# Impaired vascular reactivity following chronic ischemia in the arteries of the mouse hindlim

Trevor R. Cardinal, Thomas J Kesler, Matthew D. Yocum, Tyler S. Smith, & Nolan M. Mott
Biomedical Engineering, Cal Poly State University, SLO, CA

## Introduction

Patients with ischemic disease, such as peripheral artery disease (PAD), demonstrate impaired vascular reactivity and blood flow control. The presence of co-morbidities common in these patients, such as hypercholesterolemia, likely contribute to the impaired vascular reactivity. However, ischemia in the absence of co-morbidities (i.e. in otherwise healthy animal models) is also known to impair vascular reactivity in arterioles and reduce collateral-dependent hyperemia. Although impaired vascular reactivity and blood flow control are well described in animal models of peripheral ischemia, the cellular mechanisms of these impairments are poorly understood. Further, little is known about the behavior of individual arteries following chronic ischemia, as most of this literature focuses on arterioles in the lower leg or regional blood flow in the thigh. Therefore, the goal of our work is to determine the impact of chronic ischemia on feed artery reactivity, and to define the cellular mechanism of any observed impairments. The long-term goal of this work is to determine the relative contribution of ischemia to impaired reactivity in patients with ischemic disease.

## Mouse Hindlimb Ischemia Models

To determine the impact of ischemia on feed artery vascular reactivity, we utilized an experimental model that involved resection of the femoral artery-vein pair from just upstream of the muscular branch to the distal saphenous, **Figure 1**. This surgery removes the femoral artery supply to the muscular branch and therefore induces ischemia in the muscular branch artery.

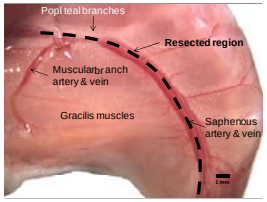


**Figure 1.** Medial aspect of the mouse hindlimb. The region of femoral artery-vein resection is indicated with a dashed line.

## Intravital Microscopy

We measured feed artery diameter using Side-stream Dark Field (SDF) intravital microscopy. SDF collects the reflected light from oblique 530nm pulses. 530nm is the isosbestic point for hemoglobin, thus the vasculature appears dark owing to the absorbance of light by red blood cells; the parenchymal and stromal tissue appears light. Our intravital imaging station is shown in **Figure 2**. Example images of the muscular branch artery (at rest and following functional vasodilation) are shown in **Figure 3**.

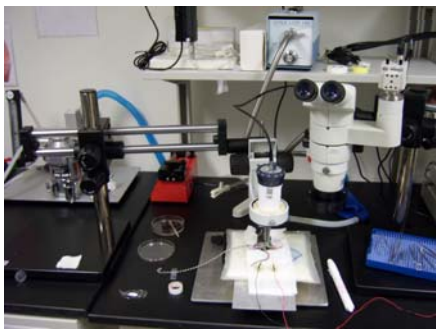

**Figure 2.** Intravital microscopy imaging station for functional vasodilation measurements.

## Intravital Microscopy

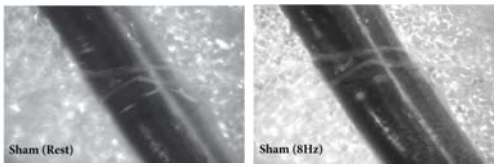


**Figure 3**. Still images of the muscular branch artery at rest (left) and following 90 seconds of 8Hz muscle contraction (right).

## Intravital Microscopy- Functional Vasodilation

We electrically stimulate muscle contraction in the gracilis muscles to assess the functionality of endogenous vasodilation pathways in the muscular branch artery, which feeds the gracilis muscles. In pilot studies, we examined both electrode placement (**Figures 4 & 5**) and stimulus parameters (**Figure 6 & 7**). Placing the stimulating electrode directly on the obturator nerve, which innervates the gracilis muscles), produced a similar magnitude of vasodilation to placing the electrode at the motor end plate in response to 1mA, 8Hz, 500µs, 90sec, **Figure 4**. However, nerve placement produced more variable dilation responses, **Figure 4**.

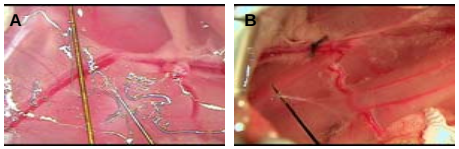


**Figure 4.** Stimulating electrode on the motor end plate of the gracilis (A) or the obturator nerve (B).

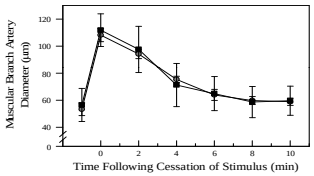


**Figure 5.** Vasodilation in the muscular branch artery in response to stimulating the gracilis motor end plate (grey circles) or the obturator nerve (black boxes).

In addition to electrode position, we also examined the impact of pulse frequency, stimulus duration, and stimulus amplitude in developing our functional vasodilation protocol. Increasing stimulus frequency leads to an increase in muscular branch vasodilation, **Figure 6**. Varying stimulus intensity had a limited affect on diameter in the muscular branch artery, **Figure 7**.

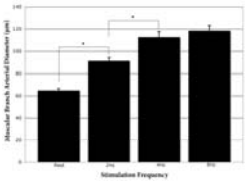


**Figure 6.** Vasodilation in the muscular branch artery in response to increasing stimulation frequency- 2, 4, & 8Hz, 500µs, 1mA, 90sec.

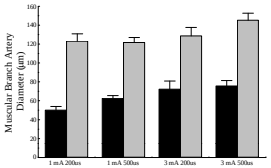


**Figure 7.** Vasodilation in the muscular branch artery in response to increasing stimulus intensity- 1 or 3mA and 200 or 500µs. All at 8Hz stimulus frequency for 90sec.

## Ischemia Eliminates Functional Vasodilation in Response to Moderate Muscle Contraction

To examine the impact of ischemia on muscular branch artery diameter, we measured functional vasodilation at day-14 following femoral artery-vein resection surgery, using the following stimulation parameters- 1mA, 200µs, 8Hz, 90sec. These stimulation parameters failed to elicit a functional vasodilation in the ischemic muscular branch artery, **Figure 8**.

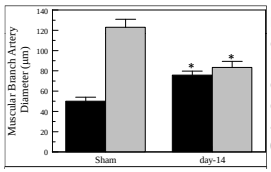


**Figure 8.** Muscular branch artery diameter at rest (black bar) and immediately following the cessation of moderate muscle contraction (grey bar), stimulated at 1mA, 200µs, 8Hz, 2min. * p < 0.05 vs sham.

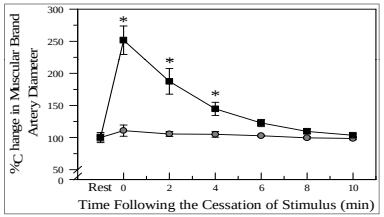


**Figure 9**. Functional vasodilation (% change from resting or 100%) of the ischemic muscular branch collateral artery with moderate intensity muscle contraction for sham (black) and day-14 ischemic (grey).

## Ischemia does not Affect Functional Vasodilation during Intense Muscle Contraction

To further examine the impact of ischemia on muscular branch artery diameter, we measured functional vasodilation at day-14 following femoral artery-vein resection surgery, using the following stimulation parameters- 1mA, 500µs, 8Hz, 90sec. The increased stimulus duration produces a more robust muscle contraction and presumably a greater stimulus for vasodilation. Functional vasodilation in ischemic arteries is not different from sham arteries in response to intense muscle contraction, **Figure 10**.

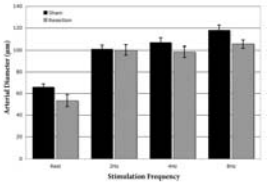


**Figure 10**. Muscular branch artery diameter at d14 following femoral artery resection in response to increasing stimulus frequency- 2, 4, or 8Hz and 1mA, 500µs, 90sec.

## Ischemia Impairs Vasoconstriction Following Intense Muscle Contraction

As seen in Figure 10, a more intense muscle contraction can cause a normal vasodilation in the ischemia muscular branch artery. However, vasoconstriction back to resting diameter seemed to be delayed in these studies. Therefore, we examined the time course of vessel diameter before and after the cessation of intense muscle contraction(1mA, 500µs, 8Hz, 90sec), **Figure 11**.

## Ischemia Impairs Vasoconstriction Follo Intense Muscle Contraction

Vasoconstriction back to resting diameter is significantly attenu the ischemic muscular branch arteries, **Figure 11**.

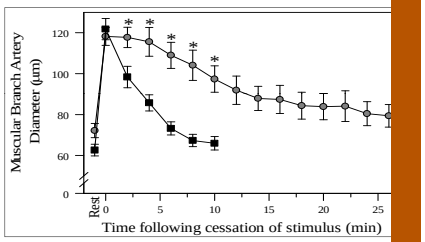


**Figure 11**. Functional vasodilation in the ischemic mus branch collateral artery following high intensity contrac Sham operated (black circles) and day-14 ischemic art (grey circles).

## Summary

- Ischemia eliminates functional vasodilation in response to mo intensity muscle contraction, **Figure 9**.
- Functional vasodilation is normal in the ischemic muscular bra artery if a more intense muscle contraction is induced, **Figure 1**
- Ischemic muscular branch arteries are refractory to vasoconstr back down to their resting diameter following the cessation of r contraction, **Figure 11**.

## Future Work

The ability of the ischemic muscular branch to vasodilate only i response to intense muscle contraction (**Figure 10**) and not mo muscle contraction (**Figure 9**) suggests that different vasodilatio pathways are operational in the ischemic artery, or that the typi pathways are less sensitive to dilating stimuli. Specifically, we hypothesize that endothelial-dependent vasodilation is impaire ischemic muscular branch artery while smooth muscle depende vasodilation is preserved. Therefore, upcoming studies will use superfusion intravital microscopy to examine cell specific vasod responses, such as those in **Figure 12**.

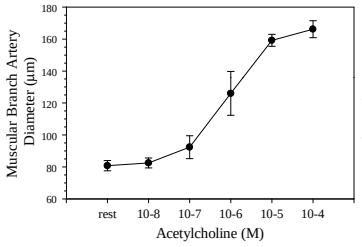


**Figure 12**. M branch arte diameter in animals in r to increasin of acetylcho The prepara was superfu with a PSS s bubbled wit $CO_2$.

Superfusion intravital microscopy will also be used to test the hypothesis that impaired vasoconstriction following intense mu contraction is due to reduced norepinephrine release by sympa neurons and/or reduced sensitivity of ischemic muscular branch to norepinephrine.

## Contact Information

tcardina@calpoly.edu

## Acknowledgements

Andrew Fuglevand, PhD; Steve Segal, PhD; Alex Moore, M