FUNCTIONAL VASODILATION AND VASCULAR REACTIVITY IN ARTERIOLAR COLLATERALS IN THE SPINOTRAPEZIUS OF MALES AND FEMALES

A Senior Project
Presented to
The Faculty of Biomedical Engineering Department
California Polytechnic State University,
San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

By
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August 2016

PROJECT INFORMATION

TITLE:	FUNCTIONAL VASODILATION AND VASCULAR REACTIVITY IN ARTERIOLAR COLLATERALS IN THE SPINOTRAPEZIUS OF MALES AND FEMALES
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DATE SUBMITTED:	AUGUST 2016
ADVISOR:	TREVOR CARDINAL, ASSOCIATE PROFESSOR

ABSTRACT

Functional Vasodilation and Vascular Reactivity in Arteriolar Collaterals in the Spinotrapezius of Males and Females

Britta Nelson

Peripheral arterial occlusive disease (PAOD) occurs when there is narrowing or blockage of the peripheral arteries that carry blood to the extremities, most commonly the legs. The most common symptom of PAOD is intermittent claudication, or ischemic pain during exercise. Women with PAOD experience a greater extremity functional impairment than men. Since impaired vasodilation might cause the ischemic pain from PAOD, we should evaluate vasodilation post ligation in males and females in collateral vessels, which connect two arterial segments to maintain blood flow to an otherwise hypoxic area. First, we need to examine collateral vasodilation in unoperated male and female animals. The goal of this study was to create a consistent protocol to measure functional vasodilation in collaterals of male and female C57Bl/6 mice, and to test the hypothesis that unoperated male and female C57Bl/6 mice exhibit equal vasodilation. The spinotrapezius muscle allows for clear visualization of intramuscular collaterals, which in animal models and patients will undergo arteriogenesis in response to an arterial occlusion. The muscle was stimulated using microelectrodes to induce endogenous vasodilation, and resting and dilated diameters were recorded. Diameters obtained from covering the preparation with plastic wrap and irrigating the preparation with a physiological salt solution (PSS) were also compared to determine the effect of the preparation of the muscle on vasodilation. As expected, there was no difference between the resting diameters or the dilated diameters of males and females when preparations were irrigated with PSS. Additionally, the preparation of the muscle had no effect on vasodilation. These findings suggest further investigation into vasodilation post ligation in males and females in collateral vessels.

Keywords: arteriogenesis, ischemia, peripheral arterial occlusive disease, vasodilation, spinotrapezius

ACKNOWLEDGMENTS

I would like to thank Dr. Trevor Cardinal for giving me the opportunity to work in his research lab and for his guidance and patience.

I would also like to thank the members of the Microcirculation and Vascular Regeneration Lab for their help throughout this project.

Many thanks to the Hannah Forbes Scholarship for funding this project.

Special thanks to Kenneth Gouin for training me on all procedures, and for being a fantastic mentor throughout this study.

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CHAPTER 1: Introduction

1.1 Clinical Relevance: Ischemic Disease

Ischemic disease is the major cause of mortality in Western civilization [1]. Ischemic disease develops when an atherosclerotic plaque prevents downstream tissue from receiving sufficient blood flow, causing tissue necrosis if the arteries become completely occluded (**Figure 1**) [2,3]. Peripheral arterial occlusive disease (PAOD) occurs when the atherosclerotic plaques affect the peripheral arteries that carry blood to the extremities, most commonly the legs [4]. The disease affects approximately 8 million people in the US, while the general population awareness of the disease is only around 25% [4]. Women with PAOD have a worse physical health status and health-related quality of life, and a greater lower extremity functional impairment compared with men [5,6].

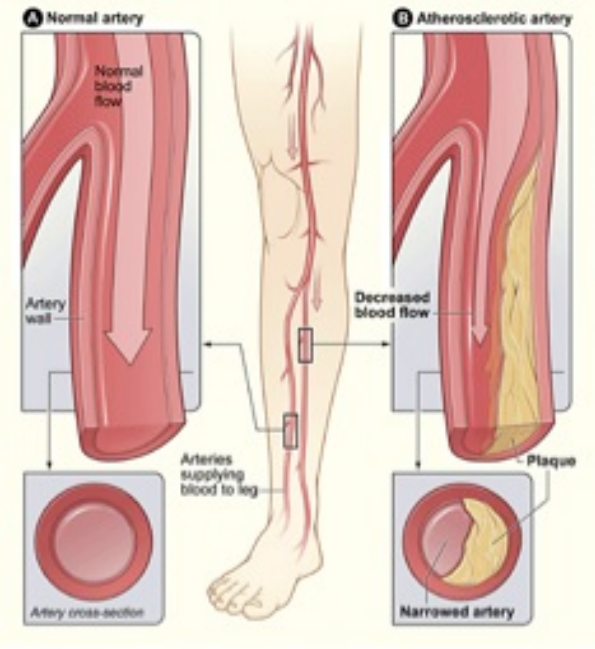


Figure 1. Peripheral Arterial Occlusive Disease. A normal artery compared to an atherosclerotic artery in a patient with PAOD [5].

1.2 Symptoms and Diagnosis of Peripheral Arterial Occlusive Disease

The most common symptom of PAOD is intermittent claudication, or ischemic pain during exercise, in the legs [4]. For example, patients may feel pain, numbness, or heaviness in the buttock, hip, thigh, or calf when walking or climbing the stairs [4]. The skin may appear cyanotic, and there may be ulcers on the toes, feet, or legs [2]. High risk factors for PAOD include smoking, high blood pressure, diabetes, high cholesterol, and age over 60 [4]. Patients who exhibit intermittent claudication are diagnosed using a non-invasive test called an ankle-brachial index, in which the systolic blood pressure in the tibial artery is compared with the systolic blood pressure in the brachial artery, before and after exercise [4,7]. A low ABI index (a comparison of the readings) is indicative of PAOD, meaning there is a lower blood pressure in the ankles than in the arms, caused by the narrowing of arteries and blockage of blood flow [8]. Women have a lower mean ABI and are more likely than men to have an ABI of less than 1.0 [9]. The extent of collateral networks is related to prognosis, which might be an explanation of why females are worse off than males [10].

1.3 Male and Female Differences in Vasodilation

There exist gender differences in vasodilation capabilities. Immediately following femoral artery ligation in mice, there is no difference in acetylcholine (Ach), a nitric oxide dependent vasodilator, induced reductions in hindlimb vascular resistance between males and females [10]. Females exhibited a greater reduction in vascular resistance than males after adding L-NAME, a nitric oxide synthase inhibitor [10]. Ten days post ligation, females exhibited less Ach induced vascular resistance than males, and adding

L-NAME inhibited Ach induced vascular resistances more in females than in males [10]. Ten days post ligation, females also exhibited less nitroglycerin, a smooth muscle cell dependent vasodilator, vascular resistance when compared to males [10]. This suggests that as collaterals remodel, males develop a more robust nitric oxide synthase vascular resistance than females [10]. Possible reasons of early dilatory impairment are impaired smooth muscle cell function, impaired endothelial cell function, or a combination of both [10]. At baseline, females have higher levels of VEFG and eNOS, but ten days post femoral artery ligation, males have higher levels of VEGF and eNOS [10]. The impaired recovery of flow of females is associated with multiple factors, including decreased collateral remodeling, ased vasoconstrictor activity [10].

Vasodilators are currently used as a therapy for peripheral arterial occlusive disease, such as cilostazol and pentoxifylline [11,12]. Pentoxifylline works by helping blood flow more easily through narrowed arteries, increasing blood flow and therefore oxygen levels to the muscles [12,13]. Cilostazol is an anticoagulant drug and a vasodilator that can be used to improve blood flow [11]. Men and women show no differences in the treatment effects of Cilostazol [6].

1.4 Previous Work

Vasodilation of collateral vessels can be used as an alternative treatment for patients who are not candidates for surgical intervention. Natural bypasses, or collateral vessels, connect two arteriolar segments [14]. Collaterals are the main site of resistance to blood flow in the ischemic zone, so it is important to study how each zone reacts to

occlusion and arteriogenesis [15]. C57Bl/6 mice have a high density of pre-existing collaterals (PECs) [16].

Collaterals consist of three zones: the reentry, close to the site of the occlusion, the stem at the distal aspect, where blood flow from another tree is entering, and the midzone, between the two regions. At rest, the net shear stress in collateral midzone is zero, but post occlusion, there is an increase in shear stress in the vessels (**Figure 4**) [17]. The amount of shear stress in the different zones potentially causes different zones to have different degrees of remodeling (ref). At rest, there is no net blood flow in the collateral midzone [14]. However, following an occlusion, there is an increase in the pressure gradient across the collaterals, which induces arteriogenesis - a shear stress-driven process in which PECs outwardly remodel to become conduit vessels, providing an alternate pathway for blood to travel around an occluded artery (**Figure 3**) [18]. When an atherosclerotic plaque blocks a vessel, there is a decrease in downstream pressure and flow is redirected through PECs, increasing the shear stress in the collaterals. The increased shear stress activates endothelial cells, which express cell-surface adhesion molecules and proinflammatory cytokines to recruit and activate monocytes to the vessel wall [19]. The extravasated macrophages secrete matrix metalloproteinases (MMPs) that degrade the extracellular matrix, vascular endothelial growth factor (VEGF) that causes endothelial proliferation, and platelet-derived growth factor (PDGF), which attracts smooth muscle cells [19,20]. As a result, vessels enlarge, resulting in a larger resting diameter (ref). This inhibits the increase in shear stress, halting arteriogenesis. It is imperative to visualize the entire collateral to understand how the different collateral zones are affected by occlusion and arteriogenesis.

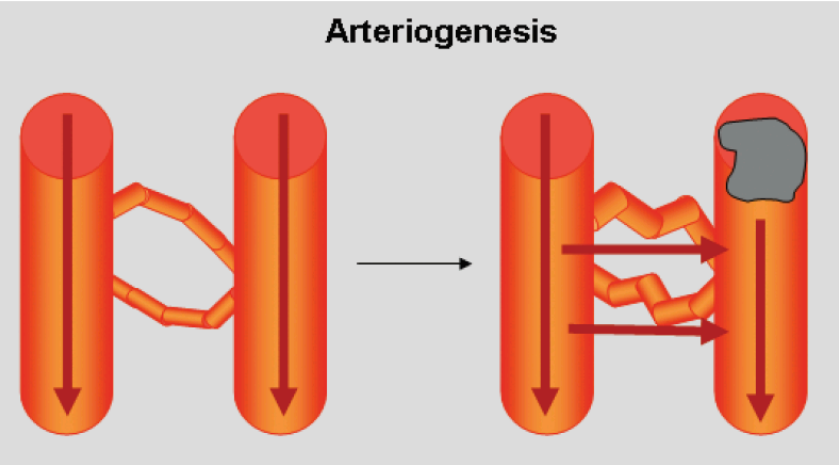


Figure 3. Arteriogenesis. Arteriogenesis occurs due to a decrease in downstream tissue following the occlusion of an artery. Blood flow is redirected through collaterals from the increased shear stress in the vessels [21].

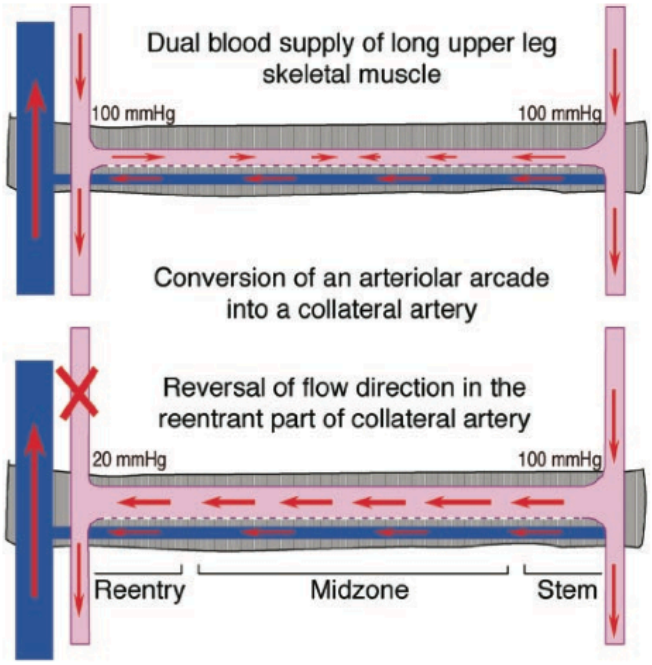


Figure 4. Collateral Blood Flow. Normal blood flow in a collateral compared to blood flow in a collateral post occlusion [17].

1.5 Purpose of Current Study

The purpose of this study was to assess vasodilation in PECs in the spinotrapezius of C57Bl/6 males and females. If vasodilation is used as a treatment for PAOD, we want to see if there are any differences in the dilatory capabilities of males and females. Unlike previous studies in the hindlimb, all zones of a complex collateral circulation can be readily visualized in the spinotrapezius. Therefore, we can study how each zone of the collateral circulation is affected by occlusion and arteriogenesis.

The specific aims of this study are as follows:

Specific Aim 1: Develop a protocol for consistent functional vasodilation in the spinotrapezius of C57Bl/6 males and females.

Corollary to Specific Aim 1: Test the hypothesis that there is no difference in resting and dilated vessel diameter measurements of female mice when irrigating the preparation with physiological salt solution and covering the preparation with plastic wrap.

Specific Aim 2: Test the hypothesis that females and males exhibit equal functional vasodilation.

CHAPTER 2: Methods

2.1 Animal Husbandry

All procedures for this study were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of California Polytechnic State University San Luis Obispo. Six male C57Bl/6 mice, with average weights of 23 ± 1 g, and six female C57Bl/6 mice with average weights of 18 ± 0.4 g, aged between 7-9 weeks were used in this study. Mice were housed in micro-isolator cages within a temperature-controlled room (69-73°F) in the University Vivarium. The room was monitored daily and maintained 12-hour light/dark cycles. Males and females were segregated by sex and each cage housed a maximum of four mice. Mice were provided with rodent chow, water, bedding, a plastic 'mouse house', a tunnel tube, and a chew toy.

2.2 Mouse Preparation

Each mouse was initially anesthetized in an induction chamber with 5% vaporized isoflurane in oxygen flowing at 0.8-1.2 $l \cdot min^{-1}$. The mice were weighed and transferred to a preparatory bench where anesthesia was maintained with 2-3% isoflurane at a flow rate of 0.8-1.2 $1 \cdot min^{-1}$. Trimming clippers and depilatory cream were used to remove the hair on the anterior dorsal aspect and the anterior ventral aspect of the mouse. The mice were then transferred to a heat pad on the surgical stage, in which core body temperature was maintained at 35°C via rectal thermister feedback. The left or right spinotrapezius was exposed by an initial skin incision at the caudal end of the muscle, followed by the removal of skin and connective tissue via blunt dissection; the contralateral side was then exposed, alternating between the left and right spinotrapezius for each procedure. The ipsilateral side was continually irrigated with physiological salt solution (PSS) containing

(in mM) 131.9 NaCl, 18 $NaHCO_3$, 4.7 KCL, 2 $CaCa_2$, and 1.17 MgSO4 to maintain a physiologic pH and temperature and ionic concentrations similar to the extracellular fluid to avoid creating any concentration gradients. Following exposure, the entire muscle was covered with plastic wrap, and the mouse was moved to supine position. An initial incision was made in the center of the chest, and identified, and after overlying connective tissue was removed by blunt dissection, was catheterized. The catheter lined with heparinized saline (1 mg/mL) was used to deliver 0.3mL of FITC-conjugated dextran (250,000 MW, Sigma Aldrich). 0.005 mL of the FITC dextran and heparinized saline solution was injected about every hour throughout the experiment.

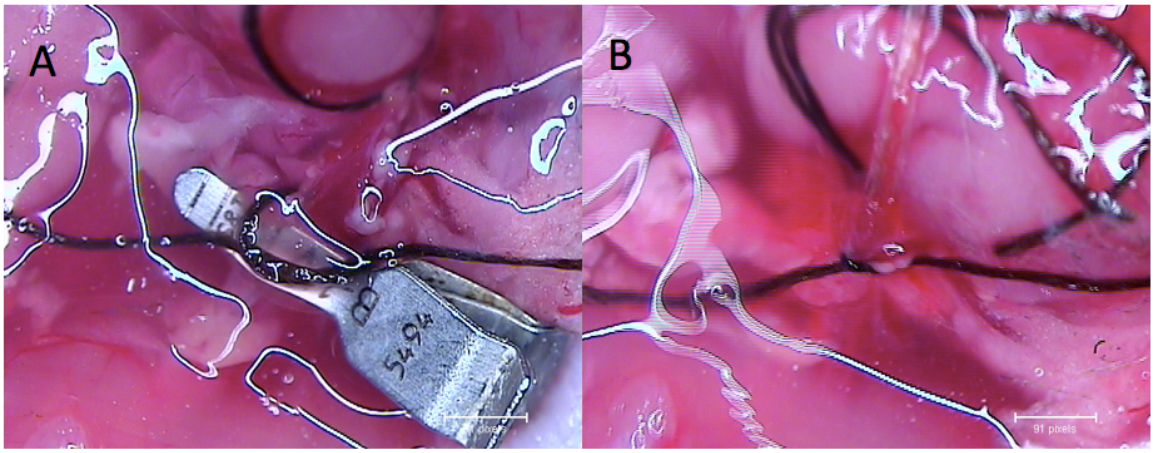
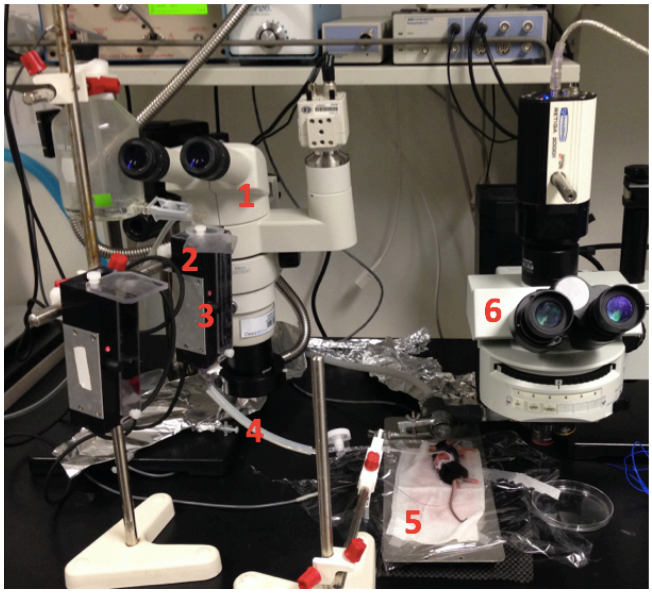


Figure 5. Catheter Placement. A) A silk suture is placed around the upstream portion of the jugular vein, and a clamp is placed around the downstream portion. B) The catheter is placed in the jugular vein and a suture is tied around it to hold it in place.

2.3 Preparation Irrigation



1. Dissecting microscope
2. Syringe heater
3. 60mL syringe
4. Tubing
5. Heat pad
6. Olympus BXFM

Figure 6. Superfusion Setup.

After the catheter was placed, the mouse was returned to the prone position. The plastic wrap was removed from the muscle. Collateral arterioles were visualized using an intravital microscope (Olympus BXFM 5x). Physiological salt solution was prepared fresh the day of each procedure from a stock solution, as described above. The PSS was bubbled with a 5% CO_2 - 95% N_2 mixture to deoxygenate the solution and maintain a pH of ~7.4 and flowed over the tissue at rate of ~2 $mL \cdot min^{-1}$ and a temperature of 35°C. The same procedure was followed for the contralateral muscle.

2.4 Functional Vasodilation

The spinotrapezius was exposed, as described above, and the overlying fascia was gently removed. Two tungsten microelectrodes were placed lateral to the spine at the caudal end of the muscle to induce contraction by electrical field stimulation. Proper

placement of the electrodes was determined with a brief set of 2mA square waves of 200 μs duration at 1 Hz (LabChart and a PowerLab Data Acquisition System, ADInstruments). Following electrode placement, the muscle was covered with plastic wrap to prevent desiccation, collateral arterioles were identified with the intravital microscope, and the preparation was given 30 minutes to equilibrate. After capturing images of the collaterals at rest, the muscle was stimulated for 90 seconds with a set of 2mA square waves of 200 μs duration at 8 Hz, and a second set of images were captured immediately following the stimulation cessation. The plastic wrap was removed from the contralateral muscle and the PSS was superfused over the muscle according to the specifications outlined in Section 2.4. The preparation was given 30 minutes to equilibrate. Images were captured at rest, and the muscle was stimulated as described above. A second set of images were captured immediately following the stimulation cessation. Upon completion of the experiment, the mouse was euthanized by cervical dislocation.

2.5 Imaging

Images were captured using an Olympus BXFM bright field microscope with a 5x objective. QCapture Pro Image and Analysis software was used to capture sequences of fifteen images.

2.6 Data Analysis

Vessel diameters were measured using ImageJ analysis software. The diameters before and after muscle stimulation were measured and the percent change was calculated

using the difference of the dilated and resting diameters divided by the resting diameter and multiplied by one hundred. Differences in resting and stimulated diameters of males and females, with PSS irrigation or plastic wrap were determined using a paired t-test. Differences in diameters of males and females, resting and dilated, were determined using an independent t-test. Differences in diameters of females, with PSS irrigation or plastic wrap, and differences in percent change of diameters, were determined using an independent t-test. A p-value of less than 0.05 was used to denote statistical significance. Data are expressed as mean ± standard error.

CHAPTER 3: Results

The goal of this study was to develop a protocol to measure functional vasodilation in the spinotrapezius collaterals of male and female C57Bl/6 mice. To accomplish this goal, we compared resting and dilated diameters of male and female C57Bl/6 mice, when the preparation was covered with plastic wrap and when it was irrigated with a physiological salt solution.

Four collateral measurements were taken in each muscle, in two collaterals per muscle. There was up to a 5 μm variability between different collaterals of the same branching order on different muscles of each sex. The anatomical variability of the collaterals was sufficiently small, meaning that similar collaterals could be measured in different animals.

As expected, there was no difference between the resting diameters of males and females when preparations were irrigated with PSS, 21 ± 2 μm and 19 ± 1 μm, respectively, (**Figure 7C**). Also as predicted, there was no difference between the dilated diameters of males and females when preparations were irrigated with PSS, 37 ± 2 μm and 33 ± 2 μm, respectively, (**Figure 7C**). Vasodilation in male mice when the preparation was irrigated with PSS involved an 81.4 ± 7.0 % above resting, which was not different from the female dilation of 77.9 ± 8.8 %, (**Figure 7D**).

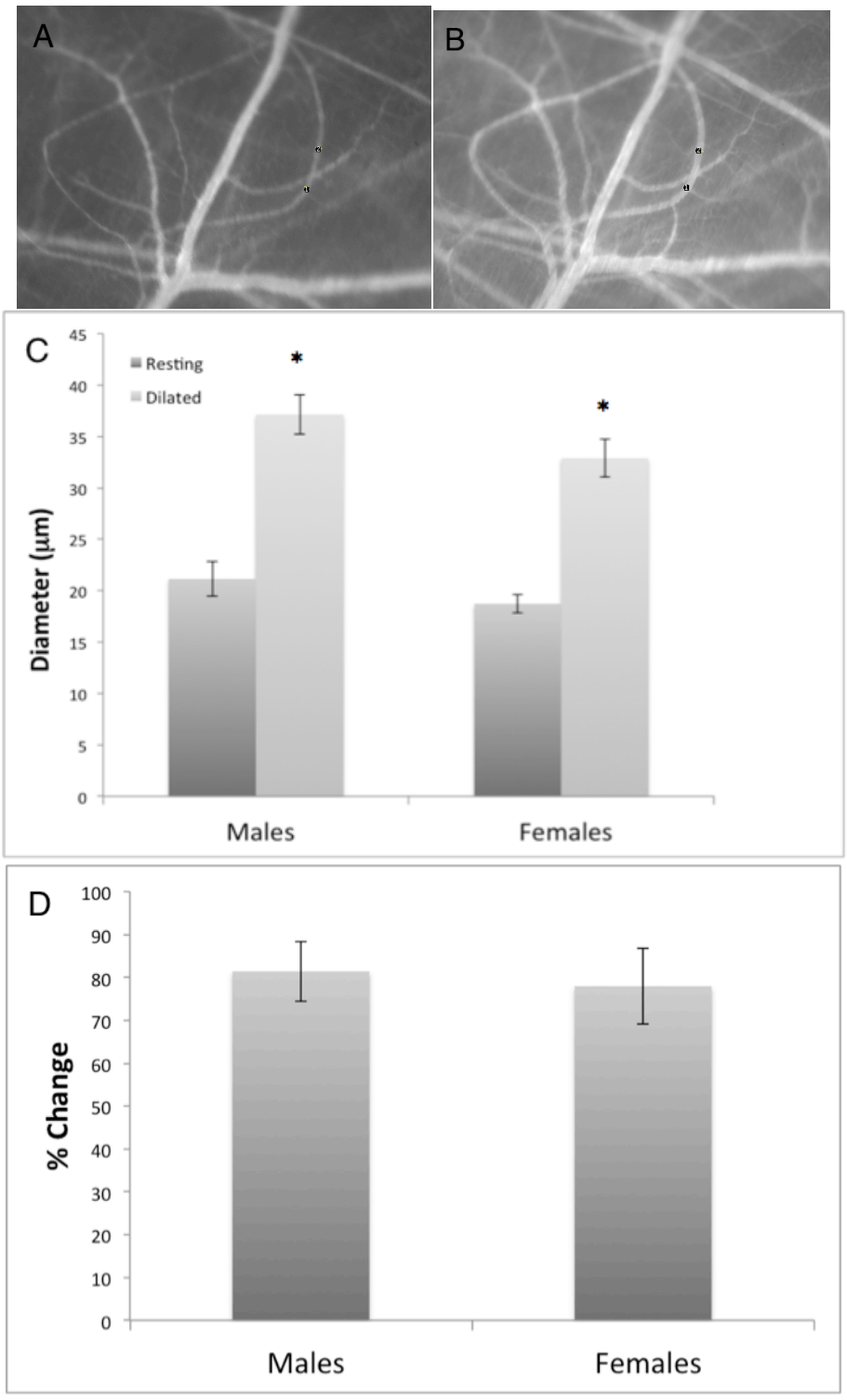


Figure 7. Spinotrapezius C57Bl/6 Collateral Diameters. Representative photomicrographs of resting (A) and dilated (B) collateral vessel measurements in ImageJ in the midzone. C) Diameters in microns pre and post muscle stimulation via electrodes with superfusion (n=8, *, $p<0.05$). D) Percent changes between collateral vessels pre and post muscle stimulation via electrodes (n=8, *, $p<0.05$).

To determine the effect of the preparation of the muscle on vasodilation, we performed functional vasodilation after covering the preparation with plastic wrap, and irrigating the preparation with PSS. There was no difference between the resting diameters of females when the preparation was superfused with PSS versus covering the preparation with plastic wrap, 19 ± 1 μm and 19 ± 1 μm, respectively (**Figure 8A**). Similarly, there was no difference between the dilated diameters of females when the preparation was superfused with PSS versus covering the preparation with plastic wrap, 33 ± 2 μm and 31 ± 1 μm, respectively, (**Figure 8A**). Vasodilation in female mice when the preparation was superfused with PSS involved a 77.9 ± 8.8 % above resting. This was not different from the female dilation when plastic wrap covered the preparation 65.7 ± 7.7% (**Figure 8B**).

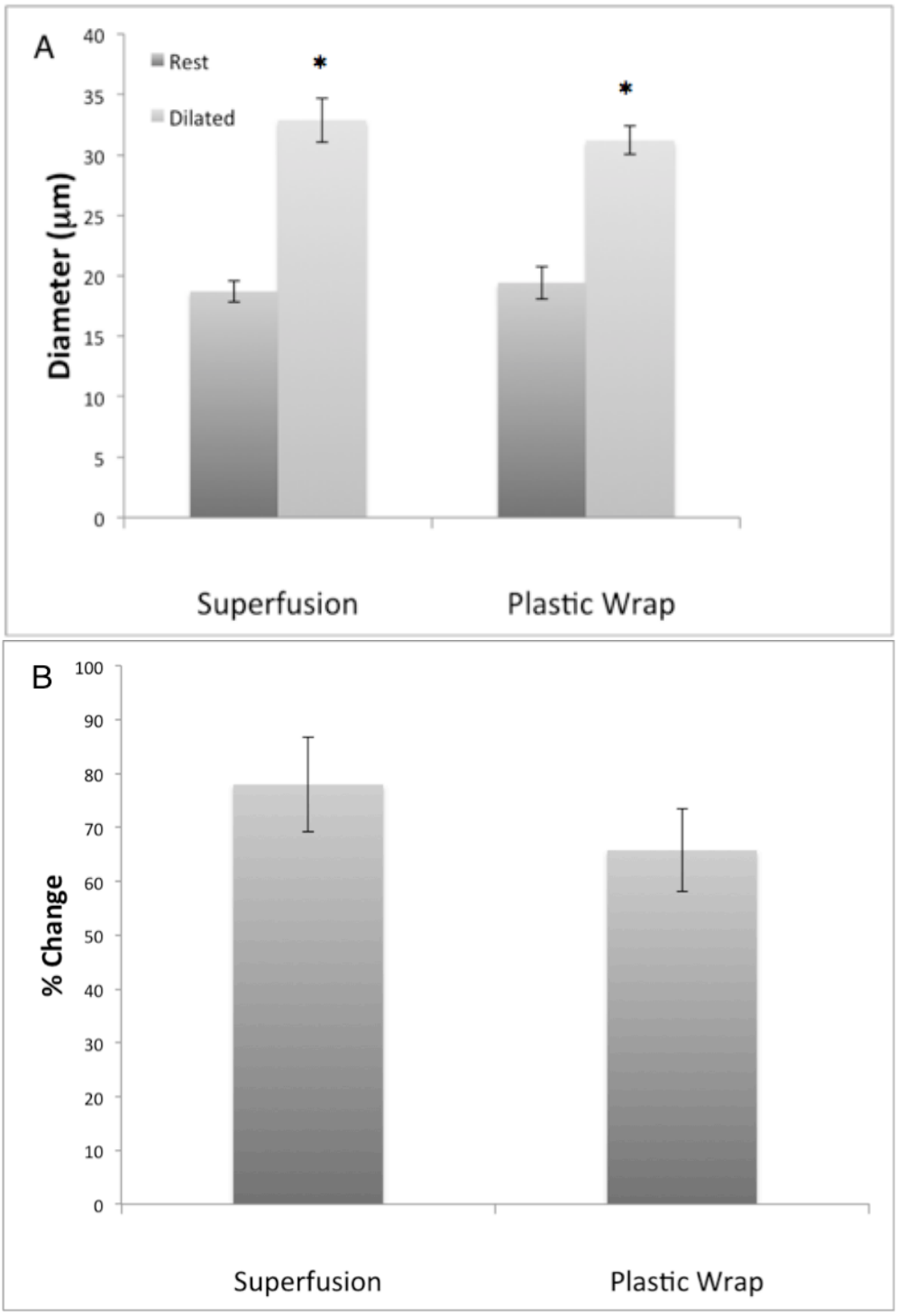


Figure 8. Female Spinotrapezius Collateral Diameters. A) Diameters in microns pre and post muscle stimulation via electrodes with superfusion and plastic wrap (n=8, *, $p<0.05$). B) Percent changes between collateral vessels pre and post muscle stimulation via electrodes with superfusion and plastic wrap (n=8, *, $p<0.05$).

CHAPTER 4: Discussion

Patients with PAOD experience intermittent claudication that is caused by the blockage of an artery, preventing downstream tissue from receiving sufficient blood flow. Collateral vessels act as natural bypasses to provide oxygen to tissue that would otherwise be hypoxic. Collateral circulation is the primary site of resistance to blood flow downstream. Impaired vasodilation of the collaterals might explain intermittent claudication.

Vasodilation occurred in collaterals of males and females. There was no difference between the diameters of male and female arterioles at rest or dilated, or in the percent change. These findings are comparable to those of an isolated vessel preparation of gracilis muscle arterioles of male and female wild type mice, where unoperated males and females exhibited comparable vasodilation [22]. The change in diameter of females was 5-7% less than that of males as pulsatile flow increased, which is comparable to the 3% difference in the change in diameter between males in females in this study [22].

PSS was superfused over the muscle to maintain physiologic conditions of the muscle. Superfusion is advantageous because it is an endogenous method of covering the muscle preparation. However, the setup of superfusion is complicated, and requires the user to maintain an end temperature of 35°C on the muscle. PSS is heated to 50°C in a volumetric flask and poured into a heated 60 mL syringe. This caused a change in temperature of the PSS in the 60 mL syringe, causing the temperature of the tubing to fluctuate throughout the experiment, yielding a temperature of 33°C - 37°C on the muscle. This could have led to inaccuracies in diameter measurements. There was a difference between the resting and dilated diameters of females when preparations were

covered with plastic wrap. There was no difference between diameters or percent change of females when preparations were covered with plastic wrap or irrigated with PSS. Using plastic wrap to cover the muscle during functional vasodilation is a reliable method to prevent tissue desiccation.

The resting and dilated diameters of males, 21 ± 2 μm and 37 ± 2 μm, and of females, 19 ± 1 μm and 33 ± 2 μm, when the preparation was superfused with PSS are similar to those of other intravital studies of functional vasodilation. Second order arterioles in the gluteus maximus muscle of unoperated male C57Bl/6 mice dilate from 20 μm to 42 μm in response to a rhythmic contraction via electrodes at 2 Hz and 8 Hz in a randomized order for 30 seconds [23]. Although the muscles are of different sizes, both are skeletal muscles that contain arterioles of similar sizes that exhibit comparable vasodilation [23].

Future work to expand this study could include comparing the baseline measurements of resting and dilated diameters to measurements following the ligation of a feed artery in male and female C57Bl/6 mice. This comparison would investigate the dilatory capabilities pre-existing collaterals in males and females post-occlusion, to determine when the vessels are able to dilate, restoring blood flow control to the ischemic area of patients with PAOD. Further, a study could be done comparing the dilatory capabilities post-occlusion of pre-existing collaterals in C57/Bl6 mice to newly formed arterialized capillaries in BALB/c mice, to investigate any differences in their dilatory capabilities. This is important to study because some patients have pre-existing collaterals while others do not.

Vasodilation of collateral vessels could be an effective treatment for patients with PAOD. It is important to investigate any differences in the dilatory capabilities of males and females to provide a better understanding of male and female differences in the prognosis of PAOD.

REFERENCES

1. "Heart and Stroke Association Statistics: 2015 Update." American Heart Association. Web. 5 Mar. 2015.

2. "Peripheral Arterial Disease." *Medline Plus*. U.S. National Library of Medicine. Web. 20 Mar. 2015.

3. "Critical Limb Ischemia (CLI)." *UC Davis Vascular Center*. UC Davis. Web. 26 Mar. 2015.

4. "Peripheral Arterial Disease (PAD) Fact Sheet." *Divison for Heart Disease and Stroke Prevention*. Centers for Disease Control and Prevention, 22 July 2014. Web. 10 Mar. 2015.

5. Dreyer, R. P., van Zitteren, M., Beltrame, J. F., Fitridge, R., Denollet, J., Vriens, P. W. "Gender Differences in Health Status and Adverse Outcomes Among Patients With Peripheral Arterial Disease." Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease, *4*(1) (2015). Print.

6. Hirsch, Alan, Matthew Allison, and Antoinette Gomes. "A Call to Action: Women and Peripheral Artery Disease." *Circulation*. American Heart Association. Web.

7. "Ankle Brachial Index." Stanford Medicine. Web. 17 Mar. 2015.

8. Khan, Tahir, Falahat Farooqui, and Khusrow Niazi. "Critical Review of the Ankle Brachial Index." *Current Cardiology Reviews*. Bentham Science Publishers Ltd. Web. 13 Mar. 2015.

9. Hiramoto, Jade S. et al. "Gender-Specific Risk Factors for Peripheral Artery Disease in a Voluntary Screening Population." *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease* 3.2 (2014): e000651. *PMC*. Web. 24 May 2016.

10. Peng, XinZhi, Wang, Jinsong, Lausanne-Soares, Roberta M., Najafi, Amir H., Sood, Subeena, Aghili, Nima, Alderman, Lee O., Panza Julio A., Faber, James E., Wang, ShenMang, Epstein, Stephen E., Burnett, Mary Susan. "Gender Differences Affect Blood Flow Recovery in a Mouse Model of Hindlimb Ischemia." Heart and Circulatory Physiology 200.6 (2011): H2027–H2034. Print.

11. "Cilostazol." *Medline Plus*. U.S. National Library of Medicine, 1 Oct. 2010. Web. 27 Mar. 2015.

12. "Pentoxifylline." *Medline Plus*. U.S. National Library of Medicine, 1 Sept. 2010. Web. 27 Mar. 2015.

13. "Peripheral Artery Disease - Legs." *Health Guide*. The New York Times, 14 May 2012. Web. 26 Mar. 2015.

14. Klabunde, Richard E. "Collateral Blood Flow." *CV Physiology:*. Web. 5 Mar. 2015.

15. Silverthorn, Dee Unglaub. Human Physiology: An Integrated Approach. 6th ed. Pearson, 2012. Print.

16. Hellstrom, Sara. "Vascular Reactivity of Immature Arterialized Capillaries." (2013). Web.

17. "Factors Regulating Arteriogenesis." Arteriosclerosis, Thrombosis, and Vascular Biology 23 (2003): 1143-151. Print.

18. Deindl, Elisabeth, and Wolfgang Schaper. "The Art of Arteriogenesis." Cell Biochemistry and Biophysics 23.1 (2005): 1-15. Springer. Print.

19. "Macrophage Activation." Macrophage Activation. R&D Systems. Web.

20. Silverthorn, Dee Unglaub. Human Physiology: An Integrated Approach. 6th ed. Pearson, 2012. Print.

21. Schaper, W., M. Hein, I. Eitenmuller, and T. Schmitz-Rixen. "Arteriogenesis versus Angiogenesis: Similarities and Differences." Journal of Cellular and Molecular Medicine 10.1 (2006): 45-55. Pub Med. Print.

22. Qin, Jun, Starath Khan, and Ghezal Froogh. "Sexually Dimorphic Phenotype of Arteriolar Responsiveness to Shear Stress in Soluble Epoxide Hydrolase-knockout Mice." *American Physiological Society* 309.11 (n.d.): H1860-1866. *PubMed*. Print.

23. Novielli, N. M. and Jackson, D. N. “Contraction-evoked vasodilation and functional hyperaemia are compromised in branching skeletal muscle arterioles of young pre-diabetic mice.” Acta Physiologica, 211 (2014): 371–384. Print.

CHAPTER 5: Apendices

Date_____ **Functional Vasodilation with Superfusion** Initials _____

Mouse Information

DOB: _____
Sex: _____
Tag: _____
Genotype/Strain: _____
Cage: _____
Weight: _____

Materials

____1. Standard pattern forceps
____2. 545s
____3. Iris scissors
____4. Microdissection scissors
____5. S&Ts (2)
____6. Volumetric flask
____7. Kim wipes
____8. 20x PSS
____9. 20x $NaHCO_3$
____10. Bubbler
____11. 60mL syringe
____12. Catheter
____13. FITC Dextran
____14. Vascular clamp
____15. Tungsten microelectrodes
____16. Plastic wrap

Equipment Preparation

____17. Turn on ultrasonic bath to 50°C
____18. Transfer 50mL of 20x PSS into 1 L volumetric flask
____19. Transfer50mL of 20x $NaHCO_3$ into 1L volumetric flask
____20. Dilute PSS & $NaHCO_3$ to 1L with 18MΩ H_20
____21. Place 1L volumetric flask in water bath
____22. Fill syringe in syringe heater with 50mL of 1x PSS & turn on syringe heater
____23. Place thermistor in syringe heater

Animal Preparation

____24. Weigh animal in weigh boat
____25. Place animal in anesthesia box
____26. Open the oxygen cylinder and set anesthesia machine flow meter to 0.8-1.2 $l \cdot min^{-1}$.
____27. Anesthetize animal with 5% isoflurane
____28. Reduce flow rate to 0.8-1.2 $l \cdot min^{-1}$. and the isoflurane to 2-3%
____29. Lay animal supine on preparation bench with nose in nose cone
____30. Use trimming clippers & depilatory cream to remove on anterial dorsal and anterior ventral aspects
____31. Transfer mouse to heat pad
____32. Insert rectal probe and set thermo-controller to 35°C

Protocol

____33. Make 1cm incision at caudal end of spinotrapezius
____34. Extend incision cranially to the fat pad
____35. Blunt dissect connective tissue
____36. Cover muscles with plastic wrap
____37. Move animal to supine position
____38. Make incision in center of chest and extend
____39. Identify jugular vein
____40. Tie silk suture around jugular vein upstream
____41. Place vascular clam around jugular vein downstream
____42. Poke hole to insert catheter between suture and clamp
____43. Deliver hepanarized saline and FITC dextran
____44. Flip mouse to supine position
____45. Open stockcock on delivery tubing to check flow rate of superfusion solution and use thermistor to measure temperature
____46. Adjust flow rate or temperature to achieve ~2mL·min^{-1} and 35°C at tip
____47. Place electrodes as close together as possible in clay and place electrodes lateral to the spine, at the caudal end of the muscle
____48. Stimulate the muscle at frequency 1 Hz, duration 200 us, and 2 mA to ensure electrode placement stimulates the muscle
____49. Place saran wrap over the exposed muscle
____50. Allow a 30 minute time period to pass between test stimulation and taking the first measurement
____51. Take rest measurements with BFXM
____52. Set frequency to 8 Hz and keep other settings the same
____53. Stimulate the muscle for 90 seconds
____54. Immediately take dilated measurements
____55. Perform the same procedure on the control limb
____56. Cervical dislocation to euthanize animal