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Skeletal Muscle Sympathetic Vasoconstrictor Control Following Short-Term Mild- and Heavy-Intensity Exercise Training

by

Nicholas G. Jendzjowsky

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Abstract

The purpose of this thesis was to investigate the effects of short-term endurance exercise training (ET) on sympathetic vasoconstriction in resting and contracting skeletal muscle. To achieve these aims, rats were exercise trained on a motorized treadmill and an anesthetised whole animal *in situ* vascular preparation was used to investigate the effects of ET on sympathetic vascular control in resting and contracting skeletal muscle.

Exercise training augmented resting sympathetic vasoconstrictor responsiveness in a training intensity-dependent manner. Concurrently, endothelium-dependent vasodilation was augmented in a training intensity-dependent manner and significantly correlated to the magnitude of sympathetic vasoconstrictor responsiveness.

During acute exercise sympathetic outflow is increased however the vascular response to sympathetic stimulation is diminished and aids in the regulation of skeletal muscle blood flow; a physiologic phenomenon termed functional sympatholysis. The inhibition of sympathetic vasoconstriction during muscular contraction was augmented in a manner influenced by the intensity of ET through a nitric oxide-dependent mechanism.

Given the increased resting sympathetic vasoconstrictor responsiveness and enhanced sympatholysis following training, it was hypothesized that ET may mediate these effects, in part, by an altered contribution of post-synaptic α_2-adrenergic receptors to the regulation of sympathetic vasoconstriction. Exercise training significantly augmented the contribution of α_2-adrenergic receptor to basal sympathetic vasoconstriction. During contraction the α_2-adrenergic

receptor was relatively resistant to inhibition. The ET induced increase in sympatholysis was mediated, in part, by the inhibition of the α_1-adrenergic receptor through a nitric oxide-dependent mechanism.

Collectively, these results highlight the integrated nature of ET mediated adaptations to sympathetic regulation of skeletal muscle vascular conductance and the plasticity of sympathetic regulation of skeletal muscle vascular conductance.

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ACh, acetylcholine; ATP, adenosine triphosphate; EDD, endothelium-dependent vasodilation; ET, chronic endurance exercise training; eNOS, endothelial nitric oxide synthase; FBF, femoral artery blood flow; FVC, femoral vascular conductance; H, heavy-intensity trained group; HR, heart rate; L-NAME, N_{ω}-Nitro-L-arginine methyl ester hydrochloride; M, mild-intensity trained group; MAP, mean arterial pressure; MCF, maximal contractile force; MSNA, muscle sympathetic nerve activity; MT, motor threshold; NE, norepinephrine; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; paO_2, arterial oxygen tension; $paCO_2$, arterial carbon dioxide tension; S, sedentary time-control group.

Chapter 1

Introduction

Cardiovascular diseases (i.e. hypertension and coronary artery disease) account for ~ 30% of deaths in Canada (11) and an estimated annual economic burden of ~ $21 billion (10). Non-modifiable (age, gender and genetics) and modifiable risk factors (hypertension, elevated serum cholesterol and glucose, smoking and a sedentary lifestyle) contribute to the development of cardiovascular diseases. Of the modifiable risk factors, a reduction of sedentary behavior by increasing physical activity has been shown reduce the severity of other modifiable risk factors such as serum cholesterol and glucose (4, 5) and in the long-term to reduce cardiovascular disease morbidity and mortality (2, 47, 52). Indeed, it has been estimated that a 10% increase in the prevalence of physical activity, in the form of chronic endurance exercise training (ET), could potentially reduce Canadian health care costs by ~ $150 million and prevent ~ 2000 premature deaths annually (36).

Despite the documented evidence that regular physical activity and ET reduce cardiovascular disease risk (2, 47, 52), our understanding of the mechanisms responsible for the cardio-protective effects of ET is not completely clear. For example, differences in cardiovascular disease risk factors only explain ~ 60% of the lower cardiovascular disease risk in physically active women (54). Therefore, other factors beyond changes in traditional risk factor profiles must contribute to the cardio-protective effects of ET. Cardiovascular diseases are characterized by arterial dysfunction and altered regulation of vascular resistance and arterial blood pressure. The vascular dysfunction is characterized by reduced endothelial function (6, 48, 82) and by

heightened efferent muscle sympathetic nerve activity (MSNA) at rest and in response to physiological stress (13, 61, 63, 95). These functional changes result in a reduced vasodilator capacity, elevated vasoconstriction, reduced tissue blood flow and increased arterial pressure.

Several studies in human and animal experimental models have demonstrated that ET can improve vascular function (3, 14, 30, 32, 40, 42, 43, 60, 96). Therefore, it is conceivable that ET may reduce cardiovascular disease risk and severity through improvement(s) in the basic physiological mechanisms that regulate arterial blood vessel function (24, 27, 35).

Consistent with this notion, ET has been repeatedly shown to improve the vasodilator function of the skeletal muscle vasculature (16, 17, 21, 37, 38, 45, 46, 50, 51, 73, 77, 101). The beneficial effect of ET on vasodilator function appears to be mediated by an increase in endothelium-dependent vasodilation (EDD) (15, 26, 43, 90, 91) and may be associated with increased nitric oxide (NO) bioavailability, and/or endothelial NO synthase (NOS) protein expression (38, 80, 81). In contrast to the documented benefits of ET on vasodilator function, relatively little is known about the effect of ET on sympathetic nervous system-mediated control of the skeletal muscle vasculature.

Sympathetic nervous system activity evokes the release of norepinephrine (NE) that binds to post-synaptic α_1- and α_2-adrenergic receptors on vascular smooth muscle to produce vasoconstriction. In the skeletal muscle vascular bed, tonic sympathetic vasoconstriction has been documented at rest and during exercise (1, 7, 8, 19, 31, 49, 53, 59, 103). Chronic endurance exercise training has the potential to alter the regulation of sympathetic vasoconstriction in the skeletal muscle vasculature by affecting efferent sympathetic nervous system outflow, neurotransmitter release and/or the vascular response to sympathetic activity. However to date, there has been relatively

little investigation of the effects of ET on the regulation of sympathetic vasoconstriction and the existing scientific literature is conflicting. For example, several studies have reported that resting MSNA (72) or plasma NE concentrations (62, 99, 100) are unchanged after ET. However in contrast, Grassi et al. (23) have reported that 10 weeks of run training (2 hours·day^{-1}) reduced basal MSNA and a cross-sectional study reported that basal MSNA was increased in trained compared to untrained adults (58).

Exercise training may also impact the ability of the vasculature to respond to sympathetic stimulation. Indeed, previous studies have reported that the vasoconstrictor response to NE or the α_1-adrenergic receptor specific agonist phenylephrine was decreased (17, 21, 78, 97), increased (39, 46, 51) or unchanged (34, 81) following ET. The vasoconstrictor response to sympathetic stimulation may be modulated by local vaso-active factors (28, 57, 71, 83, 84, 104). For example, in isolated vascular beds and blood vessels, removal of the endothelium or NOS blockade has been shown to enhance the constrictor response to sympathetic stimulation, suggesting that NO may inhibit sympathetic vasoconstriction (28, 57, 71, 83, 84, 104). Exercise training has been shown to augment NO bioavailability (33, 41, 44-46, 50, 74, 81, 93) and removal of the endothelium or NOS blockade produced a greater magnitude of α-adrenergic-mediated vasoconstriction in abdominal aorta (17, 78) and skeletal muscle arterioles (21) in trained compared to sedentary control rats. Collectively, these studies suggest that ET may increase NO bioavailability and augment NO mediated inhibition of sympathetic vasoconstriction in the skeletal muscle vascular bed.

In response to acute exercise, MSNA increases in an exercise-intensity dependent manner (18, 59). However, following 4-6 weeks of intermittent rhythmic handgrip training a smaller increase in MSNA was observed in response to acute rhythmic and isometric handgrip exercise (75, 76) suggesting that ET may blunt the

increase in MSNA during exercise. Whether similar changes in the sympathetic nervous system response to exercise occur following large-muscle mass ET remains unknown.

During exercise, local vaso-active molecules released from the endothelium and/or skeletal muscle have been shown to inhibit sympathetic vasoconstriction (12, 20, 55, 66, 67, 70, 86, 88, 89); a phenomenon termed functional sympatholysis (65). A reduced ability to inhibit sympathetic vasoconstriction during exercise appears to contribute to the dysregulation of skeletal muscle blood flow in cardiovascular and metabolic diseases (13, 64, 69, 94). Thus If ET augmented the inhibition of sympathetic vasoconstriction it may contribute to the cardio-protective effects of ET. Nitric oxide appears to be involved in the inhibition of sympathetic vasoconstriction during muscular contraction as NOS blockade has been shown to augment vasoconstriction during muscular contraction (12, 88). Genetically altered mice and Duchenne muscular dystrophy mice and humans that lack NOS, also have an inability to blunt sympathetic vasoconstriction during muscular contraction (70, 86). However, six weeks of intermittent handgrip training in humans did not reduce the vasoconstrictor response to a cold pressor test at rest or during acute rhythmic handgrip exercise (98). Similarly, five weeks of intense single-leg knee-extensor ET did not reduce the vasoconstrictor response to intra-arterial infusion of tyramine during acute single-leg knee-extensor exercise (56). A cross-sectional study comparing the vasoconstrictor response in the forearm of runners, climbers and sedentary controls reported that the vasoconstrictor response to a cold-pressor test at rest and during acute handgrip exercise was not different between groups (98). Similarly, a cross-sectional comparison of cyclists and untrained controls demonstrated that the vasoconstrictor response of the leg was not different at rest or during single-leg knee-extensor exercise in response to a cold pressor test (102). Taken together, the human and rodent studies that have investigated the

effects of ET on the control of sympathetic vasoconstrictor responses in resting and contracting muscle are conflicting and further investigation in this area appears necessary and warranted.

In addition to a lack of understanding of the effects of ET on the basic physiological regulation of arterial function, the dose response relationship between ET and vascular adaptions is poorly understood. A training program is characterized by the FITT principle and the overall duration of training. To date, investigations of the effects of ET on arterial function have utilized a variety of training programs (16, 17, 21, 25, 37, 38, 45, 46, 50, 51, 77, 79, 101) and a systematic approach to isolate the effects of the individual components of the training program on arterial function has not been undertaken. Short duration training (< 6 weeks) is believed to produce functional adaptations in the vasculature, which may gradually diminish with continued training, as structural adaptations such as vascular remodelling and growth begin to evolve (15, 26, 43). The cardiovascular system appears to be sensitive to the intensity of training (22, 29, 43). However, the relationship between the intensity of ET and vascular adaptations has not been established (26).

In summary, the effects of ET on the regulation of sympathetic vasoconstriction in resting and contracting skeletal muscle have not been established. Therefore, the purpose of this thesis was to investigate the effect of short-term mild- and heavy-intensity ET on sympathetic vasoconstriction in resting and contracting skeletal muscle. The overall hypothesis was that ET would diminish sympathetic vasoconstrictor responsiveness at rest and during contraction in a training-intensity dependent manner. To achieve these aims, rats were exercise trained on a motorized treadmill and an anesthetised whole

animal *in situ* vascular preparation was used to investigate the effects of ET on sympathetic vascular control in resting and contracting skeletal muscle.

The skeletal muscle vasculature receives a substantial proportion of resting cardiac output and therefore contributes substantially to the control of vascular resistance (68). Therefore, in the first study (chapter 2) the effects of short-term mild- and heavy-intensity ET on sympathetic vasoconstrictor responsiveness and EDD in resting skeletal muscle were investigated. It was hypothesized that ET would reduce sympathetic vasoconstrictor responsiveness and augment EDD in a manner dependent on the intensity of ET.

The regulation of skeletal muscle vascular conductance becomes increasingly complex during acute exercise as vasodilation in exercising skeletal muscle and sympathetic outflow directed to active and inactive tissues are increased (68). Additionally, the vasoconstrictor response to sympathetic outflow is blunted in contracting muscle which is thought to further aid in the redistribution of skeletal muscle blood flow (87, 92). Therefore, the subsequent investigation of this thesis (chapter 3) was designed to study the effects of short-term mild- and heavy-intensity ET on sympathetic vasoconstrictor responsiveness and the inhibition of sympathetic vasoconstriction during acute muscular contraction. It was hypothesized that ET would increase sympathetic vasoconstrictor responsiveness and concurrently increase the ability to inhibit sympathetic vasoconstriction during muscular contraction (i.e. functional sympatholysis).

α_2-Adrenergic receptors mediate a significant proportion of the magnitude of sympathetic vasoconstriction at rest (1, 19, 31, 49, 53) and are also inhibited during muscular contraction thus contributing to functional sympatholysis (7, 9, 85, 103). Therefore, the final study of this thesis (chapter 4) investigated whether the training-

mediated changes in sympathetic vasoconstrictor responsiveness and sympatholysis were mediated by post-synaptic α_2-adrenergic receptors. It was hypothesized that ET would augment the contribution of the α_2-adrenergic receptor to sympathetic vascular control in resting and contracting skeletal muscle. It was also hypothesized that ET would augment sympatholysis and that α_2-adrenergic receptor blockade would abolish sympatholysis in trained rats.

The studies in this thesis contribute to our understanding of the effects of mild- and heavy-intensity ET on sympathetic nervous system control of vascular conductance in the resting and contracting skeletal muscle. Moreover, the findings have the potential to advance our understanding of how ET may be utilized to treat and prevent vascular dysfunction in pathophysiological conditions that are characterized by malperfusion of skeletal muscle and reduced exercise tolerance.

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Chapter 2

Short-term exercise training augments sympathetic vasoconstrictor responsiveness and endothelium-dependent vasodilation in resting skeletal muscle

Introduction

The precise regulation of arterial tone is necessary for the maintenance of systemic arterial blood pressure and the adequate delivery of blood flow and oxygen to vital organs and tissues. Arterial tone is regulated by a dynamic balance between sympathetic nervous system-mediated vasoconstriction, myogenic tone (i.e. the background tone upon which other vaso-active signals act) and local vasodilator signaling (45). At rest, the skeletal muscle vascular bed receives a substantial portion of cardiac output and, therefore, is responsible for the maintenance of a large portion of systemic vascular resistance and systemic arterial blood pressure.

Efferent sympathetic nerve activity is characterized by random bursts of activity, followed by periods of quiescence. In humans and animals, there is continuous low-frequency nerve discharge at rest, with an average firing frequency of ~1 Hz (37). However, during physiological stress, such as exercise, the burst frequency of sympathetic nerve discharge increases, with intraburst single nerve discharge frequencies of 20–50 Hz (23, 26, 37). The frequency and pattern of efferent sympathetic nerve activity influence the amount and type of neurotransmitter released (4, 11, 12, 18, 22, 26, 42). Low-discharge frequencies lead to adenosine 5′ triphosphate (ATP) release followed by norepinephrine (NE), whereas midrange discharge frequencies produce both NE and ATP release,

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while high-discharge frequencies favor the release of neuropeptide Y (NPY) (26). As such, sympathetic vasoconstriction is mediated by the relative contributions of NE and the sympathetic cotransmitters ATP and NPY. Studies (4–10, 14, 22, 26, 43, 44) have demonstrated that NE, ATP, and NPY each contribute to vasoconstriction in the skeletal muscle vascular bed, and their contribution to the overall regulation of arterial tone varies with aging (13), exposure to hypoxia (12, 23, 26), and exercise (5–8).

Chronic endurance exercise training has been repeatedly shown to enhance skeletal muscle vasodilation (16, 17, 19, 24, 27, 33, 34, 39, 52) and increase skeletal muscle blood flow capacity (1, 32). A consistent vascular adaptation associated with ET is an increase in endothelium-dependent vasodilation (EDD) (17, 19, 27, 34, 52). However, vascular control mechanisms operate in an integrative manner, and as such, adaptations to exercise training are unlikely to occur in isolation. Whether training-induced changes in vasodilator function affect the regulation of skeletal muscle sympathetic vasoconstriction has not been clearly established. Indeed, ET has been shown to increase (41), decrease (20, 48), or have no effect (47) on efferent muscle sympathetic nerve activity. Furthermore, postsynaptic α-adrenergic receptor responsiveness has been shown to be increased (28), decreased (17, 19, 49, 55), or unchanged (24, 52) following ET. In summary, the available scientific literature related to the effect of ET on the regulation of skeletal muscle sympathetic vasoconstriction is limited and contradictory.

In addition to a lack of understanding of the effects of ET on the regulation of sympathetic vasoconstriction, our understanding of the training stimulus responsible for producing vascular adaptations is limited. Indeed, vascular

adaptations to ET have been demonstrated in response to training paradigms of various durations and intensities (16, 17, 19, 24, 27, 33, 34, 39, 52). To the authors' knowledge, the relationship between the intensity of ET and sympathetic vasoconstriction and EDD has not been studied.

With this background, the purpose of the present study was to investigate the effects of 4 wk of mild- and heavy-intensity ET on *1*) the magnitude of vasoconstriction in response to stimulation of the lumbar sympathetic chain and *2*) EDD in the resting skeletal muscle vascular bed. The lumbar sympathetic chain was stimulated with different patterns of impulses that have been shown to evoke the preferential, but not the exclusive, release of NE (2 Hz continuous), ATP (bursts of impulses at 20 Hz), and NPY (bursts of impulses at 40 Hz) (23, 37, 40). It was hypothesized that ET would diminish the magnitude of sympathetic vasoconstriction and augment EDD in a manner dependent on the intensity of ET.

Methods

Animals and animal care

A total of 45 male Sprague-Dawley rats (~2 mo old) were obtained from the institutional animal colony. Rats were housed in pairs in a 12:12-h light-dark cycle, environmentally controlled (22–24°C, 40–70% humidity) room. Water and rat chow (Lab Diet 5001, PMI Nutrition, Brentwood, MO) were provided ad libitum. All experiments were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Animal Care and Use Committee: Health Sciences for the University of Alberta.

Chronic endurance exercise training

All rats were habituated to the laboratory and exercise by running on a treadmill (Panlab LE8710; Panlab, Barcelona, Spain) 10 min a day for 5 days at 10 m/min, 0% grade. At this point, four rats were removed from the study because of an inability to run voluntarily. After familiarization, rats were randomly assigned to three groups: *1*) sedentary time-control (S), *2*) mild-intensity ET (M; 20 m/min, at 5% grade), or *3*) heavy-intensity ET (H; 40 m/min, at 5% grade).). Previous studies have demonstrated that VO_{2max} values in young healthy rats are achieved at treadmill speeds and grades ranging from ~50m·min^{-1} and 15% grade to ~80m·min^{-1} and 10% grade (11, 14, 46). Therefore the selected treadmill speeds and grades mild correspond to ~30-40% and ~70-80% VO_{2max} and in rats (11, 14, 46). These relative intensities correspond with mild- and heavy-intensity exercise that is typically performed at 25% and 75% VO_{2max} in humans (22).

Blocked randomization was achieved by selecting one of three labeled chips (sedentary, mild, or heavy) from a bag for each rat. ET was performed 5 days/wk for 4 wk. The S group was handled and weighed daily. Immediately following familiarization, the M group ran at 20 m/min at 5% grade for 600 m and maintained this intensity for the duration of the exercise program. The H group began running at 40 m/min at 5% grade, starting with 15 intervals of 1 min of running and 1 min of rest. Each day, interval run time was increased, while rest time was maintained at 1 min until each animal was able to run continuously at 40 m/min at 5% grade for 600 m; this was achieved within 11 ± 2 days, as described previously (25). Animals in each training group ran the same distance (600 m) at their assigned treadmill speed and grade in each training bout. Thus, by differing run times to achieve 600m run distance in each training group, the

total volume of work was matched between groups allowing the effect of ET intensity to be isolated.

Instrumentation

Twenty-four hours after the last training session, rats were anesthetized with inhalation of isoflurane (3.5%, balance O_2). The right jugular vein was then cannulated, and anesthesia was maintained with α-chloralose (8–16 $mg \cdot kg^{-1} \cdot h^{-1}$) and urethane (50–100 $mg \cdot kg^{-1} \cdot h^{-1}$). The depth of anesthesia was assessed by the stability of arterial blood pressure, heart rate (HR), and the absence of a withdrawal reflex in response to a painful stimulus (i.e., paw-pinch). A tracheotomy was performed to facilitate spontaneous respiration, and the left brachial artery was cannulated and connected to a solid state pressure transducer (Abbott, North Chicago, IL) for continuous measurement of arterial blood pressure. The left femoral artery and vein were cannulated for the delivery of pharmacology. Blood flow was measured using a flow probe (0.7 V; Transonic Systems T107, Ithaca, NY), placed around the right femoral artery, and connected to a flow-meter (T106 Transonic Systems). Core temperature was monitored by rectal probe and maintained at 36–37°C by external heating pad (Physitemp, TCAT-2, Clifton, NJ).

Lumbar sympathetic chain stimulation

Through a laparotomy, a bipolar silver-wire stimulating electrode was attached to the lumbar sympathetic chain between L3 and L4. The electrodes were secured in place and electrically isolated by embedding them in a rapidly curing silicone elastomer (Kwiksil, World Precision Instruments, Sarasota, FL). The electrodes were used to deliver constant current stimulation patterns through an isolated stimulator (Digitimer DS3, Digitimer, Welwyn City, UK).

Following surgical instrumentation, a ~20-min recovery period was utilized to allow all hemodynamic variables to stabilize, and the following experiments were conducted. Five rats died during surgical instrumentation.

The effects of short-term mild- and heavy-intensity exercise training on endothelium-dependent vasodilatation (S, n=13; M, n=12; H, n=11).

To investigate the effect of ET on EDD, the magnitude of vasodilation in response to intra-arterial bolus injections of ACh (0.005, 0.05, 0.1, 0.25, and 0.5 μg, in 0.1mL) was assessed. A 5-min resting period interspersed each injection. To minimize any flow-induced vasodilation during intra-arterial injections, small boluses (100 μl) of drug were injected over ~5 s. Vehicle injections at this volume and rate did not increase hind-limb blood flow.

The effects of short term mild- and heavy-intensity exercise training on the response to lumbar sympathetic chain stimulation (S n=13, M n=12, H n=11).

The vasoconstrictor response evoked by stimulation of the lumbar sympathetic chain was determined. Stimulation patterns included *1*) continuous stimulation at 2 Hz; *2*) 20-Hz bursts for 1 s repeated every 10 s and; *3*) 40-Hz bursts for 0.5 s repeated every 10 s. During each 1-min stimulation period, a total of 120 1-ms impulses were delivered at 1 mAmp. Sympathetic stimulations were delivered in random order and were separated by at least 5 min to allow restoration of baseline hemodynamics between stimulations. The stimulation patterns were designed to be reflective of a low resting level of MSNA and moderate- and high-frequency bursts of MSNA that occur in response to physiological stress, such as exercise (40).

Assessment of training efficacy

Upon completion of all experiments, animals were euthanized by anesthetic overdose, and the heart and soleus muscle were dissected free. Heart mass and soleus muscle citrate synthase activity were determined and used as indicators of the efficacy of ET. Citrate synthase activity was measured according to the method of Srere (50) and normalized for total protein concentration of the tissue sample, as determined by a Bradford protein assay kit.

Drugs

All drugs were purchased from Sigma-Aldrich (Oakville, ON Canada) and dissolved in 0.9% physiological saline.

Data analysis

Data were recorded using Chart 7 data acquisition software (AD Instruments Colorado Springs, CO). Arterial blood pressure and femoral artery blood flow (FBF) were recorded continuously at 100 Hz. HR was derived from the arterial blood pressure waveform, and femoral vascular conductance (FVC) was calculated. The magnitude of vasoconstriction in response to sympathetic stimulation was calculated as the difference between the integral of FVC during the 1-min lumbar sympathetic chain stimulation period and the integral of 1 min of the FVC baseline preceding the stimulation and was expressed as a percent change from the FVC baseline (data shown in Figure 2; S, n = 13, M, n =12, H, n = 11). The response to ACh was calculated as the difference between the peak FVC response (~3 s average) and the preinfusion baseline (~20 s average) and was expressed as a percentage change from the FVC baseline (Figure 3; S, n = 13, M, n = 12, H, n = 11). All data were expressed as means ± SD.

The effect of ET on body and cardiac mass, citrate synthase activity, and the response to ACh were determined by one-way ANOVA. The effect of ET on the response to sympathetic stimulation was determined by two-way repeated-measures ANOVA (SigmaPlot 11 Systat, Richmond, CA). When significant *F* ratios were found, Student-Newman-Keuls post hoc analysis was performed. The relationship between endothelium-dependent vasodilation (FVC, % change in response to 0.1 μg IA ACh injection) and the response to continuous and patterned sympathetic stimulations (FVC, % change) was assessed with Pearson product moment correlation. A *P* value <0.05 was considered statistically significant.

Results

All rats randomized to ET groups completed the prescribed training regimen.

Upon completion of the ET protocols, body mass was lower in exercise trained compared to sedentary time-control rats ($p<0.05$). Heart mass was greater in exercise trained compared to sedentary time-control rats ($p<0.05$) and the heart mass: body mass ratio was increased in a training intensity-dependent manner (Table 2.1, $p<0.05$). Soleus muscle citrate synthase activity was also greater in exercise trained compared to sedentary time-control rats (Table 2.1, $p<0.05$).

Basal HR and mean arterial blood pressure (MAP) were lower ($p<0.05$) in exercise trained compared to the sedentary time-control rats, whereas basal FBF and FVC were similar in all groups (Table 2.2).

Effect of exercise training on the response to sympathetic stimulation

The responses to lumbar sympathetic stimulation delivered continuously at 2Hz and in bursting patterns at 20 and 40Hz in a representative animal are illustrated in Figure 2.1. Each pattern of sympathetic stimulation produced a similar vasoconstriction in S, M and H groups. However, the magnitude of vasoconstriction in response to sympathetic stimulation delivered continuously at 2 Hz and in patterns at 20 and 40 Hz was increased as a function of training intensity (Figure 2.2 and Table 2.3).

Effects of exercise training on endothelium-dependent vasodilation

Short-term ET enhanced EDD in a manner dependent on the intensity of ET (Figure 2.3, $p<0.05$).

Relationship between the vascular response to dilator and constrictor stimuli

The magnitude of EDD (vasodilation to 0.1µg ACh) was correlated with the magnitude of vasoconstriction in response to lumbar sympathetic chain stimulations delivered continuously at 2Hz ($r=0.602$; $p<0.001$) and in bursting patterns at 20 ($r=0.619$; $p<0.001$) and 40 Hz ($r=0.601$; $p<0.001$).

Table 2.1. Cardiovascular and metabolic indices of training efficacy.

Group	Body Mass (g)	Heart Mass (g)	Heart : Body Mass Ratio	Soleus Citrate Synthase Activity ($\mu mol \cdot min^{-1} \cdot$ [protein] mg^{-1})
Sedentary (n=13)	445 ± 30	1.5 ± 0.2	0.34 ± 0.06	34 ± 15
Mild-Intensity (n=12)	422 ± 31	1.7 ± 0.2†	0.40 ± 0.05†	49 ± 12†
Heavy-Intensity (n=11)	393 ± 20 †	1.8 ± 0.2†	0.45 ± 0.05† ‡	48 ± 9†

Values are mean ± SD with group sample sizes in parentheses. † indicates a difference from the sedentary control group. ‡ indicates a difference between mild- and heavy-intensity trained groups. A p-value<0.05 was considered statistically significant.

Table 2.2. Basal hemodynamics.

Group	HR (beats·min^{-1})	MAP (mmHg)	FBF (mL·min^{-1})	FVC (mL·min^{-1}·mmHg^{-1})
Sedentary (n=13)	400 ± 27	99 ± 13	3.5 ± 1.1	0.036 ± 0.014
Mild-Intensity (n=12)	354 ± 35†	92 ± 14†	3.4 ± 0.6	0.038 ± 0.008
Heavy-Intensity (n=11)	366 ± 32†	90 ± 15†	3.4 ± 0.8	0.039 ± 0.011

Heart rate (HR), mean arterial blood pressure (MAP), femoral blood flow (FBF) and femoral vascular conductance (FVC). Values are mean ± SD with group sample sizes in parentheses. † indicates a difference from the sedentary control group within a condition. A p-value<0.05 was considered statistically significant.

Table 2.3. Absolute resting skeletal muscle vascular response to sympathetic stimulation.

Stimulation Pattern (Hz)	Group	MAP (I.A.U.)	FBF (I.F.U.)	FVC (I.C.U.)
2	Sedentary (n=13)	2.9± 5.2	-0.7 ± 0.4	-0.007 ± 0.004*
	Mild-Intensity (n=12)	6.2 ± 5.7	-0.9 ± 0.4	-0.013 ± 0.005*
	Heavy-Intensity (n=11)	5.9 ± 3.4	-1.5 ± 0.6† ‡	-0.018 ± 0.008*
20	Sedentary (n=13)	3.3 ± 5.4	-0.8 ± 0.5	-0.008 ± 0.005*
	Mild-Intensity (n=12)	6.5 ± 4.7	-1.0 ± 0.5	-0.012 ± 0.005*
	Heavy-Intensity (n=11)	9.1 ± 3.4	-1.5 ± 0.5†	-0.019 ± 0.007*
40	Sedentary (n=13)	3.5 ± 6.6	-0.9 ± 0.6	-0.009 ± 0.005*
	Mild-Intensity (n=12)	8.7 ± 3.7	-0.9 ± 0.3	-0.013 ± 0.004*
	Heavy-Intensity (n=11)	9.1 ± 4.0	-1.5 ± 0.6	-0.020 ± 0.007*

Absolute changes in integrated units for mean arterial blood pressure (MAP), femoral blood flow (FBF) and femoral vascular conductance (FVC). Values are mean ± SD. † indicates a significant difference from the sedentary control group. ‡ indicates a significant difference between mild-intensity and heavy-intensity trained groups. * indicates a significant difference between all groups. A p-value<0.05 was considered statistically significantly.

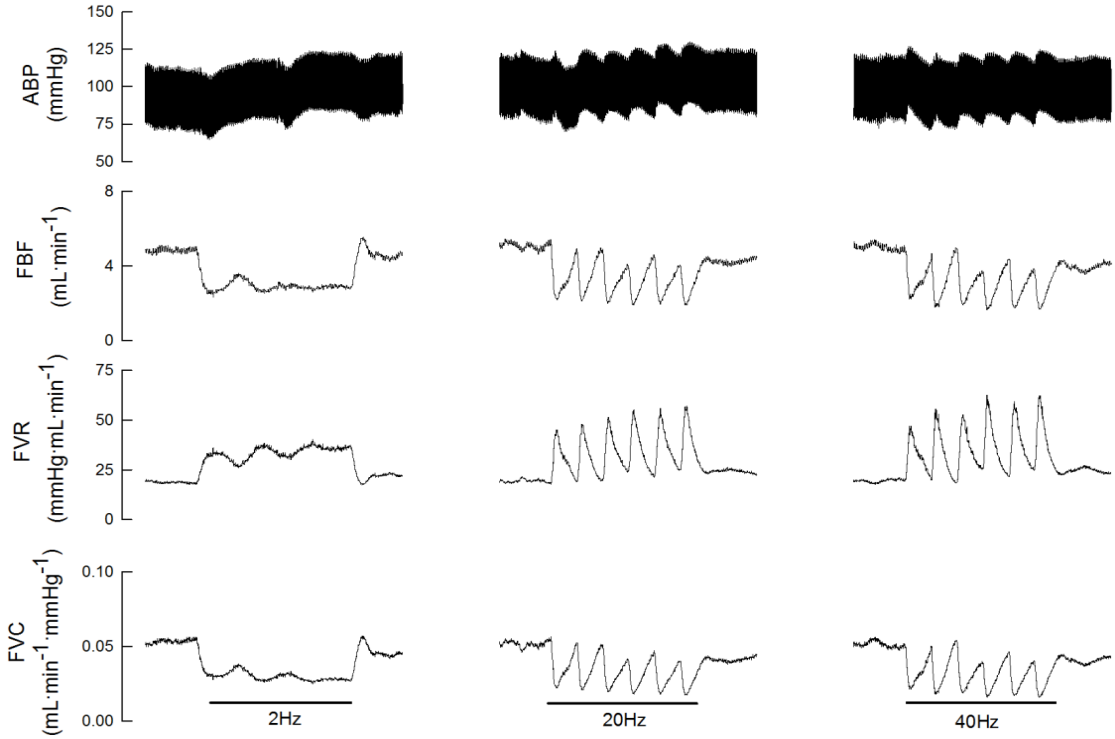

Figure 2.1. Original data tracing illustrating the response of arterial blood pressure (ABP), femoral blood flow (FBF), femoral vascular resistance (FVR) and femoral vascular conductance (FVC) to lumbar sympathetic chain stimulation delivered continuously at 2Hz and in bursting patterns at 20 and 40Hz in a representative animal.

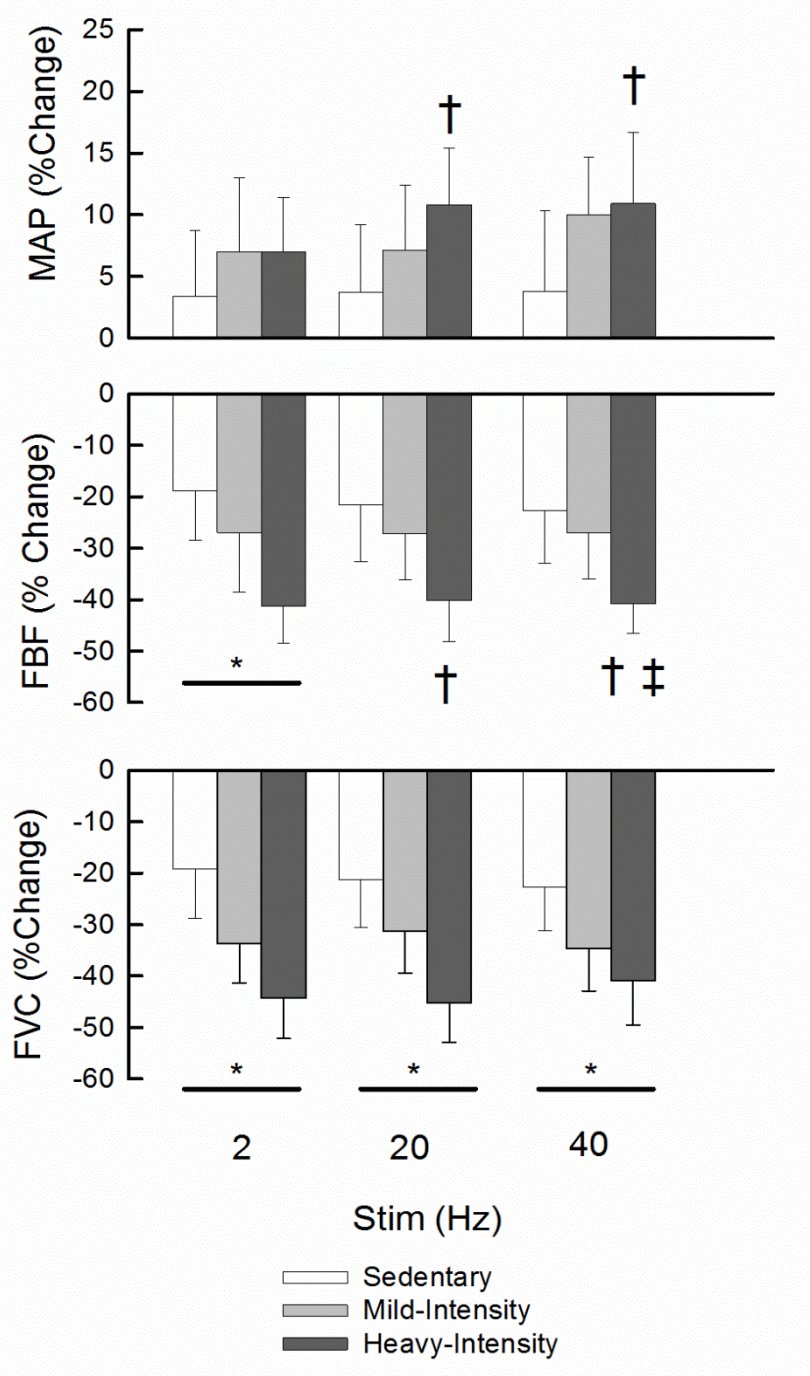

Figure 2.2. Percent change of mean arterial blood pressure (MAP), femoral blood flow (FBF) and femoral vascular conductance (FVC) in response to sympathetic stimulation at 2, 20 and 40 Hz in sedentary time-control (white bars, n=13), mild-intensity (light gray bars, n=12) and heavy-intensity trained (dark gray bars, n=11) rats. Values are mean ± SD. † indicates a significant difference from the sedentary time-control group. ‡ indicates a significant difference from the mild-intensity group. * indicates a significant difference between all groups. A p-value <0.05 was considered statistically significant.

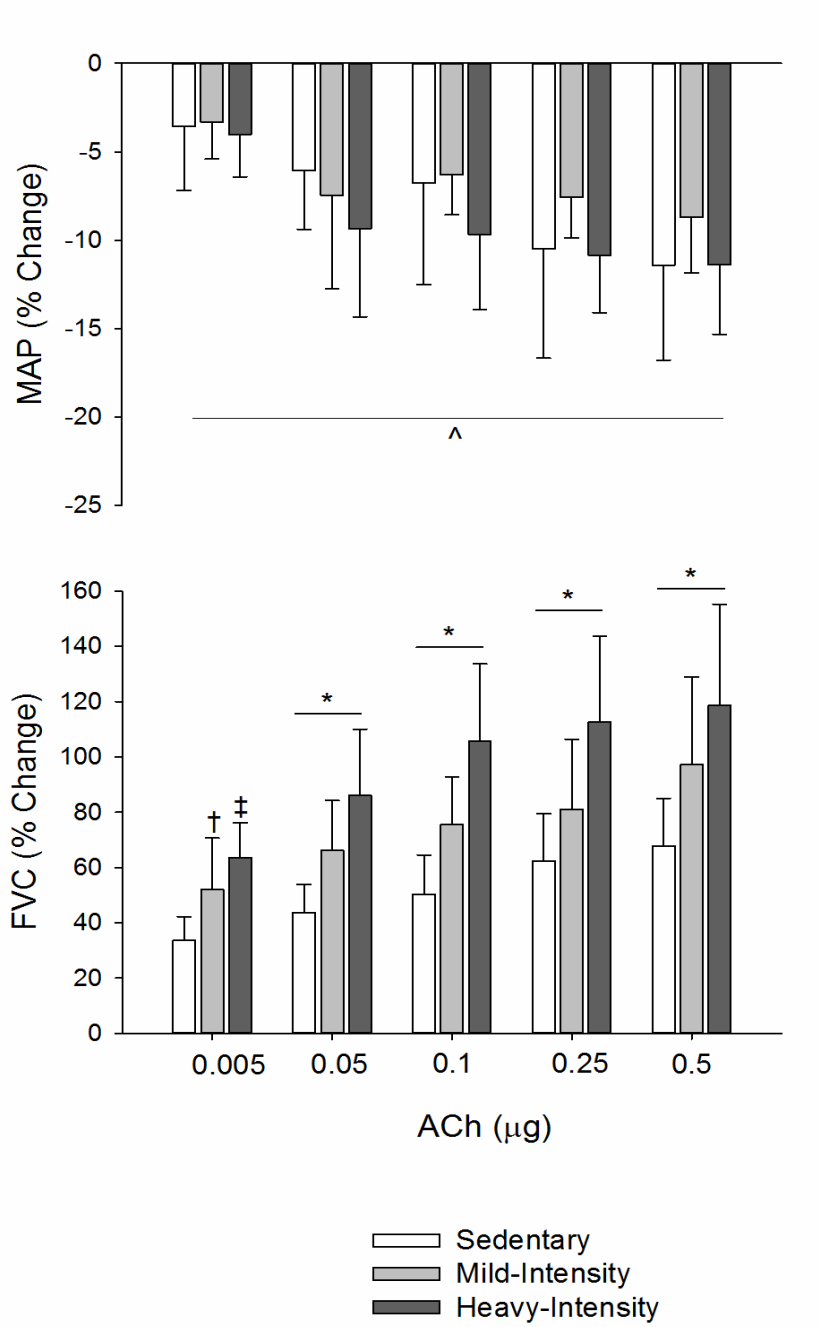

Figure 2.3. Percent change of mean arterial blood pressure (MAP) and femoral vascular conductance (FVC) in response to 100μL bolus injections of acetylcholine (ACh) in sedentary time-control (white circles, n=13), mild-intensity (light gray circles, n=12) and heavy-intensity (dark gray circles, n=11) trained rats. Values are mean ± SD. † indicates a significant difference between sedentary time-control and mild-intensity trained groups. ‡ indicates a significant difference between sedentary time-control and heavy-intensity trained groups. * indicates a significant difference between all groups. ^ indicates a significant main effect of drug dose. A p-value <0.05 was considered statistically significant.

Discussion

The purpose of this study was to investigate the effects of short-term, mild- and heavy-intensity ET on the magnitude of vasoconstriction in response to sympathetic stimulation and EDD in resting skeletal muscle. The primary new findings from this study were that *1*) short-term ET augmented the magnitude of vasoconstriction in response to sympathetic stimulation and EDD in a manner dependent on the intensity of training and *2*) the exercise-training mediated increases in sympathetic vascular responsiveness was significantly correlated with EDD.

Sympathetic vasoconstriction to lumbar sympathetic chain stimulation

The magnitude of vasoconstriction in response to all patterns of sympathetic stimulation was increased in exercise-trained rats compared with sedentary time-controls as a function of training intensity. The response to sympathetic stimulation may be altered by an increased expression or an enhanced responsiveness of postsynaptic receptors. To date, investigations of the effects of ET on the expression of postsynaptic receptors in the skeletal muscle vasculature have not been completed. Previous studies of the effect of ET on postsynaptic receptor responsiveness have utilized a variety of experimental models and training paradigms and have produced conflicting results (17, 19, 24, 28, 34, 49, 55). An important strength of the present study was the isolation of the effects of exercise-training intensity on vascular control by matching the total volume of work completed at each training intensity. To our knowledge, this is the first study to demonstrate a dose-response relationship between the intensity of ET and the response to sympathetic stimulation.

However, previous studies that have used heavy-intensity ET to investigate the effects of training on vascular responsiveness have demonstrated an enhanced vasoconstriction in response to sympathetic stimulation following training (34, 39), suggesting that training-induced adaptations may be, in part, dictated by the intensity of ET.

The overall duration of the training program may also influence the effects of ET on vascular function. Exercise training-induced vascular adaptations appear to occur in phases, where functional (i.e., vasoreactivity) changes occur during the early portion of a training program and are followed by structural adaptations (i.e., vessel growth and vascular remodeling) as training is continued (15, 21). Therefore, the short-term training duration employed in the present study may reveal early functional changes in the responsiveness of the hind-limb vascular bed as the length of training necessary to develop and sustain angiogenic growth, and develop vascular remodeling appears to be greater than 4 weeks (15, 21).

Studies that have utilized short-term training durations (1–6 weeks) have reported unchanged (52), reduced (55), or enhanced (39) responsiveness to NE following ET. Following very mild- to mild-intensity treadmill training, the response to NE was not altered in isolated gracilis muscle first-order arterioles (52). Whereas, swim training in rats reduced the response to NE in the cremaster muscle vascular bed (55). Finally, in a porcine training study, 7 days of heavy-intensity treadmill ET enhanced the response to NE in brachial and femoral arteries (39).

Evidence from investigations that have employed longer durations of ET (10–12 weeks) has also produced conflicting results. Moderate-intensity ET did

not alter the response to NE in the abdominal aorta (17, 49) and feed arteries isolated from rodent soleus muscles (24). In contrast, mild-intensity training diminished the response to NE in first-order arterioles isolated from the gastrocnemius muscle (19). Heavy-intensity sprint-interval training also reduced the response to phenylephrine in gastrocnemius feed arteries (34). In contrast, 90 min of daily moderate-intensity exercise enhanced the reactivity of the spinotrapezius vascular bed to NE (28). Finally, 10 wk of heavy-intensity sprint-interval training enhanced the response to phenylephrine in third-order arterioles from the white portion of the gastrocnemius muscle (34).

In summary, the accumulated evidence related to the effects of exercise training on sympathetic vascular responsiveness is inconclusive, with the divergent findings potentially being attributable to the intensity, duration, and frequency of individual training sessions, as well as the total volume of work completed during the training program and the vascular bed or segment being studied. However, the available literature does suggest that if ET is completed at a heavy intensity, it may be associated with increased vascular responsiveness to sympathetic stimuli, which is consistent with the training intensity-dependent increase in vasoconstriction to sympathetic stimulation in the present study.

The enhanced sympathetic response in exercise-trained animals in the present study may be associated with training adaptations in vascular beds of muscle composed of different fiber types. Progressive exercise is associated with a progressive recruitment of muscle and, in particular, the progressive recruitment of glycolytic muscle fibers (2, 29). Therefore, the dose-response relationship between the intensity of ET and the response to sympathetic stimuli may reflect the conditioning of a progressively larger vascular bed at the different

training intensities. Glycolytic muscle appears to have a heightened basal sympathetic tone compared with predominantly oxidative muscle (3) despite evidence of similar postsynaptic α_1-adrenergic receptor distribution in glycolytic and oxidative muscle (38). Whether ET preferentially increased the response of blood vessels in glycolytic muscles could not be determined in the present study; however, there is evidence that ET produces different adaptations in different segments of the vascular tree and in slow- and fast-twitch muscles (33).

Endothelium-dependent vasodilation

A consistent vascular adaptation associated with ET is an enhanced EDD (16, 17, 19, 24, 27, 33, 34, 39, 52). In agreement with previous findings (16, 17, 19, 24, 27, 33, 34, 39, 52), EDD was augmented following ET in the present study. A variety of ET paradigms ranging from mild-intensity continuous training to heavy-intensity sprint-interval training have been shown to enhance EDD (16, 17, 27, 34, 52). However, to our knowledge, the present study is the first to demonstrate a training intensity-dependent up-regulation of EDD.

As defined by Pyke and Tschakovsky: Shear stress is a function of vessel diameter, blood flow velocity at the vessel lumen and blood viscosity whereas shear rate is an estimate of shear stress without accounting for blood viscosity (48). Blood velocity increases during exercise in a manner dependent on the intensity of exercise. As such, in response to acute progressive incremental exercise, shear rate has been shown to increase as a function of exercise intensity (56) and an exercise induced increase in shear rate has been shown to be the primary mechanism for the improved EDD following ET (53). Shear rate was not measured during ET in the present study, however a greater shear rate during heavy-intensity compared to mild-intensity ET would be expected. Thus,

the present findings suggest that a training intensity-dependent increase in shear rate may lead to a proportional increase in EDD. Consistent with this notion, eNOS expression has been shown to increase as a function of shear rate in cultured endothelial cells exposed to different levels of shear stress (35, 54).

The training intensity-dependent improvement of EDD may also be related to skeletal muscle recruitment. It is well established that there is a progressive recruitment of additional muscle fibres during progressive exercise and that skeletal muscle blood flow is closely matched to skeletal muscle recruitment (30, 31). Therefore, it is conceivable that a larger proportion of the hind-limb skeletal muscle vascular bed was exposed to increased shear rates during heavy- compared to mild-intensity ET. Thus, the potential exists that the intensity-dependent increase of EDD in exercise trained animals reflects the collective vasodilation of a greater number of conditioned vessels in heavy-intensity compared to mild-intensity trained and sedentary rats.

Relationship between the vascular response to dilator and constrictor stimuli

In a study of middle-aged men, Sugawara et al. (51) reported that following 3 months of aerobic exercise training an enhanced EDD was offset by a parallel increase in sympathetic vasoconstriction. NE spillover was increased following training in the study of Sugawara et al. (51), suggesting that the increased sympathetic vasoconstriction was the result of a training induced increase in basal sympathetic outflow. In the present study, the magnitude of vasoconstriction in response to sympathetic stimulation at each frequency was correlated with the magnitude of EDD in all rats. Collectively, the present findings suggest that an increase in the response to sympathetic nerve activity may offset a training-induced increase in EDD in order to maintain basal hemodynamics.

Experimental Considerations and Limitations

A major strength of the current experimental approach is the ability to study the dynamic regulation of sympathetic vasoconstriction in an intact vascular bed as the reactivity of a single vascular segment may not be reflective of the control of an entire vascular bed. The lumbar sympathetic chain was directly stimulated in order to induce the release of endogenous neurotransmitters in the present study.

A limitation of the assessment of sympathetic vascular reactivity in the intact hind-limb skeletal muscle vascular bed is that the assessment of blood flow distribution between and within muscles was not possible. Another potential limitation of the present study was the use of juvenile aged rats (~ 2 months of age at onset of training). Although these animals were sexually mature, ET occurred during a period of growth and development where vascular growth factors may have influenced vascular responsiveness (36, 46). However, the inclusion of a sedentary time-control group minimized any confounding effects related to vascular growth and development.

Perspectives and Significance

Chronic endurance exercise training is generally associated with positive adaptations in vascular function. However, our knowledge of the training paradigm (i.e. the prescription of exercise intensity, frequency and duration) that produces changes in vascular function and the time course of vascular adaptations in response to training are limited. The findings from the present study indicate that as little as 4 weeks of chronic endurance ET augments EDD and vasoconstriction to sympathetic stimulation in a training intensity-dependent manner. I believe that the present data demonstrate that training induced adaptations to one signalling pathway do not occur in isolation but likely occur concurrently with other adaptations that are integrated by the vascular smooth

muscle. Future investigations should focus on the dose-response relationship between ET and integrated vascular adaptations.

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Chapter 3

Short-term exercise training enhances functional sympatholysis through a nitric oxide-dependent mechanism

Introduction

The transition from rest to exercise is characterized by an increase in arterial pressure and a redistribution of cardiac output away from inactive tissue toward exercising skeletal muscle (52). Skeletal muscle blood vessels dilate to facilitate the matching of local O_2 delivery to local O_2 demand (56). The robust local vasodilatation is balanced by a concomitant increase in sympathetic nerve activity which tonically constricts blood vessels in non-active tissue and exercising skeletal muscle in order to maintain arterial blood pressure (52). Indeed, several studies have demonstrated that the sympathetic nervous system tonically constricts the vasculature of exercising muscle even during intense exercise (5, 10, 44). Despite the presence of tonic vasoconstriction in active skeletal muscle, it is well established that a number of substances released from either the active skeletal muscle (38, 39, 65, 69, 71) and/or endothelium (11, 19, 46, 66) can blunt the vascular response to sympathetic nerve activity during exercise, a physiological phenomenon termed functional sympatholysis (50). While a definitive mechanism for sympatholysis has not been identified, evidence is accumulating that nitric oxide (NO) may be involved as removal of the endothelium or NO synthase (NOS) blockade enhances the vascular response to sympathetic stimulation (19, 46, 66, 71).

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Chronic endurance exercise training is known to affect the skeletal muscle vasculature in a number of ways. Following ET, it is generally believed that the vascular response to known vasodilators is augmented (8, 13, 28, 34, 62), skeletal muscle vascular resistance is reduced during submaximal exercise (49) and skeletal muscle vascular conductance at peak exercise is augmented (41, 59), however these adaptations may be dependent upon the limb and vascular segment investigated (22). In the whole hind-limb of rats, it was recently reported that the vascular response to lumbar sympathetic chain stimulation in resting skeletal muscle was augmented following four weeks of ET in a training-intensity dependent manner (25). Whether ET alters the vascular response to sympathetic stimulation and the blunting of sympathetic vasoconstriction in contracting muscle has not been established.

The understanding of how the intensity of ET affects the regulation of skeletal muscle sympathetic vasoconstriction in contracting skeletal muscle is also limited, despite some evidence that training adaptations in the cardiovascular system and in other systems appear to be sensitive to the intensity of training (17, 20, 33).

Therefore, the purpose of the present study was to investigate the effects of four weeks of mild- and heavy-intensity ET on the magnitude of vasoconstriction in response to sympathetic stimulation in resting and contracting skeletal muscle. It was hypothesized that ET would augment sympatholysis in a manner dependent on the intensity of ET. It was also hypothesized that the augmented sympatholysis would be due to an increased NO-mediated inhibition of sympathetic vasoconstriction in exercise trained rodents.

Methods

Animals and animal care

Male Sprague-Dawley rats (~8 weeks old) were obtained from the institutional breeding colony and housed in pairs in a 12:12h light-dark cycle, environmentally controlled (22-24^{0}C, 40-70% humidity) room. Water and rat chow (Lab Diet 5001, PMI Nutrition, Brentwood MO, USA) were provided ad libitum. All experiments were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Animal Care and Use Committee: Health Sciences for the University of Alberta.

Chronic endurance exercise training

All rodents were habituated to the lab and exercise by running on a treadmill (Panlab LE8710, Barcelona, Spain) 10 min·day^{-1} for 5 days at 10m·min^{-1}, 0% grade. After familiarization rats were randomly assigned one of three groups: 1) time-control sedentary (S); 2) mild-intensity ET (M; 20·min^{-1}, 5% grade) or 3) heavy-intensity ET (H; 40m·min^{-1}, 5% grade). Training volume was matched between exercise groups by having animals run the same distance (600m) during each training bout. Rats were trained 5 days·week^{-1} for 4 weeks. On the first training day, H rats ran 15 intervals of 1 minute at 40m·min^{-1} 5% grade interspersed with rest periods of equal time. With each subsequent training bout, run time was increased, while rest time was maintained. This training progression allowed all rats in the H group to run continuously at the prescribed speed and grade within 11 ± 2 days, consistent with previous studies from our lab (24). Rats randomized to the M group ran at the prescribed speed, grade and distance immediately following familiarization and maintained this for the entire

exercise program. This training protocol has previously been shown to increase heart mass, heart mass: body mass ratio, soleus citrate synthase activity and endothelium-dependent vasodilatation (25). Sedentary time control animals were handled and weighed daily.

Instrumentation

Twenty-four hours after the last training session rodents were anaesthetised by inhalation of isoflurane (3.5%, balance O_2). The right jugular vein was then cannulated and anaesthesia was maintained with α-chloralose (8-16 $mg \cdot kg^{-1} \cdot h^{-1}$) and urethane (50-100$mg \cdot kg^{-1} \cdot h^{-1}$). The depth of anaesthesia was assessed by the stability of arterial blood pressure, heart rate (HR) and the absence of a withdrawal reflex in response to a painful stimulus (i.e. paw-pinch). Core temperature was monitored by rectal probe and maintained at 36-37^{0}C by external heating pad (Physitemp, TCAT-2, Clifton, NJ USA). A tracheotomy was performed to allow spontaneous breathing of room air. The left brachial artery was cannulated and connected to a solid state pressure transducer (Abbott, North Chicago, IL USA) for the continuous measurement of arterial blood pressure. The left femoral artery and vein were cannulated for the delivery of pharmacology. Blood flow was measured using a flow probe (0.7 V; Transonic Systems, Ithaca, NY USA) placed around the right femoral artery and was connected to a flow-meter (T106 Transonic Systems, Ithaca, NY USA). Heart rate was derived from the arterial blood pressure waveform. Arterial blood samples were taken at rest and at the end of each contraction bout for the measurement of paO_2, $paCO_2$ and pH (IDEXXLaboratories, VetStat, Markham, ON Canada).

Muscle contraction

The right sciatic nerve was exposed and instrumented with a nerve cuff electrode. The triceps surae muscle group was dissected free and attached to a force transducer (Model MLT1030/D, AD Instruments Colorado Springs, CO USA) via the calcaneal tendon. Maximal contractile force (MCF) was determined by stimulation of the triceps surae muscle group with 25, 1ms impulses delivered at 100Hz, 10 X motor threshold (MT). The optimal muscle length for tension development was determined by progressively lengthening the muscle and repeating the nerve stimulation until a plateau in tension (peak – baseline) was observed. Rhythmic contractions of the triceps surae muscles were produced at 30% MCF (40Hz 0.1ms pulses in 250 ms trains at a rate of 60 trains/minute at ~ 2.0 X MT) and 60% MCF (40Hz 0.1ms pulses in 250 ms trains at a rate of 60 trains/minute at ~ 5.5 X MT).

Lumbar sympathetic chain stimulation

Following a laparotomy, the great vessels were temporarily retracted and the lumbar chain was exposed by dissection with a blunt glass pipette. A bipolar silver-wire stimulating electrode was attached to the lumbar sympathetic chain between L3 and L4. The electrodes were embedded and electrically isolated in a rapidly-curing non-toxic silicone elastomer (Kwiksil, WPI, Sarasota, FL USA). The electrodes delivered constant current stimulations through an isolated stimulator (Digitimer DS3, Welwyn City, UK).

Following a 20 minute stabilization period, the following experiments were conducted in a total of 36 rats (S n=11, M n=12, H n=13).

Series 1: The effects of exercise training on the magnitude of vasoconstriction in response to sympathetic stimulation at rest and during muscle contraction.

The skeletal muscle vascular response evoked by lumbar sympathetic chain stimulation (1 min of 1 ms, 1mAmp pulses delivered at 2 and 5Hz in random order) was determined at rest and during muscle contraction at 30 and 60% of MCF. Bouts of muscle contraction were 8 minutes in duration, completed in random order, and separated by 60 minutes of recovery. During each bout, stimulations of the lumbar sympathetic chain were delivered 3 and 6 minutes after the onset of contraction.

Series 2: The effects of exercise training on NO-mediated inhibition of sympathetic vasoconstriction at rest and during muscle contraction.

Following completion of Series 1 and a 30 minute recovery period, a bolus injection of the non-selective NOS inhibitor, N_{ω}-Nitro-L-arginine methyl ester hydrochloride was delivered (L-NAME, $5mg \cdot kg^{-1}$, IV). After ~20 minutes and stabilization of hemodynamic parameters stimulation of the lumbar sympathetic chain was repeated at rest and during contraction at 30 and 60% MCF as described above.

Time control studies

Given the relatively long duration of the experimental protocol, an additional group of animals (n=5) were used to investigate whether the vascular response to sympathetic stimulation in resting and contracting skeletal muscle was reproducible over time. Briefly, the skeletal muscle vascular response evoked by lumbar sympathetic chain stimulation was determined at rest and during muscle contraction at 30 and 60% of MCF as described above (Time 1). Following a recovery period (~30 min), stimulation of the lumbar sympathetic chain was repeated at rest and during contraction at 30 and 60% MCF (Time 2).

Upon completion of all experiments, animals were euthanized by anaesthetic overdose and the heart was dissected free for measurement of cardiac mass.

Drugs

All drugs were purchased from Sigma-Aldrich (Oakville, ON Canada) and dissolved in 0.9% physiological saline.

Data analysis

Data were recorded using Chart 7^{TM} data acquisition software (AD Instruments, Colorado Springs, CO USA). Arterial blood pressure, femoral artery blood flow (FBF) were sampled at 100 Hz and femoral vascular conductance (FVC) and heart rate were calculated. The magnitude of vasoconstriction in response to sympathetic stimulation was determined by calculating the mean of the response to sympathetic stimulation and expressing it as a percent change from the preceding 1 minute steady state value for mean arterial blood pressure (MAP), FBF and FVC. The magnitude of sympatholysis was calculated as the difference between the percent change in FVC in response to sympathetic stimulation at rest and the percent change in FVC in response to sympathetic stimulation during muscular contraction. All data are expressed as mean ± standard deviation.

Data were analyzed by three-way repeated measures ANOVA (group x contractile condition x drug condition). The effect of ET and NOS inhibition on the magnitude of sympatholysis was determined by two-way repeated measures ANOVA (group x drug condition; STATISTICA 10 Statsoft Inc., Tulsa, OK USA). When significant F-ratios were detected, Student-Newman-Keuls post-hoc analysis was performed. A p-value <0.05 was considered statistically significant.

Results

All rats randomized to ET groups completed the assigned training protocol. Body mass was lower ($p<0.05$), whereas heart mass and the heart: body mass ratios were increased ($p<0.05$) in exercise trained compared to S rodents (Table 3.1). Resting HR, MAP, FBF and FVC were similar ($p>0.05$) between S and exercise trained animals (Table 3.2). Arterial blood gases and pH were within normal limits at rest and during each contractile bout and were not different ($p>0.05$) between groups at any time point (Table 3.3).

Series 1: Magnitude of Vasoconstriction in Response to Sympathetic Stimulation in Resting and Contracting Skeletal Muscle

The response to lumbar sympathetic stimulation delivered at 2 and 5Hz in resting skeletal muscle in a representative animal is shown in Figure 3.1A. The magnitude of vasoconstriction in response to sympathetic stimulation delivered at 2Hz was increased ($p<0.05$) by ET in a training intensity-dependent manner (Figure 2). In response to 5Hz stimulation, a greater ($p<0.01$) constriction was seen in M and H compared to S rats (Figure 3.2).

The response to lumbar sympathetic chain stimulation delivered at 2 and 5Hz during skeletal muscle contraction in a representative animal is shown in Figure 3.1B. Muscle contraction at 30% MCF produced a similar ($p>0.05$) increase in skeletal muscle blood flow and vascular conductance in S, M and H groups (Table 3.4). Muscle force production was not different ($p>0.05$) between groups at 30% MCF (S: 763 ± 122g; M: 774 ± 107g; H: 745 ± 89g. The magnitude of vasoconstriction in response to lumbar sympathetic stimulation at

2Hz was greater ($p<0.05$) in M and H compared to S rats at 30% MCF. In contrast, the response to sympathetic stimulation delivered at 5Hz was similar ($p>0.05$) between trained rats and the S group at 30% MCF (Figure 3.2).

Compared to rest, the magnitude of vasoconstriction in response to sympathetic stimulation was diminished ($p<0.05$) during contraction (i.e. sympatholysis) at 30% MCF in S, M and H rats (Figure 3.2). However, the magnitude of sympatholysis (%FVC rest – contraction, Δ%FVC) was greater ($p<0.05$) in H compared to M and S groups during 2Hz sympathetic stimulation and greater ($p<0.01$) in H compared to S rats during 5Hz sympathetic stimulation (Figure 3.3).

Muscle contraction at 60% MCF produced a similar ($p>0.05$) increase in skeletal muscle blood flow and vascular conductance in S, M and H animals (Table 3.4). Muscle force production was not different ($p>0.05$) between S, M and H at 60% MCF (S: 1179 ± 131g; M: 1241 ±163g; H: 1199 ± 116g). During contraction at 60% MCF, the magnitude of vasoconstriction in response to sympathetic stimulation at 2 and 5Hz was similar ($p>0.05$) in S, M and H rats (Figure 3.2).

However, the magnitude of vasoconstriction in response to sympathetic stimulation was reduced ($p<0.05$) compared to rest in all groups (Figure 3.2). In response to sympathetic stimulation delivered at 2Hz, the magnitude of sympatholysis was increased ($p<0.05$) in a training intensity-dependent manner during contraction at 60% MCF (Figure 3.5). During sympathetic stimulation at 5Hz, sympatholysis was greater ($p<0.05$) in M and H compared to S rats during contraction at 60% MCF (Figure 3.4).

Series 2: Nitric oxide mediated inhibition of sympathetic vasoconstriction

Nitric oxide synthase blockade produced a similar decrease in resting HR, FBF and FVC and increase in MAP in S, M and H rats (Table 3.2; $p<0.05$). The vascular response to sympathetic stimulation at both 2 and 5Hz was augmented ($p<0.05$) in resting and contracting skeletal muscle in S, M and H rats in the presence of L-NAME (Figure 3.5).

L-NAME did not alter ($p>0.05$) muscle force production. The increase in FBF during contraction at 30% or 60% MCF was also not altered ($p>0.05$) by L-NAME (Table 3.4). However, the increase in FVC during contraction at 30% or 60% was reduced ($p<0.01$) by L-NAME in S, M and H rats (Table 3.4).

Nitric oxide synthase blockade augmented ($p<0.05$) sympathetic vasoconstriction during muscular contraction in all rats (Figure 3.5). In the presence of L-NAME, at 30% MCF, H rodents had a greater ($p<0.05$) vasoconstrictor response to sympathetic stimulation at 2Hz compared to S and M rodents, whereas 5Hz stimulation produced a greater ($p<0.01$) vasoconstriction in both M and H compared to S rodents (Figure 3.5). At 30% MCF, the magnitude of sympatholysis was not affected ($p>0.05$) by L-NAME in S rats and M rats during 2 or 5Hz sympathetic stimulation (Figure 3.3). However, L-NAME significantly reduced ($p<0.05$) the magnitude of sympatholysis during contractions at 30% MCF in H rats during sympathetic stimulation at both 2 and 5Hz (Figure 3.3).

During NOS blockade at 60% MCF, H rodents had an increased ($p<0.05$) vascular response to 2 and 5Hz sympathetic stimulation compared to the S group (Figure 3.5). L-NAME did not alter ($p>0.05$) the magnitude of sympatholysis in S rats during 2 or 5Hz sympathetic stimulation (Figure 3.4). However, L-NAME

reduced ($p<0.05$) the magnitude of sympatholysis during contractions at 60% MCF in M and H rats during both 2 and 5Hz sympathetic stimulation (Figure 3.4).

Time Control Studies

There was no difference ($p>0.05$) in the magnitude of vasoconstriction in response to sympathetic stimulation delivered at 2 and 5 Hz between Time 1 and Time 2 at rest (difference between in % decrease in FVC Time 1 - Time 2: 2Hz: -0.46 ± 0.53; 5Hz: 4.02 ± 0.55), 30% MCF (2Hz: -0.46 ± 0.53; 5Hz: 4.02 ± 0.55) or 60% MCF (2Hz: -1.82 ± 0.77; 5Hz: -2.20 ± 1.67).

Table 3.1. Indices of training efficacy.

Group	Body Mass (g)	Heart Mass (g)	Heart : Body Mass Ratio(g)
Sedentary Time-Control	457 ± 37	1.5 ± 0.1	0.32 ± 0.03*
Mild-Intensity Trained	414 ± 40†	1.7 ± 0.2†	0.40 ± 0.04*
Heavy-Intensity Trained	385 ± 31†^	1.7 ± 0.2†	0.43 ± 0.04*

Values are mean ± standard deviation. † indicates a significant difference from sedentary control group. ^ p=0.05 difference from mild-intensity group. * indicates a significant training-intensity dependent difference. A p-value < 0.05 was considered statistically significant.

Table 3.2. Basal hemodynamics.

Group	Drug Condition	HR (beats·min^{-1})	MAP (mmHg)	FBF (mL·min^{-1})	FVC (mL·min^{-1}·mmHg^{-1})
Sedentary Time-Control	Baseline	359 ± 36	93 ± 9	3.1 ± 0.6	0.03 ± 0.01
	L-NAME	321 ± 51^	130 ± 14^	3.3 ± 0.8	0.02 ± 0.01^
Mild-Intensity Training	Baseline	337 ± 50	94 ± 11	3.0 ± 0.6	0.03 ± 0.01
	L-NAME	312 ± 42^	131 ± 11^	3.1 ± 1.0	0.02 ± 0.01^
Heavy-Intensity Training	Baseline	335 ± 28	91 ± 10	3.0 ± 0.8	0.03 ± 0.01
	L-NAME	298 ± 49^	126 ± 13^	2.8 ± 0.8	0.02 ± 0.01^

Heart rate (HR), mean arterial blood pressure (MAP), femoral blood flow (FBF) and femoral vascular conductance (FVC). Values are mean ± standard deviation. ^ indicates a significant main effect of L-NAME. A p-value <0.05 was considered statistically significant.

Table 3.3. Arterial blood gases and pH.

		Series 1 Control			Series 2 L-NAME		
		Rest	30% MCF	60% MCF	Rest	30% MCF	60% MCF
Sedentary	P_aO_2 (mmHg)	91 ± 3	90 ± 2	89 ± 2	88 ± 3	87 ± 4	91 ± 3
Time-Control	P_aCO_2 (mmHg)	38 ± 1	39 ± 1	38 ± 1	41 ± 2	40 ± 2	38 ± 2
	pH	7.42 ± 0.01	7.43 ± 0.01	7.42 ± 0.01	7.41± 0.01	7.42 ± 0.01	7.42 ± 0.01
Mild-Intensity	P_aO_2 (mmHg)	90 ± 4	89 ± 4	87 ± 2	90 ± 4	89 ± 4	89 ± 3
Training	P_aCO_2 (mmHg)	41 ± 2	41 ± 3	40 ± 3	39 ± 3	41 ± 2	39 ± 1
	pH	7.42 ± 0.01	7.43 ± 0.01	7.41 ± 0.02	7.41 ± 0.02	7.41 ± 0.02	7.40 ± 0.03
Heavy-Intensity	P_aO_2 (mmHg)	91 ± 3	89 ± 3	90 ± 4	90 ± 4	90 ± 3	90 ± 4
Training	P_aCO_2 (mmHg)	40 ± 2	40 ± 2	39 ± 2	40 ± 2	40 ± 2	40 ± 1
	pH	7.41 ± 0.02	7.42 ± 0.02	7.40 ± 0.02	7.40 ± 0.02	7.41 ± 0.02	7.42 ± 0.02

P_aO_2, P_aCO_2 and pH for sedentary, mild- and heavy-intensity trained rats, during control and L-NAME conditions at: rest, 40% and 70% maximal contractile force (MCF).

Table 3.4. Muscle contraction induced hyperaemia during two acute intensities of sustained rhythmic contractions.

Contractile Force (% Max)	Group	Drug Condition	FBF ($mL \cdot min^{-1}$)	FVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$)
30%	Sedentary	Baseline	3.04 ± 0.75	0.032 ± 0.007*
	Time-Control	L-NAME	3.56 ± 1.21^	0.030 ± 0.010^*
	Mild-Intensity Training	Baseline	3.72 ± 1.3	0.039 ± 0.011*
		L-NAME	4.12 ± 1.32^	0.033 ± 0.011^*
	Heavy-Intensity Training	Baseline	3.72 ± 1.17	0.041 ± 0.010*
		L-NAME	3.79 ± 1.02^	0.031 ± 0.010^*
60%	Sedentary Time-Control	Baseline	5.30 ± 1.16	0.050 ± 0.010*
		L-NAME	4.90 ± 1.57^	0.042 ± 0.012^*
	Mild-Intensity Training	Baseline	5.88 ± 1.88	0.058 ± 0.015*
		L-NAME	6.04 ± 1.34^	0.051 ± 0.011^*
	Heavy- Intensity Training	Baseline	5.77 ± 1.76	0.060 ± 0.011*
		L-NAME	5.26 ± 1.70^	0.044 ± 0.015^*

The peak absolute hyperaemic increase of femoral artery blood flow (FBF) and femoral vascular conductance (FVC) in response to muscle contraction. Values are mean ± standard deviation. ^ indicates a significant main effect of L-NAME. * indicates a significant main effect of muscle contractile force. A p-value<0.05 was considered significantly different.

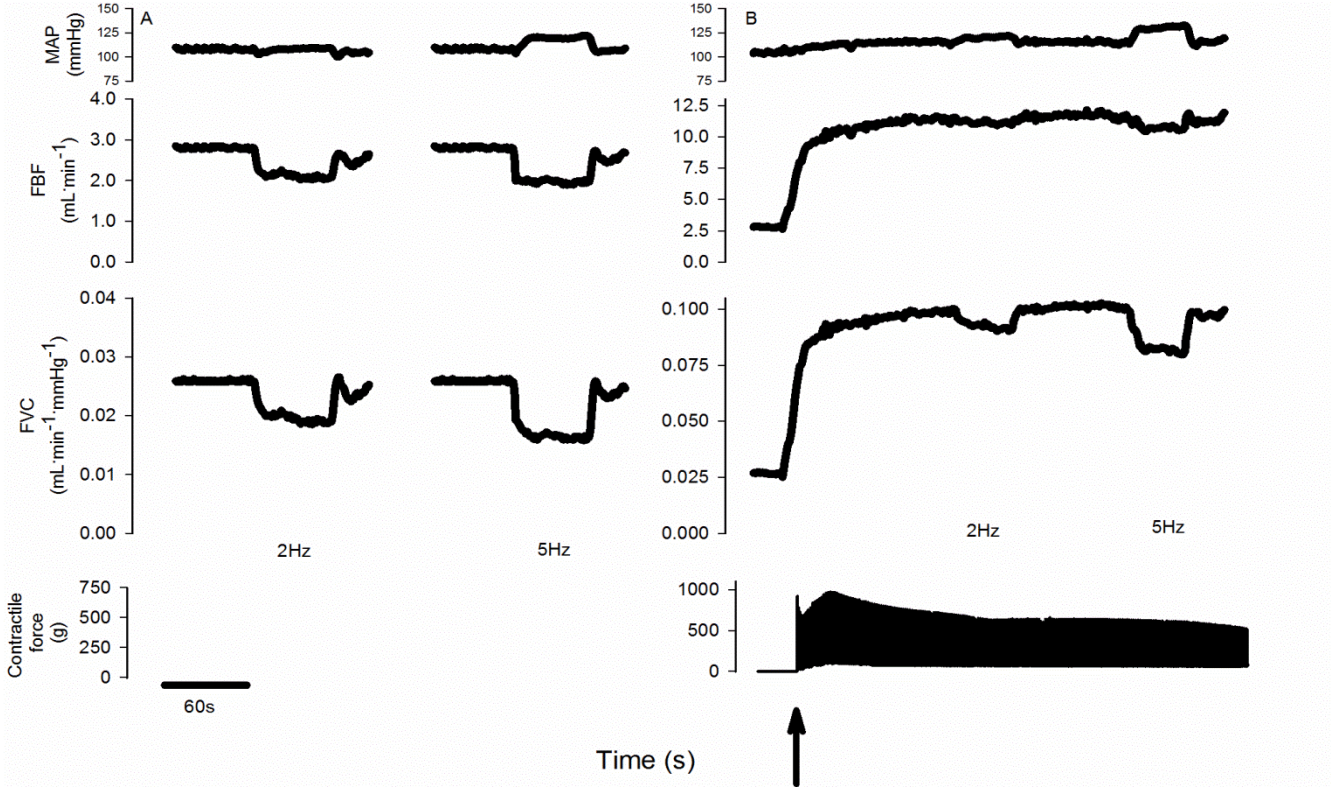

Figure 3.1. Original data from a representative animal illustrating the response of mean arterial blood pressure (MAP), femoral blood flow (FBF), femoral vascular conductance (FVC) and contractile force to lumbar sympathetic stimulations delivered at 2 and 5 Hz in resting skeletal muscle (A) and during skeletal muscle contraction at 60% of maximal contractile force (B). Arrow denotes the onset of contraction.

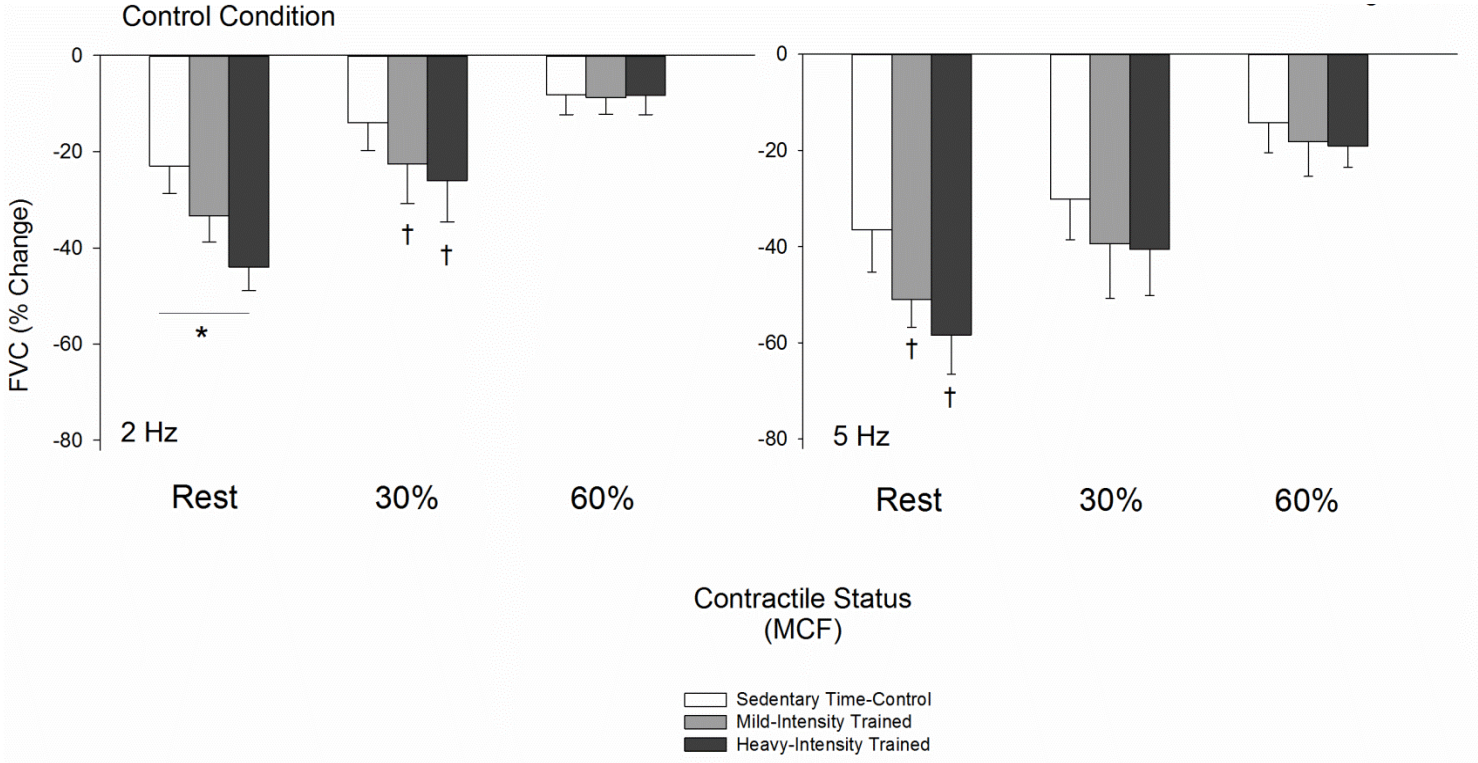

Figure 3.2. The percent change of femoral vascular conductance (FVC) in response to 2Hz (left) and 5 Hz (right) sympathetic stimulation at rest, and during contraction at 30% and 60% of maximal contractile force (MCF) in sedentary (white), mild-intensity (light gray) and heavy-intensity trained (dark gray) rats during control conditions. Values are mean ± standard deviation. * indicates a significant difference between all groups at specified contractile state. † indicates a significant difference from the sedentary control group. A p-value < 0.05 was considered statistically significant.

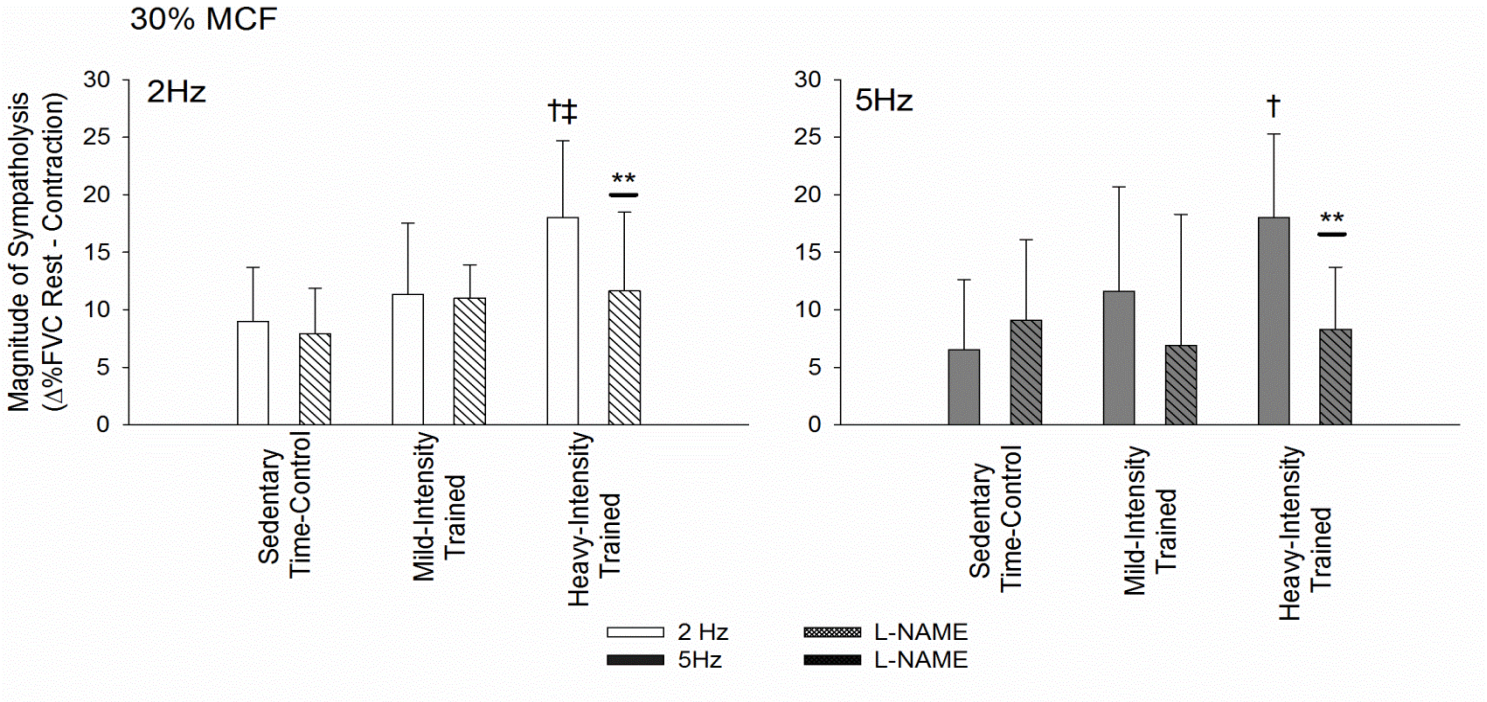

Figure 3.3. The magnitude of sympatholysis calculated as the difference between the percent change in femoral vascular conductance (FVC) in response to sympathetic stimulation at rest and during contraction at 30% maximal contractile force (MCF) in response to sympathetic stimulation at 2Hz (left) and 5Hz (right) in sedentary (white), mild-intensity (light gray) and heavy-intensity trained (dark gray) rats prior to (solid bars) and following (hatched bars) NOS blockade with L-NAME ($5 mg \cdot kg^{-1}$ IV). Values are mean ± standard deviation. † indicates a significant difference from sedentary control group. ‡ indicates a significant difference from mild-intensity trained group. ** indicates a difference between control and L-NAME conditions within the same training group. A p-value < 0.05 was considered statistically significant.

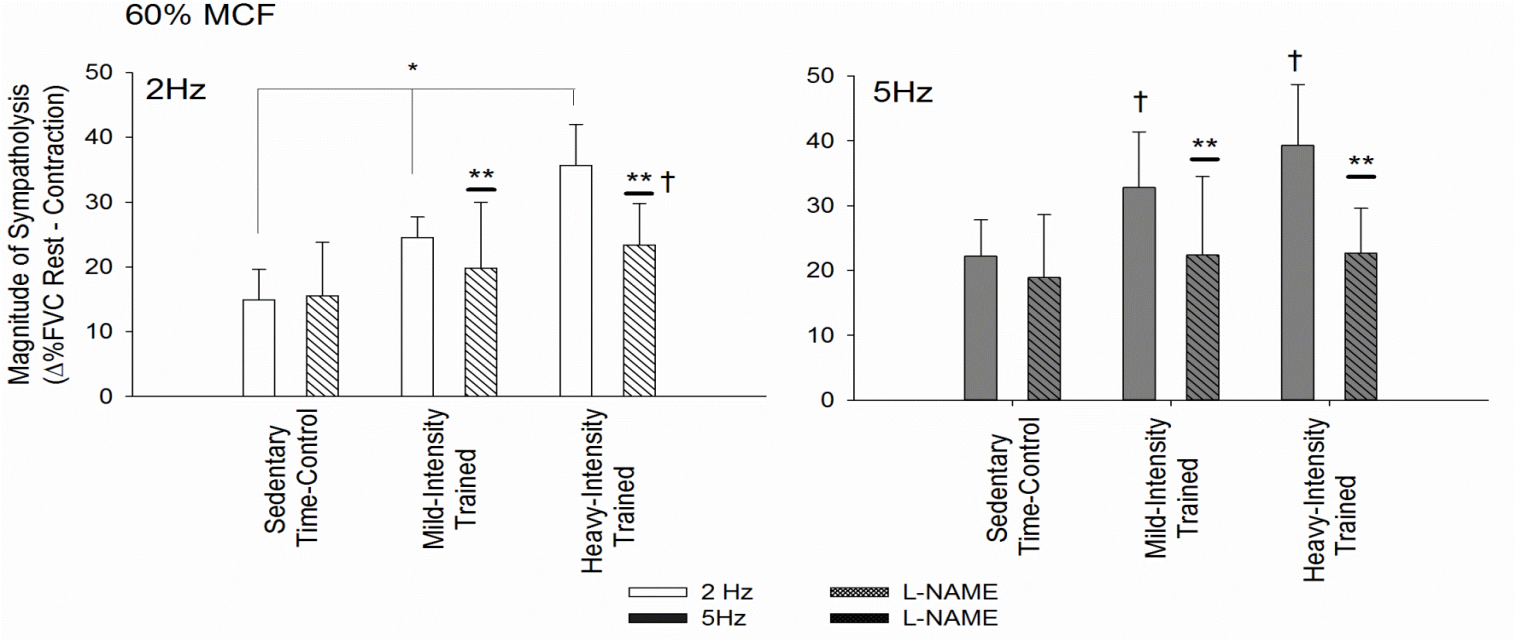

Figure 3.4. The magnitude of sympatholysis calculated as the difference between the percent change in femoral vascular conductance (FVC) in response to sympathetic stimulation at rest and during contraction at 60% maximal contractile force (MCF) in response to sympathetic stimulation at 2Hz (left) and 5Hz (right) in sedentary (white), mild-intensity (light gray) and heavy-intensity trained (dark gray) rats prior to (solid bars) and following (hatched bars) NOS blockade with L-NAME ($5 mg \cdot kg^{-1}$ IV). Values are mean ± standard deviation. * indicates a significant difference between all groups. † indicates a significant difference from sedentary control group. ** indicates a difference between control and L-NAME conditions within the same training group. A p-value < 0.05 was considered statistically significant.

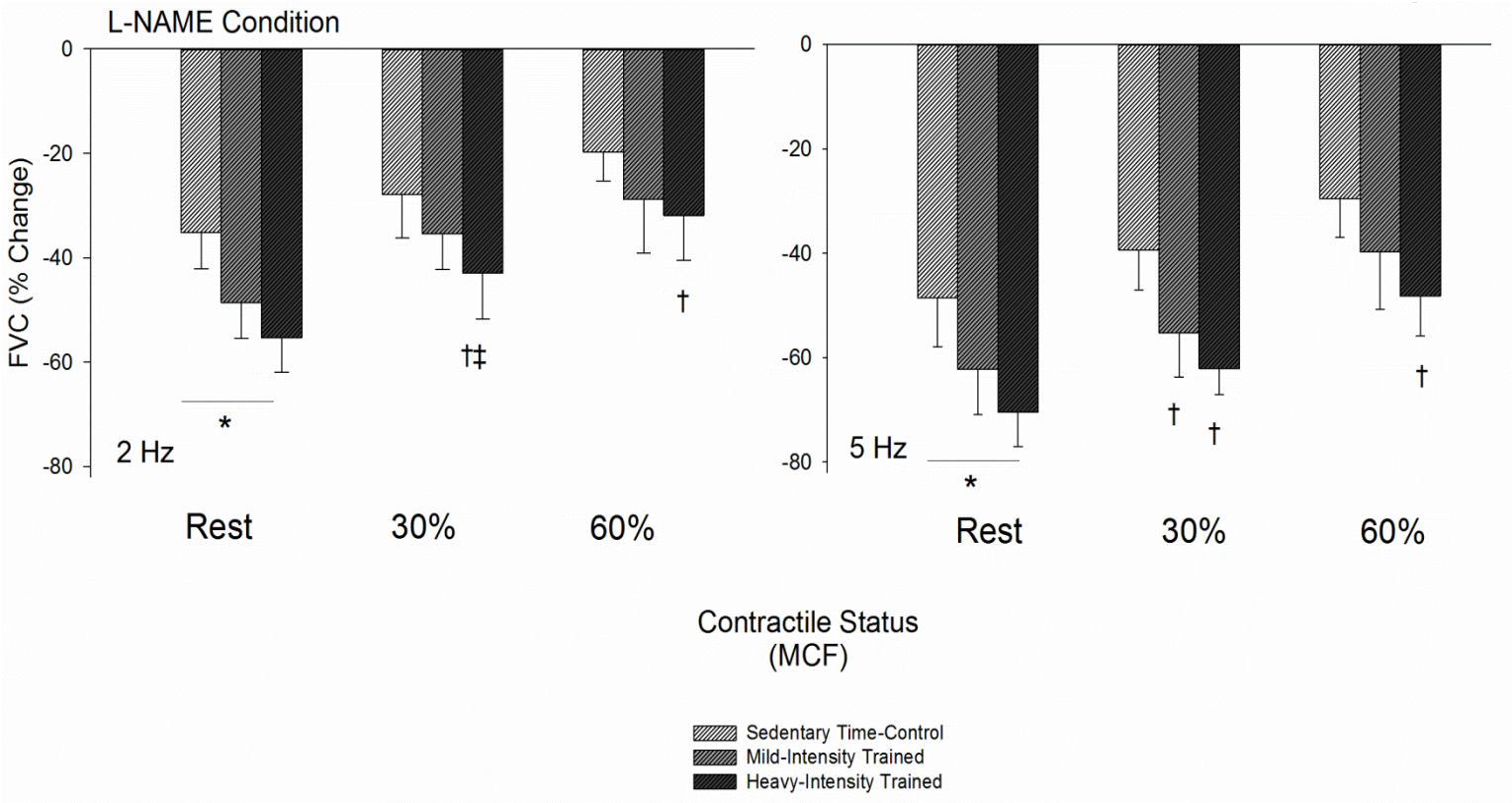

Figure 3.5. The percent change of femoral vascular conductance (FVC) in response to 2Hz (left) and 5 Hz (right) sympathetic stimulation at rest, and during contraction at 30% and 60% of maximal contractile force (MCF) in sedentary (white), mild-intensity (light gray) and heavy-intensity trained (dark gray) rats during NOS blockade with L-NAME (5mg·kg^{-1} IV). Values are mean ± standard deviation. * indicates a significant difference between all groups at specified contractile state. † indicates a significant difference from the sedentary control group. ‡ indicates a significant difference from mild-intensity trained group. A p-value < 0.05 was considered statistically significant.

Discussion

The purpose of the present study was to investigate the effects of four weeks of mild- and heavy-intensity ET on the magnitude of vasoconstriction in response to sympathetic stimulation in resting and contracting skeletal muscle. Consistent with previous findings from our laboratory (25), the present study demonstrated that short-term ET augmented the constrictor response to sympathetic stimulation at rest. The important novel finding in the present study was that ET augmented sympatholysis in contracting muscle. The training induced improvements in sympatholytic capacity appeared to be sensitive to the intensity of the training stimulus, as rats that trained at a heavy-intensity had an augmented ability to inhibit sympathetic vasoconstriction during moderate- and heavy-intensity contractions. In contrast, rats that trained at a moderate-intensity only exhibited augmented sympatholysis during heavy-intensity contractions. The mechanism responsible for the augmented sympatholysis in trained animals was an augmented NO-mediated inhibition of sympathetic vasoconstriction as NOS blockade decreased the magnitude of sympatholysis in exercise trained rats.

To our knowledge, this is the first study to demonstrate that a prospective short-term ET regimen augments sympatholysis. These results provide direct evidence of a training intensity-sensitive up-regulation of sympatholysis, mediated through an NO-dependent mechanism.

The magnitude of vasoconstriction in response to sympathetic stimulation

Previous studies which have investigated the effects of ET on sympathetic vascular responsiveness have done so in resting skeletal muscle or

in isolated vascular preparations and have produced equivocal results (9, 13, 23, 29, 34, 36, 58, 61, 62, 76). Indeed, chronic endurance ET has been shown to increase (29), decrease (9, 13, 61, 76) or not alter (23, 62) post-synaptic α-adrenergic receptor responsiveness. However, studies that have investigated sympathetic vascular responsiveness following heavy-intensity aerobic exercise (36) and sprint interval exercise training (34) have demonstrated an augmented response to exogenous administration of norepinephrine and phenylephrine in isolated arterioles (34, 36). Our laboratory also recently reported augmented sympathetic vascular responsiveness in resting skeletal muscle following heavy-intensity ET (25). Consistent with our previous investigation (25), the vascular response to sympathetic stimulation delivered at 2Hz was augmented in resting skeletal muscle in a manner dependent on the intensity of ET in the present study. These previous findings are extended by demonstrating that the response to sympathetic stimulation delivered at a higher frequency (5Hz), reflective of a higher level of sympathetic outflow, was also increased in exercise trained compared to S rats.

Compared to rest, muscular contraction diminished the magnitude of vasoconstriction in response to sympathetic stimulation in all rats. Indeed, the magnitude of vasoconstriction declined as a function of muscle contractile force in all rats. During muscular contraction at 30% MCF, exercise trained rats had a greater vasoconstriction in response to sympathetic stimulation delivered at 2Hz compared to sedentary rats, whereas the response to 5Hz sympathetic stimulation was not different between groups. This increase in vascular responsiveness to sympathetic stimulation at 2Hz suggests that short-term ET resulted in vascular smooth muscle becoming more sensitive to low

levels/frequencies of sympathetic outflow during muscle contraction at moderate intensities. However, it is possible that ET may have also altered the amount or composition of neurotransmitters released in response to stimulation of the lumbar chain or that ET altered local muscle metabolite production. During muscular contraction at 60%MCF, the vascular response to sympathetic stimulation was not different between groups at both 2 and 5Hz stimulation frequencies. These findings suggest that ET, does not alter vascular responsiveness to sympathetic stimulation during heavy-intensity contractile activity.

Proctor et al. (49) previously reported that 9-12 weeks of heavy-intensity ET decreased leg blood flow during sub-maximal cycle exercise at 70 and 140 W. The lower post-training leg blood flows were achieved at similar or reduced levels of leg NE spillover suggesting that ET may have augmented sympathetic vascular responsiveness in contracting muscle, consistent with the present findings.

Magnitude of sympatholysis

Previous studies completed in dynamically exercising humans and animals and *in situ* muscle preparations have demonstrated that sympatholysis is dependent on the intensity of exercise/ muscle contractile force (1, 2, 4-6, 45, 51, 54, 55, 73, 74, 79). In agreement with prior investigations, the ability to inhibit sympathetic vasoconstriction was related to muscle force production in all rats in the present study.

Additionally, ET augmented sympatholysis in a training intensity-dependent manner. Specifically, H trained rats had a greater degree of sympatholysis during contraction at both 30 and 60% of MCF, compared to M

trained rats where a greater magnitude of sympatholysis occurred only during contraction at 60% MCF. The commonly accepted method to assess sympatholysis is to compare the percent decrease in FVC during contraction to the response at rest, as the percent change in FVC has been shown to correspond to a similar percent reduction in blood vessel radius despite differing levels of vascular conductance between rest and exercise (3). In the present study, the magnitude of sympatholysis was calculated as the difference between the percent decrease in FVC at rest and during contraction. It could be argued that the increased constrictor response to sympathetic stimulation at rest equipped the exercise trained rats with a greater capacity for sympatholysis. However, it is difficult to reconcile how an increase in constrictor responsiveness at rest would also confer a greater ability to inhibit sympathetic vasoconstriction during contraction upon the trained animals. Indeed, the greater constrictor responsiveness at rest could in fact make it more difficult for the trained animals to inhibit constriction during contraction. Thus, while the trained animals may have a greater range over which to decrease constrictor responsiveness, I believe the important consideration is that the trained animals are able to markedly inhibit the response to sympathetic stimulation during contraction, despite an increase in constrictor responsiveness at rest.

The present findings are in contrast to recent human studies. Wimer and Baldi (77) have shown that 6 weeks of intermittent handgrip training performed at 30% maximal voluntary contraction for 8 minutes, 3 times per week did not alter sympatholysis during handgrip exercise. Similarly, Mortensen et al. (2012) recently reported no difference in sympatholysis between control and exercise trained limbs following 5 weeks of single-leg knee-extension exercise training.

Recent cross-sectional studies have also reported a similar magnitude of sympatholysis in trained compared to untrained humans (77, 78). The reason(s) for these contrasting results are not readily apparent; however they may be related to the intensity and volume of ET prescribed. As demonstrated in the current study, the intensity of training appears to influence sympathetic vascular control. Another potential explanation for the contrasting findings may be related to differences between prospective training studies and cross-sectional investigations. Functional vascular adaptations appear to occur early during training (≤6 weeks) with structural adaptations arising in response to prolonged durations of ET (7, 18). A major strength of the current study design was the ability to isolate the effect of training intensity and match the total volume of ET completed between groups. The training history of subjects in a cross sectional study can obviously not be controlled in this manner and effects of training on the vasculature are subject to a variety of factors such as training history and volume. Further studies will be required to investigate the relationships between the duration and volume of ET and functional sympatholysis and to determine if improved sympatholysis is a vascular adaption that persists with more prolonged or larger volume training regimens.

A decline in post-synaptic receptor responsiveness has been mechanistically linked to sympatholysis. Although not a universal finding, tonic α_1-adrenergic receptor mediated vasoconstriction and α_1-adrenergic receptor responsiveness have generally been shown to decline only during heavy-intensity exercise (1, 6, 79), whereas tonic α_2-adrenergic receptor mediated constriction and receptor responsiveness appears to be diminished during moderate- and heavy-intensity exercise (1, 6, 14, 38-40, 51, 65, 68, 79). It has

been argued that this intensity-dependent modulation of receptor responsiveness may be related to the distribution of receptors within the vascular tree as receptors positioned on distal branches of the vascular tree would be in closer proximity to the interstitial environment exposing them to larger concentrations of vasoactive molecules that oppose sympathetic vasoconstriction. Data from the rat cremaster muscle indicate that α_2-adrenergic receptors are localized to small, distal secondary and tertiary arterioles, wherease α_1-adrenergic receptors are primarily located on larger proximal, arterioles While differences have been shown to exist between muscles (Moore et al., 2010), α_2-adrenergic receptors are thought to be localized to small, distal secondary and tertiary arterioles (1, 15, 38, 39). However, in the mouse gluteus maximus muscle the functional distribution of α_2 receptors was greater in proximal (1A) arterioles, while the functional distribution of α_1 receptors was greater in distal (3A) arterioles, suggesting that the distribution of receptors varies between vessel branch orders and skeletal muscle type/function (40). Exercise training induced changes in the expression/distribution or responsiveness of individual post-synaptic receptors were not investigated in the present study. Given the short-term training stimulus and relatively low volume of training used in this study, I do not believe that receptor expression/distribution would be altered in the present study. However, ET has been shown to up-regulate the expression of other vaso-regulatory molecules and therefore, further studies will be required to establish the effect of ET on post-synaptic receptor expression/distribution.

Nitric oxide-dependent inhibition of sympathetic vasoconstriction

A definitive mechanism responsible for sympatholysis has not been established. However, NO has been shown to inhibit sympathetic

vasoconstriction at rest (19, 43, 66) and during muscle contraction (21, 46, 57, 69, 71). NO may mediate sympatholysis through activation of potassium sensitive ATP channels and blunting of post-synaptic α-adrenergic receptor responsiveness (64, 67). Consistent with previous studies, the present data demonstrate that NO inhibits sympathetic vasoconstriction in resting (19, 43, 46, 66) and contracting skeletal muscle (21, 57, 69, 71), regardless of training status. An important novel finding of the present study was that NO-mediated sympatholysis was augmented by ET in a training-intensity dependent manner (Figures 4 and 5).

Mechanical and chemical stimuli elicit the release of NO from NOS localized in the endothelium (eNOS) (53) and skeletal muscle sarcolemma (neuronal NOS, nNOS) (27, 42) in response to muscular contraction. Both eNOS (46) and nNOS (57, 69, 70) have been shown to inhibit sympathetic vasoconstriction in human and animal preparations (21, 46, 57, 69, 71). In the presence of NOS blockade or endothelial removal, vascular responsiveness to norepinephrine and phenylephrine in the abdominal aorta (9, 61) and gastrocnemius and soleus 1st order arterioles (13) was augmented following moderate-intensity ET, suggesting that eNOS mediated inhibition of sympathetic vasoconstriction was enhanced following ET. Indeed, ET has been shown to enhance eNOS protein expression in response to moderate- (35, 47, 60) and heavy-intensity training (34). Expression of the nNOS isoform has also been shown to increase in response to ET. Ten days of exhaustive cycle ET increased nNOS protein expression in human skeletal muscle (37) and four weeks of daily swim-training increased nNOS protein expression in the rat hind-limb (63). In contrast, run training 3 x/week for 6 weeks did not change nNOS protein

expression in human skeletal muscle (16). Thus, the available evidence, although not conclusive, suggests that the expression of both eNOS and nNOS may change in response to ET and may be involved in the training mediated up-regulation of sympatholysis. To our knowledge, the effect of ET on specific NOS isoform-mediated sympatholysis has not been established.

Another potential mechanism involved in the ET mediated augmentation of NO-mediated sympatholysis may be associated with the recruitment of skeletal muscle fibres. Increasing intensities of exercise are associated with a progressive recruitment of skeletal muscle and, in particular, the recruitment of additional glycolytic muscle fibres (30-32). Although, the present study did not assess the amount or pattern of muscle fibre recruitment during ET, it is conceivable that a larger proportion of glycolytic muscle fibres were recruited during heavy- compared to mild-intensity training (30-32). It also seems likely that ET resulted in a training intensity-dependent vascular recruitment, such that a larger volume of blood vessels and vessels from different skeletal muscles may have been "conditioned" in exercise trained compared to sedentary rats. Some evidence suggests that a greater magnitude of NO-mediated sympatholysis occurs in glycolytic compared to oxidative skeletal muscle (71). Therefore, if a larger number of blood vessels from glycolytic muscles were affected as a function of training-intensity, it is conceivable that this may have contributed to the training intensity-dependent up-regulation of sympatholysis in the present study.

Perspectives and Significance

An ET induced increase in vascular reactivity to sympathetic stimulation in resting skeletal muscle and an enhanced inhibition of sympathetic

vasoconstriction during contraction appears counter-intuitive. I suggest that ET augments the responsiveness of vascular smooth muscle to vasoactive molecules and that the skeletal muscle contractile state impacts the effectiveness and/or availability of individual vasoactive signalling molecules and influences regulation of the overall level of vascular tone. In resting skeletal muscle, when sympathetic neurotransmitters are largely unopposed by locally released vasodilators, vasoconstriction is augmented. During muscle contraction, exercise trained rats may release a larger quantity of vasoactive molecules in response to contraction, or become more responsive to vasoactive molecules that oppose sympathetic vasoconstriction (i.e. NO), resulting in an augmented sympatholysis.

The present findings demonstrate that short-term ET augmented functional sympatholysis in a training-intensity dependent manner through a NO-dependent mechanism. These novel findings advance our understanding of the effects of exercise-training on the regulation of sympathetic vasoconstriction in resting and contracting skeletal muscle.

Several previous studies have shown that the ability to inhibit sympathetic vasoconstriction may become impaired with aging, oxidative stress and disease (12, 26, 48, 72, 75, 80). The present data demonstrate that there is considerable plasticity in the regulation of sympathetic vasoconstriction and that the skeletal muscle vasculature is remarkably responsive to a relatively modest volume of ET. These data suggest that ET may be an important component in the treatment of pathophysiological conditions characterized by elevated sympathetic outflow, increased vascular resistance and a loss of sympatholytic capacity.

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Chapter 4

Short-term exercise training augments α_2-adrenergic receptor mediated sympathetic vasoconstriction in resting and contracting skeletal muscle

Introduction

Efferent sympathetic nervous system activity leads to the exocytotic release of neurotransmitters from sympathetic nerve fibers. The primary sympathetic neurotransmitter norepinephrine (NE) binds to post-synaptic α_1-and α_2-adrenergic receptors on vascular smooth muscle in the skeletal muscle vascular bed and produces vasoconstriction. Both α_1-and α_2-adrenergic receptors have been shown to produce tonic sympathetic vasoconstriction in resting (2, 12, 18, 34, 39) and contracting skeletal muscle (6, 7, 41, 61). During acute exercise, efferent muscle sympathetic nerve activity (MSNA) increases in an exercise-intensity dependent manner (11, 33, 40, 49). However, the vascular response to MSNA is blunted in contracting muscle and therefore, despite an increase in MSNA, the magnitude of tonic sympathetic vasoconstriction is reduced in contracting compared to resting skeletal muscle (8, 46-48, 56, 61). The ability of muscle contraction to inhibit sympathetic vasoconstriction is known as functional sympatholysis (44). While the precise mechanism(s) responsible for sympatholysis have not been identified, a decline in the responsiveness of post-synaptic α_1-and α_2-adrenergic receptors appears to be involved (8, 46, 56, 61). The cellular mechanism(s) responsible for a decline in adrenergic receptor responsiveness during exercise have not been fully elucidated, however the vaso-active molecule nitric oxide (NO) has been linked to reduced adrenergic receptor responsiveness in contracting muscle (57).

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Our laboratory recently reported that ET augmented the vasoconstrictor response to lumbar sympathetic chain stimulation in resting and contracting skeletal muscle of rats (23, 24). Exercise trained rats also exhibited a training-intensity dependent improvement in functional sympatholysis that appeared to be mediated by improved NO-mediated blunting of sympathetic vasoconstriction (24). Whether the training-induced increase in sympathetic vasoconstrictor responsiveness and sympatholysis was mediated by alterations to specific post-synaptic α-adrenergic receptor(s) was not investigated in our previous studies.

Previous exercise training studies have reported increased (27, 31, 35), decreased (10, 13, 52, 60) or unchanged (21, 53) α-adrenergic receptor mediated vasoconstrictor responses following a variety of exercise training paradigms. These studies have largely been conducted in isolated arterioles or isolated vascular preparations and have investigated α-adrenergic vasoconstriction with non-selective pharmacology or have focused on α_1-adrenergic receptor mediated responses. To our knowledge, the effects of exercise training on α_2-adrenergic receptor mediated vasoconstriction in resting and contracting skeletal muscle has not been investigated.

Therefore, the purpose of this study was to investigate the effects of short-term mild- and heavy-intensity ET on α_2-adrenergic receptor-mediated sympathetic vasoconstriction in resting and contracting skeletal muscle. It was hypothesized that α_2-adrenergic receptor mediated vasoconstrictor responses would be increased in resting and contracting skeletal muscle following ET. It was also hypothesized that ET would augment sympatholysis and that the greater sympatholysis in exercise trained rats would be abolished by pharmacological blockade of NO and α_2-adrenergic receptors.

Methods

Animals and animal care

Male Sprague-Dawley rats were obtained from the institutional breeding colony and housed in pairs in a 12:12h light-dark cycle, environmentally controlled (22-24^{0}C, 40-70% humidity) room. Water and rat chow (Lab Diet 5001, PMI Nutrition, Brentwood, MO USA) were freely available. All experiments were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the Animal Care and Use Committee: Health Sciences for the University of Alberta.

Chronic endurance exercise training

All rats were habituated to the lab and exercise by walking on a treadmill (Panlab LE8710, Barcelona, Spain) 10 min·day^{-1} for 5 days at 10 m·min^{-1}, 0^0 grade. Following familiarization, rats were randomly assigned to one of three groups: 1) sedentary time-control (S; n=10); 2) mild-intensity ET (M; n=10; 20 m·min^{-1}, 5^0 grade) or 3) heavy-intensity ET (H; n=10; 40 m·min^{-1}, 5^0 grade). M and H rats trained on 5 days·week^{-1} for 4 weeks, while S rats were handled and weighed daily. On the first training day, H rats completed 15, 1 minute intervals at 40 m·min^{-1} 5^0 grade interspersed with rest periods of equivalent duration. Each subsequent training day, run time was increased while rest time was maintained. This training progression allowed all H rats to run continuously for 600 m at the prescribed speed and grade within 11 ± 2 days. Rats randomized to the M group ran continuously at the prescribed speed (20 m·min^{-1}), grade (5^0 grade) and distance (600 m) immediately following familiarization and continued to do so for the entire training program. The total volume of ET was matched between M and

H groups by having all rats run the same distance during each exercise bout. This training paradigm is routinely used in our laboratory and has been shown to increase heart mass, heart mass: body mass ratio, soleus citrate synthase activity and endothelium-dependent vasodilation (22-24).

Instrumentation

Approximately twenty-four hours after the last training session anesthesia was induced by inhalation of isoflurane (3-3.5%, balance O_2). The right jugular vein was cannulated and anesthesia was maintained by infusion of α-chloralose (8-16 $mg \cdot kg^{-1} \cdot h^{-1}$) and urethane (50-100 $mg \cdot kg^{-1} \cdot h^{-1}$). The depth of anesthesia was assessed by the stability of arterial blood pressure, heart rate (HR) and the absence of a withdrawal reflex in response to painful stimuli (i.e. paw-pinch). Core temperature was monitored by rectal probe and maintained at 36-37^{0}C by an external heating pad (Physitemp, TCAT-2, Clifton, NJ USA). A tracheotomy was performed to allow spontaneous breathing of room air. The maintenance of arterial blood gases and acid base status at rest and during contraction in this preparation has been previously demonstrated (24) thus, arterial blood gases and acid base status were checked periodically to confirm the maintenance of normal values in this experiment. The left brachial artery was cannulated and connected to a solid state pressure transducer (Abbott, North Chicago, IL USA) for the continuous measurement of arterial blood pressure. Mean arterial pressure (MAP) and HR were derived from the arterial blood pressure waveform. The left femoral artery and vein were cannulated for the delivery of pharmacology. Blood flow was measured using a transit-time flow probe (0.7V; Transonic Systems, Ithaca, NY USA) placed around the right femoral artery and connected to a flow-meter (T106 Transonic Systems, Ithaca, NY USA).

Muscle contraction

The right sciatic nerve was exposed and instrumented with a cuff electrode. The triceps surae muscle group was then dissected free of all skin and connective tissue and attached to a force transducer (Model MLT1030/D, AD Instruments, Colorado Springs, CO USA) via the calcaneal tendon. Hind-limb contractions were produced by electrical stimulation of the sciatic nerve with Chart 7.2^{TM} software (AD Instruments, Colorado Springs, CO USA). Maximal contractile force (MCF) was determined by stimulation of the triceps surae muscle group with 25, 1 ms impulses delivered at 100 Hz, 10x motor threshold (MT). The optimal muscle length for tension development was determined by progressively lengthening the muscle and repeating the nerve stimulation until a plateau in tension (peak – baseline) was observed. The triceps surae muscles were stimulated (40 Hz, 0.1 ms pulses in 250 ms trains, at a rate of 60 trains/minute at ~6x MT) to contract rhythmically at 60% MCF.

Lumbar sympathetic chain stimulation

A laparotomy was performed and the aorta and vena cava were temporarily retracted in order to instrument the lumbar sympathetic chain at the L3/L4 level with a bipolar silver-wire stimulating electrode. The electrodes were embedded and electrically isolated in a rapidly-curing non-toxic silicone elastomer (Kwiksil, WPI, Sarasota, FL USA). The electrodes were used to deliver constant current stimulations through an isolated stimulator (Digitimer DS3, Welwyn City, UK). Following a 20 minute stabilization period the following experiments were conducted.

α_2-Adrenergic receptor vasoconstriction in resting and contracting skeletal muscle

The vasoconstrictor response to 1 minute of lumbar sympathetic chain stimulation (1 ms, 1 mAmp pulses) delivered at 2 and 5 Hz in random order was measured at rest and during muscle contraction under control conditions, following the bolus injection of the selective α_2-adrenergic receptor antagonist yohimbine (0.5 $mg \cdot kg^{-1}$, IV) and subsequent NO synthase blockade (combined yohimbine + L-NAME, 5 $mg \cdot kg^{-1}$, IV). Rhythmic muscle contraction was produced for a total of 8 minutes and lumbar sympathetic chain stimulation was delivered 3 and 6 minutes after the onset of contraction in random order. Control, yohimbine and combined yohimbine + L-NAME conditions were separated by 30 minutes of recovery.

Upon completion of all experiments, animals were euthanized by anaesthetic overdose and the heart was dissected free for measurement of cardiac mass.

Effectiveness of α_2-adrenergic receptor blockade

The effectiveness and selectivity of α_2-adrenergic receptor blockade was assessed by injection (100 µL) of the selective α_2-adrenergic agonist clonidine (0.1 $\mu g \cdot mL \cdot min^{-1}$, IA) and the selective α_1-adrenergic receptor agonist, phenylephrine (0.1 $\mu g \cdot mL \cdot min^{-1}$, IA) prior to and following yohimbine and combined treatment with yohimbine + L-NAME.

Pharmacology

All drugs were purchased from Sigma-Aldrich (Oakville, ON Canada) and dissolved in 0.9% physiological saline.

Data analysis

Data were recorded using Chart 7.2^{TM} software (AD Instruments, Colorado Springs, CO USA). Arterial blood pressure and femoral artery blood flow (FBF) were sampled at 100 Hz and femoral vascular conductance (FVC) was calculated as FBF ÷ MAP ($mL \cdot min^{-1} \cdot mmHg^{-1}$). Peak force production and fatigue index (([peak force – end-contraction force] ÷ peak force) x 100) were calculated for each contractile bout. The magnitude of the vasoconstrictor response to sympathetic stimulation was determined by calculating the mean of the FVC response to sympathetic stimulation and expressing it as a percentage change from FVC prior to the stimulation, during control, yohimbine and combined yohimbine + L-NAME conditions. The magnitude of sympatholysis was calculated as the difference between the percentage change in FVC in response to sympathetic stimulation at rest and the percentage change in FVC in response to sympathetic stimulation during muscular contraction for control, yohimbine and combined yohimbine + L-NAME conditions (∆%FVC). All data are expressed as mean ± standard deviation.

Statistics

The vasoconstrictor response to sympathetic stimulation was analyzed with three-way repeated measures ANOVA (training group x muscle contractile state x drug condition; STATISTICA 10 Statsoft Inc., Tulsa, OK USA). The responses to each frequency of sympathetic stimulation were analyzed separately. The effect of ET and drug condition on muscle contractile force, the magnitude of sympatholysis and basal hemodynamics were determined by two-way repeated measures ANOVA (group x drug condition; SigmaPlot 12.3 Systat, Richmond, CA USA). Indices of training efficacy were assessed by one-way

ANOVA (SigmaPlot 12.3 Systat, Richmond, CA USA). When significant F-ratios were detected, specific differences were assessed using Student Newman Keuls post-hoc analysis. A p-value<0.05 was considered statistically significant.

Results

Basal hemodynamics and indicators of training efficacy

All rats randomized to ET groups completed the assigned training. Body mass was lower ($p<0.05$) in H compared to M and S rats, heart mass was increased ($p<0.05$) in M and H compared to S rats and the heart mass: body mass ratio was increased ($p<0.05$) by ET in a training-intensity dependent manner (Table 4.1). Resting HR, MAP, FBF and FVC were not different ($p>0.05$) between groups (Table 4.2).

Effect of exercise training on sympathetic vasoconstrictor responsiveness

The vasoconstrictor response to lumbar sympathetic stimulation delivered at 2 and 5 Hz in resting and contracting skeletal muscle in a representative animal is shown in Figure 4.1. At rest, the magnitude of vasoconstriction in response to sympathetic stimulation delivered at 2 Hz was increased ($p<0.05$) by ET in a training-intensity dependent manner (Figure 4.2). In response to stimulation at 5 Hz, a greater ($p<0.05$) vasoconstriction was observed in M and H compared to S rats (Figure 4.2).

During rhythmic muscle contraction, muscle force production (S: 1232 ± 136g; M: 1243 ± 141g; H: 1187 ± 226g) and fatigue index (S: 50 ± 12%; M: 48 ± 11%; H: 44 ± 7%) were not different ($p>0.05$) between groups. Muscle contraction produced a similar increase ($p>0.05$) in FBF and FVC in S, M and H

groups (Table 4.3). In contracting muscle, lumbar sympathetic chain stimulation at 2 Hz produced a larger vasoconstriction ($p<0.05$) in M and H compared to S rats, whereas sympathetic stimulation at 5 Hz produced a similar ($p>0.05$) decrease in FVC in all groups (Figure 4.2).

The magnitude of sympatholysis (Contraction %FVC – Rest %FVC) was increased ($p<0.05$) in ET rats in a training-intensity dependent manner (Figure 4.3).

Effects of α_2-adrenergic receptor blockade on sympathetic vasoconstrictor responsiveness

Yohimbine increased ($p<0.05$) resting FBF and FVC in S, M and H rats (main effect of drug; Table 4.2). In S rats at rest, yohimbine did not alter ($p>0.05$) the constrictor response to sympathetic stimulation delivered at 2 or 5 Hz (Figure 4.2). In contrast, yohimbine decreased ($p<0.05$) the vasoconstrictor response to sympathetic stimulation delivered at 2 Hz and 5 Hz in ET rats (Figure 4.2). Following α_2-receptor blockade, the constrictor response to sympathetic stimulation was not different ($p>0.05$) between M and S rats and M and H rats, but remained greater ($p<0.05$) in H compared to S rats (Figure 4.2).

During contraction, yohimbine did not alter ($p>0.05$) muscle force production or the increase in FBF and FVC (Table 4.3). In S rats, the response to sympathetic stimulation delivered at 2 Hz and 5 Hz was not altered ($p>0.05$) by yohimbine (Figure 4.2). In contrast, the vasoconstrictor response to sympathetic stimulation delivered at 2 and 5 Hz was reduced ($p<0.05$) in M and H rats by α_2-receptor blockade (Figure 4.2). Following yohimbine, the constrictor response in contracting muscle was not different ($p>0.05$) between S, M and H rats (Figure 4.2).

During α_2-adrenergic receptor blockade, the magnitude of sympatholysis remained greater ($p<0.05$) in M and H compared to S rats (Figure 4.3) during sympathetic stimulation at both 2 and 5 Hz. In M and S rats, yohimbine did not alter ($p>0.05$) the magnitude of sympatholysis, whereas yohimbine reduced ($p<0.05$) the magnitude of sympatholysis compared to the control condition in H rats during sympathetic stimulation at both 2 and 5 Hz. (Figure 4.3).

Effects of combined α_2-adrenergic receptor and nitric oxide synthase blockade on sympathetic vasoconstrictor responsiveness

Combined treatment with yohimbine and L-NAME reduced ($p<0.05$) resting HR, FBF and FVC and increased ($p<0.05$) resting MAP in S, M and H rats (main effect of drug; Table 4.2). In resting skeletal muscle, the constrictor response to sympathetic stimulation delivered at 2 and 5 Hz was increased ($p<0.05$) in S, M and H rats during combined treatment with yohimbine and L-NAME (Figure 4.2). However, the increase in the vasoconstrictor response when L-NAME was added to α_2-adrenergic blockade was greater ($p<0.05$) in ET compared to S rats. Specifically, the vasoconstrictor response was increased in a training-intensity dependent manner to sympathetic stimulation at 2 Hz and was increased ($p<0.05$) by a greater magnitude in M and H compared to S rats to sympathetic stimulation at 5 Hz (Figure 4.2).

Muscle force production was not altered ($p>0.05$) by combined treatment with yohimbine + L-NAME. The increase in FBF in response to contraction was larger; ($p<0.05$) whereas, the increase in FVC was not different ($p>0.05$) in the combined yohimbine and L-NAME condition compared to the control and yohimbine conditions (Table 4.3). During contraction, the constrictor response to sympathetic stimulation at 2 and 5 Hz was greater ($p<0.05$) in the combined

yohimbine + L-NAME condition compared to control and yohimbine conditions in S, M and H rats (Figure 4.2). However, the increase in the vasoconstrictor response when L-NAME was added to α_2-adrenergic receptor blockade was greater ($p<0.05$) in ET compared to S rats. Specifically, the constrictor response to sympathetic stimulation at 2 Hz was increased in a training-intensity dependent manner and was increased ($p<0.05$) in response to sympathetic stimulation at 5 Hz by a greater magnitude in ET compared to S rats (Figure 4.2).

During combined inhibition of α_2-adrenergic receptors and NOS, the magnitude of sympatholysis remained greater ($p<0.05$) in ET compared to S rats. Sympatholyis was increased in a training-intensity dependent manner when sympathetic nerve stimulation was delivered at 2 Hz, whereas sympatholysis was greater in H compared to S rats when sympathetic stimulation was delivered at 5 Hz (Figure 4.3, $p<0.05$).

Compared to the yohimbine condition, combined treatment with yohimbine and L-NAME did not alter ($p>0.05$) sympatholysis in S, M or H rats when sympathetic stimulation was delivered at 2 Hz; whereas the magnitude of sympatholyis was reduced in M and H rats when sympathetic stimulation was delivered at 5 Hz (Figure 4.3).

Constrictor response to selective adrenergic receptor agonists

The vasoconstrictor response to clonidine was increased ($p<0.05$) in M (-45 ± 7%FVC) and H (-47 ± 5%FVC) rats compared to S rats (-28 ± 9%FVC) and was abolished ($p<0.05$) by α_2-adrenergic receptor blockade with yohimbine (S: -2 ± 6%FVC; M: -1± 4%FVC H: -3± 4%FVC) and by combined treatment with yohimbine + L-NAME (S: -4 ± 6%FVC; M: -1 ± 4%FVC H: -2 ± 4%FVC). The response to the selective α_1-adrenergic receptor agonist phenylephrine was

increased ($p<0.05$) in M (-50 ± 4%FVC) and H (-52 ± 5%FVC) rats compared to S rats (-29 ± 10%FVC) and was not altered ($p>0.05$) by yohimbine (S: -35 ± 9%FVC; M: -46± 2%FVC H: -49± 6%FVC) but was increased during the combined yohimbine + L-NAME condition (main effect of drug, $p<0.05$) in all groups (S: -40 ± 6%FVC; M: -57 ± 7%FVC H: -59 ± 7%FVC).

Table 4.1. Indices of training efficacy.

Group	Body Mass (g)	Heart Mass (g)	Heart : Body Mass Ratio (g)
Sedentary	455 ± 21	1.4 ± 0.2	0.31 ± 0.03*
Mild-Intensity	434 ± 39	1.7 ± 0.2†	0.39 ± 0.03*
Heavy-Intensity	374 ± 13†‡	1.6 ± 0.1†	0.43 ± 0.03*

Values are mean ± SD. † indicates a significant difference from sedentary control group. ‡ indicates a significant difference from mild-intensity group. * indicates a significant training-intensity dependent difference. A p-value < 0.05 was considered statistically significant.

Table 4.2 Basal hemodynamics.

Group	Drug Condition	HR (beats·min^{-1})	MAP (mmHg)	FBF (mL·min^{-1})	FVC (mL·min^{-1}·mmHg^{-1})
Sedentary Time-Control	Control	411 ± 42	94 ± 11	3.2 ± 0.6	0.035 ± 0.005
	Yohimbine	410 ± 38	90 ± 9	3.4 ± 0.8†	0.038 ± 0.008†
	Yohimbine + L-NAME	361 ± 35‡	109 ± 12‡	1.9 ± 0.6‡	0.018 ± 0.004‡
Mild-Intensity Trained	Control	366 ± 38	92 ± 10	3.2 ± 0.9	0.035 ± 0.007
	Yohimbine	371 ± 35	95 ± 9	4.0 ± 1.2†	0.042 ± 0.011†
	Yohimbine + L-NAME	342 ± 29‡	122 ± 12‡*	2.6 ± 1.0‡	0.021 ± 0.007‡
Heavy-Intensity Trained	Control	381 ± 34	85 ± 7	2.7 ± 0.2	0.032 ± 0.005
	Yohimbine	384 ± 30	89 ± 12	3.5 ± 0.9†	0.040 ± 0.009†
	Yohimbine + L-NAME	353 ± 25‡	112 ± 7‡	1.8 ± 0.3‡	0.017 ± 0.003‡

Heart rate (HR), mean arterial blood pressure (MAP), femoral blood flow (FBF) and femoral vascular conductance (FVC). Values are mean ± SD. † indicates a significant main effect of yohimbine. ‡ indicates a significant main effect of L-NAME. * indicates a significant difference from the heavy-intensity trained and sedentary control group in the combined yohimbine + L-NAME condition. A p-value <0.05 was considered statistically significant.

Table 3. Hemodynamic responses to muscle contraction.

Group	Drug Condition	HR (beats·min^{-1})	MAP (mmHg)	FBF (mL·min^{-1})	FVC (mL·min^{-1}·mmHg^{-1})
Sedentary Time-Control	Control	8 ± 9	7 ± 4	5.12 ± 0.56	0.053 ± 0.006
	Yohimbine	9 ± 6	6 ± 6	5.00 ± 0.76	0.052 ± 0.007
	Yohimbine + L-NAME	15 ± 10	9 ± 8	5.66 ± 1.03‡	0.048 ± 0.008
Mild-Intensity Trained	Control	9 ± 9	6 ± 6	5.00 ± 1.34	0.053 ± 0.011
	Yohimbine	11 ± 8	6 ± 1	5.14 ± 0.79	0.053 ± 0.010
	Yohimbine + L-NAME	8 ± 6	7 ± 7	5.64 ± 1.50‡	0.051 ± 0.013
Heavy-Intensity Trained	Control	8 ± 2	8 ± 7	5.03 ± 0.92	0.056 ± 0.009
	Yohimbine	11 ± 7	7 ± 2	5.56 ± 1.42	0.059 ± 0.011
	Yohimbine + L-NAME	14 ± 9	5 ± 5	5.63 ± 1.02‡	0.051 ± 0.011

The increase of heart rate (HR), mean arterial pressure (MAP), femoral artery blood flow (FBF) and femoral vascular conductance (FVC) in response to muscle contraction. Values are mean ± SD. ‡ indicates a significant main effect of L-NAME. A p-value <0.05 was considered statistically significant.

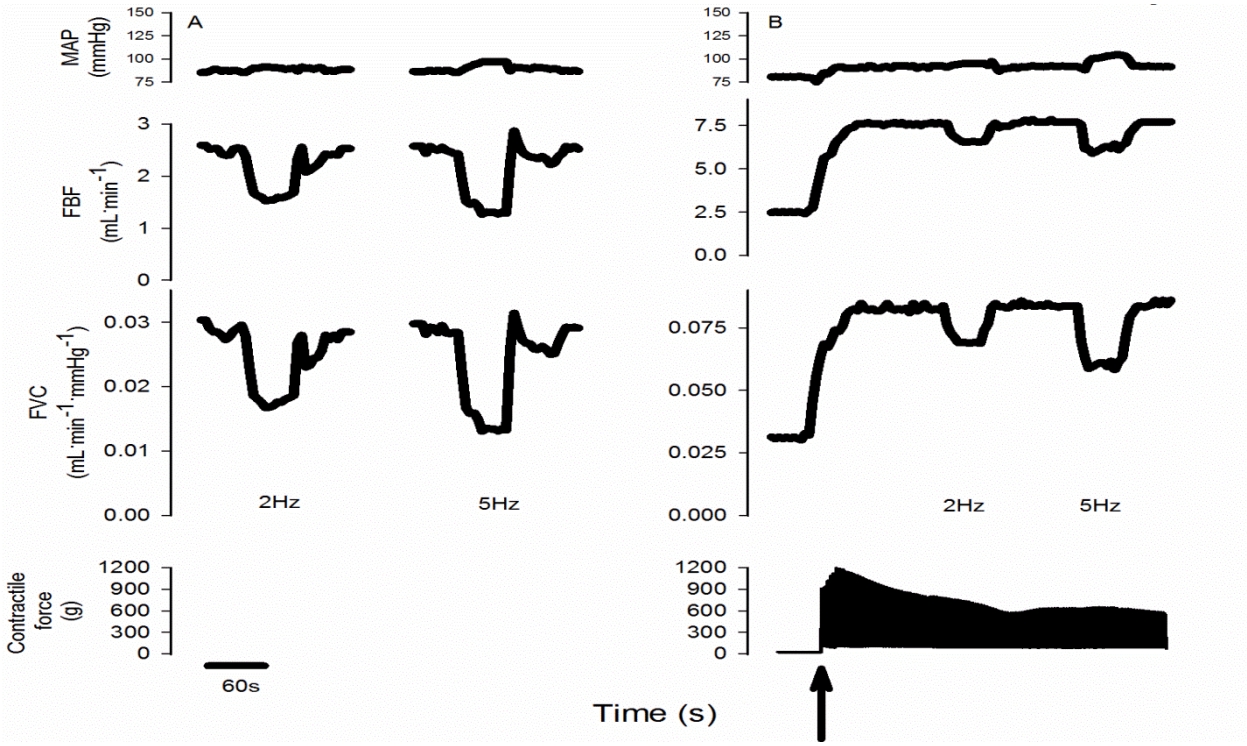

Figure 4.1. Original data from a representative animal illustrating the response of mean arterial blood pressure (MAP), femoral artery blood flow (FBF), femoral vascular conductance (FVC) and contractile force (g) at rest (A) and during 60% rhythmic maximal contractile force (MCF, B). The arrow indicates the onset of contraction. Lumbar sympathetic stimulations of 2 and 5Hz were delivered in random order during each condition in each rat.

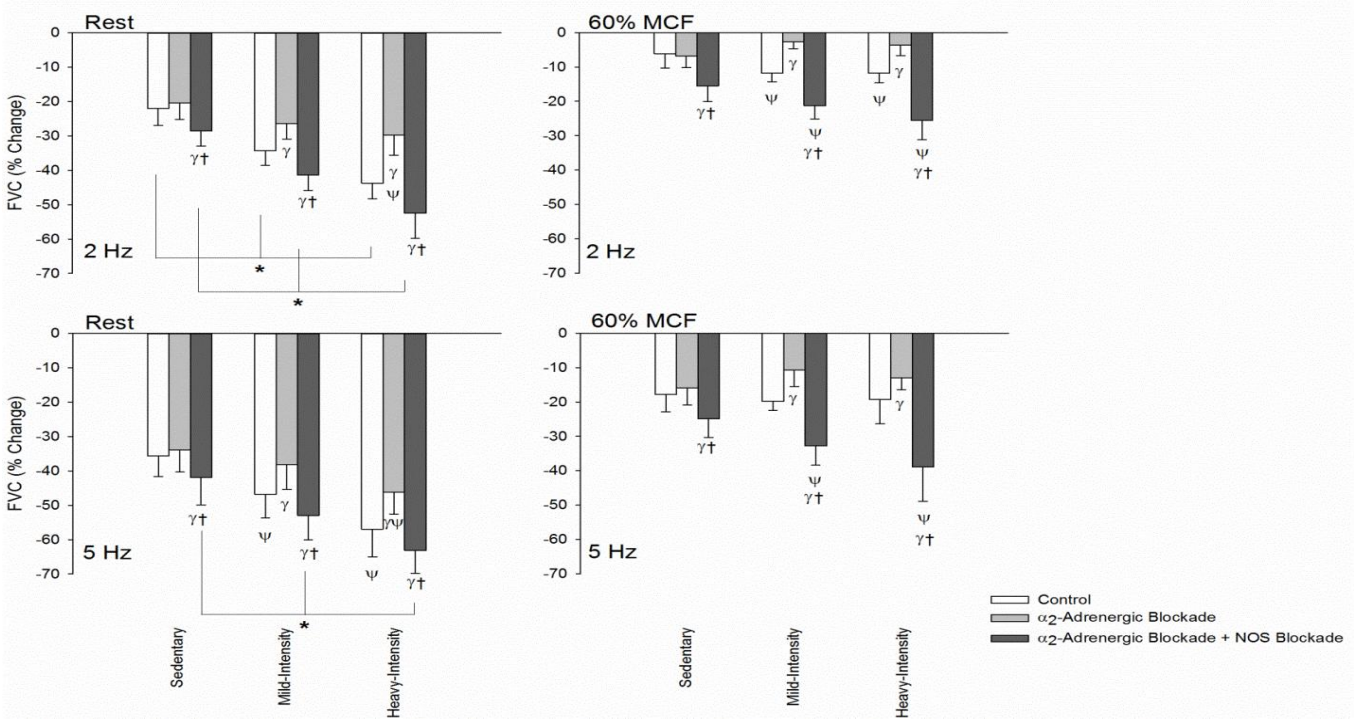

Figure 4.2. The percentage change of femoral vascular conductance (%FVC) during 2 Hz (top) and 5 Hz (bottom) sympathetic stimulation at rest (left) and 60% maximal contractile force (MCF, right) within the control condition (open bars),α_2-adrenergic receptor blockade with yohimbine (0.5mg·kg^{-1} IV, light gray bars) and combined α_2-adrenergic receptor blockade and NOS blockade (L-NAME 5mg·kg^{-1} IV, dark gray bars). Values are mean ± SD. * indicates a significant difference between all groups within specified drug condition. Ψ indicates a significant difference from the sedentary control group. γ indicates a significant difference from the control condition. † indicates a significant difference from yohimbine conditions within specified group. A p-value<0.05 was considered statistically significant.

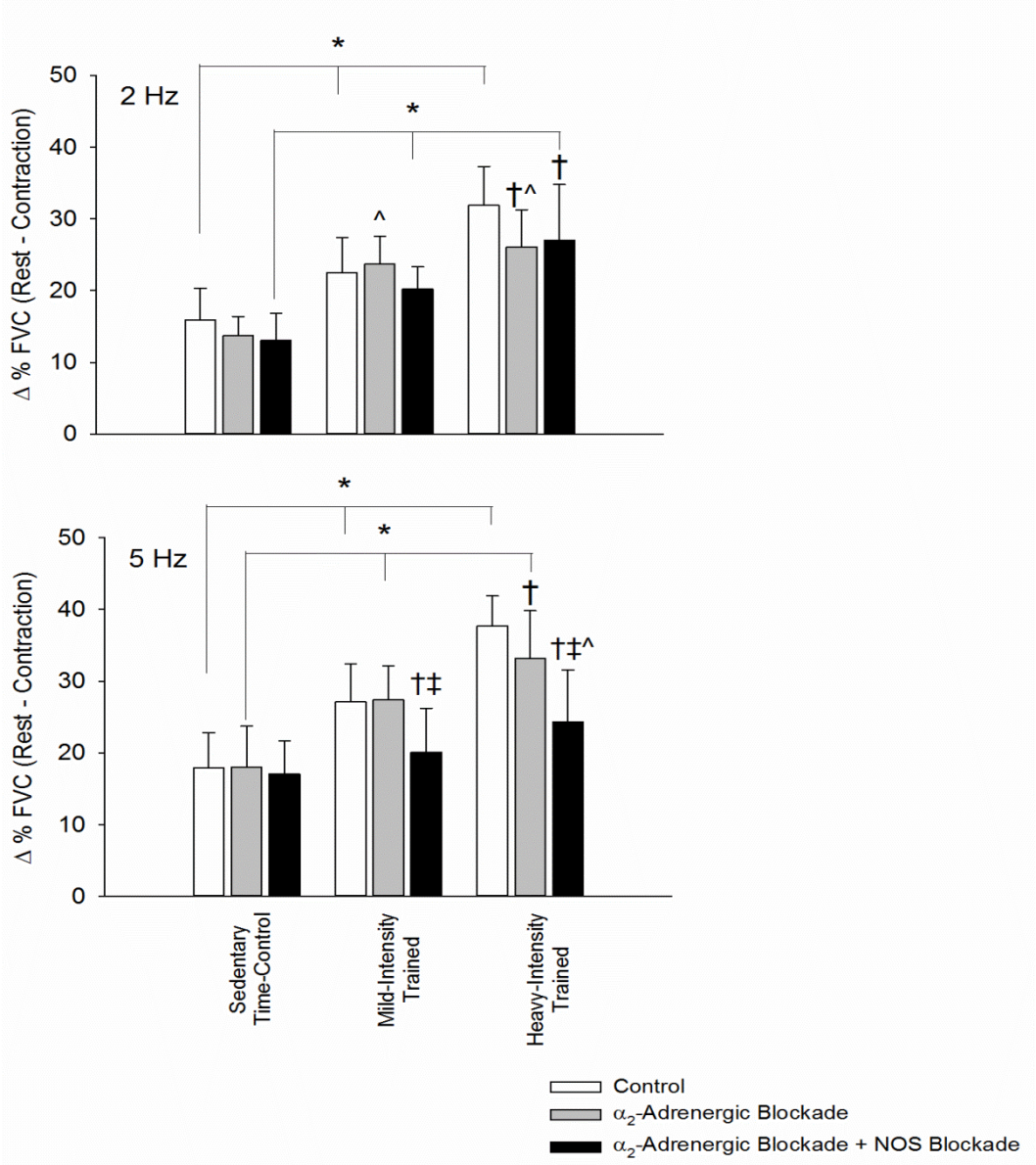

Figure 4.3. The magnitude of sympatholysis expressed as the difference between contracting and resting muscle sympathetic vasoconstrictor responsiveness (femoral vascular conductance, Δ%FVC) during 2 Hz (top) and 5 Hz (bottom) sympathetic stimulation, during control (open bars), α_2-adrenergic receptor blockade with yohimbine (0.5mg·kg^{-1} IV, gray bars) and combined α_2-adrenergic receptor blockade and NOS blockade (L-NAME 5mg·kg^{-1} IV, black bars) for sedentary time-control, mild-intensity trained and heavy-intensity trained groups. Values are mean ± SD. * indicates a significant difference between all groups. ^ indicates a significant difference from the sedentary time-control group. † indicates a significant difference from the control condition within the specified training group. ‡ indicates a significant difference from the yohimbine condition within the specified training group. A p-value<0.05 was considered statistically significant.

Discussion

The purpose of this study was to investigate the effect of short-term ET on α_2-adrenergic receptor mediated sympathetic vasoconstriction in resting and contracting skeletal muscle. Exercise training augmented α_2-adrenergic receptor mediated sympathetic vasoconstrictor responses in resting and contracting skeletal muscle. Exercise training also augmented functional sympatholysis, through improved NO mediated inhibition of α_1-adrenergic receptor mediated vasoconstriction in contracting muscle. In contrast, α_2-adrenergic receptors appeared to become resistant to inhibition during muscular contraction following ET. Short-term ET appears to induce a shift in post-synaptic α-adrenergic receptor mediated regulation of sympathetic vasoconstriction. In exercise trained rats, α_2-adrenergic receptors mediate a significant component of the vascular response to sympathetic stimulation in resting and contracting skeletal muscle that is lacking in sedentary rats. These data suggest that the regulation of sympathetic vasoconstriction becomes more complex following ET and that the relative contributions of α-adrenergic receptors to sympathetic vasoconstriction are markedly different between sedentary and exercise trained rats.

α_2-Adrenergic receptor mediated vasoconstriction at rest and during contraction

Consistent with previous studies from our laboratory (23, 24), short-term ET augmented sympathetic vasoconstrictor responsiveness in resting skeletal muscle in a manner dependent on the intensity of training. The present data demonstrate that the augmented vascular responsiveness following ET was largely mediated by increased α_2-adrenergic receptor mediated vasoconstriction. Specifically, treatment with yohimbine reduced the vasoconstrictor response to sympathetic stimulation at 2 and 5 Hz in mild-

and heavy-intensity trained rats. The decrease in the constrictor response with yohimbine indicated that ~20-30% of the vasoconstrictor response in resting skeletal muscle of exercise trained rats was attributable to α_2-adrenergic receptors. Following treatment with yohimbine, the constrictor response in mild-intensity trained rats was not different from sedentary rats suggesting that the ET-induced increase in constriction in mild-intensity trained rats was entirely attributable to the α_2-adrenergic receptor. In heavy-intensity trained rats, yohimbine did not completely abolish the increased vasoconstrictor responsiveness and the constrictor response to sympathetic stimulation delivered at both 2 and 5 Hz remained significantly higher in heavy-intensity trained compared to sedentary rats. Thus, heavy-intensity ET may have also augmented α_1-adrenergic receptor and/or non-adrenergic receptor mediated vasoconstriction. In contrast to the effects in exercise trained rats, yohimbine did not alter vasoconstriction in response to sympathetic stimulation in resting skeletal muscle of sedentary rats, suggesting that constrictor responses were mediated by the α_1-adrenergic receptor in the untrained state. The lack of an α_2-adrenergic receptor mediated response to sympathetic stimulation does not indicate that α_2-adrenergic receptors were absent or non-functional in the skeletal muscle vasculature of sedentary rats because: 1) yohimbine caused a similar increase in resting FVC in all groups indicative of tonic α_2-adrenergic vasoconstriction in resting skeletal muscle of all rats (Table 2) and; 2) injection of the selective α_2-adrenergic receptor agonist, clonidine, produced a robust vasoconstriction in all rats.

The addition of NOS inhibition to α_2-adrenergic blockade augmented the vasoconstrictor response to sympathetic stimulation at both 2 and 5 Hz in S, M and H rats demonstrating the presence of NO mediated inhibition of α_1-adrenergic receptor mediated vasoconstriction in all groups. However, the increased sympathetic

vasoconstriction was greater in M and H compared to S rats suggesting a greater NO mediated inhibition of α_1-adrenergic receptor mediated vasoconstriction in exercise trained rats.

During muscular contraction, the vasoconstrictor response to sympathetic stimulation at 2 Hz was greater ($p<0.05$) in exercise trained compared to sedentary rats, whereas the vasoconstrictor response to sympathetic stimulation at 5 Hz was not different ($p>0.05$) between groups. Thus, the increase in vascular responsiveness to low frequencies of sympathetic stimulation observed in exercise trained rats at rest appears to be maintained during muscular contraction, consistent with previous findings from our laboratory (24). Treatment with yohimbine reduced the vasoconstrictor response to sympathetic stimulation at both 2 and 5 Hz in mild- and heavy-intensity trained rats. In contracting muscle of exercise trained rats ~70% of the constrictor response to sympathetic stimulation delivered at 2 Hz and ~40% of the constrictor response to sympathetic stimulation at 5 Hz was mediated by α_2-adrenergic receptors. In the presence of yohimbine the constrictor response to sympathetic stimulation was not different between exercise trained and sedentary rats, suggesting that the increased constrictor responsiveness in contracting muscle of exercise trained rats was mediated by α_2-adrenergic receptors. In sedentary rats, yohimbine did not alter the vasoconstrictor response to sympathetic stimulation during muscular contraction. Thus, sympathetic vasoconstrictor responses in resting and contracting skeletal muscles of sedentary rats appear to be mediated by α_1-adrenergic receptors. The addition of L-NAME to α_2-adrenergic blockade augmented the vasoconstrictor response to sympathetic stimulation at both 2 and 5 Hz in S, M and H rats, demonstrating that NO inhibited α_1-adrenergic receptor mediated vasoconstriction in all groups. However, the increased sympathetic vasoconstriction was greater in M and H compared to S rats

suggesting a greater NO inhibition of α_1-adrenergic receptor mediated vasoconstriction in exercise trained rats.

The increased α_2-adrenergic receptor mediated vasoconstriction in exercise trained rats may be attributable to increased α_2-adrenergic receptor binding affinity for NE, increased receptor expression/density and/or alterations to intracellular signalling downstream from the receptor involved in the regulation of intracellular [Ca^{2+}]. To our knowledge, the effect of ET on the affinity of skeletal muscle vascular receptors for NE has not been investigated. Ten weeks of treadmill training at ~80% of VO_2 max has been shown to increase myocardial α_1-adrenergic receptor density in rats (15); however to date, ET mediated changes in skeletal muscle adrenergic receptor expression/density have not been investigated. Exercise training has been shown to increase vascular tone of coronary resistance arterioles through increased voltage gated Ca^{2+} channel activity (4, 5, 19, 45). Whether a similar adaptation occurs in the skeletal muscle vascular bed following ET has not been established. Interestingly, α_2-adrenergic receptors in the skeletal muscle vasculature regulate vascular smooth muscle contraction by controlling extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels (1, 38) and therefore an increased contribution of α_2-adrenergic receptors to sympathetic vasoconstriction through increases in membrane voltage-gated Ca^{2+} channel flux following ET appears plausible; however experimental evidence to support this contention is lacking.

The constrictor response to sympathetic stimulation delivered at 2 Hz was up-regulated following ET in a training-intensity dependent manner suggesting that the skeletal muscle vasculature became more responsive to low frequency sympathetic stimulation following ET. Given that the increased constrictor responsiveness was largely mediated by the α_2-adrenergic receptor, the present data suggest that ET may increase the sensitivity of α_2-adrenergic receptors to low frequency sympathetic

stimulation. The release of neurotransmitters from sympathetic nerves appears to be sensitive to the frequency of nerve stimulation. In particular, low discharge frequencies (~1-2 Hz) evoke release of adenosine 5' triphosphate and NE (25). Therefore, it is plausible that sympathetic stimulation at 2 Hz may have caused a greater release of NE in trained compared to sedentary rats. However, the constrictor response to intra-arterial injections of selective α_1- and α_2- adrenergic receptor agonists was also greater in trained compared to untrained rats, indicating that the training induced increase in constrictor responses were likely mediated by an increase in post-synaptic receptor responsiveness and not increased neurotransmitter release.

Sympatholysis

Consistent with previous data from our laboratory (24), sympatholysis was increased by ET in a training-intensity dependent manner in the present study. Selective α_2-adrenergic receptor antagonism did not alter sympatholysis in mild-intensity exercise trained rats, whereas α_2- adrenergic receptor blockade reduced sympatholysis in heavy-intensity exercise trained rats, indicating that in mild-intensity trained rats sympatholysis was mediated solely by inhibition of α_1-adrenergic receptors; whereas in heavy-intensity trained rats both α_1- and α_2-adrenergic receptors were inhibited during contraction. When NOS blockade was added to blockade of α_2- adrenergic receptor, sympatholysis was not different in exercise trained rats in response to sympathetic stimulation at 2 Hz, but sympatholysis was reduced in response to sympathetic stimulation at 5 Hz demonstrating that NO partially inhibited α_1-adrenergic receptors. Collectively, the present data indicate that the ET induced increase in sympatholytic capacity was primarily mediated by an improved ability to inhibit α_1-adrenergic receptor mediated vasoconstriction and that α_2-adrenergic receptors became resistant to

inhibition during contraction in exercise trained animals. Nitric oxide appears to mediate a portion of the inhibition of α_1-adrenergic receptors during contraction; however the presence of a significant amount of sympatholysis during combined α_2-adrenergic receptor and NOS blockade suggests that other factors contribute to the improved inhibition of sympathetic vasoconstriction in exercise trained rats.

A training mediated improvement in the inhibition of α_1-adrenergic receptor constriction and a relative resistance of α_2-adrenergic receptors to inhibition is in contrast to our hypothesis and appears to conflict with several prior studies that have reported a greater inhibition of α_2-adrenergic receptors and a relative resistance of α_1-adrenergic receptors to inhibition during an acute exercise bout (8, 56, 61). However, an equivalent inhibition of α_1-and α_2-adrenergic receptors during moderate-intensity contraction in the human forearm (46) has been reported and heavy-intensity contractions have been shown to readily inhibit α_1-adrenergic mediated vasoconstriction (8, 61). Nitric oxide-mediated sympatholysis was augmented following ET as recently demonstrated (24). Taken together, the results from the present study and our previous investigation suggest that short-term ET augmented the inhibition of α_1-adrenergic receptor mediated vasoconstriction and that a portion of the inhibition is attributable to NO. It has been argued that NO inhibits sympathetic vasoconstriction (57) by activating ATP-sensitive K^+ receptors and reducing α_2-adrenergic receptor mediated influx of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels (54, 55). However, this cellular mechanism has not been definitively established. The present data and other recent studies demonstrate that NO also inhibits α_1-adrenergic receptors (20, 42, 58) and that the improved

sympatholytic capacity following heavy-intensity training is partly mediated by improved NO inhibition of α_1-adrenergic receptors

Perspectives

The present study and previous investigations from our laboratory (23, 24) have shown that resting mean arterial blood pressure, skeletal muscle vascular conductance and the hyperemic response (increase in FBF and FVC) to muscular contraction were not altered by short-term ET. If ET reduced basal sympathetic outflow (17, 26, 37) and the sympathetic response to exercise (43, 50, 51); the changes in adrenergic receptor mediated vascular control may be necessary to maintain systemic arterial blood pressure at rest and during exercise.

The exercise conditioning of specific post-synaptic adrenergic receptors may be influenced by muscle recruitment patterns during training and differences in the anatomical distribution of receptors between fast and slow twitch muscles and segments of the vascular tree (2, 14, 16, 36, 59). During aerobic exercise, oxidative skeletal muscles are recruited first, followed by progressive recruitment of glycolytic muscles fibres as exercise intensity increases (28-30). Studies utilizing the rat cremaster muscle indicate that α_1-adrenergic receptors are predominantly located on larger proximal arterioles, whereas α_2-adrenergic receptors are expressed in distal arteriolar segments (2, 14, 36, 59). However, Moore et al. (39) investigated the responses of 1A and 3A arterioles from the gluteus maximus muscle to selective adrenergic receptor agonists and demonstrated that the α_1-adrenergic agonist phenylephrine caused a larger vasoconstriction in 3A compared to 1A arterioles and the α_2-adrenergic agonist UK14304 caused the greatest constrictor response in 1A compared to 3A

arterioles. Taken together, these studies indicate that the distribution of adrenergic receptors may differ between skeletal muscles and that the anatomical distribution of receptors may not be reflective of the functional contributions of each receptor type to sympathetic vasoconstriction. Nonetheless, it has been reported that blood flow increases to oxidative muscles and decreases to glycolytic muscles after ET (3, 32). Therefore, the observed changes in adrenergic vascular control may reflect the conditioning of a greater number of blood vessels in fast-twitch glycolytic muscle fibers that facilitate training-induced changes in the distribution of skeletal muscle blood flow. However, further investigation is necessary to determine muscle fiber-type specific effects of ET on adrenergic vascular control.

Experimental considerations

The current study utilized the selective α_2-adrenergic receptor antagonist yohimbine to block post-synaptic α_2-adrenergic receptors on skeletal muscle vascular smooth muscle. The effectiveness and selectivity of α_2-adrenergic receptor blockade with yohimbine was confirmed by intra-arterial injections of the selective α_1- and α_2-adrenergic agonists, phenylephrine and clonidine, respectively. However, α_2-adrenergic receptors are also expressed pre-junctionally on sympathetic neurons and on the endothelium, where they inhibit NE release and are able to produce endothelium-dependent vasodilation. An effect of yohimbine on α_2-adrenergic receptor inhibition of neuronal NE release appears unlikely as yohimbine decreased the constrictor response to sympathetic stimulation in trained animals and had no effect in sedentary rats. Blockade of α_2-adrenergic receptors on the endothelium would be expected to diminish vasodilation and augment vasoconstriction. Thus, the effect of yohimbine on

skeletal muscle vasoconstriction in response to sympathetic stimulation may have been underestimated if yohimbine up-regulated vasodilation in the present study. However, the absence of an augmented vasoconstrictor response to sympathetic stimulation in sedentary rats suggests that treatment with yohimbine did not diminish α_2-adrenergic receptor mediated vasodilation.

Finally, male Sprague-Dawley rats were utilized in the present study. Consistent with the present data, Coney et al. (9) have also reported that α_2-adrenergic blockade had no effect on vasoconstrictor responses to sympathetic stimulation in resting skeletal muscle of untrained male Wistar-Kyoto rats. In contrast, Thomas et al. (56) have reported α_2-adrenergic receptor mediated vasoconstriction in resting and contracting skeletal muscle and a preferential inhibition of α_2-adrenergic receptor mediated vasoconstriction in contracting muscle of sedentary female Sprague-Dawley rats. Thus, it is possible that α_2-adrenergic receptor control of skeletal muscle vasoconstriction differs between male and female rats and that the present findings are specific to male rats.

Conclusion

The present study demonstrated that ET augmented α_2-adrenergic receptor mediated vasoconstrictor responsiveness in resting and contracting skeletal muscle. Exercise training also augmented sympatholytic capacity through an improved ability to inhibit α_1-adrenergic receptor mediated vasoconstriction which was partially mediated by an NO-dependent mechanism. These data indicate that ET alters the relative contributions of α-adrenergic receptors to sympathetic regulation of vascular conductance in resting and contracting skeletal muscle and that the regulation of skeletal muscle sympathetic vasoconstriction becomes more complex following ET. This study

advances our understanding of the basic physiological mechanisms involved in the vascular adaptations to aerobic ET.

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Chapter 5

Summary, Experimental Considerations, Future Directions and Conclusions

Summary

This thesis investigated the effects of ET on sympathetic nervous system regulation of skeletal muscle vascular conductance. The overall purpose was to investigate the effects of short-term ET on sympathetic vasoconstrictor responses in resting and contracting skeletal muscle. The total volume of ET and the components of the training paradigm were controlled to isolate the effect of training intensity on sympathetic vasoconstrictor responsiveness and the inhibition of sympathetic vasoconstriction at rest and during skeletal muscular contraction.

Following short-term ET, sympathetic vasoconstrictor responsiveness was augmented in resting skeletal muscle in a manner dependent on the intensity of ET. Endurance ET also increased EDD in a training-intensity dependent manner and the magnitude of the increase in EDD and sympathetic vasoconstrictor responsiveness were significantly correlated. These data suggest that the training adaptations may occur in an integrative manner and that ET may concurrently alter dilator and constrictor pathways involved in the local control of skeletal muscle vascular conductance.

During acute exercise, the control of skeletal muscle vascular conductance becomes more complex as a relatively greater proportion of the

increase in cardiac output is diverted to the contracting skeletal muscle and sympathetic vasoconstriction must balance robust local vasodilation to ensure the maintenance of arterial blood pressure (66). Vasodilation in the contracting skeletal muscle facilitates the matching of blood perfusion to metabolic demand (67). Meanwhile, a concomitant increase in MSNA which signals vasoconstriction in active and inactive tissues ensures the maintenance of arterial blood pressure (13, 18, 60). Despite the increased sympathetic outflow, it is well established that a number of vaso-active substances are able to inhibit the magnitude of sympathetic vasoconstriction (79, 83).

Therefore, the effects of ET on sympathetic vasoconstrictor responsiveness and the inhibition of sympathetic vasoconstriction in contracting muscle were subsequently investigated. Consistent with the first study, ET augmented resting sympathetic vasoconstrictor responsiveness. Additionally, ET augmented sympathetic vasoconstrictor responsiveness to low frequency sympathetic stimulation during contraction. Interestingly however, short-term ET also augmented the magnitude of functional sympatholysis in a manner dependent on the intensity of ET. The training mediated increase in sympatholysis was partly mediated through an NO-dependent mechanism as NOS blockade significantly diminished the magnitude of sympatholysis in trained rats. Several previous studies have documented NO-mediated sympatholysis (15, 21, 30, 61, 68, 78, 80); however to my knowledge, this is the first study to demonstrate that ET improves NO-mediated inhibition of sympathetic vasoconstriction in resting and contracting skeletal muscle. The cellular mechanism of how NO inhibits sympathetic vasoconstriction has not been

established, however it has been argued that NO may diminish post-synaptic receptor responsiveness (75, 76).

Previous investigations of the effect of ET on post-synaptic receptor responsiveness have reported conflicting findings, with increased (41, 46, 52), decreased (17, 20, 72, 85) or unchanged (35, 74) constrictor responses to NE or the α_1-adrenergic receptor specific agonist phenylephrine reported in isolated vascular segments or tissue vascular preparations following ET. Moreover, the effects of ET on α_2-adrenergic receptor mediated vasoconstriction in resting and/or contracting skeletal muscle has not been studied, despite evidence that α_2-adrenergic receptors produce a substantial amount sympathetic vasoconstriction in the skeletal muscle vascular bed (2, 28, 50, 56) and that α_2-adrenergic receptors are inhibited during acute exercise (2, 14, 77, 86).

Therefore, the final study in this thesis investigated the effect of ET on post-synaptic α_2-adrenergic receptor mediated vasoconstriction in resting and contracting muscle. Short-term ET significantly augmented the contribution of α_2-adrenergic receptors to sympathetic vasoconstriction at rest and during contraction. The increased contribution of α_2-adrenergic receptors during contraction in trained compared to untrained rats suggested that the α_2-adrenergic receptors became resistant to inhibition during muscular contraction. ET also increased symaptholysis and the increased sympatholysis appeared to be largely mediated by improved NO-mediated inhibition of α_1-adrenergic receptors. These findings suggest that ET alters the contribution of post-synaptic adrenergic receptors to the regulation of sympathetic vasoconstriction, with the contribution of α_2-adrenergic receptors being increasingly important in the regulation of sympathetic vasoconstrictor control in resting and contracting

skeletal muscle following ET; a component of sympathetic control which appears to be lacking in sedentary control rats. The more complex sympathetic vasoconstrictor control in ET rats may improve between and within muscle distribution of blood flow. Previous studies have demonstrated that ET alters skeletal muscle blood flow distribution where during acute exercise, blood flow is preferentially increased toward oxidative muscles and decreased towards glycolytic fibers (3, 49). However; this study was unable to determine specific arteriole branch order or skeletal muscle fiber type changes to sympathetic vasoconstrictor responsiveness. Therefore, whether the changes in sympathetic vasoconstrictor control following ET contribute to improved control of blood flow distribution requires further investigation.

An increase in sympathetic vasoconstrictor responsiveness at rest and during contraction in ET rats could have important functional consequences for the regulation of arterial blood pressure and skeletal muscle vascular conductance. However, vascular conductance was not different between ET and sedentary rats at rest or during contraction. Therefore, it is conceivable that the increased vasoconstrictor responsiveness in ET rats may be necessary to maintain arterial pressure, if training also diminished resting MSNA and the response of MSNA to acute exercise (24, 40, 55, 63, 69, 70). MSNA was not measured in this thesis, however it has been argued that the sympathetic nervous system and cardiovascular system must continuously work in an integrated manner to simultaneously maintain systemic arterial blood pressure and optimize the delivery and distribution of skeletal muscle blood flow (37, 73, 83). Thus, any effect of ET on efferent MSNA may require compensatory

vascular adaptations to ensure that arterial blood pressure does not fall, while muscle blood flow is maximized.

The findings from this thesis highlight the integrated manner in which the vasculature responds to ET and the remarkable plasticity of sympathetic vasoconstrictor control. The training-intensity dependent effects of ET on sympathetic vasoconstrictor control of skeletal muscle vascular conductance indicate that vascular adaptations to short-term aerobic exercise training are a function of the training-intensity.

Experimental Considerations

The studies within this thesis utilized an *in vivo* anesthetized animal preparation to investigate the effects of ET on sympathetic vascular control in the hind-limb skeletal muscle vascular bed. The lumbar sympathetic chain was stimulated at the level of the L3/L4 spine to evoke the endogenous release of neurotransmitters in the hind-limb skeletal muscle vascular bed (71). Sympathetic stimulations were delivered at frequencies reflective of MSNA at rest and during physiological stress, such as during exercise (36, 48, 59). Although sympathetic stimulations were delivered in a consistent manner in all animals, it is possible that ET may have altered the amount and/or composition of neurotransmitters released in response to sympathetic stimulation and that the increased constrictor responses reflect training mediated effects on neurotransmitter release and not vascular responsiveness. However, ET rats also responded more vigorously to intra-arterial injections of selective α-adrenergic receptor agonists compared to sedentary control rats, suggesting that the change in vasoconstrictor responsiveness in ET rats was attributable to enhanced post-synaptic receptor responsiveness and not altered neurotransmitter release.

Adaptations of vascular post-synaptic receptor expression/density may occur in response to training and may contribute to ET-mediated alterations of vascular responsiveness (31, 43, 45, 53, 64, 84, 87). Knowledge of any training induced change in receptor protein expression/density would allow determination of what portion of the increased vasoconstrictor responses in ET rats were attributable to changes in protein expression and to changes in the responsiveness of post-synaptic receptors. However, receptor protein expression is typically measured in isolated vascular segments or whole muscle homogenates. Thus, whether receptor expression data measured in this manner would be reflective of the ET induced changes in the entire hind-limb is questionable and alignment of receptor expression data with functional data in the hind-limb may be difficult. Therefore, further investigation appears warranted in this area.

A limitation of the present studies is the inability to assess ET-mediated adaptations within specific vascular regions or within specific skeletal muscle types. Previous studies, have reported that vascular adaptations in response to training occur in a skeletal muscle fibre type and vascular branch order dependent manner, however these changes may also be influenced by the training paradigm utilized (1, 3, 16, 27, 42, 44, 46, 51, 77, 79). These studies highlight the heterogeneous manner in which different skeletal muscles and branch orders of the vasculature respond to ET. However, the function of an isolated vascular section may not reflect the function of an entire vascular bed. Therefore, investigations with alternate complementary methods may be necessary to further identify changes in sympathetic vasoconstrictor responsiveness in specific vascular segments and skeletal muscle fibre types in response to ET.

Future directions

This thesis investigated the effects of ET on the vascular response to sympathetic stimulation at rest and during muscular contraction. To date studies investigating the effects of ET on MSNA have produced variable and divergent findings (24, 40, 55, 63, 69, 70). Elucidation of the effects of ET on MSNA at rest and during contraction may provide further information and may help the understanding of why ET increased sympathetic vasoconstrictor responsiveness at rest and during contraction in the current studies.

Training augmented sympathetic vasoconstrictor responsiveness through an increased contribution of the α_2-adrenergic receptor in both mild- and heavy-intensity trained groups. α_2-Adrenergic receptor blockade diminished vasoconstrictor responsiveness of mild-intensity trained rats to a level similar to sedentary control rats. However, in the heavy-intensity trained group, α_2-adrenergic receptor blockade did not completely diminish the increased sympathetic vasoconstrictor responsiveness. Heavy-intensity ET may therefore, condition additional vaso-regulatory pathways, such as non-adrenergic receptors (10-12, 29, 36) and/or vascular smooth muscle cell Ca^{2+} regulation (8, 9, 33, 64) which may contribute to the augmented sympathetic vasoconstrictor responsiveness. Further investigation into the cellular mechanisms responsible for ET-induced alterations in sympathetic vasoconstrictor responses in resting and contracting muscle is required to fully elucidate the effects of ET on sympathetic vasoconstriction.

This thesis also demonstrated that ET increased the magnitude of sympatholysis through an increased NO-mediated inhibition of sympathetic

vasoconstriction; however NOS blockade did not abolish functional sympatholysis. Other studies have demonstrated that intra-luminally released ATP which acts on endothelial purinergic P2Y receptors (58, 65) and complementary actions of prostaglandins (19) may also be involved in sympatholysis. Additionally, vascular smooth muscle cell Ca^{2+} regulation through myo-endothelial feedback mechanisms (34, 39, 81, 88) have been shown to inhibit vasoconstriction produced in response to phenylephrine in isolated skeletal muscle arterioles. This may be an additional mechanism which mediates functional sympatholysis. Thus, further investigation is warranted to elucidate the effects of ET on additional sympatholytic mechanisms.

As discussed above, the effects of ET on sympathetic vascular control may also be dependent on vessel branch order and skeletal muscle fiber type. Thus, an understanding of the effects of ET on vascular control in specific vascular segments and in different skeletal muscles/ fiber types may further enhance our understanding of the effects of ET on the regulation of skeletal muscle vascular conductance. Investigations with modifications to the present experimental model and/or alternate complementary methods will be necessary to further identify specific changes to vascular regions and skeletal muscle fiber types in response to ET.

The focus of this thesis was to investigate skeletal muscle sympathetic vascular control in response to short-term mild- and heavy-intensity ET. The findings from this thesis advance our understanding of how training intensity affects ET mediated vascular adaptations. However, the components of a training program also include exercise duration, frequency and the overall duration of the training program. A scientific dissection of the duration, frequency

and volume of training has not been completed. Future studies should investigate the effects of the duration and frequency of the overall training program on sympathetic vasoconstrictor and vasodilator responsiveness in order to elucidate the effects of prolonged ET on sympathetic vaso-regulatory mechanisms which may aid in the preservation of vascular function and prevention of cardiovascular diseases.

Moreover, the effects of physical inactivity on vasodilator and vasoconstrictor responsiveness are not completely understood. Physical inactivity has been shown to be an independent risk factor for cardiovascular disease (4, 6, 7, 47, 54); insight into the effects of inactivity may provide further insight into the development of cardiovascular disease states. As such, studies focusing on a complete spectrum of physical activity/inactivity may provide further insight of its impact on skeletal muscle vascular regulation.

Conclusion

This thesis demonstrated that ET augmented sympathetic vasoconstrictor responsiveness and the ability to inhibit sympathetic vasoconstriction. These findings suggest that ET may promote a state of “vascular fitness” where the arterial vasculature becomes more responsive to a number of vaso-active stimuli. Increased vascular responsiveness may improve the regulation of arterial blood pressure and the distribution of blood flow within and between skeletal muscles.

It has been argued that a large proportion of the beneficial effect of ET on cardiovascular function may be mediated by alterations to arterial function (25, 26, 38) as the reduction of traditional cardiovascular risk factors do not entirely

explain the beneficial effects of ET (57). The findings from this thesis illustrate the effects of ET on sympathetic vasoconstrictor control in resting and contracting muscle of healthy rats and the mechanisms which contribute to the ET-mediated changes in sympathetic vasoconstrictor control providing a basis for the study of the effects of ET on sympathetic vascular control in cardiovascular disease.

This thesis further investigated the effects of the intensity of ET on arterial function. Current public health agency guidelines for physical activity/ET primarily recommend activities of a mild- to moderate-intensity (5, 22, 23, 32, 62); however, alterations which propose heavier-intensity exercise are beginning to be implemented (82). The data from this thesis may help to further refine public health recommendations for the promotion of cardiovascular health by elucidating the relationship between the intensity of ET and sympathetic control of arterial function.

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