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# Skeletal muscle arteriolar function following myocardial infarction: Analysis of branch-order effects

**Michael A. Tevald**, **John D. Lowman**, and **Roland N. Pittman**
Department of Physiology and Biophysics, Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, Virginia USA

## Abstract

Diminished bioavailability of nitric oxide (NO) may impair skeletal muscle arteriolar function after myocardial infarction (MI). We tested the hypotheses that chronic MI induced would diminish 1) endothelial function in large (resting diameter ~75 μm) feed arterioles, and 2) functional dilation in feed arterioles, but not smaller arcade (~25 μm) or transverse (~15 μm) arterioles, in the spinotrapezius muscle of female Sprague-Dawley rats. Additionally, we hypothesized that blockade of NO production with $N^G$-nitro-L-arginine methyl ester (L-NAME; 30 mg/kg i.v.) would have a greater blunting effect on control rats than MI rats. Endothelial function of the feed arterioles was assessed with an infusion of acetylcholine (1.5 μg i.v.) after pretreatment with indomethacin (5 mg/kg i.p.). MI blunted the response to acetylcholine in feed arterioles (p=0.037), but did not affect resting or post-contraction diameter at any branching order. L-NAME had similar effects on MI and SHAM rats; the response to acetylcholine was blunted in feed arterioles (p=0.003), resting diameter was diminished in arcade arterioles (p=0.003), and post-contraction diameter was diminished in both arcade arterioles (p=0.03) and transverse arterioles (p=0.05). In conclusion, despite endothelial dysfunction in feed arterioles, functional dilation was not affected by MI in any branching order studied. L-NAME had similar effects on MI and SHAM rats that were branch order-dependent. These branch-order effects should be considered in future studies of the control of blood flow.

### Keywords

Myocardial infarction; vasodilation; heart failure; nitric oxide; skeletal muscle

## INTRODUCTION

Peripheral vascular dysfunction is commonly associated with chronic myocardial infarction (MI) (Didion and Mayhan 1997, Thomas et al. 1998, Musch and Terrell 1992, Thomas et al.

Correspondence: Roland N. Pittman, PhD, Department of Physiology and Biophysics, Virginia Commonwealth University, Medical College of Virginia Campus, Box 980551, Richmond, VA 23298, pittman@vcu.edu, Phone – 804-828-9545, Fax – 804-828-7382.
Current Address for Michael A. Tevald, PT, PhD, Department of Rehabilitation Sciences, University of Toledo, Mail Stop 119, 2801 W. Bancroft Street, HH 2012, Toledo, OH 43606-3390, michael.tevald@utoledo.edu, phone – 419-383-3508
Current Address for John D. Lowman, PT, PhD, Department of Physical Therapy, University of Alabama at Birmingham, 344 School of Health Professions Building, 1530 3rd Avenue South, Birmingham, AL 35294-1212, jlowman@uab.edu, phone - 205-934-5892

**DISCLOSURES**
None

2001) and heart failure (Zelis et al. 1974, Kubo et al. 1991, Drexler and Lu 1992, Katz et al. 1996), and likely plays a key role in limiting exercise tolerance in this population. Indirect evidence suggests that arteriolar vasodilation is impaired in heart failure and may limit blood flow to exercising muscle (Zelis et al. 1974, LeJemtel et al. 1986), although this is not unequivocal (Wilson et al. 1986, Shoemaker et al. 1999, Magnusson et al. 1997, Wiener et al. 1986). While the mechanism of impaired arteriolar function is incompletely understood, several studies suggest that endothelial function is impaired in heart failure due to diminished bioavailability of nitric oxide (NO) (Drexler et al. 1992, Katz et al. 1999, Katz et al. 1996, Hirai et al. 1995). Furthermore, diminished responsiveness to NO-dependent vasodilators has been reported in small (resting diameter < 10 μm) (Thomas et al. 1998) and intermediate-sized (resting diameter = 38 μm) (Didion and Mayhan 1997) arterioles of rats with chronic MI. Although the role of NO in exercise hyperemia remains controversial (see for review (Tschakovsky and Joyner 2008)), there is direct evidence that NO plays a key role in arteriolar functional dilation (Hester et al. 1993). However, it is unclear if MI-related impairments in endothelial function affect functional dilation of arterioles.

Functional dilation of skeletal muscle arterioles is regulated by a complex interaction of control systems, including endothelial, neural, myogenic, and metabolic factors (see for review (Delp and Laughlin 1998)). The relative importance of each of these control systems appears to vary along the arteriolar tree, as evidenced by the heterogeneous responses of arterioles of different branching orders to hemodynamic and metabolic stimuli (Kuo et al. 1995, Sylvester et al. 2000). It has been proposed that the endothelium (Pohl et al. 2000), and NO in particular (Pohl and de Wit 1999, Hester et al. 1993), plays a greater role in the control of large arterioles, where flow-mediated dilation may serve to coordinate and magnify the hyperemic response to exercise, than in smaller, more metabolically responsive arterioles (Kuo et al. 1995).

Given the putative importance of the endothelium to functional dilation in large feed arterioles, and the evidence implicating impaired bioavailability of NO in the vascular dysfunction associated with chronic MI, we questioned whether endothelial dysfunction would lead to blunted functional dilation in large feed arterioles, and whether NO may play a role in these impairments. Furthermore, since the mechanisms controlling arteriolar diameter may vary along the arteriolar tree, we questioned whether the effects of MI on functional dilation would vary at different branching orders. Therefore, the purpose of this study was to assess the role of NO in functional dilation in three branching orders of skeletal muscle arterioles in normal rats and those with chronic MI. To accomplish this, we used in situ microscopy to measure the diameter of large feed (resting diameter ~75 μm), intermediate arcade (~25 μm), and small transverse (~15 μm) arterioles in the rat spinotrapezius muscle at rest and in response to contraction. Measurements were made before and after blockade of NO production with $N^G$-nitro-L-arginine methyl ester (L-NAME) in rats with MI and sham-operated controls. We hypothesized that, compared to control animals, rats with chronic MI would exhibit endothelial dysfunction and blunted functional dilation in feed arterioles, but preserved functional dilation in smaller arterioles. Furthermore, we hypothesized that NOS blockade would mimic the MI pheonotype (endothelial dysfunction, blunted functional dilation in feed arterioles only) in SHAM-operated control rats, but have little effect on MI rats, suggesting a role for NO in the MI-related impairments.

## METHODS

Female Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed in plastic containers at 20–23 °C on a 12:12 hour light/dark schedule, and allowed access to food and water ad libitum. Female rats are commonly used in studies of the effects of myocardial infarction on

blood flow (Behnke et al. 2004, Ferreira et al. 2006, Hirai et al. 1995, Kindig et al. 1999, McAllister et al. 1993, Musch and Terrell 1992, Richardson et al. 2003, Thomas et al. 1998, Thomas et al. 2001), and were employed in the current study because they have slower growth rates than males in adulthood and, therefore, would have thinner spinotrapezius muscles that produce better quality images in transillumination. All procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee, and the investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Unless otherwise noted, all chemicals were obtained from Sigma.

## Coronary Artery Ligation

MI was induced by coronary artery ligation (CAL), as described by Musch et al (1992). Briefly, ten-week-old rats were anesthetized with an isoflurane/oxygen mixture (Abbott Laboratories, North Chicago, IL), intubated, and mechanically ventilated. The heart was exposed via a left lateral thoracotomy, and the left coronary artery was ligated. In the SHAM procedure, a suture was placed, but the artery was not ligated. The incision was closed and the animal was extubated. Buprenex (0.3 mg/kg) was administered subcutaneously to control postoperative pain, and the animal was returned to the vivarium until the terminal experiment approximately 25 weeks later. This time was sufficient to allow recovery from the CAL surgery and to ensure that all animals had stable myocardial infarctions.

## Spinotrapezius Muscle Preparation

The spinotrapezius is a thin muscle that originates on the spinous processes of the thoracic vertebrae and inserts on the scapula. The microvascular anatomy has been described by Engelson and colleagues (1985), and we have adopted their terminology. The muscle is perfused by several large feed arterioles (~75 μm in diameter) which supply an interconnected network of intermediate arcading arterioles (~25 μm in diameter). Smaller transverse arterioles branch off the arcading network and connect, via 3 to 5 branching orders, to the capillaries (Engelson et al. 1985).

The spinotrapezius muscle was prepared in a manner similar to that described by Smith and colleagues (2004). This method leaves the nerve and vasculature intact, preserving microvascular function (Bailey et al. 2000). Briefly, the animals were initially anesthetized with an intraperitoneal injection of a mixture of ketamine (72 mg/kg; Forth Dodge Animal Health, Fort Dodge, IA) and acepromazine (3mg/kg; Vedco, St. Joseph, MO). Anethesia was maintained by continuous infusion of the anesthetic alfaxalone/alfadolone at 0.1 mg/kg/min i.v. (Saffan, Shering-Plough Animal Health, Welwyn Garden City, UK). The trachea and both jugular veins were catheterized, and the right carotid artery was initially cannulated with a 1.4 French Millar Mikro-tip pressure transducer catheter (model SPR-671, Millar Instruments, Inc., Houston, TX), to measure left ventricular end diastolic pressure (LVEDP; sampled at 5000 Hz, Biopac MP-150, Biopac Systems, Inc., Goleta, CA), followed by a fluid-filled catheter to allow monitoring of arterial pressure and blood sampling. An arterial blood sample (95 μl) was collected prior to the start of the experimental protocol, and blood gases were determined with a blood gas analyzer (ABL 705, Radiometer, Copenhagen, Denmark).

The muscle was exposed and the edges to a wire frame, and the animal was transferred to a custom-made thermostatic microscope platform for viewing (see (Golub and Pittman 2003) for description of the platform). The wire frame was fixed to the platform, and silver/silver chloride electrodes were placed under the muscle near the proximal and distal ends to allow electrically-stimulated muscle contraction. The muscle tissue was then covered with plastic

film (Saran, Dow Corning, Midland, MI) to prevent desiccation and gas exchange with the atmosphere.

### Muscle Contraction

Muscle stimulation was achieved with 200 millisecond trains of 40 Hz pulses delivered at 0.5 Hz. Pulse duration was 0.1 ms, and amplitude was 6 mA (A360 Pulse Generator and A310 Accupulser Stimulus Isolator, World Precision Instruments, Sarasota, FL). Stimulation voltage, measured by an oscilloscope, was less than 10 V. The pulse duration and voltage parameters are below the threshold for direct vascular effects (Jacobs and Segal 2000, Lash and Bohlen 1987).

### Video Microscopy

A 100 watt halogen lamp was used to transilluminate the muscle, and the microvasculature was observed with a Zeiss Axioplan 2 microscope equipped with an Achroplan objective (20X, NA=0.45). Images of the microcirculation were recorded on videotape (CCD-72 camera, MTI, Michigan City, IN; AG-D555 VCR and Viero Flat Panel Monitor, Panasonic, Secaucus, NJ) with a total magnification of 1,100X at the monitor. Vessel luminal diameter was measured with an image-shearing monitor (Model 908, Instrumentation for Physiology and Medicine, San Diego, CA). Spatial resolution of the system was 0.2 microns.

### Experimental Protocol

To avoid the potentially confounding influence of prostanoid-induced vasodilation during nitric oxide synthase (NOS) blockade with L-NAME, indomethacin (in propylene glycol, 5mg/kg i.p.) was administered prior to any measurements, as per prior studies (Hirai et al. 1995, Katz et al. 1996). The dose of indomethicin was chosen based on previous published work demonstrating its effectiveness (Gelgor et al. 1992, Thomas et al. 1988). In each muscle, feed, arcade, and transverse arterioles were studied in random order with a 20-min rest period between contraction bouts. An arteriole with good optical clarity was identified for study, and its diameter was measured before and immediately after 2 min of contraction. Arteriolar diameter immediately following contraction reflects peak diameter during the contraction, as skeletal muscle arterioles remain dilated for up to 10 seconds following the cessation of contractions before returning to resting diameter (Gorczynski and Duling 1978). In the rare instance where significant vasomotion was noted during the resting period, diameter measurements were averaged over 30 seconds. Significant vasomotion did not appear to affect post-contraction diameter measurements.

Following the three contraction periods, the vasodilatory response of the feed arteriole to a bolus i.v. infusion of 1.5 μg acetylcholine (ACh) was measured to assess endothelial function using a method adapted from previous studies (Hirai et al. 1995, Gardiner et al. 1990). L-NAME (30 mg/kg i.v.) was then administered, followed by a 10-minute rest period. The L-NAME dose was chosen based on previous published reports (Hester et al. 1993, Hirai et al. 1995, Gardiner et al. 1990), and it has been shown that the hemodynamic effects of L-NAME administration plateau within 10 minutes, and last for a minimum of 3 hours (Bellan et al. 1993, Gardiner et al. 1990). The contraction and vasodilation measurements were repeated on the same vessels, in the same order as above, followed by the ACh infusions. The animals were then euthanized (Euthasol, 200 mg/kg i.v.), and the heart and lungs were removed. The organs were blotted dry, then weighed, and organ weight/body weight ratios were calculated.

Data from arterioles were excluded from analysis if 1) mean arterial pressure dropped below 70 mm Hg during the experiment (n = 2 animals), 2) flow in the arteriole being investigated stopped during the recording period (n = 2 vessels), 3) the arteriole did not recover to within

10% of resting diameter within 10 minutes (n = 1 vessel), 4) the arteriole was unresponsive to muscle contraction (less than 1% change in diameter; n = 1 vessel), or 5) poor image quality precluded accurate assessment of arteriolar diameter within 10 s (i.e., due to movement during the contraction period; n = 4 vessels). Only arterioles with complete pre- and post-L-NAME recordings were included in the analysis.

### Statistics

Group comparisons for descriptive statistics (mass, LVEDP, and blood gases) were made using a 2-tailed t-test. Mean arterial pressure (MAP), heart rate (HR) and vessel diameter measurements made during the experiments were analyzed using a mixed-models factorial ANOVA for repeated measures. The analysis model included tests for a group effect (MI vs. SHAM), an L-NAME effect (pre-L-NAME vs. post-L-NAME), and a two-way (Group · L-NAME) interaction. *A priori* pairwise comparisons were made using an F-test. All statistical analysis was performed using JMP v4 software (SAS Institute Inc., Cary, NC), and differences were considered significant if $p \leq 0.05$. Unless otherwise indicated, data are expressed as mean (SEM).

## RESULTS

The CAL procedure was performed on 36 rats that were randomly assigned to either MI (n = 26) or SHAM (n = 10) groups. Ten animals died post-operatively (9 MI, 1 SHAM). At the time of the terminal experiment, mean age for the MI rats was 38 (SD 5.3) weeks, while that for the SHAM rats was 31 (SD 7.8) weeks. Experiments could not be completed on 4 MI animals due to deterioration of the preparation. Therefore, experiments were completed on 22 animals, 13 of which were in the MI group, and 9 in the SHAM group.

### Hemodynamics – Effects of MI and L-NAME Infusion

Baseline heart rate (HR), mean arterial pressure (MAP), and arterial blood gases were not different in MI and SHAM animals (Table 1). LVEDP was measured in 17 (MI = 11, SHAM = 6) animals due to technical difficulties in 5 of the animals. LVEDP, as well as the right ventricular- and lung mass-to-body mass ratios (RV/body and Lung/Body, respectively), were significantly higher in the MI animals (see Table 2) and consistent with moderate left ventricular dysfunction.

As expected, L-NAME infusion sharply increased MAP (pre vs. post, see Table 1). A Group · L-NAME interaction was present for MAP (p = 0.001), indicating that L-NAME had a greater pressor effect in the SHAM group. As a result, MAP after L-NAME infusion was significantly higher in the SHAM group.

### Endothelial Function

Resting diameter in the feed arterioles was not different between SHAM and MI animals. ACh infusion produced substantial vasodilation in both groups. The ANOVA revealed a main effect of MI, indicating that chronic MI blunted the vasodilatory response to ACh (p = 0.037; see Fig. 1a). Prior to L-NAME, the vasodilatory response to ACh was ~24% smaller in MI rats than in SHAM rats (p = 0.028 for pairwise comparison).

L-NAME significantly diminished the response of the feed arterioles to ACh infusion (p = 0.003; see Figure 1). Although no Group · L-NAME interaction was present (p = 0.3), pairwise comparison revealed that L-NAME abolished the difference in the response to ACh that was observed at baseline (p = 0.34); in other words, L-NAME treatment made the vasodilatory response to ACh more similar in SHAM and MI animals.

### Resting and Post-Contraction Diameter

**Effect of MI—**Resting arteriolar diameter was not different between the MI rats and SHAM controls at any branching order ($p > 0.2$ for all comparisons; Fig. 2). In response to muscle contraction, all arteriolar levels exhibited marked vasodilation. Post-contraction diameter (shown in Fig. 2) was not significantly different between MI and SHAM animals in feed ($p = 0.17$), arcade ($p = 0.76$), or transverse ($p = 0.42$) arterioles. Similarly, the percentage change in diameter was not affected by chronic MI at any branching order ($p > 0.36$ for all comparisons; data not shown).

**Effects of L-NAME—**L-NAME did not significantly affect resting diameter in the feed arterioles ($p = 0.57$; Fig. 2). Resting diameter in arcade arterioles was smaller after L-NAME ($p = 0.05$), while that of the transverse arterioles was not affected ($p = 0.9$). Post-contraction diameter in the feed arterioles was not affected by L-NAME ($p = 0.64$; Fig 2), but was significantly diminished in the arcade arterioles ($p = 0.03$) and transverse arterioles ($p = 0.05$). Finally, there were no Group · L-NAME interactions ($p > 0.2$ for all comparisons), indicating that L-NAME had a similar effect on post-contraction diameters in both MI and SHAM animals. Similarly, L-NAME did not affect the percent change in arteriole diameter at any branching order studied ($p > 0.5$ for all comparisons).

## DISCUSSION

We found that chronic, moderate MI was associated with endothelial dysfunction in skeletal muscle feed arterioles, but that functional dilation was preserved in feed, arcade, and transverse arterioles. Contrary to our hypothesis, L-NAME had similar effects on resting and post-contraction arteriolar diameter in both SHAM and MI rats; feed arteriolar diameter was unaffected, while resting diameter was smaller in arcade, and post-contraction diameter was smaller in both arcade and transverse arterioles after L-NAME.

### Endothelial Dysfunction

The elevated LVEDP and mass ratios (left ventricular and lung to body mass) in the MI rats are consistent with the presence of moderate, compensated left ventricular dysfunction (Lunde et al. 2001). The blunted response to ACh in the MI rats establishes the presence of endothelial dysfunction, and is consistent with previous reports in conduit (Katz et al. 1996, Kubo et al. 1991, Drexler and Lu 1992, Kaiser et al. 1989) vessels, as well as intermediate (resting diameter ≈ 38 μm (Didion and Mayhan 1997)) and small terminal (diameter < 10 μm (Thomas et al. 1998)) arterioles. Our finding of endothelial dysfunction in large feed arterioles is an important contribution, as it has been proposed that feed arterioles are more dependent on endothelium-mediated vasodilation than smaller arterioles, which are more dependent upon metabolic stimuli (Kuo et al. 1995).

Prior to NOS blockade with L-NAME, the vasodilatory response to ACh was blunted by 24% in MI rats compared to the SHAM rats. Given that flow varies directly with the fourth power of the radius of the vessel, this small difference in arteriolar diameter would have a substantial effect on blood flow into the muscle bed, assuming a similar pressure gradient and blood viscosity. After L-NAME infusion, the vasodilatory response to ACh was similar in SHAM and MI, suggesting that diminished NO bioavailability and/or function contributed to endothelial dysfunction in the MI rats. The fact that L-NAME blunted the response to ACh in the MI group suggests that either 1) NO function was not completely obliterated in the MI animals, or that 2) MI may blunt the ACh-induced release of other factors, such as endothelium-derived hyperpolarizing factor (EDHF). Indeed, Katz and Krum (2001) recently suggested that EDHF, rather than NO, played a larger role in the vasodilatory response to ACh in patients with heart failure. Because our focus was on the

influence of endothelial dysfunction on functional vasodilation, we did not fully investigate the mechanisms of endothelial dysfunction in these animals. Although this has been the subject of much investigation, further work in this area is warranted.

## Resting and Post-Contraction Diameter

**Effects of MI—**Similar resting diameter in MI and SHAM animals is consistent with previous studies of arcade (resting diameter ~38 μm (Didion and Mayhan 1997)), but not small terminal arterioles (diameter <10 μm (Thomas et al. 1998)). The lack of an effect of MI on post-contraction diameter highlights the robustness of the vasodilatory response to muscle contraction, and is consistent with the concept that exercise hyperemia is regulated by multiple redundant mechanisms (Clifford and Hellsten 2004). Our results suggest that additional vasodilatory mechanisms may be able to compensate for moderate endothelial dysfunction, allowing full expression of the hyperemic response to exercise in animals and humans with chronic MI and moderate left ventricular dysfunction.

Several human studies have found no effect of moderate heart failure on hyperemia during exercise employing a small muscle mass (Magnusson et al. 1997, Katz et al. 1996, Shoemaker et al. 1999, Wilson et al. 1986, Arnold et al. 1990), which is consistent with our results in rats. In contrast, capillary hemodynamics at rest (Kindig et al. 1999) and in the transition from rest to exercise (Richardson et al. 2003) are altered in rats with moderate MI. However, these alterations could be the result of impairments in downstream terminal arterioles (Thomas et al. 1998), venous congestion or structural alterations to the post-capillary vasculature (McAllister et al. 1993), or a blunted muscle pump (Shiotani et al. 2002). Furthermore, movement of the muscle during contraction precluded measurement of the kinetics of vasodilation in this study, which could alter capillary hemodynamics and oxygen delivery to the muscle (Behnke and Delp 2010). Further study in this area is warranted.

Since the primary goal was to investigate the role of NO in functional dilation, the confounding influence of vasodilator prostanoids was diminished by pretreatment with indomethacin, as per other investigations (Hirai et al. 1995, Katz et al. 1996). Lang et al (1997) found that indomethacin blunted exercise leg blood flow in heart failure patients, but had no effect on healthy control subjects. Thus, it is unlikely that the lack of difference in functional dilation observed here is the result of an artifact of indomethacin pretreatment.

**Effects of L-NAME—**L-NAME had similar effects on arteriolar diameter in MI and SHAM rats. This is consistent with previous studies of hindlimb vascular resistance at rest (Drexler and Lu 1992) and during exercise (Hirai et al. 1995), as well as the microvascular $PO_2$ response to contraction (Ferreira et al. 2006), in rats with moderate, but not severe, heart failure (Hirai et al. 1995, Ferreira et al. 2006). Further study is warranted to investigate the effects of severe heart failure on arteriolar functional dilation.

Vasoconstriction of the arcade arterioles following L-NAME infusion likely contributed to the strong pressor effect of the drug. However, systemic infusion of L-NAME is known to cause greater vasoconstriction in the mesenteric vasculature than in the hindquarters of rats (Gardiner et al. 1990), suggesting that vasoconstriction in other vascular beds also contributed to the pressor response. The reduced pressor response to L-NAME in the MI rats may reflect reduced sensitivity to NOS blockade in other vascular beds that were not examined in this study.

Our hypothesis that L-NAME would preferentially affect large feed, rather than smaller arcade and transverse, arterioles was based on the results of Hester et al (1993), who used a similar experimental design in the cremaster muscle of male hamsters. While muscle- and

species- specific difference may contribute to the discrepancy in these results, recent studies suggest gender differences may also play a role. During blockade (Wu et al. 2001) and knockout (Scotland et al. 2005) of both NOS and cyclo-oxygenase, endothelium-dependent vasodilation was abolished in males but preserved in females, demonstrating substantial gender-based difference in the regulation of vascular function. The source of these gender differences is not completely understood, but the menstrual cycle is known to have certain vascular effects, and the lack of control from the phase of the menstrual cycle may be a source of variability in our study. Additionally, our animals were pretreated with indomethacin in order to diminish the production of endothelium-derived prostanoids, which can compensate for diminished NO production during NOS blockade (Wu et al. 2001, Lamping et al. 2000). These two factors may explain the discrepancies between the current results and those of Hester and colleagues (1993).

## Limitations

Previous work has suggested that, compared to glycolytic muscles, highly oxidative muscles may be both more 1) dependent on endothelium-dependent vasodilation (Muller-Delp et al. 2002, McCurdy et al. 2000), and 2) susceptible to reductions in blood flow during exercise (Musch and Terrell 1992, McAllister et al. 1993). The fiber-type composition of the spinotrapezius muscle is ~ 40% type I, 7% type IIA, 17% type IID/X, 35% type IIB (Delp and Duan 1996), and it is therefore possible that arteriolar function in other muscles may have been affected by MI and L-NAME to a greater degree than in the spinotrapezius. Unfortunately, fiber-type differences in microvascular function are difficult to investigate with in situ microscopy due to the requirement that the muscles be thin enough for transillumination.

In our experimental set-up, the spinotrapezius muscle was covered with a plastic film (Saran) which served as a barrier to gas exchange between the tissue and the atmosphere. This arrangement has been used extensively in our laboratory (Smith et al. 2004) and others (Dodd and Johnson 1991) for microvascular studies. Despite the advantages of this arrangement, it does not allow for the convenient topical application of substances to the muscle. As a result, indomethacin, ACh and L-NAME were delivered systemically using procedures that have previously been shown to be effective (Gelgor et al. 1992, Thomas et al. 1988, Hirai et al. 1995, Gardiner et al. 1990, Hester et al. 1993).

Several studies have shown that systemic administration of L-NAME at the same or similar dose employed in the present study blunts endothelial NO release (Hester et al. 1993, Hirai et al. 1995, Gardiner et al. 1990), and de Wit and colleagues (de Wit et al. 1993) found similar blunting of ACh-induced vasodilation with systemic infusion or topical application of $N^{\omega}$-nitro-L-arginine. One limitation to the systemic infusion of L-NAME is the pressor response, which was greater in SHAM animals, and may have led to differences in arteriolar wall shear stress after L-NAME infusion. Additionally, the differences in MAP after L-NAME infusion may have led to different myogenic responses of the arteriolar wall in the two groups. While we cannot rule out these possibilities, the lack of MI · L-NAME interaction indicates that L-NAME had a similar effect on arteriolar diameter in SHAM and MI animals.

Our approach to assess endothelial function was adapted from the work of Hirai and colleagues (1995), who found that systemic infusion of ACh caused a 25% drop in mean arterial pressure that recovered rapidly (time to 50% recovery approximately 15 s). We found a similar transient drop in blood pressure that would have triggered increased sympathetic nervous activity via the baroreflex. If baroreflex sensitivity was diminished in the MI rats, as it is in humans with heart failure (Grassi et al. 1995), the reflex

vasoconstriction following the ACh-induced vasodilation may have been blunted. As a result, our approach may have underestimated endothelial dysfunction in the MI rats.

As discussed above, several previous studies have suggested that impairments in NO-dependent vasodilation play a role in the peripheral vascular dysfunction associated with chronic MI. We therefore focused our efforts on the NO-dependent pathway and did not directly investigate other pathways of endothelium-dependent vasomotion. Further study is needed to investigate possible branch order effects of additional vasoactive substances.

### Conclusions

Chronic MI, in the absence of decompensated heart failure, produced endothelial dysfunction in large feed arterioles of the rat spinotrapezius muscle, which are thought to depend more on endothelium-dependent mechanisms than in smaller arterioles. However, functional vasodilation was preserved in feed arterioles in the MI rats, indicating that, under these conditions, impaired endothelial function does not necessarily blunt functional dilation. Functional vasodilation was also preserved in smaller arcade and transverse arterioles, further highlighting the robustness of the arteriolar response to muscle contraction. L-NAME reduced resting diameter in arcade arterioles, as well as post-contraction diameter in arcade and transverse arterioles. However, feed arterioles were not affected. These differential responses should be considered in future studies of the control of arteriolar function.

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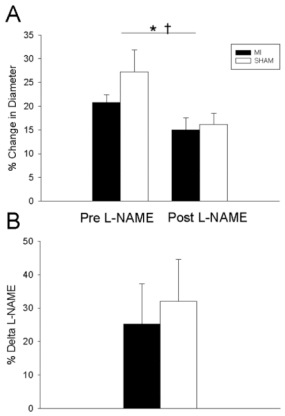


**Figure 1. Effects of MI and L-NAME on feed arteriole response to ACh infusion**

A) Feed arteriolar vasodilation in response to ACh was significantly blunted by MI ($p = 0.037$) and L-NAME ($p = 0.003$). Prior to L-NAME, the vasodilatory response to ACh was diminished in MI rats ($p = 0.028$ for pairwise comparison), while after L-NAME, the vasodilatory response to ACh was not different in MI and SHAM animals. (* effect of MI, † effect of L-NAME). B) The effect of L-NAME on the vasodilatory response, calculated as (Pre L-NAME % change – Post L-NAME % change)/(Pre L-NAME % change).

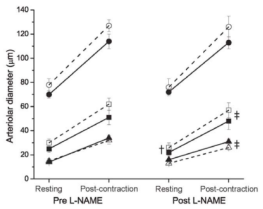


**Figure 2. Arteriolar Diameter in 3 branching orders before and after NOS blockade with L-NAME**

Resting and post-contraction diameter was not different between SHAM (open symbols) and MI (closed symbols) animals in feed (circles), arcade (squares), or transverse (triangles) arterioles. However, L-NAME diminished resting diameter in arcade arterioles, as well as post-contraction diameter in arcade and transverse arterioles.

## Table 1

Descriptive statistics.

| | SHAM (SD) | MI (SD) | p |
|---|---|---|---|
| **Body Mass (g)** | 270 (20) | 280 (5) | 0.24 |
| **Muscle Mass (mg)** | 286 (54) | 288 (36) | 0.91 |
| **pH** | 7.34 (0.08) | 7.36 (0.08) | 0.48 |
| **$P_aCO_2$ (mm Hg)** | 39 (11) | 38 (8) | 0.94 |
| **$P_aO_2$ (mm Hg)** | 90 (14) | 94 (12) | 0.53 |
| **$MAP_{pre}$ (mm Hg)** | 91 (5) | 88 (7) | 0.51 |
| **$MAP_{post}$ (mm Hg)** | 134 (8) | 109 (16) | <0.0001 |

pH = arterial pH, $P_aCO_2$ = arterial $PCO_2$, $P_aO_2$ = arterial $PO_2$, $MAP_{pre}$ and $MAP_{post}$ = mean arterial pressure before (pre) and after (post) L-NAME infusion

**Table 2**

Indices of left ventricular dysfunction.

| | SHAM (SD) | MI (SD) | p |
|---|---|---|---|
| **LVEDP (mm Hg)** | 4 (3) | 12 (5) | **0.005** |
| **RV/Body (mg/g)** | 0.59 (0.08) | 0.80 (0.21) | **0.011** |
| **Lung/Body (mg/g)** | 5.2 (1.8) | 8.1 (3.3) | **0.004** |

LVEDP = left ventricular end diastolic pressure, RV/Body = right ventricular mass/body mass, Lung/Body = lung mass/body mass