EFFECTS OF AGE ON SYMPATHETIC VASOCONSTRICCTOR
RESPONSIVENESS IN EXERCISING LEG MUSCLES

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ABSTRACT

Vasoconstrictor responsiveness to acute sympathetic stimulation declines with advancing age in resting skeletal muscle. However, the effect of age on sympathetic vasoconstrictor responsiveness in exercising skeletal muscle has not been examined previously. Therefore, the purpose of these studies was to examine the balance between sympathetic vasoconstriction and metabolic vasodilation in the leg vasculature, both during large and small muscle exercise.

The purpose of the first study was to determine if age-related reductions in sympathetic vasoconstrictor responsiveness also occur in exercising skeletal muscle. Thirteen younger (20-30 yr) and seven older (62-74 yr) healthy non-endurance trained men performed cycle ergometer exercise at ~60% of maximal oxygen uptake while leg blood flow (femoral vein thermodilution), mean arterial blood pressure (radial artery catheter), and plasma adrenaline and noradrenaline concentrations were measured. After steady state was reached (i.e. ~4 minutes), acute sympathetic stimulation was achieved by immersing a hand in ice water for 2-4 minutes (cold pressor test, CPT). CPT tended to cause a larger increase in mean arterial blood pressure in older men (O:16±3 vs. Y:10±2 mmHg) during exercise, but increases in arterial noradrenaline were similar (O:2.56±0.96 vs. Y:1.98±0.40 nM). However, the older men demonstrated a larger percentage reduction in exercising leg vascular conductance (leg blood flow/mean arterial pressure) during CPT compared to younger men (O: -13.6±3.1% vs. Y: -1.5±4.3%; P=0.04). Leg blood flow tended to increase in the younger men, but not in the older men (P=0.10). These results suggest, in contrast to what has been observed in resting skeletal muscle,
that vasoconstrictor responsiveness to sympathetic stimulation is not reduced, but may be augmented in exercising muscle of healthy older humans.

The results of the first study raised the possibility that aging enhances sympathetic vasoconstrictor responsiveness in exercising legs. However, age-associated reductions in cardiac reserve or age group differences in the absolute workload performed may have contributed to the increased vasoconstriction observed in older men in that study. In the second study, then, the topic was revisited. In this study, small muscle leg exercise (single leg knee extension) exercise was used to minimize cardiac output limitations, and all subject groups were matched for absolute exercise intensity. Therefore, 10 young (20-30 yrs) and 9 older (60-79 yrs) healthy, recreationally active men performed both active and passive single leg knee extension exercise before, during, and after acute sympathetic stimulation (ischemic handgrip at 40% maximal voluntary contraction to exhaustion, followed by 2 minutes of post-handgrip circulatory arrest). Blood pressure (Finometer), femoral artery blood flow (Doppler ultrasound), femoral conductance, and plasma catecholamines were measured at baseline, during exercise, and during exercise with sympathetic stimulation. No age group differences were found in the femoral vascular conductance response to sympathetic stimulation during passive exercise ($P=0.56$), but smaller decreases in conductance were noted in older versus younger men during active exercise (Y: 14±3%; O: 4±3%, $P=0.045$). In a subset analysis of 4 younger and 4 older men, sympatholytic responses were augmented in the older men ($P=0.002$). These results suggest that there is not an age-associated impairment in functional sympatholysis in healthy men under these conditions.
Collectively, the results of these studies suggest that primary aging is unlikely to be associated with enhanced vasoconstrictor responsiveness in exercising leg muscles in healthy, normally-active men. Briefly, the results of the first study were not duplicated in the second study in which younger and older subject groups were matched on absolute exercise intensity.
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LIST OF ABBREVIATIONS

ABI Ankle-brachial index
A-D Analog to digital
ATP Adenosine triphosphate
BMI Body mass index
cm Centimeters
cm/sec Centimeters per second
CPT Cold pressor test
DXA Dual energy x-ray absorptiometry
EKG Electrocardiography
EMG Electromyography
Epi Epinephrine
FBF Femoral blood flow
FVC Femoral vascular conductance
HbO₂ Oxyhemoglobin
HHb Deoxyhemoglobin
Hz Hertz
HDL High-density lipoprotein
kg Kilograms
L/min Liters per minute
LBF Leg blood flow
LDL Low-density lipoprotein
<table>
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<th>Abbreviation</th>
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<tr>
<td>LVC</td>
<td>Leg Vascular Conductance</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal voluntary contraction</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>m</td>
<td>Meters</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliters</td>
</tr>
<tr>
<td>mL/min</td>
<td>Milliliters per minute</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimeters of mercury</td>
</tr>
<tr>
<td>MSNA</td>
<td>Muscle sympathetic nerve activity</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometers</td>
</tr>
<tr>
<td>nM</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>NIRS</td>
<td>Near infrared spectroscopy</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>VE</td>
<td>Minute ventilation</td>
</tr>
<tr>
<td>VCO₂</td>
<td>Carbon dioxide release</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen uptake</td>
</tr>
<tr>
<td>VO₂max</td>
<td>Maximal oxygen uptake during treadmill graded exercise test</td>
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<tr>
<td>VO₂peak</td>
<td>Peak oxygen uptake during leg cycle ergometer graded exercise test</td>
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<td>W</td>
<td>Watts</td>
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Chapter 1

INTRODUCTION

Background and Significance

Whole body exercise places tremendous regulatory demands on the cardiovascular system. For muscular activity to continue for more than a few seconds, increased blood flow is required by active muscles to supply the oxygen and nutrients needed to meet the increased metabolic demand. During light exercise, withdrawal of parasympathetic outflow to the heart triggers an increase in heart rate to help meet the increased demand for blood flow to active muscles. Once parasympathetic withdrawal is complete, stimulation of the sympathetic nervous system produces increases in cardiac output and a redistribution of blood flow to the exercising muscles. This redistribution of cardiac output is achieved by constricting vascular beds supplying resting skeletal muscle and other “inactive” vascular beds and dilating blood vessels supplying blood to active muscle (Rowell, 1993, 1997). Paradoxically, these opposing changes in vascular tone in resting vs. exercising muscles occur even though sympathetic outflow is directed to both resting (O’Hagan et al., 1993) and active skeletal muscle (Savard et al., 1987; Pawelczyk et al., 1992; Hansen et al., 1994; DiCarlo et al., 1996). During exercise, metabolites and heat accumulate in active muscles and may reduce vasoconstrictor responses to sympathetic outflow in these vascular beds, a phenomenon known as “functional sympatholysis” (Remensnyder et al., 1962). Therefore, vasoconstriction occurs in less
active tissues where there are fewer metabolites available to interfere with sympathetic vasoconstriction, while dilation occurs in active tissues where more metabolites are produced, causing oxygenated blood to be distributed to where it is needed.

The balance of sympathetic vasoconstriction and local vasodilatory mechanisms reflects competing demands to deliver adequate oxygen and nutrients to the exercising muscle while maintaining systemic blood pressure (Buckwalter & Clifford, 2001). As exercise intensity increases, a greater proportion of cardiac output is directed to active muscles, with as much as 80-90% of cardiac output being directed to active muscles during peak exercise (Rowell, 1993). Under these conditions, vascular tone in vasculature of active muscles plays a significant role in blood pressure regulation. Given the tremendous vasodilatory capacity of human skeletal muscle (Andersen & Saltin, 1985; Richardson et al., 1993), sympathetic restraint of muscle blood flow is necessary for the maintenance of blood pressure and perfusion of vital organs (Rowell, 1993; Buckwalter & Clifford, 2001).

Currently, little is known about how advancing age alters the balance between sympathetic vasoconstriction and metabolic vasodilation in the leg vasculature during dynamic exercise. On one hand, greater sympathetic restraint of exercise hyperemia in older subjects compared to young controls, if present, could underlie reduced leg blood flow (Wahren et al., 1974; Proctor et al., 1998b; Beere et al., 1999; Lawrenson et al., 2003; Poole et al., 2003; Proctor et al., 2003a) and/or elevated blood pressure responses to exercise (Stratton et al., 1994; Fleg et al., 1995; Proctor et al., 2003a; Proctor et al., 2003b). In this regard, increased sympathetic responsiveness in active muscles may contribute to the well-documented declines in aerobic capacity and exercise tolerance
with age (Hodgson & Buskirk, 1977; Buchner et al., 1992; Wagner et al., 1992; Jackson et al., 1995; Jackson et al., 1996; NIA, 1998a; Van Heuvelen et al., 1998).

Conversely, the need for sympathetic restraint of active muscle blood flow may be exacerbated in older individuals due to age-associated reductions in cardiac output reserve (Ogawa et al., 1992; Stratton et al., 1994; Fleg et al., 1995; ACSM, 1998), although there is some contention as to the effect of age on cardiac output at a given absolute exercise intensity (Rodeheffer et al., 1984; Proctor et al., 1998a). In this context, increased sympathetic responsiveness in active muscles in older subjects may be a beneficial response that allows them to maintain higher vascular resistance that would be required to compensate for reduced cardiac output (Taylor et al., 1992).

It is also possible that sympathetic vasoconstrictor responsiveness in active muscles may be reduced or unchanged in older versus younger men. In a previous study in our laboratory, we found well-preserved leg blood flow responses in healthy, normally active older men across a wide range of submaximal exercise intensities, but a greater percentage of cardiac output was directed to the legs (Proctor et al., 2003b). This could reflect decreased sympathetic vasoconstriction in these subjects. For this to be true, the augmented blood pressure responses observed in that study suggest that vasoconstriction must have been augmented in other vascular beds, such as the renal, splanchnic, or cutaneous circulations or non-active skeletal muscle.

Currently, the effect of advancing age on sympathetic responsiveness in active muscle has not been systematically examined. The major question addressed by this dissertation, then, is whether sympathetic vasoconstrictor responsiveness in contracting leg muscles is altered with age in men.
Mechanistically, available evidence in humans generally suggests that sympathetic outflow is augmented (Seals & Esler, 2000), but α-adrenoreceptor responsiveness is reduced (Elliott et al., 1982; Hogikyan & Supiano, 1994; Sugiyama et al., 1996; Davy et al., 1998b) with age under resting conditions. It is possible that this age-associated decline in vascular responsiveness is mediated primarily by reductions in α1-, but not α2-adrenoreceptor responsiveness (Dinenno et al., 2002a). In this regard, reduced sympathetic vasoconstrictor responsiveness in the leg vasculature during exercise would not be surprising. However, sympathetic responsiveness during exercise is attenuated during exercise. Although the exact mechanisms underlying functional sympatholysis are not known, they may involve nitric oxide (Hansen et al., 2000b; Chavoshan et al., 2002; Dinenno & Joyner, 2003; Buckwalter et al., 2004c; Dinenno & Joyner, 2004), vasodilator prostaglandins (Dinenno & Joyner, 2004), adenosine (Nishigaki et al., 1991), hypoxia (Hansen et al., 2000a), ATP (Rosenmeier et al., 2004), neuropeptide Y (Buckwalter et al., 2004a; Buckwalter et al., 2005), and/or K_ATP channels (Keller et al., 2004). Therefore, age-related changes in any of these or other possible mediators could reconcile observations of impaired sympathetic vasoconstriction at rest, but augmented vasoconstrictor responsiveness during exercise. A secondary goal of this dissertation is to gain insight into age-related differences in potential mechanisms underlying sympathetic responsiveness by measuring sympathetic vasoconstrictor responsiveness during both large and small muscle exercise and examining potential correlations with catecholamines and ATP.
Specific Aims and Hypotheses

**SPECIFIC AIM 1.** The purpose of this study, “Augmented leg vasoconstriction in dynamically exercising older men during acute sympathetic stimulation,” was to determine the effect of age on leg vasoconstrictor responsiveness in healthy, dynamically exercising men during an acute sympathetic stimulus.

**Hypothesis 1.** Sympathetic vasoconstrictor responsiveness, as evaluated by the percent decrease in leg vascular conductance when acute sympathetic stimulation is superimposed during ongoing moderate-intensity exercise, will be reduced in older versus younger healthy men, similar to what has been observed under resting conditions (Elliott *et al.*, 1982; Hogikyan & Supiano, 1994; Sugiyama *et al.*, 1996; Davy *et al.*, 1998b).

**SPECIFIC AIM #2.** The results of study #1 supported an age-related increase in sympathetic vasoconstrictor responsiveness in active muscles during leg cycle ergometer exercise at the same relative intensity. However, it was unclear whether or not age group differences in the absolute exercise intensity or cardiac output reserve may have contributed to these results. Accordingly, the purpose of study #2, “Is functional sympatholysis impaired in older men during dynamic leg exercise?” was to determine the effect of age on “functional sympatholysis” in healthy men during small muscle dynamic leg exercise at the same absolute intensity.

**Hypothesis 2a.** Femoral vascular conductance will decrease to a greater extent (i.e. greater vasoconstriction) in an actively exercising leg in older compared to...
younger normally active men during acute sympathetic stimulation by post-handgrip forearm occlusion.

**Hypothesis 2b.** Normally active older men will exhibit a smaller percent decrease in femoral vascular conductance (i.e. less vasoconstriction) in a passively exercising leg compared to younger men during acute sympathetic stimulation by post-handgrip forearm occlusion.

**Hypothesis 2c.** Functional sympatholysis will be impaired in older versus younger normally active men.
Chapter 2

REVIEW OF LITERATURE

This chapter will review the literature regarding topics relevant to this dissertation, including 1) the relevance of aging and leg blood flow responses to exercise, 2) a brief review on the effects of age on leg blood flow responses to exercise, 3) potential mechanisms that may underlie age-related changes in leg blood flow and its regulation, 4) the role of the sympathetic nervous system in regulating vascular tone in active muscles, 5) age-related changes in the sympathetic nervous system, 6) potential mechanisms of sympatholysis and the effects of age on these potential mechanisms.

Age and Limb Blood Flow During Large Muscle Exercise in Men

Maximal aerobic work capacity and submaximal exercise tolerance decline with advancing age in humans, ultimately contributing to functional impairment and loss of independence (Buchner et al., 1992; Holloszy & Kohrt, 1995; NIA, 1996; Delp, 1998). Active muscles require an adequate local blood supply to meet the functional and metabolic demands of exercise and other daily physical activities. In this context, age-associated changes in exercising muscle blood flow could contribute to reduced functional capacity in older adults (Strandell, 1964; Wahren et al., 1974; Saltin, 1986; Davy & Seals, 1994; NIA, 1998b; Proctor et al., 1998b). Understanding the effects of
age on vascular responsiveness in exercising limbs also has important implications for systemic blood pressure regulation and cardiovascular disease risk.

The literature is replete with seemingly contradictory results concerning the effect of age on leg exercise hyperemia in men. Some studies support age-associated reductions in whole leg blood flow responses to exercise during upright, two-leg cycle ergometer exercise (Wahren et al., 1974; Proctor et al., 1998b; Beere et al., 1999; Poole et al., 2003), while a recent study from our laboratory suggests preserved hyperemic responses (Proctor et al., 2003b). These varying results may depend on the training state of the subjects examined, as our study (Proctor et al., 2003b) was the only one to examine submaximal leg blood flow responses in healthy, recreationally active (i.e. not sedentary or highly trained) men (Koch et al., 2005; Proctor & Parker, 2006).

During dynamic leg exercise, most of the cardiovascular adjustments are aimed at increasing leg blood flow to supply the oxygen and nutrients necessary for active muscles to meet their increased energy needs. Accordingly, there are tight correlations leg blood flow and exercise intensity, workload, and oxygen consumption across a variety of experimental models. Blood flow to an exercising limb can be changed by altering cardiac output or its distribution by changes in regional vascular tone in the exercising limb and other vascular beds (Rowell, 1993; Saltin et al., 1998). Therefore, age-associated alterations in exercising limb blood flow could result from age differences in one or more of these mechanisms. *The studies in this dissertation will focus on the control of vascular tone in exercising leg muscles in young and older men.*
Altered Vasodilatory Responses in Exercising Muscles of Older Humans?

There is compelling evidence in the literature that vasodilation in exercising limbs is reduced in some populations of older compared to younger adults. During submaximal cycle ergometer exercise, leg vascular conductance was reduced in older vs. younger chronically endurance-trained men (Proctor et al., 1998b) at all workloads. In sedentary older men (Poole et al., 2003), conductance was reduced compared to younger controls except at low workloads. The reduced vascular conductance responses were caused solely by reduced leg blood flow responses in older endurance-trained men (Proctor et al., 1998b), while sedentary older men had reduced leg blood flow responses despite increased mean arterial pressure (Poole et al., 2003).

Leg vascular conductance was also reduced relative to younger controls in sedentary older men (Lawrenson et al., 2003) and moderately trained middle-aged men (Magnusson et al., 1994) performing small muscle dynamic exercise. In the study of sedentary men (Lawrenson et al., 2003), decreased leg vascular conductance responses in older subjects were due to both reduced leg blood flow and augmented mean arterial pressure responses. In moderately trained middle-aged men (Magnusson et al., 1994), leg blood flow was maintained compared to younger men, but higher mean arterial pressure was required to achieve this response.

Vascular tone represents a balance between vasoconstrictor and vasodilator pathways, integrated at the level of the vascular smooth muscle (Laughlin & Korzick, 2001). The primary regulators of vascular tone are the sympathetic nervous system and local vascular control (Laughlin et al., 1996). Therefore, age-related changes in vascular
conductance in exercising limbs imply a change in the balance of sympathetic vasoconstriction versus locally-mediated vasodilatory pathways. A heightened degree of vasoconstriction or a lesser degree of vasodilation would be necessary to maintain blood pressure in the face of an age-related decline in cardiac output. To date, the literature on the effects of age on leg vascular conductance during large muscle dynamic exercise consistently reports that blood pressure is at least maintained (Proctor et al., 1998b), and usually augmented (Beere et al., 1999; Poole et al., 2003; Proctor et al., 2003b) in older vs. younger men. It is likely that this is due to an age-related alteration in endothelial function and/or increased sympathetic vasoconstrictor tone.

**Blunted Endothelium-Mediation Vasodilation?**

The vascular endothelium plays a pivotal role in the regulation of vascular tone through the synthesis and release of both vasodilator (nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors) and vasoconstrictor (endothelin-1 and thromboxane) substances. Under basal conditions, endothelial vasodilator function appears to decline with age. In vitro, age-associated reductions in endothelium-dependent vasodilator responsiveness to acetylcholine and flow have been reported using soleus feed arteries (Woodman et al., 2002, 2003, 2004) and first order arterioles (Muller-Delp et al., 2002b; Spier et al., 2004) isolated from male Fischer rats. Nitric oxide-mediated (Woodman et al., 2003; Spier et al., 2004) and prostacyclin-mediated (Woodman et al., 2003) endothelial dilation appear to be responsible for these changes. Moreover, these responses appear to be muscle-specific, as vasodilation induced by acetylcholine was not found in the gastrocnemius (Muller-Delp et al., 2002b; Woodman
There is also in vitro evidence of age-associated reductions in conducted endothelium-dependent vasodilation (Bearden et al., 2004). In humans, pharmacological studies have suggested decreased forearm vascular responsiveness to acetylcholine with age during resting conditions (Taddei et al., 1995; Gerhard et al., 1996; Taddei et al., 1996; Taddei et al., 1997; DeSouza et al., 2000; Taddei et al., 2000; DeSouza et al., 2002). This age-related endothelial dysfunction can be reversed or prevented, at least in part, by regular aerobic exercise training (DeSouza et al., 2000; Taddei et al., 2000).

Currently, the effects of age on vascular endothelium-mediated vasodilator responses to exercise have not been addressed. In fact, there is still considerable debate as to whether the vascular endothelium plays a major role in exercise hyperemia. Exercising forearm blood flow was either unchanged or reduced compared to control exercise bouts when N\textsuperscript{G} monomethyl-L-arginine (L-NMMA, nitric oxide synthase inhibitor) was infused intra-arterially (Wilson & Kapoor, 1993; Endo et al., 1994; Gilligan et al., 1994; Dyke et al., 1995; Katz et al., 1996; Shoemaker et al., 1997a; Brock et al., 1998; Duffy et al., 1999). Infusions of L-NMMA into the femoral artery had no effect on leg blood flow during knee extensor exercise (Bradley et al., 1999; Radegran & Saltin, 1999; Frandsen et al., 2000). However, Hillig et al. (2003) recently reported that infusions of both L-NMMA and sulfaphenazole, a proposed endothelium-derived hyperpolarizing factor inhibitor, into the femoral artery during knee extensor exercise lowered leg blood flow by approximately 16%. This raised the possibility of redundant vasodilator systems. Finally, Schrage et al. (2004b) found that when both N\textsuperscript{G} nitro-L-arginine methyl ester (L-NAME, nitric oxide synthase inhibitor) and ketorolac (cyclooxygenase inhibitor) were infused intra-arterially during steady state forearm
exercise, blood flow transiently dropped to 70% of the steady state exercise level, and then stabilized at approximately 80%. This suggests that the contribution of the vascular endothelium to exercise hyperemia was also obscured because previous studies had delivered drugs before exercise began.

**Augmented Sympathetic Vasoconstriction?**

Basal sympathetic outflow to skeletal muscle increases with age in healthy men (Ng et al., 1993; Jones et al., 1997a; Jones et al., 1997b; Davy et al., 1998a; Davy et al., 1998b; Dinenna et al., 1999; Niimi et al., 2000). However, there is both in vitro (Hatake et al., 1992; Nielsen et al., 1992) and in vivo (Elliott et al., 1982; Hogikyan & Supiano, 1994; Sugiyama et al., 1996; Davy et al., 1998a) evidence of decreased α-adrenergic sensitivity, leading to blunted vasoconstriction. Despite this, Dinenna et al. (2002b) demonstrated that the reduced resting leg blood flow and conductance responses in older men are normalized by local α-adrenergic blockade, suggesting that elevated sympathetic tone is an underlying cause.

Currently, little is known about the effects of age sympathetic outflow during dynamic exercise. Mazzeo et al. (1997) reported that whole body norepinephrine (NE) spillover did not differ between older and younger men at rest or during exercise at 50% of VO$_{2\text{peak}}$. Furthermore, changes in hepatomesenteric NE spillover from rest to exercise were similar between age groups, but the absolute level was higher in older men due to an elevated baseline. In chronically endurance-trained men, estimated leg NE spillover rates were augmented in the older cohort during high-intensity leg cycle ergometer exercise (210 watts) (Proctor et al., 1998b). However, this did not differ between age
groups during exercise at the same relative intensity. Similarly, estimates of leg norepinephrine spillover were similar between normally active younger and older men exercising at 60% of VO$_{2peak}$ (Proctor et al., 2003b). However, in this study, older subjects exercised at a much lower absolute workload. Collectively, these results suggest that it is likely that whole body, hepatomesenteric, and leg norepinephrine spillover would be elevated in older subjects during exercise at the same absolute workload.

To date, no studies have been published that directly address the effects of age on sympathetic tone in exercising leg muscles through pharmacological blockade of adrenergic receptors or leg vascular responsiveness to sympathetic stimulation. Pharmacologically blocking adrenergic receptors during large muscle dynamic exercise would probably make it impossible for subjects to maintain blood pressure. The effects of age on leg vascular responsiveness to sympathetic stimulation, however, may have implications for understanding sympathetic tone. This dissertation will address this gap in our understanding of the regulation of exercising leg blood flow by focusing on the effects of advancing age on leg vascular responsiveness to sympathetic stimulation during leg exercise.

**Sympathetic Control of the Circulation During Exercise**

The sympathetic nervous system is crucial for the proper regulation of heart rate, ventricular contractility, and vascular tone during exercise. At the onset of exercise, heart rate and stroke volume increase, first through withdrawal of vagal tone and then as a result of epinephrine and norepinephrine stimulating $\beta_1$-adrenoreceptors in the sinoatrial
node and the ventricles. In resting skeletal muscle and non-muscle vascular beds, the predominant effect of sympathetic activation is vasoconstriction. Accordingly, sympathetic denervation or α-adrenoreceptor blockade leads to a marked increase in blood flow under these conditions (Delp & Armstrong, 1988; Laughlin et al., 1996; Dinenno et al., 2002b). In active skeletal muscle, however, the role of sympathetic vascular tone is less clear.

Over 70 years ago, it was suggested that muscular contractions can decrease vascular responsiveness to sympathetic stimulation (Rein, 1930). This phenomenon was dubbed “functional sympatholysis” by Remensnyder et al. (1962) when they observed reduced vasoconstriction in contracting canine hindlimbs during direct sympathetic nerve stimulation, reflex sympathetic activation, and norepinephrine infusion. While there are a number of studies that support sympatholysis (Remensnyder et al., 1962; Kjellmer, 1965; Anderson & Faber, 1991; Thomas et al., 1994; Richardson et al., 1995a; Hansen et al., 1996; Hansen et al., 1999; Ruble et al., 2000; Buckwalter et al., 2001; Ruble et al., 2002; Rosenmeier et al., 2003), there are several others that suggest that sympathetic restraint of blood flow is maintained (Secher et al., 1977; Joyner et al., 1990; O’Leary et al., 1991; Joyner et al., 1992; Shoemaker et al., 1997b; Buckwalter & Clifford, 1999) or even augmented in exercising muscles (Thompson & Mohrman, 1983; O’Leary et al., 1991).

Underlying the controversy as to the importance of the sympathetic nervous system for regulating vascular tone in exercising muscle is the observation that vascular tone in active skeletal muscle decreases dramatically during exercise even though sympathetic outflow to exercising muscles increases (Savard et al., 1987; Pawelczyk et al., 1992; Hansen et al., 1994; DiCarlo et al., 1996). One interpretation of these
observations is that the sympathetic nervous system does not play a role in regulating vascular tone in exercising muscle. In this context, one of the classic papers on the subject (Donald et al., 1970) reported no differences in hindlimb blood flow in canine skeletal muscle following chronic surgical ablation of sympathetic nerves, and several studies have reported no effect of systemic administration of $\alpha$-adrenoreceptor antagonists (Hartling & Trap-Jensen, 1983; Longhurst et al., 1986). In another study (Laughlin & Armstrong, 1987), systemic administration of an $\alpha$-antagonist decreased total blood flow to exercising muscles and caused a redistribution of blood flow to less oxidative fiber types within skeletal muscle, but because oxidative muscles are recruited first during exercise, this may reflect decreased vasoconstriction in inactive muscles. Another attractive hypothesis would be that the sympathetic nervous system contributes to vasodilation in active muscles. However, while some species have sympathetic cholinergic nerves in skeletal muscle vasculature that cause vasodilation, this does not appear to be the case in humans (Joyner & Dietz, 2003). Moreover, while binding of epinephrine or norepinephrine to $\beta$-adrenoreceptors causes vasodilation in exercising muscles (Hartling & Trap-Jensen, 1982), experimental evidence suggests that this is not a major action of sympathetic nerve activity in active muscle (Buckwalter et al., 1997a).

Recently, progress has been made in understanding opposing results as to the effect of exercise on sympathetic responsiveness. One key insight has been the recognition of the importance of using the appropriate index of vascular tone, vascular resistance or vascular conductance (Lautt & Legare, 1991; O'Leary, 1991; Rowell, 1993). In fact, Rowell (1993) demonstrated that a study that relied on changes in vascular resistance to support sympatholysis (Kjellmer, 1965) would not support a decreased
sympathetic responsiveness if conductance was used as the index of vascular tone rather than resistance. When expressing changes in vascular tone as changes in vascular conductance, it is most appropriate to express them as percent changes in vascular tone because this accounts for differences in baseline flow. Therefore, similar percent changes in vascular vessel diameter result in similar percent changes in vascular conductance regardless of the baseline blood flow (Buckwalter & Clifford, 2001). Experimental differences in the strength of sympathetic stimulus, exercise duration, exercise modality, and exercise intensity are also likely to be important modulators of sympatholysis (Thomas et al., 1994; Hansen et al., 2000b).

Currently, there is a growing consensus in the literature that sympathetic nervous outflow to exercising muscles causes vasoconstriction in exercising skeletal muscle and can help offset metabolic vasodilation. One line of evidence comes from disease states that compromise the normal functioning of the sympathetic nervous system, such as primary autonomic failure (peripheral failure) and Shy-Drager syndrome (central failure). Patients with these conditions have hypotensive responses and exaggerated muscle blood flow responses to exercise compared to healthy controls (Marshall et al., 1961; Smith et al., 1995; Joyner & Wieling, 1997; Puvi-Rajasingham et al., 1997; Schrage et al., 2004a), suggesting that the sympathetic nervous system restricts blood flow to active muscles to maintain blood pressure.

Outside of these patient populations, several experimental models have been employed to show that skeletal muscle hyperemia is limited during intense exercise. Several studies have reported increases in vascular conductance in active limbs when sympathetic outflow to exercising limbs was both acutely and specifically antagonized,
either pharmacologically (Joyner et al., 1992; Buckwalter et al., 1997b; O'Leary et al., 1997; Buckwalter & Clifford, 1999) or surgically (Peterson et al., 1988). A number of researchers have reported evidence of vasoconstriction in active muscles when the sympathetic nervous system was activated reflexively (Strandell & Shepherd, 1967; Secher et al., 1977; Joyner et al., 1990; O'Leary et al., 1991; Joyner et al., 1992; Saito et al., 1992; Kagaya, 1993; Kagaya et al., 1994; Shoemaker et al., 1997b; Shoemaker et al., 1999; Strange, 1999) or directly (Thompson & Mohrman, 1983), with some reporting decreases in blood flow to active muscles when sympathetic outflow was increased (Secher et al., 1977; Kagaya, 1993; Kagaya et al., 1994). Collectively, these studies have provided convincing evidence for sympathetic restraint of blood flow to exercising muscle.

Although it is becoming evident that sympathetic restraint of exercise hyperemia exists, the metabolic effects of this restraint are unclear. It has been suggested that the balance of local metabolic vasodilation and sympathetic outflow creates a situation in which blood flow is reduced primarily to the least active fibers, maximizing oxygen extraction to preserve muscle oxygen consumption (Strandell & Shepherd, 1967; Nellis et al., 1980). Although some data support this idea (Strandell & Shepherd, 1967; Flaim et al., 1979; Nellis et al., 1980), other studies refute it (Thompson & Mohrman, 1983). Studies addressing this question in humans also conflict on this issue. At least one study suggests that sympathetic constriction of exercise hyperemia can limit muscle oxygen uptake (Joyner et al., 1992), while another found no evidence of sympathetically-mediated reductions in tissue oxygenation during rhythmic handgrip exercise (Hansen et al., 1996).
In summary, convincing evidence of sympathetic restraint of blood flow during exercise comes from a variety of experimental models. The results of these experiments are consistent with the theoretical argument suggesting that it is necessary for the maintenance of blood pressure during exercise. Furthermore, it appears that sympathetic vasoconstriction may limit muscle oxygen uptake, perhaps compromising exercise tolerance. However, questions still exist as to the site of vasoconstriction within the arterial tree and the relationship between exercise intensity and sympathetic vasoconstriction (Buckwalter & Clifford, 2001).

What is the Relationship Between Sympatholysis and Blood Pressure Control?

Although functional sympatholysis may not present a problem during small muscle exercise, whole body exercise may create a situation in which cardiac output limitations present a pressing need for sympathetic restraint in active muscles (Joyner & Thomas, 2003). Therefore, the amount of muscle mass recruited may modulate the effects of increased sympathetic outflow on sympatholytic responses. For this reason, this dissertation will examine the effects of age on sympathetic vasoconstrictor responsiveness both during leg cycle ergometer (large muscle mass) and single leg knee extension (small muscle mass) exercise.

A key question, then, is how the body balances metabolic vasodilation and sympathetic vasoconstriction during large muscle exercise (Joyner & Thomas, 2003). How can blood flow to exercising muscles be preserved without threatening systemic blood pressure? In this regard, a number of studies support marked sympatholysis
responses in $\alpha_2$-adrenoreceptors, but relatively preserved $\alpha_1$-mediated responsiveness during exercise (McGillivray-Anderson & Faber, 1990, 1991; Thomas et al., 1994; Buckwalter et al., 2001; VanTeeffelen & Segal, 2003). It has been reported that there are differences in the distribution of $\alpha_1$- and $\alpha_2$-adrenoreceptors in human limbs. Specifically, proximal feed arteries have both receptor subtypes, while distal arteries have predominantly $\alpha_2$-adrenoreceptors (Flavahan et al., 1987). Therefore, it is possible that during intense exercise involving large muscle mass, $\alpha_2$-mediated sympathetic vasoconstrictor responses are attenuated in distal arterioles, while more proximal arteries have relatively maintained $\alpha_1$-responsiveness to maintain systemic blood pressure (Hansen et al., 2000b; Buckwalter & Clifford, 2001; Joyner & Thomas, 2003; VanTeeffelen & Segal, 2003). However, it has also been suggested that $\alpha_1$ adrenoreceptors may be more important than $\alpha_2$ in mediating sympathetic vasoconstriction in human forearms (Jie et al., 1985; van Brummelen et al., 1986). Specifically, infused tyramine or norepinephrine produced vasoconstriction that was sensitive to both $\alpha_1$ and $\alpha_2$-adrenoreceptor antagonists, while the vasoconstriction induced by LBNP responded only to an $\alpha_1$-antagonist. Collectively, this may suggest that $\alpha_1$-adrenoreceptors are located near the neurovascular junction, while $\alpha_2$ receptors are located away from the synaptic cleft. Because of this, we chose to use sympathoexcitatory maneuvers that cause endogenous catecholamine release for the studies in this dissertation, as opposed to pharmacologic techniques.
Potential Mechanisms of Functional Sympatholysis

Many potential mechanisms of functional sympatholysis have been proposed, but the phenomenon is not clearly understood. Since sympathetic vasoconstrictor responsiveness is not impaired during hyperemia caused by infusion of vasodilator drugs, (Thomas et al., 1994, 1997; Tschakovsky et al., 2002; Rosenmeier et al., 2003), sympatholysis is believed to reflect direct cross-talk between metabolic vasodilation and sympathetic vasoconstriction (Hansen et al., 2000b). Several studies have linked nitric oxide to sympatholysis (Thomas et al., 1998; Thomas & Victor, 1998; Hansen et al., 2000b; Sander et al., 2000; Chavoshan et al., 2002; Buckwalter et al., 2004c). Observations of impaired sympatholysis responses in dystrophin-deficient rats (Thomas et al., 1998) and humans (Sander et al., 2000), who have greatly reduced neuronal nitric oxide synthase (nNOS) expression but normal endothelial nitric oxide synthase expression, advanced the hypothesis that the nNOS is the source of the nitric oxide that inhibits sympathetic vasoconstriction during exercise. However, Dinenno and Joyner argue that nitric oxide is not obligatory in producing sympatholysis, probably because of a redundant role of vasodilator prostaglandins (Dinenno & Joyner, 2003, 2004). In humans, studies have alternatively suggested that reducing nitric oxide is (Sander et al., 2000; Chavoshan et al., 2002) and is not sufficient to produce sympatholysis (Dinenno & Joyner, 2003, 2004). It is unclear whether these disparate findings can be explained by the technique used to measure vascular responses (NIRS versus Doppler ultrasound) or the sympathetic maneuver (LBNP or physiologic stimulation versus pharmacologic stimulation).
A second mechanism of sympatholysis that has received a great deal of attention is the role of ATP-sensitive potassium ($K_{\text{ATP}}$) channels. These channels, located in the vascular smooth muscle, produce hyperpolarization when activated, thus contributing to reduced vascular tone by reducing the probability of voltage-gated calcium-channel opening (Quast et al., 1994). These channels respond to a variety of stimuli that may be found in exercising muscles, including intracellular ATP and nucleotide diphosphates, as well as extracellular oxygen, hydrogen ions, adenosine, nitric oxide, and prostaglandins PGI$_2$ and PGE$_2$ (Quayle & Standen, 1994; Nelson & Quayle, 1995). In this respect, these channels represent a potential mediator of reported sympatholytic responses to adenosine (Nishigaki et al., 1991), hypoxia (Hansen et al., 2000a), and the nitric oxide and prostacycin-mediated mechanism described above. Inhibition of $K_{\text{ATP}}$ channels reduced sympatholysis in both rat (Thomas et al., 1997) and human (Keller et al., 2004) subjects.

Thirdly, it is now known that ATP is coreleased, along with norepinephrine, from sympathetic nerve terminals (Kennedy et al., 1986; Hopwood & Burnstock, 1987; Martin et al., 1991a). ATP can bind to P2X purinergic receptors on vascular smooth muscle, producing vasoconstriction, or P2Y receptors on the vascular endothelium, where it produces vasodilation. P2X receptors are ideally located to be stimulated by ATP from sympathetic nerve terminals, and recent studies in dogs suggest that stimulation of these receptors produces vasoconstriction that is progressively attenuated by exercise of increasing intensity (Buckwalter et al., 2003). Blockade of P2X receptors increased blood flow and vascular conductance (Buckwalter et al., 2004b). Finally, the constriction produced by binding to P2X receptors is independent of nitric oxide (Buckwalter et al., 2003), but is attenuated in response to acidosis (Kluess et al., 2005a) and temperature
elevation (Kluess et al., 2005b), providing plausible mechanistic links to exercise-induced attenuation. These results have yet to be confirmed in humans.

Aside from a potential role for ATP in the interstitium, circulating ATP binding to P2Y purinergic receptors on the vascular endothelium has been proposed as an alternative explanation for functional sympatholysis (Rosenmeier et al., 2004). In this potential mechanism, erythrocytes sense ambient oxygen, and then release ATP from hemoglobin during deoxygenation (Ellsworth et al., 1995; Jagger et al., 2001; Gonzalez-Alonso et al., 2002; Ellsworth, 2004). This intravascular ATP binds to P2Y receptors, causing release of nitric oxide, prostaglandins, and endothelium-derived hyperpolarization factors that cause vasodilation at the vascular smooth muscle (Ellsworth et al., 1995; Wihlborg et al., 2003). Rosenmeier and colleagues (2004) provided experimental support for this hypothesis when they reported that leg exercise and intra-arterial infusion of ATP both abolish tyramine-induced vasoconstriction. Based on studies showing that ATP does not readily cross over the endothelium (Mo & Ballard, 2001) and that interstitial ATP would likely produce P2X-mediated vasoconstriction (Buckwalter et al., 2003), it was concluded that the sympatholytic effects of infused ATP probably do not represent a direct alteration of α-adrenoreceptor activity (Rosenmeier et al., 2004).

Finally, neuropeptide Y, another sympathetic cotransmitter, produces vasoconstriction in resting and exercising muscles (Buckwalter et al., 2004a; Buckwalter et al., 2005). Exercise blunted the vasoconstrictor responses to neuropeptide Y in an intensity-dependent fashion that does not appear to be dependent on nitric oxide (Buckwalter et al., 2004a). At this point, little is known on the effect of age on any of these potential mechanisms of sympatholysis in humans.
Chapter 3

AUGMENTED LEG VASOCONSTRICTION IN DYNAMICALLY EXERCISING OLDER MEN DURING ACUTE SYMPATHETIC STIMULATION

Introduction

Under resting conditions, sympathetic vasoconstrictor outflow to skeletal muscle increases progressively with advancing age in humans, as evidenced by elevated basal systemic noradrenaline spillover rates and muscle sympathetic nerve activity (MSNA) (Seals & Esler, 2000). However, there appears to be a corresponding desensitization of arterial $\alpha$-adrenergic receptors, such that blunted vasoconstrictor responses to acute sympathetic stimulation are observed. Although in vitro studies of human arterial $\alpha$-adrenergic responsiveness have given mixed results, with some showing no change (Scott & Reid, 1982; Docherty, 1990) and others showing decreased sensitivity in arteries isolated from older individuals (Hatake et al., 1992; Nielsen et al., 1992), studies using in vivo approaches generally support an age-related decrease in responsiveness (Elliott et al., 1982; Hogikyan & Supiano, 1994; Sugiyama et al., 1996; Davy et al., 1998b). Recent evidence in humans (Dinenno et al., 2002a) suggests that a major component of
this age-associated decrease in vascular responsiveness involves blunted \( \alpha_1 \), but not \( \alpha_2 \), adrenergic receptor responsiveness.

During large muscle dynamic exercise, the sympathetic nervous system mediates vasoconstriction in non-active muscle and in visceral regions, which contributes to an increase in arterial perfusion pressure and facilitates redistribution of blood flow to exercising muscles (Rowell, 1993). However, sympathetic vasoconstrictor outflow is also directed to active skeletal muscle to balance active muscle vasodilation with the rise in cardiac output so that systemic arterial pressure can be maintained. Although the vasculature of exercising muscle displays a reduced sensitivity to \( \alpha \)-adrenergic stimuli, a phenomenon referred to as “functional sympatholysis”, sympathetic restraint of active muscle blood flow is still a quantitatively important contributor to systemic blood pressure maintenance during large muscle exercise in humans (Buckwalter & Clifford, 2001).

In this context, most healthy older (>60 yr) adults demonstrate a reduced absolute cardiac output response during submaximal and especially during maximal exercise (Fagard et al., 1993), but a relatively well-preserved skeletal muscle vasodilator capacity (Martin et al., 1991b; Jasperse et al., 1994). These results suggest that sympathetic restraint of active muscle vasodilation during exercise may become even more important for older adults. Thus the need for increased sympathetic restraint of active muscle blood flow in older adults vs. the blunted vasoconstrictor responses seen in aged limbs is a potential paradox that has not previously been explored.

To gain insight into this issue, we measured changes in leg blood flow and vascular conductance during cycle ergometer exercise at a similar relative intensity (60%
VO_{2\text{peak}}) in non-endurance trained younger and older men before, during, and after immersion of one hand in ice water (cold pressor test, CPT). Local cold stimulation was chosen because it is a robust sympathetic stimulus capable of doubling MSNA within 1-2 minutes (Victor et al., 1987; Seals, 1990), and its effects appear to be independent of age (Ng et al., 1994). We hypothesized that during moderate intensity exercise, the leg vasculature of older men would exhibit less vasoconstriction in response to acute sympathetic stimulation in comparison with younger men, similar to what has been observed under resting conditions.

**Methods**

**Subject Screening and Preliminary Tests**

Thirteen younger (20-30 yr) and seven older (62-74 yr) men from State College, PA and surrounding communities completed all phases of this study. Each subject was informed of potential risks and discomforts and signed an informed consent form approved by the Institutional Review Board of the Pennsylvania State University and the General Clinical Research Center (GCRC) at the University Park campus. All studies were performed in accordance with the Declaration of Helsinki. All subjects were recreationally active, but none participated in moderate or high intensity aerobic exercise more than 3 days per week during past 12 months or had a treadmill VO_{2\text{max}} greater than the 80th percentile according to age group norms (ACSM, 2000). Additionally, lower body strength-trained subjects (more than 1 day per week during past 12 months) were excluded from participation.
All subjects were non-obese (≤30% body fat and BMI≤30), non-smokers, and had clinically normal hemoglobin concentrations (12.5-16.6 g/dL) and resting supine ankle-brachial index ratings (ABI <0.90; (Isselbacher et al., 1980; ACSM, 2000). No subject had a history or symptoms of cardiac, vascular, pulmonary, metabolic, or neurological disease. Hypertensive individuals (resting blood pressure ≥140/90 mmHg) were also excluded because their central (Fagard et al., 1995) and peripheral (Tanaka et al., 1998) hemodynamic responses to exercise differ compared with normotensive age-matched controls. No subjects were taking medications having significant hemodynamic effects, but four older men did take aspirin on a regular basis. Subjects underwent a treadmill test to maximal exertion to rule out exercise-induced electrocardiograph or blood pressure abnormalities.

After screening, subjects returned to the laboratory on two separate days, once for a preliminary cycle ergometer exercise session and once for the invasive leg blood flow study. All exercise testing for both sessions was performed in the upright posture using a Lode™ electronically braked cycle ergometer with toe clips. A padded forearm rest was attached above the handlebars to prevent the subject from leaning forward and to facilitate blood sampling from the radial artery catheter. Pulmonary gas exchange (VO$_2$, VCO$_2$, and minute ventilation, $V_E$) was measured on both days using the TrueMax 2400 metabolic system (Parvomedics, Salt Lake City, UT; (Bassett et al., 2001). Heart rate was recorded from an electrocardiograph, and ratings of perceived exertion were assessed using the Borg 6 to 20-point scale. Room temperature was maintained between 19 and 22°C, and subjects were encouraged to drink water between exercise bouts to remain well hydrated.
The purpose of the preliminary cycle ergometer exercise session was to familiarize the subject with the cycle ergometer and pulmonary gas exchange apparatus (i.e., mouthpiece, nose clip). Subjects completed two incremental tests to establish submaximal and peak VO\textsubscript{2}, heart rate, and rating of perceived exertion responses.

**Subject Preparation for Invasive Leg Blood Flow Study**

Subjects were instructed to abstain from products containing caffeine or aspirin for 12 hours prior to testing. Subjects were provided a standardized dinner the evening before (~1800 hr) and a breakfast the morning of (0600 hr) the study. Therefore, all subjects were tested in the post-absorptive state. Subjects were also encouraged to drink 6-8 glasses of water the day before the study.

At the beginning of the study day, indwelling catheters were placed in the femoral vein and the radial artery for direct measurement of leg blood flow, mean arterial pressure (MAP), blood lactate and O\textsubscript{2} content, and plasma adrenaline and noradrenaline concentrations. Preparation for catheter placements typically began between 0700 and 1000 hr. Subjects shaved their right groin region and applied a topical anesthetic (Emla\textsuperscript{TM} crème). Catheters were placed by a physician using aseptic procedures and local anesthetic (2% lidocaine). A thermister wire (IT-18, Physitemp Instruments, Clifton, NJ) and an 18-gauge infusion catheter with 10 side ports (Cook royal flush II 4.0 Fr) were placed approximately 15 cm apart in the right femoral vein (anterograde and retrograde, respectively) for leg blood flow measurement and blood sampling. A 20-gauge Teflon catheter (Arrow arterial catheterization set FA-04020) was inserted into the radial artery for MAP measurements and blood sampling.
**Experimental Protocol**

At the beginning of the protocol, subjects performed 2 submaximal exercise bouts to collect data for another study (Proctor *et al.*, 2003b). Briefly, subjects began with an incremental protocol with workloads ranging from 20-100 watts. Next, subjects rested in the supine position for an hour to allow sympathetic and hemodynamic variables to return to baseline. At the end of the resting period, subjects were placed back on the cycle ergometer and exercised at a moderate intensity eliciting a VO$_2$ of approximately 1.1 L/min (60-70 watts) for 6 minutes, followed by 10 minutes of active recovery at a very light workload (20 watts). Subjects resumed exercise at a workload eliciting 60% VO$_2$peak until steady state heart rate and VO$_2$ were reached (~4 minutes). Next, subjects were asked to perform a cold pressor test (CPT), which consisted of placing the left hand in ice water (0-1°C) for 2-4 minutes while continuing to exercise at the same workload. Subjects continued pedaling at the same workload for 4 minutes after removing their hand from the ice water (post-CPT). Leg blood flow, MAP, lactate, and catecholamines were measured before, during, and after the CPT. Specifically, leg blood flow and MAP were measured 2 to 3 times before the CPT (during the 3rd and 4th minutes or 2nd, 3rd, and 4th minutes), 2 to 3 times during the CPT (within the first 30 seconds and then approximately every minute thereafter) and 2 to 3 times following the CPT (during the 1st and 3rd minutes or during the 1st, 2nd, and 3rd minutes). Arterial and venous blood samples were drawn for measurement of lactate and catecholamines during the 30 seconds immediately before the CPT (pre-CPT), during the last 30 seconds of the CPT (CPT), and during the last 30 seconds of the exercise bout (post-CPT). Following an
hour of rest, subjects performed a maximal graded exercise test on the cycle ergometer, and peak plasma adrenaline and noradrenaline responses were measured.

**Measurements**

*Measurement of leg blood flow and arterial pressure.* Whole leg blood flow was measured during exercise by using the constant infusion, femoral vein thermodilution technique as described previously (Proctor *et al.*, 2003b). Leg blood flow was calculated by using the thermal balance equation detailed by Andersen and Saltin (1985) and doubled to estimate two-leg blood flow (L/min). Simultaneous recordings from the radial artery pressure transducer (Baxter PX-MK099) were displayed, recorded, and analyzed using WinDaq software. The transducer was zeroed at the aortic arch (4th intercostal space) for each subject. Leg vascular conductance was calculated as leg blood flow x 2/MAP.

*Measurement of catecholamines and lactate.* Arterial and venous plasma catecholamine concentrations (5 mL each) were measured using high-performance liquid chromatography with electrochemical detection (Weicker, 1988). Arterial and venous lactate concentrations were measured using a commercially available analyzer (Yellow Springs Instruments 2300 stat-plus).
Body composition. Total body fat, fat-free mass, and leg tissue composition were estimated on a separate laboratory visit using dual-energy x-ray absorptiometry (DXA; Hologic QDR 4500-W, software version 9.80D, Waltham, Mass). Weekly calibrations were performed on the DXA scanner to ensure accuracy.

Data Analysis

For hemodynamic variables (i.e. leg blood flow, MAP, and leg conductance) the values reported for steady state cycling exercise (pre-CPT) represent the average of the last two measurements before the hand was immersed in ice water. Responses reported during CPT represent the highest MAP measurement and its corresponding leg blood flow measurement. Age group comparisons of subject characteristics (Table 3-1) and various hemodynamic and blood variables (Tables 3-2 and 3-3) during exercise and CPT were analyzed using two-tailed two-sample t-tests assuming unequal variances (Mini-tab version 13.1). All data are presented as means ± S.E.M. Statistical significance was accepted when \( P<0.05 \).

Results

Subject Characteristics

Table 3-1 presents subject characteristics of both the older and younger (control) men. Older men had higher percentage of body fat and lower arterial hemoglobin concentration and treadmill VO\(_{2\text{max}}\) \( (P<0.05) \). Height, weight, and plasma cholesterol did not differ between age groups, although the older men tended to be heavier \( (P=0.14) \).
Baseline Resting Measurements

At rest, there were no age group differences observed for blood pressure (Table 3-1). Although arterial noradrenaline concentrations were ~75% higher in the older men, these differences were not significant (Table 3-1).

Responses to Submaximal Leg Cycling

Absolute responses to steady state cycle ergometer exercise are shown in Table 3-2. Although younger and older men exercised at similar relative workloads eliciting 61±2% and 63±3% of peak oxygen uptake respectively (P>0.05), the older men cycled at a significantly lower absolute workload and systemic VO$_2$ due to their lower peak oxygen uptake. Heart rate and lactate concentrations were also significantly lower in the older men. There was a strong trend toward lower leg blood flow responses in the older men (Y: 9.2±0.5, O: 7.2±0.4 L/min; P=0.07) (Figure 1), but MAP responses were identical (Y: 116±3, O: 116±6 mmHg). Leg vascular conductance was not different between young and older men (Y:80±5, O: 63±9 mL min$^{-1}$·mmHg$^{-1}$, P=0.14). It should be noted that the absolute MAP values measured for one of our older subjects were much higher than expected (Figure 3-1B). These data were retained in this analysis because the primary focus of this study was on the change in MAP during CPT rather than absolute values and
because no systematic drift of the blood pressure transducer was noted during this subject's study. Also, this subject's resting MAP was normotensive.

Increases in MAP from baseline during submaximal cycling exercise averaged 20-25 mmHg in both age groups, while heart rate increased to a lesser extent in older men. Arterial noradrenaline increased by $3.0\pm0.5$ nM and $4.1\pm0.9$ nM in younger and older men respectively ($P>0.05$).

**Responses to Local Cold Stimulation**

The sympathetic responses to local cold stimulation are shown in Table 3. When subjects immersed their hand in ice water, arterial noradrenaline rose to a similar degree in both age groups. Systemic VO$_2$ increased slightly in both age groups. Arterial lactate increased significantly more in the younger men, while venous lactate showed a trend in the same direction (Table 3-3).

The hemodynamic responses to local cold stimulation are highlighted in Figure 3-1. Leg vascular conductance showed almost no change in the younger men (-1.5±4%), whereas it decreased by ~14% in the older men ($P=0.04$ between age groups). In the younger men, there was little change in leg vascular conductance because MAP increased by 10±2 mmHg, but leg blood flow x 2 also increased by 0.5±0.3 L/min. In contrast, MAP tended to increase to a larger degree in older men than in young ($P=0.08$), but there was no corresponding increment in leg blood flow. Finally, the percent change in leg vascular conductance per unit change in arterial noradrenaline concentration was much more pronounced in the older men, whether expressed in absolute terms (Figure 3-2A) or
expressed as a percentage of the peak noradrenaline response found during maximal exercise (Figure 3-2B).

**Discussion**

There were three major new findings in the present study. First, cold pressor stimulation applied during submaximal dynamic leg exercise evoked a rapid and marked increase in arterial blood pressure in healthy older men and younger men. Increases in mean arterial pressure (~10-15 mmHg) were physiologically significant, but consistently less than those previously reported under resting conditions (~20 mmHg; Victor *et al.*, 1987; Ng *et al.*, 1994). Second, increases in sympathetic outflow to the exercising legs during local cold stimulation, as estimated by arterial noradrenaline concentrations, were similar in the younger and older men. Our third, and most significant finding was that the older men demonstrated a larger percentage reduction in leg vascular conductance in response to local cold stimulation applied during exercise. These results suggest that vasoconstrictor responsiveness to acute sympathetic stimulation is not reduced, but may be augmented in the exercising legs of healthy non-endurance trained older men. These findings have important implications for the regulation of active limb vasomotor tone and systemic blood pressure regulation during dynamic exercise in older humans.
**Age and vasoconstrictor responsiveness in exercising muscle**

Previous studies have attempted to alter vascular tone in exercising muscles through acute sympathetic stimulation. Strange (1999) found a reduction in leg vascular conductance in young men during one leg knee extensor exercise at both light and moderate intensities when the sympathetic nervous system was stimulated by ischemic handgrip. Leg blood flow was not affected by the sympathetic stimulus, but MAP was significantly elevated (Strange, 1999). Pawelczyk et al. (1992) reported a decrease in leg vascular conductance during cardiac output reductions achieved through $\beta_1$-adrenoreceptor blockade. To the best of our knowledge, however, the present study is the first to test for possible age-associated differences in responsiveness to sympathetic stimulation in active skeletal muscle. Our major new finding was that older men demonstrated a larger percentage reduction in exercising leg vascular conductance during cold pressor stimulation than was observed in younger men. The percentage change in vascular conductance was used because it is the most appropriate index for comparing changes in vascular tone when differences in baseline blood flow exist (e.g. pre-cold stimulation) (Buckwalter & Clifford, 2001). When evaluated in relation to individual changes in arterial noradrenaline concentrations (Table 3-3), the augmented vasoconstrictor responsiveness to sympathetic stimulation in the older men was even more evident. Collectively, these results provide the first evidence of an age-related augmentation of vascular responsiveness to sympathoexcitation in active limbs in healthy humans.
The decrease in leg vascular conductance in response to local cold stimulation in our older subjects relative to their younger counterparts could be due to age differences in the sensitivity, density, or distribution of various adrenergic receptor subtypes. \( \beta_2 \)-adrenoreceptors cause local vasodilation in vascular smooth muscle through both cAMP and nitric oxide mechanisms (Dawes et al., 1997). Because adrenaline readily binds to \( \beta_2 \)-adrenoreceptors, a larger increase in circulating adrenaline in younger men could offset a potential \( \alpha \)-mediated vasoconstriction. However, increases in both arterial and venous adrenaline concentration with cold stimulation did not significantly differ between younger and older men in this study (Table 3-3). If anything, increases in circulating adrenaline during CPT were larger in older men (Table 3-3). We cannot exclude the possibility that an age-related decline in vascular \( \beta_2 \)-responsiveness could explain our results (van Brummelen et al., 1981; Pan et al., 1986).

Another possible explanation for the reduced leg vascular conductance seen in our older men during dynamic leg exercise would be an increase in the density or sensitivity of \( \alpha \)-adrenoreceptors relative to younger men. In this context, Rudner et al. (1999) found an increased \( \alpha_1 \)-adrenoreceptor density in mammary arteries isolated from older patients. However, Dinennon and colleagues (2002a) reported a decrease in \( \alpha_1 \) responsiveness in the forearms of older men at rest, evidenced by a smaller decrement in forearm blood flow in response to phenylephrine (a specific \( \alpha_1 \) agonist). If these results can be extrapolated to the legs, this would argue against an increase in density or sensitivity of \( \alpha \)-adrenoreceptors in our older men.
Our results could also be due to age group differences in myogenic responsiveness. Although an age-related increase in myogenic responsiveness to a large change in MAP could explain the augmented vasoconstriction in older men, there is recent evidence suggesting that myogenic responsiveness is diminished, rather than augmented, in rat soleus and gastrocnemius arterioles (Muller-Delp et al., 2002a).

Although this was not directly tested in the current investigation, an age-related decrease in metabolic modulation of sympathetic vasoconstriction could also explain the decrease in exercising leg vascular conductance in older men during cold stimulation. Recently, Chavoshan et al. (2002) provided evidence that nitric oxide plays a major role in modulating vasoconstrictor responsiveness to acute sympathetic stimulation in the microcirculation of exercising forearm muscle. In the context of ageing, Taddei et al. (2000) found that healthy untrained older humans demonstrate reduced nitric oxide-mediated vasodilation in the forearm. If there is a similar age-related reduction in nitric oxide availability in exercising leg muscles, this could impair the metabolic modulation of sympathetic activity and increase the vasoconstrictor responsiveness of exercising leg muscles to an acute sympathetic stimulus.

**Implications for blood pressure regulation during exercise in older adults**

Why would the leg vasculature of older non-endurance trained men demonstrate an augmented responsiveness to sympathetic stimulation during dynamic exercise? As mentioned in INTRODUCTION, vasoconstriction in active skeletal muscle is necessary for maintaining arterial blood pressure during large muscle dynamic exercise in humans.
Cardiac output measured in these subjects at an intensity of 60% of VO2peak averaged 3.7 L/min lower in the older than in the younger men (unpublished data). Therefore, to achieve the same arterial blood pressure response, the older men would need to maintain a higher level of systemic vascular resistance than the younger men. Taylor et al. (1992) speculated that inactive muscle (i.e., forearm muscle) might be an important contributor to the augmented systemic vascular resistance seen in older men during moderate intensity leg exercise. The results of the present study suggest that active leg muscle is likely to be another major target for sympathetic vasoconstriction to generate a pressor response of this magnitude during large muscle moderate intensity exercise.

**Experimental considerations**

One alternative explanation for the augmented vasoconstrictor responses seen in our older subjects is that the sympathoexcitatory stimulus was greater than that of the younger subjects. This possibility cannot be excluded because calculations of leg noradrenaline spillover (Savard et al., 1987) were negative in several subjects due to limitations associated with our plasma adrenaline measurements (i.e. venous adrenaline concentrations higher than arterial). We also decided against the use of deep venous noradrenaline concentrations as a measure of the sympathetic stimulus because two of these samples from our older men could not be obtained, and another showed a questionable 2.5 nM reduction during cold pressor stimulation. For these reasons, we opted to estimate the strength of the sympathetic stimulus using arterial noradrenaline concentrations. It should be noted that this provided similar estimates to what would
have been obtained using venous noradrenaline concentrations after missing and questionable measurements were excluded.

Although arterial noradrenaline concentrations were used to estimate sympathetic outflow, we believe that local cold stimulation evoked a similar degree of sympathetic outflow in our younger and older subjects for the following reasons: First, although there were trends toward higher arterial noradrenaline concentrations in the older men pre-stimulation (i.e., 60% VO\textsubscript{2peak}), the absolute increases in both adrenaline and noradrenaline concentrations with cold stimulation were similar between groups (Table 3-3). Secondly, under resting conditions, Ng \textit{et al.} (1994) reported similar absolute increases in MSNA in similar groups of younger and older men during 2.5 minutes of hand immersion in ice water. Together, these findings suggest that the increase in sympathetic outflow to the legs under the conditions of the present study was equivalent between the two age groups. Our results are also in agreement with accumulating evidence indicating that sympathoadrenal responsiveness to several types of acute laboratory stress is not exaggerated with age in healthy humans (Ng \textit{et al.}, 1994; Mazzeo \textit{et al.}, 1997).

It is also possible that our results could be explained by differences in the amount of metabolites available to interfere with sympathetic vasoconstriction. In an attempt to match the degree of sympathetic activation between age groups prior to the application of local cold stress, we had subjects exercise at the same relative oxygen consumption (~60% VO\textsubscript{2peak}) (Fleg \textit{et al.}, 1985; Lehmann & Keul, 1986; Mazzeo & Grantham, 1989). This resulted in our older subjects exercising at a lower absolute workload (Table 3-2). Presumably, then, the active muscle mass was smaller in the older men, which would
account for the tendency toward lower absolute limb blood flows in the older men during exercise at 60% VO$_{2\text{peak}}$. The lower arterial and venous lactate concentrations observed in the older men during exercise at 60% VO$_{2\text{peak}}$ both before (Table 3-2) and during cold stimulation (data not shown) suggest that the amount of metabolites available to interfere with sympathetic vasoconstriction may have been lower in the older men. It cannot be determined whether similar age group differences in leg vascular conductance would be observed if subjects were tested at the same absolute workload.

Despite the acknowledged limitations of this investigation, we believe the key consideration is that the cold pressor test produced a similar increase in sympathetic vasoconstrictor neural outflow (i.e., increase in arterial noradrenaline concentrations, Table 3-3) without increasing the metabolic demand appreciably in either subject group (i.e. systemic VO$_2$, Table 3-3). Because metabolic demand showed a similar small increase in both subject groups, while conductance declined only in the older men, it is reasonable to conclude that the larger percentage reduction in leg vascular conductance seen in the older compared to younger men in this study reflects an age-associated increase in vascular responsiveness to sympathetic stimulation in exercising skeletal muscle.

**Conclusions**

In summary, the present study demonstrated an augmented vasoconstrictor response to sympathoexcitation in the arterial vasculature of exercising skeletal muscle of healthy older compared to younger men. These findings add to the accumulating
evidence in the literature suggesting that active limb vasomotor tone is actively regulated by the sympathetic nervous system during exercise. Moreover, these findings have important implications for blood pressure regulation in older humans and support the need for further study of the underlying mechanisms.

Acknowledgements

The authors thank the subjects for participating. We also thank Sean Newcomer, Khoi Le, Kristin Shay, Benjamin Tu, and Jaime Platts for assistance with data collection, Sandy Smithmyer for recruiting and scheduling subjects, Fred Weyandt, Don Fink, and Doug Johnson for technical assistance, and the GCRC nursing staff for their assistance during in-patient studies.

This work was supported by National Institute of Health Grants R01-AG-18246 (to D. N. Proctor), M01-RR-10732 (GCRC), and T32-GM-08619.
Table 3-1. Subject Characteristics. Data are expressed as group means ± S.E.M. for 13 younger and 7 older men except where noted in parentheses. Body fat % was estimated by DXA as described in METHODS. Resting BP indicates seated blood pressure (average of 2-3 visits) measured by auscultation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23 ± 1</td>
<td>67 ± 2</td>
<td>0.00</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.8 ± 1.8</td>
<td>180.2 ± 2.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.5 ± 3.1</td>
<td>85.3 ± 3.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19.2 ± 1.6</td>
<td>26.6 ± 1.4</td>
<td>0.00</td>
</tr>
<tr>
<td>Arterial hemoglobin, g/dl</td>
<td>15.1 ± 0.3</td>
<td>14.0 ± 0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol, g/dl</td>
<td>169 ± 5</td>
<td>182 ± 13</td>
<td>0.37</td>
</tr>
<tr>
<td>Resting systolic BP, mmHg</td>
<td>123 ± 3 (12)</td>
<td>126 ± 5 (6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Resting diastolic BP, mmHg</td>
<td>76 ± 2 (12)</td>
<td>80 ± 2 (6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Treadmill VO$_{2max}$, ml/kg/min</td>
<td>44.9 ± 1.4 (12)</td>
<td>31.5 ± 1.9</td>
<td>0.00</td>
</tr>
<tr>
<td>Resting Arterial Noradrenaline, nM</td>
<td>1.43 ± 0.3 (12)</td>
<td>2.49 ± 0.6</td>
<td>0.15</td>
</tr>
<tr>
<td>Resting Venous Noradrenaline, nM</td>
<td>1.33 ± 0.3</td>
<td>2.65 ± 0.8</td>
<td>0.18</td>
</tr>
<tr>
<td>Resting Arterial Adrenaline, nM</td>
<td>0.35 ± 0.1 (11)</td>
<td>0.56 ± 0.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Resting Venous Adrenaline, nM</td>
<td>0.34 ± 0.1 (11)</td>
<td>0.39 ± 0.1 (6)</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Table 3-2. Responses to submaximal leg cycling prior to local cold stimulation. Data are expressed as group means ± S.E.M. for 13 younger and 7 older men except where noted in parentheses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workload, watts</td>
<td>120 ± 7</td>
<td>86 ± 6</td>
<td>0.00</td>
</tr>
<tr>
<td>Systemic VO₂, L/min</td>
<td>1.70 ± 0.1</td>
<td>1.34 ± 0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Systemic VO₂, % peak</td>
<td>60.6 ± 1.6</td>
<td>63.3 ± 3.0</td>
<td>0.44</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>143.2 ± 4.1</td>
<td>110.6 ± 3.3</td>
<td>0.00</td>
</tr>
<tr>
<td>Leg O₂ extraction, %</td>
<td>66.4 ± 1.6 (12)</td>
<td>71.8 ± 1.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Arterial lactate, mmol</td>
<td>2.76 ± 0.2 (12)</td>
<td>1.74 ± 0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Venous lactate, mmol</td>
<td>3.23 ± 0.3</td>
<td>1.88 ± 0.2</td>
<td>0.00</td>
</tr>
<tr>
<td>Arterial noradrenaline, nM</td>
<td>4.44 ± 0.8 (12)</td>
<td>6.60 ± 1.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Venous noradrenaline, nM</td>
<td>4.43 ± 0.9</td>
<td>7.63 ± 1.8 (6)</td>
<td>0.15</td>
</tr>
<tr>
<td>Arterial adrenaline, nM</td>
<td>0.89 ± 0.1 (11)</td>
<td>1.05 ± 0.2</td>
<td>0.57</td>
</tr>
<tr>
<td>Venous adrenaline, nM</td>
<td>0.75 ± 0.1</td>
<td>0.87 ± 0.1 (6)</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 3. Responses to local cold stimulation during submaximal cycling. Data are expressed as group mean changes (Δ) ± S.E.M. for 13 younger and 7 older men except where noted in parentheses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Systemic VO$_2$, l/min</td>
<td>0.16 ± 0.1</td>
<td>0.08 ± 0.1</td>
<td>0.40</td>
</tr>
<tr>
<td>Δ Systemic VO$_2$, % peak</td>
<td>5.7 ± 0.8</td>
<td>4.0 ± 0.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Δ Heart Rate, beats/min</td>
<td>7.3 ± 1.5 (12)</td>
<td>6.6 ± 1.8 (5)</td>
<td>0.79</td>
</tr>
<tr>
<td>Δ O$_2$ Extraction, %</td>
<td>3.0 ± 1.5 (11)</td>
<td>1.0 ± 1.3</td>
<td>0.32</td>
</tr>
<tr>
<td>Δ Arterial Lactate, mmol</td>
<td>0.59 ± 0.1 (12)</td>
<td>0.30 ± 0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Δ Venous Lactate, mmol</td>
<td>0.61 ± 0.1</td>
<td>0.36 ± 0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Δ Arterial noradrenaline, nM</td>
<td>1.97 ± 0.4 (12)</td>
<td>2.56 ± 1.0</td>
<td>0.59</td>
</tr>
<tr>
<td>Δ Venous noradrenaline, nM</td>
<td>2.09 ± 0.4</td>
<td>1.38 ± 1.3 (5)</td>
<td>0.63</td>
</tr>
<tr>
<td>Δ Arterial adrenaline, nM</td>
<td>0.06 ± 0.1 (11)</td>
<td>0.20 ± 0.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Δ Venous adrenaline, nM</td>
<td>0.21 ± 0.1</td>
<td>0.24 ± 0.3 (5)</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Figure 3-1. Leg blood flow (A), mean arterial pressure (B), and leg vascular conductance (C) before (Pre-CPT) and during (CPT) cold pressor test in younger and older men. Light lines represent individual subject responses, and bold lines represent group means ± S.E.M. for 13 younger and 7 older men.
Figure 3-2. Change (Δ) leg vascular conductance with addition of cold pressor stimulation to steady state leg cycling exercise at 60% VO\textsubscript{2peak} as a function of the absolute change in arterial noradrenaline (A) and the change in arterial noradrenaline expressed as a percentage of peak (B) in 13 younger (○) and 7 older (●) men.
Chapter 4

IS LEG VASCULAR RESPONSIVENESS TO SYMPATHETIC STIMULATION AUGMENTED IN OLDER MEN DURING SMALL MUSCLE DYNAMIC LEG EXERCISE?

Introduction

A considerable body of work has been aimed at understanding the role of the sympathetic nervous system in checking metabolic vasodilation in active muscles to maintain blood pressure and perfusion of vital organs (Buckwalter & Clifford, 2001; Thomas & Segal, 2004; Secher & Volianitis, 2006), yet relatively little is known about how aging modifies this control in adult humans. Recent studies have demonstrated age-related impairments in the modulation of sympathetic vasoconstrictor responsiveness by forearm exercise in women and men (Dinenno et al., 2005). However, previous studies suggest that both vasodilator (Newcomer et al., 2004; Newcomer et al., 2005) and sympathetic vasoconstrictor (Dinenno et al., 1999; Dinenno et al., 2002a; Dinenno & Joyner, 2006) responses differ in the arm and the leg (Proctor & Newcomer, 2006).

In the first study to directly address the effect of age on sympathetic responsiveness in exercising legs, we obtained evidence for augmented sympathetic vasoconstrictor responsiveness in older versus younger men in response to a cold pressor
test performed during upright cycle ergometry at a similar relative workload (Koch et al., 2003). However, it is possible that these results could be explained by older men exercising at a lower absolute intensity, and therefore having fewer metabolites available to interfere with sympathetic vasoconstriction. Furthermore, during leg cycle ergometry, age-associated reductions in cardiac output reserve (Ogawa et al., 1992; Stratton et al., 1994; Fleg et al., 1995; ACSM, 1998) may alter sympathetic outflow through a baroreflex-mediated mechanism (Secher et al., 1977; Rowell, 1997; Secher & Volianitis, 2006). In the current study, sympathetic responsiveness was examined in younger and older men at the same absolute workloads during single leg knee extension exercise. Moreover, in an attempt to isolate the effects of increased metabolic rate from the mechanical effects of exercise, we also examined sympathetic responsiveness in a passively exercising leg. Based on the results of our previous study (Koch et al., 2003), we hypothesized that active leg exercise would significantly reduce sympathetic responsiveness in younger, but not older men.

A key additional consideration when making age group comparisons of sympathetic responsiveness is the whether or not the strength of the sympathetic stimulus is similar between younger and older subject groups. Therefore, the current study included measurements of interstitial norepinephrine (NE), epinephrine (Epi), and adenosine triphosphate (ATP) in a subset of subjects using microdialysis. Interstitial concentrations of these neurotransmitters may be better estimates of the concentration of sympathetic neurotransmitters acting at the neurovascular junction than plasma or spillover measurements (Yamazaki et al., 1997).
Methods

Subjects and Subject Screening

A convenience sample of healthy younger (i.e. 20 to 30 years old) and older (i.e. 60 to 80 years old) men was recruited from Hershey, PA and surrounding communities via word of mouth, fliers, and newspaper advertisements. Each potential subject was prescreened using a telephone interview, and potentially eligible subjects reported to the General Clinical Research Center at the Milton S. Hershey Medical Center for further screening. Each of these subjects was informed of the potential risks and discomforts associated with participation in this study and signed an informed consent form that had been approved by the Institutional Review Boards of the Pennsylvania State University at the Hershey and University Park campuses.

All of the subjects included in this study were apparently healthy. Men with a history or symptoms of cardiac, vascular, pulmonary, metabolic, renal, or neurological disease were excluded from the study. Subjects were also excluded from participation if their resting blood pressure consistently exceeded 140/90 mmHg. None of the subjects had participated in moderate- or high-intensity aerobic exercise for more than 90 minutes per week during the previous 12 months or had a VO$_{2\text{peak}}$ that exceeded the 80$^{\text{th}}$ percentile for their age group based on published norms (ACSM, 2005). Subjects who were lower-body strength trained (>1 day per week for the previous 12 months) were also excluded. None of the subjects took any medications that are known to have significant cardiovascular effects, and they were asked to refrain from alcohol, strenuous exercise, caffeine, medications, and herbal supplements for at least 12 hours prior to all tests.
The screening techniques included a medical history, a physical examination, a physical activity questionnaire (Yale physical activity questionnaire for older men, Baeke physical activity questionnaire for younger men), a blood sample (complete blood count, lipid profile, renal profile, and plasma glucose), and a graded exercise test. Blood samples were drawn by a trained nurse following an overnight fast (at least 12 hours). For the older men, a 12-lead electrocardiogram was monitored by a physician at rest, during, and post graded exercise testing. In the younger subjects, heart rate was monitored using a Polar heart rate monitor (Polar Electro Inc., Lake Success, NY). All graded exercise tests were conducted using a ramping protocol with 1-minute increments in workload on a Lode™ electronically-braked cycle ergometer with toe clips. Pulmonary gas exchange (VO$_2$, VCO$_2$, and minute ventilation, V$_E$) was measured using a TrueMax 2400 metabolic system (Parvomedics, Salt Lake City, UT; Bassett et al., 2001), and ratings of perceived exertion were assessed using the Borg 6 to 20-point scale.

A dual energy X-ray analysis (DXA) scan was performed on all subjects who screened into the study by trained technicians to analyze percent body fat, total muscle mass, and limb muscle masses. Specifically, we were interested in the thigh muscle mass of the leg used for single leg knee extension exercise (described below) in each subject. The same technician evaluated all of the scans and set regions of interest.

Before beginning studies, each subject’s static maximum volitional contraction (MVC) was measured for handgrip in the non-dominant using a Stoelting handgrip dynamometer (Wood Dove, IL). The highest force measured in 3 attempts was taken as the MVC and was used to determine workloads for ischemic handgrip (40% MVC).
Study Procedures

Single Leg Knee Extension Exercise

After screening, all of the testing was done on a single leg knee extension ergometer (Krogh ergometer) which consisted of an adjustable seat and a modified Monark cycle ergometer. The seat was placed in a near-supine position (approximately 15 degrees above supine) for all subjects to minimize baroreflex-mediated changes in sympathetic outflow. The subject’s legs were strapped to the seat just above the knee using adjustable velcro straps, and one foot was placed in a boot which was connected to the crank arm on the Monark ergometer by a metal rod. When the subject extended his knee from the starting position (90° knee flexion), it pedaled the Monark. The subject was instructed to extend his knee at a cadence of 40 per minute, and to let the momentum of the flywheel bring his knee back to the flexed starting position. All subjects were familiarized with single leg knee extension exercise before any blood flow data was collected. During active exercise, subjects received visual feedback on their cadence from a digital tachometer and verbal reminders from the researchers if their cadence deviated substantially from the proper cadence (40 rpm). Near the end of steady state exercise, shortly before the beginning of the sympathetic stimulus, subjects were asked to rate their perceived exertion on a Borg 6-20 scale. The resistance on the ergometer was usually set so that the 40 rpm cadence produced an external workload of 20 watts. However, 2 older subjects had difficulty with the 20 watt workload, so for them resistance was set at 10 or 15 watts. In these cases, we asked 2 younger subjects to repeat the experiment at the reduced workloads to match these older subjects.
Sympathetic Stimulation

Physiological sympathetic stimulation was achieved in this study through ischemic static handgrip exercise (Stoelting handgrip dynamometer) performed with the non-dominant hand at 40% of MVC. This intensity of handgripping was utilized because Seals (1993) observed maximal increases in muscle sympathetic outflow during static handgrip at this intensity. A visual display was calibrated to give the subject visual feedback on the handgrip force. Upon commencement of the sympathetic stimulus to begin, the subject began squeezing the handgrip dynamometer at the target force, followed by a rapid inflation of a blood pressure cuff on the upper arm to 250 mmHg to occlude blood flow to the arm. When the subject was no longer able to maintain the appropriate force despite verbal encouragement (usually 1.5 to 4.5 minutes), he was instructed to relax his hand, and a 2-minute period of post handgrip occlusion began (Post HGCA). Near the end of Post HGCA, the subject was asked to rate the pain or discomfort associated with the forearm ischemia on a 0-10 scale, with 0 representing no discomfort and 10 signifying extreme pain or discomfort. After Post HGCA, the blood pressure cuff was released and blood was allowed to flow back into the arm.

Experimental Protocol

Each study consisted of 2 bouts of single leg knee extension exercise. During 1 bout (active exercise), the subject performed single leg knee extension exercise as described above, and during the other (passive leg movement), each subject was instructed to leave his leg completely relaxed as a researcher moved his leg at 40 rpm.
Compliance was evaluated using non-quantitative electromyography (EMG) electrodes placed over the vastus lateralis of the “exercising” quadriceps. Whenever a researcher noted evidence of EMG activity in the leg, the subject was reminded to keep the leg relaxed. A 30-minute rest period was provided between the 2 exercise bouts, and the order of the exercise bouts was arranged so that half of each age group performed active exercise first, and the other half performed passive exercise first.

A schematic diagram of the experimental protocol is provided in Figure 4-1. Each exercise bout began with a baseline period (BASE) of 2 to 5 minutes, followed by 5 minutes of active or passive leg extension exercise (EX). Next, subjects continued leg extension exercise, but began simultaneous handgrip at 40% MVC, and a blood pressure cuff was inflated on the upper arm to occlude blood flow (i.e. sympathetic stimulation, described above). When subjects had completed ischemic handgrip exercise to exhaustion and the 2-minute Post HGCA, the blood pressure cuff was released and the subject continued leg exercise for another 5 minutes (REC). Most subjects repeated the entire experiment, except for blood draws for plasma catecholamine measurements, on at least 2 days.

**Measurements**

Data were collected and stored using a Powerlab system (AD Instruments, Castle Hill, Australia) operating at a sampling rate of 200 Hz throughout the protocol. Heart rate was measured using electrocardiography, and mean arterial blood pressure was measured using a Finometer Pro (Finapres Medical Systems, Amsterdam, Netherlands) or
Finapres (Ohmeda, Madison, WI) system. Briefly, a finger blood pressure cuff was placed on the middle or ring finger of each subject’s dominant hand (non-gripping hand) before the start of each experiment to non-invasively monitor beat-to-beat changes in mean arterial pressure throughout the experiment. The hand with the finger cuff was kept warm by contact with a bag of warm water to improve the accuracy of measurements. Mean arterial pressure values obtained by Finapres or Finometer were corrected to baseline mean arterial pressure measurements obtained by a Dinamap device (Criticon, Tampa, FL). Single leg knee extension cadence was recorded through a force transducer arranged in series with the rod connecting the subject’s foot to the Krogh ergometer.

Doppler ultrasound (HDI 5000, ATL Ultrasound, Bothell, WA) was used to measure femoral artery blood flow. The common femoral artery was scanned distal to the inguinal ligament, but above the bifurcation into the deep and superficial femoral arteries. Most of the measurements were made using a 4 to 7 Mhz linear array transducer operating at an output frequency of 4 Mhz, although some measurements were made using a 5 to 12 Mhz transducer operating at 6 Mhz. The angle of insonation was ≤ 60 degrees for all studies. Blood velocity was recorded continuously throughout the protocol by filtering the angle-corrected, intensity-weighted audio output from the ultrasound system through a custom-made filter and A-D converter that was sampled by Powerlab. Extensive pilot work established the validity of the custom-made converter (Herr et al., manuscript in preparation). Also, each study was recorded on super-VHS cassettes, which allowed errors of insonation to be detected, and the corresponding blood velocity data to be excluded on Powerlab.
Several studies have relied on near-infrared spectroscopy (NIRS) to examine microvascular responsiveness to sympathetic stimulation (Hansen et al., 1996; Hansen et al., 2000a; Chavoshan et al., 2002; Fadel et al., 2004a; Fadel et al., 2004b). In the present study, therefore, NIRS (LEDI; NIM, Inc., Philadelphia, PA) was used to estimate changes in microvascular blood flow. Briefly, the NIRS unit emits light at two different wavelengths (730 nm and 850 nm). The NIRS probe contains two separate light sources that are separated from the detectors by 30 mm. The absorption of light at 730 nm (deoxygenated hemoglobin, HHb) and 850 nm (oxygenated hemoglobin, HbO$_2$) by the tissue is measured in six detectors within the probe, and produces eight measurements of HHb and HbO$_2$. For this experiment, the NIRS probe was wrapped in clear plastic wrap and placed longitudinally over the vastus lateralis muscle of the leg that was going to be used for passive and active exercise before each study. The probe was held in place using an elastic adhesive bandage wrapped around the probe and the leg. The NIRS unit was calibrated and balanced before each study, and data were collected continuously throughout each study and stored at 3 Hz on a laptop computer to be analyzed. At the conclusion of each study, a blood pressure cuff was inflated around the subject’s upper thigh to suprasystolic pressure (250-300 mmHg) to occlude blood flow to the leg. The researchers waited until the measured microcirculatory oxygenation values reached a steady-state minimum value (usually 6 to 10 minutes) and then released the cuff to determine the maximum physiologic range of oxygenation (total labile signal).

Venous blood samples were taken through a catheter placed in an antecubital vein of the dominant hand at 4 different times during the protocol (see Figure 4-1) for analysis of plasma epinephrine and norepinephrine. Venous blood samples were immediately
placed in ice, and then spun in a refrigerated centrifuge at 3000 rpm for 20 minutes. Plasma was stored in an ultralow freezer at approximately –80°C until analysis. Samples were analyzed using ion-pairing high performance liquid chromatography with electrochemical detection.

To better characterize the true sympathetic stimulus that adrenergic receptors on the vascular smooth muscle in the active leg were exposed to, interstitial catecholamines and ATP were measured during a separate visit in a subset of 5 younger and 4 older subjects. Microdialysis techniques have been described previously (MacLean et al., 2000; Lott & Sinoway, 2004). Four custom-made microdialysis probes were placed into the vastus lateralis muscle of the leg that the subject would be using for single leg knee extension exercise. First, the skin was sterilized, and then the skin and subcutaneous sites were anesthetized by local injections of 0.5-1.0 mL of Lidocaine. Next, the probes were inserted through a 20-gauge cannula, and then attached to perfusion pumps (CMA model 102). The probes were perfused with saline solution at a rate of 5 μl/minute. After probe insertion, the subject was allowed to rest at least 1 hour to allow clearance of products of cellular disruption caused by probe insertion (MacLean et al., 1998; MacLean et al., 2000). Samples were collected during BASE, EX, ischemic handgrip, Post HGCA, and REC during each bout. Dialysate catecholamines and ATP were analyzed using high performance liquid chromatography.
**Data Analysis**

To measure femoral artery diameter, images from the VHS were digitized at approximately 15 frames per second using Brachial Imager software (Medical Imaging Applications, Iowa City, IA) and then analyzed using automated edge-detection software (Brachial Analyzer Software, Medical Imaging Applications). A region of interest was selected along the arterial wall, and the software detected the edge of each vessel wall. A technician performed visual inspections of all frames to ensure that the diameter measurements were made at the intima-lumen border and that images affected by motion artifact were excluded from the analysis. Each diameter measurement represents the average 10-20 seconds during rest.

Femoral blood flow was calculated by using femoral artery diameter and blood flow velocity data according to the following formula:

\[
\text{FBF (mL/min)} = \text{blood velocity (cm/s)} \cdot \pi \cdot \left(\frac{\text{femoral artery diameter (cm)}}{2}\right)^2 \cdot 60,
\]

where 60 is used to convert from mL/second to mL/minute. Femoral artery vascular conductance was calculated as femoral blood flow/mean arterial pressure.

All heart rate, mean arterial pressure, femoral blood flow, femoral vascular conductance, and cadence data was averaged over 20 second (or 20\% of time to fatigue for ischemic handgrip) increments and plotted individually for each subject (i.e. Figures 4-2 and 4-3). When valid data were available for 2 or more trials of the experiment on a subject, responses from the separate trials were averaged. Multiple trials were available for all subjects during passive exercise and for all except 3 young and 3 older men during active exercise. All heart rate, mean arterial pressure, femoral blood flow, and femoral
vascular conductance values used for statistical purposes represented steady state responses averaged over 40 seconds to 1 minute near the end of each condition.

To assess vascular responsiveness to sympathetic stimulation during passive leg movement and active single leg exercise, age group comparisons were made of the percent change in femoral vascular conductance from steady state EX to Post HGCA. For subjects who had a larger percent decrease during passive exercise compared to active exercise, an index of the degree of sympatholysis was calculated using the formula:

\[
\text{Sympatholysis index} = \frac{\% \Delta \text{FVC}_{\text{passive}} - \% \Delta \text{FVC}_{\text{active}}}{\% \Delta \text{FVC}_{\text{passive}}} \times 100
\]

where % Δ FVC represents the percent change femoral vascular conductance from EX to Post HGCA.

For NIRS measurements, calculations of concentrations of HHb and HbO\(_2\) from the absorption of electromagnetic radiation were made using a program provided by NIM, Inc. (led_brain9w.m) run in Matlab (Matlab version 7; The Mathworks, Natick, MA). This program used the Beer-Lambert law to estimate the concentrations of HHb and HbO\(_2\) relative to baseline. HbO\(_2\) data were expressed as percentages of the range between baseline (0%) and the minimum HbO\(_2\) signal obtained during the total labile signal (-100%). These data were averaged over the same 40 second to 1 minute period as the mean arterial pressure, femoral blood flow, and femoral vascular conductance values for each condition.

Statistical analyses were performed using SPSS software (SPSS 13.0, Chicago, IL). Data are reported as group means ± S.E.M. Statistical significance was set at \(P<0.05\). Age group comparisons were made using two-tailed independent sample t-tests. Levene’s test was used to determine whether or not to assume equal variances. One
sample t-tests were used to evaluate if changes in variables were significant within each subject group. Linear regression analysis was used to evaluate the relationships between changes in femoral vascular conductance and changes in norepinephrine, epinephrine, and ATP with sympathetic stimulation.

Results

**Subject Characteristics**

Data were collected on 11 older and 10 younger men, but the results from two older subjects were excluded, one because the subject was physically unable to complete the knee extension protocol, and the other because a run of ventricular tachycardia was detected during a resting period of a protocol. Therefore, the results presented were obtained in 10 younger (24±2 years) and 9 older (71±6 years) men. Subject characteristics are shown in Table 4-1. Body mass index, plasma cholesterol concentration, and plasma LDL concentrations were significantly higher in the older vs. the younger (control) men (\(P<0.05\)). Height, weight, and percent body fat did not differ significantly between age groups. \(\text{VO}_{2\text{peak}}\) was significantly lower in the older subjects (\(P<0.01\)).
Resting and Baseline Measurements

Resting and baseline measurements are highlighted in Table 4-2. During the baseline measurements before the active and passive exercise bouts, plasma and interstitial catecholamine concentrations, ATP concentrations, mean arterial pressure, femoral blood flow, femoral vascular conductance did not differ significantly between age groups before either bout (P>0.1).

Responses to Passive and Active Single Leg Knee Extension

Physiologic responses to passive leg movement (Table 4-3) did not differ substantially between age groups. However, two strong trends emerged: older men tended to have larger absolute increases in blood pressure (P=0.05) and relative changes in interstitial epinephrine (P=0.08) compared to younger men.

During active single leg knee extension exercise (Table 4-4), RPE was similar in younger and older men, but mean arterial pressure responses were elevated in the older men. Older men also tended to have higher femoral blood flow (P=0.07) and greater increases in femoral blood flow from baseline (P=0.02). Venous norepinephrine concentrations tended to be higher in older men (P=0.062), but no other age group differences were noted in catecholamine or ATP responses.
Responses to Sympathetic Stimulation

Passive Single Leg Knee Extension (Table 4-5, Figure 4-4)

During passive single leg knee extension, sympathetic stimulation produced larger increases in mean arterial pressure in young men. Femoral blood flow and vascular conductance did not change significantly in either age group. Venous norepinephrine increased significantly in older ($P=0.001$), but not younger men. Accordingly, there was a trend towards an age-group difference in the increases in venous norepinephrine in response to sympathetic stimulation (Y: 0.22±0.25 nM; O: 0.75±0.16 nM; $P=0.09$). No significant age group differences were observed in perceived pain or interstitial catecholamines or ATP in response to Post HGCA in either group.

Active Single Leg Knee Extension (Table 4-6, Figure 4-5)

When Post HGCA was superimposed on active single leg knee extension, sympathetic stimulation caused a marked drop in femoral vascular conductance in young but not in older men (Y: -14±3%; O: -4±3%, $P=0.045$). Mean arterial pressure increased in both groups, but the increases were approximately twofold higher in young versus older men, while femoral blood flow responses were similar between age groups. Similar to the femoral blood flow responses, relative changes in NIRS-derived HbO$_2$ did not differ between age groups. Perceived pain or discomfort was similar in young and older men. Importantly, no age group differences were noted or plasma or interstitial
catecholamine and ATP concentrations. However, interstitial epinephrine dropped in young subjects and increased in older men, producing age group differences in interstitial epinephrine that nearly reached significance ($P=0.06$). Finally, a strong correlation was noted between the change in interstitial norepinephrine with sympathetic stimulation and the change in femoral vascular conductance in young men, but no relationship between these variables was noted in older men (Figure 4-8B).

**Functional Sympatholysis (Figures 4-6 and 4-7)**

In a subset of 4 young and 4 older subjects, vasoconstriction (decrease in leg vascular conductance) was observed in response to sympathetic stimulation during both passive and active single leg knee extension, and the vasoconstriction was larger during the passive bout, allowing calculation of the sympatholysis index described in METHODS. In this analysis, the percent decrease in femoral vascular conductance was similar in young and older men during passive single leg knee extension (Y: -34±5%; O: -32±9%, $P=0.91$), but the constrictor response was markedly attenuated in the older men during active exercise (Y: -22±2%; O: -9±1%, $P=0.00$) (Figure 4-6). Stated differently, active exercise eliminated 66±7% of the decrease in femoral vascular conductance with sympathetic stimulation in older men, compared to only 32±5% in the young (i.e. greater sympatholysis in older men, $P=0.01$, Figure 4-7). Blood pressure responses in this sub-analysis are similar to those observed in the overall sample, with substantially smaller increases in blood pressure in the older men (data not shown). Although sample sizes were very small (n=2 young and 2 old), interstitial ATP concentrations were
approximately threefold higher in older versus younger subjects throughout passive single leg knee extension ($P=0.02$ to $0.07$).

**Discussion**

Recent studies have suggested that the ability of dynamic exercise to reduce vasoconstrictor responsiveness to sympathetic stimulation (i.e. functional sympatholysis; (Remensnyder et al., 1962) may be blunted in the forearm vasculature of older versus younger men (Dinenno et al., 2005) and women (Fadel et al., 2004b). Moreover, studies from our laboratory have provided preliminary evidence for increased vasconstrictor responsiveness during metabolic stimuli in the legs of older men (Koch et al., 2003). In the present investigation, young and older men performed ischemic handgrip with post-handgrip forearm occlusion to robustly stimulate the sympathetic nervous system. The sympathetic stimulus was superimposed on both active and passive single leg knee extension in an attempt to isolate the effect of metabolic rate from the mechanical effects of muscle contraction on leg vascular responsiveness to sympathetic stimulation. This experimental approach produced three major findings. First, sympathetic stimulation by post handgrip ischemia produced a robust increase in sympathetic outflow during passive single leg knee extension, but this was not associated with a consistent directional change in femoral vascular conductance in either age group. Secondly, during active exercise, there were smaller percentage reductions in femoral vascular conductance in older versus younger men in response to this sympathetic stimulus. Finally, although a sympatholysis index could only be calculated in 4 younger and 4 older subjects in this model, active
exercise eliminated 66±7% of the constriction observed during passive exercise in older men, compared to only 32±5% in younger men (Figure 4-7). In this model, our results do not support an age-associated impairment, but rather an enhancement, in the ability of active exercise to blunt leg vasoconstrictor responsiveness to sympathetic stimulation.

**Age and Adrenergic Responsiveness During Passive Leg Movement**

Most previous studies have examined the effect of exercise on sympathetic responsiveness by comparing vascular responses to acute sympathetic stimulation during exercise with those obtained at rest (Strange, 1999; Jacob et al., 2000; Fadel et al., 2004b; Dinenno et al., 2005; Parker et al., 2007). In this study, we hoped to isolate the effect of increased metabolic rate from the mechanical effects of exercise by comparing vascular responses during active exercise to those obtained during passive movement at the same speed and through the same range of motion. In this context, a recent study suggested that mechanical factors per se do not explain reduced vasoconstrictor responses in the forearm vasculature during exercise (Kirby et al., 2005), but this had never been examined in the leg vasculature, nor had it been examined in older subjects. Interestingly, femoral vascular conductance did not decrease in young or older subjects in response to ischemic handgrip (Table 4-5). Other studies have reported larger vasoconstrictor responses to ischemic handgrip under resting conditions than we found in the current study during passive leg movement (Saito et al., 1990; Strange, 1999; Pellinger & Halliwill, 2007). One possible explanation is that the passive movement may have caused shear-induced release of vasoactive substances that counteracted
sympathetic vasoconstriction (Tanaka et al., 2006). Secondly, it is possible that some subjects inadvertently began recruiting leg muscles during handgrip and/or post-handgrip ischemia, and the increased metabolic demand led to increases in femoral vascular conductance. In support of this possibility, some elevation in leg EMG activity was noted during post-handgrip ischemia in the four subjects (2 young, 2 older) whose femoral vascular conductance increased to the largest extent during the passive bout. The extent of muscle contraction cannot be determined because our EMG was not setup for quantitative analysis. In general, though, we found no age group differences in femoral vascular responses to post-handgrip ischemia during passive single leg knee extension. When viewed in combination with a strong trend toward greater increases in plasma norepinephrine in older subjects (Table 4-5) and smaller increases in blood pressure in older men during sympathetic stimulation superimposed on passive leg movement (Table 4-5), our results are consistent with an age-associated decrease in sympathetic responsiveness in non-exercising vascular beds (Hogikyan & Supiano, 1994; Davy et al., 1998b; Dinenno et al., 2002a).

In a subset analysis (n=4 young and 4 old), we compared vascular responsiveness during passive leg movement in subjects for whom we could calculate the magnitude of sympatholysis (Dinenno et al., 2005). In these 8 subjects, the magnitude of sympathetic vasoconstrictor responses was greater than in the overall sample, averaging 35±14% in younger men and 34 ± 11% in older men. Again, coupled with a strong trend towards higher plasma norepinephrine responses in this subset of older men (P = 0.08), this is consistent with decreased sympathetic responsiveness in the leg vasculature of older men during passive leg movement.
Age and Adrenergic Responsiveness During Active Leg Exercise

Younger and older men averaged similar workloads in the active single leg knee extension protocol. A previous study reported that a similar group of younger and older men did not differ in their peak work capacity in this exercise model (Parker et al., 2008a). Therefore, it is not surprising that in the present study, RPE did not differ between age groups during steady state active exercise either before (Y: 11.9±0.5; O: 11.8±0.5, \( P=0.915 \)) or after (Y: 12.5±0.6; O: 13.0±0.6, \( P=0.527 \)) sympathetic stimulation. However, femoral blood flow tended to be higher \( (P=0.07) \) and mean arterial was significantly higher \( (P=0.011) \) in older vs. younger men during steady state leg exercise without sympathetic stimulation (Table 4-4). Therefore, femoral vascular conductance responses to similar single leg knee extension exercise did not differ between age groups (Table 4-4), thereby facilitating direct comparisons of vasoconstrictor responsiveness to subsequent sympathetic stimulation.

Vasoconstrictor responses were significantly smaller in older vs. younger men during active single leg knee extension exercise \( (P = 0.045) \). In the subset analysis described above, this age difference became even stronger \( (P = 0.002) \). Finally, active exercise blunted vasoconstrictor responses to a greater degree in older versus young men in the subset analysis \( (P = 0.006) \). Although these results differ from the results of previous studies suggesting that dynamic exercise is less able to oppose sympathetic vasoconstriction in older men (Koch et al., 2003; Dinenna et al., 2005), any of several experimental differences between these studies and the current one could explain these differences.
One key factor that differed between studies on the effect of age on sympathetic responsiveness during exercise in men is the type of exercise that was performed. The study by Dinenno et al. (2005) superimposed sympathetic stimulation on forearm exercise, while Koch et al. (2003) and the current study both used leg exercise. This is a key consideration because previous studies suggest that both vasodilator (Newcomer et al., 2004; Newcomer et al., 2005) and sympathetic vasoconstrictor (Dinenno et al., 1999; Dinenno et al., 2002a; Dinenno & Joyner, 2006) responses differ in the arm and the leg (Proctor & Newcomer, 2006). The current study and our previous study (Koch et al., 2003) both examined the balance between sympathetic vasoconstrictor and metabolic vasodilator responses in the leg vasculature, but there were two key differences in the leg exercise performed in these studies. First, in our previous study, subjects exercised on a leg cycle ergometer (large muscle mass involved), while in the current study single leg knee extension exercise was used. In light of reduced cardiac reserve in older versus younger men (Ogawa et al., 1992; Stratton et al., 1994; Fleg et al., 1995; ACSM, 1998), it is possible that the augmented leg vasoconstrictor responsiveness in older men during leg cycling was an adaptive response to help them maintain blood pressure. In the single leg exercise model used in the current study, cardiac reserve would not be a major limitation to leg blood flow (Davies & Sargeant, 1974; Andersen & Saltin, 1985). This was not likely to explain the diverging results between our previous study and the current one because a follow-up analysis of data from our initial study (Koch et al., 2003) suggests that older men had greater cardiac output reserve at the workload used in that study (60% of VO$_{2\text{max}}$). Specifically, young men were exercising at approximately 85±3% of peak cardiac output, while older men were only at 71±2% of their peak cardiac.
output ($P=0.002$). The most likely explanation for the differing results of the current study and our previous study is that subjects in the previous study exercised at a similar relative workload (60% of VO$_{2\max}$). Because of the age-associated differences in aerobic fitness between the subjects in that study, the older men exercised at a lower absolute workload. Therefore, it is possible that the older men vasoconstricted to a larger degree than younger men because they had fewer metabolites available to interfere with sympathetic vasoconstriction. In support of this interpretation, older men had smaller increases in blood lactate concentrations compared to their younger counterparts (Koch et al., 2003). In contrast, all subjects exercised at the same absolute workload in the current study.

Finally, previous studies on this topic have used different sympathetic stimuli, specifically cold pressor stimulation (Koch et al., 2003) and pharmacological techniques (Dinenno et al., 2005). The current study is the only study to date on this topic that employed ischemic handgrip and Post HGCA to stimulate the sympathetic nervous system.

**Potential Mechanisms**

There are several potential mechanisms that may explain the reduced vasoconstrictor responsiveness in older versus younger men during active single leg exercise. One potential explanation lies in the balance between vasoconstriction in response to $\alpha$-adrenoreceptor stimulation and $\beta_2$-mediated vasodilation. Accordingly, a recent study found an age-associated reduction in $\alpha_1$ and $\alpha_2$ adrenergic vasoconstrictor
responsiveness in the leg vasculature at rest (Smith et al., 2007). The mechanism for this impaired responsiveness is unknown, but may reflect age-associated reductions in \(\alpha\)-adrenoreceptor density, decreased \(\alpha\)-adrenoreceptor binding affinity, or impaired downstream signaling (Seals & Dineno, 2004). In this study, the change in interstitial norepinephrine was highly correlated with the degree of constriction with sympathetic stimulation in active muscles in young men, but not in older men (Figure 4-8B). However, interstitial epinephrine decreased in young and increased in older men with sympathetic stimulation (Table 4-6), raising the possibility that \(\alpha\)-adrenergic responsiveness was similar or even augmented in older men, but this was masked by \(\beta_2\)-mediated vasodilation. Although previous studies suggest that \(\beta_2\)-adrenoreceptor signaling plays a minor role in the leg vascular response to sympathetic stimulation via ischemic handgrip (Saito et al., 1990; Pellinger & Halliwill, 2007), to our knowledge this issue had never been explored previously in older subjects.

Another potential explanation for our results is that interstitial ATP concentrations, although not significantly different due to small sample sizes and large intersubject variability, were approximately twofold higher throughout both active and passive exercise in older men. A recent study by Keller and colleagues (2004) demonstrated a role for ATP-sensitive potassium channels (\(K_{ATP}\) channels) in producing sympatholysis responses, confirming previous findings in rats (Thomas et al., 1997). The opening of these potassium channels generally produces hyperpolarization of the vascular smooth muscle, and thus would be expected to enhance dilation and oppose constriction (Quast et al., 1994). Furthermore, these receptors have been shown to be sensitive to a
variety of signals, including nitric oxide, ATP, ADP, oxygen, adenosine (Quayle & Standen, 1994; Nelson & Quayle, 1995). Therefore, enhanced opening of K\textsubscript{ATP} channels in older men, whether via increased ATP or other mechanisms, may underlie the sympatholysis responses observed in the older men in the current study.

Finally, whole leg blood flow did not change substantially during passive or active exercise in younger or older men (average approximately 0.1 L/min increase), despite large increases in mean arterial pressure. Given this observation, the age group differences in vasoconstrictor responses would be secondary to the significant differences observed in the blood pressure responses to ischemic handgrip and post-handgrip circulatory occlusion. To gain insight into the relative contributions of autoregulation and sympathetic vasoconstriction in determining vascular responses to stress, Momen and colleagues (2005) recently examined renal vascular resistance responses to ischemic handgrip in healthy control subjects and kidney transplant patients, whose kidneys are functionally denervated. Although renal vascular resistance increased in both groups of subjects, it was fourfold higher in control subjects. This suggests that, at least in the renal circulation, vasoconstrictor responses depend more on sympathetic activation than on local autoregulation. Whether this is true in contracting skeletal muscles in healthy younger or older humans has not been examined. Therefore, we cannot exclude the possibility that well-preserved metabolic autoregulation produced the vasoconstrictor responses observed in this study, rather than differences in sympathetic vasoconstrictor responsiveness per se.
Magnitude of Sympathetic Stimulation

Although greater changes in sympathetic outflow in response to sympathetic stimulation in young versus older men may explain our results, several lines of evidence suggest that the sympathetic stimulus (i.e. post exhaustive handgrip forearm ischemia) was similar between age groups, or possibly greater in older men. First, ratings of perceived pain or discomfort (0-10 scale) did not differ between groups at the end of Post HGCA in either the passive (Y:6.5 ± 0.6; O: 7.5 ± 0.5; \( P=0.23 \)) or active (Y:6.8 ± 0.4; O: 7.6 ± 0.6; \( P=0.27 \)) bouts. Secondly, there were no age group differences in the absolute or relative changes in interstitial norepinephrine from leg exercise to Post HGCA in either the passive (Table 4-5) or active (Table 4-6) bouts. Also, previous work suggests that, at least during rest, MSNA responses to ischemic handgrip are similar between younger and older men (Ng et al., 1994). If anything, the sympathetic stimulus may have been greater in the older men, based on the observations that the increase in plasma norepinephrine with sympathetic stimulation tended to be greater in older men during passive exercise (\( P=0.09 \); Table 4-5), and a similar trend in interstitial epinephrine was measured during active exercise (\( P=0.07 \); Table 4-6). Notably, although this did not approach statistical significance, the average absolute concentrations of norepinephrine, epinephrine, and ATP in the interstitium were higher in the older men in both bouts, lending further credence to the suggestion that the sympathetic stimulus was not greater in the younger men in this study.
**Physiological Implications**

In a previous study from our laboratory (Proctor et al., 2003b), we used a sample of young and older healthy, normally-active men (similar to the sample used in the current study) to examine age-related changes in leg blood flow during leg cycle ergometer exercise. Notably, the results from that study suggested that leg blood flow responses to large muscle dynamic exercise were not impaired in older versus young men. However, it appears that the older men were able to distribute a greater percentage of cardiac output to the exercising legs, enabling them to compensate for reduced absolute cardiac output responses at a given submaximal workload. If the results from the current study can be extrapolated to large muscle exercise at the same absolute workload, then augmented sympatholysis in exercising muscles may explain, in part, the increased distribution of cardiac output to the legs we previously observed in older men.

**Conclusions**

The results of the current investigation suggest that vasoconstrictor responses to sympathetic stimulation via ischemic handgrip are not augmented, and may be attenuated in the femoral vasculature of healthy older versus young men during single leg knee extension exercise. The mechanism(s) responsible for this reduced vascular responsiveness are complex, but could involve age-associated differences in the balance of $\alpha$ versus $\beta$-mediated-responses at the vascular smooth muscle, local autoregulation, or $K_{\text{ATP}}$ channels.
Figure 4-1. Schematic diagram of experimental protocol. BASE = baseline resting measurements. EX = single leg knee extension before sympathetic stimulus, Post HGCA = forearm circulatory arrest following exhaustive handgrip. REC = single leg knee extension after sympathetic stimulus. Half of the subjects performed active single leg knee extension first, while half performed passive single leg knee extension first.
Figure 4-2. Time course (20 second increments) of femoral blood flow (top panel), mean arterial pressure (center panel), and femoral vascular conductance (bottom panel) in a representative young subject.
Figure 4-3. Time course (20 second increments) of femoral blood flow (top panel), mean arterial pressure (center panel), and femoral vascular conductance (bottom panel) in a representative older subject.
Figure 4-4. Femoral artery blood flow (A), mean arterial pressure (B), and femoral vascular conductance (C) during passive single leg knee extension before (EX) and during (Post HGCA) sympathetic stimulation. Thin lines represent individual subject responses, and thick lines represent group means ± S.E.M.
Figure 4-5. Femoral artery blood flow (A), mean arterial pressure (B), and femoral vascular conductance (C) during active single leg knee extension before (EX) and during (Post HGCA) sympathetic stimulation. Thin lines represent individual subject responses, and thick lines represent group means ± S.E.M.
Figure 4-6. Percent change in femoral vascular conductance in response to sympathetic stimulation by post-exhaustive handgrip forearm occlusion during passive and active single leg knee extension in a subset of 4 younger and 4 older men. Data are expressed as group means ± S.E.M. # Significant difference compared to passive bout. * Significant difference between young and older men.
Figure 4-7. Sympatholysis index (Percentage of vasoconstriction in response to sympathetic stimulation during passive single leg knee extension that is abolished during active leg exercise) calculated in a subset of 4 younger and 4 older men, expressed as group means ± S.E.M. $P=0.002$. 
Figure 4-8. Change in femoral vascular conductance with sympathetic stimulation (forearm ischemia following exhaustive handgrip) during passive (A) and active (B) single leg knee extension exercise, plotted as a function of the change in interstitial norepinephrine in 5 younger and 4 older men.
Table 4-1. Subject characteristics. Values are mean ± S.E.M. for 10 younger and 9 older men. Fat % and lean mass were estimated by DXA as described in METHODS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24 ± 1</td>
<td>71 ± 2</td>
<td>0.00</td>
</tr>
<tr>
<td>Height, cm</td>
<td>182 ± 2</td>
<td>178 ± 2</td>
<td>0.18</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78 ± 2</td>
<td>83 ± 3</td>
<td>0.19</td>
</tr>
<tr>
<td>Body Mass Index (kg/m^2)</td>
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<td>25.9 ± 0.4</td>
<td>0.01</td>
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<tr>
<td>Body fat, %</td>
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<td>0.14</td>
</tr>
<tr>
<td>Thigh lean mass, kg</td>
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<td>8.3 ± 0.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Thigh fat, %</td>
<td>23.6 ± 2.2</td>
<td>23.4 ± 1.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Venous hemoglobin, g/dl</td>
<td>14.5 ± 0.2</td>
<td>14.0 ± 0.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>142 ± 8</td>
<td>188 ± 8</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>49 ± 3</td>
<td>47 ± 3</td>
<td>0.72</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>82 ± 9</td>
<td>120 ± 8</td>
<td>0.01</td>
</tr>
<tr>
<td>Cycle ergometer VO_{2peak}, ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>38.5 ± 1.8</td>
<td>24.5 ± 1.5</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 4-2. Resting and baseline measurements. Values are means ± S.E.M. for 10 younger and 9 older men unless indicated in parentheses. Resting BP indicates seated blood pressure (average of 2-3 visits) measured by auscultation. Baseline BP indicates blood pressure measured by Finometer or Finapres during the baseline period at the beginning of the active and passive exercise bouts. NE stands for norepinephrine. Epi stands for epinephrine. MAP stands for mean arterial pressure. FBF stands for femoral blood flow. FVC stands for femoral vascular conductance. * means significantly different from active baseline ($P<0.05$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate, bpm</td>
<td>64±3</td>
<td>56±2</td>
<td>0.03</td>
</tr>
<tr>
<td>Resting systolic BP, mmHg</td>
<td>121±3</td>
<td>119±4</td>
<td>0.60</td>
</tr>
<tr>
<td>Resting diastolic BP, mmHg</td>
<td>72±2</td>
<td>76±1</td>
<td>0.08</td>
</tr>
<tr>
<td>Passive Baseline Venous NE, nM</td>
<td>2.14±0.35 (9)</td>
<td>2.50±0.45 *</td>
<td>0.54</td>
</tr>
<tr>
<td>Passive Baseline Venous Epi, nM</td>
<td>0.90±0.17 (9)</td>
<td>0.70±0.10</td>
<td>0.31</td>
</tr>
<tr>
<td>Passive Baseline Interstitial NE, nM</td>
<td>2.32±1.28 (5)</td>
<td>3.37±0.78 (4)</td>
<td>0.53</td>
</tr>
<tr>
<td>Passive Baseline Interstitial Epi, nM</td>
<td>3.92±2.18 (5)</td>
<td>3.73±1.53 (4)</td>
<td>0.95 *</td>
</tr>
<tr>
<td>Passive Baseline Interstitial ATP, nM</td>
<td>0.12±0.04 (5)</td>
<td>0.16±0.06 (4)</td>
<td>0.55 *</td>
</tr>
<tr>
<td>Passive Baseline MAP, mmHg</td>
<td>88±3</td>
<td>93±3</td>
<td>0.26</td>
</tr>
<tr>
<td>Passive Baseline FBF, l/min</td>
<td>0.35±0.03</td>
<td>0.34±0.04</td>
<td>0.85</td>
</tr>
<tr>
<td>Passive Baseline FVC, ml/min^-1*mmHg^-1</td>
<td>3.9±0.3</td>
<td>3.6±0.4</td>
<td>0.63</td>
</tr>
<tr>
<td>Active Baseline Venous NE, nM</td>
<td>1.94±0.30 (9)</td>
<td>2.83±0.50</td>
<td>0.14</td>
</tr>
<tr>
<td>Active Baseline Venous Epi, nM</td>
<td>0.65±0.09 (9)</td>
<td>0.94±0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>Active Baseline Interstitial NE, nM</td>
<td>2.61±1.14 (5)</td>
<td>3.55±1.18 (4)</td>
<td>0.59</td>
</tr>
<tr>
<td>Active Baseline Interstitial Epi, nM</td>
<td>2.01±1.12 (5)</td>
<td>3.04±1.44 (4)</td>
<td>0.58</td>
</tr>
<tr>
<td>Active Baseline Interstitial ATP, nM</td>
<td>0.19±0.07 (5)</td>
<td>0.31±0.07 (4)</td>
<td>0.25</td>
</tr>
<tr>
<td>Active Baseline MAP, mmHg</td>
<td>87±3</td>
<td>92±3</td>
<td>0.22</td>
</tr>
<tr>
<td>Active Baseline FBF, l/min</td>
<td>0.37±0.04</td>
<td>0.38±0.06</td>
<td>0.88</td>
</tr>
<tr>
<td>Active Baseline FVC, ml/min^-1*mmHg^-1</td>
<td>4.2±0.4</td>
<td>4.2±0.6</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Table 4-3. Responses to passive single leg knee extension. Values are mean ± S.E.M. for 10 younger and 9 older men unless indicated in parentheses. Δ indicates the change in the value from baseline to steady state during passive single leg knee extension. NE stands for norepinephrine. Epi stands for epinephrine. MAP stands for mean arterial pressure. FBF stands for femoral blood flow. FVC stands for femoral vascular conductance. # represents a significant change within a subject group (P<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate – Passive Ex, bpm</td>
<td>68 ± 4</td>
<td>57 ± 2</td>
<td>0.03</td>
</tr>
<tr>
<td>Δ Heart rate, bpm</td>
<td>4 ± 1 #</td>
<td>1 ± 1</td>
<td>0.01</td>
</tr>
<tr>
<td>Δ Heart rate, %</td>
<td>7 ± 2 #</td>
<td>1 ± 1</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP – Passive Ex, mmHg</td>
<td>90 ± 2</td>
<td>96 ± 3</td>
<td>0.10</td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>1 ± 1</td>
<td>4 ± 1 #</td>
<td>0.05</td>
</tr>
<tr>
<td>Δ MAP, %</td>
<td>2 ± 1</td>
<td>4 ± 1 #</td>
<td>0.10</td>
</tr>
<tr>
<td>FBF – Passive Ex, l/min</td>
<td>0.44 ± 0.04</td>
<td>0.41 ± 0.04</td>
<td>0.53</td>
</tr>
<tr>
<td>Δ FBF, l/min</td>
<td>0.10 ± 0.02 #</td>
<td>0.07 ± 0.02 #</td>
<td>0.40</td>
</tr>
<tr>
<td>Δ FBF, %</td>
<td>30.1 ± 7.5 #</td>
<td>22.5 ± 8.4 #</td>
<td>0.51</td>
</tr>
<tr>
<td>FVC – Passive Ex, ml/min⁻¹·mmHg⁻¹</td>
<td>4.9 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Δ FVC, ml/min⁻¹·mmHg⁻¹</td>
<td>1.0 ± 0.2 #</td>
<td>0.6 ± 0.2 #</td>
<td>0.23</td>
</tr>
<tr>
<td>Δ FVC, %</td>
<td>27.7 ± 6.2 #</td>
<td>19.4 ± 7.8 #</td>
<td>0.41</td>
</tr>
<tr>
<td>Venous NE – Passive Ex, nM</td>
<td>2.18 ± 0.41 (8)</td>
<td>2.63 ± 0.48</td>
<td>0.50</td>
</tr>
<tr>
<td>Δ Venous NE, nM</td>
<td>-0.10 ± 0.28</td>
<td>0.13 ± 0.07</td>
<td>0.45</td>
</tr>
<tr>
<td>Δ Venous NE, %</td>
<td>0.0 ± 11.8</td>
<td>4.8 ± 3.0</td>
<td>0.71</td>
</tr>
<tr>
<td>Venous Epi – Passive Ex, nM</td>
<td>0.78 ± 0.12 (9)</td>
<td>0.84 ± 0.15</td>
<td>0.74</td>
</tr>
<tr>
<td>Δ Venous Epi, nM</td>
<td>-0.17 ± 0.13</td>
<td>0.14 ± 0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>Δ Venous Epi, %</td>
<td>-7.1 ± 11.0</td>
<td>23.5 ± 20.9</td>
<td>0.23</td>
</tr>
<tr>
<td>Interstitial NE – Passive Ex, nM</td>
<td>3.55 ± 2.06 (5)</td>
<td>7.28 ± 2.28 (4)</td>
<td>0.25</td>
</tr>
<tr>
<td>Δ Interstitial NE, nM</td>
<td>1.23 ± 0.79 (5)</td>
<td>3.91 ± 1.58 (4)</td>
<td>0.20</td>
</tr>
<tr>
<td>Δ Interstitial NE, %</td>
<td>27.7 ± 19.7 (5)</td>
<td>107.1 ± 28.0 (4) #</td>
<td>0.05</td>
</tr>
<tr>
<td>Interstitial Epi – Passive Ex, nM</td>
<td>4.79 ± 2.67 (5)</td>
<td>6.28 ± 2.55 (4)</td>
<td>0.70</td>
</tr>
<tr>
<td>Δ Interstitial Epi, nM</td>
<td>0.87 ± 0.58 (5)</td>
<td>2.55 ± 1.04 (4)</td>
<td>0.18</td>
</tr>
<tr>
<td>Δ Interstitial Epi, %</td>
<td>31.1 ± 14.5 (5)</td>
<td>68.7 ± 6.5 (4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Interstitial ATP – Passive Ex, nM</td>
<td>0.75 ± 0.23 (5)</td>
<td>1.28 ± 0.32 (4)</td>
<td>0.20</td>
</tr>
<tr>
<td>Δ Interstitial ATP, nM</td>
<td>0.63 ± 0.25 (5)</td>
<td>1.13 ± 0.30 (4) #</td>
<td>0.23</td>
</tr>
<tr>
<td>Δ Interstitial ATP, %</td>
<td>1738 ± 1378 (5)</td>
<td>870 ± 282 (4)</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table 4-4. Responses to active single leg knee extension. Values are mean ± S.E.M. for 10 younger and 9 older men unless indicated in parentheses. Δ indicates the change in the value from baseline to steady state during single leg knee extension exercise. NE stands for norepinephrine. Epi stands for epinephrine. MAP stands for mean arterial pressure. FBF stands for femoral blood flow. FVC stands for femoral vascular conductance. # represents a significant change within a subject group ($P<0.05$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate – Active Ex, bpm</td>
<td>87 ± 4</td>
<td>74 ± 3</td>
<td>0.03</td>
</tr>
<tr>
<td>Δ Heart rate, bpm</td>
<td>22 ± 3 #</td>
<td>17 ± 1 #</td>
<td>0.12</td>
</tr>
<tr>
<td>Δ Heart rate, %</td>
<td>36 ± 5 #</td>
<td>29 ± 2 #</td>
<td>0.27</td>
</tr>
<tr>
<td>MAP – Active Ex, mmHg</td>
<td>102 ± 2</td>
<td>114 ± 4</td>
<td>0.01</td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>15.3 ± 2.2 #</td>
<td>21.9 ± 3.5 #</td>
<td>0.12</td>
</tr>
<tr>
<td>Δ MAP, %</td>
<td>18.4 ± 3.0 #</td>
<td>24.4 ± 4.4 #</td>
<td>0.27</td>
</tr>
<tr>
<td>FBF – Active Ex, l/min</td>
<td>2.08 ± 0.14</td>
<td>2.42 ± 0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Δ FBF, l/min</td>
<td>1.71 ± 0.11 #</td>
<td>2.03 ± 0.06 #</td>
<td>0.02</td>
</tr>
<tr>
<td>Δ FBF, %</td>
<td>503 ± 59 #</td>
<td>616 ± 72 #</td>
<td>0.24</td>
</tr>
<tr>
<td>FVC – Active Ex, ml/min⁻¹:mmHg⁻¹</td>
<td>20.3 ± 1.2</td>
<td>21.5 ± 1.3</td>
<td>0.50</td>
</tr>
<tr>
<td>Δ FVC, ml/min⁻¹:mmHg⁻¹</td>
<td>16.1 ± 0.9 #</td>
<td>17.4 ± 0.9 #</td>
<td>0.31</td>
</tr>
<tr>
<td>Δ FVC, %</td>
<td>408 ± 43 #</td>
<td>477 ± 55 #</td>
<td>0.34</td>
</tr>
<tr>
<td>Venous NE – Active Ex, nM</td>
<td>2.11 ± 0.30 (8)</td>
<td>3.51 ± 0.61</td>
<td>0.06</td>
</tr>
<tr>
<td>Δ Venous NE, nM</td>
<td>0.02 ± 0.06 (7)</td>
<td>0.68 ± 0.40</td>
<td>0.17</td>
</tr>
<tr>
<td>Δ Venous NE, %</td>
<td>1.1 ± 4.8 (7)</td>
<td>31.4 ± 19.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Venous Epi – Active Ex, nM</td>
<td>0.63 ± 0.12 (9)</td>
<td>0.88 ± 0.15</td>
<td>0.22</td>
</tr>
<tr>
<td>Δ Venous Epi, nM</td>
<td>0.00 ± 0.06 (8)</td>
<td>-0.06 ± 0.17</td>
<td>0.74</td>
</tr>
<tr>
<td>Δ Venous Epi, %</td>
<td>5.6 ± 11.7 (8)</td>
<td>-0.6 ± 13.9</td>
<td>0.74</td>
</tr>
<tr>
<td>Interstitial NE – Active Ex, nM</td>
<td>6.08 ± 3.29 (5)</td>
<td>8.83 ± 3.04 (4)</td>
<td>0.57</td>
</tr>
<tr>
<td>Δ Interstitial NE, nM</td>
<td>3.47 ± 2.24 (5)</td>
<td>5.28 ± 2.03 (4)</td>
<td>0.58</td>
</tr>
<tr>
<td>Δ Interstitial NE, %</td>
<td>207 ± 164 (5)</td>
<td>166 ± 36 (4) #</td>
<td>0.83</td>
</tr>
<tr>
<td>Interstitial Epi – Active Ex, nM</td>
<td>5.54 ± 2.79 (5)</td>
<td>6.33 ± 2.58 (4)</td>
<td>0.85</td>
</tr>
<tr>
<td>Δ Interstitial Epi, nM</td>
<td>3.53 ± 1.69 (5)</td>
<td>3.28 ± 1.30 (4) #</td>
<td>0.92</td>
</tr>
<tr>
<td>Δ Interstitial Epi, %</td>
<td>133 ± 63 (5)</td>
<td>115 ± 59 (4)</td>
<td>0.84</td>
</tr>
<tr>
<td>Interstitial ATP – Active Ex, nM</td>
<td>2.28 ± 0.64 (5)</td>
<td>5.91 ± 2.69 (4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Δ Interstitial ATP, nM</td>
<td>2.09 ± 0.58 (5) #</td>
<td>5.60 ± 2.63 (4)</td>
<td>0.27</td>
</tr>
<tr>
<td>Δ Interstitial ATP, %</td>
<td>1544 ± 567 (5)</td>
<td>1533 ± 532 (4)</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Table 4-5. Responses to sympathetic stimulus during passive single leg knee extension. Values are means ± S.E.M. for 10 younger and 9 older men unless indicated in parentheses. Δ indicates the change in the value from steady state passive single leg knee extension to steady state passive single leg knee extension with superimposed post handgrip forearm ischemia. NE stands for norepinephrine. Epi stands for epinephrine. MAP stands for mean arterial pressure. FBF stands for femoral blood flow. FVC stands for femoral vascular conductance. # represents a significant change within a subject group (P<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perceived pain (0-10), units</td>
<td>6.5 ± 0.6</td>
<td>7.5 ± 0.5</td>
<td>0.23</td>
</tr>
<tr>
<td>Heart rate – Post HGCA, bpm</td>
<td>69 ± 2</td>
<td>58 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>Δ Heart rate, bpm</td>
<td>1 ± 2</td>
<td>1 ± 2</td>
<td>0.93</td>
</tr>
<tr>
<td>Δ Heart rate, %</td>
<td>2 ± 3</td>
<td>2 ± 3</td>
<td>0.99</td>
</tr>
<tr>
<td>MAP – Post HGCA, mmHg</td>
<td>125 ± 2</td>
<td>116 ± 3</td>
<td>0.04</td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>35.1 ± 2.2 #</td>
<td>19.8 ± 2.8 #</td>
<td>0.00</td>
</tr>
<tr>
<td>Δ MAP, %</td>
<td>39.8 ± 3.4 #</td>
<td>21.1 ± 3.4 #</td>
<td>0.00</td>
</tr>
<tr>
<td>FBF – Post HGCA, l/min</td>
<td>0.60 ± 0.10</td>
<td>0.49 ± 0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>Δ FBF, l/min</td>
<td>0.16 ± 0.07 #</td>
<td>0.08 ± 0.06</td>
<td>0.45</td>
</tr>
<tr>
<td>Δ FBF, %</td>
<td>31.8 ± 12.4 #</td>
<td>24.4 ± 15.6</td>
<td>0.71</td>
</tr>
<tr>
<td>Δ HbO₂, %</td>
<td>14.3 ± 4.9</td>
<td>8.4 ± 2.2</td>
<td>0.30</td>
</tr>
<tr>
<td>FVC – Post HGCA, ml·min⁻¹·mmHg⁻¹</td>
<td>4.7 ± 0.7</td>
<td>4.3 ± 0.7</td>
<td>0.70</td>
</tr>
<tr>
<td>Δ FVC, ml·min⁻¹·mmHg⁻¹</td>
<td>-0.2 ± 0.5</td>
<td>0.0 ± 0.1</td>
<td>0.76</td>
</tr>
<tr>
<td>Δ FVC, %</td>
<td>-6.5 ± 9.0</td>
<td>3.2 ± 14.1</td>
<td>0.56</td>
</tr>
<tr>
<td>Venous NE – Post HGCA, nM</td>
<td>2.28 ± 0.40 (9)</td>
<td>3.38 ± 0.45</td>
<td>0.09</td>
</tr>
<tr>
<td>Δ Venous NE, nM</td>
<td>0.22 ± 0.25 (8)</td>
<td>0.75 ± 0.16 #</td>
<td>0.09</td>
</tr>
<tr>
<td>Δ Venous NE, %</td>
<td>17.5 ± 15.0 (8)</td>
<td>40.6 ± 13.3 #</td>
<td>0.27</td>
</tr>
<tr>
<td>Venous Epi – Post HGCA, nM</td>
<td>1.18 ± 0.21 (9)</td>
<td>0.94 ± 0.13</td>
<td>0.35</td>
</tr>
<tr>
<td>Δ Venous Epi, nM</td>
<td>0.46 ± 0.27 (8)</td>
<td>0.10 ± 0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>Δ Venous Epi, %</td>
<td>95.7 ± 60.1 (8)</td>
<td>19.9 ± 12.3</td>
<td>0.21</td>
</tr>
<tr>
<td>Interstitial NE – Post HGCA, nM</td>
<td>3.27 ± 1.56 (5)</td>
<td>7.06 ± 2.31 (4)</td>
<td>0.20</td>
</tr>
<tr>
<td>Δ Interstitial NE, nM</td>
<td>-0.28 ± 0.51 (5)</td>
<td>-0.22 ± 0.60 (4)</td>
<td>0.94</td>
</tr>
<tr>
<td>Δ Interstitial NE, %</td>
<td>48.1 ± 33.0 (5)</td>
<td>-1.3 ± 6.6 (4)</td>
<td>0.23</td>
</tr>
<tr>
<td>Interstitial Epi – Post HGCA, nM</td>
<td>5.66 ± 3.16 (5)</td>
<td>6.59 ± 2.74 (4)</td>
<td>0.84</td>
</tr>
<tr>
<td>Δ Interstitial Epi, nM</td>
<td>0.87 ± 0.51 (5)</td>
<td>0.31 ± 0.49 (4)</td>
<td>0.46</td>
</tr>
<tr>
<td>Δ Interstitial Epi, %</td>
<td>11.9 ± 5.3 (5)</td>
<td>3.8 ± 4.8 (4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Interstitial ATP – Post HGCA, nM</td>
<td>0.64 ± 0.22 (5)</td>
<td>1.32 ± 0.36 (4)</td>
<td>0.14</td>
</tr>
<tr>
<td>Δ Interstitial ATP, nM</td>
<td>-0.19 ± 0.07 (5)</td>
<td>-0.15 ± 0.08 (4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Δ Interstitial ATP, %</td>
<td>-24.9 ± 8.9 (5) #</td>
<td>-7.7 ± 5.4 (4)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Table 4-6. Responses to sympathetic stimulus during active single leg knee extension. Values are mean ± S.E.M. for 10 younger and 9 older men unless indicated in parentheses. Δ indicates the change in the value from steady state single leg knee extension exercise to steady state during single leg knee extension exercise with superimposed post handgrip forearm ischemia. NE stands for norepinephrine. Epi stands for epinephrine. MAP stands for mean arterial pressure. FBF stands for femoral blood flow. FVC stands for femoral vascular conductance. # represents a significant change within a subject group (P<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Younger Men</th>
<th>Older Men</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perceived pain (0-10), units</td>
<td>6.8 ± 0.4</td>
<td>7.6 ± 0.6</td>
<td>0.27</td>
</tr>
<tr>
<td>Heart rate – Post HGCA, bpm</td>
<td>84 ± 4</td>
<td>75 ± 3</td>
<td>0.08</td>
</tr>
<tr>
<td>Δ Heart rate, bpm</td>
<td>-2 ± 2</td>
<td>1 ± 2</td>
<td>0.26</td>
</tr>
<tr>
<td>Δ Heart rate, %</td>
<td>-2 ± 2</td>
<td>2 ± 3</td>
<td>0.23</td>
</tr>
<tr>
<td>MAP – Post HGCA, mmHg</td>
<td>125 ± 2</td>
<td>126 ± 3</td>
<td>0.85</td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>22.7 ± 1.8 #</td>
<td>11.4 ± 2.3 #</td>
<td>0.00</td>
</tr>
<tr>
<td>Δ MAP, %</td>
<td>22.5 ± 2.0 #</td>
<td>10.3 ± 2.1 #</td>
<td>0.00</td>
</tr>
<tr>
<td>FBF – Post HGCA, l/min</td>
<td>2.21 ± 0.17</td>
<td>2.52 ± 0.09</td>
<td>0.14</td>
</tr>
<tr>
<td>Δ FBF, l/min</td>
<td>0.14 ± 0.06 #</td>
<td>0.11 ± 0.06</td>
<td>0.75</td>
</tr>
<tr>
<td>Δ FBF, %</td>
<td>6.2 ± 3.3</td>
<td>4.9 ± 2.6</td>
<td>0.77</td>
</tr>
<tr>
<td>FVC – Post HGCA, ml/min/mmHg⁻¹</td>
<td>17.5 ± 1.2</td>
<td>20.4 ± 1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Δ FVC, ml/min⁻¹/mmHg⁻¹</td>
<td>-2.9 ± 0.6</td>
<td>-1.1 ± 0.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Δ FVC, %</td>
<td>-14.2 ± 3.1</td>
<td>-4.5 ± 3.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Venous NE – Post HGCA, nM</td>
<td>2.93 ± 0.50 (8)</td>
<td>4.12 ± 0.46 (7) #</td>
<td>0.10</td>
</tr>
<tr>
<td>Δ Venous NE, nM</td>
<td>0.98 ± 0.27 (7) #</td>
<td>0.61 ± 0.45 (7) #</td>
<td>0.52</td>
</tr>
<tr>
<td>Δ Venous NE, %</td>
<td>46.7 ± 14.6 (7) #</td>
<td>32.5 ± 13.7 (7) #</td>
<td>0.49</td>
</tr>
<tr>
<td>Venous Epi – Post HGCA, nM</td>
<td>0.89 ± 0.16 (8)</td>
<td>1.09 ± 0.17 (8)</td>
<td>0.41</td>
</tr>
<tr>
<td>Δ Venous Epi, nM</td>
<td>0.24 ± 0.14 (7)</td>
<td>0.22 ± 0.06 (7) #</td>
<td>0.90</td>
</tr>
<tr>
<td>Δ Venous Epi, %</td>
<td>43.0 ± 31.0 (7)</td>
<td>27.9 ± 6.7 (7) #</td>
<td>0.60</td>
</tr>
<tr>
<td>Interstitial NE – Post HGCA, nM</td>
<td>8.42 ± 3.78 (5)</td>
<td>9.56 ± 2.75 (4) #</td>
<td>0.82</td>
</tr>
<tr>
<td>Δ Interstitial NE, nM</td>
<td>-0.46 ± 0.76 (5)</td>
<td>0.73 ± 0.37 (4) #</td>
<td>0.24</td>
</tr>
<tr>
<td>Δ Interstitial NE, %</td>
<td>33 ± 42 (5)</td>
<td>14 ± 6 (4)</td>
<td>0.67</td>
</tr>
<tr>
<td>Interstitial Epi – Post HGCA, nM</td>
<td>4.62 ± 2.45 (5)</td>
<td>7.25 ± 3.00 (4) #</td>
<td>0.51</td>
</tr>
<tr>
<td>Δ Interstitial Epi, nM</td>
<td>-0.92 ± 0.51 (5)</td>
<td>0.93 ± 0.67 (4) #</td>
<td>0.06</td>
</tr>
<tr>
<td>Δ Interstitial Epi, %</td>
<td>-13.3 ± 8.5 (5)</td>
<td>12.1 ± 7.7 (4) #</td>
<td>0.06</td>
</tr>
<tr>
<td>Interstitial ATP – Post HGCA, nM</td>
<td>2.07 ± 0.85 (5)</td>
<td>4.88 ± 1.96 (4) #</td>
<td>0.26</td>
</tr>
<tr>
<td>Δ Interstitial ATP, nM</td>
<td>-0.21 ± 0.28 (5)</td>
<td>-1.03 ± 0.93 (4) #</td>
<td>0.38</td>
</tr>
<tr>
<td>Δ Interstitial ATP, %</td>
<td>-17.2 ± 9.9 (5)</td>
<td>-4.5 ± 20.1 (4) #</td>
<td>0.56</td>
</tr>
</tbody>
</table>
Chapter 5

CONCLUSIONS AND FUTURE DIRECTIONS

The studies in this dissertation explored the influence of augmented sympathetic outflow on leg vascular responsiveness during steady state submaximal exercise in younger versus older men. The key findings were that 1) physiological sympathetic stimulation superimposed on large muscle dynamic exercise at the same relative intensity produced a greater decrease in leg vascular conductance (i.e. augmented vasoconstriction) with age and 2) older men vasoconstricted less than young controls in response to sympathetic stimulation during small muscle exercise at the same absolute intensity.

Age-Related Changes in Sympathetic Vasoconstrictor Responsiveness in Active Legs

The two studies in this dissertation are the only studies to date to examine the effects of age on sympathetic vasoconstrictor responsiveness in exercising leg muscles in men, and they produced opposite results. Specifically, in the first study (Koch et al., 2003), young and older men performed leg cycle ergometer exercise at 60% of VO2peak before and during acute sympathetic stimulation by a cold pressor test (Chapter 3). In this model, sympathetic stimulation produced greater vasoconstriction in the older men. In Study #2 (Chapter 4), larger vasoconstrictor responses were observed in younger men when acute sympathetic stimulation by ischemic handgrip and post-handgrip forearm
ischemia was superimposed on single leg knee extension exercise at the same absolute workload. Importantly, these differing responses probably cannot be explained by study differences in subject characteristics. Independent sample, two-tailed t-tests comparing older subjects in the two studies revealed no significant differences in age, VO$_2$peak, weight, percent body fat, blood cholesterol, or systolic or diastolic blood pressure ($P>0.1$ for all comparisons). Younger subjects in Study #1 (Chapter 3) had significantly higher blood cholesterol levels ($P=0.01$), but there were no other differences in any of these variables ($P>0.1$ for all comparisons). Collectively, this implies that the sample characteristics were similar between the two studies.

A number of experimental differences may explain the opposite results obtained in the two studies. One major difference is that subjects performed large muscle exercise in Study #1 (leg cycling exercise) and small muscle leg exercise in Study #2 (single leg knee extension). This is a potentially important factor because cardiac output limits large muscle exercise (Davies & Sargeant, 1974; Secher et al., 1977; Mortensen et al., 2005), and older men have less cardiac output reserve than their young counterparts (Ogawa et al., 1992; Stratton et al., 1994; Fleg et al., 1995; ACSM, 1998). Conversely, cardiac output is not believed to be an important determinant of leg blood flow during small muscle mass exercise (Davies & Sargeant, 1974; Andersen & Saltin, 1985; Richardson et al., 1995b). Baroreflex-mediated sympathetic restraint of metabolic vasodilation is presumably more important when a large amount of muscle is dilated. In this regard, patients with primary autonomic failure or Shy-Drager syndrome become progressively hypotensive with increasing intensity of exercise (and presumably increased muscle recruitment) (Marshall et al., 1961; Smith et al., 1995; Joyner & Wieling, 1997; Puvi-
Rajasingham et al., 1997; Schrage et al., 2004a). Moreover, during exercise involving large muscle mass, augmented baroreflex-mediated sympathetic outflow may actively restrain blood flow when metabolic demands increase, sometimes creating muscle blood flow “steal” phenomena when cardiac output cannot increase further without compromising blood pressure control (Secher et al., 1977; Harms et al., 1997; Harms et al., 1998; Secher & Volianitis, 2006). In designing Study #2, a single leg knee extensor model was chosen to explore potential age-associated differences in local mechanisms of functional sympatholysis.

While it is unlikely that central mechanisms affected the results of Study #2, it is possible that they affected Study #1. Additional insight can be gained by examining the cardiac output measurements obtained in Study #1. Specifically, an acetylene rebreathing technique was used to estimate cardiac output responses to leg cycle ergometer exercise at 60% VO$_2$peak and at or near 100% VO$_2$peak on a different day from the cold pressor test study (data not shown). The results of this analysis suggest that the young men were at a higher percentage of peak cardiac output during exercise at 60% VO$_2$peak (Y: 85±3%; O: 71±2%; $P=0.00$). Examined differently, young men theoretically could have increased cardiac output by another 3.0±0.7 L/min, while older subjects had another 4.1±0.3 L/min of cardiac output reserve ($P=0.24$). These data suggest that the increased sympathetic vasoconstrictor responsiveness in exercising legs observed in older men in Study #1 probably was not necessary to maintain systemic blood pressure in the face of reduced peak cardiac output.

A more likely explanation for the larger vasoconstrictor responsiveness in older subjects in Study #1 is that, due to age-group differences in aerobic fitness (treadmill
VO_2max averaged 45±1 ml·kg^{-1}·min^{-1} in younger men compared to 32±2 ml·kg^{-1}·min^{-1} for older men, (P=0.00), the lower men were exercising at a lower absolute workload (Y: 120±7; O: 86±6 watts, P=0.00). Mechanistically, the higher metabolic demands in young compared to older men could have produced more metabolites that were present to interfere with sympathetic vasoconstriction. In support of this view, arterial (Y: 2.8±0.2; O: 1.7±0.2 mM; P=0.01) and femoral venous (Y: 3.2±0.3; O: 1.9±0.2 mM; P=0.00) lactate concentrations were higher in young men during leg cycling exercise at 60% VO_2peak (Table 3-2), and the elevations in both were also larger in the young men when cold pressor stimulation was superimposed on ongoing leg exercise (Table 3-3). Two potential mechanisms are known through which increased lactate and decreased pH may reduce sympathetic vasoconstrictor responsiveness. First, the binding of ATP to P2X purinergic receptors on the vascular smooth muscle causes vasoconstriction that is blunted by acidosis (Kluess et al., 2005a). Secondly, lactate and hydrogen ions can activate K_{ATP} channels (Quayle & Standen, 1994; Nelson & Quayle, 1995), causing hyperpolarization of the vascular smooth muscle that reduces the opening of voltage-gated calcium channels (Quast et al., 1994). A role of K_{ATP} channels in mediating sympathetic responsiveness in exercising human thigh muscles was implicated when Keller et al. (2004) partially restored sympathetic vasoconstriction during single leg knee extension exercising by giving a K_{ATP} channel inhibitor.

During single leg knee extension exercise, the older men in Study #2 vasoconstricted less than their young counterparts in response to acute sympathetic stimulation by exhaustive ischemic handgrip, followed by post-handgrip occlusion. There
are several potential mechanisms that may explain the reduced vasoconstrictor responsiveness observed in older versus younger men during active leg exercise. One possibility is that $\alpha$-adrenoreceptor-mediated vasoconstriction was either blunted (Elliott et al., 1982; Hogikyan & Supiano, 1994; Dinenno et al., 2002a; Smith et al., 2007) or masked by $\beta_2$-mediated vasodilation. In support of this view, older men had a strong tendency towards greater increases in interstitial epinephrine in response to sympathetic stimulation during active exercise ($P=0.06$). A second potential mechanism involves ATP. In Study #2, interstitial ATP concentrations were approximately twice as high in older men throughout active exercise, although the age group differences were not significant. It is possible that increased ATP concentrations could impair sympathetic vasoconstriction in older men by activating $K_{ATP}$ channels as discussed above. Alternatively, ATP may reduce vasoconstrictor responsiveness to sympathetic outflow during exercise by binding to P2Y purinergic receptors (Rosenmeier et al., 2004). While this is an attractive hypothesis, we do not have strong evidence in support of it. Similarly, nNOS may play a role in sympatholysis (Thomas et al., 1998; Sander et al., 2000; Fadel et al., 2003). Although the effects of advancing age on nNOS are not known in humans, nNOS expression is upregulated in senescent rats (Capanni et al., 1998).

Finally, it is possible that metabolic autoregulation or the myogenic response could explain the age-group differences in vasoconstriction in active muscles observed in Study #2 independent of local sympathetic responsiveness per se. Blood flow responses did not change appreciably in either age group during sympathetic stimulation (~0.1 to 0.2 L/min increase in both age groups), and mean arterial pressure increased by about twice as much in younger men with sympathetic stimulation (Table 4-6). However,
studies performed at the Hershey Medical Center suggest that, at least in the brachial artery, myogenic responsiveness is greater in older subjects (Lott et al., 2004).

To summarize, it is likely that the increased sympathetic vasoconstriction observed in the exercising legs of older versus younger men in Study #1 were due to age group differences in the absolute workload at which the subjects exercised. The results from Study #2 suggest that there are not significant local impairments in sympatholysis. Therefore, the new working hypothesis that arises from these studies is that sympatholysis responses will not be impaired, and may be augmented, in healthy, normally-active older versus young men performing large muscle dynamic exercise at the same absolute workload unless the intensity is high enough to challenge the limits of cardiac output in these older subjects.

**Physiological Implications**

Understanding the effects of advancing age on the regulation of blood flow to exercising muscles has important implications for muscle oxygen delivery and systemic blood pressure regulation. The current results complement previous work in our lab suggesting that leg hyperemic responses to exercise are well-preserved with advancing age in healthy, normally-active men (Proctor et al., 2003b; Parker et al., 2008a). During large muscle dynamic exercise, healthy, normally-active older men appear to compensate for reduced cardiac output responses by augmenting the percentage of cardiac output that is delivered to the legs (Proctor et al., 2003b). In this regard, decreased sympathetic
vasoconstrictor responsiveness in older men during large muscle dynamic exercise, if present, could help facilitate this enhanced redistribution.

With respect to blood pressure regulation, most studies indicate that blood pressure is higher at any given absolute submaximal workload in older versus younger subjects (Martin et al., 1991b; Fleg et al., 1995). In view of the results of Study #2, it is unlikely that impaired sympatholysis responses in the leg underlie the augmented blood pressure responses we observed in healthy, normally-active older men. This is particularly true during small muscle dynamic exercise where increasing workload is not associated with marked increases in sympathetic outflow (Saito & Mano, 1991). Given the strong trend towards increased muscle blood flow in response to single leg knee extension exercise at 20 watts observed in older men in Study #2 ($P=0.07$), it is likely that the elevated blood pressure responses observed in the older men were due to increased vascular resistance in inactive muscles and/or other regional circulations, like the splanchnic, renal, or cutaneous circulations.

**Future Directions**

Several new questions were raised by the studies in this dissertation. The first questions pertain to the applicability of the results of these studies to large muscle leg exercise at the same absolute workload. Will sympatholysis be attenuated in older men in this model? If not, how can these healthy older men maintain similar to slightly higher leg blood flow responses compared to their younger counterparts (Proctor et al., 2003b)
despite similar or reduced cardiac output and similar or augmented sympatholysis responses?

Additional questions raised by these studies pertain to the mechanisms underlying age-associated differences in sympathetic responsiveness in exercising legs. Would blocking β-adrenoreceptors in the leg vasculature have substantially altered the results? Does age affect α-adrenoreceptor responsiveness in the legs during exercise? Does ATP underlie age-associated differences in leg sympathetic responsiveness via P2X or P2Y purinergic receptors or K_{ATP} channels? What would happen if nNOS was blocked by L-NAME infusion or if nitric oxide bioavailability was increased via sodium nitroprusside or ascorbic acid infusion?

Another question raised by the studies in this dissertation pertains to the potential arm versus leg differences in responsiveness to sympathetic vasoconstrictor stimuli during exercise in older men. Specifically, while sympatholysis appears to be augmented in older men in the leg vasculature (Study #2), Dinenno and colleagues (2005) found evidence for impaired sympatholysis in the forearms of older, sedentary men when they infused tyramine and α-agonists during rhythmic handgrip exercise and a non-metabolic dilator stimulus (adenosine). Were the subjects similar to the subjects in the current studies? Can differences in the mode of sympathetic stimulation (i.e. ischemic handgrip versus infusions of exogenous sympathomimetic agents) explain the difference in the results? Of course, it is possible that sympatholysis could be impaired with age in forearms and maintained or even augmented in the legs. Studies done under resting conditions suggest that both vasodilator (Newcomer et al., 2005) and sympathetic vasoconstrictor (Dinenno et al., 1999; Dinenno et al., 2002a; Dinenno & Joyner, 2006)
responses differ between the arm and leg of younger and older men under resting conditions (Proctor & Newcomer, 2006). Although Dinanno et al. (2005) speculated that the impaired sympatholysis they observed in the forearms of older men may be due to age-associated reductions in endothelial function, there is less evidence to support endothelial dysfunction in the leg vasculature of older adults (Newcomer et al., 2005).

Finally, questions remain as to the effect of age on functional sympatholysis in women. Two studies have been published supporting impaired metabolic modulation of sympathetic vasoconstriction in older versus younger women (Fadel et al., 2004b; Parker et al., 2007). However, neither study directly addressed the ability of dynamic exercise to blunt sympathetic vasoconstriction in the legs. Previous studies from our laboratory highlight important sex differences in the effect of age on leg blood flow and its regulation, and they generally suggest that aging is associated with substantial declines in leg vasodilator capacity in healthy, normally-active women, but less so in men (Proctor et al., 2003a; Proctor et al., 2003b; Koch et al., 2005; Proctor & Parker, 2006; Parker et al., 2008a). It is certainly tempting to speculate that sympatholysis would be impaired in healthy older women.

**Conclusion**

The results of the studies in this dissertation suggest that sympathetic vasoconstrictor responsiveness is not augmented, and may be attenuated, in actively contracting leg muscles during submaximal exercise in healthy older men. In this context, the current study is consistent with accumulating evidence from our laboratory
suggesting leg blood flow and its regulation are not substantially impaired in healthy, recreationally active older men (Proctor et al., 2003b; Newcomer et al., 2005; Parker et al., 2008b). However, this does not preclude the possibility of impaired sympatholytic responses in older women or extremely sedentary or endurance-trained men in whom attenuated leg blood flow responses have been reported (Wahren et al., 1974; Proctor et al., 1998b; Beere et al., 1999; Lawrenson et al., 2003; Poole et al., 2003).
Figure 5-1. Relationship between cardiac output and leg blood flow leg cycle ergometer exercise in young (○) and older (●) men. Reprinted from figure 4A from *J Appl Physiol* 94: 1859-1869.
BIBLIOGRAPHY


Appendix A

CHAPTER 3 INFORMED CONSENT FORM

Title of Project: “Regulation of Muscle Blood Flow in Old Age” (Protocol 1) IRB# 990755

Principal Investigator:
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Phone: 814-863-0724

Other Investigators:
Urs Leuenberger, M.D.
Office: Section of Cardiology, Penn State College of Medicine
Phone: 717-531-6853

This is to certify that I, __________________________, have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Dr. Proctor, Assistant Professor of Kinesiology.

Purpose of the study:
The purpose of this study is to determine if the amount of blood flowing through the muscles of the legs is less in older (compared to younger) people during exercise on a stationary exercise bike. Volunteers will be healthy men and women who are taking no medications that affect the function of the heart and blood vessels and who are not involved in a vigorous exercise program. We are planning to study a total of 60 adult volunteers (15 young women, 15 older women, 15 young men, 15 older men).

Procedures to be followed:
Completion of this study will require a series of initial health screening and exercise tests followed by one 6-hour experiment to measure leg blood flow. For this leg blood flow experiment you will be admitted to the General Clinical Research Center (GCRC) either the night before or the morning of the study so that diet, fluid intake, and activity can be closely controlled. You will also remain in the GCRC for at least one hour after the leg blood flow study is completed.

The initial screening tests will include taking a small blood sample (1/2 ounce) from a vein in your arm, questioning you about your physical activity habits and food preferences, a physical examination by the GCRC Medical Staff, a body composition scan, and a leg volume measurement. These tests will be followed (on different days) by:

- one treadmill exercise-screening test
- two bike exercise tests (each on different days)
- one additional bike test for subjects taking eye drop medication
- one leg blood flow experiment (also involves bike exercise)
Older volunteers will perform the treadmill test with 10 electrodes taped to their chest (electrocardiogram) and an arm blood pressure cuff so that a physician can monitor their heart and blood pressure. Young volunteers will wear a portable heart rate monitor for this test. Both young and older volunteers will wear a mouthpiece and noseclip during the test so that their oxygen consumption can be measured.

Your body composition (% fat, muscle, and bone) will be estimated by dual energy x-ray absorptiometry or “DXA” scanning. This procedure requires that you lie flat on a padded table without moving for 6 minutes while an x-ray scanner moves across your body. You will need to change into a short sleeve shirt, shorts, underwear, and socks when you arrive. Total visit lasts approximately 20 minutes.

Your leg muscle volume will be estimated by water displacement. For this test, you will be asked to place one of your legs into a tank filled with room temperature water. This will be done twice. Skinfold thicknesses and bone width measurements will also be taken with special calipers. These measurements will last approximately 5 minutes.

During all three bike exercise tests (including the leg blood flow experiment), you will be asked to exercise at 3 to 4 different work efforts, including one as hard as you can. You will wear a mouthpiece, noseclip, and adhesive electrodes on your chest. For the second bike exercise test you will also be asked periodically to breathe into and out of a rubber bag containing a mixture of air and a very small amount of a gas (acetylene) that is absorbed into the blood. This gas is not harmful in these very small quantities and is quickly (< 3 minutes) removed from the body.

At the beginning of bike test #2, there will be a 30-minute rest period during which blood flow to your right leg will be measured with a non-invasive technique (ultrasound scanning). This involves placing an ultrasound transducer (hand-held wand or probe) firmly against your skin directly over the blood vessels being measured. Blood flow will be measured in two different blood vessels (behind your knee and just below your groin) using high frequency sound waves that you will not be able to feel. A jelly like substance will be applied to the skin over these areas immediately prior to scanning. You will lie quietly on a padded table during these measurements.

On the afternoons before bike exercise tests #2 and #3, you will be asked to pick up a prepared bag meal at the GCRC kitchen and to eat the entire contents of the meal by 5PM that evening. You will also be encouraged to consume a total of 6 to 8 glasses of fluid during the day before these two bike tests.

Some subjects will complete the leg blood flow study in the morning. These subjects will be required to arrive at the GCRC by at least 9PM on the evening before this study. These subjects will be given a small 7AM breakfast. The rest of the subjects will complete the leg blood flow study in the afternoon. These afternoon subjects will not be required to stay overnight at the GCRC, but will be asked to arrive at 9AM on the morning of the study.

Approximately one hour before the leg blood flow study you will be instructed on how to shave a small area of your right groin and to apply a numbing lotion to the shaved area. You will then be given the opportunity to do this privately. Next, you will be moved to a sterile preparation room in the GCRC where the groin area can be thoroughly cleansed (sterilized). While you are lying
on a cushioned examination table, a physician will inject several small amounts of numbing medicine (anesthetic) in and around the shaved area of your groin. Once the anesthetic has taken effect the physician will insert two soft plastic tubes (catheters) into the leg vein that runs through the groin. We place these two catheters in the groin region so we can directly measure how fast blood flows through your leg muscles during exercise. After these catheters are taped into place, an area of one wrist will be numbed and another soft plastic catheter will be inserted into the wrist artery to measure blood pressure. Small blood samples will then be withdrawn from both the groin vein and wrist artery while you lie on the table for approximately 45 minutes. These samples will be used to measure the oxygen content, blood flow sensitive hormones, and in younger females, reproductive hormones.

You will then be pushed in a wheel chair to the exercise laboratory located next to the GCRC. For the actual exercise tests you will be asked to ride a stationary exercise bike at several different effort levels including one as hard as you can. During each workload, blood and expired air will be obtained and leg blood flow determined. Leg blood flow is determined at each work level by pumping ice-cooled, sterile salt water into your leg vein catheter. You usually cannot tell when this ice water is being injected. The total amount of blood withdrawn during this study will be approximately 8 ounces. During one of the moderate workloads on the bike, you will be asked to place your left hand in a bucket of ice water for 2 to 4 minutes. During other workloads, you may have a sensor placed over your left wrist to measure blood pressure.

After completion of the exercise the soft catheters in your groin and wrist will be removed, these areas bandaged, and you will be moved back to the GCRC for at least 1 hour of observation (and lunch or dinner). You may leave the GCRC when the physician is satisfied that you have recovered from the exercise and the catheter sites show no signs of continued bleeding. You will be asked to return to the GCRC 1-2 days later to ensure that the catheter sites are healing properly.

**Discomforts and risks:**

There is discomfort associated with bike or treadmill testing to maximum effort, including temporary muscle fatigue and shortness of breath. These feelings go away very quickly after exercise is stopped. It is also possible for a participant to stumble or fall on the treadmill or to become lightheaded during the cool-down period. A research assistant will closely monitor you throughout exercise and recovery and will assist you on and off the treadmill. Very rarely (less than once in 2500 tests) will life-threatening problems (such as a heart attack) occur during laboratory exercise testing. However, precautions are taken to avoid these problems including periodic monitoring of the participant’s heart (electrocardiogram) and blood pressure. Investigators are trained in CPR, and medical staff and emergency equipment are in close proximity to the exercise laboratory should an emergency situation arise. Overall, the risks of these exercise tests are minimal, and probably less than if you were to exercise outside of a medical facility by yourself.

The procedures associated with the leg blood flow study also have potential discomforts and risks. A “prickling” sensation often occurs during injection of the numbing medication. This lasts only a few seconds. Mild discomfort can also occur during insertion of the catheters. A feeling of pressure is the most common sensation, but this goes away almost immediately once the catheter is in position. There is a risk of puncturing the leg artery (approximately 1 out of every 10 subjects) or causing nerve irritation (approximately 1 out of every 25 subjects) while attempting to place these leg catheters. There is a risk that insertion of the catheter in the wrist
artery may cause it to spasm (twitch) temporarily (approximately 1 out of 20 subjects). There is a very small risk that a blood clot may form in either the leg vein or wrist artery (less than 1 in 5000 subjects). A blood clot could form in the plastic catheters themselves, but this is unlikely because we frequently “flush” the catheters with a sterile salt-water solution (saline). If a blood clot were to form in the leg or wrist blood vessels, it could stay there leading to swelling of the vessel (thrombophlebitis), or the clot could travel to other blood vessels, including the lungs (thromboembolism). If either of these conditions would develop, you would immediately receive emergency treatment and if necessary you would be transported to Centre Community Hospital for additional medical care. Any of the three catheters used for the leg blood flow study may also cause a bruise, but this black and blue mark usually disappears within 7 days. With these procedures there is also a risk of infection. This risk is minimized by our use of sterile techniques. After completing the leg blood flow study, you will be given written and verbal instructions to take home with you regarding activity restrictions and signs/symptoms of potential problems to watch out for. Overall, the risks associated with leg and arm catheters are extremely small in healthy younger and older people who have been carefully screened for underlying medical problems.

The body composition (DXA) scan and leg volume measurements are virtually risk-free. The total amount of radiation received during the DXA scan is much less than is received during a routine dental x-ray. Subjects will be asked to remove any jewelry from their body prior to the scan.

This study may be hazardous to an unborn or nursing child. There is currently insufficient medical information to determine whether there are significant risks to a fetus carried by a mother participating in this study. Therefore, nursing mothers will not be allowed to participate. Also, women who are still menstruating or who have not been surgically sterilized must have a negative pregnancy test prior to participating in this study. These women will have a urine pregnancy test done at the beginning of each visit. The results of the pregnancy test will be made available to the participant prior to the start of the study. Women who plan to become pregnant within six months after completion of the study should not participate.

**Abnormal test results:**
In the event that abnormal test results are obtained, either during the initial screening tests or during the bike exercises testing, you will be apprised of the results and recommended to contact your medical provider for follow-up.

**Benefits to subjects:**
None.

**Potential benefits to society:**
The ability to exercise declines as we age. The results of these studies will tell us whether reduced blood flow to the leg muscles is in part responsible for this.

**Time duration of the procedures and study:**
The initial blood draw and physical examination will require approximately one-hour. Exercise screening tests (treadmill test, first bike test) will require approximately one-hour each. The body composition scan takes approximately 20 total minutes. For some subjects, one overnight stay in the GCRC will be required the evening before the leg blood flow study. The leg blood flow study itself (including catheter insertions, exercise testing, and the post-test observation period) will end
at approximately 1-2 PM the following day. For the other (afternoon) subjects, the leg blood flow study will end at approximately 5-6 PM. Overall, these studies will take place over approximately a 4 to 6 week period.

**For older subjects who use eye drop medications:**
Older volunteers who use eye drop medications (example: Timoptic) will be required to perform one additional bike test 24 hours after discontinuing their medication (with prior approval from their personal physician). If their heart rate responses are significantly higher during this bike test, then they will be excluded from further study. In either case, these subjects will resume taking their eye drops once this bike test is completed.

**For older subjects who have mildly elevated blood pressure and/or cholesterol:**
Older volunteers with mildly elevated resting blood pressure (systolic pressure between 140 and 159 mmHg and/or diastolic pressure between 90 and 99 mmHg) and/or elevated blood cholesterol (200-239 mg/dl) will also be given the option to participate in these studies. These individuals will need to sign a second consent form.

**Statement of confidentiality:**
Your participation in this research is confidential. Only the investigator, and his/her assistants will have access to your identity and to information that can be associated with your identity. In the event of publication of this research, no personally identifying information will be disclosed.

**Right to ask questions:**
The principal investigator, Dr. Proctor, telephone 814-863-0724 (office) or 814-867-1584 (home), may be contacted at any time about the nature, conduct, or problems with the study. The Office for Research Protections can be contacted at 212 Kern Building, University Park, PA 16802 or by calling 1-814-865-1775 with regards to questions about the rights of research participants. In the event of a research-related emergency, you may contact the GCRC medical staff (7AM-5PM) at 814-865-7103. You will be told of significant new findings or any changes in the study or procedures that may occur.

I have been given an opportunity to ask any questions I may have, and all such questions or inquiries have been answered to my satisfaction.

**Compensation:**
The cost of all tests and procedures directly related to participation in this study will be paid for by the study. These tests and procedures include the initial blood screening tests, the physical exam, the treadmill test, the bike tests, and the leg muscle scan. You will be paid $10 for completing the treadmill test, $20 for completing the first bike test, $50 for completing the second bike test, and $150 for completing the leg blood flow study. Therefore, if you complete all of these tests, you will earn a total of $230. There will be no compensation for the body composition scan, but you will be given a printed report and explanation of your results. If you need to withdraw from the study before completing all tests, you will be paid only for the tests you completed. These payments compensate you for the inconvenience and time associated with participating in this study. If you live out of town (>30 miles driving distance from State College), we will pay for any travel expenses incurred or provide transportation if needed.

If you are an employee of Penn State University, the compensation you receive for participation will be treated as taxable income and therefore taxes will be taken from the total amount. If you
are not employed by Penn State University, total payments within one calendar year that exceed $600 will require the University to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

**In the event of injury:**
I understand that medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. I also understand that I am not waiving any rights that I may have against the University for injury resulting from negligence of the University or investigators.

**Voluntary participation:**
I understand that my participation in this study is voluntary, and that I may withdraw from this study at any time by notifying the investigator. My withdrawal from this study or my refusal to participate will in no way affect my care or access to medical services. I also understand that I may decline to answer specific questions if I so chose.

The investigators may stop your involvement at any time if it is in your best interest, if you do not follow the study requirements, or if the study is stopped.

This is to certify that I consent to and give permission for my participation as a volunteer in this program of investigation. I understand that I will receive a signed copy of this consent form. I have read this form, and understand the content of this consent form.

Volunteer ___________________________ Date __________

I, the undersigned, have defined and explained the studies involved to the above volunteer.

Investigator ___________________________ Date __________
Appendix B

CHAPTER 4 INFORMED CONSENT FORM

Title of Project: Influence of age and sex on acute sympathetic vasoconstrictor responsiveness during small muscle dynamic exercise.

Principal Investigator: Urs Leuenberger, MD

Other Investigators: Dennis Koch, BS, Cynthia Hogeman, BA, BSN, RN, Mary Lott, PhD, MSN, Lawrence Sinoway, MD, David Proctor, PhD, Shelly Silber, BS, RN, Cheryl Blaha, BSN, Samuel Ridout, BS, Beth Parker, BS

Participant’s Printed Name: _____________________________

This is a research study. Research studies include only people who voluntarily choose to take part. This consent form gives you information about this research, which will be discussed with you. This consent form may contain words or procedures that you do not understand. You are urged to ask questions about anything that is unclear to you. Discuss it with your family and friends and take your time to make your decision.

1. Purpose of the Research:
You are being offered the opportunity to take part in this research because you are a healthy volunteer. The capacity to perform the physical activity of daily living and the ease with which physical activity is performed declines with advancing age. This may be due in part to changes in the regulation of blood flow to the legs. The purpose of this research is to determine whether there are age-related changes in the control of blood flow to exercising muscles by the sympathetic nervous system (i.e. fight or flight system). Understanding the factors regulating blood flow to active muscles also has important implications for chronic diseases associated with old age. Approximately 100 people will take part in this research, all at the Hershey Medical Center.

2. Procedures to be followed:
For every visit to the GCRC, you will be asked to refrain from caffeine after 8pm on the evening before the visit.

Screening Procedures:
If you agree to be a subject in this research, you will have several screening procedures to determine if you meet the entry requirements for this research.

Pregnancy Testing: It is important that a fetus (developing unborn baby) not be exposed to any unnecessary risks. If you are a female capable of becoming pregnant, you must not be pregnant at the beginning of this investigation or become pregnant during the research. A urine pregnancy test will be required before you begin the research. If at any point during the research you believe
there is any possibility that you may be pregnant, you must notify the investigator immediately. You may be required to take another pregnancy test.

**Medical History Questionnaire:** You will be asked to fill out a form about your medical history (i.e. major injuries, illnesses, etc.)

**Physical Activity Questionnaire:** You will be asked to fill out a form about your daily/weekly physical activity (i.e. walking, running, lifting, yard work, etc.)

**Physical Examination:** A clinician at the General Clinical Research Center (GCRC) will review your medical history and conduct a brief physical examination.

**Blood Analysis:** You will be asked to provide a small sample of your blood (approximately than 2.5 tablespoons) during the screening visit. These will be common blood tests to determine your health status (including cholesterol and fats in your blood). Blood will be drawn from your arm using a sterile needle. Please note that you will have to fast for at least 12 hours before this blood test to give accurate results. You may drink water during the 12 hour fast.

**Graded Exercise Test:** You will be asked to perform an exercise test on a stationary bicycle to assess your cardiovascular fitness and to rule out any blood pressure or heart abnormalities. During the test, the tension on the pedals will gradually increase every minute until you reach maximal effort. If you are 60-80 years old, your heart will be continuously monitored via a 12 lead ECG (electrical tracing of your heart’s activity). If you are 20-30 years old, your heart rate will be continuously monitored using either a 12 lead ECG or a Polar heart rate monitor. Your blood pressure will be monitored regularly during the test, and you will be asked to indicate how hard you are working. You will also be asked to wear a mouthpiece and a nose clip during this test so that your exhaled air can be measured. You may not have to perform this test if you have performed one within the past year for our lab.

The study doctor will review the results of the screening tests to determine if you qualify for the research. If you are eligible to continue the research you will return to the GCRC for additional studies. For each of these additional studies, you will be asked to refrain from eating and drinking for at least 2 hours before the study. The major purpose of these visits will be to measure the blood flow through blood vessels supplying your legs during rest and exercise with or without activation of the sympathetic nervous system (fight or flight system).

**Familiarization Visit:**

**Body Composition Test:** Your body composition (% fat, muscle, bone) will be estimated using a Dual-Energy X-Ray Absorptiometry (DEXA) test. This whole body scan requires that you lie flat on a padded table without moving for approximately 10 minutes while an X-ray scanner moves over your body.

**Preparation:** You will be strapped into the single leg knee kick device. The single leg knee kick device is a special chair that will be placed in a reclined position, and one of your legs will be strapped into a “boot” through which resistance can be applied. You will be asked to perform tests to measure the strength of your forearm and leg muscles. Handgrip strength will be determined by having you squeeze a handgrip device as hard as you can with your non-dominant hand. This test will be performed three times. Leg strength will be measured by having you attempt to extend your leg against resistance. This test will also be performed three times. Next, ECG probes will be placed
on your chest to measure your heart rate and the electrical activity of your heart throughout the tests. Probes that measure the electrical activity of your muscles (EMG probes) will be placed on your leg and arm to make sure that you keep certain muscles relaxed during the exercise. A blood pressure cuff will be placed on your arm to measure blood pressure at various times during the tests. Another device will be placed on a finger of the same arm to measure blood pressure continuously throughout the tests. Near infrared spectroscopy (NIRS) probes will be placed on the surface of the skin on the outer muscle on each of your thighs. The probes emit and receive near infrared light to measure oxygen in your body. They will be held in place by a bandage. Ultrasound probes will be placed firmly against your skin to painlessly measure blood flow through a vessel to your legs. You will perform two tests, and you will have at least 20 minutes of rest between each test. In one test, you will perform single leg knee kick exercise at a moderate workload (active knee kick), while in another test you will relax your leg as we move it through the same range of motion (passive knee kick). You will be asked to rest quietly for 10 minutes prior to each test.

Single Leg Knee Kick Exercise: At the end of this rest period, we will make our baseline measurements of heart rate, blood pressure, EMG, oxygen, and blood flow. Next, we will have you will perform single leg knee kick (active or passive). For the active knee kick, you will be asked to perform a repetitive kicking motion by extending your leg against resistance. We will ask you to try to use only the muscles on the front of your thigh (quadriceps) for this exercise, and to keep your non-kicking leg completely relaxed. We will check if you are doing, and we will coach you if necessary. Measurements of heart rate, blood pressure, oxygen, and blood flow will then be repeated. The passive knee kick test will be identical except you will keep both legs relaxed, and we will move one of your legs through the same range of motion as the active knee kick test.

Sympathetic Stimulus: We will ask you to continue doing knee kick exercise (active or passive), but we will then have you perform handgrip exercise to stimulate the sympathetic nervous system (fight or flight system). For this handgrip exercise, we will inflate a blood pressure cuff around your non-dominant arm to block blood flow to that arm. You will then squeeze a handgrip device at a target force until exhaustion. Next, you will relax your hand, but continue to do knee kick exercise (active or passive), and measurements of heart rate, blood pressure, oxygen, and blood flow will be repeated before the blood pressure cuff is released to allow blood to flow back to your arm (about 2-3 minutes after exhaustion).

Recovery From Sympathetic Stimulus: Once the sympathetic stimulus is over, (i.e. when the blood pressure cuff blocking blood flow to your arm is released or your hand is removed from ice water), you will continue doing single leg knee kick exercise (active or passive). Measurements of leg blood flow, heart rate, oxygen, and blood pressure will be repeated.

Near Infrared Adjustment: After all other protocols have been completed a short test will be performed to provide the proper adjustment range for the NIRS machine. A blood pressure cuff will be placed on the upper thigh of one leg and inflated to a pressure above your resting blood pressure. During this time NIRS data will be acquired. After several minutes the cuff will be deflated and NIRS data will be recorded for a short time (1 minute).

Experimental Visit 1:
Procedures followed for this experiment will be very similar to those described above for the familiarization visit. One difference is that on this day, a small plastic tube (IV catheter) will be placed in one of your forearm veins for collection of blood samples. The study will require collection of 8 blood samples (~ 8 tablespoons total) to measure chemicals naturally produced by
your exercising muscle. A blood sample will be taken at the end of the rest period, at the end of knee kick exercise, at the end of the sympathetic stimulus, and at the end of the recovery from the sympathetic stimulus.

Sub-Experiments:
On different days, you may be asked to perform one or more of the following protocols:
1. Reproducibility Study. The procedures for this study would be identical to those described for Experimental Visit 1 above. This would allow us to determine how much your responses vary from one day to another.
2. Second Workload. The procedures for this study would be identical to those described for Experimental Visit 1 above, except that the single leg knee kick exercise would be at a lower workload.
3. Time Control. The procedures for this study would be identical to those described for Experimental Visit 1 above, except that you would not perform the sympathetic stimulus.

Optional Microdialysis Visit:
On a different day, if you choose to participate in this section of the experiment, you will undergo a procedure called microdialysis that involves the insertion of up to 6 tiny plastic tubes in your thigh muscle. A salt-water (saline) solution called microdialysis solution will be pumped through the microdialysis probes. To insert the microdialysis probes, the skin over the muscle tissue will be cleaned and a local anesthetic (10 ml of 1% Lidocaine or 100 mg) will be injected into the skin to numb the area. A sterile hollow needle (about the thickness of a paperclip wire) will be inserted through the skin at the entrance site, pushed through the muscle and out through the skin at the exit site. A sterile thin plastic tube (the microdialysis probes) will be inserted through the hollow needle, and the needle will be removed, leaving the microdialysis probe in the muscle. The total amount of solution (described above) to be pumped through the microdialysis probes will be less than 10 mL. During the course of the study, the solution that passes through the microdialysis probe will be collected.

Aside from the microdialysis probes, the study will be very similar to that described above for experimental visit 1. The only differences are that we may choose not to measure blood flow using the Ultrasound probe and/or oxygen in the muscle. In addition, on this day the blood flow to the arm performing handgrip exercise (sympathetic stimulus) will remain blocked for about 5 minutes after you reach exhaustion in the handgrip exercise.

Optional Limb Compression Visit:
Resting measurements: ECG, blood pressure, and resting leg blood flow will be measured using the same techniques as those described for the First and Second Experimental Visits above. In addition, blood flow through your heart (cardiac output) will be estimated by placing an ultrasound probe (small pen like device; similar to that used for measuring leg blood flow) against the left side of your chest with light pressure. There is no blood sampling (venous catheter) during this visit.

Exercising measurements: Blood flow to the exercising leg will be measured as described in the second experimental visit (one light and one moderate workload). However, we will not measure blood flow in the non-exercising leg. Instead, cardiac output will be measured.

Limb compression: The effects of mild leg compression (usually your left leg) will be determined at rest and during submaximal exercise with your opposite leg. This will involve having you place a
nylon inflatable sleeve over your left leg (extends from groin to ankle). This sleeve will be inflated three times at rest (approx. 2 min each with 5 min recovery) and two times during exercise.

3. Discomfort and Risks:

Blood Analysis: The discomfort associated with removing blood by venipuncture (by needle from a vein) is a slight pinch or pin prick when the sterile needle enters the skin. The risks include mild discomfort and/or a black-and-blue mark at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.

Graded Exercise Test: There is discomfort associated with graded exercise testing to maximum effort, including temporary muscle fatigue and shortness of breath. These feelings go away quickly after exercise is stopped. It is possible that you may also experience lightheadedness, chest discomfort, cramping in the legs or stomach, irregular heart beats, and irregular blood pressures during this test. The risk of life-threatening problems (such as a heart attack) is very small (1 in 2500 tests). Other potential risks, including fainting, nausea, muscle strain, and muscle soreness, will be minimized by proper warm-up, familiarization procedures, and cool-down. A research assistant will watch you closely throughout exercise and recovery. Overall, the risks for this exercise test are minimal and are probably less than if you were to exercise outside of a medical facility by yourself.

Body Composition Test: The DEXA bone density procedure results in a small amount of x-ray radiation exposure. The dose to the whole body is approximately 0.5 mrad (mrad is a measure of the radiation dose) and, the dose where the very narrow x-ray beam crosses the femur (upper leg bone) is approximately 24 mrad. When averaged over the entire body, this amount of radiation poses no more risk than the natural background radiation (continuous radiation exposure from cosmic rays, radioactive materials present in the earth and building materials and radioactive materials normally present within the human body) that is received each day from living in south-central Pennsylvania. For further comparison purposes, this is less radiation than is received from a routine chest x-ray or from cosmic rays during a coast-to-coast flight.

Heart Rate, Blood Pressure, EMG Measurements: Other than skin irritation from the sticky electrodes, there are no known risks associated with these procedures.

Strength Testing: There is minimal risk involved with strength testing. You may experience cramping, tightness, and temporary weakness in the muscles required for the test. Additionally, muscle strains or delayed muscle soreness (24-48 hours after testing) may occur. To reduce these risks, warm-up and stretching will be done prior to testing. All procedures will be demonstrated prior to testing.

Resting Leg Blood Flow Tests: The risk associated with measuring leg blood flow and cardiac output using Doppler Ultrasound is minimal. You may experience minor redness at the point where the Ultrasound probe is pressed against your skin. The redness is due to the pressure on your skin from the probe. It is temporary and goes away quickly.

Venous Catheter: The discomfort associated with removing blood by intravenous catheter is slight pinch or pinprick when the sterile needle enters the skin. The risks include mild discomfort and/or black and blue mark at the site of puncture. Less common risks include a small blood clot,
infection or bleeding at the puncture site, or on rare occasions lightheadedness during the procedure.

**Exercising Leg Blood Flow Tests:** Again, the risk associated with Doppler Ultrasound is minimal. There are also minor risks involved with single leg exercise. You may experience cramping, tightness, or temporary weakness in the exercising leg. Additionally, muscle strains or delayed muscle soreness (24-48 hours after testing) may occur. To reduce these risks, warm-up and stretching will be done prior to testing. All procedures will be demonstrated prior to testing. Discomforts for the handgrip exercise include fatigue and slight bruising from the cuff used to block blood flow. Also, you may experience discomfort and/or a numb, tingling sensation in your arm (like when your hand or foot "falls asleep." ) These sensations go away quickly after the cuff is released and blood flow is restored. You may experience very mild tingling during the leg compression experiment (third visit). However, the inflation pressure in the leg cuff is well below that used for the handgrip exercise test (second visit) and the inflation time is brief (about 2 min). Consequently, the risks of numbness or bruising are extremely small.

**Near Infrared Spectroscopy:** There are no known risks associated with near infrared spectroscopy measurements. Discomforts for the near infrared adjustment procedure include slight bruising from the cuff used to block blood flow. Also, you may experience discomfort and/or a numb, tingling sensation in your leg (like when your hand or foot "falls asleep." ) These sensations go away quickly after the cuff is released and blood flow is restored.

**Microdialysis (optional experiment):** The discomfort associated with insertion of microdialysis probes is a slight pinch or pinprick sensation when the sterile needle enters the skin and/or the muscle. You may possibly experience a drop in blood pressure that could result in lightheadedness or fainting. There may be a black and blue mark, some bleeding after the needle placement, and rarely an infection may occur. However, sterile needle will be used so this risk is quite small. Some subjects have discomfort at the site of the probe placement that can last for weeks. There is a slight chance that the microdialysis probe could break and a portion of the fiber stay in the body. However, this has only occurred in 1 study out of over 150 (and most studies insert up to 6 probes each) and there were no adverse reactions since the fibers are made out of a material that is absorbed into the body.

**4. Possible Benefits:**

a. Possible benefits to the participant:
You will not benefit from taking part in this research study.

b. Possible benefits to society:
This research may help to clarify the impact of age on the regulation of blood flow to the legs.

**5. Other Options That Could be Used Instead of this Research:**
You do not have to take part in this research study.

**6. Time Duration of the Procedures and Study:**
The initial screening visit, including the blood draw, physical exam, questionnaires, and the bike screening test will take approximately two hours. Each experimental visit using single leg knee extensor exercise will take approximately two or three hours. The optional microdialysis visit will take approximately four hours. Overall, all visits will be completed within approximately two months.
7. Statement of Confidentiality:

a. Privacy and Confidentiality Measures

Your research records that are reviewed, stored, and analyzed at The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) will be labeled with a code. The list that matches your name with the code will be kept in a locked file in Dr. Leuenberger's office. The research records will be kept in file cabinets in locked rooms at the HMC. In the event of any publication resulting from the research, no personally identifiable information will be shared.

b. The Use of Private Health Information

Health information about you will be collected if you choose to be part of this research study. Health information is protected by law as explained in the HMC Privacy Notice. If you have not received this notice, please request a copy from the researcher. At The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) your information will only be used or shared as explained and authorized in this consent form or when required by law. It is possible that some of the people/groups who receive your health information may not be required by Federal privacy laws to protect your information and may share it without your permission.

To participate in this research you must allow the research team to use your health information. If you do not want us to use your protected health information, you may not participate in this research. Your permission for the use, retention, and sharing of your identifiable health information will continue indefinitely. Any research information in your medical record will be kept indefinitely.

If you choose to participate, you are free to withdraw your permission for the use and sharing of your health information at any time. You must do this in writing as indicated in the HMC privacy notice. Write to Dr. Leuenberger and let him know that you are withdrawing from the research study. His mailing address is Milton S. Hershey Medical Center, Cardiology H047, 500 University Dr, Hershey, PA 17033.

If you withdraw your permission, we will no longer use or share medical information about you for the reasons covered by your written authorization, except when the law allows us to do so. We are unable to take back anything we have already done or any information we have already shared with your permission. We may continue using and sharing the information obtained prior to your withdrawal if it is necessary to maintain the soundness of the overall research. We are required to keep our records of the care that we provided to you as long as the law requires.

The research team may use the following sources of health information:

All data collected during your participation in this research.

Representatives of the following people/groups within HMC/PSU are allowed to use your health information and share it with other specific groups in connection with this research study:

The principal investigator, Dr. Leuenberger
The HMC/PSU Institutional Review Board
The HMC/PSU Human Subjects Protection Office
The research team.

The people or groups listed in the above paragraph may share your health information with the following persons and organizations outside HMC/PSU for their use in connection with this research.
study. These groups, while monitoring the research study, may also review and/or copy your original PSU/HMC records:

The Office of Human Research Protections in the U. S. Department of Health and Human Services
The National Institutes of Health.

8. Costs for Participation:
 Costs: The costs of all tests and procedures directly related to participation in this study will be paid for by the study. These tests and procedures include the initial blood screening tests, the physical exam, and the bicycle test.

Treatment and compensation for injury: Every effort to prevent injury as a result of your participation will be taken. It is possible, however, that you could develop complications or injuries as a result of participating in this research study. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. Costs for the treatment of research-related injuries will be charged to your insurance carrier or to you. Some insurance companies may not cover costs associated with research studies. If for any reason these costs are not covered by your insurance, they will be your responsibility.

You are not waiving any legal rights you may have by signing this form.

9. Compensation for Participation:
You will be paid $20 for completing the screening procedures, $40 for completing the familiarization visit, $40 for experimental visit 1, $40 for each of the sub-studies you participate in, $200 for completing the optional microdialysis study, and another $40 for completing the additional experimental visit (optional limb compression test). In addition, if you live within 25 miles of the Hershey Medical Center, or if you use the Penn State Shuttle service to transport you from State College, PA to the Hershey Medical Center, you will be reimbursed an additional $5 per visit to the Hershey Medical Center for travel expenses. If you live more than 25 miles from the Hershey Medical Center and do not use the Penn State Shuttle service, you will be reimbursed travel expenses based on your mileage traveled, and reimbursement for a hotel will be provided if it is necessary in order for you to participate in the study. If you need to withdraw from the study before completing all tests, you will be paid only for the tests you completed. These payments will compensate you for the inconvenience and time associated with participating in this study. To receive a check for payment, you will have to provide your social security number and address for tax reporting purposes.

10. Research Funding:
The institution and investigators are receiving a grant from the National Institute of Health to support the activities that are required to conduct this research.

11. Voluntary Participation:
Taking part in this research study is voluntary. If you choose to take part in this research, your major responsibilities will include fasting for 12 hours (except water) before the screening test and abstaining from caffeine on the evening before (after 8pm) and morning of each visit. Also, for each additional visit, you should refrain from eating for at least 2 hours before you arrival at the GCRC. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in
the research at a later date, there will be no penalty or loss of benefits to which you are entitled. In other words, your decision not to participate in this research or to stop taking part in the research will not affect your access to medical care, academic standing, or job status.

The research team may take you out of the research study without your permission. Possible reasons for this are failure to meet the criteria for participation in the study, failure to follow the study doctor’s instructions, side effects, or pregnancy. Also, the sponsor of the research may end the research study early. If your taking part in the research ends early, you may be asked to visit the research doctor for a final visit.

12. **Contact Information for Questions or Concerns:**
You have the right to ask any questions you may have about this research. If you have questions or concerns or believe you may have developed an injury that is related to this research, contact Dr. Leuenberger at 717-531-6853. If you have questions regarding your rights as a research participant or about your privacy and the use of your personal health information, you may contact the research protection advocate in the HMC Human Subjects Protection Office at 717-531-5687.

**Signature and Consent/Permission to be in the Research**
Before making the decision regarding enrollment in this research, you should have:
- Discussed this study with an investigator,
- Reviewed the information in this form, and
- Had the opportunity to ask any questions you may have.
Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

**Participant:** By signing this consent form, you indicate that you are voluntarily choosing to take part in this research.

<table>
<thead>
<tr>
<th>Signature of Participant</th>
<th>Date</th>
<th>Time</th>
<th>Printed Name</th>
</tr>
</thead>
</table>

Please initial on this line ONLY if you agree to participate in the OPTIONAL microdialysis visit: ____________

Please initial on this line ONLY if you agree to participate in the OPTIONAL limb compression visit: __________

**Person Explaining the Research:** Your signature below means that you have explained the research to the participant/participant representative and have answered any questions he/she has about the research.

<table>
<thead>
<tr>
<th>Signature of person who explained this research*</th>
<th>Date</th>
<th>Time</th>
<th>Printed Name</th>
</tr>
</thead>
</table>

(*Only approved investigators for this research may explain the research and obtain informed consent.*)
Chapter 3 of this dissertation is an article that has been published in the Journal of Physiology. Copyright permission has been obtained for the inclusion of the manuscript in this dissertation. In addition, parts of Chapters 2 and 5 have been published in a review article published in the Canadian Journal of Applied Physiology. Permission to use this text has also been obtained. Finally, Figure 5-1 was published in the Journal of Applied Physiology. Permission to use this figure has been obtained.
VITA

Dennis William Koch

Education
December 2008 Ph.D., Physiology, The Pennsylvania State University
May 1999 B.S., Biology, Minor in Exercise Science, Canisius College

Fellowship
1999-2002 National Institute of Health Stress Physiology Training Fellow

Awards
2005-2006 The Chancellor’s List Graduate Student Honoree
2004 Caroline tume Suden/ Frances Hellebrandt Professional Opportunity Award for Excellence in Research
2002 Pennsylvania State University Physiology Program Poster Contest Winner

Publications


