)), SOD-1 and ecSOD using immunoblot analysis. When these results revealed that ecSOD content was increased by exercise training, we did a series of experiments to determine whether the improvement in endothelium-dependent dilation could be produced simply by increasing antioxidant levels by adding exogenous antioxidants.

2.2.2 Animals

All of the protocols used in the present study were approved by the Animal Care and Use Committees at the University of Missouri and Texas A&M

University. To determine the efficacy of exercise training to improve endothelium-dependent dilation in aged SFA, male Fischer 344 rats (2 and 22 mo of age; *n* = 20/age group) were purchased from a commercial dealer **(**Harlan Sprague-Dawley, Indianapolis, IN**)** and housed in the University of Missouri College of Veterinary Medicine's Animal Care Facility. One week after arrival, rats were exercise trained (Ex) or remained sedentary (Sed) for 10-12 weeks. Thus, at the end of the training program, the ages of the young and old rats were 4-5 mo or 24-25 mo respectively. The resulting experimental design consisted of four groups of rats: 1) young Sed (n = 10), 2) young Ex (n = 10), 3) old Sed (n = 10), and 4) old Ex (n = 10). To determine whether exogenous antioxidants produce exercise-like effects on aged SFA, a separate group of male Fischer 344 rats (4 and 24 mo of age) was purchased and housed at the Texas A&M University Comparative Medicine Program Facility. Both animal facilities were maintained at 24^{o} C with a 12:12-h light-dark cycle. Food and water were provided ad libitum, and the rats were examined daily by the investigators and by veterinarians affiliated with their respective institutions.

2.2.3 Training program

The exercise training protocol used in the present study has been published previously in detail (107). In brief, rats were familiarized with running on a motorized treadmill and randomly assigned to an Ex or Sed group for 10-12 weeks. Rats assigned to the Ex group ran 60 min/day, 5 days/week, at 15 m/min (15^{o} incline). Rats assigned to the Sed group were restricted to their cages and did not exercise. The efficacy of the exercise-training protocol was assessed

from measurements of citrate synthase activity in the vastus lateralis muscle (109).

2.2.4 Isolation of feed arteries

Procedures used to isolate SFA have been published previously (132-135). In brief, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50-60 mg/kg body wt, ip). Soleus muscles from the left and right hindlimb were removed and placed in MOPS-buffered physiological saline solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, 25.0 MOPS, at pH 7.4. SFA from one hindlimb were dissected free of paired veins and connective tissue under a dissection microscope, cut on both ends and transferred to a Lucite chamber containing MOPS-PSS (4^o C) for cannulation. SFA from the contra-lateral hindlimb were dissected free, transferred to a microcentifuge tube, snap frozen and stored at -80^oC for subsequent immunoblot analysis.

2.2.5 Determination of vasodilator responses

Preparation of Arteries. SFA were prepared for functional analysis as described previously (19, 42). Specifically, arteries were cannulated with two resistance matched glass micropipettes and secured with 11-0 surgical silk. The micropipettes were subsequently attached to separate pressure reservoirs filled with MOPS-PSS supplemented with albumin (1g/100ml). The height of each reservoir was initially adjusted to set intraluminal pressure in each SFA to 60 cm H_2O (1 mmHg = 1.36 cm H_20) for 20 min. After 20 min, intraluminal pressure was raised to 90 cm H_2O and the feed arteries were allowed to equilibrate for an

additional 40 min at 37^{o}C. At the end of the 60-min equilibration period, feed arteries that did not develop at least 25% spontaneous tone were constricted with phenylephrine. All experimental protocols were subsequently conducted at an intraluminal pressure of 90 cm H_2O to approximate in vivo intraluminal pressure (129).

Endothelium-Dependent Dilation. Endothelium-dependent dilation was assessed in feed arteries by adding increasing concentrations of ACh to the bath solution in cumulative concentrations over the range of 10^{-9} - 10^{-4} M in whole log increments as described previously (58, 132, 133). A total of 4 SFA were studied in parallel from each rat. In *SFA 1*, ACh-induced vasodilator responses were assessed in the absence of enzyme inhibitors. In *SFA 2*, vasodilator responses were assessed in the presence of L-NNA (300 µM) to inhibit NOS. In *SFA 3*, vasodilator responses were assessed in the presence of Indo (5 µM) to inhibit COX. In *SFA 4*, vasodilator responses were assessed in the presence of L-NNA + Indo to inhibit NOS and COX.

In a separate series of experiments, endothelium-dependent dilation was assessed in SFA from young Sed and old Sed rats in the absence and presence of exogenous antioxidants. In these studies, a total of 3 SFA were studied in parallel from each rat. In *SFA 1*, ACh-induced vasodilator responses were assessed in the absence of exogenous antioxidants. In *SFA 2*, vasodilator responses were assessed in the presence of superoxide dismutase (SOD; 120 U/mL) to scavenge superoxide. In *SFA 3*, vasodilator responses were assessed in the presence SOD and catalase (CAT; 100 U/mL) to scavenge superoxide and

hydrogen peroxide. When results revealed that exogenous antioxidants improved endothelium-dependent dilation, a subsequent set of experiments was performed to determine whether NO mediated the improvement in endothelial function. In these studies 3 SFA were studied in parallel from each rat. In *SFA 1*, ACh-induced vasodilator responses were assessed in the absence of exogenous antioxidants. In *SFA 2*, vasodilator responses were assessed in the presence of SOD. In *SFA 3*, vasodilator responses were assessed in the presence SOD and L-NNA.

Endothelium-Independent Dilation. Endothelium-independent dilation was assessed by adding increasing concentrations of sodium nitroprusside (SNP) to the bath solution in cumulative concentrations over the range of 10^{-9} - 10^{-4} M in whole log increments (58, 132, 133). SNP-induced dilation was also assessed in SFA from young and old Sed rats in the presence of SOD, SOD+CAT and SOD+L-NNA.

Passive Diameter. At the end of each experiment, SFA were incubated for 30 min in Ca^{2+}-free PSS to determine passive diameter at an intraluminal pressure of 90 cmH_2O.

2.2.6 Solutions and drugs

All reagents used in concentration-response experiments were obtained from Sigma Chemical Co. (St. Louis, MO). Reagents were prepared on the day of the experiment.

2.2.7 Quantification of eNOS, p-eNOS(ser1177), SOD-1 and ecSOD protein content.

Relative differences in eNOS, p-eNOS(ser1177), SOD-1 and ecSOD protein contents were assessed in feed arteries using immunoblot analysis as described previously in detail (58). eNOS protein content was evaluated with a monoclonal antibody (1:1250; catalog no. 610297, BD Transduction Laboratories). p-eNOS(ser1177) protein content was assessed with a monoclonal antibody (1:250; catalog no. 612393, BD Transduction Laboratories). SOD-1 protein content was assessed with a polyclonal antibody (1:3300; catalog no. SOD-100, Stressgen). ecSOD protein content was assessed with a polyclonal antibody (1:1000; catalog no. SOD-105, Stressgen). Immunoblots were evaluated by enhanced chemiluminescence (ECL, Amersham) and densitometry by using a LAS-3000 Luminescent Image Analyzer and Multi-Gauge Image Analysis Software (FUJIFILM Medical Systems). All protein data were expressed relative to GAPDH to control for small differences in protein loading. GAPDH protein content was assessed with a monoclonal antibody (1:10,000; catalog no. AB374, Millipore). To determine whether the ratio of p-eNOS(ser1177)-to-total eNOS protein content was altered with aging or exercise training, immunoblots were probed with the p-eNOS(ser1177) antibody, stripped with Restore Western Blot Stripping Buffer (Thermo catalog no. 21059) and re-probed with the eNOS antibody.

2.2.8 Statistical analysis

All values are means ± SEM. Between-group differences in body mass, citrate synthase activity, percent tone, relative protein content, and passive diameter were assessed using one-way ANOVA. Concentration response curves were analyzed by two-way ANOVA with repeated measures on one factor (Concentration) to determine whether vasodilator responses to ACh and SNP differed by group. In addition, IC_{50} values were determined by non-linear regression sigmodial concentration-reponse equations for each curve. Between group differences in IC_{50} values and response to the final concentration of ACh (10^{-4} M) were assessed with one way ANOVA. Concentration-response data were expressed as a percentage of maximal possible dilation. Percent possible dilation was calculated as $[D_{concentration}\text{-}D_B)/D_P\text{-}D_B] \times 100$ where $D_{concentration}$ is measured diameter for a given concentration, D_B is baseline diameter before an intervention was started, and D_P is maximal passive diameter. A total of 230 SFA were used in experiments to assess vasodilator function. Fifteen young (8 Sed; 7 Ex) and 21 old (11 Sed; 10 Ex) SFA required phenylephrine to achieve 25% tone. Deletion of these arteries from the statistical analyses did not alter interpretation of the results; therefore, all 230 SFA were included in the final analyses. When a significant *F* value was obtained, post hoc analyses were performed with Duncan's multiple-range test and Fisher's LSD test. Statistical significance was set at the $p \leq 0.05$ probability level.

2.3 Results

2.3.1 Characteristics of rats and SFA

Skeletal muscle citrate synthase activity was increased by training in young and old rats confirming the efficacy of the exercise training program (Table 2.1). Body weight of old Sed rats was significantly greater than young Sed rats (Table 2.1). Exercise training lowered body weight in the old rats such that the body weight of the old Ex rats was not significantly different from young Sed or young Ex rats. Maximal passive diameter was similar in all groups of arteries (Tables 2.2 & 2.3).

2.3.2 ACh-induced dilation

ACh-induced dilation was significantly blunted in the old Sed arteries relative to the young Sed arteries (Fig. 2.1). Ex improved ACh-induced dilation in old (not young, p=0.21) SFA, such that ACh-induced dilation was significantly greater in old Ex SFA than in old Sed SFA (Fig. 2.1). In addition, ACh-induced dilation of old Ex SFA was not different from that of young Sed and young Ex SFA (Fig. 2.1).

ACh-induced dilation was inhibited by L-NNA (Fig. 2.2) and L-NNA + Indo (Fig. 2.3) in young Sed, young Ex, and old Ex arteries. In contrast, ACh-induced dilation was not significantly inhibited by L-NNA (Fig. 2.2) or L-NNA + Indo (Fig. 2.3) in old Sed arteries. In the presence of L-NNA, or L-NNA + Indo, ACh-induced dilation of old Ex SFA was no longer greater than that of old Sed SFA (Figs. 2.2 and 2.3). ACh-induced dilation was not significantly inhibited by Indo alone in any group of arteries (data not shown). Alterations in the response to

Table 2.1. Body weight and citrate synthase activity in the vastus lateralis red muscle.

	Young Sed	Young Ex	Old Sed	Old Ex
Body weight, g	391 ± 9	353 ± 8^{acd}	425 ± 12^{a}	402 ± 12
Citrate synthase activity; µmol·min^{-1}·g wet wt^{-1}	29.9 ± 0.7	39.7 ± 3.7^{ac}	25.6 ± 1.8	38.7 ± 3.0^{ac}

Values are means ± SEM; *n* = 8-10 rats/group. Sed, sedentary; Ex, exercise trained. Significantly different from ^{a}young Sed, ^{b}young Ex, ^{c}old Sed, ^{d}old Ex, $p \leq 0.05$.

Table 2.2. Characteristics of soleus muscle feed arteries from young and old rats used in the exercise training study.

Parameter	Con	L-NNA	Indo	L-NNA + Indo
Maximal diameter, μm				
Young Sed	199 ± 5	193 ± 7	195 ± 11	180 ± 13
Young Ex	196 ± 12	178 ± 16	191 ± 12	184 ± 15
Old Sed	170 ± 8	173 ± 10	174 ± 17	187 ± 12
Old Ex	200 ± 10	194 ± 8	171 ± 11	171 ± 10
Initial tone pre-ACh, %				
Young Sed	48.6 ± 4.1	73.9 ± 2.8^{acd}	45.3 ± 6.9^{b}	57.6 ± 4.5^{b}
Young Ex	40.1 ± 4.3	60.4 ± 4.7^{ac}	34.4 ± 4.1^{bd}	54.7 ± 6.1^{ac}
Old Sed	44.0 ± 6.4	67.5 ± 5.2^{acd}	32.0 ± 4.0^{b}	45.6 ± 4.9^{b}
Old Ex	46.4 ± 3.9	55.8 ± 4.9^{cd}	33.4 ± 2.3^{abd}	44.1 ± 2.7^{bc}

Values are means ± SEM; n = 8-10 rats/group. Sed, sedentary; Ex, exercise trained; Con, control; L-NNA, N^{ω}-nitro-L-arginine; Indo, indomethacin. Significantly different from ^{a}Con, ^{b}L-NNA, ^{c}Indo, ^{d}L-NNA+ Indo, $p \leq 0.05$.

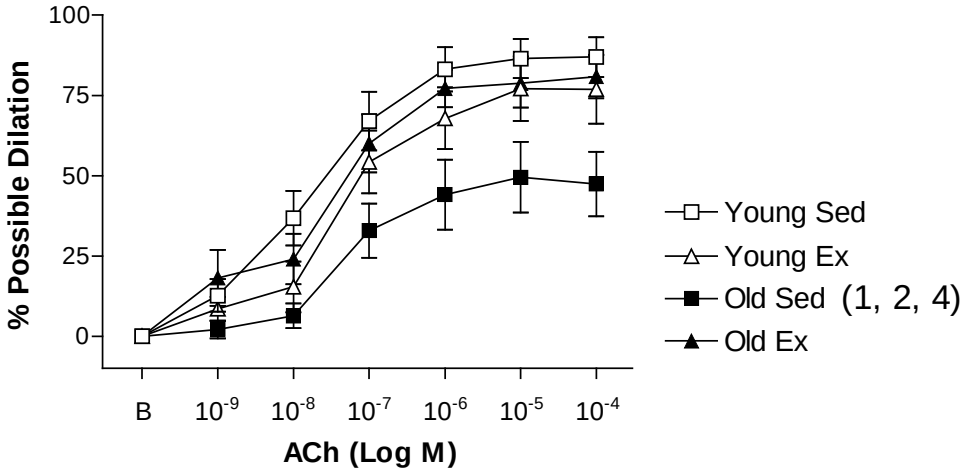


Figure 2.1. ACh-induced dilation in soleus muscle feed arteries (SFA). Sed, sedentary; Ex, exercise trained. B, baseline diameter before the first concentration of ACh. Values are means ± SEM; *n* = 8-10 rats per group. Concentration-response curve significantly different from [1]Young Sed, [2]Young Ex, and [4]Old Ex, $p \leq 0.05$.

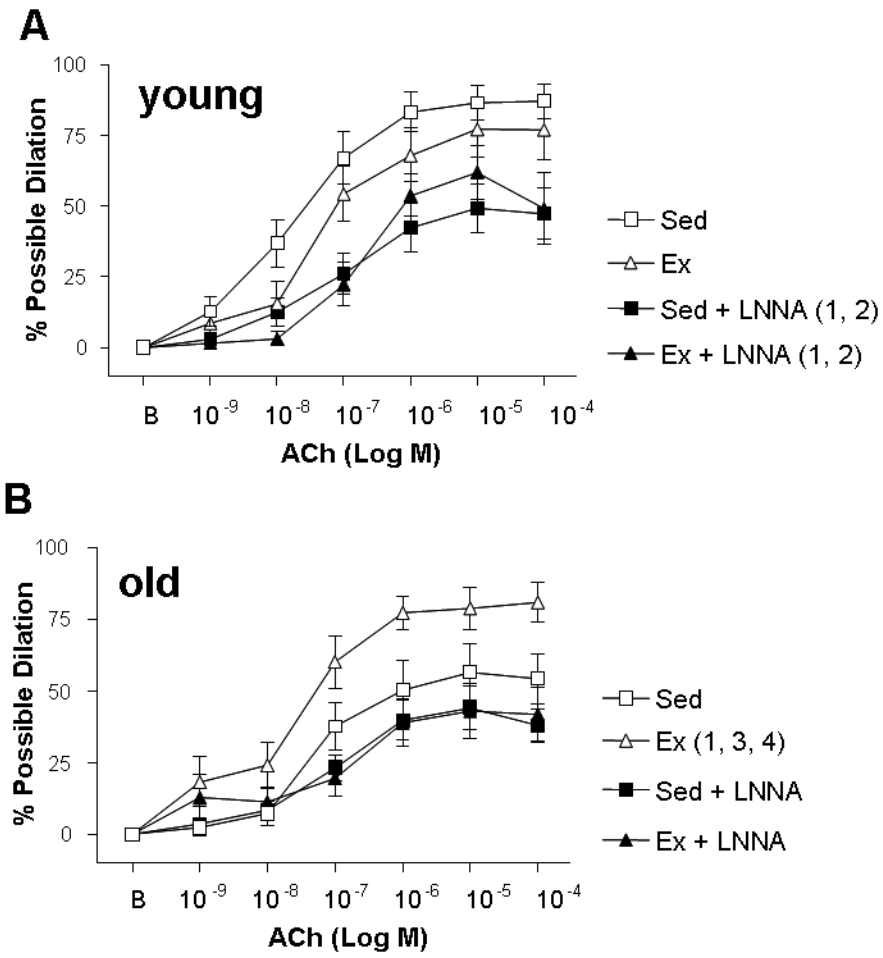


Figure 2.2. ACh-induced dilation in young (A) and old (B) SFA in the absence or presence of N^{ω}-nitro-L-arginine (L-NNA*;* 300 µM) to inhibit nitric oxide synthase. B, baseline diameter before the first concentration of ACh. Values are means ± SEM; *n* = 8-10 rats/group. Concentration-response curve significantly different from [1]Sed, [2]Ex, [3]Sed + L-NNA, and [4]Ex + L-NNA, $p \leq 0.05$.

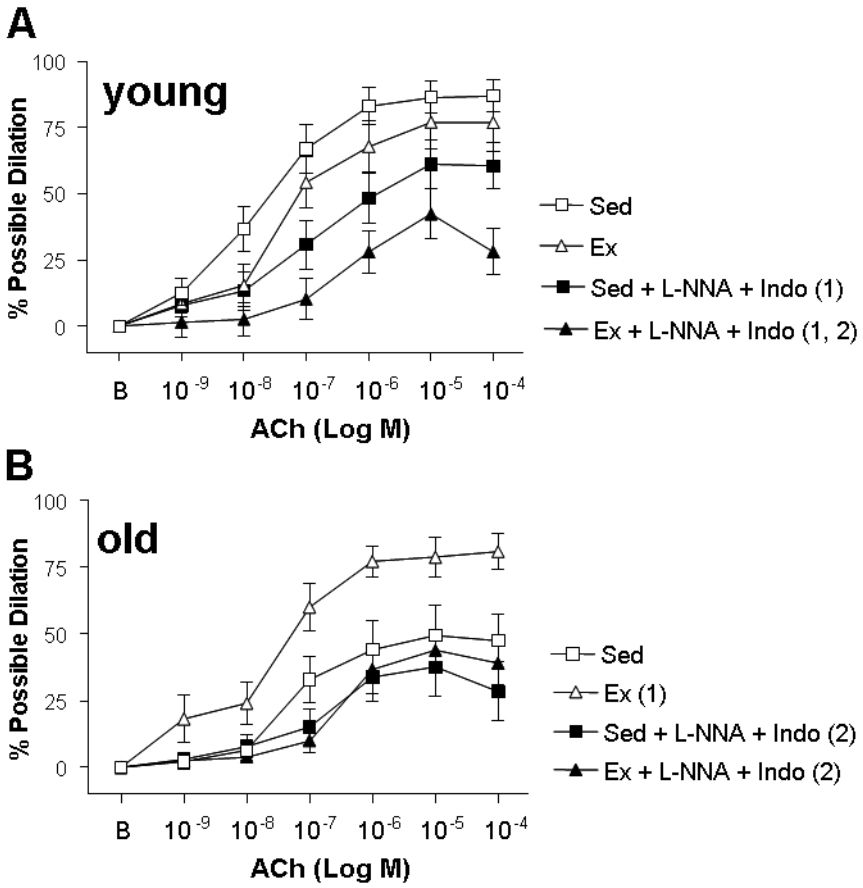


Figure 2.3. ACh-induced dilation in young (A) and old (B) SFA in the absence or presence of L-NNA (300 µM) and indomethacin (Indo; 5 µM) to inhibit nitric oxide synthase and cyclooxygenase. B, baseline diameter before the first concentration of ACh. Values are means ± SEM; *n* = 8-10 rats/group. Concentration-response curve significantly different from [1]Sed and [2]Ex, $p \leq 0.05$.

the maximal concentration of ACh (10^{-4} M) followed a similar pattern to the alterations observed in the concentration response curves. Sensitivity (IC_{50}) to ACh was not altered by age, training status or treatment (Table 2.4).

To determine if exogenous antioxidants restore ACh-induced dilation in a manner similar to exercise, experiments were carried out in the absence and presence of SOD or SOD + CAT. In the absence of antioxidants, the response to ACh was significantly attenuated in the old Sed SFA compared to the young Sed SFA ($p=0.003$) (Fig. 2.4). SOD and SOD+CAT significantly improved ACh-induced dilation in old Sed SFA (Fig. 2.4). SOD improved ACh-induced dilation in old Sed SFA such that the dilator response was greater than that seen in young Sed SFA while addition of SOD+CAT improved dilation in old Sed SFA to the extent that dilation was comparable to young Sed SFA (Fig. 2.4). In young Sed SFA, addition of SOD, or SOD+CAT, did not alter the ACh concentration response curve (Fig. 2.4); however, SOD did enhance the response to the maximal concentration of ACh (Table 2.5). In old Sed SFA, the SOD-induced improvement in ACh-induced dilation was abolished in the presence of SOD + L-NNA (Fig. 2.5). In young Sed, SOD + L-NNA tended ($p=0.12$) to inhibit ACh-induced dilation (Fig. 2.5) and significantly attenuated sensitivity (IC_{50}) to ACh (Table 2.5).

2.3.4 SNP-induced dilation

SNP elicited a concentration-dependent dilation of all arteries. Statistical analysis revealed no significant between-group differences (data not shown).

2.3.5 eNOS, p-eNOS(ser1177), SOD-1 and ecSOD protein content

Immunoblot analysis revealed that eNOS (Fig. 2.6A), p-eNOS(ser1177) (Fig. 2.6B), and SOD-1 (Fig. 2.7A) protein contents were not significantly altered by age or exercise training. The p-eNOS(ser1177)-to-total eNOS protein content ratio was also not altered by age or training status (Fig. 2.6C). In contrast, ecSOD protein content was significantly increased by exercise training in young and old SFA such that ecSOD content was greater in young Ex and old Ex arteries than in young Sed and old Sed arteries (Fig. 2.7B).

Table 2.3. Characteristics of soleus muscle feed arteries from young and old rats used in the antioxidant studies.

Parameter	Con	SOD	SOD+CAT	SOD + L-NNA
Maximal diameter, µm				
Young	162.6 ± 5.5	178.1 ± 8.0	175.8 ± 7.3	181.9 ± 8.7
Old	158.3 ± 7.6	183.2 ± 9.4	175.3 ± 13.9	169.0 ± 14.2
Initial Tone pre-ACh, %				
Young	41.1 ± 2.3	38.3 ± 2.9	38.2 ± 3.9	47.4 ± 5.2
Old	40.8 ± 3.2	39.0 ± 2.8	37.1 ± 4.1	42.4 ± 4.9

Values are means ± SEM; *n* = 6-21 rats/group. Con, control; SOD, Superoxide dismutase; SOD+CAT, Superoxide dismutase + catalase; SOD +L-NNA, Superoxide dismutase, N^{ω}-nitro-L-arginine.

Table 2.4. IC_{50} –(log) M values and response to 10^{-4} M ACh of soleus muscle feed arteries from young and old rats used in the exercise training study.

Parameter	Con	L-NNA	Indo	L-NNA+Indo
IC_{50}, -(log) M ACh				
Young Sed	-7.75 ± 0.27	-6.97 ± 0.26	-7.37 ± 0.26	-7.06 ± 0.38
Young Ex	-7.53 ± 0.30	-6.91 ± 0.18	-7.25 ± 0.29	-6.47 ± 0.45
Old Sed	-7.52 ± 0.35	-7.13 ± 0.16	-6.55 ± 0.24	-6.5 ± 0.73
Old Ex	-7.83 ± 0.55	-7.15 ± 0.26	-7.20 ± 0.60	-6.55 ± 0.60
Response to final concentration of ACh (10^{-4} M), % possible dilation				
Young Sed	87.0 ± 6.2	52.4 ± 8.2[a]	64.6 ± 9.3	60.6 ± 8.8[a]
Young Ex	76.9 ± 10.7	49.1 ± 12.6[a]	73.6 ± 9.4[d]	28.1 ± 8.6[ac]
Old Sed	54.2 ± 8.7[f]	37.9 ± 6.0	67.4 ± 9.0[bd]	28.5 ± 11.1[c]
Old Ex	80.9 ± 6.8[eb]	41.8 ± 9.5[a]	61.8 ± 11.3	39.1 ± 8.3[a]

Values are means ± SEM; *n* = 8-10 rats/group. Sed, sedentary; Ex, exercise trained; Con, control; L-NNA, N^{ω}-nitro-L-arginine; Indo, indomethacin. Significantly different from [a]Con, [b]L-NNA, [c]Indo, [d]L-NNA+ Indo, [e]Sed, [f]Young, $p \leq 0.05$

Table 2.5. IC_{50} –(log) M values and response to 10^{-4} M ACh of soleus muscle feed arteries from young and old rats used in the antioxidant studies.

Parameter	Con	SOD	SOD+CAT	SOD+ L-NNA
IC_{50}, -(log) M ACh				
Young	-6.61 ± 0.20	-7.28 ± 0.26	-6.72 ± 0.20	-8.18 ± 0.62[ac]
Old	-7.01 ± 0.18	-7.39 ± 0.14	-7.44 ± 0.35	-7.33 ± 0.46
Response to final concentration of ACh (10^{-4} M), % possible dilation				
Young	72.5 ± 4.7	78.7 ± 5.4[d]	64.9 ± 9.3	52.7 ± 13.2[b]
Old	44.1 ± 5.3[bce]	72.9 ± 6.6[ad]	72.8 ± 7.1[ad]	27.2 ± 4.5[bc]

Values are means ± SEM; *n* = 6-21 rats/group. Con, control; SOD, Superoxide dismutase; SOD+CAT, Superoxide dismutase + catalase; SOD+L-NNA, Superoxide dismutase + N^{ω}-nitro-L-arginine. Significantly different from [a]Con, [b]SOD, [c]SOD+CAT, [d]SOD+L-NNA ,[e]Young, $p \leq 0.05$.

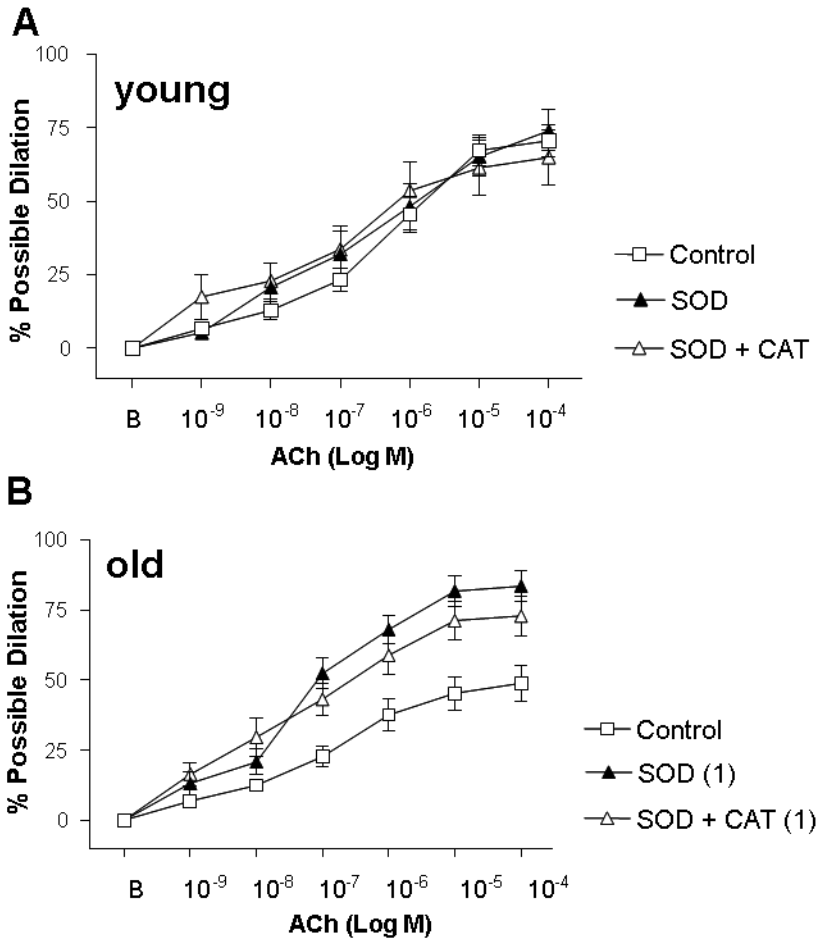


Figure 2.4. ACh-induced dilation in young (A) and old (B) SFA in the absence or presence of exogenous antioxidants Superoxide dismutase (SOD, 120 U/ml) or SOD + Catalase (CAT, 100 U/ml). B, baseline diameter before the first concentration of ACh. Values are means ± SEM; *n* = 6-14 rats per group. Concentration-response curve significantly different from [1]Control, $p \leq 0.05$.

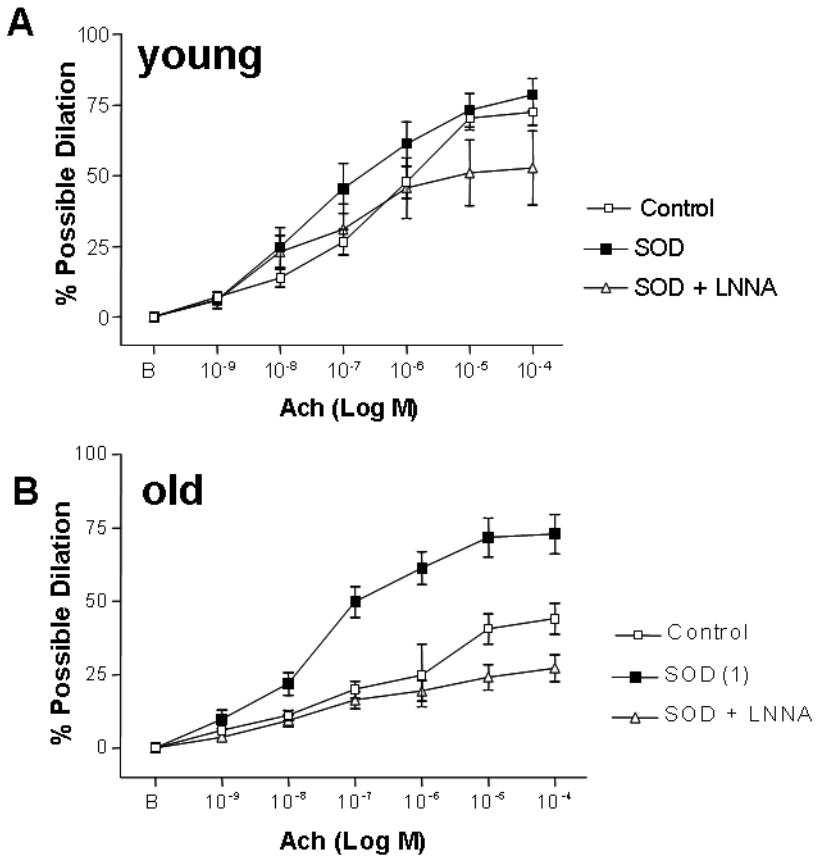


Figure 2.5. ACh-induced dilation in young (A) and old (B) SFA in the absence or presence of SOD or SOD + L-NNA. B, baseline diameter before the first concentration of ACh. Values are means ± SEM; *n* = 8-21 rats per group. Concentration-response curve significantly different from [1]Control, $p \leq 0.05$.

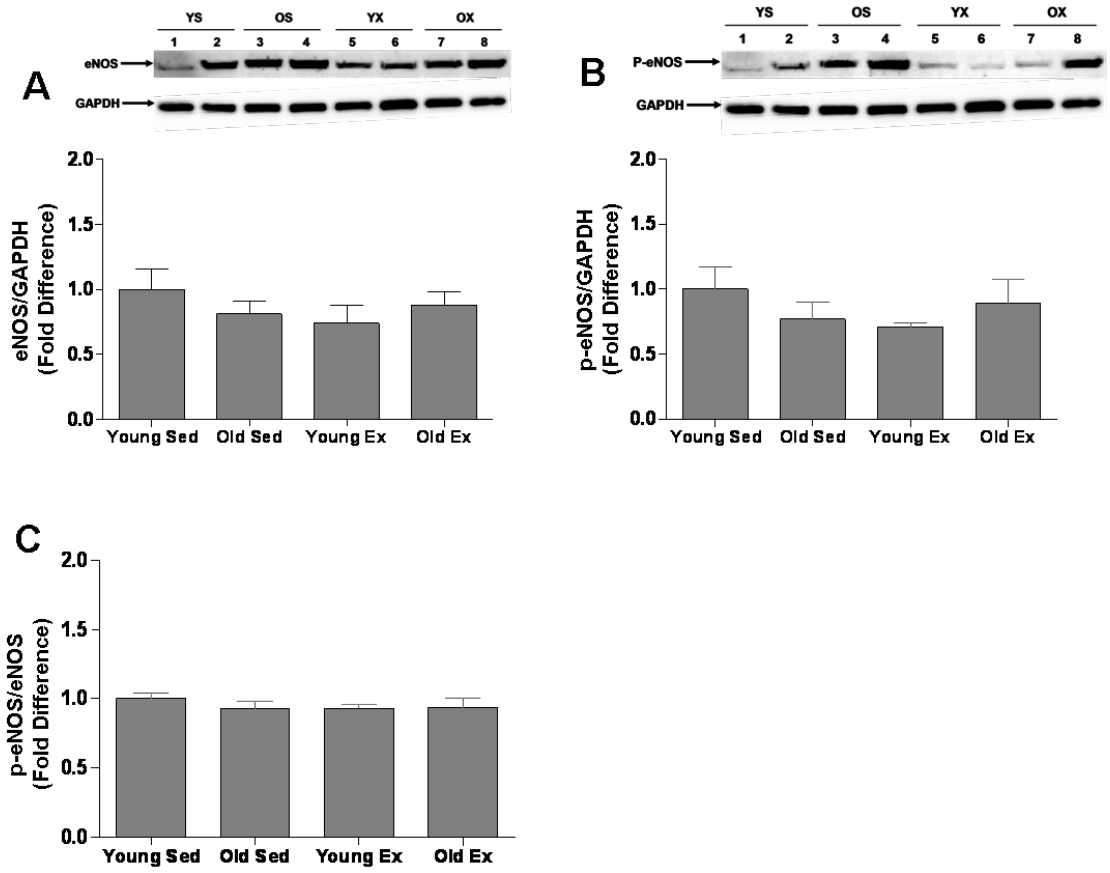


Figure 2.6. Comparison of endothelial nitric oxide synthase (eNOS) (A), P-eNOS(ser1177) (B) protein content and p-eNOS(ser1177)-to-eNOS ratio (C) in SFA from young and old rats. Insets: representative blots for the target protein (top image) and the same blot reprobed for (bottom image). YS, young sedentary; OS, old sedentary, YX, young exercise trained; OX, old exercise trained. Values are means ± SEM; *n* = 9-10 rats/group. Statistical analysis revealed no significant differences.

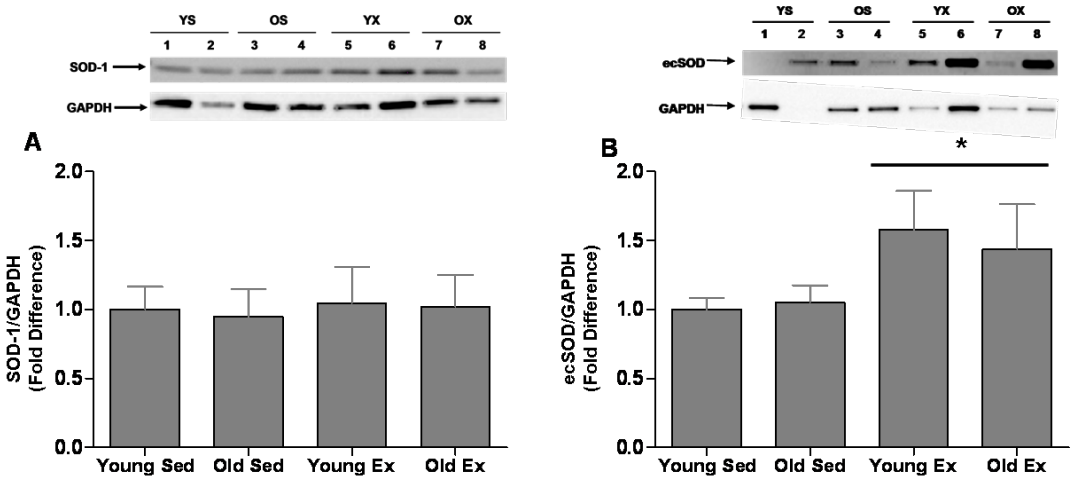


Figure 2.7. Comparison of Cu-Zn-dependent superoxide dismutase (SOD-1) (A) and extracellular superoxide dismutase (ecSOD) (B) protein content in SFA from young and old rats. Insets: representative blots for the target protein (top image) and the same blot reprobed for (bottom image). YS, young sedentary; OS, old sedentary, YX, young exercise trained; OX, old exercise trained. Values are means ± SEM; n = 8 rats/group. *Significantly different from Sed rats, $p \leq 0.05$.

2.4 Discussion

The purpose of this study was to test the hypothesis that exercise training reverses age-related decrements in endothelium-dependent dilation in SFA by increasing NO bioavailability due to increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content. The primary new findings of this study are that: 1) Exercise training improved ACh-induced dilation in old SFA such that vasodilator responses in old Ex SFA were similar to young Sed and young Ex SFA. 2) ecSOD protein content was increased by training in SFA from both young and old rats whereas the SFA protein content of eNOS, p-eNOS(ser1177), and SOD-1 were not altered by training. 3) Exogenous SOD, and SOD + CAT, restored ACh-induced dilation in old SFA in an NO-dependent manner similar to the effects of exercise training. Results also demonstrate that treatment with L-NNA, or L-NNA + Indo, inhibited ACh-induced dilation in old Ex SFA such that the response was not greater than the response in the old Sed SFA. Also, ACh-induced dilation was not significantly inhibited by Indo alone in young or old rats. Collectively, these results indicate that exercise training reverses the detrimental effects of aging on endothelium-dependent dilation in SFA and that increased vascular ecSOD protein content may contribute to this improvement. To our knowledge, this is the first study to demonstrate that exercise training reverses age-induced endothelial dysfunction in SFA or any skeletal muscle feed artery. Given that skeletal muscle feed arteries contribute importantly to regulating *total* skeletal muscle blood flow during exercise (47), this exercise induced adaptation may have substantial functional significance.

Reduced exercise capacity with age is well documented in humans and animals (4, 10, 75). The mechanism(s) for the age-related decline in exercise tolerance is not fully understood; however, an impaired ability to increase skeletal muscle blood flow during exercise may contribute to the reduction in exercise capacity (4, 55, 83, 91, 98). There are numerous potential mechanisms for the impairment in the blood flow response to exercise, including age-related increases in vasoconstrictor responsiveness (23, 28, 29, 98, 120) and blunted NO-mediated endothelium-dependent dilation (98). The role of NO in exercise hyperemia is somewhat controversial. In some studies NOS inhibition blunts exercise hyperemia (98, 102), whereas in other studies NOS inhibition has no effect on exercise hyperemia (40, 93). Importantly, Schrage et al. (98) have shown that the contribution of NO to exercise hyperemia is reduced in older subjects.

The decreased ACh-induced dilation observed in SFA from old Sed rats in the present study (Figs. 2.1, 2.4, 2.5) is in accord with previous studies indicating that endothelium-dependent dilation is impaired in feed arteries (132, 133) as well as 1A arterioles (81) of the soleus muscle of aged rats. Also, previous studies indicate that exercise training attenuates the detrimental effects of aging on endothelium-dependent dilation of skeletal muscle 1A arterioles (107, 108). Present results indicate that exercise improved ACh-induced dilation in old SFA such that vasodilator responses in old Ex SFA were similar to young Sed and young Ex SFA (Fig. 2.1). The exercise-induced improvement in endothelial function in SFA may be functionally significant given that feed arteries serve as

the primary control point for regulating *total* muscle blood flow to soleus muscle at rest and during exercise (129). In addition to enhancing NO-mediated dilation in aged skeletal muscle arterioles, exercise training has also been shown to attenuate vasoconstrictor responses in skeletal muscle arterioles (28, 29, 107, 108). Thus, it is likely that the exercise-induced improvement in endothelium-dependent vasodilator responses in SFA observed in the present study works in concert with enhanced vasodilator, and attenuated vasoconstrictor, responses in skeletal muscle arterioles to enhance the capacity to increase skeletal muscle blood flow and to distribute blood flow to the actively contracting muscle fibers during exercise.

2.4.1 Mechanisms responsible for increased vasodilator responses

Results of the present study indicate that the beneficial effect of exercise training on ACh-induced vasodilator function is primarily due to increases in NO bioavailability, since the exercise effect was eliminated in the presence of L-NNA (Fig. 2.2B). Importantly, the improvement in NO-mediated dilation could not be attributed to an exercise-induced improvement in the ability of vascular smooth muscle cells to respond to NO, since vasodilator responses to SNP (an NO donor) were similar in all groups of arteries. To determine whether the beneficial effect of exercise on endothelium-dependent dilation also involved COX products, ACh-induced dilation was assessed in the presence of Indo to block COX. ACh-induced dilation was not significantly inhibited by Indo alone in young or old rats regardless of training status (data not shown). Thus, the beneficial

effect of exercise training on vasodilator responses in aged SFA does not to appear to be mediated by altered COX signaling.

To determine whether enhancement of a NOS- and COX-independent vasodilator mechanism contributed to the beneficial effect of exercise training, ACh-induced dilation was assessed in the presence of L-NNA + Indo (double blockade). In the presence of L-NNA + Indo, residual dilation to ACh can be attributed to vasodilators other than NO and prostacyclin (PGI_2), primarily endothelium-derived hyperpolarizing factors (EDHF). Double blockade had no statistically significant impact on old Sed arteries (Fig., 2.3B) suggesting that endothelium-dependent dilation in Old Sed SFA is mediated entirely by non-NOS, non-COX mechanisms. Double blockade inhibited, but did not eliminate, ACh-induced dilation in Old Ex SFA (Fig. 2.3B). Equally important was the finding that ACh-induced dilation in old Ex SFA in the presence of L-NNA + Indo, was not different from the old Sed arteries in the presence of double blockade (Fig. 2.3B). These data indicate that a vasodilator pathway independent of NOS and COX contributed to ACh-induced dilation in young and old arteries; however, the beneficial effect of exercise training in aged arteries can not be attributed to enhancement of this pathway. In conclusion, our pharmacological experiments indicate that exercise training reverses the detrimental effects of aging on endothelial function of SFA primarily by enhancing NO bioavailability. This conclusion is similar to previous reports for 1A arterioles of soleus muscle of aged rats (107, 108).

Results of the present study also indicate that exercise training did not induce increases in eNOS protein content in young or old SFA (Fig. 2.6A). Nor did exercise training increase basal levels of p-eNOS(ser1177) (Fig. 2.6B) or alter the p-eNOS(ser1177)-to-total eNOS ratio (Fig. 2.6C). These data suggest that enhanced NO-mediated, endothelium-dependent dilation in old Ex SFA is not due to increased eNOS protein content or eNOS phosphorylation on Ser 1177. Taken together with previous results in solues 1As exercise training increases NO bioavailability in SFA (Figure 2.6) and 1As (107, 108) of aged soleus muscle; however, the vascular adaptation induced by exercise in SFA is not the same as that reported in soleus 1A arterioles by Spier et al. where exercise training increased eNOS protein content (107). Thus, while exercise training improves endothelial function in SFA and 1A arterioles perfusing soleus muscle, the mechanism by which exercise improves NO-mediated endothelium-dependent dilation is different in SFA and 1A arterioles of this muscle (107, 108). Exercise training has also been shown to increase eNOS mRNA and protein content in aged rat aorta (117). The differences in aging and exercise training induced alterations in different vessels suggest that findings in one vessel cannot necessarily be applied to other vessels.

Another mechanism whereby exercise training could increase NO bioavailability in aged SFA is through an exercise-induced reduction in the rate of NO degradation. The primary mechanism for NO degradation in blood vessels is rapid interaction with superoxide anion, leading to the formation of peroxynitrite (48). This mechanism has been implicated in aging-induced endothelial

dysfunction (48, 126). Vascular superoxide dismutases play a role in preserving the bioactivity of NO by scavenging superoxide. Consequently, an exercise-induced increase in the expression of superoxide dismutases could lead to the exercise-induced improvement in NO-mediated, endothelium-dependent dilation in aged SFA. Therefore we tested the hypothesis that exercise training increases the expression of SOD-1 and ecSOD in old SFA. The rationale for this hypothesis was based on experimental evidence indicating that exercise training increases expression of SOD-1 and ecSOD in the aorta of young mice and pigs (42, 96). Our results reveal that ecSOD (not SOD-1) content was increased by training in SFA from both young and old rats (Fig. 2.7B). These results support the hypothesis that the beneficial effect of exercise on endothelium-dependent dilation observed in the old SFA is, in part, the result of an enhanced capacity of ecSOD to scavenge superoxide, resulting in an increase in the bioavailability of NO. Because ecSOD activity is regulated by a number of factors including protein folding, reactions with NO and hydrogen peroxide and copper availability (42, 51, 59, 89), further study is needed to fully elucidate the role of ecSOD in the beneficial effects of exercise training in aged SFA.

In light of the observation that ecSOD protein content was increased in SFA with exercise training, we reasoned that the beneficial effects of exercise on endothelium-dependent dilation of SFA may be mimicked by treatment with exogenous antioxidant enzymes. To test this hypothesis we chose SOD (non-cell permeable) rather than PEG-SOD (cell permeable) because the primary exercise-induced alteration in antioxidant protein content occurred with the

extracellular isoform of SOD (ecSOD). Results indicated that addition of exogenous SOD, or SOD + CAT, did not alter the ACh concentration response in feed arteries from young rats but resulted in greater ACh-induced dilation in old Sed SFA when compared to old Sed SFA without antioxidants (Fig. 2.4). In addition, ACh-induced dilation in old Sed SFA was either equal to (in the presence of SOD + CAT) or greater than (in the presence of SOD alone) ACh-induced dilation in young Sed SFA (Fig. 2.4). Also, L-NNA abolished the improvement in ACh-induced dilation in old Sed SFA observed in the presence of SOD (Fig. 2.5). These results demonstrate that exogenous antioxidant enzymes mimic the effects of exercise in old SFA, consistent with our hypothesis. Based on the observation that exercise training increased ecSOD protein content and that exogenous SOD mimicked the effects of exercise on ACh-induced dilation in senescent SFA, we conclude that exercise training-induced improvements in NO-mediated, endothelium-dependent dilation in senescent SFA may be in part the result of enhanced scavenging of superoxide by ecSOD, resulting in increased stability of NO. These results are consistent with those obtained in human subjects where older athletes exhibited enhanced endothelium-dependent dilation and antioxidant capacity when compared to their sedentary counterparts (41); also, addition of exogenous antioxidants enhanced endothelium-dependent dilation in old sedentary subjects (35). Complimenting these observations, our study is the first to demonstrate enhanced ecSOD protein content after exercise training in the aged vasculature.

2.4.2 Conclusion

The results of this study indicate that exercise induces improvement in endothelium-dependent dilation in soleus muscle feed arteries of aged rats. The beneficial effect of exercise training in aged feed arteries was mediated by enhanced NO bioavailability that appears to be the result of increased ecSOD protein content in the aged SFA. The improved NO bioavailability was not associated with increased content or phosphorylation of eNOS or increased SOD-1 protein content. Exogenous SOD treatment of SFA from old Sed rats mimicked the effects of exercise training. Collectively, these results suggest that exercise training reverses the detrimental effects of aging on endothelial function in skeletal muscle feed arteries by enhancing the capacity to scavenge superoxide, increasing the bioavailability of NO. The exercise training-induced improvement in endothelial function in SFA may work in concert with enhanced endothelial function in skeletal muscle arterioles to improve skeletal muscle blood flow and increase exercise tolerance in the elderly.

CHAPTER III

NAD(P)H OXIDASE-DERIVED REACTIVE OXYGEN SPECIES CONTRIBUTE TO AGE-RELATED IMPAIRMENTS OF ENDOTHELIUM-DEPENDENT DILATION IN RAT SOLEUS FEED ARTERIES

3.1 Introduction

Aging is associated with a decline in maximal exercise capacity (32, 54, 67, 86) which is due in part to impaired exercise hyperemia (4, 31, 55, 62, 83, 91, 92). Emerging evidence in humans and animals suggests that with age, endothelial dysfunction limits vasodilation in the skeletal muscle vascular beds, particularly in oxidative muscle (62, 81, 107, 133). Indeed, our laboratory has demonstrated impaired endothelial function in the rat soleus muscle feed artery (SFA) which is primarily due to attenuated nitric oxide (NO)-bioavailability (123, 132-135). The feed artery is of particular importance as it is a primary control point for regulating total blood flow to the soleus muscle at rest and during exercise (129).

NO bioavailability is determined by the balance of NO production and NO degradation. Decreased NO production by endothelial nitric oxide synthase (eNOS) and increased NO degradation by superoxide anion ($O_2^{\cdot -}$) have both been implicated as mechanisms for age-related endothelial dysfunction (16, 26, 104-106, 123, 126). A number of potential sources of $O_2^{\cdot -}$ exist in the aged vasculature including: NAD(P)H oxidase, xanthine oxidase and mitochondrial

sources (16, 56, 125). In addition, eNOS itself, in the absence of its cofactor tetrahyrobiopterin (BH_4), can produce $O_2^{\cdot -}$ (6, 17, 36, 103). We have recently reported that scavenging $O_2^{\cdot -}$ with exogenous superoxide dismutase (SOD) improves, NO-mediated, endothelium-dependent dilation in senescent rat SFA (123). Similarly, exogenous antioxidants improve vasodilation and exercise hyperemia in the human forearm vasculature (35, 62). Interestingly, Sindler et al. recently reported that inhibition of $O_2^{\cdot -}$ production or scavenging of $O_2^{\cdot -}$ attenuated flow-induced dilation in soleus first order (1A) arterioles from both young and old rats (103). In addition, these investigators reported that hydrogen peroxide (H_2O_2) plays a role in flow-induced dilation in soleus 1A (103). H_2O_2 is the product of SOD scavenging of $O_2^{\cdot -}$ and has been implicated in some studies as a vasodilator in the aged vasculature (61, 103) whereas other studies suggest that H_2O_2 contributes to age-related endothelial dysfunction (118, 119, 125, 141).

Due to the apparent contrast between our data and that of Sindler et al., the ambiguous role of H_2O_2 in endothelial function with age and the differing location and roles of the SFA (outside the muscle, regulation of total muscle blood flow) and the 1A arteriole (inside the muscle, regulation of blood flow within the muscle), we sought to determine whether reactive oxygen species (ROS) play a role in age-related endothelial dysfunction in rat SFA. We hypothesized that age-related endothelial dysfunction in rat SFA is mediated, in part, by NAD(P)H oxidase-derived ROS.

3.2 Methods

3.2.1 Animals

The methods used in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee. Male Fischer 344 rats [4 mo (n = 40) and 24 mo of age (n = 36)] acquired from the National Institute of Aging (NIA) and housed at the College of Veterinary Medicine's Comparative Medicine Program Facility. Rats were housed under a 12:12-h light-dark cycle and food and water were provided *ad libitum*. The rats were examined daily by Comparative Medicine Program veterinarians. Fischer 344 rats were chosen, in part, because of the absence of atherosclerosis or hypertension with age (67); thus, we could examine the effect of aging in the absence of other cardiovascular risk factors.

3.2.2 Isolation of feed frteries

The protocol for SFA isolation has been described previously in detail (132-135). Briefly, rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg body wt. ip). The soleus/gastrocnemius muscle complex was dissected from both hindlimbs and was placed in a MOPS buffered physiological saline solution (PSS), containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA and 25.0 MOPS (pH 7.4). SFA were dissected free and transferred to a Lucite chamber containing MOPS-PSS (2 ml) for cannulation. SFA not used for isolated artery studies were dissected, transferred to a microcentifuge tube, snap frozen and stored at -80^{o}C for subsequent biochemical analyses.

3.2.3 Determination of vasodilator responses

Preparation of arteries. SFA were cannulated with two resistance-matched glass micropipettes and secured with a single strand of surgical thread. The micropipettes were attached to separate reservoirs filled with MOPS-PSS supplemented with albumin (1g/100ml). The height of each reservoir was adjusted to set intraluminal pressure in each feed artery to 60 cmH_2O (1 mmHg = 1.36 cm H_2O) for 20 min. SFA were checked for leaks by verification that intraluminal diameter was maintained after closing the pressure reservoirs. After 20 min, intraluminal pressure was raised to 90 cmH_2O and the SFA were allowed to equilibrate for an additional 40 min at 37^oC. At the end of the equilibration period, arteries that did not develop at least 25% spontaneous tone were discarded. All experimental protocols were conducted at an intraluminal pressure of 90 cmH_2O to approximate *in vivo* SFA pressure (129).

Assessment of vasodilation. Endothelium-dependent, flow-induced dilation was assessed by establishing intraluminal flow in the SFA by raising and lowering the heights of the pressure reservoirs in equal but opposite directions while maintaining constant pressure at the midpoint of the artery (66). Vasodilator responses to flow were assessed at pressure gradients of 0, 2, 4, 6, 8, 10, 15, 20, 30 and 40 cmH_2O, corresponding to flow rates of 0-62 µl/min (57). Each flow rate was maintained for 5 min to allow SFA to reach a steady diameter. Endothelium-dependent, acetylcholine (ACh)-induced dilation was assessed in SFA by adding cumulative, increasing, whole log concentrations of ACh over the range of 10^{-9}-10^{-4} M. Endothelium-independent dilation was assessed in SFA by

addition of cumulative, increasing, whole log concentrations of sodium nitroprusside (SNP) over the range of 10^{-9}-10^{-4} M.

Passive Diameter. At the end of each experiment, SFA were incubated for 30 minutes in Ca^{2+}-free PSS to determine passive diameter at an intraluminal pressure of 90 cmH_2O.

3.2.4 Effects of ROS on endothelium-dependent dilation

To determine the role and source of vascular $O_2^{\cdot-}$, endothelium-dependent vasodilator responses were assessed in the absence and presence of tempol (100 μM, a cell permeable SOD mimetic) and apocynin (100 μM), an NAD(P)H oxidase inhibitor (100). Tempol or apocynin was added to the vessel bath 30 minutes before assessing the dilator responses.

To determine the role of H_2O_2 in endothelium-dependent dilations, vasodilator responses were assessed in the absence and presence of catalase (100 U/ml, a non cell-permeable H_2O_2 scavenger) and PEG-catalase (200 U/ml, a cell-permeable H_2O_2 scavenger). Catalase or PEG-catalase was added to the vessel bath for 30 minutes before assessing the dilator responses.

3.2.5 Immunoblotting

Relative protein content of NAD(P)H oxidase subunits, SOD isoforms and catalase were assessed in single SFA using immunoblotting techniques described previously in detail (58). The following NAD(P)H subunit protein contents were assessed using monoclonal antibodies: gp91phox (1:1,000, BD Biosciences, catalog no.G95320), p47phox (1:1,000, BD Biosciences, catalog no.P33720), p67phox (1:250, BD Biosciences, catalog no.610912), Nox-1 (1:500,

Santa Cruz Biotechnology, catalog no.sc-25545). The following SOD isoform protein contents were assessed using polyclonal antibodies: Cu/Zn SOD (1:3,333, Assay Designs, catalog no.SOD-100), MnSOD (1:6,000, Assay Designs, catalog no.SOD-110), ecSOD (1:1,000, Assay Designs, catalog no.SOD-105). Catalase protein contents were assessed using a monoclonal antibody (1:1000, Sigma-Aldrich, catalog no.C-0979). In addition, relative SFA Nitrotyrosine content was assessed using a polyclonal antibody (1:1000, Millipore, catalog no.AB5411). Immunoblots were evaluated by enhanced chemiluminescence (ECL, Amersham) and densitometry by using a LAS-4000 Luminescent Image Analyzer and Multi-Gauge Image Analysis Software (FUJIFILM Medical Systems). Total protein data were expressed relative to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to control for small differences in protein loading. GAPDH protein content was assessed with a monoclonal antibody (1:10,000, Millipore, catalog no.AB374).

3.2.6 SOD activity assay

Single SFA were solubilized in 20 µl of 20 mM HEPES buffer using repeated freeze thaw cycles. SFA SOD activity was assessed using a commercially available SOD activity assay kit (Cayman Chemical) as previously described (33) and assessed colormetrically.

3.2.7 Drugs

All drugs were obtained from Sigma-Aldrich and with the exception of apocynin were dissolved in PSS. Apocynin was dissolved in dimethyl sulfoxide (DMSO) such that the concentration of DMSO in the vessel bath did not exceed

0.1%. Pilot studies revealed that this concentration did not alter endothelium-dependent or –independent dilations.

3.2.8 Statistical analysis

All data are presented as means ± SEM. Between-group differences in body mass, maximal diameters, % spontaneous tone, relative protein content and SOD activity were assessed by using student's *t*-test or one-way ANOVA where appropriate. Vasodilator response data were assessed as percent possible dilation calculated as $[(D_{concentration} - D_B)/(D_P - D_B)] \times 100$ where $D_{concentration}$ is the measured diameter for a give concentration/flow rate, D_B is the baseline diameter before the concentration response curve and D_P is maximal passive diameter. Two-way repeated-measures ANOVA with repeated measures on one factor (concentration/flow rate) was used to determine differences in dilator responses. Statistical significance was set a $P \leq 0.05$ probability level.

3.3 Results

3.3.1 Characteristics of rats and SFA

Body weight and maximal vessel diameter were significantly greater in old rats compared to young (Table 3.1). Spontaneous myogenic tone was not altered with either age or pharmacological treatment (Table 3.1).

3.3.2 Vasodilator responses

Both flow- and ACh-induced dilations were attenuated with age (Fig. 3.1). SNP-induced (endothelium-independent) dilation was not altered with age (data not shown).

3.3.3 Role of $O_2^{\cdot -}$ in endothelium-dependent dilation

In SFA from young rats, scavenging of $O_2^{\cdot -}$ with tempol did not alter flow-induced dilation (Fig. 3.2A). In SFA from old rats, tempol significantly improved flow-induced dilation (Fig. 3.2B). Similarly, the presence of tempol did not alter ACh-induced dilation in SFA from young rats and improved dilation in SFA from old rats (Fig. 3.2C, D).

Inhibition of NAD(P)H oxidase by apocynin did not alter flow-induced dilation in SFA from young rats (Fig. 3.3A) whereas NAD(P)H oxidase inhibition significantly improved flow-induced dilation in SFA from old rats (Fig. 3.3B). Apocynin attenuated ACh-induced dilation in SFA from young rats (Fig. 3.3C) and improved ACh-induced dilation in SFA from old rats (Fig. 3.3D).

3.3.4 Role of H_2O_2 in endothelium-dependent dilation

Neither catalase (extracellular H_2O_2 scavenging) nor PEG-catalase (intracellular H_2O_2 scavenging) significantly altered flow-induced dilation in SFA

from young rats (Fig. 3.4A); however, both catalase and PEG-catalase blunted ACh-induced dilation in SFA from young rats (Fig. 3.4C). In SFA from old rats, catalase significantly improved flow-induced dilation (Fig. 3.4B) and PEG-catalase significantly improved ACh-induced dilation (Fig. 3.4D).

3.3.5 Immunoblotting

Immunoblot analysis for NAD(P)H oxidase subunits revealed that age resulted in increased gp91phox protein content (Fig. 3.5C) whereas age did not alter p47phox, p67phox or Nox-1 protein content (Fig. 3.5A, B&D). Immunoblots for SOD isoform protein content revealed that SOD-1 and ecSOD protein contents from SFA from young and old rats were similar (Fig. 3.6A & C). MnSOD protein content was greater in SFA from old rats compared to young (Fig. 3.6B). SOD enzyme activity was also greater in SFA from old rats (Fig. 3.6D). Catalase protein content was not altered with age (Fig. 3.6E). Nitrotyrosine expression was not different with age (Fig. 3.7).

Table 3.1 Animal and vessel characteristics.

	Young (n = 40)	Old Rats (n = 36)
Body weight, g	377 ± 6	414 ± 7*
Vessel Characteristics		
Maximal Diameter, μm	160 ± 4	173 ± 5*
Spontaneous tone, %		
Pre-Flow		
Control	41 ± 3	40 ± 2
Tempol	46 ± 4	37 ± 3
Apocynin	46 ± 5	49 ± 7
Catalase	45 ± 4	34 ± 2
PEG-Catalase	40 ± 3	38 ± 4
Pre-Acetylcholine		
Control	38 ± 2	40 ± 3
Tempol	37 ± 3	37 ± 3
Apocynin	41 ± 4	49 ± 8
Catalase	38 ± 3	37 ± 2
PEG-catalase	35 ± 2	33 ± 2

Values are means ± SEM. *significantly different from young, $p \leq 0.05$.

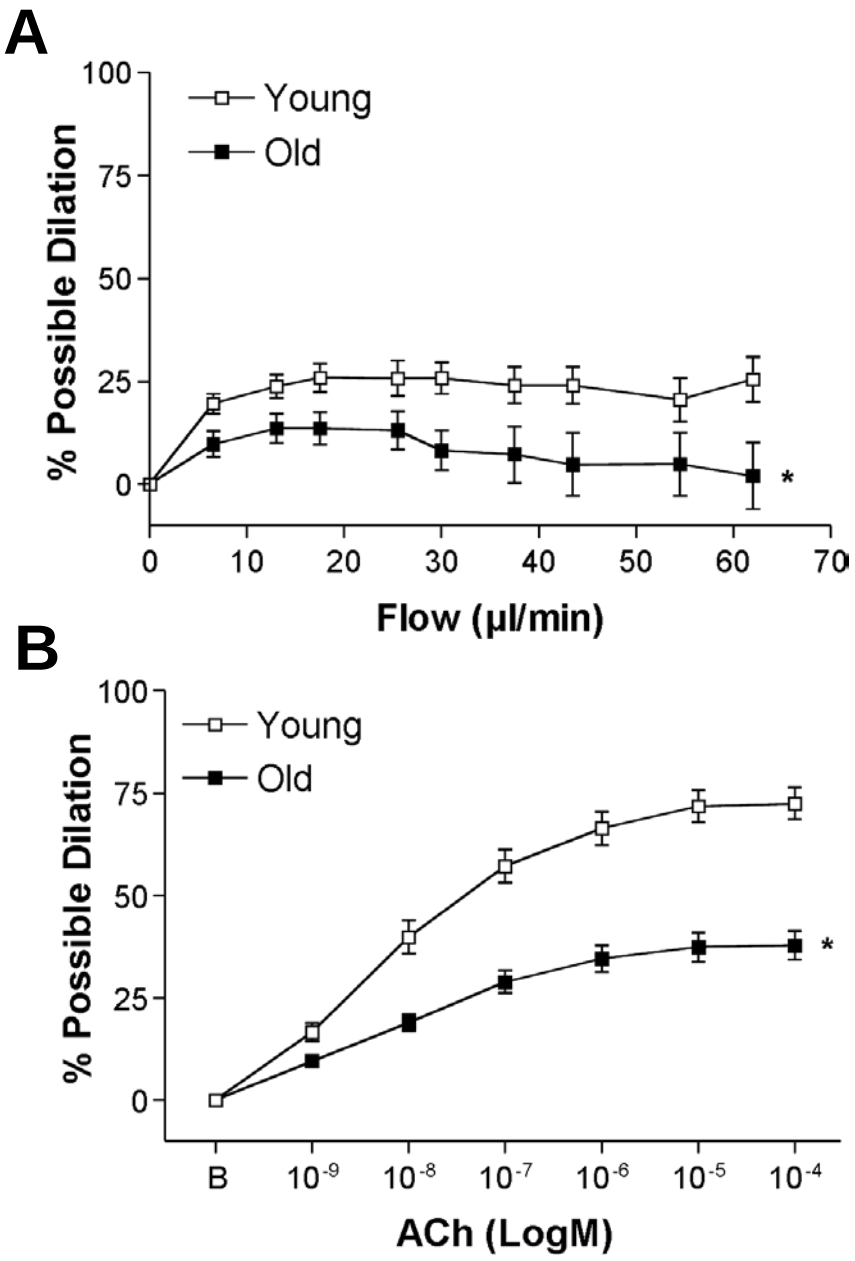


Figure 3.1. Flow-induced (A) and ACh-induced (B) dilation in soleus muscle feed arteries (SFA). B, baseline diameter before the first concentration of ACh. Values are means ± SEM. n sizes: young flow (n = 34), old flow (n = 36), young ACh (n = 37), old ACh (n = 34). *concentration-response curve significantly different from young, $p \leq 0.05$.

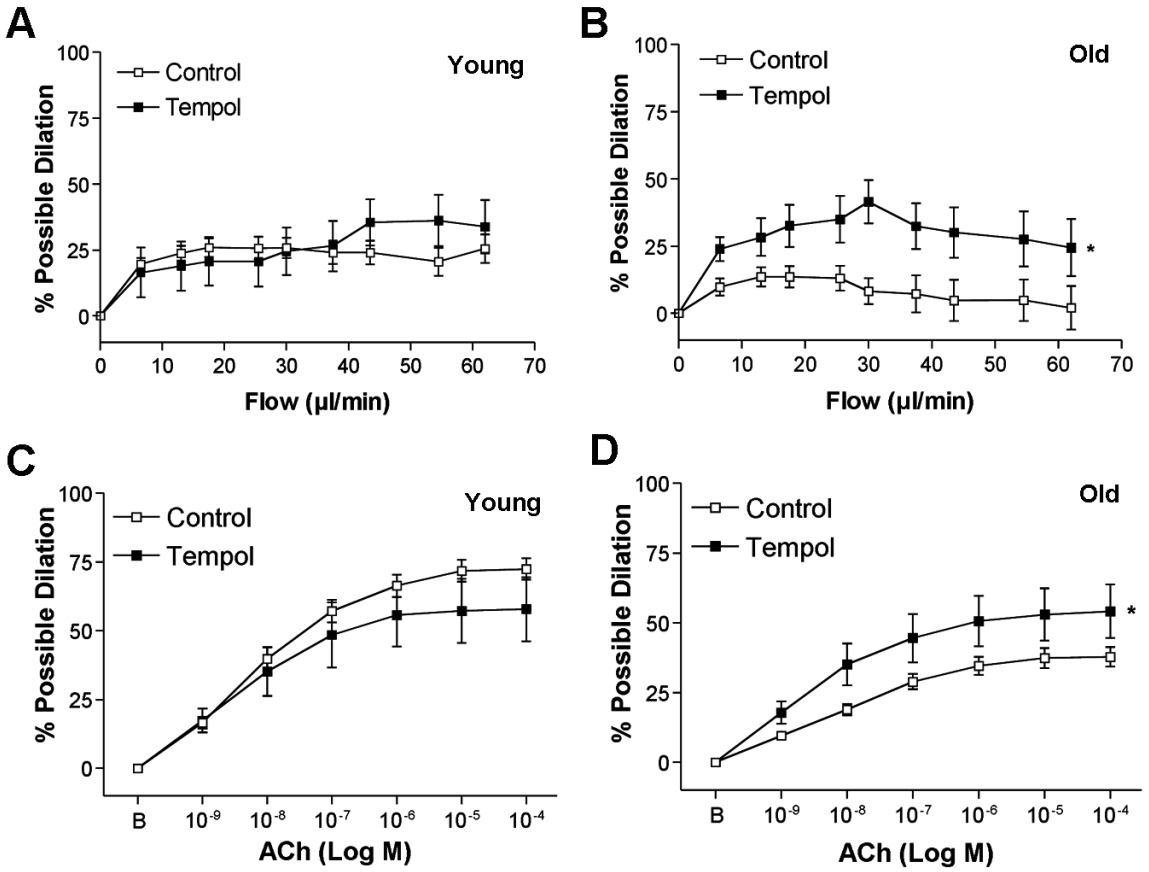


Figure 3.2. Flow-induced (A & B) dilation in soleus muscle feed arteries (SFA) in the absence or presence of SOD mimetic Tempol (100µM). n sizes: young control (n = 24), young + Tempol (n = 8), old control (n = 29), old + Tempol (n = 10). ACh-induced (C & D) dilation in SFA in the absence or presence of Tempol. n sizes: young control (n = 28), young + Tempol (n = 9), old control (n = 30), old + Tempol (n = 11). B, baseline diameter before the first concentration of ACh. Values are means ± SEM. *concentration-response curve significantly different from control, $p \leq 0.05$.

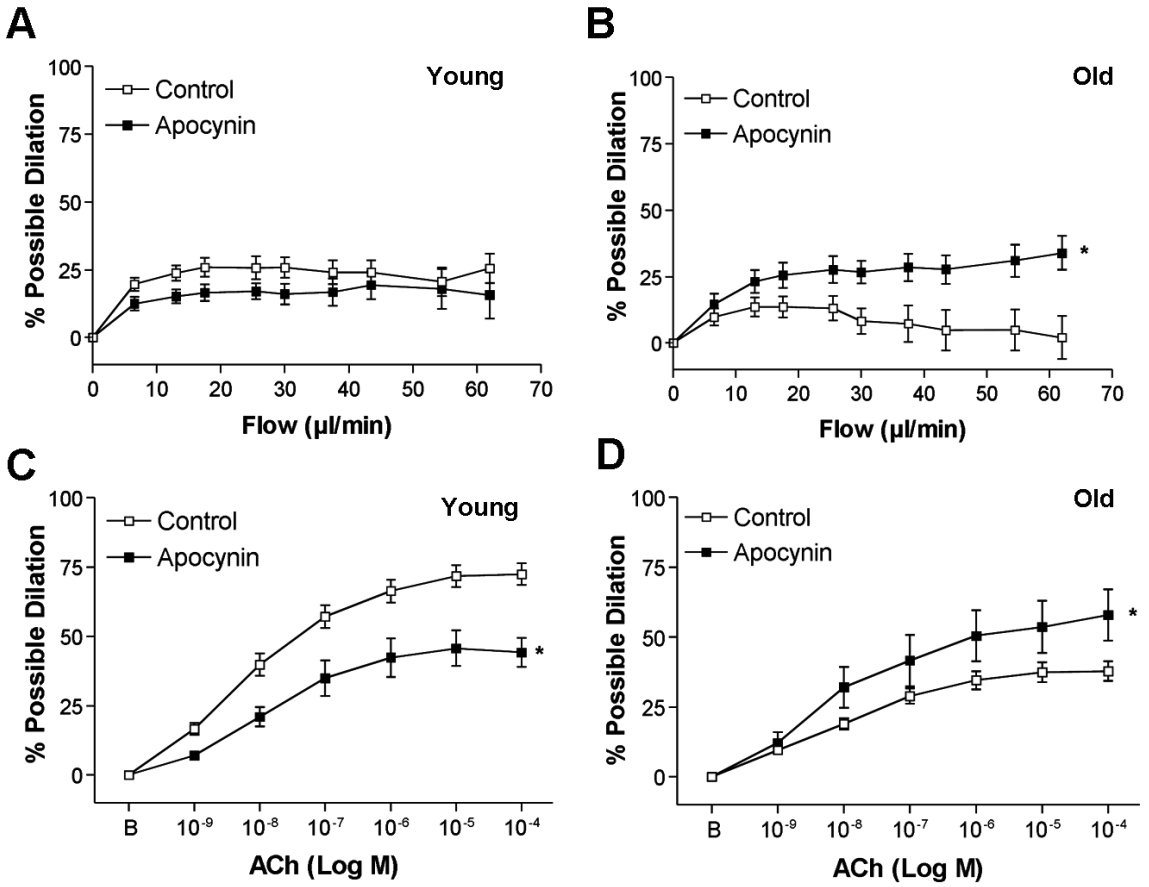


Figure 3.3. Flow-induced (A & B) in soleus muscle feed arteries (SFA) in the absence or presence of Apocynin (100µM). n sizes: young control (n = 24), young + Apocynin (n = 11), old control (n = 29), old + Apocynin (n = 8). ACh-induced (C & D) dilation in SFA in the absence or presence of apocynin. n sizes: young control (n = 28), young + Apocynin (n = 11), old control (n = 29), old + Apocynin (n = 8). B, baseline diameter before the first concentration of ACh. Values are means ± SEM. *concentration-response curve significantly different from control, $p \leq 0.05$.

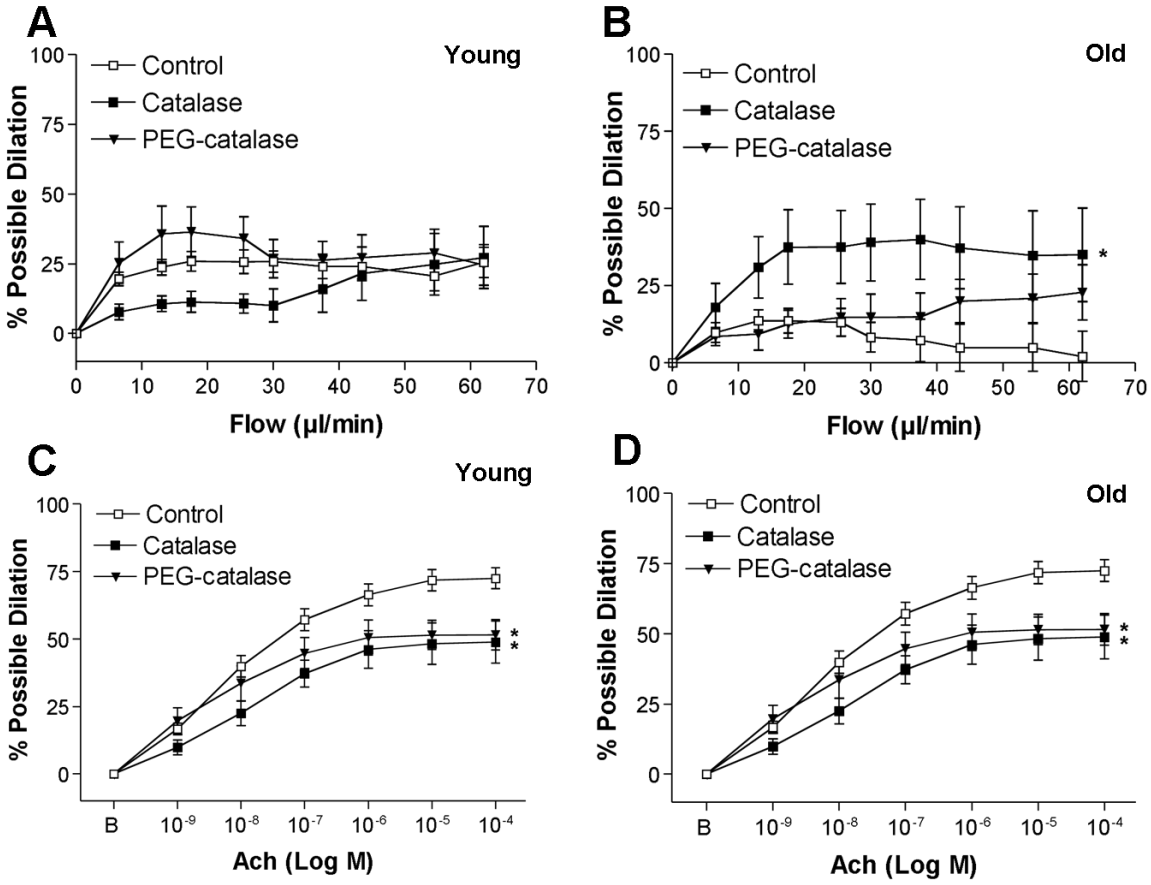


Figure 3.4. Flow-induced (A & B) dilation in soleus muscle feed arteries (SFA) in the absence or presence of catalase (an extracellular H_2O_2 scavenger, 100 U/ml) or PEG-catalase (an intracellular H_2O_2 scavenger, 200 U/ml). n sizes: young control (n = 24), young + Catalase (n = 8), young + PEG-catalase (n = 8), old control (n = 29), old + Catalase (n = 7), old + PEG-catalase (n = 8). ACh-induced (C & D) dilation in SFA in the absence or presence of catalase or PEG-catalase. n sizes: young control (n = 28), young + catalase (n = 8), young + PEG-catalase (n = 9), old control (n = 29), old + catalase (n = 8), old + PEG-catalase (n = 8). B, baseline diameter before the first concentration of ACh. Values are means ± SEM. *concentration-response curve significantly different from control, $p \leq 0.05$.

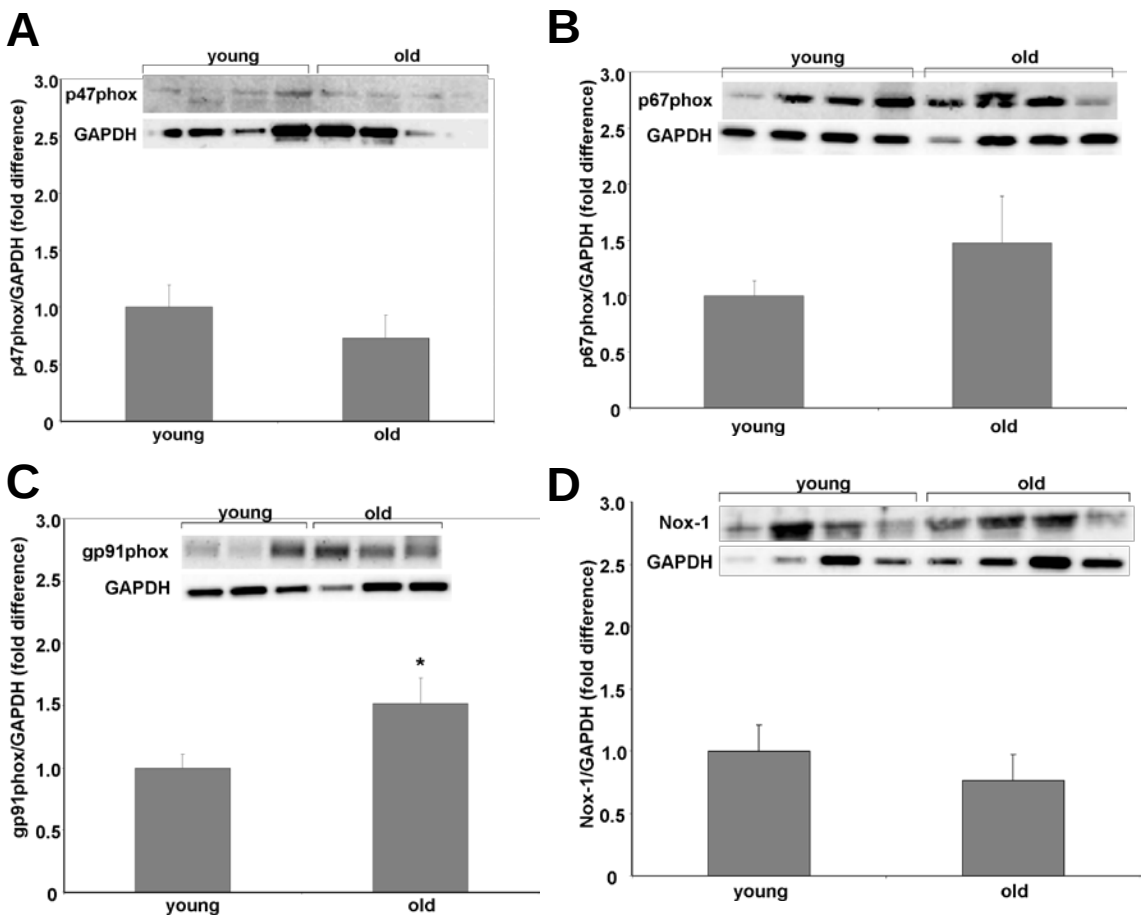


Figure 3.5. Comparison of NAD(P)H oxidase subunit protein contents p47phox (A) p67phox (B) gp91phox (C) Nox-1 (D) in SFA from young and old rats. Insets: representative blots for the target protein (top image) and the same blot reprobed for GAPDH (bottom image). Values are means ± SEM; *n* = 8-12 rats/group. *significantly different from young, $p \leq 0.05$.

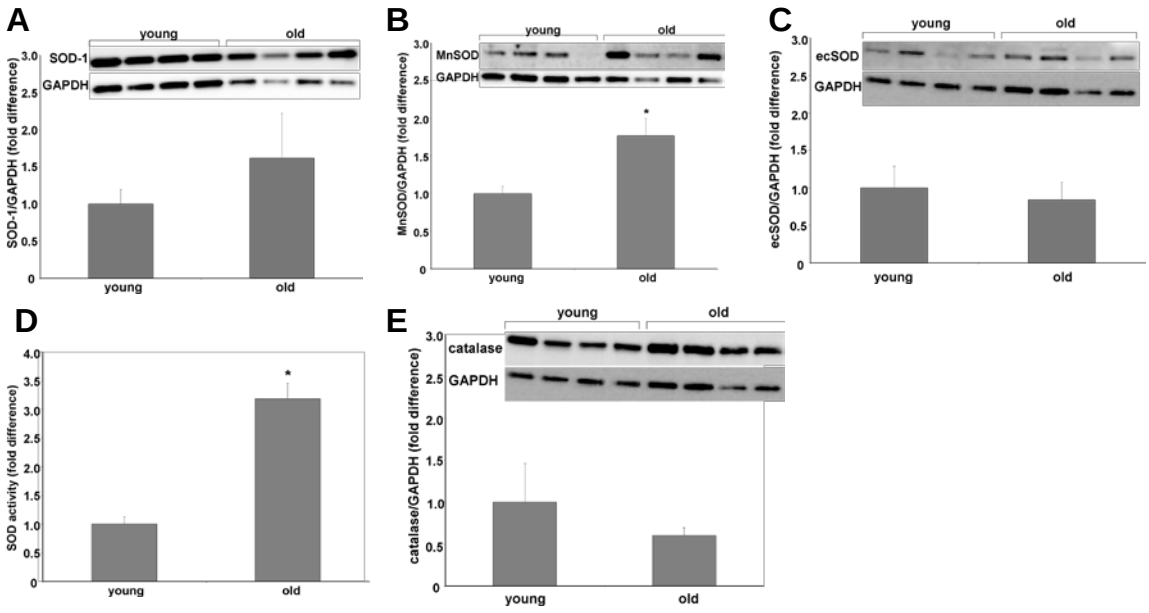


Figure 3.6. Comparison of Cu-Zn-dependent superoxide dismutase (SOD-1) protein content (A), mitochondrial superoxide dismutase (mnSOD) protein conent (B), extracellular superoxide dismutase (ecSOD) protein content (C), total superoxide dismutase activity (D) and catalase protein content (E) in SFA from young and old rats. Insets: representative blots for the target protein (top image) and the same blot reprobed for GAPDH (bottom image). Values are means ± SEM; n = 8-12 rats/group. *significantly different from young, $p \leq 0.05$.

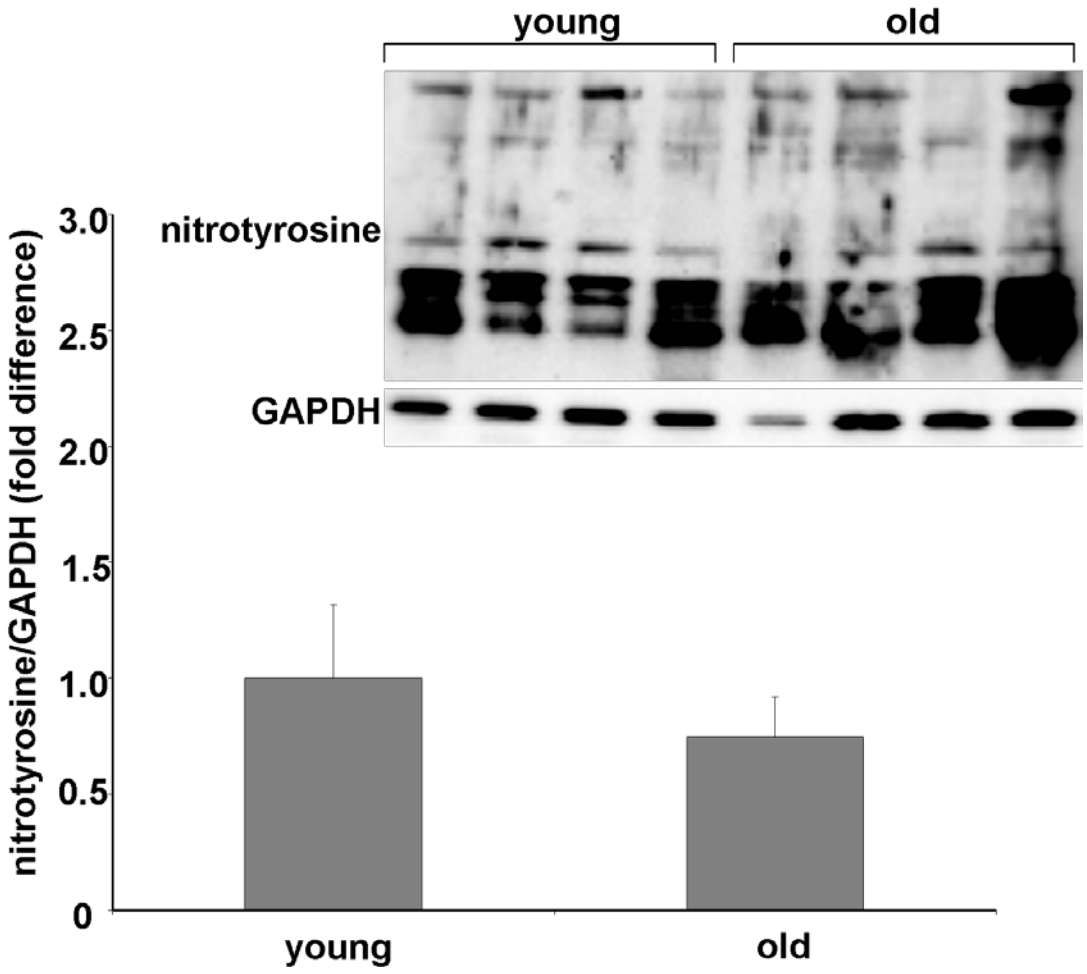


Figure 3.7. Comparison of nitrotyrosine expression in SFA from young and old rats. Inset: representative blots for nitrotyrosine (top image) and the same blot reprobed for (bottom image). Values are means ± SEM; n = 12 rats/group.

3.4 Discussion

The purpose of this study was to test the hypothesis that age-related endothelial dysfunction in rat SFA is mediated in part by NAD(P)H oxidase-derived ROS. The major new findings of this study are as follows: 1) $O_2^{\cdot-}$ scavenging and inhibition of NAD(P)H oxidase improved endothelium-dependent dilation in SFA from old rats. 2) Inhibition of NAD(P)H oxidase attenuated ACh-induced dilation in SFA from young rats. 3) Scavenging of H_2O_2 improved endothelium-dependent dilations in SFA from old rats. 4) Scavenging of H_2O_2 attenuated ACh-induced dilation in SFA from young rats 5) NAD(P)H oxidase subunit, gp91phox protein content was greater in SFA from old compared to young rats. 6) MnSOD protein content and SOD enzyme activity were greater in SFA from old compared to young rats. Collectively, these results suggest that ROS contribute to impaired endothelium-dependent dilation in old SFA; whereas, ROS appear to play a role in ACh-mediated dilation in SFA from young rats.

Age is associated with impairment in endothelial function in conduit and resistance arteries in humans and animals including the SFA (3, 6, 12, 16, 17, 81, 116, 126, 132). SFA were used in this study because the SFA is a primary control point for regulating total muscle blood flow to the soleus muscle (129) and because, exercise hyperemia to oxidative muscle (including the soleus) is attenuated with age (83). An impaired ability of the SFA to dilate during physical activity may contribute to attenuated exercise hyperemia and exercise capacity. In the present study, the finding that age attenuates both flow- and ACh-induced

dilation are in accord with previous studies from our laboratory demonstrating endothelial dysfunction with age in the SFA (123, 132-135).

We have previously shown that exogenous SOD improved ACh-induced dilation in a NO-dependent manner in senescent SFA (123). This led us to hypothesize that with age, vascular $O_2^{\cdot -}$ limits endothelium-dependent dilation. We found that scavenging of $O_2^{\cdot -}$ with the SOD mimetic tempol or inhibition of NAD(P)H oxidase (a major source of $O_2^{\cdot -}$) with apocynin improved endothelium-dependent dilations in SFA from old rats. Interestingly, Sindler et al. (103), found that in senescent soleus 1A arterioles both $O_2^{\cdot -}$ scavenging with tempol and inhibition of $O_2^{\cdot -}$ production with apocynin attenuated flow-induced dilation. This difference may be explained by the difference in anatomical location of the vessel as the SFA is external to the soleus whereas the 1A arteriole is internal. As contracting muscle releases ROS (94), the apparently ROS-mediated dilations of the 1A arterioles suggest that muscle-derived ROS may mediate dilation of these vessels during exercise. In contrast, the feed artery is external to the muscle and may be exposed to different concentrations and/or respond differently to extravascular ROS.

Since H_2O_2 has been implicated as an endothelium-derived hyperpolarizing factor (EDHF) in some studies (61, 80, 103) but contributes to endothelial dysfunction in others (118, 119, 125, 141), we also sought to determine the role of H_2O_2 in endothelial dysfunction. We utilized both extracellular (catalase) and intracellular (PEG-catalase) H_2O_2 scavengers and found that scavenging of H_2O_2 did not significantly alter flow-induced dilation, but

blunted ACh-induced dilation in SFA from young rats. In SFA from old rats, catalase treatment improved flow-induced dilation, whereas PEG-catalase improved ACh-induced dilation. These results suggest that with age H_2O_2 impairs endothelium-dependent dilation in the rat SFA. These results are in contrast to those observed in soleus 1A arterioles (103) and underscores the important differences in mechanisms regulating vascular reactivity in feed arteries versus arterioles despite their proximity. The finding that extracellular H_2O_2 scavenging improved flow-induced dilation and intracellular H_2O_2 scavenging improved ACh-dilation in SFA from aged rats indicates the location of H_2O_2 may be important in the regulation of vascular function. This finding is somewhat surprising as H_2O_2 is an uncharged molecule which should be able to freely traverse the cell membrane. The apparent importance of the precise location of vascular H_2O_2 is an important topic for further investigation.

The finding that scavenging of H_2O_2 and inhibition of NAD(P)H oxidase blunt dilation in SFA from young rats (Fig. 3.4) is intriguing as it suggests that ROS contribute to ACh-induced dilation in young vessels. Importantly, H_2O_2 has been implicated as an EDHF and also appears to play a role in eNOS phosphorylation and activation (80, 121). We have previously shown that a significant amount of ACh-induced dilation in rat SFA is EDHF-dependent (123, 133), whereas flow-induced dilation is primarily NO- and prostacyclin-mediated (133). This difference may explain why H_2O_2 scavenging only blunted ACh-induced dilation in SFA from young rats. Inhibition of NAD(P)H oxidase also blunted ACh-induced dilation (Fig. 3.3C). NAD(P)H oxidase is a major source of

vascular H_2O_2, both from dismutation of O_2^- and direct H_2O_2 production (21, 138). This may explain the inhibitory effect of apocynin on ACh-induced dilation in SFA from young rats. Together these observations suggest that NAD(P)H oxidase-derived H_2O_2 contribute to ACh-mediated dilation in young rat SFA.

The observation that scavenging of H_2O_2 improves endothelial function in senescent SFA but blunts ACh-induced dilation in SFA from young rats suggests that with age there is an alteration in the role of H_2O_2. It is conceivable that in the young SFA, ROS contribute to endothelium-dependent dilation and with age the production and/or scavenging of ROS is altered resulting in excessive vascular ROS, blunting endothelium-dependent dilation.

With age there appear to be multiple sources of vascular ROS. These sources include NAD(P)H oxidase, xanthine oxidase, mitochondrial sources and eNOS (in the absence of it's cofactor BH_4) (6, 16, 17, 36, 56, 103, 125). NAD(P)H oxidase is a membrane bound enzyme complex with several subunits (100). The finding that NAD(P)H subunit gp91phox protein content is increased with age is of significance as gp91phox is homologous to the catalytic subunit Nox-2, which facilitates the transfer of electrons from NAD(P)H to molecular oxygen yielding $O_2^{\cdot -}$ (100). Nox-1, a different isoform of the catalytic subunit, was not altered with age. p47phox and p67phox bind to the catalytic subunit and facilitate electron flow through the catalytic subunit. In the present study, these protein contents were not altered with age. This is in contrast with findings in conduit vessels from aged humans and mice demonstrating increased p47phox and p67phox respectively (26, 33, 90). The age-related increase in protein content of the

catalytic subunit gp91phox is in accord with the finding that inhibition of NAD(P)H oxidase improved endothelium-dependent dilation, suggesting that NAD(P)H oxidase-derived ROS play a role in age-induced endothelial dysfunction in the SFA. In addition to NAD(P)H oxidase, it appears that eNOS is also a source of $O_2^{\cdot -}$ in the skeletal muscle resistance vasculature. In soleus 1A a precursor of BH_4 improved endothelial function and inhibition of eNOS resulted in lower vascular $O_2^{\cdot -}$ concentrations (17, 103). It is conceivable that in the aged skeletal muscle vasculature, these two mechanisms are linked in that $O_2^{\cdot -}$ derived from NAD(P)H oxidase may oxidize BH_4 limiting it's availability and resulting in eNOS uncoupling and further $O_2^{\cdot -}$ production.

In addition to hypothesizing that with age ROS production contributes to endothelial dysfunction, we hypothesized that decreased ROS scavenging would also contribute to the observed decline in endothelium-dependent dilation. Previously we have shown that exercise training improved endothelial function in senescent SFA and that this improvement was mediated by an increase in ecSOD protein content (123). In the present study we found that SOD-1, ecSOD and catalase protein contents were not altered with age. Contrary to our hypothesis, we found that both MnSOD protein and total SOD activity were greater in SFA from old rats compared to young. This finding may be reflective of a compensatory cellular response in attempt to combat greater vascular $O_2^{\cdot -}$ concentrations. Further, this may result in increased vascular H_2O_2 which appears to blunt dilation in senescent SFA (Fig. 3.4B,D). In addition, it is interesting to note that the mitochondrial isoform of SOD (MnSOD) was

enhanced with age and mitochondrial-derived ROS appear to play a role in vascular aging in rat conduit arteries (125). The increased MnSOD protein content and greater SOD activity may also explain the finding that nitrotyrosine was not altered with age in SFA. Nitrotyrosine is an index of peroxynitrite, the product of NO and $O_2^{\cdot -}$. Increased SOD activity may facilitate scavenging of $O_2^{\cdot -}$ and reduce formation of peroxynitrite. In addition, there are a number of potential mechanisms by which NO production may be reduced with age (74, 104-106, 136). It is conceivable that reduced NO production with age reduces the availability of NO for reaction with $O_2^{\cdot -}$.

In summary, we tested the hypothesis that age-related endothelial dysfunction in rat SFA is mediated, in part by NAD(P)H oxidase-derived ROS. The findings of the present study suggest that NAD(P)H oxidase-derived ROS attenuate endothelium-dependent dilation in senescent rat SFA. In contrast, it appears that NAD(P)H oxidase-derived H_2O_2 play a role in ACh-induced dilation in young rat SFA. Contrary to our hypothesis, MnSOD protein content and total SOD activity were increased with age suggesting a compensatory response in attempt to scavenge $O_2^{\cdot -}$. These results suggest that ROS contribute to impaired endothelium-dependent dilation in old SFA; however, ROS appear to play a role in ACh-induced dilation in SFA from young rats.

CHAPTER IV

INFLUENCE OF AGING ON PI3 KINASE/AKT-DEPENDENT eNOS PHOSPHORYLATION(SER1177) IN RAT SOLEUS MUSCLE FEED ARTERIES

4.1 Introduction

Age is associated with impairments in endothelial function in humans and animals (3, 12, 19, 81, 132). This impairment likely contributes to increased risk for cardiovascular diseases with age. In addition, endothelial dysfunction in the skeletal muscle resistance vasculature appears to contribute to impairments in muscle blood flow and oxygen delivery (11, 16-18, 24). These alterations likely contribute to the decline in exercise capacity observed in the elderly (10, 32, 54).

In old rats, oxidative muscles exhibit attenuated exercise hyperemia in comparison to muscles from young rats (83). In soleus muscle, a primary control point regulating total muscle blood flow at rest and during exercise is the soleus feed artery (SFA) (129). We have previously shown that with age endothelium-dependent dilation is impaired in the SFA and that this impairment is primarily mediated by decreased nitric oxide (NO)-bioavailability (132, 133). NO bioavailability is determined by the balance of production of NO (primarily by the endothelium) and degradation of NO which is primarily due to NO reacting with superoxide anion (48). We have recently reported that exogenous superoxide

dismutase improves NO-dependent dilation in senescent SFA, suggesting that excess superoxide plays a role in decreasing NO bioavailability with age (123).

Whether decreased production of NO also contributes to impairments in NO bioavailability in SFA is unknown. Endothelial nitric oxide synthase (eNOS) is the primary enzyme responsible for synthesis of NO in the vasculature and several post-translational modifications of eNOS act to facilitate enzyme activation and NO production. Phosphorylation of eNOS on serine residue 1177 (p-eNOSser1177), mediated by the PI3-kinase(PI3K)/Protein Kinase B (Akt) pathway appears to play and important role in eNOS activation and NO production (22, 38). In mouse conduit vessels, a single bout of exercise induces increased phosphorylation of Akt(ser473), and eNOS(ser1177) as well as eNOS activity, suggesting that this pathway may contribute to NO-production and subsequent dilation of vessels perfusing skeletal muscle (140). In rat and mouse conduit vessels basal p-eNOS(ser1177) is attenuated with age (33, 104-106). In old mice, wheel running augments basal phosphorylation at this site (33). Recently, LeBlanc et al. (74) have reported that impairment of flow-induced dilation in coronary arterioles with age is related to alterations of the PI3K/Akt pathway. In SFA, we have reported no change in basal p-eNOS(ser1177) with age or exercise training status (123); however, whether there are age-related alterations in the ability of the PI3K/Akt pathway to phosphorylate eNOS in response to vasodilatory stimuli is unknown. Therefore, the purpose of this study is to test the hypothesis that impaired endothelium-dependent dilation in

senescent SFA is due to an age-related impairment in PI3K/Akt dependent phosphorylation of eNOS on serine residue 1177.

4.2 Methods

4.2.1 Animals

The methods used in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee. Male Fischer 344 rats [4 mo (n = 25) and 24 mo (n = 23)] were obtained from the National Institute of Aging (NIA) and housed at the College of Veterinary Medicine's Comparative Medicine Program Facility. Rats were housed under a 12:12-h light-dark cycle, food and water were provided *ad libitum*. The rats were examined daily by Comparative Medicine Program veterinarians. Fischer 344 rats were chosen, in part, because of the absence of atherosclerosis or hypertension with age (67).

4.2.2 Isolation of feed arteries

The protocol for SFA isolation has been described previously in detail (132-135). Briefly, rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg body wt. ip). The soleus/gastrocnemius muscle complex was dissected out and placed in a MOPS buffered physiological saline solution (PSS), containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA and 25.0 MOPS (pH 7.4). SFA were then transferred to a Lucite chamber containing MOPS-PSS for cannulation.

4.2.3 Determination of vasodilator responses

Preparation of arteries. Both ends of the SFA were cannulated with resistance-matched glass micropipettes and secured with a single strand of

surgical thread. The pipettes were attached to separate reservoirs filled with MOPS-PSS supplemented with albumin (1 g/100 ml). The height of each reservoir was adjusted to set intraluminal pressure in each feed artery to 60 cmH_2O for 20 min. The SFA were checked for leaks by verification that the intraluminal diameter was maintained after closing the pressure reservoirs. After 20 min, intraluminal pressure was raised to 90 cmH_2O and the SFA were allowed to equilibrate for an additional 40 min at 37^oC. At the end of the equilibration period, arteries that did not develop at least 25% spontaneous tone were constricted with phenylephrine. All experimental protocols were conducted at an intraluminal pressure of 90 cmH_2O, the approximate in vivo SFA pressure (129).

Assessment of vasodilation. Endothelium-dependent, flow-induced dilation was assessed by establishing intraluminal flow in the SFA by raising and lowering the heights of the pressure reservoirs in equal but opposite directions while maintaining constant pressure at the midpoint of the artery (66). Vasodilator responses to flow were assessed at pressure gradients of 0, 2, 4, 6, 8, 10, 15, 20, 30 and 40 cmH_2O, corresponding to flow rates of 0-62 µl/min. Each flow rate was maintained for 5 min to facilitate SFA reaching a steady diameter. Endothelium-dependent, acetylcholine (ACh)-induced dilation was assessed in SFA by adding cumulative, increasing, whole log concentrations of ACh over the range of 10^{-9}-10^{-4} M. Endothelium-independent dilation was assessed in SFA by adding cumulative, increasing, whole log concentrations of sodium nitroprusside (SNP) over the range of 10^{-9}-10^{-4} M. After the SNP concentration reponse curve

SFA were incubated in Ca^{2+} free PSS for 30 minutes to assess maximal passive diameter.

4.2.4 Role of PI3K

In order to determine the role of PI3K, endothelium-dependent and independent dilations were assessed in the presence and absence of a PI3K inhibitor LY-294002 (25μM). LY-294002 was added to the vessel bath at least 30 minutes before the assessment of dilations and remained in the bath for the duration of the experiment.

4.2.5 Role of Akt

In a separate series of experiments the role of Akt in endothelium-dependent and –independent dilation was assessed by performing concentration-response curves in the presence and absence of an Akt inhibitor 1L6-Hydroxymethyl-chiro-inositol-2-(R)-2-O-methyl-3-O-octadecyl-*sn*-glycerocarbonate, 5μM (Akt inhibitor). The Akt inhibitor was added to the vessel bath at least 30 minutes before the assessment of dilations and remained in the bath for the duration of the experiment. In this series of experiments, after the assessment of flow-, ACh- or SNP-induced dilation, the SFA were immediately removed from the pipettes and snap frozen on dry ice for subsequent immunoblot analysis (as described below). Because these vessels were not incubated with Ca^{2+} free PSS, true maximal diameter was not determined. Maximal diameter was estimated from the initial diameter of the vessel immediately after cannulation which, in our experience, is similar to the true maximal diameter.

4.2.6 Akt and eNOS phosphorylation

To determine whether basal and agonist-stimulated p-Akt(ser473) and p-eNOS(ser1177) were altered with age, SFA not used for dilator response studies were isolated and placed in 200µl of PSS in a microcentrifuge tube and allowed to equilibrate for 1 hour at 37^{o}C in the absence and presence of the PI3K inhibitor LY-294002 (25 µM). After the equilibration period, a group of SFA were treated with 10^{-5} M ACh for 1 min. After this treatment, the PSS was removed and 20 µl of Laemmli buffer was added to the tube and the vessels were snap frozen in the Laemmli buffer. Phosphorylation of Akt(ser473) and eNOS(ser1177) as well as total Akt, and eNOS protein contents were assessed in single SFA using immunbloting techniques described previously described in detail (58). p-Akt(ser473) was assessed using a monoclonal antibody (1:1,000, Cell Signaling catalog no. 4058), p-eNOS(ser1177) was assessed using a monoclonal antibody (1:250, BD Biosciences catalog no. 612393), total Akt protein content was assessed using a polyclonal antibody (1:1,000, Cell Signaling catalog no. 9272) and total eNOS protein content was assessed using a monoclonal antibody (1:1,250, BD Biosciences catalog no. 610297). Immunoblots were evaluated by enhanced chemiluminescence (ECL, Amersham) and densitometry by using a LAS-4000 Luminescent Image Analyzer and Multi-Gauge Image Analysis Software (FUJIFILM Medical Systems). Protein phosphorylation data were expressed as a ratio of phosphorylated-to-total protein where the blot was first probed for the phosphorylated protein then stripped (Restore Western Blot Stripping Buffer, Thermo) and probed for the total protein. Total protein data were

expressed relative to GAPDH to control for small differences in protein loading. GAPDH protein content was assessed with a monoclonal antibody (1:10,000, Millipore catalog no. AB374).

4.2.7 Drugs

LY-294002 was obtained from Sigma-Aldrich and Akt Inhibitor was obtained from Calbiochem. Both drugs were dissolved in dimethyl sulfoxide (DMSO). The concentration of DMSO in the vessel bath did not exceed 0.25%. Pilot studies (n = 9) revealed that this concentration did not alter dilator responses.

4.2.8 Statistical analysis

All data are presented as means ± SEM. Between group differences in body mass, maximal diameters, % spontaneous tone and relative phosphorylation/ total protein content were assessed by using student's *t*-test or one-way ANOVA where appropriate. Vasodilator response data were assessed as percent possible dilation calculated as $[(D_{concentration} - D_B)/(D_P - D_B)] \times 100$ where $D_{concentration}$ is the measured diameter for a give concentration/flow rate, D_B is the baseline diameter before the concentration response curve and D_P is maximal passive diameter. One-way ANOVA with repeated measures on one factor (concentration/flow rate) was used to determine differences in dilator responses. Statistical significance was set a $P \leq 0.05$ probability level.

4.3 Results

4.3.1 Characteristics of rats and SFA

Body weight, maximal vessel diameter and myogenic tone values for the studies with LY-294002 and the Akt inhibitor are shown in Tables 4.1 & 4.2, respectively. Body weights were significantly greater in old rats compared to young (Tables 4.1 & 4.2). Spontaneous myogenic tone was blunted in SFA from old rats in the presence of the Akt inhibitor before the flow-induced dilation experiments compared to SFA from young rats (Table 4.2). A total of 64 arteries were used in functional studies; 10 were preconstricted with phenylephrine, removing these vessels did not alter statistical analysis so they were included in all final analyses.

4.3.2 Effect of age on endothelium-dependent and –independent dilations.

Both flow- (Fig. 4.1A) and ACh-induced (Fig. 4.1B) vasodilations were attenuated with age. SNP-induced dilation was not altered with age, PI3K inhibition or Akt inhibition (data not shown).

4.3.3 Roles of PI3K and Akt in endothelium-dependent dilation.

In the presence of the PI3K inhibitor, LY-294002, both flow- and ACh-induced dilations were blunted in SFA from young rats (Fig. 4.2A & C). In SFA from old rats, LY-294002 did not alter flow-induced dilation (Fig. 4.2B), whereas ACh-induced dilation was blunted (Fig. 4.2D). The presence of LY-294002 eliminated the age difference in flow-induced dilation, whereas the difference in ACh-induced dilation remained. Akt inhibition did not alter flow- or ACh-induced dilation in SFA regardless of age (Fig. 3).

4.3.4 Immunoblotting

Phosphorylation of eNOS(ser1177) (Fig. 4.4A) and Akt(ser473) (Fig. 4.4B) were not altered with age, ACh stimulation or treatment with LY-294002. Total eNOS (Fig. 4.4C) and Akt (Fig. 4.4D) protein contents were not altered with age or treatment.

Table 4.1 Animal and vessel characteristics LY-294002 studies.

	Young (n = 11)	Old Rats (n = 10)
Body weight, g	359 ± 9	418 ± 5*
Maximal Diameter, μm	184 ± 6	186 ± 10
Spontaneous tone, %		
Pre-Flow		
Control	44 ± 5	53 ± 6
LY-294002	40 ± 2	43 ± 6
Pre-Acetylcholine		
Control	48 ± 4	41 ± 4
LY-294002	36 ± 2	45 ± 7

Values are means ± SEM. *significantly different from young, $p \leq 0.05$.

Table 4.2. Animal and vessel characteristics Akt Inhibitor studies.

	Young (n = 14)	Old Rats (n = 13)
Body weight, g	375 ± 7	440 ± 16*
Maximal Diameter Flow, μm	162 ± 6	176 ± 6
Maximal Diameter ACh, μm	164 ± 6	172 ± 6
Spontaneous tone pre-Flow, %		
Control	47 ± 4	45 ± 4
Akt Inhibitor	51 ± 4	38 ± 3*
Spontaneous tone Pre-ACh, %		
Control	44 ± 3	43 ± 4
Akt Inhibitor	44 ± 3	42 ± 2

Values are means ± SEM. *significantly different from young, $p \leq 0.05$.

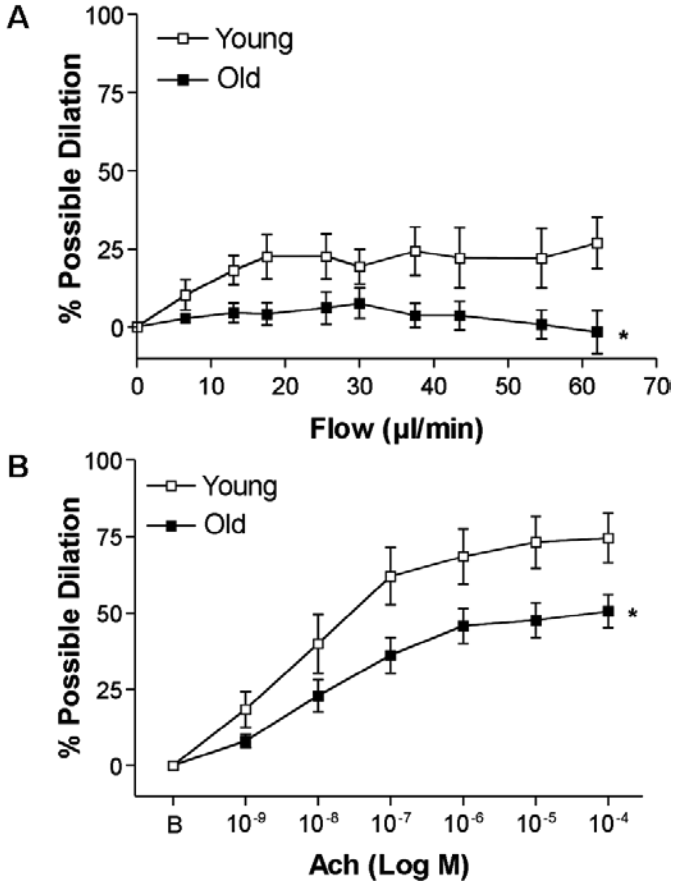
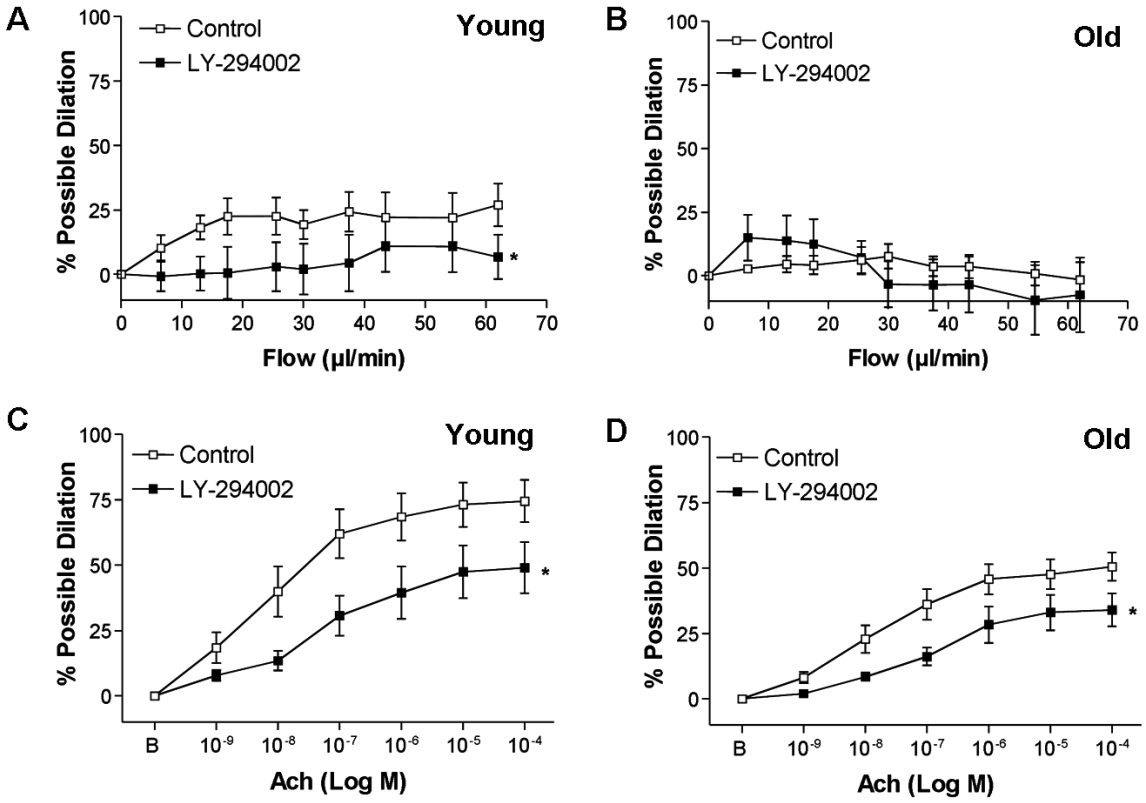


Figure 4.1. Flow-induced (A) and ACh-induced (B) dilation in soleus muscle feed arteries (SFA). B, baseline diameter before the first concentration of ACh. Values are means ± SEM. n sizes: young flow (n = 11), old flow (n = 9), young ACh (n = 8), old ACh (n = 9). *concentration-response curve significantly different from young, $p \leq 0.05$.



ure 4.2. Flow-induced (A & B) dilation in soleus muscle feed arteries (SFA) in the absence or presence of PI3K inhibitor LY-204002 (25µM). n sizes: young control (n = 11), young + LY-294002 (n = 10), old control (n = 9), old + LY-294002 (n = 7). ACh-induced (C & D) dilation in SFA in the absence or presence of LY-294002. n sizes: young control (n = 8), young + LY-294002 (n = 9), old control (n = 9), old + LY-294002 (n = 7). B, baseline diameter before the first concentration of ACh. Values are means ± SEM. *concentration-response curve significantly different from control, $p \leq 0.05$.

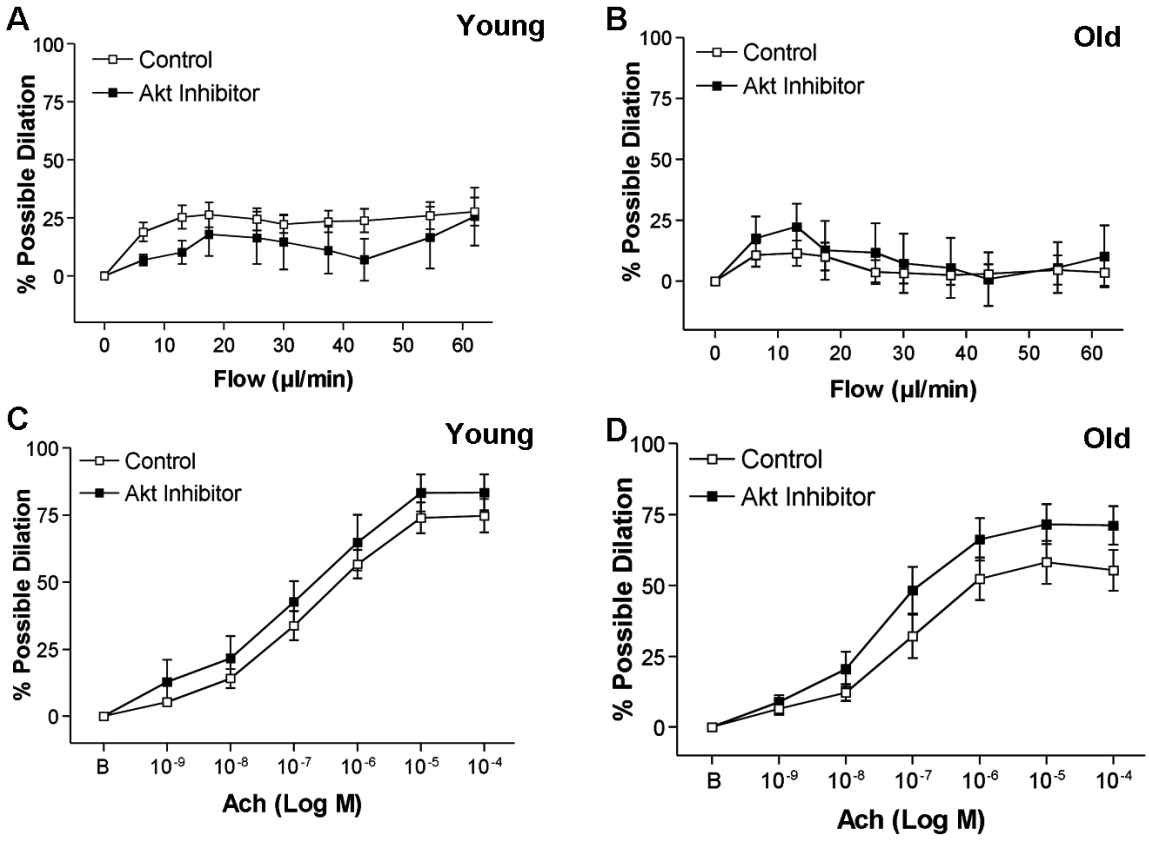


Figure 4.3. Flow-induced (A & B) dilation in soleus muscle feed arteries (SFA) in the absence or presence of Akt Inhibitor (5µM). n sizes: young control (n = 10), young + Akt Inhibitor (n = 10), old control (n = 13), old + Akt Inhibitor (n = 10). ACh-induced (C & D) dilation in SFA in the absence or presence of Akt Inhibitor. n sizes: young control (n = 14), young + Akt Inhibitor (n = 10), old control (n = 13), old + Akt Inhibitor (n = 10). B, baseline diameter before the first concentration of ACh. Values are means ± SEM.

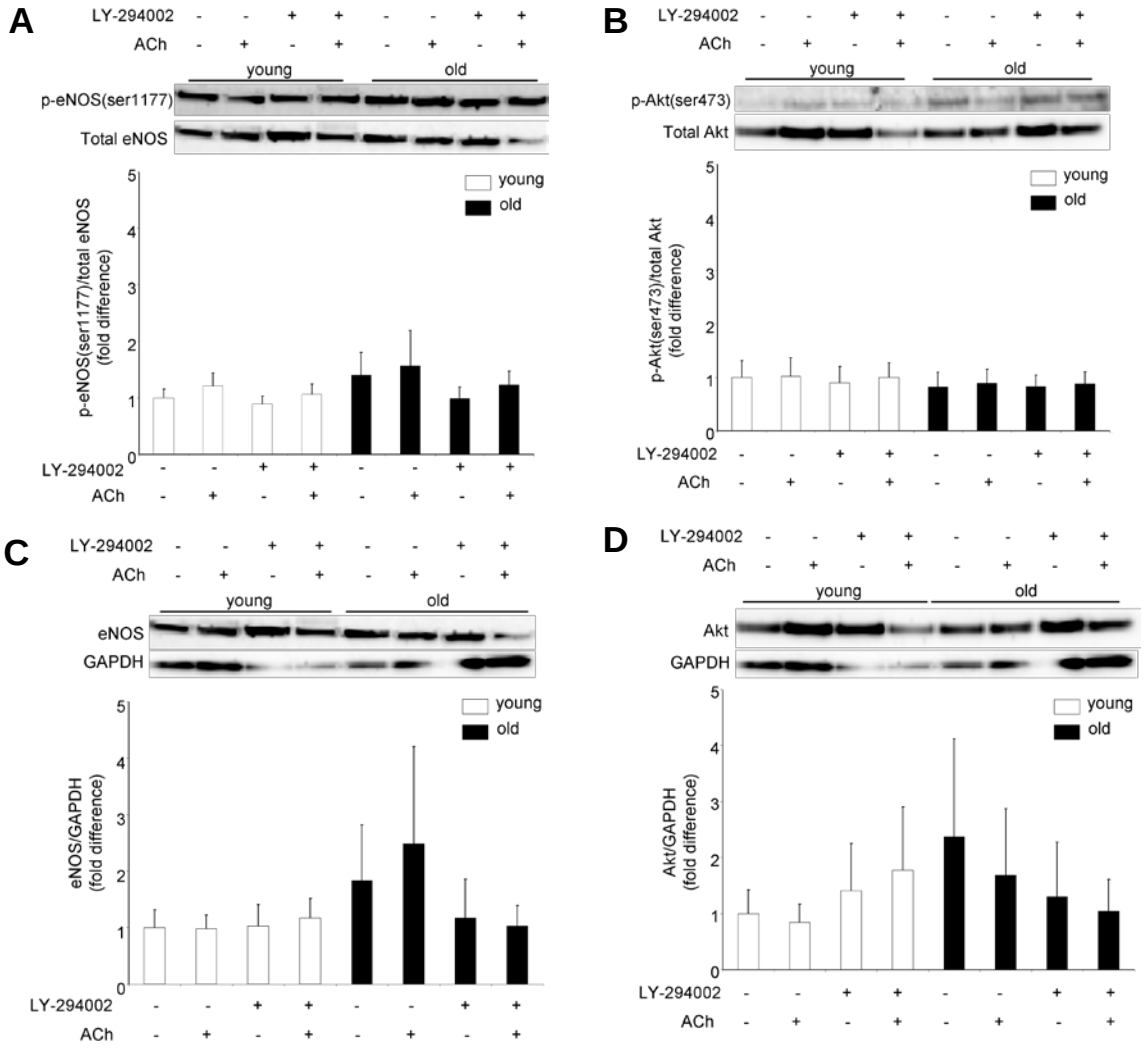


Figure 4.4. Comparison of p-eNOS(ser1177) (n = 9 rats/group) (A); p-Akt(ser473) (n = 6 rats/group) (B); total eNOS (n = 9 rats/group) (C); total Akt (n = 6 rats/group) (D) in SFA from young and old rats. Sample blots shown represent SFA from young and old animals treated with or without ACh (10^{-5} M) and with or without LY-294002 (25μM). Values are means ± SEM.

4.4 Discussion

The purpose of this study was to test the hypothesis that impaired endothelium-dependent dilation in senescent SFA is due to an age-related impairment in PI3K/Akt-dependent phosphorylation of eNOS on serine residue 1177. The major new findings of this study are as follows: 1) Inhibition of PI3K blunts flow- and ACh-induced dilation in SFA from young rats. 2) Inhibition of PI3K blunts ACh-induced dilation in SFA from old rats. 3) Inhibition of Akt did not alter endothelium-dependent dilation regardless of age. 4) Treatment of SFA with ACh in the presence or absence of a PI3K inhibitor did not alter p-Akt(ser473) or p-eNOS(ser1177).

Aging has been shown to impair endothelium-dependent dilation in a variety of animal models and vessels (3, 6, 11, 16, 17, 33, 81, 116, 126, 132). We chose SFA for use this study because it is a primary control point for regulating total soleus muscle blood flow at rest and during exercise (129). Exercise hyperemia is blunted with age in oxidative muscles like the soleus (83) and this may be, in part, explained by impaired dilation of the vessels perfusing skeletal muscle. We have previously shown that aging blunts endothelium-dependent dilation in the SFA (123, 132-135). In the present study, both flow- and ACh-mediated dilations are impaired in accordance with our previous findings. Also in accordance with previous studies, SNP-induced (endothelium-independent) dilation was not altered with age.

In previous investigations from our laboratory we found that the age-related deficit in endothelium-dependent dilation is primarily mediated by

attenuated NO bioavailability (123, 133-135). Scavenging of superoxide with exogenous SOD improved endothelium-dependent dilation in senescent SFA (123) suggesting that with age, NO bioavailability is blunted by enhanced superoxide-mediated NO degradation; however, whether mechanisms influencing the ability of the endothelium to produce NO are altered with age in the SFA is unknown. This led us to hypothesize that impaired endothelium-dependent dilation in senescent SFA is due to an impaired potential for agonist stimulated p-eNOSser1177.

The PI3K/Akt pathway is important in contributing to the activation of eNOS and NO production (22, 38). In the present study, we found that inhibition of PI3K resulted in blunted dilation in response to flow and ACh in SFA from young rats, consistent with the concept that PI3K plays a role in endothelium-dependent dilation. In senescent SFA, inhibition of PI3K blunted ACh-mediated dilation but not flow-mediated dilation. The observation that PI3K inhibition blunted ACh-induced dilation in SFA from both young and old rats suggests that the role of PI3K is preserved with age. Similarly, in coronary arterioles from both young and old rats, PI3K inhibition blunted vascular endothelial growth factor (VEGF)-induced dilation (74). Together these results suggest that PI3K signaling is preserved with age.

As PI3K has been shown to activate Akt and Akt to phosphorylate and activate eNOS (22, 38), we sought to determine if impaired Akt signaling contributes to age-related endothelial dysfunction. In the present study, inhibition of Akt did not alter flow- or ACh-induced dilation regardless of age. Kobayashi et

al. (63) recently reported that Akt inhibition did not alter ACh-induced relaxation of rat aortic rings, but did blunt insulin-induced relaxation. These results support the concept that the signaling pathways involved in endothelium-dependent dilation are agonist-specific. Additionally, it has been reported that PI3K can activate PKA in addition to Akt and that PKA can also phosphorylate and activate eNOS (7, 8). This may explain why PI3K, but not Akt inhibition blunted endothelium-dependent dilation in SFA in the present study.

We have previously reported that neither aging nor exercise training altered basal p-eNOS(ser1177) in rat SFA (123). This is in contrast to observations in rat conduit vessels where p-eNOS(ser1177) was attenuated with age (104-106). In the human brachial artery p-eNOS(ser1177) was greater in old subjects compared to young (27). In rat coronary arterioles stimulation with either flow or VEGF increased p-eNOS(ser1177) to a similar extent regardless of age despite age-related impairments in dilation in response to these stimuli (74). In this same study VEGF-induced p-Akt(ser473) was blunted in vessels from old rats compared to young (74). In rat mesenteric arteries flow-induced p-eNOS(ser1777) was blunted with age (113). ACh has been shown to induce increases in p-eNOS(ser1177) and p-Akt(ser473) in rat aorta (139). Therefore, in the present study, we sought to determine if the ability of ACh to stimulate p-Akt(ser473) and p-eNOS(ser1177) in rat SFA was altered with age. Additionally, we used the PI3K inhibitor LY-294002 to determine if this response was PI3K-dependent. Results revealed that age did not alter p-eNOS(ser1177) in untreated vessels, in accord with our previous study (123). Treatment with ACh did not

significantly alter p-Akt(ser473) or p-eNOS(ser1177) regardless of age, or PI3K inhibition. Interestingly, PI3K inhibition did blunt ACh-induced dilation in SFA from both young and old rats, suggesting that PI3K may mediate alternative pathways of vasodilator production. In addition to p-eNOS(ser1177), PI3K can act upstream of PKA to facilitate p-eNOS(ser633), another activation site (7). Thus, it is possible that PI3K inhibition can blunt p-eNOS(ser633) and as result, NO production, explaining the blunting of endothelium dependent-dilation but not p-eNOS(ser1177) observed in the presence of LY-294002. The differences in our study compared to others may be reflective of differing vessel, stimuli and downstream PI3K targets. Overall, the finding that p-eNOS(ser1177) and p-Akt(ser473) were not altered with age under either basal or ACh-stimulated SFA, suggests that the PI3K/Akt pathway is preserved with age. This is consistent with the observation that inhibition of PI3K blunted ACh-induced dilation to a similar extent.

In the present study we also found that total eNOS protein content was not altered with age. Aging appears to have variable effects on eNOS protein content depending on species and vessel studied. In SFA, we have reported both decreased and unchanged eNOS protein content with age (123, 132, 135). Despite varying age-related changes in eNOS protein content, we have consistently observed an age-related decline in NO-mediated endothelium-dependent dilation. These observations suggest eNOS protein content alone does not mediate age-related endothelial dysfunction. Total Akt protein content

was also not altered with age in the present study. This is consistent with observations in rat aorta and coronary arterioles (74, 104-106).

In summary, we tested the hypothesis that impaired endothelium-dependent dilation in senescent SFA is due to an age-related impairment in PI3K/Akt dependent phosphorylation of eNOS on serine residue 1177. The findings of the present study suggest that PI3K mediates flow- and ACh-induced dilation in rat SFA. Since inhibition of PI3K blunts ACh-induced dilation to a similar extent in SFA from young and old rats, and p-eNOS(ser1177) is preserved with age, these data suggest that the PI3K/Akt/eNOS pathway is preserved with age and is not a mechanism accounting for the age-related decline in NO-mediated endothelium-dependent dilation.

CHAPTER V

SUMMARY AND CONCLUSION

5.1 Summary

The overall purpose of this dissertation was to determine the mechanisms that mediate age-related endothelial dysfunction in rat SFA and to determine whether exercise training ameliorates this impairment in endothelial function.

In the first investigation we tested the hypothesis that exercise training reverses age-related decrements in endothelium-dependent dilation in SFA and that improved endothelium-dependent dilation is the result of increased NO bioavailability due to increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content. The major findings of the study were as follows: 1) Exercise training improved ACh-induced dilation in old SFA such that vasodilator responses in old Ex SFA were similar to young Sed and young Ex SFA. 2) ecSOD protein content was increased by training in old SFA whereas the SFA protein content of eNOS, p-eNOS(ser1177), and SOD-1 were not altered by training. 3) Exogenous SOD, and SOD + CAT, restored ACh-induced dilation in old SFA in an NO-dependent manner similar to the effects of exercise training. These findings suggest that exercise training reverses the detrimental effects of aging on endothelial function in skeletal muscle feed arteries by enhancing the capacity to scavenge superoxide, increasing the bioavailability of NO and are summarized in the scheme in Figure 5.1.

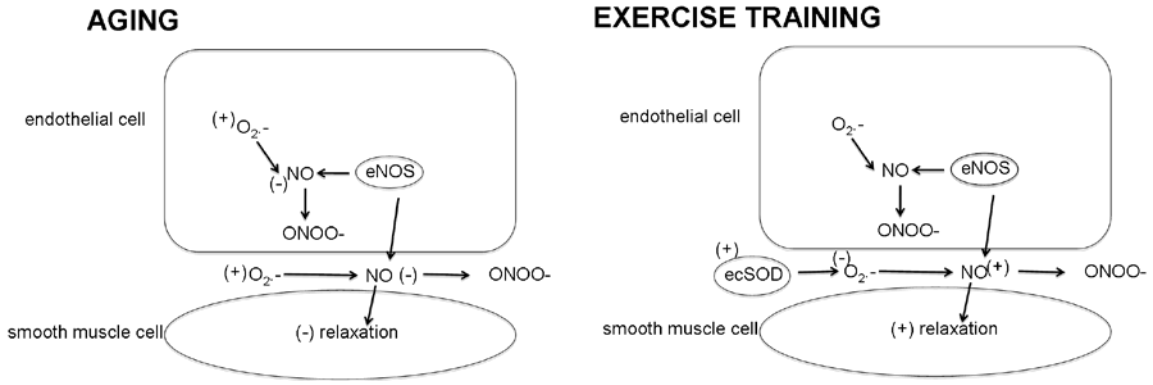


Figure 5.1 Proposed alterations in endothelial phenotype with aging and exercise training. With age greater vascular $O_2^{\cdot -}$ concentrations react with NO to reduce NO bioavailability. Exercise training increases ecSOD protein content, increasing the capacity to scavenge extracellular $O_2^{\cdot -}$ and improving NO bioavailability.

Second, we tested the hypothesis that age-related endothelial dysfunction in rat SFA is mediated in part, by NAD(P)H oxidase-derived ROS. The major findings of the study were as follows: 1) $O_2^{\cdot -}$ scavenging and inhibition of NAD(P)H oxidase improved endothelium-dependent dilation in SFA from old rats. 2) Inhibition of NAD(P)H oxidase attenuated ACh-induced dilation in SFA from young rats. 3) Scavenging of H_2O_2 improved endothelium-dependent dilations in SFA from old rats. 4) Scavenging of H_2O_2 attenuated ACh-induced dilation in SFA from young rats. 5) NAD(P)H oxidase subunit, gp91phox protein content was greater in SFA from old compared to young rats. 6) MnSOD protein content and SOD enzyme activity were greater in SFA from old compared to young rats. Collectively, these results suggest that ROS are detrimental and contribute to impaired endothelium-dependent dilation in old SFA; whereas, ROS appear to play a beneficial role, contributing ACh-mediated dilation in SFA from young rats. The potential alterations in ROS influence on vascular function are summarized in Figure 5.2.

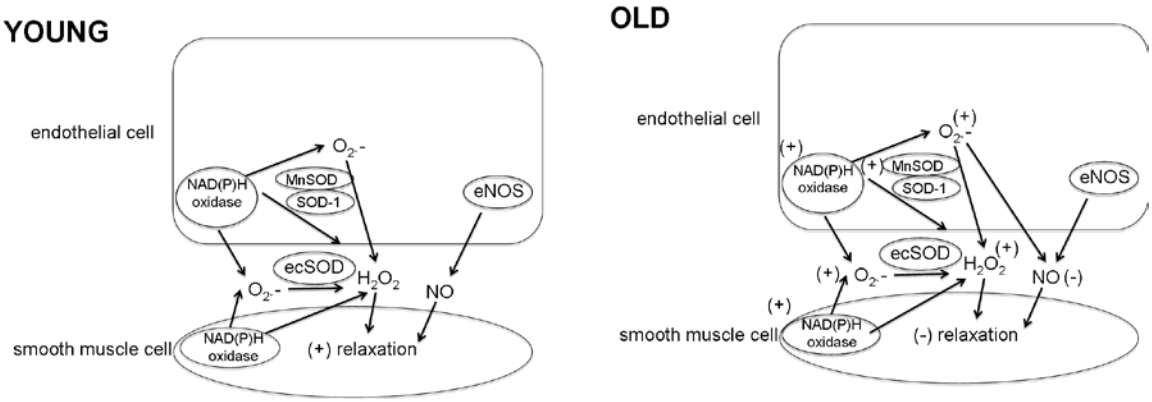


Figure 5.2. Proposed model of ROS regulation with age in the SFA. With age increased NAD(P)H oxidase activity results in greater vascular $O_2^{\cdot -}$ and H_2O_2 production. $O_2^{\cdot -}$ reacts with NO, reducing NO bioavailability. Aging also results in increased MnSOD protein content and SOD activity increasing $O_2^{\cdot -}$ scavenging and H_2O_2 concentrations. With age the role of H_2O_2 appears to shift, mediating ACh-induced dilation in young SFA but blunting dilation in old SFA. This may be a result of altered concentrations of H_2O_2 or altered downstream effects of H_2O_2.

Lastly, we tested the hypothesis that impaired endothelium-dependent dilation in senescent SFA is due an age-related impairment in PI3K/Akt dependent phosphorylation of eNOS on serine residue 1177. The major new findings from the study were as follows: 1) Inhibition of PI3K blunts flow- and ACh-induced dilation in SFA from young rats. 2) Inhibition of PI3K blunts ACh-induced dilation in SFA from old rats. 3) Inhibition of Akt did not alter endothelium-dependent dilation regardless of age. 4) Treatment of SFA with ACh in the presence or absence of a PI3K inhibitor did not alter p-Akt(ser473) or p-eNOS(ser1177). The findings of this study suggest that PI3K mediates flow- and ACh-induced dilation in rat SFA. Since inhibition of PI3K blunted ACh-induced dilation to a similar extent in SFA from young and old rats and p-eNOS(ser1177) was persevered with age, these data suggest that the PI3K/Akt/eNOS pathway is preserved with age. Importantly, PI3K inhibition blunted endothelium-dependent dilation but not alter p-eNOS(ser1177). It is possible that PI3K mediates other pathways of eNOS activation, specifically p-eNOS(ser633) (7, 8). In addition, PKA may serve as an alternative pathway to mediate phosphorylation/activation of eNOS (7, 8, 25). Whether these pathways are altered with age in SFA is unknown. This is an important topic for further study and a potential scheme of regulation of eNOS phosphorylation is presented in Figure 5.3.

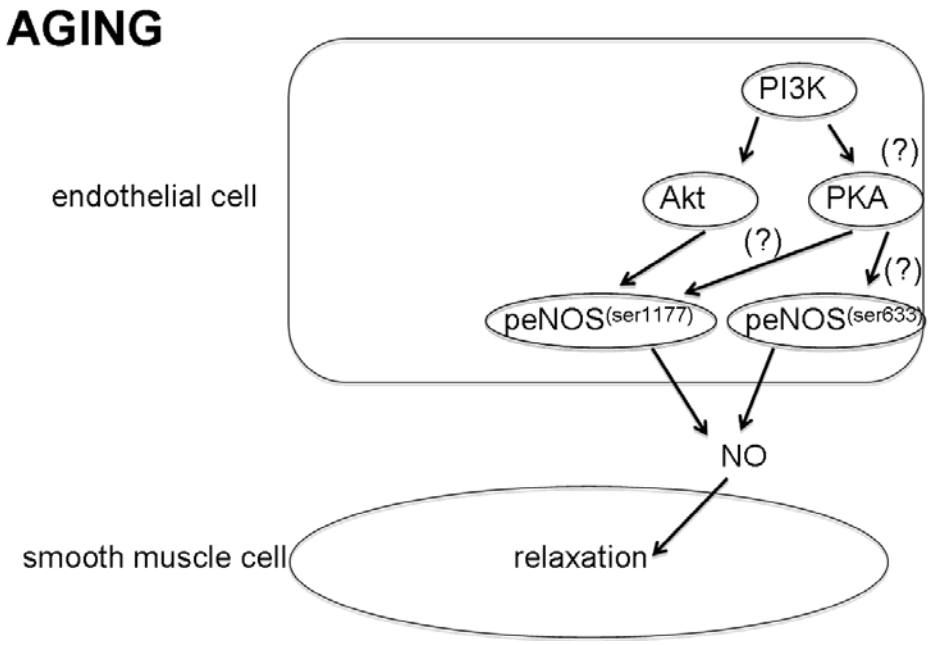


Figure 5.3 Potential alterations in PI3K-mediated endothelial cell signaling. Data from the present study suggest that the PI3K/Akt/p-eNOS(ser1177) pathway is preserved with age; however whether there are age-related alterations in PKA-dependent phosphorylation of eNOS on ser633 or 1177 is unknown.

In accordance with our hypotheses, we found that scavenging of $O_2^{\cdot -}$, scavenging of H_2O_2, or inhibition of NAD(P)H oxidase-derived ROS improved endothelium-dependent dilation in senescent SFA. In addition, we found that exercise training increased ecSOD protein content in SFA. Together these data strongly suggest that oxidative stress plays a role in age-related endothelial dysfunction and that exercise training results in an improved antioxidant capacity. Future studies are required to elucidate the source(s) of vascular ROS in SFA, the precise roles of $O_2^{\cdot -}$ and H_2O_2 in age-related endothelial dysfunction, and whether ROS contribute to eNOS uncoupling in the SFA.

Interestingly, we also found that scavenging of H_2O_2 and inhibition of NAD(P)H oxidase blunted ACh-induced dilation in SFA from young animals. These observations are in accordance with the observation that H_2O_2 can act as an EDHF and phosphorylate eNOS (80, 121). H_2O_2 also appears to play a role in dilation in soleus 1A arterioles (103). The observation that ROS contribute to dilation in SFA from young rats but impair dilation in SFA from old rats suggest that aging results in an alteration of the role of vascular ROS.

Contrary to our hypothesis, we found that inhibition of PI3K blunted endothelium-dependent dilation to a similar extent in SFA from young and old rats. In addition, p-eNOS(ser1177) and p-AKt(ser473) appear to be unaltered with age. These results suggest that the PI3K/Akt/eNOS pathway is preserved with age. Interestingly, inhibition of PI3K blunted endothelium-dependent dilation but not did not alter p-eNOS(ser1177) or p-Akt(ser473). This suggests that PI3K

may mediate other mechanisms of eNOS activation. PI3K has been shown to activate PKA which mediates phosphorylation of eNOS at ser(1177) and ser(633) (7, 8, 25). In addition PKA may mediate phosphorylation at those sites independently of PI3K (see Figure 5.3) (7). It is possible that other mechanisms of eNOS activation and NO production beyond the scope of these studies are altered with age. Importantly, eNOS phosphorylation may be blunted on other activation sites or phosphorylation may be enhanced on inhibitory sites. Also, the state of eNOS protein—protein interactions with Hsp90, CaM, Akt and Cav-1 may change with age. Lastly, eNOS activity may be well preserved with age, but it may be in an uncoupled state due to decreased BH_4 synthesis and/or increased BH_4 oxidation. This mechanism may link the observed ROS-mediated impairments in endothelial function to impairments in NO production, further exacerbating age-related endothelial dysfunction.

In conclusion, in the rat SFA ROS appear to play a role in age-related endothelial dysfunction. Exercise training appears to ameloriate this observed endothelial dysfunction partly by increasing antioxidant capacity. Finally, it appears that PI3K-dependent activation of eNOS is preserved with age. These results contribute to the understanding of how aging and exercise training influence endothelial phenotype and underscore the importance of physical activity for maintaining optimal vascular function.

5.2 Limitations

There are several limitations that must be considered when interpreting the present studies. First, the isolated artery technique used for the functional

assessment of endothelium-dependent dilation is an *in vitro* preparation. This provides the major advantage of isolating mechanisms that regulate endothelial function that may be altered with age, however, *in vivo* there are numerous factors that influence vascular tone (see section 1.3). For example, in the isolated artery technique there is no innervation of the vessels, no release of vasoactive molecules from surrounding tissue and no flow running through the vessel (with the exception of flow-induced dilation experiments).

Second, the flow rates used to assess endothelium-dependent dilation (0-62 µl/min) may be lower than those predicted *in vivo* (95 µl/min standing-225 µl/min running) (57). Thus, the flow-induced dilation experiments conducted in the present studies may be limited in their applicability to exercise hyperemia. Despite this limitation important insight can be gained from these experiments. Flow-induced dilation is a non-agonist mediated dilator response this is in contrast to ACh-mediated dilation which is mediated through the muscarinic receptor. Our results indicate both flow- and ACh-induced dilations are blunted in aged arteries suggesting that cell signaling event(s) impaired by aging are downstream of the receptor level. In addition, although the flow rates in these experiments differ from those *in vivo,* these studies provide insight into the basic mechanisms that regulate endothelial function with age.

Finally, these studies have examined numerous mechanisms that regulate NO bioavailability and ROS concentrations however, we have not directly assessed vascular NO and ROS concentrations. Direct assessment of the concentration of these molecules would add considerable clarity to the

mechanisms regulating endothelial function with aging and exercise training. As these molecules have short half-lives and the amount of SFA tissue is limited these experiments have proved to be technically challenging.

5.3 Clinical relevance

The results from these studies are of particular importance as they provide mechanistic insight into findings observed in human subjects. In several studies infusion of exogenous antioxidants improve endothelium-dependent dilation old subjects (30, 35, 62, 115). Recently, increased p47phox protein content and nitrotyrosine expression has been reported in the aged human vasculature (26). These results suggest that similar to the present results ROS contribute to age-related endothelial dysfunction. Importantly, infusion of exogenous antioxidants results in greater forearm blood flow during handgrip exercise compared to exercise without antioxidants (62). This suggests that exogenous antioxidants improve endothelial function in the skeletal muscle vasculature, complimenting the observations in the present studies. Exercise training improves endothelial function in old subjects (20, 30) and physically active older subjects exhibit greater endothelium-dependent dilation compared to their sedentary counterparts (35, 114). In elderly subjects, exercise-induced improvements in endothelial function are not further improved by exogenous antioxidants (30, 35). This suggests that exercise improves vascular antioxidant capacity, in accord with the finding that exercise increase ecSOD protein content in SFA. Lastly, in the present studies, mechanisms that regulate eNOS activation do not appear to be altered with age. Studies in human subjects examining these mechanisms are

limited, however one study has shown an age-related increase in p-eNOS(ser1177) in endothelial cells from the brachial artery (27).

Interestingly, in young subjects exogenous antioxidants resulted in blunted endothelium-dependent dilation during handgrip exercise (30). This report compliments the observation in the present studies that inhibition of NAD(P)H oxidase or scavenging of H_2O_2 blunts dilation in SFA from young rats and suggests that in both rats and humans ROS play a role in mediating dilation of the skeletal muscle vasculature.

The present studies provide mechanistic insight into the regulation of endothelial phenotype with age and exercise training. In particular these studies: 1) Clarify the roles and sources of ROS in the vasculature. 2) Provide insight into the mechanisms of exercise-induced improvement in the aged vasculature. 3) Underscore the importance of physical activity in maintenance of vascular health. The results from these studies also provide direction for future study to further examine age-related endothelial dysfunction and identify targets for lifestyle and/or pharmacological interventions to preserve vascular health with age.

REFERENCES

1. American College of Sports Medicine Position Stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc* 30: 992-1008, 1998.

2. **Armstrong RB, Delp MD, Goljan EF, and Laughlin MH.** Distribution of blood flow in muscles of miniature swine during exercise. *J Appl Physiol* 62: 1285-1298, 1987.

3. **Barton M, Cosentino F, Brandes RP, Moreau P, Shaw S, and Luscher TF.** Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. *Hypertension* 30: 817-824, 1997.

4. **Beere PA, Russell SD, Morey MC, Kitzman DW, and Higginbotham MB.** Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. *Circulation* 100: 1085-1094, 1999.

5. **Berkowitz DE, White R, Li D, Minhas KM, Cernetich A, Kim S, Burke S, Shoukas AA, Nyhan D, Champion HC, and Hare JM.** Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* 108: 2000-2006, 2003.

6. **Blackwell KA, Sorenson JP, Richardson DM, Smith LA, Suda O, Nath K, and Katusic ZS.** Mechanisms of aging-induced impairment of endothelium-dependent relaxation: role of tetrahydrobiopterin. *Am J Physiol Heart Circ Physiol* 287: H2448-2453, 2004.

7. **Boo YC, Hwang J, Sykes M, Michell BJ, Kemp BE, Lum H, and Jo H.** Shear stress stimulates phosphorylation of eNOS at Ser(635) by a protein kinase A-dependent mechanism. *Am J Physiol Heart Circ Physiol* 283: H1819-1828, 2002.

8. **Boo YC, Sorescu G, Boyd N, Shiojima I, Walsh K, Du J, and Jo H.** Shear stress stimulates phosphorylation of endothelial nitric-oxide synthase at Ser1179 by Akt-independent mechanisms: role of protein kinase A. *J Biol Chem* 277: 3388-3396, 2002.

9. **Busse R, Pohl U, Kellner C, and Klemm U.** Endothelial cells are involved in the vasodilatory response to hypoxia. *Pflugers Arch* 397: 78-80, 1983.

10. **Cartee GD and Farrar RP.** Muscle respiratory capacity and VO2 max in identically trained young and old rats. *J Appl Physiol* 63: 257-261, 1987.

11. **Celermajer DS, Sorensen KE, Bull C, Robinson J, and Deanfield JE.** Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 24: 1468-1474, 1994.

12. **Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, and Deanfield JE.** Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 24: 471-476, 1994.

13. **Cernadas MR, Sanchez de Miguel L, Garcia-Duran M, Gonzalez-Fernandez F, Millas I, Monton M, Rodrigo J, Rico L, Fernandez P, de Frutos T, Rodriguez-Feo JA, Guerra J, Caramelo C, Casado S, and Lopez F.** Expression of constitutive and inducible nitric oxide synthases in the vascular wall of young and aging rats. *Circ Res* 83: 279-286, 1998.

14. **Chauhan A, More RS, Mullins PA, Taylor G, Petch MC, and Schofield PM.** Aging associated endothelial dysfunction in humans is reversed by L-arginine. *J Am Coll Cardiol* 28: 1796-1804, 1996.

15. **Cosentino F and Katusic ZS.** Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. *Circulation* 91: 139-144, 1995.

16. **Csiszar A, Ungvari Z, Edwards JG, Kaminski P, Wolin MS, Koller A, and Kaley G.** Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ Res* 90: 1159-1166, 2002.

17. **Delp MD, Behnke BJ, Spier SA, Wu G, and Muller-Delp JM.** Ageing diminishes endothelium-dependent vasodilatation and tetrahydrobiopterin content in rat skeletal muscle arterioles. *J Physiol* 586: 1161-1168, 2008.

18. **Delp MD, McAllister RM, and Laughlin MH.** Exercise training alters aortic vascular reactivity in hypothyroid rats. *Am J Physiol* 268: H1428-1435, 1995.

19. **DeSouza CA, Clevenger CM, Greiner JJ, Smith DT, Hoetzer GL, Shapiro LF, and Stauffer BL.** Evidence for agonist-specific endothelial vasodilator dysfunction with ageing in healthy humans. *J Physiol* 542: 255-262, 2002.

20. **DeSouza CA, Shapiro LF, Clevenger CM, Dinenno FA, Monahan KD, Tanaka H, and Seals DR.** Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 102: 1351-1357, 2000.

21. **Dikalov SI, Dikalova AE, Bikineyeva AT, Schmidt HH, Harrison DG, and Griendling KK.** Distinct roles of Nox1 and Nox4 in basal and angiotensin II-stimulated superoxide and hydrogen peroxide production. *Free Radic Biol Med* 45: 1340-1351, 2008.

22. **Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, and Zeiher AM.** Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601-605, 1999.

23. **Dinenno FA, Dietz NM, and Joyner MJ.** Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. *Circulation* 106: 1349-1354, 2002.

24. **Dinenno FA, Jones PP, Seals DR, and Tanaka H.** Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and declines in oxygen demand. *Circulation* 100: 164-170, 1999.

25. **Dixit M, Loot AE, Mohamed A, Fisslthaler B, Boulanger CM, Ceacareanu B, Hassid A, Busse R, and Fleming I.** Gab1, SHP2, and protein kinase A are crucial for the activation of the endothelial NO synthase by fluid shear stress. *Circ Res* 97: 1236-1244, 2005.

26. **Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE, and Seals DR.** Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. *Circ Res* 100: 1659-1666, 2007.

27. **Donato AJ, Gano LB, Eskurza I, Silver AE, Gates PE, Jablonski K, and Seals DR.** Vascular endothelial dysfunction with aging: endothelin-1 and endothelial nitric oxide synthase. *Am J Physiol Heart Circ Physiol* 297: H425-432, 2009.

28. **Donato AJ, Lesniewski LA, and Delp MD.** Ageing and exercise training alter adrenergic vasomotor responses of rat skeletal muscle arterioles. *J Physiol* 579: 115-125, 2007.

29. **Donato AJ, Lesniewski LA, and Delp MD.** The effects of aging and exercise training on endothelin-1 vasoconstrictor responses in rat skeletal muscle arterioles. *Cardiovasc Res* 66: 393-401, 2005.

30. **Donato AJ, Uberoi A, Bailey DM, Wray DW, and Richardson RS.** Exercise-induced brachial artery vasodilation: effects of antioxidants and exercise training in elderly men. *Am J Physiol Heart Circ Physiol* 298: H671-678, 2010.

31. **Donato AJ, Uberoi A, Wray DW, Nishiyama S, Lawrenson L, and Richardson RS.** Differential effects of aging on limb blood flow in humans. *Am J Physiol Heart Circ Physiol* 290: H272-278, 2006.

32. **Drinkwater BL, Horvath SM, and Wells CL.** Aerobic power of females, ages 10 to 68. *Journal of Gerntol* 30: 385-394, 1975.

33. **Durrant JR, Seals DR, Connell ML, Russell MJ, Lawson BR, Folian BJ, Donato AJ, and Lesniewski LA.** Voluntary wheel running restores endothelial function in conduit arteries of old mice: direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase. *J Physiol* 587: 3271-3285, 2009.

34. **Eskurza I, Kahn ZD, and Seals DR.** Xanthine oxidase does not contribute to impaired peripheral conduit artery endothelium-dependent dilatation with ageing. *J Physiol* 571: 661-668, 2006.

35. **Eskurza I, Monahan KD, Robinson JA, and Seals DR.** Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J Physiol* 556: 315-324, 2004.

36. **Eskurza I, Myerburgh LA, Kahn ZD, and Seals DR.** Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. *J Physiol* 568: 1057-1065, 2005.

37. **Feletou M and Vanhoutte PM.** EDHF: an update. *Clin Sci (Lond)* 117: 139-155, 2009.

38. **Fisslthaler B, Dimmeler S, Hermann C, Busse R, and Fleming I.** Phosphorylation and activation of the endothelial nitric oxide synthase by fluid shear stress. *Acta Physiol Scand* 168: 81-88, 2000.

39. **Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, and Busse R.** Phosphorylation of Thr(495) regulates Ca(2+)/calmodulin-dependent endothelial nitric oxide synthase activity. *Circ Res* 88: E68-75, 2001.

40. **Frandsenn U, Bangsbo J, Sander M, Hoffner L, Betak A, Saltin B, and Hellsten Y.** Exercise-induced hyperaemia and leg oxygen uptake are not altered during effective inhibition of nitric oxide synthase with N(G)-nitro-L-arginine methyl ester in humans. *J Physiol* 531: 257-264, 2001.

41. **Franzoni F, Ghiadoni L, Galetta F, Plantinga Y, Lubrano V, Huang Y, Salvetti G, Regoli F, Taddei S, Santoro G, and Salvetti A.** Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men. *Am J Hypertens* 18: 510-516, 2005.

42. **Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, and Harrison DG.** Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. *J Clin Invest* 105: 1631-1639, 2000.

43. **Furchgott RF and Zawadzki JV.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373-376, 1980.

44. **Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, and Sessa WC.** Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature* 392: 821-824, 1998.

45. **Goumas G, Tentolouris C, Tousoulis D, Stefanadis C, and Toutouzas P.** Therapeutic modification of the L-arginine-eNOS pathway in cardiovascular diseases. *Atherosclerosis* 154: 255-267, 2001.

46. **Govers R and Rabelink TJ.** Cellular regulation of endothelial nitric oxide synthase. *Am J Physiol Renal Physiol* 280: F193-206, 2001.

47. **Greif DM, Kou R, and Michel T.** Site-specific dephosphorylation of endothelial nitric oxide synthase by protein phosphatase 2A: evidence for crosstalk between phosphorylation sites. *Biochemistry* 41: 15845-15853, 2002.

48. **Gryglewski RJ, Palmer RM, and Moncada S.** Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. *Nature* 320: 454-456, 1986.

49. **Hammer LW and Boegehold MA.** Functional hyperemia is reduced in skeletal muscle of aged rats. *Microcirculation* 12: 517-526, 2005.

50. **Harris MB, Slack KN, Prestosa DT, and Hryvniak DJ.** Resistance training improves femoral artery endothelial dysfunction in aged rats. *Euro Journal Appl Physiol* 108: 533-540, 2010.

51. **Hink HU, Santanam N, Dikalov S, McCann L, Nguyen AD, Parthasarathy S, Harrison DG, and Fukai T.** Peroxidase properties of extracellular superoxide dismutase: role of uric acid in modulating in vivo activity. *Arterioscler Thromb Vasc Biol* 22: 1402-1408, 2002.

52. **Ignarro LJ.** Nitric oxide. A novel signal transduction mechanism for transcellular communication. *Hypertension* 16: 477-483, 1990.

53. **Ignarro LJ, Buga GM, Wood KS, Byrns RE, and Chaudhuri G.** Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A* 84: 9265-9269, 1987.

54. **Inbar O, Oren A, Scheinowitz M, Rotstein A, Dlin R, and Casaburi R.** Normal cardiopulmonary responses during incremental exercise in 20- to 70-yr-old men. *Med Sci Sports Exerc* 26: 538-546, 1994.

55. **Irion GL, Vasthare US, and Tuma RF.** Age-related change in skeletal muscle blood flow in the rat. *J Gerontol* 42: 660-665, 1987.

56. **Jacobson A, Yan C, Gao Q, Rincon-Skinner T, Rivera A, Edwards J, Huang A, Kaley G, and Sun D.** Aging enhances pressure-induced arterial superoxide formation. *Am J Physiol Heart Circ Physiol* 293: H1344-1350, 2007.

57. **Jasperse JL and Laughlin MH.** Flow-induced dilation of rat soleus feed arteries. *Am J Physiol* 273: H2423-2427, 1997.

58. **Jasperse JL and Laughlin MH.** Vasomotor responses of soleus feed arteries from sedentary and exercise-trained rats. *J Appl Physiol* 86: 441-449, 1999.

59. **Jeney V, Itoh S, Wendt M, Gradek Q, Ushio-Fukai M, Harrison DG, and Fukai T.** Role of antioxidant-1 in extracellular superoxide dismutase function and expression. *Circ Res* 96: 723-729, 2005.

60. **Joyner MJ and Wilkins BW.** Exercise hyperaemia: is anything obligatory but the hyperaemia? *J Physiol* 583: 855-860, 2007.

61. **Kang LS, Reyes RA, and Muller-Delp JM.** Aging impairs flow-induced dilation in coronary arterioles: role of NO and H(2)O(2). *Am J Physiol Heart Circ Physiol* 297: H1087-1095, 2009.

62. **Kirby BS, Voyles WF, Simpson CB, Carlson RE, Schrage WG, and Dinenno FA.** Endothelium-dependent vasodilatation and exercise hyperaemia in ageing humans: impact of acute ascorbic acid administration. *J Physiol* 587: 1989-2003, 2009.

63. **Kobayashi T, Taguchi K, Nemoto S, Nogami T, Matsumoto T, and Kamata K.** Activation of the PDK-1/Akt/eNOS pathway involved in aortic endothelial function differs between hyperinsulinemic and insulin-deficient diabetic rats. *Am J Physiol Heart Circ Physiol* 297: H1767-1775, 2009.

64. **Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, and Sone H.** Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA* 301: 2024-2035, 2009.

65. **Kuchan MJ and Frangos JA.** Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *Am J Physiol* 266: C628-636, 1994.

66. **Kuo L, Chilian WM, and Davis MJ.** Interaction of pressure- and flow-induced responses in porcine coronary resistance vessels. *Am J Physiol Heart Circ Physiol* 261: H1706-1715, 1991.

67. **Lakatta EG.** Cardiovascular system. In: *Handbook of Physiology: Aging*, edited by Masoro EJ. New York: Oxford Univ. Press, 1995, p. 413-474.

68. **Lane P and Gross SS.** Disabling a C-terminal autoinhibitory control element in endothelial nitric-oxide synthase by phosphorylation provides a molecular explanation for activation of vascular NO synthesis by diverse physiological stimuli. *J Biol Chem* 277: 19087-19094, 2002.

69. **Lash JM and Bohlen HG.** Functional adaptations of rat skeletal muscle arterioles to aerobic exercise training. *J Appl Physiol* 72: 2052-2062, 1992.

70. **Laughlin MH.** Endothelium-mediated control of coronary vascular tone after chronic exercise training. *Med Sci Sports Exerc* 27: 1135-1144, 1995.

71. **Laughlin MH, Pollock JS, Amann JF, Hollis ML, Woodman CR, and Price EM.** Training induces nonuniform increases in eNOS content along the coronary arterial tree. *J Appl Physiol* 90: 501-510, 2001.

72. **Laughlin MH, Woodman CR, Schrage WG, Gute D, and Price EM.** Interval sprint training enhances endothelial function and eNOS content in some arteries that perfuse white gastrocnemius muscle. *J Appl Physiol* 96: 233-244, 2004.

73. **Lawrenson L, Poole JG, Kim J, Brown CF, Patel PM, and Richardson RS.** Vascular and metabolic response to isolated small muscle mass exercise: the effect of age. *Am J Physiol Heart Circ Physiol* 285: H1023-H1031, 2003.

74. **LeBlanc AJ, Shipley RD, Kang LS, and Muller-Delp JM.** Age impairs Flk-1 signaling and NO-mediated vasodilation in coronary arterioles. *Am J Physiol Heart Circ Physiol* 295: H2280-2288, 2008.

75. **Mazzeo RS, Brooks GA, and Horvath SM.** Effects of age on metabolic responses to endurance training in rats. *J Appl Physiol* 57: 1369-1374, 1984.

76. **McAllister RM, Jasperse JL, and Laughlin MH.** Nonuniform effects of endurance exercise training on vasodilation in rat skeletal muscle. *J Appl Physiol* 98: 753-761, 2005.

77. **McAllister RM and Laughlin MH.** Short-term exercise training alters responses of porcine femoral and brachial arteries. *J Appl Physiol* 82: 1438-1444, 1997.

78. **McCabe TJ, Fulton D, Roman LJ, and Sessa WC.** Enhanced electron flux and reduced calmodulin dissociation may explain "calcium-independent" eNOS activation by phosphorylation. *J Biol Chem* 275: 6123-6128, 2000.

79. **Michel JB, Feron O, Sacks D, and Michel T.** Reciprocal regulation of endothelial nitric-oxide synthase by Ca2+-calmodulin and caveolin. *J Biol Chem* 272: 15583-15586, 1997.

80. **Morikawa K, Shimokawa H, Matoba T, Kubota H, Akaike T, Talukder MA, Hatanaka M, Fujiki T, Maeda H, Takahashi S, and Takeshita A.** Pivotal role of Cu,Zn-superoxide dismutase in endothelium-dependent hyperpolarization. *J Clin Invest* 112: 1871-1879, 2003.

81.**Muller-Delp JM, Spier SA, Ramsey MW, and Delp MD.** Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. *Am J Physiol Heart Circ Physiol* 283: H1662-1672, 2002.

82. **Muller JM, Meyers PR, and Laughlin MH.** Vasodilator response of coronary resistance arteries of exercise-trained pigs. *Circulation* 89: 2308-2314, 1994.

83. **Musch TI, Eklund KE, Hageman KS, and Poole DC.** Altered regional blood flow responses to submaximal exercise in older rats. *J Appl Physiol* 96: 81-88, 2004.

84. **Musicki B, Kramer MF, Becker RE, and Burnett AL.** Age-related changes in phosphorylation of endothelial nitric oxide synthase in the rat penis. *J Sex Med* 2: 347-355, 2005.

85. **Newaz MA, Yousefipour Z, and Oyekan A.** Oxidative stress-associated vascular aging is xanthine oxidase-dependent but not NAD(P)H oxidase-dependent. *J Cardiovasc Pharmacol* 48: 88-94, 2006.

86. **Ogawa T, Spina RJ, Martin WH, 3rd, Kohrt WM, Schechtman KB, Holloszy JO, and Ehsani AA.** Effects of aging, sex, and physical training on cardiovascular responses to exercise. *Circulation* 86: 494-503, 1992.

87. **Ortiz PA, Hong NJ, and Garvin JL.** Luminal flow induces eNOS activation and translocation in the rat thick ascending limb. II. Role of PI3-kinase and Hsp90. *Am J Physiol Renal Physiol* 287: F281-288, 2004.

88. **Parker JL, Oltman CL, Muller JM, Meyers PR, Adams HR, and Laughlin MH.** Effects of exercise training on regulation of tone in coronary arteries and arterioles. *Med Sci Sports Exerc* 26: 1252-1261, 1994.

89. **Petersen SV, Valnickova Z, Oury TD, Crapo JD, Chr Nielsen N, and Enghild JJ.** The subunit composition of human extracellular superoxide dismutase (EC-SOD) regulates enzymatic activity. *BMC Biochem* 8: 19, 2007.

90. **Pierce GL, Lesniewski LA, Lawson BR, Beske SD, and Seals DR.** Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation* 119: 1284-1292, 2009.

91. **Proctor DN, Koch DW, Newcomer SC, Le KU, and Leuenberger UA.** Impaired leg vasodilation during dynamic exercise in healthy older women. *J Appl Physiol* 95: 1963-1970, 2003.

92. **Proctor DN, Shen PH, Dietz NM, Eickhoff TJ, Lawler LA, Ebersold EJ, Loeffler DL, and Joyner MJ.** Reduced leg blood flow during dynamic exercise in older endurance-trained men. *J Appl Physiol* 85: 68-75, 1998.

93. **Radegran G and Saltin B.** Nitric oxide in the regulation of vasomotor tone in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 276: H1951-1960, 1999.

94. **Reid MB.** Nitric oxide, reactive oxygen species, and skeletal muscle contraction. *Med Sci Sports Exerc* 33: 371-376, 2001.

95. **Remensnyder JP, Mitchell JH, and Sarnoff SJ.** Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* 11: 370-380, 1962.

96. **Rush JWE, Laughlin MH, Woodman CR, and Price EM.** SOD-1 expression in pig coronary arterioles is increased by training. *Am J Physiol* 279: H2068-H2076, 2000.

97. **Rywik TM, Blackman MR, Yataco AR, Vaitkevicius PV, Zink RC, Cottrell EH, Wright JG, Katzel LI, and Fleg JL.** Enhanced endothelial vasoreactivity in endurance-trained older men. *J Appl Physiol* 87: 2136-2142, 1999.

98. **Schrage WG, Eisenach JH, and Joyner MJ.** Ageing reduces nitric-oxide- and prostaglandin-mediated vasodilatation in exercising humans. *J Physiol* 579: 227-236, 2007.

99. **Schrage WG, Joyner MJ, and Dinenno FA.** Local inhibition of nitric oxide and prostaglandins independently reduces forearm exercise hyperaemia in humans. *J Physiol* 557: 599-611, 2004.

100. **Selemidis S, Sobey CG, Wingler K, Schmidt HH, and Drummond GR.** NADPH oxidases in the vasculature: molecular features, roles in disease and pharmacological inhibition. *Pharmacology & therapeutics* 120: 254-291, 2008.

101. **Sessa WC, Pritchard K, Seyedi N, Wang J, and Hintze TH.** Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial nitric oxide synthase gene expression. *Circ Res* 74: 349-353, 1994.

102. **Sheriff DD, Nelson CD, and Sundermann RK.** Does autonomic blockade reveal a potent contribution of nitric oxide to locomotion-induced vasodilation? *Am J Physiol Heart Circ Physiol* 279: H726-732, 2000.

103. **Sindler AL, Delp MD, Reyes R, Wu G, and Muller-Delp JM.** Effects of ageing and exercise training on eNOS uncoupling in skeletal muscle resistance arterioles. *J Physiol* 587: 3885-3897, 2009.

104. **Smith AR, Visioli F, Frei B, and Hagen TM.** Age-related changes in endothelial nitric oxide synthase phosphorylation and nitric oxide dependent vasodilation: evidence for a novel mechanism involving sphingomyelinase and ceramide-activated phosphatase 2A. *Aging Cell* 5: 391-400, 2006.

105. **Smith AR, Visioli F, and Hagen TM.** Plasma membrane-associated endothelial nitric oxide synthase and activity in aging rat aortic vascular endothelia markedly decline with age. *Arch Biochem Biophys* 454: 100-105, 2006.

106. **Soucy KG, Ryoo S, Benjo A, Lim HK, Gupta G, Sohi JS, Elser J, Aon MA, Nyhan D, Shoukas AA, and Berkowitz DE.** Impaired shear stress-induced nitric oxide production through decreased NOS phosphorylation contributes to age-related vascular stiffness. *J Appl Physiol* 101: 1751-1759, 2006.

107. **Spier SA, Delp MD, Meininger CJ, Donato AJ, Ramsey MW, and Muller-Delp JM.** Effects of ageing and exercise training on endothelium-dependent vasodilatation and structure of rat skeletal muscle arterioles. *J Physiol* 556: 947-958, 2004.

108. **Spier SA, Delp MD, Stallone JN, Dominguez JM, 2nd, and Muller-Delp JM.** Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI2 and nitric oxide. *Am J Physiol Heart Circ Physiol* 292: H3119-3127, 2007.

109. **Srere PA.** Citrate synthase. *Methods Enzymol* 13: 3-5, 1969.

110. **Sun D, Huang A, Koller A, and Kaley G.** Adaptation of flow-induced dilation of arterioles to daily exercise. *Microvasc Res* 56: 54-61, 1998.

111. **Sun D, Huang A, Koller A, and Kaley G.** Enhanced NO-mediated dilations in skeletal muscle arterioles of chronically exercised rats. *Microvasc Res* 64: 491-496, 2002.

112. **Sun D, Huang A, Koller A, and Kaley G.** Short term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol* 76: 2241-2247, 1994.

113. **Sun D, Huang A, Yan EH, Wu Z, Yan C, Kaminski PM, Oury TD, Wolin MS, and Kaley G.** Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am J Physiol Heart Circ Physiol* 286: H2249-2256, 2004.

114. **Taddei S, Galetta F, Virdis A, Ghiadoni L, Salvetti G, Franzoni F, Giusti C, and Salvetti A.** Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. *Circulation* 101: 2896-2901, 2000.

115. **Taddei S, Virdis A, Ghiadoni L, Magagna A, and Salvetti A.** Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 97: 2222-2229, 1998.

116. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, Sudano I, and Salvetti A.** Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation* 91: 1981-1987, 1995.

117. **Tanabe T, Maeda S, Miyauchi T, Iemitsu M, Takanashi M, Irukayama-Tomobe Y, Yokota T, Ohmori H, and Matsuda M.** Exercise training improves ageing-induced decrease in eNOS expression of the aorta. *Acta Physiol Scand* 178: 3-10, 2003.

118. **Tanguy S, Boucher F, Toufektsian MC, Besse S, and de Leiris J.** Aging exacerbates hydrogen peroxide-induced alteration of vascular reactivity in rats. *Antioxid Redox Signal* 2: 363-368, 2000.

119. **Thengchaisri N, Hein TW, Wang W, Xu X, Li Z, Fossum TW, and Kuo L.** Upregulation of arginase by H2O2 impairs endothelium-dependent nitric oxide-mediated dilation of coronary arterioles. *Arterioscler Thromb Vasc Biol* 26: 2035-2042, 2006.

120. **Thijssen DH, Rongen GA, van Dijk A, Smits P, and Hopman MT.** Enhanced endothelin-1-mediated leg vascular tone in healthy older subjects. *J Appl Physiol* 103: 852-857, 2007.

121. **Thomas SR, Chen K, and Keaney JF, Jr.** Hydrogen peroxide activates endothelial nitric-oxide synthase through coordinated phosphorylation and dephosphorylation via a phosphoinositide 3-kinase-dependent signaling pathway. *J Biol Chem* 277: 6017-6024, 2002.

122. **Topper JN, Cai J, Falb D, and Gimbrone MA, Jr.** Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci U S A* 93: 10417-10422, 1996.

123. **Trott DW, Gunduz F, Laughlin MH, and Woodman CR.** Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. *J Appl Physiol* 106: 1925-1934, 2009.

124. **Uematsu M, Ohara Y, Navas JP, Nishida K, Murphy TJ, Alexander RW, Nerem RM, and Harrison DG.** Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. *Am J Physiol* 269: C1371-1378, 1995.

125. **Ungvari Z, Orosz Z, Labinskyy N, Rivera A, Xiangmin Z, Smith K, and Csiszar A.** Increased mitochondrial H2O2 production promotes endothelial NF-kappaB activation in aged rat arteries. *Am J Physiol Heart Circ Physiol* 293: H37-47, 2007.

126. **van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, Palacios-Callender M, Erusalimsky JD, Quaschning T, Malinski T, Gygi D, Ullrich V, and Luscher TF.** Enhanced peroxynitrite formation is associated with vascular aging. *J Exp Med* 192: 1731-1744, 2000.

127. **Vasquez-Vivar J, Kalyanaraman B, and Martasek P.** The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications. *Free Radic Res* 37: 121-127, 2003.

128. **Wahren J, Saltin B, Jorfeldt L, and Pernow B.** Influence of age on the local circulatory adaptation to leg exercise. *Scand J Clin Lab Invest* 33: 79-86, 1974.

129. **Williams DA and Segal SS.** Feed artery role in blood flow control to rat hindlimb skeletal muscles. *J Physiol* 463: 631-646, 1993.

130. **Williams DA and Segal SS.** Microvascular architecture in rat soleus and extensor digitorum longus muscles. *Microvascular Res* 43: 192-204, 1992.

131. **Woodman CR, Muller JM, Laughlin MH, and Price EM.** Induction of nitric oxide synthase mRNA in coronary resistance arteries isolated from exercise-trained pigs. *Am J Physiol Heart Circ Physiol* 273: H2575-H2579, 1997.

132. **Woodman CR, Price EM, and Laughlin MH.** Aging induces muscle-specific impairment of endothelium-dependent dilation in skeletal muscle feed arteries. *J Appl Physiol* 93: 1685-1690, 2002.

133. **Woodman CR, Price EM, and Laughlin MH.** Selected contribution: Aging impairs nitric oxide and prostacyclin mediation of endothelium-dependent dilation in soleus feed arteries. *J Appl Physiol* 95: 2164-2170, 2003.

134. **Woodman CR, Price EM, and Laughlin MH.** Shear stress induces eNOS mRNA expression and improves endothelium-dependent dilation in senescent soleus muscle feed arteries. *J Appl Physiol* 98: 940-946, 2005.

135. **Woodman CR, Trott DW, and Laughlin MH.** Short-term increases in intraluminal pressure reverse age-related decrements in endothelium-dependent dilation in soleus muscle feed arteries. *J Appl Physiol* 103: 1172-1179, 2007.

136. **Yang YM, Huang A, Kaley G, and Sun D.** eNOS uncoupling and endothelial dysfunction in aged vessels. *Am J Physiol Heart Circ Physiol* 297: H1829-1836, 2009.

137. **Yazdanyar A and Newman AB.** The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. *Clinics Ger Med* 25: 563-577, vii, 2009.

138. **Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, and Griendling KK.** Role of NADH/NADPH oxidase-derived H2O2 in angiotensin II-induced vascular hypertrophy. *Hypertension* 32: 488-495, 1998.

139. **Zecchin HG, Priviero FB, Souza CT, Zecchin KG, Prada PO, Carvalheira JB, Velloso LA, Antunes E, and Saad MJ.** Defective insulin and acetylcholine induction of endothelial cell-nitric oxide synthase through insulin receptor substrate/Akt signaling pathway in aorta of obese rats. *Diabetes* 56: 1014-1024, 2007.

140. **Zhang QJ, McMillin SL, Tanner JM, Palionyte M, Abel ED, and Symons JD.** Endothelial nitric oxide synthase phosphorylation in treadmill-running mice: role of vascular signalling kinases. *J Physiol* 587: 3911-3920, 2009.

141. **Zhou X, Bohlen HG, Unthank JL, and Miller SJ.** Abnormal nitric oxide production in aged rat mesenteric arteries is mediated by NAD(P)H oxidase-derived peroxide. *Am J Physiol Heart Circ Physiol* 297: H2227-2233, 2009.

APPENDIX

RAW DATA TABLES

Table 1-Exercise Training Studies Acetylcholine Data (Figs. 2.1-2.3)

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
115	Young Sed Con	424	191	50.0
101	Young Sed Con	369	192	40.0
153	Young Sed Con	374	197.2	55.0
151	Young Sed Con	395	213.4	44.0
105	Young Sed Con	400	206.1	35.0
149	Young Sed Con	358	173.6	46.0
107	Young Sed Con	426	216.2	73.0
142	Young Sed Con	382	205.7	46.0
	Mean		199.4	48.6
	Standard Error		4.9	4.1
107	Young Sed LNNA		184.3	67
142	Young Sed LNNA		192.9	75
101	Young Sed LNNA		207.5	81
149	Young Sed LNNA		219.6	78
97	Young Sed LNNA		176.3	89
151	Young Sed LNNA		191.8	69
105	Young Sed LNNA		224.9	75
153	Young Sed LNNA		210.3	63
116	Young Sed LNNA		171	82
115	Young Sed LNNA		158.4	60
	Mean		193.7	73.9
	Standard Error		6.9	2.9
115	Young Sed INDO		198.7	34
151	Young Sed INDO		221.5	41
149	Young Sed INDO		168.2	48
101	Young Sed INDO		143.3	28
107	Young Sed INDO		234.3	38
116	Young Sed INDO		170.1	34
105	Young Sed INDO		225.3	85
97	Young Sed INDO		174.4	26
142	Young Sed INDO		219.2	74
	Mean		195.0	45.3
	Standard Error		10.7	6.9

Table 1, continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
151	Young Sed Both		202.5	39
97	Young Sed Both		187.1	34
105	Young Sed Both		247.6	68
142	Young Sed Both		170.1	60
115	Young Sed Both		138	58
153	Young Sed Both		122.9	49
116	Young Sed Both		182.1	63
101	Young Sed Both		137.5	53
107	Young Sed Both		228.3	80
	Mean		179.9	57.6
	Standard Error		12.6	4.5
156	Young Ex Con	370	156.2	19
103	Young Ex Con	341	221	49
99	Young Ex Con	353	178	47
143	Young Ex Con	310	211.6	33
110	Young Ex Con	366	243.4	53
152	Young Ex Con	374	190.7	41
113	Young Ex Con	360	173.2	39
	Mean		196.3	40.1
	Standard Error		11.5	4.3
113	Young Ex LNNA		158.3	52
103	Young Ex LNNA		215	66
143	Young Ex LNNA		169.1	46
110	Young Ex LNNA		243.6	83
152	Young Ex LNNA		113.3	51
156	Young Ex LNNA		167.6	61
99	Young Ex LNNA		175.9	64
	Mean		177.5	60.4
	Standard Error		15.8	4.7

Table 1, continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
103	Young Ex INDO		215	26
143	Young Ex INDO		196.5	25
99	Young Ex INDO		153.9	40
113	Young Ex INDO		149.9	37
110	Young Ex INDO		231.1	36
152	Young Ex INDO		180.4	54
	Mean		187.8	36.3
	Standard Error		12.3	4.0
110	Young Ex Both		197.9	65
103	Young Ex Both		207	45
152	Young Ex Both		219.7	47
99	Young Ex Both		195.9	46
113	Young Ex Both		110	33
143	Young Ex Both		148.1	79
156	Young Ex Both		208.4	68
	Mean		183.9	54.7
	Standard Error		15.0	6.1
98	Old Sed Con	474	147	82
145	Old Sed Con	385	176.2	52
138	Old Sed Con	424	171.3	25
111	Old Sed Con	436	179.5	34
148	Old Sed Con	412	195.2	43
106	Old Sed Con	426	153.1	44
102	Old Sed Con	459	194.8	27
150	Old Sed Con	380	138.9	45
	Mean		169.5	44.0
	Standard Error		7.5	6.4

Table 1, continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
150	Old Sed LNNA		136.8	61
138	Old Sed LNNA		178.7	82
111	Old Sed LNNA		201.6	93
145	Old Sed LNNA		137.9	60
102	Old Sed LNNA		188.6	61
148	Old Sed LNNA		190.9	47
106	Old Sed LNNA		152.8	61
	Mean		173.3	67.4
	Standard Error		9.5	5.2
106	Old Sed INDO		245	27
102	Old Sed INDO		199.5	50
138	Old Sed INDO		109.7	28
148	Old Sed INDO		115.4	18
150	Old Sed INDO		208.7	30
98	Old Sed INDO		153.1	28
111	Old Sed INDO		199.3	27
145	Old Sed INDO		162.2	48
	Mean		174.1	32.0
	Standard Error		16.7	3.9
145	Old Sed Both		199.8	68
138	Old Sed Both		272.1	58
150	Old Sed Both		190.2	47
148	Old Sed Both		109.2	46
98	Old Sed Both		140.9	23
106	Old Sed Both		233.9	49
102	Old Sed Both		156.5	33
111	Old Sed Both		190.7	41
	Mean		186.7	45.6
	Standard Error		18.3	4.9

Table 1, continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
100	Old Ex Con	400	167	45
147	Old Ex Con	346	183.1	45
112	Old Ex Con	464	166.3	38
154	Old Ex Con	403	233.1	42
109	Old Ex Con	410	197	54
114	Old Ex Con	411	248	74
144	Old Ex Con	354	193.9	45
104	Old Ex Con	402	182	36
155	Old Ex Con	425	232.4	39
	Mean		200.3	46.4
	Standard Error		10.1	3.9
109	Old Ex LNNA		190.2	61
104	Old Ex LNNA		168	71
147	Old Ex LNNA		183	65
144	Old Ex LNNA		193.1	36
155	Old Ex LNNA		222.8	41
154	Old Ex LNNA		167.5	30
112	Old Ex LNNA		158.8	53
100	Old Ex LNNA		202.1	78
108	Old Ex LNNA		232.8	58
114	Old Ex LNNA		223.2	65
	Mean		194.2	55.8
	Standard Error		8.2	5.0
114	Old Ex INDO		232.4	29
144	Old Ex INDO		153.3	27
155	Old Ex INDO		122.6	28
100	Old Ex INDO		173.7	34
154	Old Ex INDO		179.3	24
108	Old Ex INDO		123.2	42
112	Old Ex INDO		187.4	44
109	Old Ex INDO		183.1	38
104	Old Ex INDO		184	33
	Mean		171.0	33.2
	Standard Error		11.4	2.3

Table 1, continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
114	Old Ex Both		228	44
104	Old Ex Both		163.8	45
154	Old Ex Both		145	28
147	Old Ex Both		155.3	59
144	Old Ex Both		153.2	47
155	Old Ex Both		209.6	40
100	Old Ex Both		159	50
109	Old Ex Both		180.8	34
108	Old Ex Both		126.6	45
112	Old Ex Both		183.8	49
	Mean		170.5	44.1
	Standard Error		9.7	2.7

Table 1, continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
115	Young Sed Con	0	7.9	29.8	75.3	92.9	97.9	99.3
101	Young Sed Con	0	8.7	13.6	69.9	91.1	94.4	94.7
153	Young Sed Con	0	15.3	55.1	84.2	94.5	95.5	95.5
151	Young Sed Con	0	3.1	3	7.5	38.3	48.7	48.9
105	Young Sed Con	0	12.9	27.7	62.3	72.1	72.3	72.4
149	Young Sed Con	0	0	36.1	66.9	90.7	90.7	90.9
107	Young Sed Con	0	7.7	50.9	80.3	92.2	98.1	99
142	Young Sed Con	0	46.3	78	89.6	94.1	94.1	95.6
	Mean	0.0	12.7	36.8	67.0	83.2	86.5	87.0
	Standard Error	0.0	5.1	8.5	9.1	6.9	6.1	6.2
107	Young Sed LNNA	0	2.8	4.4	4.4	24.4	13.1	13.1
142	Young Sed LNNA	0	7	51.8	56.7	66.9	70.4	70.3
101	Young Sed LNNA	0	0	0	2.5	21.3	28.1	21.6
149	Young Sed LNNA	0	0	21.2	7.1	56.6	48.4	40
97	Young Sed LNNA	0	0	0	0	0	0	0
151	Young Sed LNNA	0	1.5	11.6	27.5	57.6	59.8	59.8
105	Young Sed LNNA	0	9.4	13.5	63.1	85.9	67.5	87.7
153	Young Sed LNNA	0	0	0	24.2	9.8	82.7	71.6
116	Young Sed LNNA	0	0	1.4	42.3	52.2	53.1	44.1
115	Young Sed LNNA	0	9.6	20.1	33.1	48.4	69.7	63.7
	Mean	0.0	3.0	12.4	26.1	42.3	49.3	47.2
	Standard Error	0.0	1.3	5.1	7.2	8.6	8.6	9.0
115	Young Sed INDO	0	2.8	11.8	63.1	89.3	93	98.6
151	Young Sed INDO	0	43.7	53.6	68.2	87.5	89.8	89.8
149	Young Sed INDO	0	0	2.1	30.4	45.2	46.4	53.8
101	Young Sed INDO	0	24.3	26.2	72.4	74.3	78.1	81.8
107	Young Sed INDO	0	6.4	11.9	59.6	80.2	81.3	81.9
116	Young Sed INDO	0	28.9	26.2	33.1	54.7	62.9	43.9
105	Young Sed INDO	0	3.2	3.2	5.8	7.9	11.1	11.1
97	Young Sed INDO	0	0	33.2	57.3	63.9	67.6	74.7
142	Young Sed INDO	0	0	0	10.6	25.5	46.4	46.4
	Mean	0.0	12.1	18.7	44.5	58.7	64.1	64.7
	Standard Error	0.0	5.4	5.9	8.4	9.4	8.7	9.3

Table 1, continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
149	Young Sed Both	0	0	0	0	7.9	32.2	34.4
151	Young Sed Both	0	3.2	4.6	9	27.5	47.7	47.7
97	Young Sed Both	0	6.9	31.1	31.1	19.2	19.2	17.1
105	Young Sed Both	0	7.3	10.7	66.2	90.1	92.2	93.3
142	Young Sed Both	0	0	0	50.5	79.6	87.9	77.1
115	Young Sed Both	0	11.8	8.9	12.3	17.7	19.5	23.5
153	Young Sed Both	0	46.3	73.3	84.1	87.9	89.6	89.6
116	Young Sed Both	0	3.5	3.5	43.1	51.3	77.5	81.9
101	Young Sed Both	0	0	2.1	10.2	43.6	73.5	66.1
107	Young Sed Both	0	0	0	1.5	58.4	72.7	75.3
	Mean	0.0	7.9	13.4	30.8	48.3	61.2	60.6
	Standard Error	0.0	4.4	7.3	9.2	9.6	9.2	8.8
156	Young Ex Con	0	24.9	-5.4	62.4	63	85.4	85.4
103	Young Ex Con	0	18	58.1	78.5	78.5	83.2	83.2
99	Young Ex Con	0	0	0	73.7	96	100	100
143	Young Ex Con	0	5.7	13.1	16.3	60.8	84.5	86.9
110	Young Ex Con	0	10.1	19.7	66.7	88.4	89.3	90
152	Young Ex Con	0	1.2	18	18	18	18	14.2
113	Young Ex Con	0	0	4.2	64.6	71	79.4	78.9
	Mean	0.0	8.6	15.4	54.3	68.0	77.1	76.9
	Standard Error	0.0	3.7	7.9	9.8	9.6	10.2	10.7
113	Young Ex LNNA	0	0	-8.8	-8.5	23.2	20.9	0
103	Young Ex LNNA	0	0	2.2	31.2	71.9	84.9	79.5
143	Young Ex LNNA	0	0	0	1.9	51.8	77.7	84.2
110	Young Ex LNNA	0	3.7	7.9	18.5	37.3	41.5	42.5
152	Young Ex LNNA	0	0	11.4	24.8	48	47	12.5
156	Young Ex LNNA	0	2.4	2.4	39.9	77.4	83.1	76.8
99	Young Ex LNNA	0	4.3	6.4	47.4	66.5	78.6	48.1
	Mean	0.0	1.5	3.1	22.2	53.7	62.0	49.1
	Standard Error	0.0	0.7	2.5	7.6	7.4	9.5	12.6

Table 1, continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
103	Young Ex INDO	0	13.1	33.5	80.1	85.8	85.8	86.8
143	Young Ex INDO	0	18.6	45	66.7	72	88.3	88.3
99	Young Ex INDO	0	5.2	21.9	43.2	77.8	83.7	84.3
113	Young Ex INDO	0	0	0	54.5	75.4	91.6	83.2
110	Young Ex INDO	0	2.7	2.7	25.3	53.8	59.9	64.8
152	Young Ex INDO	0	1.3	1.3	1.3	9.4	18.8	20
	Mean	0.0	6.8	17.4	45.2	62.4	71.4	71.2
	Standard Error	0.0	2.8	7.2	10.8	10.6	10.6	10.0
110	Young Ex Both	0	0	3.7	3.7	28.2	42.1	40.3
103	Young Ex Both	0	33.7	37.6	44.6	61.2	69.1	46.8
152	Young Ex Both	0	-18.9	-18.9	-18.9	6.8	20.7	1.2
99	Young Ex Both	0	0	0	0	24.4	31	6.8
113	Young Ex Both	0	-3.9	-3.9	29	43.9	73.7	57.5
143	Young Ex Both	0	0	0	0	0	5.9	6.3
156	Young Ex Both	0	0	0	12.9	32.5	54.2	37.9
	Mean	0.0	1.6	2.6	10.2	28.1	42.4	28.1
	Standard Error	0.0	6.0	6.5	7.9	7.9	9.5	8.6
98	Old Sed Con	0	4.3	4.4	5.4	5.9	7.4	7.4
145	Old Sed Con	0	-10.8	-9.7	26.8	27.4	54.7	54.7
138	Old Sed Con	0	0	0	0	0	0	0
111	Old Sed Con	0	7.7	14.8	62	52.5	45.1	45.1
148	Old Sed Con	0	-2.4	6	17.2	43.1	56.2	62.4
106	Old Sed Con	0	15.4	26.1	48.7	70.9	70.9	70.9
102	Old Sed Con	0	2.3	9.7	43.1	69.9	73.7	75.4
150	Old Sed Con	0	0	0	60.1	83.1	87.6	63.4
	Mean	0.0	2.1	6.4	32.9	44.1	49.5	47.4
	Standard Error	0.0	2.7	3.8	8.5	10.9	11.0	10.1
98	Old Sed LNNA	0	2.7	6.3	26.7	20.4	24.6	24.6
150	Old Sed LNNA	0	6.8	13.1	39.7	56.3	66.6	55.2
138	Old Sed LNNA	0	0	1.1	0	12.8	15.5	15.4
111	Old Sed LNNA	0	5.3	13.6	32.7	40.2	48.4	41.1
145	Old Sed LNNA	0	3	3.2	19.5	35.8	33.8	23.6
102	Old Sed LNNA	0	0	3.7	18.5	36.9	29.8	29.7
148	Old Sed LNNA	0	6.8	19.5	31.2	39.2	59.1	59.2
106	Old Sed LNNA	0	4.5	7.6	15.4	76.9	74.5	54.6
	Mean	0.0	3.6	8.5	23.0	39.8	44.0	37.9
	Standard Error	0.0	1.0	2.2	4.4	7.0	7.5	6.0

Table 1, continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
106	Old Sed INDO	0	2.3	20.9	57.9	81.7	98.26	120.16
102	Old Sed INDO	0	15.4	42.4	47.8	84.7	98.6	118.78
138	Old Sed INDO	0	5.3	8.7	19.6	45.8	47.65	58.24
148	Old Sed INDO	0	0	0	0	10.6	8.48	10.6
150	Old Sed INDO	0	27.9	27.9	39.6	83.7	89.55	107.46
98	Old Sed INDO	0	8.1	10.4	12.6	31.1	32.45	39.12
111	Old Sed INDO	0	0	0	8.5	9.5	11.85	14.6
145	Old Sed INDO	0	0	3.9	54.2	89.4	99.4	122.7
	Mean	0.0	7.4	14.3	30.0	54.6	60.8	74.0
	Standard Error	0.0	3.5	5.3	8.0	12.2	14.2	17.2
145	Old Sed Both	0	0	-14.2	-11.4	47	41.7	25.3
138	Old Sed Both	0	6.3	12.3	12.3	15.5	15.5	15.5
150	Old Sed Both	0	-9.8	2.6	2.6	2.3	2.3	-26.3
148	Old Sed Both	0	0	0	0	5.7	5.8	13.6
98	Old Sed Both	0	12.5	19.6	28.5	31.2	31.2	31.2
106	Old Sed Both	0	4.8	4.8	11.7	41.7	54.7	33.1
102	Old Sed Both	0	7.7	11.8	29.1	43.3	55.2	55.2
111	Old Sed Both	0	2.6	25.1	48.8	85.2	95.4	80.6
	Mean	0.0	3.0	7.8	15.2	34.0	37.7	28.5
	Standard Error	0.0	2.3	4.3	6.8	9.5	11.0	11.1
100	Old Ex Con	0	23.5	26.9	46.9	66.6	69.7	71
147	Old Ex Con	0	17.9	23.4	88.9	92.3	92.3	96.6
112	Old Ex Con	0	-1.5	7.7	42.5	74.9	74.6	74.9
154	Old Ex Con	0	0	5.6	87.7	94.4	94.4	96.3
109	Old Ex Con	0	6.8	19.5	37.8	62.5	63.4	56.8
114	Old Ex Con	0	0	6.6	16.9	44.6	29.9	44.3
144	Old Ex Con	0	83.3	82	86.1	87.4	88.2	88.2
104	Old Ex Con	0	13	17.6	48.1	75.4	100	100
155	Old Ex Con	0	20.6	27.5	86.4	96.9	96.7	100
	Mean	0.0	18.2	24.1	60.1	77.2	78.8	80.9
	Standard Error	0.0	8.7	7.8	9.1	5.8	7.5	6.8

Table 1, continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
109	Old Ex LNNA	0	2.4	2.5	36.7	54.7	60	61.9
104	Old Ex LNNA	0	6	9.6	43	45.4	31.3	23
147	Old Ex LNNA	0	1.8	2.7	10.7	45.7	52.1	38.4
144	Old Ex LNNA	0	84	45.6	-3.3	55.4	79.1	76.7
155	Old Ex LNNA	0	19.1	25.6	16.5	35.3	41.4	41.3
154	Old Ex LNNA	0	0	-11.1	-11.1	-23.1	-21.5	-16.3
112	Old Ex LNNA	0	0	4.2	10.1	63.1	85.3	86.1
100	Old Ex LNNA	0	7.8	14.8	17.5	25.5	27.8	28
108	Old Ex LNNA	0	2.6	7.1	25.9	23	23	23
114	Old Ex LNNA	0	4.5	11.6	50.5	63.9	50.4	55.9
	Mean	0.0	12.8	11.3	19.7	38.9	42.9	41.8
	Standard Error	0.0	8.1	4.9	6.2	8.2	9.7	9.5
114	Old Ex INDO	0	0	0	28.7	32.6	45.6	39
144	Old Ex INDO	0	0	14.5	67.4	88.1	88.1	90.3
155	Old Ex INDO	0	0	0	0	34.6	77.5	77.5
100	Old Ex INDO	0	27.4	43.6	96.8	97.1	96.8	97.1
154	Old Ex INDO	0	0	11.9	43.6	63.4	63.4	63.9
108	Old Ex INDO	0	9.1	9.1	13.8	21.8	22.9	28
112	Old Ex INDO	0	0	-1.4	-3.2	-3.2	-3.8	-3.8
109	Old Ex INDO	0	0	4.3	59.1	77.4	77.4	77.4
104	Old Ex INDO	0	5.6	5.6	38.1	87	87	86.7
	Mean	0.0	4.7	9.7	38.3	55.4	61.7	61.8
	Standard Error	0.0	3.0	4.6	10.9	11.7	11.3	11.3
114	Old Ex Both	0	0	3.5	5.8	71.4	53.6	39.8
104	Old Ex Both	0	4.8	8.7	33.7	61.9	71.8	71.8
154	Old Ex Both	0	0	0	1.9	1.9	1.9	1.9
147	Old Ex Both	0	0	0	0	35.5	65.8	43.2
144	Old Ex Both	0	1.1	1.1	5.1	42.7	44.7	44.8
155	Old Ex Both	0	0	0	0	3.4	10.3	10.3
100	Old Ex Both	0	0	3.6	17.2	77.7	74.8	78.6
109	Old Ex Both	0	18.6	22.2	36.5	47.4	57.5	60.5
108	Old Ex Both	0	0	0	0	5.2	25.4	25.6
112	Old Ex Both	0	0	0	0	21.2	33.3	14.2
	Mean	0.0	2.5	3.9	10.0	36.8	43.9	39.1
	Standard Error	0.0	1.9	2.2	4.5	9.0	8.1	8.3

Table 2-Exogenous SOD Acetylcholine Data (Figs. 2.4 & 2.5)

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
53	young control	326	161	57.8
56	young control	344	154	52.6
65	young control	354	194	32.0
66	young control	334	138	37.0
68	young control	300	166	42.2
70	young control	280	114	39.5
72	young control	234	181	32.5
74	young control	256	138	36.2
76	young control	321	140	37.9
78	young control	324	198	36.9
82	young control	331	154	27.3
84	young control	389	174	35.6
89	young control	302	151	35.1
90	young control	340	166	50.6
91	young control	330	177	36.7
143	young control	392	192	61.4
147	young control	371	166	48.2
	Mean		162.6	41.1
	Standard Error		5.3	2.3
78	young + SOD		191	34.6
80	young + SOD		116	41.4
82	young + SOD		170	31.8
84	young + SOD		140	35.7
86	young + SOD		170	45.9
89	young + SOD		185	62.2
90	young + SOD		214	37.9
91	young + SOD		202	41.1
135	young + SOD		201	35.8
137	young + SOD		175	21.7
139	young + SOD		198	41.9
141	young + SOD		175	29.7
	Mean		178.1	38.3
	Standard Error		8.8	3.2

Table 2- continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
78	young + SOD & catalase		157	50.3
82	young + SOD & catalase		144	59.7
84	young + SOD & catalase		167	28.7
86	young + SOD & catalase		185	38.9
88	young + SOD & catalase		214	27.6
89	young + SOD & catalase		157	44.6
90	young + SOD & catalase		179	39.7
91	young + SOD & catalase		197	27.4
92	young + SOD & catalase		182	26.9
	Mean		175.8	38.2
	Standard Error		7.3	3.9
135	young SOD & L-NNA		152	59.9
137	young SOD & L-NNA		160	40.0
139	young SOD & L-NNA		213	39.9
141	young SOD & L-NNA		180	50.0
143	young SOD & L-NNA		199	27.1
145	young SOD & L-NNA		156	59.0
147	young SOD & L-NNA		183	69.9
149	young SOD & L-NNA		212	33.5
	Mean		181.9	47.4
	Standard Error		8.7	5.2

Table 2- continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
50	old control	413	212	34.9
51	old control	412	184	26.6
52	old control	460	187	41.7
54	old control	400	174	35.0
55	old control	478	124	88.7
57	old control	448	158	54.0
58	old control	424	203	47.3
59	old control	376	179	31.8
73	old control	429	155	39.4
75	old control	418	118	31.4
77	old control	372	112	25.0
79	old control	438	152	33.6
81	old control	482	182	64.8
85	old control	454	189	36.0
87	old control	468	154	35.7
136	old control	470	206	55.3
140	old control	413	104	35.6
142	old control	475	170	35.3
144	old control	440	142	28.2
148	old control	415	113	38.1
	Mean		160.9	40.9
	Standard Error		7.5	3.3
70	old + SOD		174	35.6
73	old + SOD		185	33.0
75	old + SOD		219	35.2
77	old + SOD		116	29.3
81	old + SOD		205	41.5
83	old + SOD		185	60.0
85	old + SOD		174	36.2
87	old + SOD		167	37.7
136	old + SOD		202	57.4
140	old + SOD		227	38.8
142	old + SOD		208	31.7
148	old + SOD		136	31.6
	Mean		183.2	39.0
	Standard Error		9.5	2.8

Table 2- continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
70	old + SOD & catalase		149	48.3
73	old + SOD & catalase		136	33.1
75	old + SOD & catalase		201	31.8
81	old + SOD & catalase		224	29.9
83	old + SOD & catalase		155	28.4
85	old + SOD & catalase		187	51.3
	Mean		175.3	37.1
	Standard Error		13.9	4.1
134	old + SOD & L-NNA		167	26.9
136	old + SOD & L-NNA		197	40.6
138	old + SOD & L-NNA		171	28.7
140	old + SOD & L-NNA		224	36.2
142	old + SOD & L-NNA		199	61.3
144	old + SOD & L-NNA		171	61.4
146	old + SOD & L-NNA		114	33.3
148	old + SOD & L-NNA		109	50.5
	Mean		169.0	42.4
	Standard Error		14.2	4.9

Table 2- continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
53	young sed control	0	1.1	2.2	32.3	88.2	92.5	97.8
56	young sed control	0	2.5	6.2	27.2	60.5	55.6	58.0
65	young sed control	0	0.0	4.8	12.9	38.7	95.2	95.2
66	young sed control	0	0.0	-2.0	-2.0	-2.0	76.5	100.0
68	young sed control	0	10.0	11.4	12.9	17.1	74.3	70.0
70	young sed control	0	2.2	2.2	4.4	57.8	77.8	60.0
72	young sed control	0	4.5	4.5	7.6	46.0	82.0	83.7
74	young sed control	0	8.0	8.0	14.0	32.0	44.0	44.0
76	young sed control	0	6.0	36.9	49.1	78.6	80.2	86.6
78	young sed control	0	9.6	32.9	35.6	39.7	84.9	97.3
82	young sed control	0	28.6	31.0	40.5	54.8	69.0	69.0
84	young sed control	0	9.7	12.9	16.1	74.2	82.3	82.3
89	young sed control	0	0.0	1.9	9.4	20.8	50.9	50.9
90	young sed control	0	1.2	2.4	45.2	45.2	42.9	45.2
91	young sed control	0	15.4	24.6	43.1	46.2	61.5	66.2
143	young sed control	0	11.9	16.1	32.2	36.4	42.4	42.4
147	young sed control	0	10.0	40.0	70.0	76.3	83.8	83.8
	Mean	0.0	7.1	13.9	26.5	47.7	70.3	72.5
	Standard Error	0.0	1.7	3.3	4.5	5.6	4.1	4.7
78	young + SOD	0	15.2	40.9	62.1	77.3	87.9	89.4
80	young + SOD	0	0.0	6.3	16.7	20.8	31.3	31.3
82	young + SOD	0	9.3	9.3	11.1	24.1	61.1	83.3
84	young + SOD	0	4.0	64.0	68.0	80.0	86.0	94.0
86	young + SOD	0	9.0	33.3	43.6	41.0	61.5	82.1
89	young + SOD	0	0.9	0.9	5.2	36.5	63.5	63.5
90	young + SOD	0	4.9	9.9	48.1	48.1	54.3	70.4
91	young + SOD	0	0.0	1.2	2.4	57.8	75.9	78.3
135	young + SOD	0	8.3	65.3	84.7	88.9	88.9	80.6
137	young + SOD	0	7.9	15.8	73.7	94.7	100.0	100.0
139	young + SOD	0	6.0	24.1	60.2	67.5	67.5	71.1
141	young + SOD	0	7.7	25.0	69.2	98.1	100.0	100.0
	Mean	0.0	6.1	24.7	45.4	61.2	73.2	78.7
	Standard Error	0.0	1.4	7.2	9.3	8.7	6.5	6.0

Table 2- continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
78	young + SOD & CAT	0	1.3	1.3	7.6	11.4	11.4	11.4
82	young + SOD & CAT	0	3.5	34.9	36.0	80.2	89.5	96.5
84	young + SOD & CAT	0	29.2	39.6	50.0	83.3	87.5	87.5
86	young + SOD & CAT	0	4.2	5.6	51.4	55.6	58.3	66.7
88	young + SOD & CAT	0	6.8	20.3	35.6	67.8	71.2	71.2
89	young + SOD & CAT	0	72.9	55.7	64.3	94.3	98.6	98.6
90	young + SOD & CAT	0	19.7	19.7	18.3	22.5	49.3	49.3
91	young + SOD & CAT	0	0.0	3.7	13.0	35.2	44.4	46.3
92	young + SOD & CAT	0	20.4	26.5	26.5	32.7	42.9	57.1
	Mean	0	17.5	23.0	33.6	53.7	61.5	65.0
	Standard Error	0.0	7.7	6.1	6.4	9.8	9.3	9.3
135	young SOD & L-NNA	0	1.1					
137	young SOD & L-NNA	0	0.0	4.4	4.4	5.5	6.6	6.6
139	young SOD & L-NNA	0	8.2	12.5	12.5	89.1	92.2	92.2
141	young SOD & L-NNA	0	-8.9	28.2	44.7	44.7	88.2	88.2
143	young SOD & L-NNA	0	14.8	22.2	35.6	47.8	53.3	53.3
145	young SOD & L-NNA	0	5.4	29.6	29.6	29.6	31.5	31.5
147	young SOD & L-NNA	0	13.3	16.3	37.0	57.6	57.6	41.3
149	young SOD & L-NNA	0	12.7	58.6	80.5	80.5	69.5	100.0
	Mean	0.0	5.8	12.7	4.2	9.9	8.5	8.5
	Standard Error	0.0	2.9	23.1	31.1	45.6	50.9	52.7

Table 2- continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
50	old control	0	8.1	5.9	8.9	10.7	11.7	13.2
51	old control	0	2.0	10.8	17.6	54.1	66.2	70.3
52	old control	0	6.4	2.0	10.2	18.4	28.6	28.6
54	old control	0	13.2	12.8	39.7	94.9	100.0	100.0
55	old control	0	1.8	18.2	20.7	28.2	33.3	36.7
57	old control	0	5.0	4.5	34.5	22.7	35.5	37.3
58	old control	0	6.3	14.5	34.8	49.2	70.6	75.2
59	old control	0	7.0	11.5	12.5	21.9	26.0	26.0
73	old control	0	1.6	15.8	29.8	38.6	38.6	38.6
75	old control	0	0.0	4.9	9.8	37.7	45.9	50.8
77	old control	0	28.6	8.1	13.5	16.2	24.3	24.3
79	old control	0	5.9	28.6	46.4	50.0	50.0	57.1
81	old control	0	5.1	9.8	9.8	9.8	11.8	15.7
85	old control	0	10.3	9.3	12.7	17.8	23.7	32.2
87	old control	0	5.5	11.8	11.8	38.2	47.1	58.8
136	old control	0	0.0	5.5	5.5	16.4	18.2	18.2
140	old control	0	5.4	-3.5	10.5	19.3	23.7	23.7
142	old control	0	3.3	24.3	24.3	27.0	29.7	29.7
144	old control	0	10.0	5.0	8.3	-165.0	26.7	31.7
148	old control	0	2.3	10.0	15.0	30.0	35.0	40.0
	Mean	0.0	6.4	7.0	7.0	9.3	27.9	32.6
	Standard Error	0.0	1.4	10.5	18.7	21.7	38.1	41.4
70	old + SOD	0	37.1	50.0	53.2	74.2	93.5	93.5
73	old + SOD	0	9.8	19.7	39.3	49.2	55.7	57.4
75	old + SOD	0	19.5	23.4	48.1	72.7	88.3	94.8
77	old + SOD	0	17.6	23.5	73.5	88.2	100.0	100.0
81	old + SOD	0	3.5	10.6	50.6	61.2	67.1	67.1
83	old + SOD	0	1.8	8.1	62.2	64.9	81.1	81.1
85	old + SOD	0	7.9	19.0	66.7	82.5	96.8	100.0
87	old + SOD	0	7.9	12.7	25.4	50.8	71.4	74.6
136	old + SOD	0	5.2	5.2	19.8	25.0	31.0	31.0
140	old + SOD	0	6.8	13.6	58.0	46.6	40.9	40.9
142	old + SOD	0	0.0	48.5	54.5	69.7	78.8	78.8
148	old + SOD	0	0.0	27.9	46.5	51.2	55.8	55.8
	Mean	0.0	9.8	21.9	49.8	61.3	71.7	72.9
	Standard Error	0.0	3.1	4.2	4.5	5.1	6.4	6.6

Table 2- continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
70	old + SOD & catalase	0	27.8	55.6	55.6	51.4	94.4	100.0
73	old + SOD & catalase	0	17.8	37.8	53.3	75.6	75.6	77.8
75	old + SOD & catalase	0	25.0	32.8	40.6	40.6	51.6	56.3
81	old + SOD & catalase	0	19.4	26.9	37.3	56.7	71.6	71.6
83	old + SOD & catalase	0	2.3	4.5	52.3	81.8	81.8	79.5
85	old + SOD & catalase	0	6.3	19.8	19.8	46.9	52.1	52.1
	Mean	0.0	16.4	29.6	43.1	58.8	71.2	72.9
	Standard Error	0.0	4.2	7.0	5.6	6.7	6.9	7.1
134	old + SOD & L-NNA	0	0.0	11.1	24.4	35.6	42.2	42.2
136	old + SOD & L-NNA	0	2.5	3.8	3.8	6.3	10.0	17.5
138	old + SOD & L-NNA	0	4.1	12.2	12.2	12.2	14.3	14.3
140	old + SOD & L-NNA	0	3.7	8.6	8.6	8.6	11.1	12.3
142	old + SOD & L-NNA	0	3.3	3.3	12.3	21.3	20.5	20.5
144	old + SOD & L-NNA	0	6.7	10.5	18.1	20.0	32.4	38.1
146	old + SOD & L-NNA	0	0.0	5.3	26.3	26.3	26.3	28.9
148	old + SOD & L-NNA	0	9.1	20.0	25.5	25.5	36.4	43.6
	Mean	0.0	3.7	9.3	16.4	19.5	24.1	27.2
	Standard Error	0.0	1.1	1.9	3.0	3.5	4.3	4.5

Table 3-Desitometry Values for Training Study eNOS Immunoblots (Fig. 2.6)

vessel number	group	Proteins of Interest		
peNOS blot 1		eNOS	p-eNOSser1177	GAPDH
23709	YS	4998723	7228988	
23794	YS	5090219	7211414	
23613	OS	5867279	7835130	
23632	OS	5434181	7475966	
23688	YX	5036215	6793499	
23785	YX	4860031	6623776	
23664	OX	6312930	7345963	
23645	OX	6567813	7164256	
peNOS blot 2		eNOS	p-eNOSser1177	GAPDH
23820	YS	4977932	7499624	9789577
23856	YS	4816153	6604414	9004931
23740	OS	6103856	6285078	13975713
23759	OS	5799298	6190660	9294305
23875	YX	5647010	6548838	9959375
23897	YX	5439298	6364247	10498490
24158	OX	5150488	5948714	12252349
24139	OX	5875610	5751678	8401590
peNOS blot 5		eNOS	p-eNOSser1177	GAPDH
24471	YS	6129884	7815646	3696631
24392	YS	6058036	7504981	4428264
24435	OS	6079195	7491252	6891347
24318	OS	6288743	7612480	10984296
24293	YX	6032894	7375786	9406547
24274	YX	6052944	7251417	10566159
24333	OX	6043645	7253376	8439670
24275	OX	6088757	7525020	4530894
peNOS blot 6		eNOS	p-eNOSser1177	GAPDH
23995	YS	8378006	9486751	13879710
23915	YS	10711401	10020489	11905185
24054	OS	12543049	10942206	12954551
24016	OS	14113708	15720294	15857342
23952	YX	7753653	8988628	13315754
23931	YX	8322186	8467184	20050872
24237	OX	9486927	8752504	16321779
24199	OX	11731664	12222744	13826367

Table 3- continued

vessel number	group	Proteins of Interest		
peNOS blot 7		eNOS	p-eNOSser1177	GAPDH
24372	YS	5776349	8109234	3547762
24355	YS	5388825	7452216	4946577
24177	OS	5999296	9337652	5246277
24080	OS	5696306	7979945	4561492
24122	YX	5901194	7760329	4650936
24103	YX	5818339	7569452	3647610
24259	OX	7678377	11946974	5170605
24220	OX	5318531	9015379	4415438
peNOS blot 1		p-eNOS(ser1177)/eNOS		eNOS/GAPDH
23709	YS	1.103167142		
23794	YS	1.080704218		
23613	OS	1.018667269		
23632	OS	1.04943635		
23688	YX	1.028992333		
23785	YX	1.039655566		
23664	OX	0.887647733		
23645	OX	0.832095556		
peNOS blot 2		p-eNOS(ser1177)/eNOS		eNOS/GAPDH
23820	YS	1.149247098		0.490674529
23856	YS	1.046060732		0.516093473
23740	OS	0.785469413		0.421442944
23759	OS	0.814299958		0.602097726
23875	YX	0.884644081		0.547135574
23897	YX	0.892538706		0.499947608
24158	OX	0.881043995		0.405636944
24139	OX	0.746731529		0.674838666
peNOS blot 5		p-eNOS(ser1177)/eNOS		eNOS/GAPDH
24471	YS	0.972602787		1.600127673
24392	YS	0.945019201		1.320099987
24435	OS	0.940007281		0.851236891
24318	OS	0.923390077		0.552459061
24293	YX	0.932621661		0.618876435
24274	YX	0.913858824		0.552787222
24333	OX	0.915512187		0.691006228
24275	OX	0.942761637		1.296740936

Table 3- continued

vessel number	**group**	**Proteins of Interest**	
peNOS blot 6		p-eNOS(ser1177)/eNOS	eNOS/GAPDH
23995	YS	0.86377319	0.582463554
23915	YS	0.713617551	0.86819766
24054	OS	0.665464133	0.934306209
24016	OS	0.849654616	0.85885375
23952	YX	0.884320986	0.561887123
23931	YX	0.776112119	0.400509327
24237	OX	0.703768209	0.5608756
24199	OX	0.794752651	0.818766409
peNOS blot 7		p-eNOS(ser1177)/eNOS	eNOS/GAPDH
24372	YS	1.070900923	1.571113023
24355	YS	1.054907158	1.0512301
24177	OS	1.187299485	1.103462437
24080	OS	1.068635192	1.20502172
24122	YX	1.003143646	1.224356835
24103	YX	0.992403537	1.539214355
24259	OX	1.186892464	1.432968195
24220	OX	1.293049585	1.162321805

Table 4-Desitometry Values for Training Study SOD Immunoblots (Fig. 2.7)

Vessel number	group	Protein of Interest		
SOD Blot 1		SOD-1	GAPDH	SOD-1/GAPDH
23997	YS	6114444	6954248	0.879238704
24008	OS	2654121	3013078	0.880867007
24092	YX	3338317	5578766	0.598397029
24133	OX	3289056	4599574	0.715078396
24364	YS	2991377	4022436	0.743672988
24059	OS	3639076	6664649	0.546026655
24116	YX	3135245	4828235	0.649356338
24149	OX	2773153	3953929	0.701366413
SOD Blot 2		SOD-1	GAPDH	SOD-1/GAPDH
24364	YS	1812269	2226884	0.813813831
24384	YS	1992520	3576350	0.557137864
24070	OS	2233017	4376915	0.510180572
24169	OS	2020135	3564231	0.566780043
24286	YX	2410656	4135120	0.582971232
24287	YX	2922523	5021531	0.581998398
24189	OX	2345711	5860142	0.40028228
24209	OX	3549115	8935615	0.397187547
SOD Blot 3		SOD-1	GAPDH	SOD-1/GAPDH
23860	YS	5274950	4732164	1.114701435
23908	YS	3844191	3029255	1.269021921
23755	OS	3518444	2604045	1.351145622
24306	OS	2924918	2088665	1.400376796
23947	YX	2839963	2225507	1.276097087
24325	YX	2349232	2153891	1.090692147
24252	OX	2325682	2227751	1.043959581
24229	OX	2945301	2786973	1.056810023
SOD Blot 4		SOD-1	GAPDH	SOD-1/GAPDH
23702	YS	5307256	2235368	2.374220263
23801	YS	4657825	2680497	1.737672156
23615	OS	5038577	2998046	1.680620311
23622	OS	6415424	2435762	2.633846821
23680	YX	7294675	3555647	2.05157458
23775	YX	9717232	2978804	3.262125336
23640	OX	7255625	2697343	2.689915595
23658	OX	4336949	1913205	2.266850128

Table 4-Continued

Vessel number	group	Protein of Interest		
SOD Blot 5		SOD-1	GAPDH	SOD-1/GAPDH
23812	YS	4093717	4418229	0.926551566
23736	OS	2659298	3107444	0.855783081
23871	YX	3327690	4091016	0.813414076
23886	OX	2019570	1498504	1.34772413
SOD Blot 7				
24365	YS	GAPDH	ecSOD	ecSOD/GAPDH
24385	YS	1780270	5333161	2.995703461
24073	OS	2327248	9245513	3.972723577
24170	OS	2454684	4984811	2.030734302
24269	YX	1838122	4749193	2.583720232
24290	YX	2118379	5519178	2.60537798
24190	OX	3968498	5959649	1.501739197
24210	OX	2515577	5097815	2.026499288
		4634050	7889073	1.702414303
SOD Blot 8				
23861	YS	GAPDH	ecSOD	ecSOD/GAPDH
23909	YS	4853178	9585694	1.975137528
23763	OS	3219135	11155929	3.465505175
24307	OS	3248116	9031383	2.780498911
23948	YX	2172685	11051624	5.086620472
24326	YX	5366689	45202076	8.42271203
24253	OX	2267273	9230730	4.071291812
24230	OX	2562991	24778865	9.667948502
SOD blot 10				
23814	YS	GAPDH	ecSOD	ecSOD/GAPDH
23704	YS	3601388	8993924	2.497349355
23747	OS	2558072	6196828	2.422460353
23624	OS	2527035	10442179	4.132186139
23872	YX	4356896	8571842	1.967419466
23686	YX	3838317	19287050	5.024871578
23887	OX	2423923	6700781	2.764436412
23671	OX	2042546	6913793	3.384889741

Table 4-Continued

Vessel number	group		Protein of Interest	
SOD blot 11				
22450	YS	GAPDH	ecSOD	ecSOD/GAPDH
22565	YS	1824616	5213675	2.857409449
22442	OS	1838552	5418100	2.946938678
22425	OS	1890301	7674333	4.059847083
22444	YX	1880511	5015739	2.667221303
22495	YX	2011839	7235169	3.596296224
22454	OX	2451368	15659451	6.388045777
22462	OX	2173489	4940113	2.272895331

Table 5-NAD(P)H oxidase Studies, Flow Data (Figs. 3.1-3.4)

Animal	Group	Body Weight(g)	Maximal Diameter (μm)	% Spontaneous Tone
172	young control	410	212	37.7
174	young control	420	185	38.9
176	young control	426	233	40.3
183	young control	411	180	31.7
186	young control	450	144	33.3
195	young control	365	214	33.6
197	young control	328	149	30.2
199	young control	427	220	58.6
210	young control	311	99	59.6
216	young control	368	126	33.3
224	young control	342	89	33.7
225	young control	349	205	63.4
230	young control	404	215	27.9
231	young control	410	171	36.3
235	young control	360	115	25.2
261	young control	372	186	33.9
263	young control	310	115	25.2
297	young control	305	108	76.9
305	young control	396	182	59.9
308	young control	354	137	52.6
309	young control	407	172	32.6
310	young control	410	119	43.7
318	young control	376	163	40.5
	Mean		162.6	41.3
	Standard Error		9.4	2.2
297	young tempol		136	53.7
300	young tempol		156	41.7
302	young tempol		188	35.6
307	young tempol		229	51.1
308	young tempol		178	28.7
309	young tempol		233	35.2
313	young tempol		159	70.4
318	young tempol		176	50.0
	Mean		181.9	45.8
	Standard Error		12.1	4.7

Table 5-continued

Animal	Group	Body Weight(g)	Maximal Diameter (μm)	% Spontaneous Tone
297	young apocynin		133	32.3
300	young apocynin		118	47.5
302	young apocynin		167	35.9
305	young apocynin		136	41.9
307	young apocynin		103	57.3
308	young apocynin		180	33.9
309	young apocynin		200	70.5
310	young apocynin		193	25.4
313	young apocynin		161	75.8
318	young apocynin		136	41.9
	Mean		152.7	46.2
	Standard Error		9.3	4.8
235	young catalase		136	64.0
250	young catalase		155	40.6
255	young catalase		158	33.5
256	young catalase		159	42.8
258	young catalase		93	38.7
259	young catalase		153	57.5
261	young catalase		159	44.7
263	young catalase		160	38.1
	Mean		146.6	45.0
	Standard Error		8.1	3.7
250	young pegcat		157	30.6
255	young pegcat		171	42.1
256	young pegcat		172	40.1
257	young pegcat		119	48.7
258	young pegcat		135	43.7
259	young pegcat		198	51.5
261	young pegcat		185	25.4
263	young pegcat		133	39.8
	Mean		158.8	40.3
	Standard Error		9.8	3.1

Table 5-continued

Animal	Group	Body Weight(g)	Maximal Diameter (μm)	% Spontaneous Tone
173	old control	438	125	62.4
175	old control	453	173	24.9
177	old control	450	174	35.6
181	old control	471	165	41.8
189	old control	395	139	44.6
196	old control	452	180	30.0
203	old control	433	145	42.8
208	old control	331	110	47.3
213	old control	396	147	32.7
217	old control	401	177	27.1
222	old control	400	101	22.8
229	old control	410	100	28.0
232	old control	443	117	33.3
248	old control	384	238	42.4
249	old control	309	190	30
252	old control	317	219	33.3
253	old control	407	190	33.2
254	old control	373	126	42.1
260	old control	385	116	44.8
262	old control		133	38.3
265	old control	449	151	51.0
294	old control	407	127	46.5
298	old control	409	159	61.0
303	old control	429	136	40.4
304	old control	420	162	32.7
306	old control	404	191	74.9
312	old control	414	241	35.3
314	old control	463	128	40.6
319	old control	419	137	46.0
	Mean		155.1	40.2
	Standard Error		7.0	2.2

Table 5-continued

Animal	Group	Body Weight(g)	Maximal Diameter (μm)	% Spontaneous Tone
294	old tempol		198	25.8
298	old tempol		168	28.6
301	old tempol		206	53.9
303	old tempol		250	28.8
304	old tempol		199	35.7
306	old tempol		224	46.0
311	old tempol		124	42.7
312	old tempol		192	34.4
314	old tempol		212	40.6
317	old tempol		278	29.9
	Mean		205.1	36.6
	Standard Error		13.3	2.9
294	old apocynin			
298	old apocynin		201	52.7
301	old apocynin		165	43.0
303	old apocynin		222	33.8
304	old apocynin		244	29.1
306	old apocynin		155	56.8
312	old apocynin		165	49.7
314	old apocynin		197	30.5
317	old apocynin		205	45.9
	Mean		232	42.7
	Standard Error		198.4	42.7
			10.5	3.3

Table 5-continued

Animal	Group	Body Weight(g)	Maximal Diameter (μm)	% Spontaneous Tone
248	old catalase		227	33.9
249	old catalase		183	27.3
252	old catalase		201	35.8
262	old catalase		143	30.8
264	old catalase		106	33.0
265	old catalase		132	40.9
268	old catalase		175	33.7
	Mean		166.7	33.6
	Standard Error		15.9	1.6
234	old pegcat			
248	old pegcat		189	31.2
249	old pegcat		124	42.7
252	old pegcat		179	29.6
254	old pegcat		203	25.1
260	old pegcat		207	63.3
262	old pegcat		115	41.7
264	old pegcat		156	30.8
	Mean		147	38.1
	Standard Error		165.0	37.8

Table 5-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
172	young control	0	17.5	20.0	18.8	18.8
174	young control	0	41.7	11.1	12.5	18.1
176	young control	0	19.1	25.5	29.8	26.6
183	young control	0	12.3	12.3	52.6	59.6
186	young control	0	8.3	29.2	29.2	29.2
195	young control	0	5.6	9.7	9.7	9.7
197	young control	0	17.8	24.4	24.4	24.4
199	young control	0	16.3	22.5	22.5	28.7
210	young control	0	22.0	35.6	35.6	33.9
216	young control	0	21.4	4.8	4.8	-7.1
224	young control	0	13.3	30.0	30.0	30.0
225	young control	0	19.2	20.8	21.5	21.5
230	young control	0	25.0	25.0	28.3	28.3
231	young control	0	16.1	19.4	19.4	19.4
235	young control	0	55.2	62.1	62.1	69.0
261	young control	0	30.2	42.9	58.7	58.7
263	young control	0	6.9	10.3	13.8	17.2
297	young control	0	1.2	1.2	3.6	3.6
305	young control	0	35.8	35.8	35.8	35.8
308	young control	0	18.1	19.4	19.4	5.6
309	young control	0	8.9	14.3	14.3	10.7
310	young control	0	23.1	23.1	-5.8	-15.4
318	young control	0	16.7	42.4	47.0	59.1
	Mean	0.0	19.6	23.6	25.6	25.5
	Standard Error	0.0	2.1	2.0	2.9	3.6
297	young tempol	0	-1.4	2.7	2.7	4.1
300	young tempol	0	23.1	24.6	18.5	7.7
302	young tempol	0	3.0	7.5	7.5	7.5
307	young tempol	0	0.9	1.7	1.7	1.7
308	young tempol	0	82.4	84.3	84.3	88.2
309	young tempol	0	18.3	22.0	22.0	22.0
313	young tempol	0	0.0	0.9	2.7	8.0
318	young tempol	0	5.7	8.0	26.1	26.1
	Mean	0.0	16.5	19.0	20.7	20.7
	Standard Error	0.0	9.9	9.9	9.7	10.1

Table 5-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
297	young apocynin	0	2.3	0.0	0.0	0.0
300	young apocynin	0	12.5	14.3	14.3	25.0
302	young apocynin	0	1.7	8.3	8.3	11.7
305	young apocynin	0	22.8	26.3	26.3	33.3
307	young apocynin	0	18.6	18.6	18.6	18.6
308	young apocynin	0	6.6	13.1	14.8	16.4
309	young apocynin	0	14.9	19.9	19.9	12.8
310	young apocynin	0	20.4	24.5	24.5	24.5
313	young apocynin	0	5.7	7.4	7.4	9.8
318	young apocynin	0	19.3	19.3	31.6	17.5
	Mean	0.0	12.5	15.2	16.6	17.0
	Standard Error	0.0	2.3	2.3	2.8	2.7
235	young catalase	0	0.0	6.9	11.5	17.2
250	young catalase	0	23.8	23.8	23.8	15.9
255	young catalase	0	0.0	0.0	-9.4	-7.5
256	young catalase	0	11.8	13.2	13.2	13.2
258	young catalase	0	8.3	8.3	11.1	8.3
259	young catalase	0	10.2	18.2	22.7	22.7
261	young catalase	0	4.2	11.3	14.1	12.7
263	young catalase	0	3.3	3.3	3.3	3.3
	Mean	0.0	7.7	10.6	11.3	10.7
	Standard Error	0.0	2.8	2.8	3.8	3.3
250	young pegcat	0	25.0	33.3	33.3	33.3
255	young pegcat	0	29.2	58.3	58.3	58.3
256	young pegcat	0	33.3	43.5	49.3	49.3
257	young pegcat	0	0.0	0.0	15.5	15.5
258	young pegcat	0	5.1	5.1	3.4	3.4
259	young pegcat	0	64.7	65.7	48.0	34.3
261	young pegcat	0	36.2	70.2	74.5	63.8
263	young pegcat	0	9.4	9.4	9.4	15.1
	Mean	0.0	25.4	35.7	36.5	34.1
	Standard Error	0.0	7.4	10.0	9.0	7.7

Table 5-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
173	old control	0	0.0	17.9	24.4	24.4
175	old control	0	58.1	74.4	74.4	74.4
177	old control	0	0.0	6.5	6.5	-37.1
181	old control	0	0.0	-10.1	-24.6	-24.6
189	old control	0	6.5	9.7	11.3	37.1
196	old control	0	0.0	0.0	0.0	-20.4
203	old control	0	0.0	0.0	-1.6	1.6
208	old control	0	0.0	0.0	0.0	0.0
213	old control	0	4.2	-2.1	-8.3	-8.3
217	old control	0	2.1	2.1	2.1	2.1
222	old control	0	43.5	43.5	-21.7	-21.7
228	old control	0	17.9	3.6	3.6	3.6
232	old control	0	2.6	2.6	2.6	2.6
248	old control	0	13.9	13.9	10.9	10.9
249	old control	0	15.8	24.6	24.6	24.6
252	old control	0	5.5	12.3	35.6	39.7
253	old control	0	11.1	11.1	11.1	20.6
254	old control	0	5.7	17.0	15.1	15.1
260	old control	0	0.0	23.1	34.6	50.0
262	old control	0	3.9	9.8	19.6	19.6
265	old control	0	6.5	9.1	24.7	29.9
294	old control	0	0.0	-1.7	-1.7	-1.7
298	old control	0	1.0	12.4	14.4	19.6
303	old control	0	5.5	10.9	10.9	10.9
304	old control	0	0.0	0.0	3.8	3.8
306	old control	0	-0.7	20.3	30.1	30.1
312	old control	0	15.3	27.1	27.1	27.1
314	old control	0	65.4	65.4	65.4	46.2
319	old control	0	0.0	-7.9	0.0	0.0
	Mean	0.0	9.8	13.6	13.6	13.1
	Standard Error	0.0	3.2	3.6	4.0	4.5

Table 5-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
294	old tempol	0	15.7	19.6	27.5	27.5
298	old tempol	0	22.9	29.2	29.2	33.3
301	old tempol	0	0.9	20.7	42.3	42.3
303	old tempol	0	47.2	-11.1	-11.1	-11.1
304	old tempol	0	29.6	29.6	29.6	29.6
306	old tempol	0	9.7	29.1	31.1	31.1
311	old tempol	0	34.0	35.8	35.8	41.5
312	old tempol	0	13.6	22.7	22.7	22.7
314	old tempol	0	27.9	27.9	27.9	32.6
317	old tempol	0	37.3	79.5	90.4	100.0
	Mean	0.0	23.9	28.3	32.5	34.9
	Standard Error	0.0	4.4	7.0	7.8	8.6
294	old apocynin	0	6.6	14.2	14.2	14.2
298	old apocynin	0	4.2	21.1	22.5	36.6
301	old apocynin	0	8.0	12.0	12.0	13.3
303	old apocynin	0	11.3	26.8	26.8	28.2
304	old apocynin	0	25.0	26.1	27.3	27.3
306	old apocynin	0	40.2	53.7	53.7	56.1
312	old apocynin	0	11.7	11.7	11.7	11.7
314	old apocynin	0	20.2	25.5	42.6	42.6
317	old apocynin	0	4.0	17.2	19.2	19.2
	Mean	0.0	14.6	23.1	25.5	27.7
	Standard Error	0.0	4.0	4.3	4.8	5.0

Table 5-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
248	old catalase	0	9.1	9.1	6.5	7.8
249	old catalase	0	36.0	42.0	68.0	64.0
252	old catalase	0	30.6	79.2	90.3	91.7
262	old catalase	0	15.9	20.5	25.0	25.0
264	old catalase	0	45.7	45.7	45.7	45.7
265	old catalase	0	-11.1	13.0	18.5	20.4
268	old catalase	0	0.0	6.8	8.5	8.5
	Mean	0.0	18.0	30.9	37.5	37.6
	Standard Error	0.0	7.7	10.0	12.1	11.8
234	old pegcat	0	20.3	20.3	20.3	13.6
248	old pegcat	0	0.0	9.4	22.6	43.4
249	old pegcat	0	13.2	15.1	13.2	7.5
252	old pegcat	0	13.7	13.7	9.8	7.8
254	old pegcat	0	4.6	6.1	6.9	13.7
260	old pegcat	0	0.0	0.0	-12.5	-12.5
262	old pegcat	0	16.7	29.2	29.2	33.3
264	old pegcat	0	0.0	-19.6	10.7	10.7
	Mean	0.0	8.6	9.3	12.5	14.7
	Standard Error	0.0	3.0	5.2	4.5	6.0

Table 5-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
172	young control	18.8	11.3	11.3	11.3	11.3
174	young control	19.4	19.4	19.4	30.6	30.6
176	young control	27.7	27.7	9.6	-26.6	-29.8
183	young control	59.6	63.2	63.2	64.9	64.9
186	young control	29.2	29.2	29.2	25.0	31.3
195	young control	9.7	9.7	6.9	5.6	15.3
197	young control	15.6	20.0	28.9	33.3	33.3
199	young control	29.5	44.2	44.2	41.9	41.9
210	young control	42.4	42.4	42.4	42.4	42.4
216	young control	-9.5	-9.5	-9.5	-9.5	35.7
224	young control	30.0	13.3	13.3	-6.7	3.3
225	young control	23.8	23.8	29.2	30.8	43.8
230	young control	33.3	33.3	21.7	21.7	21.7
231	young control	19.4	17.7	17.7	17.7	17.7
235	young control	69.0	65.5	72.4	72.4	72.4
261	young control	58.7	58.7	58.7	44.4	55.6
263	young control	17.2	17.2	6.9	-6.9	-6.9
297	young control	15.7	15.7	21.7	21.7	38.6
305	young control	34.9	34.9	34.9	34.9	34.9
308	young control	5.6	-4.2	2.8	-19.4	-25.0
309	young control	1.8	7.1	8.9	12.5	12.5
310	young control	11.5	13.5	19.2	21.2	26.9
318	young control	16.7	-18.2	-18.2	-15.2	-13.6
	Mean	25.2	23.3	23.3	19.5	24.3
	Standard Error	3.9	4.3	4.5	5.6	5.2
297	young tempol	4.1	0.0	32.9	49.3	50.7
300	young tempol	7.7	-1.5	-1.5	-12.3	-12.3
302	young tempol	13.4	17.9	25.4	25.4	10.4
307	young tempol	12.0	20.5	48.7	48.7	45.3
308	young tempol	88.2	90.2	90.2	90.2	90.2
309	young tempol	22.0	36.6	36.6	36.6	24.4
313	young tempol	22.3	22.3	23.2	23.2	23.2
318	young tempol	26.1	26.1	28.4	28.4	38.6
	Mean	24.5	26.5	35.5	36.2	33.8
	Standard Error	9.5	10.2	9.3	10.3	10.8

Table 5-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
297	young apocynin	-11.6	-11.6	-11.6	-11.6	-11.6
300	young apocynin	25.0	14.3	32.1	37.5	39.3
302	young apocynin	11.7	11.7	15.0	15.0	15.0
305	young apocynin	36.8	52.6	52.6	66.7	66.7
307	young apocynin	18.6	23.7	25.4	25.4	25.4
308	young apocynin	19.7	19.7	19.7	-9.8	-34.4
309	young apocynin	12.8	7.8	7.8	3.5	10.6
310	young apocynin	16.3	18.4	18.4	18.4	18.4
313	young apocynin	13.1	13.1	13.1	8.2	0.8
318	young apocynin	17.5	17.5	21.1	26.3	26.3
	Mean	16.0	16.7	19.4	18.0	15.6
	Standard Error	3.5	4.6	4.7	6.6	7.9
235	young catalase	31.0	64.4	72.4	75.9	79.3
250	young catalase	15.9	11.1	15.9	11.1	23.8
255	young catalase	-7.5	9.4	39.6	39.6	37.7
256	young catalase	13.2	25.0	25.0	25.0	29.4
258	young catalase	13.9	11.1	8.3	8.3	8.3
259	young catalase	22.7	14.8	19.3	52.3	53.4
261	young catalase	12.7	12.7	12.7	12.7	12.7
263	young catalase	-21.3	-21.3	-21.3	-26.2	-26.2
	Mean	10.1	15.9	21.5	24.8	27.3
	Standard Error	5.9	8.4	9.5	11.0	11.1
250	young pegcat	33.3	33.3	58.3	58.3	18.8
255	young pegcat	36.1	33.3	47.2	55.6	55.6
256	young pegcat	49.3	49.3	30.4	30.4	31.9
257	young pegcat	15.5	17.2	17.2	20.7	20.7
258	young pegcat	-6.8	-5.1	-5.1	-3.4	-3.4
259	young pegcat	21.6	16.7	2.9	2.9	4.9
261	young pegcat	51.1	51.1	51.1	51.1	51.1
263	young pegcat	15.1	15.1	15.1	15.1	17.0
	Mean	26.9	26.4	27.2	28.8	24.6
	Standard Error	6.9	6.7	8.3	8.5	7.3

Table 5-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
173	old control	24.4	28.2	30.8	33.3	33.3
175	old control	46.5	46.5	46.5	48.8	48.8
177	old control	-37.1	-37.1	-75.8	-75.8	-75.8
181	old control	-24.6	17.4	42.0	50.7	50.7
189	old control	37.1	37.1	17.7	11.3	-1.6
196	old control	-27.8	-27.8	-33.3	-16.7	-16.7
203	old control	1.6	1.6	1.6	1.6	11.3
208	old control	0.0	40.4	23.1	1.9	-13.5
213	old control	-12.5	-12.5	-33.3	-33.3	-41.7
217	old control	2.1	-14.6	-14.6	-4.2	-4.2
222	old control	-21.7	-34.8	-34.8	-34.8	-39.1
228	old control	-50.0	-132.1	-132.1	-139.3	-157.1
232	old control	-5.1	-5.1	0.0	10.3	10.3
248	old control	10.9	12.9	18.8	18.8	18.8
249	old control	24.6	24.6	68.4	68.4	45.6
252	old control	39.7	39.7	39.7	39.7	47.9
253	old control	20.6	30.2	30.2	30.2	28.6
254	old control	9.4	9.4	9.4	9.4	9.4
260	old control	50.0	57.7	57.7	34.6	25.0
262	old control	-19.6	-31.4	-39.2	-43.1	-49.0
265	old control	31.2	28.6	28.6	24.7	24.7
294	old control	-1.7	-1.7	3.4	3.4	3.4
298	old control	35.1	40.2	41.2	43.3	43.3
303	old control	14.5	14.5	14.5	32.7	38.2
304	old control	3.8	3.8	3.8	3.8	7.5
306	old control	36.4	36.4	1.4	-2.1	-7.7
312	old control	21.2	21.2	17.6	17.6	17.6
314	old control	32.7	19.2	13.5	13.5	9.6
319	old control	-1.6	-1.6	-6.3	-6.3	-7.9
	Mean	8.3	7.3	4.8	4.9	2.1
	Standard Error	4.8	6.9	7.7	7.7	8.1

Table 5-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
294	old tempol	27.5	0.0	-17.6	-27.5	-27.5
298	old tempol	33.3	25.0	25.0	25.0	16.7
301	old tempol	38.7	38.7	33.3	33.3	26.1
303	old tempol	72.2	29.2	29.2	5.6	0.0
304	old tempol	29.6	29.6	29.6	38.0	38.0
306	old tempol	22.3	15.5	15.5	15.5	13.6
311	old tempol	41.5	41.5	41.5	41.5	41.5
312	old tempol	16.7	12.1	12.1	12.1	3.0
314	old tempol	32.6	32.6	32.6	32.6	32.6
317	old tempol	100.0	100.0	100.0	100.0	100.0
	Mean	41.4	32.4	30.1	27.6	24.4
	Standard Error	8.1	8.5	9.4	10.3	10.6
294	old apocynin	17.9	19.8	23.6	34.0	34.0
298	old apocynin	25.4	25.4	25.4	25.4	25.4
301	old apocynin	13.3	16.0	16.0	16.0	16.0
303	old apocynin	28.2	28.2	33.8	43.7	54.9
304	old apocynin	28.4	28.4	28.4	35.2	35.2
306	old apocynin	58.5	59.8	59.8	63.4	69.5
312	old apocynin	21.7	36.7	20.0	20.0	20.0
314	old apocynin	24.5	4.3	2.1	1.1	9.6
317	old apocynin	22.2	37.4	40.4	40.4	40.4
	Mean	26.7	28.4	27.7	31.0	33.9
	Standard Error	4.3	5.2	5.4	6.0	6.4

Table 5-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
248	old catalase	7.8	7.8	7.8	-2.6	-2.6
249	old catalase	74.0	78.0	78.0	78.0	86.0
252	old catalase	91.7	95.8	91.7	91.7	91.7
262	old catalase	25.0	31.8	31.8	31.8	15.9
264	old catalase	45.7	37.1	37.1	40.0	45.7
265	old catalase	20.4	20.4	5.6	-3.7	0.0
268	old catalase	8.5	8.5	8.5	8.5	8.5
	Mean	39.0	39.9	37.2	34.8	35.0
	Standard Error	12.4	13.0	13.2	14.4	15.1
234	old pegcat	13.6	8.5	5.1	5.1	5.1
248	old pegcat	50.9	54.7	54.7	47.2	47.2
249	old pegcat	7.5	7.5	-1.9	-13.2	-18.9
252	old pegcat	7.8	7.8	7.8	23.5	39.2
254	old pegcat	13.7	13.7	15.3	15.3	21.4
260	old pegcat	-20.8	-20.8	18.8	18.8	18.8
262	old pegcat	33.3	33.3	45.8	56.3	60.4
264	old pegcat	12.5	14.3	14.3	14.3	8.9
	Mean	14.8	14.9	20.0	20.9	22.8
	Standard Error	7.4	7.7	7.0	7.8	9.0

Table 6-NAD(P)H oxidase Studies Acetylcholine Data (Figs. 3.1-3.4)

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
172	young control	410	212	32.5
174	young control	420	185	30.3
176	young control	426	233	33.5
184	young control	400	167	38.3
186	young control	450	159	34.0
193	young control	380	209	54.1
195	young control	365	214	34.1
197	young control	328	149	38.3
199	young control	427	220	48.6
210	young control	311	99	45.5
218	young control	364	140	50.0
224	young control	342	89	34.8
225	young control	349	205	45.9
228	young control	377	163	32.5
231	young control	410	171	32.7
233	young control	381	138	36.2
235	young control	360	115.0	26.1
250	young control	359	113.0	25.7
259	young control	403	188	39.4
261	young control	372	186	25.3
263	young control	310	115	25.2
296	young control	335	128	61.7
297	young control	305	108	50.9
305	young control	396	182	36.8
308	young control	354	137	29.9
309	young control	407	172	37.8
310	young control	410	119	40.3
318	young control	376	163	49.7
	Mean		160.0	38.2
	Standard Error		7.5	1.8

Table 6-continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
296	young tempol		168	39.9
297	young tempol		136	27.2
300	young tempol		156	26.3
302	young tempol		188	30.9
307	young tempol		229	37.1
308	young tempol		178	42.1
309	young tempol		233	31.3
313	young tempol		159	52.8
318	young tempol		176	49.4
	Mean		180.3	37.5
	Standard Error		10.8	3.2
296	young apocynin		204	32.4
297	young apocynin		133	38.3
300	young apocynin		118	38.1
302	young apocynin		167	37.7
305	young apocynin		136	29.4
307	young apocynin		103	49.5
308	young apocynin		180	33.9
309	young apocynin		200	41.5
310	young apocynin		193	32.1
313	young apocynin		161	76.4
318	young apocynin		136	45.6
	Mean		157.4	41.4
	Standard Error		10.4	3.9
235	young catalase		136	30.9
250	young catalase		155	40.0
255	young catalase		158	59.5
256	young catalase		159	32.1
258	young catalase		93	31.2
259	young catalase		153	41.8
261	young catalase		159	39.0
263	young catalase		160	36.3
	Mean		146.6	38.8
	Standard Error		8.1	3.3

Table 6-continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
233	young pegcat		146	43.8
250	young pegcat		157	38.2
255	young pegcat		171	36.3
256	young pegcat		172	26.2
257	young pegcat		119	41.2
258	young pegcat		135	32.6
259	young pegcat		198	34.8
261	young pegcat		185	24.9
263	young pegcat		133	37.6
	Mean		157.3	35.1
	Standard Error		8.7	2.1

Table 6-continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
173	old control	438	125	33.6
175	old control	453	173	30.1
177	old control	450	174	57.5
181	old control	470	181	33.7
189	old control	395	139	29.5
192	old control	402	163	36.2
196	old control	452	180	35.6
203	old control	433	145	34.5
209	old control	357	110	46.4
211	old control	434	140	37.1
213	old control	396	147	34.0
217	old control	401	177	24.9
222	old control	400	101	31.7
248	old control	384	238	29.4
249	old control	309	190	28.4
252	old control	317	219	32.9
253	old control	407	190	37.4
254	old control	373	126	34.9
260	old control	385	116	40.5
262	old control	455	133	53.4
265	old control	449	151	39.7
294	old control	407	127	37.0
295	old control	454	234	29.9
298	old control	409	159	52.2
303	old control	429	136	27.9
304	old control	420	162	35.2
306	old control	404	191	58.1
312	old control	414	241	52.7
314	old control	463	128	41.4
319	old control	419	137	47.4
	Mean		161.1	42.4
	Standard Error		6.9	1.7

Table 6-continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
295	old tempol		195	29.7
298	old tempol		168	29.2
301	old tempol		206	51.9
303	old tempol		250	37.2
304	old tempol		199	33.7
306	old tempol		224	52.7
311	old tempol		124	31.5
312	old tempol		192	33.9
314	old tempol		212	42.9
317	old tempol		278	31.7
319	old tempol		166	35.5
	Mean		201.3	37.3
	Standard Error		12.6	2.5
222	old apocynin		222	30.2
298	old apocynin		165	38.8
303	old apocynin		244	61.1
304	old apocynin		155	42.6
306	old apocynin		165	25.5
311	old apocynin		197	30.4
314	old apocynin		205	49.3
317	old apocynin		232	43.5
	Mean		198.1	40.2
	Standard Error		10.1	3.5
232	old catalase		201	30.3
248	old catalase		227	40.5
252	old catalase		201	41.3
260	old catalase		221	37.1
262	old catalase		143	40.6
264	old catalase		106	25.5
265	old catalase		132	41.7
268	old catalase		175	39.4
	Mean		175.8	37.1
	Standard Error		15.7	2.1

Table 6-continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
234	old pegcat		189	31.7
248	old pegcat		124	42.7
249	old pegcat		179	27.4
252	old pegcat		203	24.6
254	old pegcat		207	42.0
260	old pegcat		115	35.7
262	old pegcat		156	28.8
264	old pegcat		147	33.3
	Mean		165.0	33.3
	Standard Error		12.4	2.3

Table 6-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
172	young control	0	30.4	69.6	85.5	89.9	89.9	89.9
174	young control	0	7.1	10.7	23.2	37.5	37.5	42.9
176	young control	0	6.4	10.3	12.8	38.5	42.3	61.5
184	young control	0	10.9	46.9	71.9	81.3	92.2	92.2
187	young control	0	9.3	22.2	63.0	64.8	64.8	66.7
193	young control	0	0.0	38.9	65.5	86.7	90.3	90.3
195	young control	0	6.8	56.2	56.2	87.7	91.8	91.8
197	young control	0	3.5	22.8	31.6	31.6	52.6	52.6
199	young control	0	38.3	32.7	69.2	69.2	92.5	92.5
210	young control	0	6.7	15.6	60.0	71.1	55.6	55.6
218	young control	0	11.4	22.9	37.1	58.6	54.3	62.9
224	young control	0	16.1	22.6	25.8	38.7	48.4	51.6
225	young control	0	8.5	13.8	78.7	88.3	93.6	93.6
228	young control	0	3.8	15.1	28.3	28.3	41.5	41.5
231	young control	0	26.8	28.6	46.4	46.4	51.8	57.1
233	young control	0	38.0	62.0	68.0	80.0	86.0	86.0
235	young control	0	23.3	46.7	50.0	53.3	80.0	80.0
250	young control	0	24.1	65.5	79.3	89.7	100.0	100.0
259	young control	0	32.4	59.5	70.3	71.6	71.6	71.6
261	young control	0	6.4	55.3	57.4	68.1	68.1	34.0
263	young control	0	24.1	62.1	65.5	75.9	75.9	75.9
296	young control	0	38.0	88.6	93.7	96.2	96.2	100.0
297	young control	0	12.7	20.0	25.5	29.1	30.9	34.5
305	young control	0	7.5	58.2	71.6	74.6	76.1	76.1
308	young control	0	22.0	56.1	75.6	80.5	87.8	87.8
309	young control	0	18.5	38.5	72.3	80.0	80.0	80.0
310	young control	0	25.0	35.4	77.1	93.8	93.8	93.8
318	young control	0	8.6	39.5	39.5	46.9	65.4	65.4
	Mean	0.0	16.7	39.9	57.2	66.4	71.8	72.4
	Standard Error	0.0	2.2	3.9	4.0	4.0	3.8	3.8

Table 6-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
296	young tempol	0	4.5	6.0	13.4	23.9	23.9	23.9
297	young tempol	0	13.5	81.1	81.1	91.9	91.9	91.9
300	young tempol	0	19.5	24.4	26.8	31.7	31.7	31.7
302	young tempol	0	3.4	6.9	6.9	13.8	13.8	17.2
307	young tempol	0	21.2	55.3	84.7	88.2	88.2	88.2
308	young tempol	0	44.0	46.7	88.0	94.7	94.7	97.3
309	young tempol	0	27.4	57.5	83.6	86.3	91.8	91.8
313	young tempol	0	4.8	8.3	9.5	16.7	20.2	20.2
318	young tempol	0	18.4	31.0	42.5	55.2	58.6	58.6
	Mean	0	17.4	35.2	48.5	55.8	57.2	57.9
	Standard Error	0	4.4	8.9	11.9	11.6	11.7	11.6
296	young apocynin	0	4.5	6.1	3.0	13.6	18.2	27.3
297	young apocynin	0	2.0	11.8	19.6	19.6	19.6	19.6
300	young apocynin	0	6.7	11.1	22.2	33.3	33.3	33.3
302	young apocynin	0	11.1	23.8	69.8	76.2	77.8	77.8
305	young apocynin	0	15.0	22.5	65.0	77.5	77.5	50.0
307	young apocynin	0	2.0	37.3	45.1	60.8	60.8	66.7
308	young apocynin	0	6.6	24.6	27.9	31.1	42.6	50.8
309	young apocynin	0	8.4	41.0	55.4	63.9	63.9	48.2
310	young apocynin	0	9.7	16.1	16.1	21.0	29.0	30.6
313	young apocynin	0	4.1	8.1	26.8	33.3	35.0	35.0
318	young apocynin	0	8.1	29.0	33.9	35.5	45.2	46.8
	Mean	0	7.1	21.0	35.0	42.3	45.7	44.2
	Standard Error	0.0	1.2	3.5	6.4	7.0	6.5	5.2
235	young catalase	0	7.1	19.0	42.9	66.7	73.8	73.8
250	young catalase	0	0.0	4.8	56.5	75.8	80.6	82.3
255	young catalase	0	9.6	40.4	43.6	51.1	51.1	47.9
256	young catalase	0	7.8	13.7	23.5	29.4	29.4	29.4
258	young catalase	0	17.2	31.0	34.5	37.9	41.4	48.3
259	young catalase	0	23.4	39.1	53.1	57.8	59.4	59.4
261	young catalase	0	1.6	17.7	22.6	22.6	22.6	22.6
263	young catalase	0	12.1	13.8	20.7	27.6	27.6	27.6
	Mean	0.0	9.9	22.5	37.2	46.1	48.2	48.9
	Standard Error	0.0	2.7	4.6	5.0	7.0	7.7	7.8

Table 6-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
233	young pegcat	0	18.8	37.5	45.3	46.9	46.9	40.6
250	young pegcat	0	3.3	3.3	33.3	40.0	40.0	40.0
255	young pegcat	0	3.2	25.8	40.3	74.2	67.7	67.7
256	young pegcat	0	15.6	24.4	51.1	60.0	57.8	57.8
257	young pegcat	0	44.9	55.1	67.3	69.4	69.4	69.4
258	young pegcat	0	22.7	40.9	43.2	45.5	54.5	54.5
259	young pegcat	0	36.2	55.1	55.1	52.2	52.2	59.4
261	young pegcat	0	26.1	52.2	58.7	58.7	58.7	58.7
263	young pegcat	0	6.0	8.0	8.0	8.0	16.0	16.0
	Mean	0.0	19.6	33.6	44.7	50.5	51.5	51.6
	Standard Error	0.0	4.9	6.5	5.7	6.5	5.4	5.6

Table 6-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
173	old control	0	9.5	9.5	11.9	23.8	28.6	28.6
175	old control	0	5.8	7.7	9.6	11.5	13.5	13.5
177	old control	0	2.0	15.0	18.0	22.0	22.0	27.0
181	old control	0	4.9	8.2	14.8	18.0	18.0	18.0
189	old control	0	19.5	41.5	41.5	43.9	46.3	46.3
193	old control	0	20.3	20.3	37.3	25.4	25.4	18.6
196	old control	0	15.6	26.6	51.6	60.9	60.9	50.0
203	old control	0	8.0	12.0	16.0	22.0	28.0	30.0
208	old control	0	0.0	25.5	35.3	70.6	78.4	78.4
211	old control	0	11.5	13.5	15.4	15.4	15.4	19.2
213	old control	0	6.0	10.0	26.0	32.0	32.0	32.0
217	old control	0	2.3	2.3	2.3	11.4	11.4	11.4
222	old control	0	31.3	53.1	53.1	53.1	56.3	56.3
248	old control	0	2.9	14.3	14.3	14.3	17.1	17.1
249	old control	0	18.5	29.6	29.6	29.6	37.0	37.0
252	old control	0	13.9	31.9	54.2	54.2	54.2	62.5
253	old control	0	2.8	22.5	32.4	32.4	35.2	35.2
254	old control	0	0.0	20.5	27.3	34.1	34.1	34.1
260	old control	0	6.4	14.9	29.8	38.3	38.3	38.3
262	old control	0	2.8	5.6	9.9	12.7	12.7	12.7
265	old control	0	8.3	15.0	16.7	20.0	20.0	25.0
294	old control	0	10.6	14.9	23.4	36.2	36.2	34.0
296	old control	0	22.9	28.6	45.7	68.6	68.6	68.6
298	old control	0	14.5	26.5	44.6	45.8	45.8	45.8
303	old control	0	0.0	28.9	34.2	55.3	84.2	84.2
304	old control	0	12.3	12.3	59.6	61.4	61.4	61.4
306	old control	0	16.2	27.0	33.3	37.8	41.4	41.4
312	old control	0	5.5	10.2	21.3	22.8	27.6	28.3
314	old control	0	9.4	11.3	32.1	41.5	49.1	49.1
319	old control	0	3.1	9.2	27.7	21.5	24.6	30.8
	Mean	0.0	10.5	18.8	35.8	43.4	48.8	49.3
	Standard Error	0.0	1.4	2.1	2.7	3.2	3.6	3.5

Table 6-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
295	old tempol	0	25.9	36.2	37.9	50.0	50.0	50.0
298	old tempol	0	16.3	26.5	28.6	28.6	30.6	30.6
301	old tempol	0	4.7	1.9	4.7	8.4	8.4	12.1
303	old tempol	0	26.9	86.0	90.3	90.3	95.7	95.7
304	old tempol	0	3.0	32.8	52.2	61.2	62.7	68.7
306	old tempol	0	11.9	14.4	16.1	22.0	20.3	20.3
311	old tempol	0	15.4	25.6	30.8	38.5	38.5	38.5
312	old tempol	0	7.7	10.8	13.8	16.9	24.6	24.6
314	old tempol	0	5.5	35.2	69.2	72.5	72.5	62.6
317	old tempol	0	43.2	48.9	64.8	80.7	80.7	92.0
319	old tempol	0	35.6	67.8	81.4	88.1	98.3	100.0
	Mean	0.0	17.8	35.1	44.5	50.7	52.9	54.1
	Standard Error	0.0	4.0	7.5	8.7	9.0	9.4	9.6
222	old apocynin	0	25.4	49.3	68.7	82.1	85.1	85.1
298	old apocynin	0	1.6	25.0	25.0	35.9	39.1	56.3
303	old apocynin	0	6.7	8.7	16.8	28.9	28.9	28.9
304	old apocynin	0	7.6	15.2	24.2	31.8	33.3	33.3
306	old apocynin	0	19.0	35.7	45.2	57.1	64.3	76.2
311	old apocynin	0	3.3	8.3	13.3	18.3	21.7	26.7
314	old apocynin	0	4.0	59.4	85.1	87.1	92.1	92.1
317	old apocynin	0	29.7	54.5	54.5	62.4	64.4	64.4
	Mean	0.0	12.2	32.0	41.6	50.5	53.6	57.9
	Standard Error	0.0	3.3	6.2	7.9	7.7	8.0	7.8
232	old catalase	0	18.0	26.2	26.2	26.2	32.8	42.6
248	old catalase	0	8.7	22.8	31.5	38.0	38.0	38.0
252	old catalase	0	2.4	8.4	41.0	41.0	41.0	33.7
260	old catalase	0	9.8	6.1	19.5	20.7	20.7	20.7
262	old catalase	0	3.4	19.0	24.1	24.1	24.1	24.1
264	old catalase	0	7.4	-7.4	0.0	11.1	29.6	33.3
265	old catalase	0	14.5	30.9	45.5	54.5	58.2	58.2
268	old catalase	0	5.8	24.6	36.2	53.6	66.7	66.7
	Mean	0.0	8.8	16.3	28.0	33.7	38.9	39.7
	Standard Error	0.0	1.9	4.5	5.0	5.6	5.7	5.6

Table 6-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
234	old pegcat	0.0	3.3	26.7	53.3	56.7	56.7	36.7
248	old pegcat	0.0	20.8	32.1	75.5	83.0	83.0	79.2
249	old pegcat	0.0	38.8	46.9	55.1	61.2	69.4	69.4
252	old pegcat	0.0	10.0	10.0	18.0	24.0	34.0	34.0
254	old pegcat	0.0	16.1	59.8	88.5	94.3	94.3	97.7
260	old pegcat	0.0	24.4	34.1	43.9	53.7	53.7	65.9
262	old pegcat	0.0	11.1	20.0	26.7	35.6	31.1	31.1
264	old pegcat	0	10.2	28.6	46.9	46.9	49.0	49.0
	Mean	0.0	16.8	32.3	51.0	56.9	58.9	57.9
	Standard Error	0.0	3.9	5.5	8.2	8.2	7.9	8.5

Table 7-Desitometry Values for NAD(P)H oxidase subunits (Fig 3.5)

vessel number	group	Proteins of Interest		
p47phox blot 1		p47phox	GAPDH	p47phox/GAPDH
370	young	5864595	4407352	1.330639123
364	young	5879287	5334629	1.102098571
352	young	5930326	3455044	1.716425608
333	young	6304112	17451502	0.36123607
255	old	5838244	16751141	0.348528139
244	old	5691892	11514471	0.494325098
227	old	5620591	2786015	2.017430272
203	old	5516961	2298337	2.4004143
p47phox blot 3		p47phox	GAPDH	p47phox/GAPDH
381	young	7258894	6616260	1.097129496
378	young	7203141	4639246	1.552653384
375	young	7422915	8781398	0.845299917
297	young	7147970	2492305	2.868015752
305	old	7139554	10037328	0.711300258
298	old	7041274	9479873	0.742760372
290	old	7216947	11161924	0.646568369
281	old	7353907	13091304	0.561739839
p47phox blot 5		p47phox	GAPDH	p47phox/GAPDH
414	young	8253038	2453106	3.364321803
407	young	8572007	2102344	4.077356988
400	young	7896528	2832937	2.78739979
393	young	8513270	4665754	1.824628988
341	old	7121896	2667032	2.670345163
336	old	7087578	1996620	3.549788142
325	old	7704696	2553266	3.017584537
315	old	7634665	3252081	2.34762449
p67phox blot 1		p67phox	GAPDH	p67phox/GAPDH
429	young	9222415	2281773	4.041775847
512	young	13959631	4409076	3.166112582
505	young	10192716	3372015	3.022737443
479	young	11106077	4540184	2.446173327
399	old	28007162	2418743	11.57922193
367	old	11338234	4138319	2.739816336
346	old	12323674	2517112	4.895957748
293	old	12399374	2372261	5.226816948

Table 7-continued

vessel number	group	Proteins of Interest		
p67phox blot 2		p67phox	GAPDH	p67phox/GAPDH
570	young	10022428	5940240	1.687209271
562	young	12417851	6715549	1.84911926
555	young	12625117	7006019	1.802038647
550	young	16836974	5752366	2.926965009
531	old	12252584	3223456	3.801070652
528	old	15287598	7792248	1.961898287
524	old	14557091	7181606	2.026996608
517	old	11029394	7646110	1.442484348
p67phox blot 3		p67phox	GAPDH	p67phox/GAPDH
590	young	7368272	15367078	0.479484258
586	young	7716745	4923909	1.567198947
580	young	7814225	4063643	1.922960506
574	young	9652465	13675397	0.705827041
578	old	8345864	6547088	1.274744436
564	old	16781173	11411190	1.47058922
558	old	12760400	15492131	0.823669771
552	old	9678698	16666449	0.580729464
gp91phox blot 2		gp91phox	GAPDH	gp91phox/GAPDH
383	young			
379	young	14980223	6549204	2.287334919
376	young	13959503	8226749	1.696843188
372	young	19315715	5283699	3.655718276
289	old	20144615	3831059	5.258236691
279	old	18181005	9369393	1.940467755
270	old	18301921	9450283	1.93665322
262	old	13211984	7347843	1.798076524
gp91phox blot 3		gp91phox	GAPDH	gp91phox/GAPDH
413	young	59477230	13945115	4.265094264
406	young	21766735	8929929	2.437503702
399	young	30923712	12914238	2.394544068
392	young	34197399	16430688	2.081312663
323	old	33225930	7532210	4.411179455
314	old	19266565	4295835	4.484940646
306	old	27956508	7351330	3.802918383
299	old	35202780	5620353	6.263446442

Table 7-continued

vessel number	group	Proteins of Interest		
gp91phox blot 4		gp91phox	GAPDH	gp91phox/GAPDH
571	young	25704780	9052276	2.839593048
563	young	63883966	14729185	4.337236989
556	young	19500525	9346475	2.086404233
515	young	25457601	13842869	1.839040809
532	old	26834993	3252672	8.25013804
525	old	37806172	9614222	3.932317352
518	old	36315744	12740311	2.850459773
509	old	73823130	16212479	4.553475752
Nox-1 blot 1		Nox-1	GAPDH	Nox-1/GAPDH
617	young	3449531	6762095	0.510127557
600	young	3501441	4030159	0.868809642
592	young	3495560	3451827	1.012669523
582	young	4649583	3330833	1.395921981
605	old	4375273	9919854	0.441062237
602	old	5065153	12166826	0.416308493
596	old	4377509	4471742	0.978927004
579	old	3390951	14550423	0.233048276
Nox-1 blot 2		Nox-1	GAPDH	Nox-1/GAPDH
584	young	3059447	25065185	0.122059622
924	young	4258001	2227435	1.911616276
920	young	8241894	2891117	2.850764601
917	young	5654112	7723003	0.732113143
830	old	4370977	3623294	1.206354494
739	old	5328255	3653720	1.458309613
766	old	6800507	5254701	1.294175825
778	old	6984966	13643696	0.511955558
Nox-1 blot 3		Nox-1	GAPDH	Nox-1/GAPDH
630	young	8446913	2702301	3.125822401
635	young	16410521	4039540	4.062472707
646	young	14907351	3563976	4.182786584
653	young	7665346	15820195	0.484529173
648	old	14021366	2772433	5.057422848
665	old	7488559	2937544	2.549258496
672	old	7870742	3656841	2.152333667
674	old	7527670	5430358	1.38621984

Table 8-Desitometry Values for Antioxidant Enzyme Blots (Fig 3.6)

vessel number	**group**	**Proteins of Interest**		
SOD-1 blot 1				
vessel		SOD-1	GAPDH	SOD-1/GAPDH
464	young	12104554	9915949	1.220715637
452	young	16059374	23152544	0.693633235
427	young			
334	young	11376696	10121845	1.123974532
282	old	14434554	29530290	0.48880502
272	old	10906705	18319706	0.595353714
228	old	28217431	23023611	1.225586681
204	old	4620910	3510003	1.31649745
SOD-1 blot 2				
vessel		SOD-1	GAPDH	SOD-1/GAPDH
504	young	9546494	4190601	2.278072763
500	young	7770925	5066725	1.533717539
496	young	9562603	4551842	2.100820503
478	young	3520548	9758433	0.360769808
355	old	4330903	6468534	0.669533931
345	old	6946114	4369510	1.589678019
301	old	9794227	3124982	3.134170693
292	old	23354586	2804949	8.326207001
SOD-1 blot 3				
Vessel		SOD-1	GAPDH	SOD-1/GAPDH
413	young	9111078	13945115	0.653352661
406	young	6643978	8929929	0.74401241
399	young	6834618	12914238	0.529231225
392	young	6281407	16430688	0.38229726
323	old	4688900	7532210	0.62251318
314	old	3258808	4295835	0.758597106
306	old	4554832	7351330	0.619592917
299	old	6449425	5620353	1.147512443

Table 8-continued

vessel number	group	Proteins of Interest		
MnSOD blot 1		MnSOD	GAPDH	MnSOD/GAPDH
468	young	3398644	7842486	0.433363094
453	young	3744627	10346037	0.361938296
428	young	3640163	9416785	0.386561125
476	young	3002041	6153413	0.487866002
300	old	5902089	6129809	0.962850392
291	old	3496525	4231260	0.826355506
283	old	3494639	6607240	0.528910559
229	old	4448641	4574988	0.972383097
MnSOD blot 2		MnSOD	GAPDH	MnSOD/GAPDH
499	young	6898605	17774747	0.388112697
495	young	3968572	7442447	0.533234835
492	young	4532549	23272870	0.19475677
477	young	4315838	13432100	0.321307763
354	old	5390392	15024802	0.358766259
344	old	4500649	7718471	0.583101109
327	old	3917999	6856429	0.571434343
ecSOD blot 1		ecSOD	GAPDH	ecSOD/GAPDH
464	young	4141479	9915949	0.41765836
452	young	5070826	23152544	0.219018091
427	young			
334	young	4640671	10121845	0.458480741
282	old	3637493	29530290	0.12317837
272	old	4433383	18319706	0.242000772
228	old	7931129	23023611	0.344478066
204	old	4692795	3510003	1.33697749
ecSOD blot 2		ecSOD	GAPDH	ecSOD/GAPDH
548	young	16670697	5786059	2.881183375
545	young	19744484	5790890	3.409576766
514	young	14106639	6285480	2.244321675
506	young	15629995	5139987	3.040862749
460	old	16509289	7671240	2.152101746
450	old	18468999	8258413	2.236385974
443	old	12620068	4192132	3.010417611
463	old	14875133	5410702	2.749205741

Table 8-continued

vessel number	**group**	**Proteins of Interest**		
ecSOD blot 3		ecSOD	GAPDH	ecSOD/GAPDH
548	young	16670697	5786059	2.881183375
545	young	19744484	5790890	3.409576766
514	young	14106639	6285480	2.244321675
506	young	15629995	5139987	3.040862749
460	old	16509289	7671240	2.152101746
450	old	18468999	8258413	2.236385974
443	old	12620068	4192132	3.010417611
463	old	14875133	5410702	2.749205741
catalase blot 1		Catalase	GAPDH	Catalase/GAPDH
547	young	21742978	1827179	11.89975257
543	young	4816450	1800401	2.675209578
542	young	2769842	1691305	1.637695153
513	young	2933310	2159447	1.358361655
434	old	2706803	1690353	1.601324102
437	old	2813523	2568708	1.095306668
411	old	2730667	1697684	1.608466004
390	old	6136334	1742953	3.520653741
catalase blot 2		Catalase	GAPDH	Catalase/GAPDH
548	young	11447265	5786059	1.978421755
545	young	6456596	5790890	1.114957459
514	young	5662084	6285480	0.900819667
506	young	6482470	5139987	1.261184124
460	old	13733423	7671240	1.790248122
450	old	10645388	8258413	1.289035557
443	old	6752264	4192132	1.610699281
463	old	6357568	5410702	1.174998734

Table 9-SOD Activity Assay Data (Fig. 3.6)

Sample #	Group	Mean Absorbance	SOD activity
465	young	0.0475	0.075464427
534	young	0.0365	0.122170953
486	old	0.0125	0.509410513
527	old	0.0125	0.509410513
475	young	0.0555	0.05312486
536	young	0.038	0.114209613
502	old	0.017	0.353518117
529	old	0.0125	0.509410513
503	young	0.038	0.114209613
546	young	0.0285	0.178784923
510	old	0.0185	0.318407216
530	old	0.0195	0.298000881
520	young	0.032	0.150533225
146	young	0.0275	0.188177696
516	old	0.0165	0.366640372
540	old	0.019	0.307935545

Table 10-Desitometry Values for Nitrotyrosine (NT) blots (Fig. 3.7)

vessel number	**group**	**Proteins of Interest**		
NT blot 1		NT	GAPDH	NT/GAPDH
370	young	9579240	4407352	2.173468332
364	young	83535635	5334629	15.65912737
352	young	51776323	3455044	14.9857203
333	young	24134929	17451502	1.382971449
255	old	8790950	16751141	0.524797087
244	old	30864941	11514471	2.680534868
227	old	16166290	2786015	5.8026572
203	old	7284254	2298337	3.169358541
NT blot 2		NT	GAPDH	NT/GAPDH
384	young	39824448	2001457	19.8977285
380	young	43178603	2078275	20.77617399
377	young	47498401	2210296	21.48961089
374	young	56131860	3478339	16.13754726
289	old	60379359	1930523	31.27616661
280	old	45951849	2451471	18.74460232
271	old	65226792	1884293	34.616056
264	old	39069201	2096791	18.6328542
NT blot 3		NT	GAPDH	NT/GAPDH
414	young	10180947	2453106	4.1502271
407	young	9587358	2102344	4.560318387
400	young	24699347	2832937	8.718636172
393	young	12313067	4665754	2.639030476
341	old	18654585	2667032	6.994511127
336	old	9795294	1996620	4.905938035
325	old	14782672	2553266	5.789710904
315	old	27973224	3252081	8.601638151
NT blot 4		NT	GAPDH	NT/GAPDH
570	young	28745336	5940240	4.839086636
562	young	24441521	6715549	3.639541756
555	young	20787591	7006019	2.967104571
550	young	28063496	5752366	4.878600562
531	old	25145434	3223456	7.800768492
528	old	26537579	7792248	3.405638399
524	old	31751116	7181606	4.421172089
517	old	77024236	7646110	10.07365

Table 11-PI3K Inhibitor Studies Flow Data (Fig. 4.1 & 4.2)

Animal	Group	Body Weight(g)	Maximal Diameter (µm)	% Spontaneous Tone
131	young control	282	165	32.7
133	young control	359	223	42.6
151	young control	345	210	27.6
154	young control	350	195	47.7
156	young control	352	136	75.7
158	young control	375	157	31.2
164	young control	370	140	35.7
165	young control	358	177	72.3
166	young control	367	199	27.6
168	young control	401	213	39.4
170	young control	390	159	54.1
	Mean		179.5	43.3
	Standard Error		9.1	5.1
151	young + LY-294002		216	36.1
154	young + LY-294002		148	41.2
156	young + LY-294002		139	50.4
158	young + LY-294002		205	46.3
160	young + LY-294002		199	46.7
164	young + LY-294002		195	30.8
165	young + LY-294002		203	43.8
166	young + LY-294002		210	41.0
168	young + LY-294002		218	25.2
170	young + LY-294002		158	42.4
	Mean		189.1	40.4
	Standard Error		9.3	40.8
112	old control	445	149	75.8
132	old control	406	178	68.0
153	old control	432	207	39.1
155	old control	420	187	39.6
157	old control	422	158	72.2
161	old control	402	117	28.2
163	old control	425	237	49.8
167	old control	410	235	72.3
169	old control	400	208	30.8
	Mean		186.2	52.9
	Standard Error		13.4	6.4

Table 11-continued

Animal	Group	Body Weight (g)	Maximal Diameter (µm)	% Spontaneous Tone
153	old + LY-294002		201	28.9
155	old + LY-294002		166	51.8
157	old + LY-294002		98	32.7
161	old + LY-294002		171	71.3
163	old + LY-294002		214	43.5
167	old + LY-294002		247	33.2
169	old + LY-294002		198	38.4
	Mean		185.0	42.8
	Standard Error		17.8	5.6

Table 11-continued

Animal	Group	Start	Δ2cmH_20	Δ4cmH_20	Δ6cmH_20	Δ8cmH_20
131	young control	0	0.0	16.7	16.7	16.7
133	young control	0	7.4	8.4	8.4	9.5
151	young control	0	0.0	10.3	10.3	10.3
154	young control	0	10.8	10.8	10.8	14.0
156	young control	0	0.0	32.0	82.5	82.5
158	young control	0	55.1	57.1	44.9	44.9
164	young control	0	10.0	24.0	30.0	28.0
165	young control	0	14.8	17.2	17.2	18.8
166	young control	0	3.6	3.6	5.5	0.0
168	young control	0	10.7	19.0	19.0	19.0
170	young control	0	1.2	1.2	2.3	4.7
	Mean	0.0	10.3	18.2	22.5	22.6
	Standard Error	0.0	4.8	4.7	7.0	7.0
151	young + LY-294002	0	1.3	3.8	20.5	17.9
154	young + LY-294002	0	0.0	-6.6	-23.0	-36.1
156	young + LY-294002	0	0.0	-4.3	-2.9	-2.9
158	young + LY-294002	0	12.6	12.6	0.0	0.0
160	young + LY-294002	0	0.0	-3.2	-15.1	-23.7
164	young + LY-294002	0	0.0	0.0	-26.7	-26.7
165	young + LY-294002	0	0.0	0.0	18.0	25.8
166	young + LY-294002	0	14.0	19.8	19.8	19.8
168	young + LY-294002	0	-49.1	-49.1	-49.1	-9.1
170	young + LY-294002	0	13.4	29.9	64.2	64.2
	Mean	0.0	-0.8	0.3	0.6	2.9
	Standard Error	0.0	-0.3	0.0	-0.4	0.5
112	old control	0	0.0	0.0	2.7	7.1
132	old control	0	1.7	4.1	5.0	8.3
153	old control	0	0.0	0.0	8.6	9.9
155	old control	0	0.0	0.0	-10.8	-10.8
157	old control		10.5	23.7	23.7	42.1
161	old control	0	0.0	-9.1	-9.1	-9.1
163	old control	0	4.2	4.2	0.8	0.8
167	old control	0	0.6	2.4	1.2	1.2
169	old control	0	7.8	15.6	15.6	6.3
	Mean	0	2.8	4.5	4.2	6.2
	Standard Error	0.0	1.3	3.2	3.6	5.1

Table 11-continued

Animal	Group	Start	Δ2cmH_20	Δ4cmH_20	Δ6cmH_20	Δ8cmH_20
153	old + LY-294002	0	5.2	5.2	5.2	0.0
155	old + LY-294002	0	64.0	67.4	64.0	32.6
157	old + LY-294002	0	28.1	28.1	31.3	31.3
161	old + LY-294002	0	0.0	0.0	1.6	4.1
163	old + LY-294002		0.0	-6.5	-6.5	-6.5
167	old + LY-294002		7.3	2.4	-7.3	-7.3
169	old + LY-294002		0.0	0.0	-1.3	-3.9
	Mean	0	14.9	13.8	12.4	7.2
	Standard Error	0.0	9.0	9.9	9.9	6.6

Table 11-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
131	young control	13.0	13.0	-20.4	-20.4	68.5
133	young control	9.5	10.5	11.6	14.7	29.5
151	young control	10.3	10.3	-15.5	-15.5	-24.1
154	young control	14.0	14.0	15.1	18.3	30.1
156	young control	66.0	68.0	68.0	66.0	65.0
158	young control	40.8	79.6	79.6	79.6	32.7
164	young control	18.0	16.0	40.0	40.0	40.0
165	young control	18.8	18.8	18.8	18.8	18.8
166	young control	3.6	18.2	21.8	21.8	9.1
168	young control	14.3	14.3	14.3	8.3	8.3
170	young control	4.7	4.7	10.5	10.5	18.6
	Mean	19.4	24.3	22.1	22.0	26.9
	Standard Error	5.5	7.5	9.2	9.1	7.9
151	young + LY-294002	17.9	21.8	21.8	21.8	21.8
154	young + LY-294002	-54.1	-70.5	-62.3	-59.0	-39.3
156	young + LY-294002	-1.4	22.9	30.0	32.9	32.9
158	young + LY-294002	0.0	-5.3	-5.3	-5.3	-14.7
160	young + LY-294002	-10.8	-10.8	-10.8	-8.6	-8.6
164	young + LY-294002	-38.3	-30.0	13.3	8.3	-6.7
165	young + LY-294002	34.8	36.0	46.1	53.9	46.1
166	young + LY-294002	19.8	31.4	36.0	36.0	26.7
168	young + LY-294002	3.6	7.3	7.3	3.6	-14.5
170	young + LY-294002	49.3	41.8	32.8	23.9	23.9
	Mean	2.1	4.5	10.9	10.8	6.7
	Standard Error	0.2	0.9	3.1	3.1	1.7
112	old control	6.2	8.8	20.4	20.4	20.4
132	old control	9.9	9.9	14.0	14.0	14.0
153	old control	9.9	9.9	6.2	6.2	6.2
155	old control	-10.8	-12.2	-17.6	-6.8	-4.1
157	old control	42.1	24.6	20.2	14.9	13.2
161	old control	3.0	3.0	9.1	0.0	0.0
163	old control	0.8	0.8	0.8	-9.3	-47.5
167	old control	1.2	1.2	-7.6	-19.4	-19.4
169	old control	6.3	-12.5	-12.5	-12.5	3.1
	Mean	7.6	3.7	3.7	0.8	-1.6
	Standard Error	4.8	3.9	4.6	4.6	6.9

Table 11-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
153	old + LY-294002	0.0	3.4	0.0	-19.0	-32.8
155	old + LY-294002	-47.7	-55.8	-59.3	-57.0	-57.0
157	old + LY-294002	34.4	34.4	37.5	37.5	37.5
161	old + LY-294002	4.1	4.1	8.2	9.8	9.8
163	old + LY-294002	-6.5	-6.5	-6.5	-57.0	-51.6
167	old + LY-294002	-3.7	-1.2	3.7	14.6	14.6
169	old + LY-294002	-3.9	-3.9	-7.9	3.9	26.3
	Mean	-3.3	-3.6	-3.5	-9.6	-7.6
	Standard Error	9.1	10.1	10.9	13.8	14.6

Table 12-Akt Inhibitor Studies Acetylcholine Data (Fig. 4.1 & 4.2)

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
131	young control	282	165	52.7
133	young control	359	223	45.7
151	young control	345	210	40.0
156	young control	352	136	53.7
165	young control	358	177	64.4
166	young control	367	199	25.6
168	young control	401	213	53.1
170	young control	390	159	50.3
	Mean		185.3	48.2
	Standard Error		10.8	4.1
151	young + LY-294002		216	30.6
154	young + LY-294002		148	39.2
156	young + LY-294002		139	36.0
158	young + LY-294002		205	43.4
160	young + LY-294002		199	41.2
165	young + LY-294002		203	41.4
166	young + LY-294002		210	31.9
168	young + LY-294002		218	25.2
170	young + LY-294002		158	34.8
	Mean		188.4	36.0
	Standard Error		10.3	2.0
112	old control	445	149	47.7
132	old control	406	178	43.3
153	old control	432	207	46.4
155	old control	420	187	43.9
157	old control	422	158	62.0
161	old control	402	117	24.8
163	old control	425	237	31.2
167	old control	410	235	37.0
169	old control	400	208	32.7
	Mean		186.2	41.0
	Standard Error		13.4	3.7

Table 12-continued

Animal	Group	Body Weight (g)	Maximal Diameter (μm)	% Spontaneous Tone
153	old + LY-294002		201	50.2
155	old + LY-294002		166	74.1
157	old + LY-294002		98	23.5
161	old + LY-294002		171	59.1
163	old + LY-294002		214	41.6
167	old + LY-294002		247	30.0
169	old + LY-294002		198	35.9
	Mean		185.0	44.9
	Standard Error		17.8	6.6

Table 12-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
131	young control	0	16.1	80.5	74.7	80.5	80.5	80.5
133	young control	0	6.9	34.3	54.9	63.7	63.7	63.7
151	young control	0	11.9	11.9	19.0	29.8	39.3	42.9
156	young control	0	13.7	61.6	89.0	94.5	94.5	94.5
165	young control	0	11.4	14.9	31.6	34.2	37.7	42.1
166	young control	0	29.4	54.9	54.9	62.7	78.4	78.4
168	young control	0	3.5	6.2	83.2	91.2	97.3	100.0
390	young control	0	55.0	55.0	88.8	91.3	93.8	93.8
	Mean	0	18.5	39.9	62.0	68.5	73.2	74.5
	Standard Error	0	5.9	9.6	9.4	9.0	8.5	8.1
151	young + LY-294002	0	15.2	28.8	34.8	60.6	66.7	69.7
154	young + LY-294002	0	1.7	1.7	1.7	3.4	3.4	10.3
156	young + LY-294002	0	6.0	24.0	68.0	94.0	96.0	96.0
158	young + LY-294002	0	0.0	-2.2	0.0	5.6	16.9	18.0
160	young + LY-294002	0	9.8	22.0	25.6	29.3	45.1	45.1
165	young + LY-294002	0	6.0	9.5	51.2	54.8	70.2	72.6
166	young + LY-294002	0	10.4	10.4	16.4	16.4	16.4	16.4
168	young + LY-294002	0	18.2	23.6	29.1	30.9	52.7	52.7
170	young + LY-294002	0	3.6	3.6	49.1	60.0	60.0	60.0
	Mean	0	7.9	13.5	30.7	39.4	47.5	49.0
	Standard Error	0	2.0	3.8	7.6	10.0	10.0	9.8

Table 12-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
112	old control	0	1.4	9.9	18.3	46.5	54.9	54.9
132	old control	0	0.0	3.9	26.0	36.4	40.3	45.5
153	old control	0	0.0	6.3	10.4	13.5	16.7	17.7
155	old control	0	11.0	20.7	41.5	47.6	51.2	54.9
157	old control	0	11.2	31.6	31.6	46.9	55.1	61.2
161	old control	0	20.7	37.9	65.5	79.3	79.3	75.9
163	old control	0	9.5	16.2	29.7	39.2	37.8	37.8
167	old control	0	10.3	27.6	49.4	49.4	40.2	54.0
169	old control	0	8.8	51.5	52.9	52.9	52.9	52.9
	Mean	0	8.1	22.8	36.2	45.7	47.6	50.5
	Standard Error	0	2.2	5.3	5.9	5.7	5.7	5.4
153	old + LY-294002	0	9.9	11.9	20.8	50.5	54.5	54.5
155	old + LY-294002	0	0.0	7.3	22.0	43.9	48.0	48.0
157	old + LY-294002	0	4.3	8.7	4.3	4.3	4.3	4.3
161	old + LY-294002	0	1.0	2.0	4.0	8.9	10.9	16.8
163	old + LY-294002	0	0.0	4.5	4.5	5.6	11.2	11.2
167	old + LY-294002	0	0.0	12.2	18.9	18.9	28.4	28.4
169	old + LY-294002	0	1.4	12.7	26.8	42.3	45.1	45.1
	Mean	0	2.4	8.5	14.5	24.9	28.9	29.8
	Standard Error	0	1.4	1.6	3.7	7.6	7.7	7.5

Table 13-Akt Inhibitor Studies Acetylcholine Data (Fig. 4.1 & 4.2)

Animal	Group	Body Weight (g)	Initial Diameter (µm)	% Spontaneous Tone
23	young control	321	103	44.7
26	young control	321	172.5	38.3
28	young control	332	155	26.5
29	young control	320	177.5	52.1
30	young control	345	129	63.4
32	young control	325	149.5	41.7
34	young control	328	140	53.6
36	young control	332	180	74.0
38	young control	358	153	47.1
109	young control	362	173	29.5
	Mean		153.3	47.1
	Standard Error		7.7	4.6
93	young Akt Inhibitor		160	43.8
95	young Akt Inhibitor		196	69.4
98	young Akt Inhibitor		171	56.7
100	young Akt Inhibitor		140	59.3
102	young Akt Inhibitor		160	45.0
104	young Akt Inhibitor		205	66.8
106	young Akt Inhibitor		133	45.1
109	young Akt Inhibitor		187	50.8
110	young Akt Inhibitor		176	38.6
111	young Akt Inhibitor		170	38.8
	Mean		169.8	51.4
	Standard Error		7.2	3.5

Table 13-continued

Animal	Group	Body Weight (g)	Initial Diameter (µm)	% Spontaneous Tone
19	old control	425	154.5	36.3
21	old control	446	161.5	31.2
22	old control	430	173	55.0
24	old control	423	160	33.8
25	old control	424	186	55.4
27	old control	425	169	35.6
31	old control	383	206	77.2
33	old control	429	217.5	38.6
35	old control	361	202	40.1
37	old control	424	185	43.8
49	old control	383	202	29.7
94	old control	455	187	43.9
99	old control	453	132	68.2
	Mean		179.7	45.3
	Standard Error		6.7	4.1
49	old Akt Inhibitor		115	36.5
94	old Akt Inhibitor		157	33.8
96	old Akt Inhibitor		206	31.6
97	old Akt Inhibitor		225	25.3
99	old Akt Inhibitor		135	43.0
101	old Akt Inhibitor		193	45.1
103	old Akt Inhibitor		188	33.5
105	old Akt Inhibitor		147	39.5
107	old Akt Inhibitor		162	35.2
108	old Akt Inhibitor		185	57.8
	Mean		171.3	38.1
	Standard Error		17.1	3.6

Table 13-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
23	young control	0	7.1	14.1	14.7	13.6
26	young control	0	23.4	26.4	31.0	30.1
28	young control	0	22.0	56.1	63.4	51.2
29	young control	0	44.2	47.9	45.0	39.7
30	young control	0	6.6	14.5	28.7	37.4
32	young control	0	17.0	23.6	24.7	26.2
34	young control	0	30.7	29.3	16.0	12.0
36	young control	0	8.5	9.8	6.5	3.5
38	young control	0	26.4	26.4	20.8	16.7
109	young control	0	3.9	5.9	13.7	13.7
	Mean	0.0	19.0	25.4	26.4	24.4
	Standard Error	0.0	4.1	5.1	5.4	4.8
93	young Akt Inhibitor	0	7.1	7.1	31.4	47.1
95	young Akt Inhibitor	0	19.9	46.3	91.2	92.6
98	young Akt Inhibitor	0	0.0	-5.2	-5.2	-9.3
100	young Akt Inhibitor	0	18.1	25.3	25.3	18.1
102	young Akt Inhibitor	0	0.0	13.9	13.9	13.9
104	young Akt Inhibitor	0	2.2	2.2	-2.2	-1.5
106	young Akt Inhibitor	0	1.7	1.7	21.7	38.3
109	young Akt Inhibitor	0	0.0	-8.4	-15.8	-31.6
110	young Akt Inhibitor	0	8.8	8.8	8.8	5.9
111	young Akt Inhibitor	0	10.6	10.6	10.6	-9.1
	Mean	0	6.8	10.2	18.0	16.5
	Standard Error	0	2.4	5.0	9.3	11.2

Table 13-continued

Animal	Group	Start	Δ2cmH_2O	Δ4cmH_2O	Δ6cmH_2O	Δ8cmH_2O
19	old control	0	8.2	3.0	4.9	0.5
21	old control	0	4.2	-4.6	-23.6	-37.0
22	old control	0	-0.5	-1.1	12.6	4.3
24	old control	0	-7.4	-5.6	-9.3	-11.1
25	old control	0	12.6	15.5	23.3	-1.0
27	old control	0	17.5	20.6	24.2	23.0
31	old control	0	1.9	1.9	0.9	2.8
33	old control	0	18.2	18.4	1.2	1.8
35	old control	0	17.3	23.5	18.5	6.2
37	old control	0	61.7	65.4	61.7	40.7
49	old control	0	5.0	5.0	0.0	0.0
94	old control	0	1.2	7.3	14.6	14.6
99	old control	0	0.0	1.1	3.3	3.3
	Mean	0	10.8	11.6	10.2	3.7
	Standard Error	0	4.8	5.2	5.7	4.9
49	old Akt Inhibitor	0	14.3	16.7	26.2	28.6
94	old Akt Inhibitor	0	9.4	9.4	-11.3	-11.3
96	old Akt Inhibitor	0	1.5	6.2	6.2	15.4
97	old Akt Inhibitor	0	0.0	-1.8	-29.8	-19.3
99	old Akt Inhibitor	0	34.5	34.5	34.5	17.2
101	old Akt Inhibitor	0	10.3	10.3	-3.4	9.2
103	old Akt Inhibitor	0	15.9	34.9	34.9	31.7
105	old Akt Inhibitor	0	91.4	100.0	100.0	100.0
107	old Akt Inhibitor	0	0.0	0.0	-7.0	-19.3
108	old Akt Inhibitor	0	0.0	13.1	-23.4	-34.6
	Mean	0	17.7	22.3	12.7	11.8
	Standard Error	0	7.7	8.4	10.4	10.5

Table 13-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
23	young control	13.9	2.8	-0.4	4.1	8.5
26	young control	26.9	28.5	26.9	17.2	14.9
28	young control	26.8	14.6	7.3	9.8	9.8
29	young control	38.0	45.6	50.1	62.6	67.9
30	young control	40.3	39.5	38.6	39.8	39.8
32	young control	27.8	25.0	20.0	41.3	42.7
34	young control	10.7	25.3	32.0	33.3	38.7
36	young control	2.0	0.7	5.8	6.6	11.5
38	young control	20.8	34.7	34.7	22.2	22.2
109	young control	15.7	17.6	23.5	23.5	21.6
	Mean	22.3	23.4	23.9	26.1	27.7
	Standard Error	3.8	4.7	5.1	5.8	6.1
93	young Akt Inhibitor	55.7	55.7	52.9	64.3	64.3
95	young Akt Inhibitor	92.6	69.1	56.6	52.9	52.9
98	young Akt Inhibitor	-15.5	-21.6	-21.6	62.9	62.9
100	young Akt Inhibitor	7.2	4.8	-6.0	71.1	71.1
102	young Akt Inhibitor	5.6	-1.4	-1.4	-1.4	-12.5
104	young Akt Inhibitor	-1.5	-1.5	-1.5	-0.7	-0.7
106	young Akt Inhibitor	38.3	26.7	11.7	0.0	-6.7
109	young Akt Inhibitor	-32.6	-32.6	-32.6	-35.8	60.0
110	young Akt Inhibitor	5.9	10.3	10.3	-14.7	-19.1
111	young Akt Inhibitor	-9.1	1.5	1.5	-33.3	-16.7
	Mean	14.7	11.1	7.0	16.5	25.6
	Standard Error	11.8	10.0	9.0	13.3	12.4

Table 13-continued

Animal	Group	Δ10cmH_2O	Δ15cmH_2O	Δ20cmH_2O	Δ30cmH_2O	Δ40cmH_2O
19	old control	9.6	13.1	7.7	13.8	1.6
21	old control	-36.1	-33.1	-28.2	-29.4	-30.5
22	old control	6.1	3.6	7.8	8.5	5.7
24	old control	0.0	0.0	-3.7	-25.9	-29.6
25	old control	-8.7	-7.8	-5.8	40.8	23.3
27	old control	21.9	26.7	28.5	27.3	31.6
31	old control	2.3	2.8	0.9	0.9	1.9
33	old control	1.3	-3.0	-3.7	-12.0	3.6
35	old control	6.2	4.9	22.2	33.3	37.0
37	old control	25.9	16.0	6.2	-4.9	-1.2
49	old control	0.0	-1.7	-1.7	-1.7	-1.7
94	old control	14.6	14.6	14.6	14.6	11.0
99	old control	0.0	-3.3	-5.6	-5.6	-5.6
	Mean	3.3	2.5	3.0	4.6	3.6
	Standard Error	4.2	4.0	4.0	5.9	5.5
49	old Akt Inhibitor	28.6	31.0	33.3	42.9	42.9
94	old Akt Inhibitor	-24.5	-34.0	-20.8	-20.8	-45.3
96	old Akt Inhibitor	15.4	10.8	-13.8	-13.8	-3.1
97	old Akt Inhibitor	-14.0	-14.0	-14.0	-8.8	8.8
99	old Akt Inhibitor	6.9	0.0	-8.6	-8.6	-15.5
101	old Akt Inhibitor	8.0	9.2	9.2	0.0	-20.7
103	old Akt Inhibitor	12.7	7.9	7.9	-3.2	66.7
105	old Akt Inhibitor	100.0	100.0	81.0	81.0	79.3
107	old Akt Inhibitor	-24.6	-24.6	-38.6	14.0	1.8
108	old Akt Inhibitor	-34.6	-31.8	-27.1	-27.1	-12.1
	Mean	7.4	5.5	0.9	5.6	10.3
	Standard Error	10.5	10.7	9.5	9.0	11.0

Table 14-Akt Inhibitor Studies Acetylcholine Data (Fig 4.3)

Animal	Group	Body Weight (g)	Initial Diameter(μm)	% Spontaneous Tone
20	young control	310	119.5	47.6
23	young control	321	133	40.6
26	young control	321	148	47.3
28	young control	332	135	46.7
29	young control	320	167.5	43.2
30	young control	345	145	57.1
32	young control	325	186	43.5
34	young control	328	141	47.7
36	young control	332	190	32.1
38	young control	358	151	40.4
100	young control	382	166.0	31.9
102	young control	381	193.0	28.0
104	young control	400	204	29.4
109	young control	362	189	76.2
	Mean		162.0	43.7
	Standard Error		7.1	3.3
93	young Akt Inhibitor		178	59.6
95	young Akt Inhibitor		154	37.0
98	young Akt Inhibitor		174	39.7
100	young Akt Inhibitor		159	53.5
102	young Akt Inhibitor		199	46.2
104	young Akt Inhibitor		204	46.6
106	young Akt Inhibitor		127	49.6
109	young Akt Inhibitor		211	41.7
110	young Akt Inhibitor		129	41.9
111	young Akt Inhibitor		131	25.2
	Mean		166.6	44.1
	Standard Error		10.0	5.3

Table 14-continued

Animal	Group	Body Weight (g)	Initial Diameter(μm)	% Spontaneous Tone
19	old control	425	163	43.6
21	old control	446	150	42.0
22	old control	430	164	33.5
24	old control	423	174	35.9
25	old control	424	190	55.2
27	old control	425	201	68.2
31	old control	383	207	64.1
33	old control	429	225	29.3
35	old control	361	161	29.8
37	old control	424	179	52.1
49	old control	383	176	26.7
94	old control	455	107	28.0
99	old control	453	136	48.5
	Mean		171.7	42.8
	Standard Error		8.6	3.9
49	old Akt Inhibitor		142	39.4
94	old Akt Inhibitor		226	33.2
96	old Akt Inhibitor		200	49.0
97	old Akt Inhibitor		168	47.0
99	old Akt Inhibitor		133	28.6
101	old Akt Inhibitor		142	43.0
103	old Akt Inhibitor		196	37.8
105	old Akt Inhibitor		193	43.0
107	old Akt Inhibitor		133	42.9
108	old Akt Inhibitor		160	51.3
	Mean		169.3	41.5
	Standard Error		10.4	2.2

Table 14-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
20	young control	0	14.0	22.2	22.8	41.0	73.6	81.2
23	young control	0	1.9	7.4	9.3	57.4	92.6	100.0
26	young control	0	4.3	5.7	28.6	48.6	55.7	58.6
28	young control	0	3.2	42.9	74.6	81.0	95.2	96.8
29	young control	0	5.8	8.5	25.0	55.5	78.8	73.1
30	young control	0	12.8	30.5	54.3	73.2	75.6	76.0
32	young control	0	4.9	4.9	60.5	67.9	95.1	95.1
34	young control	0	3.8	6.9	35.3	85.7	96.3	96.3
36	young control	0	6.6	32.8	45.9	72.1	83.6	83.6
38	young control	0	1.6	1.6	6.6	65.6	100.0	100.0
100	young control	0	0.0	0.0	39.6	52.8	54.7	71.7
102	young control	0	5.6	5.6	7.4	13.0	53.7	33.3
104	young control	0	6.7	21.7	25.0	38.3	38.3	38.3
109	young control	0	3.5	7.6	38.2	41.0	41.7	41.7
	Mean	0.0	5.3	14.2	33.8	56.6	73.9	74.7
	Standard Error	0.0	1.1	3.6	5.4	5.2	5.7	6.3
93	young Akt Inhibitor	0.0	1.9	1.9	27.4	98.1	101.9	98.1
95	young Akt Inhibitor	0.0	0.0	14.0	19.3	26.3	80.7	78.9
98	young Akt Inhibitor	0.0	5.8	5.8	21.7	89.9	100.0	100.0
100	young Akt Inhibitor	0.0	0.0	0.0	56.5	67.1	96.5	98.8
102	young Akt Inhibitor	0.0	84.8	84.8	52.2	90.2	92.4	93.5
104	young Akt Inhibitor	0.0	20.0	21.1	77.9	91.6	98.9	98.9
106	young Akt Inhibitor	0.0	0.0	42.9	79.4	93.7	96.8	96.8
109	young Akt Inhibitor	0.0	0.0	11.4	45.5	14.8	69.3	69.3
110	young Akt Inhibitor	0.0	3.7	3.7	7.4	24.1	35.2	38.9
111	young Akt Inhibitor	0.0	12.1	30.3	39.4	51.5	60.6	60.6
	Mean	0.0	12.8	21.6	42.7	64.7	83.2	83.4
	Standard Error	0.0	7.6	7.6	7.8	11.0	6.9	6.6

Table 14-continued

Animal	Group	Start	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
19	old control	0.0	16.2	14.8	16.2	42.8	45.7	39.7
21	old control	0.0	1.6	9.5	46.0	88.9	100.0	96.8
22	old control	0.0	7.3	3.6	1.8	63.6	70.9	41.8
24	old control	0.0	-1.6	-2.2	13.9	19.3	26.7	21.5
25	old control	0.0	11.8	15.4	29.7	53.5	50.1	50.8
27	old control	0.0	-0.7	7.3	40.9	55.5	55.5	54.0
31	old control	0.0	3.0	5.5	11.3	48.1	61.3	53.3
33	old control	0.0	4.5	15.2	75.8	81.8	86.4	86.4
35	old control	0.0	4.2	10.4	12.5	18.8	16.7	18.8
37	old control	0.0	-0.4	17.4	61.0	69.7	77.1	68.0
49	old control	0.0	23.4	42.6	85.1	95.7	97.9	100.0
96	old control	0.0	13.3	13.3	13.3	16.7	40.0	50.0
103	old control	0.0	1.5	6.1	9.1	25.8	27.3	37.9
	Mean	0.0	6.5	12.2	32.0	52.3	58.1	55.3
	Standard Error	0.0	2.1	3.0	7.6	7.5	7.5	7.2
49	old Akt Inhibitor	0.0	8.9	14.3	73.2	82.1	82.1	82.1
94	old Akt Inhibitor	0.0	10.7	17.3	17.3	54.7	73.3	73.3
96	old Akt Inhibitor	0.0	0.0	6.1	88.8	88.8	98.0	100.0
97	old Akt Inhibitor	0.0	2.5	3.8	32.9	89.9	81.0	51.9
99	old Akt Inhibitor	0.0	5.3	13.2	18.4	23.7	28.9	39.5
101	old Akt Inhibitor	0.0	8.2	8.2	70.5	78.7	88.5	91.8
103	old Akt Inhibitor	0.0	17.6	21.6	31.1	58.1	67.6	68.9
105	old Akt Inhibitor	0.0	14.5	14.5	25.3	31.3	34.9	38.6
107	old Akt Inhibitor	0.0	0.0	70.2	63.2	78.9	80.7	84.2
108	old Akt Inhibitor	0.0	22.0	35.4	62.2	75.6	80.5	80.5
	Mean	0.0	9.0	20.5	48.3	66.2	71.6	71.1
	Standard Error	0.0	2.3	6.2	8.2	7.4	7.1	6.7

Table 15-Desitometry Values for p-eNOS(ser1177) blots (Fig 4.4)

vessel number	group	Proteins of Interest				
blot 1		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
382	y	7408680	7447292	0.9948	1656455	4.4959
387	y Ach	7959395	5642016	1.4107	1602557	3.5206
384	y LY	7590584	7902917	0.9605	1476825	5.3513
385	y Ach+LY	7281761	8179579	0.8902	1603313	5.1017
367	o	8462115	17469276	0.4844	1365042	12.798
368	o Ach	13876679	29853080	0.4648	1407419	21.211
369	o LY	7808729	12643643	0.6176	1456319	8.6819
370	o Ach+LY	13409370	8436240	1.5895	1806765	4.6693
blot 2		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
390	y	11198519	9187268	1.2189	19683303	0.4668
391	y Ach	9719172	9718827	1	40197976	0.2418
392	y LY	10140945	12810150	0.7916	10252691	1.2494
393	y Ach+LY	10364975	8314426	1.2466	7877169	1.0555
374	o	13190514	10395349	1.2689	13793269	0.7537
375	o Ach	15288222	9926141	1.5402	17736137	0.5597
376	o LY	12234362	10459555	1.1697	38604319	0.2709
377	o Ach+LY	12031177	6384390	1.8845	42320688	0.1509
blot 3		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
394	y	5683721	3041450	1.8688	3682114	0.826
395	y Ach	5990936	3030274	1.977	2029616	1.493
388	y LY	5501770	2969006	1.8531	2036801	1.4577
389	y Ach+LY	5780938	2979854	1.94	2129324	1.3994
378	o	6653068	2968156	2.2415	2011812	1.4754
379	o Ach	5283683	2931090	1.8026	1973988	1.4849
380	o LY	4909418	2932646	1.6741	2426508	1.2086
381	o Ach+LY	6304192	2953566	2.1344	2059928	1.4338

Table 15-continued

vessel number	group	Proteins of Interest				
blot 4		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
400	y	9337798	3972113	2.3508	3667693	1.083
401	y Ach	34140402	8269143	4.1287	4918160	1.6813
402	y LY	8584119	5458386	1.5726	8956243	0.6095
403	y Ach+LY	8403718	3776342	2.2254	17809540	0.212
371	o	11472268	4837065	2.3717	6154697	0.7859
372	o Ach	30718455	7830403	3.923	8168749	0.9586
373	o LY	17626748	7161532	2.4613	46654032	0.1535
396	o Ach+LY	8383565	3562629	2.3532	13008620	0.2739
blot 5		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
411	y	9460399	3011847	3.1411	1619513	1.8597
404	y Ach	11999689	3322466	3.6117	1710332	1.9426
405	y LY	8789380	3450595	2.5472	3897531	0.8853
400	y Ach+LY	11983173	3385375	3.5397	1882632	1.7982
397	o	24229920	3690103	6.5662	2208937	1.6705
398	o Ach	44838856	3792600	11.823	2002837	1.8936
399	o LY	13001741	3141452	4.1388	9206665	0.3412
417	o Ach+LY	10651009	3115486	3.4187	1522905	2.0458
blot 6		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
400	y	9178516	2929085	3.1336	1416759	2.0675
401	y Ach	11852347	2925138	4.0519	1466925	1.9941
402	y LY	9900878	3364048	2.9431	1952634	1.7228
403	y Ach+LY	11361797	3095563	3.6703	1455174	2.1273
371	o	18202965	3034313	5.999	1949940	1.5561
372	o Ach	8478081	2881495	2.9423	1460780	1.9726
373	o LY	7995237	2901947	2.7551	2289473	1.2675
396	o Ach+LY	16862244	3156488	5.3421	3876466	0.8143
blot 7		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
423	y	5464725	2478584	2.2048		
412	y Ach	5801752	2417608	2.3998		
424	y LY	5557277	2362460	2.3523		
425	y Ach+LY	5814818	2419041	2.4038		
413	o	9072443	2470268	3.6727		
414	o Ach	6057580	2351192	2.5764		
415	o LY	5559974	2350452	2.3655		
416	o Ach+LY	5390344	2311371	2.3321		

Table 15-continued

vessel number	group	Proteins of Interest				
blot 9		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
445	y	6073726	5922650	1.0255	7315307	0.8096
446	y Ach	6234384	6084092	1.0247	11250496	0.5408
447	y LY	5969619	5243951	1.1384	7237773	0.7245
448	y Ach+LY	5780728	4944243	1.1692	2565754	1.927
430	o	7392863	13364047	0.5532	5894046	2.2674
431	o Ach	6535303	8653963	0.7552	10647092	0.8128
432	o LY	6226722	7924107	0.7858	4769255	1.6615
433	o Ach+LY	6150897	6355877	0.9677	2439936	2.6049
blot 12		peNOS	eNOS	peNOS/totaleNOS	GAPDH	eNOS/GAPDH
473	y	4424511	3871073	1.143		
474	y Ach	4396688	3997036	1.1		
482	y LY	4368992	3872843	1.1281		
483	y Ach+LY	4422787	4030981	1.0972		
438	o	5120972	6272253	0.8164		
439	o Ach	4747311	4996045	0.9502		
440	o LY	4578583	4520437	1.0129		
441	o Ach+LY	4330747	3966102	1.0919		

Table 16-Desitometry Values for p-Akt(ser473) blots (Fig. 4.5)

vessel number	group	Proteins of Interest				
blot 1		pAKt	Akt	pAkt/totalAkt	GAPDH	Akt/GAPDH
382	y	7285671	7927624	0.919023279	1656455	4.785897595
387	y Ach	7337869	5908718	1.241871587	1602557	3.687056373
384	y LY	7278021	13037187	0.558250871	1476825	8.827848256
385	y Ach+LY	7274726	18709879	0.388817373	1603313	11.66951119
367	o	7510196	24003815	0.312875099	1365042	17.58467139
368	o Ach	6935577	16780377	0.413314731	1407419	11.92280124
369	o LY	6525074	14185311	0.459988082	1456319	9.740524569
370	o Ach+LY	6728913	10866551	0.619231714	1806765	6.014368775
blot 2		pAKt	Akt	pAkt/totalAkt	GAPDH	Akt/GAPDH
390	y	7170858	11643112	0.615888433	19683303	0.591522266
391	y Ach	7487861	23175682	0.32309129	40197976	0.57653853
392	y LY	7364645	18334194	0.401689052	10252691	1.788232377
393	y Ach+LY	7435792	8867929	0.838503781	7877169	1.125776151
374	o	7918201	11862535	0.667496534	13793269	0.860023465
375	o Ach	7586109	12007759	0.63176726	17736137	0.677022229
376	o LY	8109844	19770722	0.41019463	38604319	0.512137567
377	o Ach+LY	8152303	13002491	0.626980092	42320688	0.307237231
blot 4		pAKt	Akt	pAkt/totalAkt	GAPDH	Akt/GAPDH
400	y	11048256	1804488	6.122654182	3667693	0.491995377
401	y Ach	11808770	1788708	6.601843342	4918160	0.363694552
402	y LY	10500358	1859717	5.646212838	8956243	0.207644768
403	y Ach+LY	9924539	1902964	5.215305702	17809540	0.106850823
371	o	9387858	1930621	4.862610528	6154697	0.313682542
372	o Ach	9292320	1912356	4.859095273	8168749	0.234106348
373	o LY	8320875	2477763	3.358220701	46654032	0.0531093
396	o Ach+LY	8340748	2010510	4.148573248	13008620	0.154552135

Table 16-continued

vessel number	group	Proteins of Interest				
blot 5		pAKt	Akt	pAkt/totalAkt	GAPDH	Akt/GAPDH
411	y	6541972	2395163	2.731326427	1619513	1.478940274
404	y Ach	6499782	2392857	2.716326968	1710332	1.399059949
405	y LY	7012669	2433472	2.881754547	3897531	0.624362449
400	y Ach+LY	7487979	2458875	3.045286564	1882632	1.306083717
397	o	7000656	2460720	2.84496245	2208937	1.113983785
398	o Ach	7283498	2449256	2.973759378	2002837	1.222893326
399	o LY	8308189	2448274	3.393488229	9206665	0.265924089
417	o Ach+LY	6975795	2357518	2.958957259	1522905	1.548040094
blot 6		pAKt	Akt	pAkt/totalAkt	GAPDH	Akt/GAPDH
400	y	6732725	2369260	2.841699518	1416759	1.672309828
401	y Ach	7273056	2347559	3.098135553	1466925	1.600326533
402	y LY	7116820	2530656	2.81224315	1952634	1.296021681
403	y Ach+LY	6293363	2381658	2.64242935	1455174	1.636682624
371	o	7640595	2607349	2.930407475	1949940	1.337143194
372	o Ach	6991886	2409675	2.901588803	1460780	1.649581046
373	o LY	7464697	2517247	2.965420954	2289473	1.099487524
396	o Ach+LY	8056500	2893050	2.784777311	3876466	0.746311202
blot 9		pAKt	Akt	pAkt/totalAkt	GAPDH	Akt/GAPDH
445	y	7156570	3333335	2.146969927	7315307	0.45566577
446	y Ach	7758308	4375789	1.773007794	11250496	0.388941874
447	y LY	6877093	4569164	1.505109687	7237773	0.631294184
448	y Ach+LY	7976596	2451504	3.253756062	2565754	0.955471179
430	o	7658172	7446393	1.028440481	5894046	1.263375447
431	o Ach	7107878	3623226	1.961753973	10647092	0.340301934
432	o LY	7056558	3312569	2.130237287	4769255	0.694567391
433	o Ach+LY	6727069	2719641	2.4735136	2439936	1.114636204

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PEER-REVIEWED PUBLICATIONS:

1) Woodman CR, Trott DW, Laughlin MH. Short-term increases in intraluminal pressure reverse age-related decrements in endothelium-dependent vasodilation in soleus muscle feed arteries. *J Appl Physiol*, 103:1172-1179, 2007.

2) Trott DW, Gunduz F, Laughlin MH, Woodman CR. Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. *J Appl Physiol* 109:1925-34, 2009.

3) Trott DW and Seawright JW. Rethinking the role of superoxide in the ageing skeletal muscle vasculature. *J Physiol,* 588:397-398, 2010.

4) Trott DW, Seawright JW, Woodman CR. NAD(P)H oxidase-derived reactive oxygen species contribute to age-related impairments of endothelium-dependent dilation in rat soleus feed arteries. *J Appl Physiol* In Revision