SEX DIFFERENCES AND THE EFFECTS OF EXERCISE TRAINING ON FUNCTIONAL VASODILATION
FOLLOWING ARTERIAL OCCLUSION IN THE BALB/C MOUSE SPINOTRAPEZIUS

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Britta Caroline Nelson
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COMMITTEE MEMBERSHIP

TITLE: Sex Differences and the Effects of Exercise Training on Functional Vasodilation Following Arterial Occlusion in the BALB/c Mouse Spinotrapezius

AUTHOR: Britta Caroline Nelson

DATE SUBMITTED: September 2017

COMMITTEE CHAIR: Trevor Cardinal, Ph.D.
Associate Professor of Biomedical Engineering

COMMITTEE MEMBER: Lily Laiho, Ph.D.
Associate Professor of Biomedical Engineering

COMMITTEE MEMBER: Jason Blank, Ph.D.
Associate Professor of Biology

ABSTRACT

Sex Differences and the Effects of Exercise Training on Functional Vasodilation Following Arterial Occlusion in the BALB/c Mouse Spinotrapezius

Britta Caroline Nelson

Peripheral arterial occlusive disease (PAOD) often presents as intermittent claudication, which may be caused by impaired vasodilation. Impairment of resistance vessels may contribute to the pathogenesis of PAOD, and explain the poor correlation between resting blood flow and limb function. Collateral function following arterial occlusion is not well defined, however collaterals and arterialized collateral capillaries (ACCs) in male and female animal models exhibit impaired vasodilation following arterial occlusion, which can potentially be improved with exercise training. Furthermore, resistance vessels in the ischemic tree and stem are likely involved in the pathogenesis of PAOD, however the relative importance of each is unknown. Therefore, we measured functional vasodilation in pre-existing collaterals, ACCs, the ischemic tree, and the stem region, 7 and 21-days following spinotrapezius feed artery ligation in male and female BALB/c mice, and with exercise therapy. Vasodilation in ACCs was more impaired in female mice than in males. Generally, vasodilation was impaired at day-7, likely due to impaired endothelium-dependent and smooth muscle-dependent vasodilation in maturing collaterals, and recovered by day-21. Exercise training appears to enhance collateral reactivity, more in ACCs in males than in females, suggesting that its therapeutic benefits are linked not only to structural adaptation but also to vessel functionality. Therefore, future research is required to determine the cause of sex differences in exercise therapy to treat peripheral arterial occlusive disease.

Keywords: collateral circulation, ischemia, exercise training, vasodilation, spinotrapezius, sex differences, stem region, arterialized collateral capillaries, pre-existing collaterals

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“It always seems impossible until its done” – Nelson Mandela

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Introduction

Peripheral arterial occlusive disease (PAOD) most commonly presents as intermittent claudication, or ischemic pain during exercise [1,2]. Intermittent claudication is thought to be due to a mismatch between oxygen demand and supply that results from reduced perfusion pressure due to atherosclerotic plaque(s) that obstructs the arteries supplying the lower extremities [1]. However, the ankle-brachial index (ABI) (a diagnostic test for PAOD that compares arterial pressure in the arm and the ankle, and a measure of perfusion pressure) is not associated with pain-free walking distance [3,4,5], and over half of patients with PAOD have no symptoms at all [2]. Furthermore, an increase in resting calf blood flow has only a slight association with pain-free walking distance or time, suggesting that the pathophysiology of intermittent claudication is more complex than insufficient resting blood flow [5]. Impairment of resistance vessels, which dilate in response to exercise to allow a greater volume of blood flow to meet the increased metabolic demand, may contribute to the pathogenesis of PAOD, and explain the poor correlation between resting blood flow and limb function. Vasodilation is impaired in the upper [6] and lower [7] extremities in patients with PAOD, which appears to limit the effectiveness of the collateral circulation when metabolic demand is increased [8,9,10]. This suggests that impaired vasodilation of collateral vessels may be involved in the pathophysiology of PAOD, and may contribute to intermittent claudication [5]. Vasodilation is also impaired in immature collaterals in animal models [11], but restored after maturation [11,12,13]. At day-7 following spinotrapezius feed artery ligation, pre-existing collaterals (PECs, large arterioles that anastomose (i.e. connect) two different first or second-order arterioles and enlarge to serve as a natural bypass for blood flow following an arterial occlusion) have impaired endothelial-independent and endothelial-dependent dilation, and no impairment at day-21 [12]. Because the impairment is temporary, it is likely due to the immaturity of vascular smooth muscle cells (SMCs) during the outward remodeling phase because the cells need time to arrange and mature into contractile SMCs, which likely impairs their ability to effectively relax and contract [12]. Estrogen also has an effect on SMC functionality, however sex differences in collateral functional vasodilation following arterial occlusion are unknown [14].

Therefore, studying collateral functional vasodilation following arterial occlusion will allow us to interrogate the impact of arteriogenesis on functional hyperemia [12].

Even if their vasodilation is impaired, collaterals function as an important bypass following arterial occlusion to maintain tissue viability [2,9]. However, some patients with cardiovascular disease never develop collaterals, and only 33% of patients have angiographic evidence of coronary collaterals at the onset of acute myocardial infarction [14,15]. In mice with fewer or absent collaterals, bypass collateral connections form in response to arterial occlusion via collateral capillary arteriogenesis (CCA) to normalize shear stress across the collateral capillary bed [11,16]. Arterialized collateral capillaries (ACCs) reperfuse the downstream ischemic tissue in 2-7 days following feed artery ligation, and represent a possible therapeutic target for patients with PAOD who never develop arteriolar collaterals [17]. First, it is necessary to identify when vasodilation is restored in ACCs at to determine when ACCs are capable of maintaining normal tissue function. Seven-days following spinotrapezius feed artery ligation, vasodilation in ACCs is impaired, probably due to vascular smooth muscle cell dysfunction [12,16]. By day-21, ACCs regain the capacity to dilate in response to muscle contraction, and utilize similar vasodilation pathways as control vessels [12]. Even if ACCs may be a possible therapeutic target for patients with PAOD, sex differences in vasodilation capabilities of ACCs may exist. The pathophysiology of PAOD is dependent on sex, and women have worse prognosis than men, so it is likely that sex differences in ACC behavior exist. However, sex differences in vasodilation capabilities in ACCs have not been studied [4,23].

Although the collateral circulation is necessary to perfuse tissue downstream of a vascular occlusion and maintain viability [20], it is not sufficient to maintain normal tissue function [21]. For example, *Rac2^{-/-}* and *Nox2^{-/-}* mice have impaired hind paw perfusion following femoral artery ligation, yet surprisingly, no impairment in collateral enlargement [21]. This suggests that vascular resistance in the ischemic microcirculation is a major contributor to perfusion of the ischemic tree (the vascular tree directly downstream of the occlusion site) [21]. Similarly, despite improvements in muscle blood flow and perfusion pressure, endothelial-dependent vasodilation is impaired in the microcirculation of ischemic muscles in rat skeletal muscles

[19,20]. Despite the benefits of collaterals, a reduced vasodilation in the ischemic zone is problematic following arterial occlusion. Clearly both collateral vasodilation and ischemic tree vasodilation could be involved in the pathogenesis of PAOD, but the relative importance of each is unclear. Characterizing both collateral and ischemic tree reactivity in the same preparation would allow for future work evaluating the relative contribution of each to tissue blood flow and function following arterial occlusion.

In addition to differences in collateral formation, the pathophysiology of PAOD is also dependent on sex [24]. There is a similar prevalence of PAOD among men and women, but women with PAOD have a worse health status and a greater near-term mortality rate compared with men [4,23]. For example, there is a higher prevalence of critical limb ischemia, the most severe form of PAOD, among women [24,25]. Also, if the diagnosis of PAOD is based on ABI value alone, more women have PAOD than men, and women are more likely to have asymptomatic PAOD than men, potentially going undiagnosed [25]. In animal models, females have less blood flow recovery, and a higher vascular resistance in the hindlimb following arterial ligation, which could be a result of impaired revascularization or decreased vasodilation [27]. In general, there is a lack of scientific studies on collaterals in female mice, and studying vasodilation in collateral vessels of female animal models may explain the sexual dimorphism in PAOD [27].

Although the pathogenesis of PAOD is unclear, there is abundant evidence that exercise therapy is one of the best treatment strategies [28]. Therefore, to ease symptom severity and help patients with PAOD resume a normal lifestyle, exercise therapy, typically administered as a single oral recommendation without any supervision or follow up, is included in the guidelines for management of PAOD, but patient adherence is poor [2,27]. Specifically, up to a third of patients do not follow the recommended intensity, frequency, or duration of supervised training programs, limiting their effectiveness [2]. Compounding the adherence problem is the fact that the benefits of exercise therapy are probably due to multiple, poorly-understood mechanisms, including reducing arterial obstruction, reducing endothelial dysfunction, and

shortening the time to onset of inflammatory activation [5]. Clinical attempts have been made to enhance arteriogenesis, such as the delivery of growth factors such as VEGF and FGF that increase collateral blood flow following occlusion [29], and cell transplantation to the ischemic zone to produce the growth factors necessary for collateral enlargement [30]. While these clinical attempts increase collateral blood flow both pre- and post-occlusion, it is unclear whether this is due to enhanced arteriogenesis or improved vasodilation [2,28]. Regardless of the mechanism of action in collaterals, arteriolar reactivity is restored in ischemic muscles after mild, intermittent stimulation [32]. Furthermore, long-term supervised exercise training increases pain-free walking distance without increasing ABI or number of arteries, suggesting that the reduction of intermittent claudication is not a result of improved collateralization [33]. Because vasodilation is transiently impaired during arteriogenesis [17] and impaired vasodilation may contribute to intermittent claudication, improving vasodilation may be a more sensible treatment for PAOD than enhancing arteriogenesis. The benefits of exercise therapy in improving intermittent claudication symptoms in male patients with PAOD are well-defined, but there is a lack of data concerning female patients, as females represent a small portion of participants in existing exercise rehabilitation studies for PAOD [34]. However, based on these studies, women are less responsive to exercise training than men, and have a lower absolute claudication distance (walking distance until pain threshold is reached) following exercise therapy than men [2,34]. However, exercise generally improves claudication symptoms in both sexes, as absolute claudication distance increased by 264% in men and 209% in women following 12-months of exercise therapy [35]. Further comparison is necessary to determine sex differences in vasodilation following ischemia and exercise training, to determine if sex differences in IC and response to exercise therapy might be explained by differences in vasodilation. Since exercise is a known effective treatment for PAOD, inducing functional vasodilation will allow us to interrogate vasodilation in multiple vessel types within a single preparation following exercise treatment to determine the therapeutic effects of exercise training along the vascular tree [2,27]. If exercise therapy improves functional vasodilation in animal models, then the mechanism of exercise therapy may include improved vasodilation.

Therefore, we tested the hypothesis that following arterial occlusion male BALB/c mice have

impaired vasodilation, and female BALB/c mice have a greater impairment in vasodilation, by measuring functional vasodilation in ACCs, PECs, the ischemic tree, and the stem region, 7 and 21-days following spinotrapezius feed artery ligation. Additionally, we introduced voluntary exercise to test the hypothesis that exercise accelerates the recovery of functional vasodilation in male and female BALB/c mice at day-7.

Methods

Animal Husbandry

All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of California Polytechnic State University. Male ($n = 28$) and female ($n = 24$) BALB/c mice (The Jackson Labs), aged 7-9 weeks, and weighing $26 \pm 0.4\text{g}$ and $20 \pm 0.3\text{g}$, respectively, were used for these studies. Mice were housed in micro-isolator cages within a temperature-controlled room (20-23 C) in the University Vivarium. The room was inspected daily and maintained on 12-hour light/dark cycles. Males and females were segregated by sex, and each cage housed a maximum of four mice and was provided with rodent feed, water, and enrichment (bedding, a plastic 'mouse house', and a plastic tube). Mice receiving voluntary exercise therapy were provided with a mouse running wheel with a Hall-effect sensor to record wheel revolutions (Model # 0297-0521,0501, Columbus Instruments).

Spinotrapezius Ligation

Mice were anesthetized in an induction chamber with 5% vaporized isoflurane in oxygen flowing at $0.8\text{-}1.2\text{ l}\cdot\text{min}^{-1}$, weighed, and transferred to a preparatory bench where anesthesia was maintained with 2-3% isoflurane at a flow rate of $0.8\text{-}1.2\text{ l}\cdot\text{min}^{-1}$. Prior to skin disinfection, trimming clippers and depilatory cream were used to remove the hair bilaterally on the dorsal aspect of the mouse on the spinotrapezius muscle. Mice were then transferred to a heating pad on the surgical stage, where core body temperature was maintained at 35 C with rectal-thermistor feedback (Model TC-1000 Temperature Controller, CWE Inc.). An initial incision was made above the lateral, cranial edge of the left or right spinotrapezius muscle (alternating order), and the superficial connective tissue was blunt dissected. The dorsal fat pad was blunt dissected from the spinotrapezius muscle, and the lateral feed artery was identified on the lateral edge of the spinotrapezius muscle. The artery was separated from its paired vein and ligated with two individual strands from 6-0 silk suture; the feed artery was transected between the ligations. The skin incision was closed with 7-0 polypropylene suture. A sham surgery was performed on the contralateral muscle involved blunt dissecting the fat pad from the

spinothrapezius and locating the artery and vein pair, but not separating or ligating the feed artery.

Voluntary Exercise

Mice in the exercise therapy group were placed in cages containing four mice following spinothrapezius ligation. Each cage received a mouse exercise wheel with a magnetic indicator and a Hall-effect sensor that output to software to record wheel revolutions (Model number 0297-0050,0051, Columbus Instruments). Wheel revolutions were recorded hourly and grouped by 12-hour light/dark cycles. Wheel revolutions were then converted to running distance in kilometers, using the following equation:

$$\text{Running distance} = \# \text{ of revolutions} * \text{wheel circumference (km)}$$

Functional Vasodilation

The left or right spinothrapezius (alternating order) was exposed by an initial skin incision at the caudal end of the muscle, followed by removal of the skin, then removal of superficial fascia and connective tissue via blunt dissection; the contralateral side was then exposed. Following exposure, the entire muscle was covered with plastic wrap, and the mouse was moved into a supine position. Following a skin incision on the ventral neck, 100 μ l of FITC-conjugated dextran (12.5mg/mL, 250,000 MW, 490 nm excitation, 520 nm emission, Sigma Aldrich) was injected in the jugular vein for contrast. The mouse was returned to the prone position, and two tungsten microelectrodes were placed lateral to the spine at the caudal end of the spinothrapezius 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 μ s duration at 1 Hz (LabChart and a PowerLab Data Acquisition System, ADInstruments). Following a 30-minute equilibration period, vessels were imaged using an intravital microscope (Olympus BXFM) and a Retiga 2000R mono camera (QImaging). Images were captured (QCapture Pro Image and Analysis) from the intravital fluorescence microscope with 5x and 10x objectives. The muscle was stimulated for 90 seconds with a set of 2mA square waves of 200 μ s duration at 8 Hz, and a second set of

images were captured immediately following stimulation. The same procedure was repeated for the contralateral muscle. After vasodilation measurements, mice were euthanized by cervical dislocation while anesthetized.

Data Analysis

Vessel diameters before and after muscle stimulation were measured using Image/J and the percent change was calculated. To minimize user error in Image/J measurements, each diameter was measured three times in Image/J and averaged, and the average value was recorded. Nine females and 13 male Balb/C mice used in this study had preexisting collaterals; all of these females and 9 males also developed ACCs. PECs exhibited similar dilations when ACCs were or were not present; ACCs exhibited similar dilatory behavior when PECs were or were not present. Therefore, ACC and PEC diameters from mice with both vascular structures (i.e. PECs and ACCs, n = 18) or only 1 (i.e. PECs, n = 4 or ACCs, n = 28) were analyzed irrespective of mouse anatomical variability. Differences between resting and dilated diameters were assessed using paired t-tests. Percent changes in diameter were calculated as the difference between the dilated and resting diameters divided by the resting diameter and multiplied by 100%. Differences in means of ACCs, PECs, ischemic tree, and stem tree measurements based on time point (i.e. day-7 or day 21), sex, vessel order, ligation status (i.e. operated or sham-operated), and vessel status (i.e. resting or dilated), were determined by ANOVA, Welch, and Wilcoxon tests. ANOVA tests were used for normal data with equal variances, utilizing Shapiro Wilk normality tests and F-tests for equal variances. Welch tests were used for normal data with unequal variances, and Wilcoxon tests with Levene's tests for equal variances were used for data that violated the normality test. Data are expressed as mean $\pm$ standard error.

Results

To better understand the effects of arterial obstruction on vasodilation in male and female mice, we evaluated functional vasodilation following spinotrapezius feed artery ligation. The spinotrapezius muscle allows for visualization of pre-existing collaterals (PECs), arterialized collateral capillaries (ACCs), the ischemic zone, and the stem region, all in the same tissue preparation.

Functional Vasodilation in Pre-existing Collaterals

Pre-existing collaterals (PECs), large arterioles that anastomose (i.e. connect) two different first or second-order arterioles and enlarge to serve as a natural bypass for blood flow following an arterial occlusion, are widely studied [11,35,36,37], and help to preserve downstream tissue viability following arterial occlusion (**Figure 1**) [20]. PECs occur less frequently in BALB/c mice in the hindlimb [11], spinotrapezius [17], and cerebral vasculature [39] than in other strains, and exhibit a delayed impairment in enlargement, with comparable enlargement to C57Bl/6 mice at day-7 and reduced enlargement at day-21 [11,15]. PECs in the spinotrapezius of male BALB/c mice have impaired endothelial-independent and endothelial-dependent dilation at day-7 [12]. Further, vasodilation capabilities are not completely restored at day-21, possibly due to a variation in smooth-muscle cell phenotype [12], while the vasodilation capabilities of PECs in female BALB/c mice remains unknown.

Figure 1. Representative Photomontage of Remodeled Vasculature with Pre-existing Collaterals in BALB/c Spinotrapezius Following Feed Artery Ligation. Boxed areas of interest include **A)** the re-entry vascular tree (ischemic zone), **B)** the midzone (arrow illustrates a PEC in the field of view) and **C)** the stem vascular tree. Blood flow moves from stem (C) to re-entry (A) following arterial occlusion.

PECs appeared in 9 of 24 females and 13 of 24 males (**Figures 2AB, 3AB**). Arterioles with similar dilated diameters in contralateral sham-operated muscles were used for comparisons. PECs consist of three zones: the stem (the site at which blood flow from another tree is entering the collateral bypass), the midzone (the region near the middle of the collateral bypass), and the re-entry (the site at which blood flow re-enters the ischemic tree) [40]. Following occlusion, blood flow and shear stress increase across the regions of the PEC, such that the stem increases from high to very high flow rates, the midzone increases from oscillatory flow to very high flow rates, and the reentry increases from high to very high flow rates in the opposite direction. Because arteriogenesis is induced by increases in shear stress, the differences between final and initial conditions at each collateral zone causes different amounts of remodeling in each zone [40,41]. Since remodeling likely impacts vasodilation, we evaluated vasodilation in each collateral zone.

By day-7 following femoral artery ligation, PEC remodeling is largely complete in the C57BL/6 strain, and max diameters do not appear to increase further [34,38]. We studied vasodilation in the different zones of PECs to test the hypothesis that by day-7 following feed artery ligation PECs are fully enlarged but not mature, and that vasodilation is more impaired in the midzone than in the stem and re-entry, since relative remodeling of the stem and re-entry are less. Reduction in vasodilation in the PEC stem were only significant in female mice, compared to control, $27 \pm 1.7 \mu\text{m}$ to $40 \pm 4.4 \mu\text{m}$ and $17 \pm 2.7 \mu\text{m}$ to $34 \pm 4.8 \mu\text{m}$, respectively; the male PEC stem dilated from $34 \pm 5.9 \mu\text{m}$ to $56 \pm 6.8 \mu\text{m}$, similar to $21 \pm 3.1 \mu\text{m}$ to $41 \pm 5 \mu\text{m}$ in control arterioles (**Figure 2**). Similar to the stem, PECs in the re-entry region in males and females were of similar resting diameter and dilated to a similar extent, $39 \pm 4 \mu\text{m}$ to $60 \pm 2.2 \mu\text{m}$ and $39 \pm 10 \mu\text{m}$ to $60 \pm 14.7 \mu\text{m}$, respectively (**Figure 2**). Although there was no difference between male and female re-entry PECs, they dilated less than control arterioles from males and females, $24 \pm 4.6 \mu\text{m}$ to $61 \pm 5.4 \mu\text{m}$ and $26 \pm 3.4 \mu\text{m}$ to $50 \pm 5.9 \mu\text{m}$, respectively (**Figure 2**). Unlike the stem and reentry, PECs in the midzone dilated less in males and females as compared to control, which dilated from $36 \pm 3.6 \mu\text{m}$ to $51 \pm 3.4 \mu\text{m}$ and $28 \pm 2.4 \mu\text{m}$ to $41 \pm 6.5 \mu\text{m}$, respectively, compared to $23 \pm 2.0 \mu\text{m}$ to $47 \pm 4.1 \mu\text{m}$ and $23 \pm 3.3 \mu\text{m}$ to $44 \pm 5.4 \mu\text{m}$, respectively, in control arterioles (**Figure 2**).

Figure 2. Functional Vasodilation in BALB/c Spinotrapezius Pre-existing Collaterals at Day-7. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a PEC in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation in the C) re-entry, D) midzone, and E) stem regions. F) Diameter increases from C, D, and E expressed as a percent change p<.05 vs. resting *, vs. sham &, vs. re-entry %, vs. midzone @ (n=5 males, n=4 females).

At day-21, after the presumptive maturation period, resting and dilated PEC measurements generally more closely resembled those of contralateral sham operated-muscles than at day-7 (**Figure 3**). Although general trends were more similar to the control, resting diameters of re-entry PECs in operated males were larger than midzone and stem arterioles, and percent change in diameter was greater in males than females in re-entry and midzone PECs, but females had a greater percent change in diameter in stem region PECs (**Figure 3**). Specifically, PECs in the stem region in males and females dilated from $21 \pm 3.7 \mu\text{m}$ to $58 \pm 5.1 \mu\text{m}$ and $18 \pm 4.1 \mu\text{m}$ to $48 \pm 12.5 \mu\text{m}$, respectively, compared to $22 \pm 3.2 \mu\text{m}$ to $43 \pm 5.5 \mu\text{m}$ and $17 \pm 1.7 \mu\text{m}$ to $40 \pm 4 \mu\text{m}$, respectively, in control arterioles (**Figure 3**). Pre-existing collaterals in the re-entry region in males and females dilated from $30 \pm 2.8 \mu\text{m}$ to $59 \pm 5.7 \mu\text{m}$ and $27 \pm 5.7 \mu\text{m}$ to $50 \pm 11 \mu\text{m}$, respectively, compared to $31 \pm 2.9 \mu\text{m}$ to $58 \pm 5.3 \mu\text{m}$ and $29 \pm 2.1 \mu\text{m}$ to $65 \pm 4.2 \mu\text{m}$, respectively, in control arterioles (**Figure 3**). Pre-existing collaterals in the midzone in males and females dilated from $23 \pm 5.4 \mu\text{m}$ to $49 \pm 8.6 \mu\text{m}$ and $19 \pm 3.1 \mu\text{m}$ to $41 \pm 9.8 \mu\text{m}$, respectively, compared to $28 \pm 2.4 \mu\text{m}$ to $53 \pm 3.9 \mu\text{m}$ and $23 \pm 2.9 \mu\text{m}$ to $51 \pm 5.7 \mu\text{m}$, respectively, in control arterioles (**Figure 3**).

Figure 3. Functional Vasodilation in BALB/c Spinotrapezius Pre-existing Collaterals at Day-21. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a PEC in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) twenty-one days following spinotrapezius feed artery ligation in the C) re-entry, D) midzone, and E) stem regions. F) Diameter increases from C, D, and E expressed as a percent change p<.05 vs. resting *, vs. sham &, vs. day-7 \$, vs. re-entry %, vs. midzone @, vs. stem ! (n=6 males, n=3 females).

Functional Vasodilation in ACCs

Because exercise training was only performed with a subset of mice, there was an insufficient number of PECs in exercise-trained mice to include in analysis.

Although PECs can help maintain tissue viability following arterial occlusion [2,9], some patients never develop collaterals, a condition that is mimicked in laboratory mice, such as the BALB/C strain [14,16]. Therefore, we studied functional vasodilation in ACCs to determine how collateral capillary arterialization (CCA) affects vasodilation in arterialized capillaries in males and females when enlargement is expected to be complete (day-7), when ACCs are expected to be largely mature (day-21), and following exercise therapy at day-7, to test the hypotheses that female BALB/c mice have a greater impairment in functional vasodilation in ACCs than male BALB/c mice, and that female mice are less responsive to exercise therapy [34].

Arterialized collateral capillaries reliably form in BALB/c mice, and have negligible reactivity in males seven days following feed artery ligation, while the reactivity of ACCs in female BALB/c mice is unknown. Before evaluating functional vasodilation, intravital-assessed morphology confirmed the presence of ACCs, i.e. tortuous and enlarged capillaries connecting two terminal arterioles from adjacent arteriolar trees [17]. Arterialized capillaries appeared in 24 of 24 females and 24 of 28 males at day-7 or day-21 following spinotrapezius artery ligation (**Figure 4**); contralateral sham muscles exhibited no sign of CCA (not shown).

Figure 4. Representative Photomontage of Remodeled Vasculature in BALB/c Spinotrapezius Following Feed Artery Ligation. Representative image of a female BALB/c spinotrapezius 21-days following feed artery ligation. Boxed areas of interest include **A)** the re-entry vascular tree (ischemic zone), **B)** the midzone with ACCs, and **C)** the stem vascular tree. **D)** Arrow illustrates an ACC in the field of view.

At day-7, ACCs in males were larger than in females, and exhibited greater vasodilation, $7.9 \pm 0.4 \mu\text{m}$ to $8.7 \pm 0.6 \mu\text{m}$ and $6.1 \pm 0.2 \mu\text{m}$ to $6.4 \pm 0.3 \mu\text{m}$, respectively (**Figure 5**). However, the vasodilation of male and female ACCs was markedly less than in sham-operated terminal arterioles of comparable resting diameter, which nearly doubled in size following muscle contraction in males and females, $7.4 \pm 0.3 \mu\text{m}$ to $13.7 \pm 0.4 \mu\text{m}$ and $5.8 \pm 0.3 \mu\text{m}$ to $11 \pm 0.5 \mu\text{m}$, respectively (**Figure 5**). Consistent with ACCs, diameters of terminal arterioles in sham-operated females were smaller than in males.

Figure 5. Functional Vasodilation in BALB/c Spinotrapezius Arterialized Capillaries at Day-7. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates an ACC in the field of view. C) Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation. D) Diameter increases from C expressed as a percent change $p < 0.05$ vs. resting *, vs. sham &, and vs. males # (n=9 males, n=9 females).

At day-21, after the presumptive maturation period, ACCs in males still had larger diameters than females. However, unlike day-7, there was no difference in vasodilation between sham and operated muscles, and resting diameters were smaller in day-21 ACCs (Figure 6). However, dilated diameters were greater for both males and females at day-21 as compared to day-7, $6.4 \pm 0.4 \mu\text{m}$ to $11.9 \pm 0.7 \mu\text{m}$ and $5.1 \pm 0.3 \mu\text{m}$ to $10.2 \pm 0.7 \mu\text{m}$, respectively, compared to $6.9 \pm 0.5 \mu\text{m}$ to $12.4 \pm 0.8 \mu\text{m}$ and $6.0 \pm 0.4 \mu\text{m}$ to $11.3 \pm 0.8 \mu\text{m}$, respectively, in control (Figure 6). Interestingly, percent change in diameter was greater in operated females than operated males at day-21.

Figure 6. Functional Vasodilation in BALB/c Spinotrapezius Arterialized Capillaries at Day-21. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates an ACC in the field of view. C) Diameters (μm) at rest and following muscle contraction (dilated) twenty-one days following spinotrapezius feed artery ligation. D) Diameter increases from C expressed as a percent change $p < 0.05$ vs. resting *, vs. males #, vs. day-7 \$ (n=12 males, n=8 females).

Although vasodilation was restored by day-21, if the natural history of ACC development is similar or longer in humans, the clinical relevance of this potential therapeutic target may be reduced because patient adherence and follow-up may be poor. It is possible that ACC development can be accelerated, by a mechanism such as exercise. Exercise is a widely-used effective therapy for patients with PAOD, but it's mechanism of action is unclear [2]. Therefore, we introduced exercise therapy to determine if exercise could accelerate the restoration of vasodilation by day-7, and to determine if there is some support for improved collateral vasodilation, which could possibly explain some of the benefits of exercise. Voluntary exercise increased vasodilation capacity in ACCs for both males and females, $7.3 \pm 0.4 \mu\text{m}$ to 9.4 ± 0.5

μm and $6.4 \pm 0.6 \mu\text{m}$ to $7.6 \pm 0.7 \mu\text{m}$, respectively (**Figure 7**). Although vasodilation was greater than in the sedentary group, it was still less than in terminal arterioles of the sham-operated males and females, to $7.2 \pm 0.3 \mu\text{m}$ to $14.3 \pm 0.7 \mu\text{m}$ and $6.6 \pm 0.4 \mu\text{m}$ to $12.2 \pm 0.7 \mu\text{m}$, respectively (**Figure 7**). Interestingly, a smaller percent change in diameter was seen in operated females than operated males.

Figure 7. Functional Vasodilation in BALB/c Spinotrapezius Arterialized Capillaries at Day-7 with Voluntary Exercise Therapy. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates an ACC in the field of view. C) Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation with voluntary exercise therapy with running wheel. D) Diameter increases from C expressed as a percent change $p < 0.05$ vs. resting *, vs. sham &, vs. males #, vs. day-7 \$ (n=8 males, n=7 females).

Functional Vasodilation in the Ischemic Tree

Although the collateral circulation is the primary site of blood flow resistance, the microcirculation in the ischemic zone has a major contribution to the overall resistance to blood flow in the ischemic tree. Understanding the natural history of vasodilation in the ischemic zone will help identify potential therapeutic targets for improving its function. Therefore, we tested the hypothesis that the ischemic tree has significantly impaired vasodilation at day-7, improved vasodilation at day-7 with exercise therapy, and restored vasodilation at day-21.

PECs originating from ischemic arteriolar trees likely influence the vascular reactivity of the ischemic tree because they provide an alternate route for blood flow to reach the ischemic tree. To study the arteriolar tree arising from the ligated feed artery independent from any influence of PECs, measurements from vascular trees where PECs originated were not included in the analysis. All orders of arterioles in the ischemic tree in males and females dilated less than in the contralateral sham-operated muscles at day-7, and there were no differences between male and female dilation (**Figure 8**). First-order arterioles from the ischemic tree in males and females dilated from $48 \pm 4.4 \mu\text{m}$ to $53 \pm 3.6 \mu\text{m}$ and $46 \pm 14 \mu\text{m}$ to $54 \pm 13 \mu\text{m}$, respectively, compared to $34 \pm 4.5 \mu\text{m}$ to $61 \pm 5.3 \mu\text{m}$ and $26 \pm 3.4 \mu\text{m}$ to $50 \pm 5.9 \mu\text{m}$, respectively, in control arterioles (**Figure 8**). Second-order arterioles from the ischemic tree in males and females dilated from $40 \pm 2.1 \mu\text{m}$ to $45 \pm 2.8 \mu\text{m}$ and $32 \pm 3.8 \mu\text{m}$ to $35 \pm 3.6 \mu\text{m}$, respectively, compared to $23 \pm 2.1 \mu\text{m}$ to $47 \pm 4.1 \mu\text{m}$ and $23 \pm 3.3 \mu\text{m}$ to $44 \pm 5.4 \mu\text{m}$, respectively, in control arterioles (**Figure 8**). Third-order arterioles from the ischemic tree in males and females dilated from $24 \pm 7.6 \mu\text{m}$ to $28 \pm 9.2 \mu\text{m}$ and $24 \pm 1.4 \mu\text{m}$ to $32 \pm 3.5 \mu\text{m}$, respectively, compared to $21 \pm 3.1 \mu\text{m}$ to $41 \pm 5 \mu\text{m}$ and $17 \pm 2.7 \mu\text{m}$ to $34 \pm 4.8 \mu\text{m}$, respectively, in control arterioles (**Figure 8**).

Figure 8. Functional Vasodilation in BALB/c Spinotrapezius Arterioles from the Ischemic Tree at Day-7. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a second-order ischemic tree vessel in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation in C) first, D) second, and E) third-order vessels. F) Diameter increases from C, D, and E expressed as a percent change $p < .05$ vs. resting *, vs. sham & (n=9 males, n=9 females).

By day-21, vasodilation significantly improved in arterioles from the ischemic tree compared to day-7 (**Figure 9**). Percent change in diameter of first-order arterioles in operated males, first-order arterioles in sham-operated females, and second and third-order arterioles in operated males and females were larger than at day-7. At day-21, first-order arterioles from the ischemic tree in males and females dilated from $39 \pm 4.7 \mu\text{m}$ to $77 \pm 9.4 \mu\text{m}$ and $30 \pm 4 \mu\text{m}$ to $60 \pm 8.4 \mu\text{m}$, respectively compared to $31 \pm 2.9 \mu\text{m}$ to $58 \pm 5.3 \mu\text{m}$ and $29 \pm 2.1 \mu\text{m}$ to $65 \pm 4.2 \mu\text{m}$, respectively in control arterioles (**Figure 9**). Second-order arterioles from the ischemic tree in males and females dilated from $29 \pm 2.8 \mu\text{m}$ to $60 \pm 2.1 \mu\text{m}$ and $20 \pm 3.3 \mu\text{m}$ to $43 \pm 5.5 \mu\text{m}$, respectively compared to $28 \pm 2.4 \mu\text{m}$ to $53 \pm 3.9 \mu\text{m}$ and $23 \pm 2.9 \mu\text{m}$ to $51 \pm 5.7 \mu\text{m}$, respectively in control arterioles (**Figure 9**). Resting and dilated diameters in females were smaller than respective diameters in males. Third-order arterioles from the ischemic tree in males and females dilated from $23 \pm 2.5 \mu\text{m}$ to $50 \pm 4.9 \mu\text{m}$ and $20 \pm 3.8 \mu\text{m}$ to $42 \pm 7.1 \mu\text{m}$, respectively compared to $22 \pm 3.2 \mu\text{m}$ to $43 \pm 5.5 \mu\text{m}$ and $17 \pm 1.7 \mu\text{m}$ to $40 \pm 4 \mu\text{m}$, respectively in control arterioles (**Figure 9**).

Figure 9. Functional Vasodilation in BALB/c Spinotrapezius Arterioles from the Ischemic Tree at Day 21. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a second-order ischemic tree vessel in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) twenty-one days following spinotrapezius feed artery ligation in C) first, D) second, and E) third-order vessels. F) Diameter increases from C, D, and E expressed as a percent change p<.05 vs. resting *, vs. males #, vs. day-7 [§] (n=12 males, n=8 females).

Exercise therapy only slightly increased functional vasodilation in ischemic tree arterioles. At day-7 with exercise therapy, first-order arterioles from the ischemic tree in males and females dilated from $60 \pm 3.5 \mu\text{m}$ to $71 \pm 4.2 \mu\text{m}$ and $51 \pm 2.1 \mu\text{m}$ to $57 \pm 2 \mu\text{m}$, respectively, compared to $35 \pm 2.7 \mu\text{m}$ to $63 \pm 4.2 \mu\text{m}$ and $35 \pm 1.3 \mu\text{m}$ to $57 \pm 2.7 \mu\text{m}$, respectively, in control arterioles (**Figure 10**). Additionally, first-order dilated arteriole diameters in operated males were almost $15 \mu\text{m}$ larger than those in females. Exercise therapy increased vasodilation in second-order arterioles in males and females as compared to sedentary day-7, $42 \pm 1.8 \mu\text{m}$ to $47 \pm 1.6 \mu\text{m}$ and $37 \pm 1.7 \mu\text{m}$ to $44 \pm 1.3 \mu\text{m}$, respectively compared to $24 \pm 1.3 \mu\text{m}$ to $49 \pm 2.1 \mu\text{m}$ and $21 \pm 2.1 \mu\text{m}$ to $39 \pm 2.7 \mu\text{m}$, respectively, in control arterioles (**Figure 10**). Third-order arterioles were larger in dilated diameter in day-7 with exercise therapy in males than in sedentary day-7 males, however, they were smaller in diameter in day-7 with exercise therapy in females compared to sedentary day-7. Exercise therapy increased vasodilation in third-order arterioles in males and females as compared to sedentary animals, $26 \pm 2.5 \mu\text{m}$ to $34 \pm 3.2 \mu\text{m}$ and $29 \pm 1.4 \mu\text{m}$ to $35 \pm 1.3 \mu\text{m}$, respectively, compared to $16 \pm 1.1 \mu\text{m}$ to $32 \pm 2 \mu\text{m}$ and $16 \pm 1.1 \mu\text{m}$ to $28 \pm 2.3 \mu\text{m}$, respectively, in control arterioles (**Figure 10**).

Figure 10. Functional Vasodilation in BALB/c Spinotrapezius Arterioles from the Ischemic Tree at Day-7 with Voluntary Exercise Therapy. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a third-order ischemic tree vessel in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation with voluntary exercise therapy with running wheel in C) first, D) second, and E) third-order vessels. F) Diameter increases from C, D, and E expressed as a percent change p<.05 vs. resting *, vs. sham &, vs. males #, vs. day-7 sedentary \$ (n=8 males, n=7 females).

Functional Vasodilation in the Stem Region

Although collaterals are the primary site of resistance, flow in collaterals is dependent upon input from the stem region, because collaterals usually materialize from the second or third-order of the stem tree. Following feed artery ligation, the significant increase in shear stress in the stem region leads to flow-induced vasodilation and subsequently outward remodeling [43]. We tested the hypothesis that the stem region exhibits impaired functional vasodilation at day-7 in sedentary mice, which will be similar to sham-operated mice day-21, and partially recovered at day-7 with exercise therapy. Diameters in operated muscles were compared to first, second, and third-order arterioles in the re-entry tree in sham-operated muscles. Female stem arterioles had a larger percent change in diameter across all orders when compared to males. At day-7, first-order arterioles from the stem region in males and females dilated from $49 \pm 16 \mu\text{m}$ to $80 \pm 14 \mu\text{m}$ and $42 \pm 4.6 \mu\text{m}$ to $81 \pm 6.7 \mu\text{m}$, respectively, compared to $34 \pm 4.5 \mu\text{m}$ to $61 \pm 5.3 \mu\text{m}$ and $26 \pm 3.4 \mu\text{m}$ to $50 \pm 5.9 \mu\text{m}$, respectively, in control arterioles (**Figure 11**). Second-order arterioles from the stem region in males and females dilated from $51 \pm 9.4 \mu\text{m}$ to $74 \pm 13.6 \mu\text{m}$ and $27 \pm 3 \mu\text{m}$ to $56 \pm 5.9 \mu\text{m}$, respectively, compared to $23 \pm 2.1 \mu\text{m}$ to $47 \pm 4.1 \mu\text{m}$ and $23 \pm 3.3 \mu\text{m}$ to $44 \pm 5.4 \mu\text{m}$, respectively, in control arterioles (**Figure 11**). Third-order arterioles from the stem region in males and females dilated from $33 \pm 5.3 \mu\text{m}$ to $45 \pm 8.1 \mu\text{m}$ and $20 \pm 2.1 \mu\text{m}$ to $44 \pm 4.9 \mu\text{m}$, respectively, compared to $21 \pm 3.1 \mu\text{m}$ to $41 \pm 5 \mu\text{m}$ and $17 \pm 2.7 \mu\text{m}$ to $34 \pm 4.8 \mu\text{m}$, respectively, in control arterioles (**Figure 11**).

Figure 11. Functional Vasodilation in BALB/c Spinotrapezius Arterioles from the Stem Region at Day-7. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a second-order stem region vessel in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation in (C) first, (D) second, and (E) third-order vessels. (F) Diameter increases from C, D, and E expressed as a percent change $p < .05$ vs. resting *, vs. sham &, vs. males # (n=9 males, n=9 females).

In general, resting and dilated diameters were smaller in first, second, and third-order arterioles from the stem region at day-21 compared to day-7 (**Figure 12**). Arterioles in operated muscles were compared to similar sized first, second, and third-order arterioles in sham-operated muscles. At day-21, dilated diameters in first-order arterioles in males were larger than at day-7, and diameters in females were smaller, $40 \pm 46.9 \mu\text{m}$ to $83 \pm 11.9 \mu\text{m}$ and $35 \pm 0.1 \mu\text{m}$ to $74 \pm 1.8 \mu\text{m}$, respectively compared to $31 \pm 2.9 \mu\text{m}$ to $58 \pm 5.3 \mu\text{m}$ and $29 \pm 2.1 \mu\text{m}$ to $65 \pm 4.2 \mu\text{m}$, respectively in control arterioles (**Figure 12**). Second-order arterioles in males and females had greater functional vasodilation than control arterioles. Second-order arterioles from the stem region in males and females dilated from $26 \pm 3.5 \mu\text{m}$ to $62 \pm 7 \mu\text{m}$ and $22 \pm 2 \mu\text{m}$ to $57 \pm 2 \mu\text{m}$, respectively compared to $28 \pm 2.4 \mu\text{m}$ to $53 \pm 3.9 \mu\text{m}$ and $23 \pm 2.9 \mu\text{m}$ to $51 \pm 5.7 \mu\text{m}$, respectively in control arterioles (**Figure 12**). Third-order arterioles from the stem region in males and females were not difference from control or each other, and dilated from $22 \pm 2.4 \mu\text{m}$ to $41 \pm 4.6 \mu\text{m}$ and $18 \pm 2.1 \mu\text{m}$ to $41 \pm 9.8 \mu\text{m}$, respectively compared to $22 \pm 3.2 \mu\text{m}$ to $43 \pm 5.5 \mu\text{m}$ and $17 \pm 1.7 \mu\text{m}$ to $40 \pm 4 \mu\text{m}$, respectively in control arterioles (**Figure 12**).

Figure 12. Functional Vasodilation in BALB/c Spinotrapezius Arterioles from the Stem Region at Day-21. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a second-order stem region vessel in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) twenty-one days following spinotrapezius feed artery ligation in C) first, D) second, and E) third-order vessels. F) Diameter increases from C, D, and E expressed as a percent change $p < .05$ vs. resting *, vs. sham &, vs. day-7 \$, (n=12 males, n=8 females).

Arterioles from the stem region in males and females at day-7 with exercise treatment closely resembled those of sedentary males and females at day-21 at all vascular tree diameter orders (**Figure 13**). Males and females with exercise therapy had similar first-order arteriole resting and dilated diameters to respective diameters in sedentary males and females at day-21. Arterioles in operated muscles were compared to similarly-sized first, second, and third-order arterioles in sham-operated muscles. Exercise increased dilated first-order arteriole diameters in males and females when compared to sedentary day-7, $45 \pm 3.4 \mu\text{m}$ to $86 \pm 3.4 \mu\text{m}$ and $45 \pm 1.9 \mu\text{m}$ to $88 \pm 2.6 \mu\text{m}$, respectively, compared to $35 \pm 2.7 \mu\text{m}$ to $63 \pm 4.2 \mu\text{m}$ and $35 \pm 1.3 \mu\text{m}$ to $57 \pm 2.7 \mu\text{m}$, respectively, in control arterioles (**Figure 13**). Exercise reduced resting and dilated diameters in second-order arterioles in males, and increased vasodilation compared to sedentary day-7 males, so diameters appeared more like day-21 diameters. Also, exercise increased resting and dilated diameters in second-order arterioles in females compared to sedentary day-7 females, and did not affect percent change of diameters. Exercise decreased resting and dilated diameters in second-order arterioles in day-7 males, and did not change diameters in females, $32 \pm 2.3 \mu\text{m}$ to $67 \pm 1.9 \mu\text{m}$ and $35 \pm 2.6 \mu\text{m}$ to $74 \pm 4.5 \mu\text{m}$, respectively, compared to $24 \pm 1.3 \mu\text{m}$ to $49 \pm 2.1 \mu\text{m}$ and $21 \pm 2.1 \mu\text{m}$ to $39 \pm 2.7 \mu\text{m}$, respectively, in control arterioles (**Figure 13**). Exercise decreased resting diameters and did not change dilated diameters in third-order arterioles in male compared to sedentary day-7 males, although exercise enhanced vasodilation. Exercise did not change third-order arteriole diameters or percent change in diameter in females. Exercise slightly decreased resting diameters in third-order arterioles in males compared to day-7, and did not change diameters in females, $24 \pm 1.3 \mu\text{m}$ to $46 \pm 2.6 \mu\text{m}$ and $21 \pm 2.2 \mu\text{m}$ to $49 \pm 4.4 \mu\text{m}$, respectively, compared to $16 \pm 1.1 \mu\text{m}$ to $32 \pm 2 \mu\text{m}$ and $16 \pm 1.1 \mu\text{m}$ to $28 \pm 2.3 \mu\text{m}$, respectively, in control arterioles (**Figure 13**). Exercise yielded a larger percent change in diameter females when compared to sham-operated diameters (**Figure 13**).

Figure 13. Functional Vasodilation in BALB/c Spinotrapezius Arterioles from the Stem Region at Day-7 with Voluntary Exercise Therapy. Representative images before (A) and after (B) 90 seconds of muscle contraction as visualized by intravital microscopy. Red arrow indicates a second-order stem region vessel in the field of view. Diameters (μm) at rest and following muscle contraction (dilated) seven days following spinotrapezius feed artery ligation with voluntary exercise therapy with running wheel in C) first, D) second, and E) third-order vessels. F) Diameter increases from C, D, and E expressed as a percent change $p < .05$ vs. resting *, vs. sham &, vs. day-7 sedentary \$ (n=8 males, n=7 females).

Exercise Therapy

Because exercise therapy was voluntary and mice in each cage had access to a communal running wheel, we recorded distance run per cage to determine if distance run correlated with the amount of functional vasodilation at day-7, and how distance run correlates with vessel maturity with time (**Figure 14**). As distance run increased, average percent change in diameter generally increased (**Figure 14**). The two cages with male mice ran 84.8 km and 55.7 km, while the two cages with female mice ran 59.4 km and 70.4 km in the 7-day period (**Figure 15**). Mice ran more during daily 12-hour dark cycles (~5 km to 14 km per cycle) when compared to 12-hour light cycles (~0.2 km to 2.5 km per cycle) (**Figure 16**).

Figure 14. Distance Run vs. Percent Change in Diameter. Distance run per cage versus average percent change in diameter in first order stem arterioles at day-7 with exercise (n=16).

Figure 15. Estimated Total Running Distance per Mouse. Total running distance in kilometers estimated per mouse per cage and total running distance per cage of four mice over the seven-day period following spinotrapezius feed artery ligation (n=16).

Figure 16. Distance Run Over 12 Hour Light/Dark Cycles. Distance run in kilometers of each cage over seven-day period following spinotrapezius feed artery ligation. Distance measurements taken for each 12-hour light/dark cycle (7AM-7PM light, 7PM-7AM dark) (n=16).

Discussion

Peripheral artery disease often presents as intermittent claudication, which may be caused by impaired vasodilation of collateral vessels. Collateral vessel functionality following arterial occlusion is not well defined [11,13,43] and there is a lack of studies in female collaterals [27], despite the fact that females with PAOD have a worse health status than males [4,23]. Even if their vasodilation is impaired, collaterals function as an important bypass following arterial occlusion to preserve downstream tissue integrity. However, some mice do not have collaterals, and are at greater risk for ischemic injury. These mice depend on arterialized collateral capillaries to reperfuse downstream tissue following feed artery ligation [17], which represents a possible therapeutic target for patients who never develop collaterals. The ischemic microcirculation also plays an important role in perfusion following occlusion because it is a major contribution to the overall resistance to blood flow in the ischemic tree [21]. Studying vasodilation in the ischemic microcirculation in relation to the collateral in a single preparation will provide insight into the relative importance of each of these resistance networks. Although collaterals are the primary site of resistance, flow in collaterals is dependent upon input from the stem region. Because collaterals usually materialize from the 2nd or 3rd order of the stem tree, vasodilation responsiveness in the stem region could impact the potential utility of collaterals, necessitating an understanding of upstream vascular function. Finally, given that blood flow is reduced following occlusion, exercise therapy, a known effective treatment for PAOD, increases collateral blood flow [31], but it remains unknown if exercise improves blood flow by enhancing structural adaptations or vascular reactivity. Therefore, we assessed functional vasodilation in ACCs, PECs, the ischemic tree, and the stem region, in immature and mature vessels, and with and without exercise therapy.

Almost half of mice (9 of 24 females and 13 of 24 males) had PECs at day-7 or day-21 following arterial ligation. The reason for the greater number of PECs in males is unknown, but in general, variation in the number of PECs is genetic [45]. Interestingly, all female mice had ACCs, while some males did not. This could be due to more robust remodeling in males, because PECs in males were able to remodel more robustly to become a sufficiently low resistance pathway that

shear stress did not appreciably increase in the ACC, while females may always form ACCs due to sufficiently high resistances in the PEC circulation. This hypothesis is supported by the observation that when mice did not have any PECs, they always developed ACCs. In general, diameters in females (average weight $20 \pm 0.3\text{g}$) were less than in males (average weight $26 \pm 0.4\text{g}$), again, possibly because females have less robust remodeling than males [27].

At day-7, vasodilation was impaired in the re-entry, midzone, and stem regions of PECs, however, the magnitude of the impairment was far less than in ACCs. This is likely because SMCs surround PECs prior to arterial occlusion, and must proliferate and reorganize in response to occlusion, whereas SMCs must first be recruited in ACCs, potentially causing a variation in reactivity between the vessel types at day-7 [9,12]. Dilation was more impaired in the midzone than in the stem or re-entry regions of PECs, which was expected because relative remodeling is the greatest in the midzone. Additionally, impaired arteriogenesis in collaterals from other muscles may further impair the reactivity of PECs in the spinotrapezius. To test this, we could measure functional vasodilation at day-7 in the spinotrapezius of a C57BL/6, a strain with 'normal' arteriogenesis, and compare to functional vasodilation in a spinotrapezius in a BALB/c muscle. By day-21, vasodilation of PECs in operated muscles surpassed that of contralateral sham-operated muscles in all regions except for the re-entry and midzone of females, indicating that most PECs exhibit mature vasodilation responses by day-21. Following arterial occlusion, males have a more robust eNOS-mediated vasodilation than females in response to acetylcholine, an endothelial-dependent dilator, which could explain why vasodilation in operated re-entry and midzone PECs in females was less than in males at day-7 [27]. Interestingly, females have higher levels of eNOS under baseline conditions, suggesting that eNOS activity is somehow decoupled from eNOS abundance [27]. But following surgery, a switch occurs and males have higher levels of eNOS [27], which may explain why males had greater vasodilation in collaterals following surgery. To test this hypothesis, an eNOS inhibitor could be applied after assessing functional vasodilation, and the amounts of vasodilation can be compared, to confirm that males have a more robust eNOS-mediated vasodilation than females.

As expected, functional vasodilation in ACCs was impaired at day-7, restored by day-21, and slightly improved with exercise, as determined by percent change in diameter. The decrease in functional vasodilation at day-7 was not surprising, based on previous reports of impaired endothelium-dependent and smooth muscle-dependent vasodilation in maturing collaterals [20,45]. However, since ACC reactivity is comparable in both endothelial-dependent and endothelial-independent vasodilation at day-7, this indicates that immature SMCs are likely responsible for the impairment, as they need time to arrange and mature into contractile SMCs [12]. Vessels regained vasodilation functionality by day-21, consistent with previous work from our group, demonstrating that functional vasodilation was impaired in immature vessels, but vessels exhibited mature responses by day-21 [12]. Capillaries generally are not surrounded by SMCs because dilation and constriction to regulate blood flow are typically not capillary functions, so most SMCs in ACCs are new to the area or recently differentiated from perivascular cells and likely need sufficient time to remodel and mature [12]. Exercise slightly improved vasodilation in ACCs, likely because exercise training improves endothelial-dependent dilation of collateral vessels by acutely elevating NO [29,47]. This could be confirmed by applying an eNOS inhibitor and determining if the effect disappears. There was a greater percent change in diameter in males than females at day-7 with exercise therapy, which is consistent with clinical data, suggesting that females respond less efficaciously to exercise therapy than males [35]. This may be due to NO-dependent endothelial dysfunction in females, which may not be overcome by acute elevation of NO with exercise [31].

In general, males and females had comparable vasodilation, but vasodilation was more impaired in females than males at day-7, as seen in ACCs, as males had a greater percent change in diameter at day-7 and at day-7 with exercise therapy, due to greater SMC relaxation. At baseline, there is no difference in the number of alpha-smooth muscle actin (alpha-SMA) positive vessels in female and male mice [27]. However, by day-7 post ligation, there is no change from baseline in the number of alpha-SMA positive vessels in females, but there is a significant increase in males [27]. The greater number of alpha-SMA-positive vessels in males may mean more arteriogenesis and capillary arterialization occurred, which may help explain

why vasodilation was more impaired in females than in males. Another important factor to consider relating to sex differences is estrogen. Estrogen activates and upregulates endothelial nitric oxide synthase (eNOS), increasing nitric oxide (NO) production that promotes smooth muscle cell relaxation [21]. Despite the protective role of estrogen, females with cardiovascular disease have a higher mortality rate than males, possibly because females are more susceptible to NO-dependent endothelial dysfunction than males, which may overpower the beneficial effects of estrogen [11]. Because arteriogenesis is regulated by NO, and NO significantly impacts vasomotor tone to enhance conductance of newly formed vessels, impaired eNOS signaling could explain the impairment in vasodilation in female mice at day-7 [48]. This impairment in females is further supported by the fact that female mice had smaller resting diameters than males. However, by day-21, the percent change in diameter was greater in females than in males. Because shear induced vasodilation plays a large role in determining resting diameter [49], the small resting diameter but large percent change in females could be due to less basal NO and high levels of NO upon stimulation [27]. This could be confirmed by using an eNOS inhibitor, and measure functional vasodilation to compare diameters in females and males at rest and upon stimulation.

Although the collateral circulation is the primary site of blood flow resistance, the microcirculation in the ischemic zone has a major contribution to the overall resistance to blood flow in the ischemic tree. Vasodilation was significantly impaired in the ischemic zone at day-7 and resting diameters were elevated when compared to day-21. This outcome was expected, because ischemia impairs feed artery vasodilation by impairing the responsiveness of the vascular wall to vasodilation stimuli [50]. Because vessels had appropriate time to mature, vasodilation was recovered by day-21, and in some cases even surpassed that of control vessels, such as in first, second, and third-order arterioles in males. Interestingly, exercise had no impact on vasodilation in the ischemic zone at day-7. The loss of vascular tone at day-7 and day-7 with exercise is likely a result of high shear stress following occlusion, which increased NO production and caused vasodilation. To confirm the loss of vascular tone, we could apply a vasoconstrictor at day-7 to see if vessels are capable of constriction. If vasoconstriction

capabilities are normal, and vessels have larger resting diameters than at day-21, vessels are likely actively vasodilating. Since the microcirculation in the ischemic zone has a major contribution to the overall resistance to blood flow in the ischemic tree, we can apply these results to better understand the time for recovery relative to other ischemic areas.

Flow in collaterals is dependent upon input from the stem region because collaterals usually materialize from the second or third-order of the stem tree. As expected, functional vasodilation was impaired at day-7 in sedentary mice, but was similar to sham-operated mice at day-21. We expected vasodilation to be partially recovered at day-7 with exercise therapy, however, vasodilation was almost completely recovered, which might indicate that exercise therapy is more beneficial in the stem region than we predicted. This is not completely surprising, because following feed artery ligation, the significant increase in shear stress in the stem region leads to flow-induced vasodilation and subsequently outward remodeling [43], which might be enhanced with exercise, by providing additional NO, expediting arteriogenesis. Because following occlusion, all blood flow must go through the stem before reaching upstream vasculature in the spinotrapezius, we expect more relative remodeling. Exercise increased dilated first-order arteriole diameters in males and females, but it decreased both resting and dilated diameters in second-order arterioles in males, and resting diameters in third-order arterioles in males. This may be because first order stem arterioles are in a constant state of high flow for the first week following occlusion, which is further increased with exercise, because exercise increases levels of NO [29,51]. Second and third-order arterioles may have an opportunity to mature to a greater extent relative to first-order arterioles, since they are downstream of the stem vascular tree, and the blood flow is distributed among more vessels, which would allow them a chance to mature [51]. Because second and third-order arteriole resting diameters decreased with exercise only in males, it seems that exercise had a greater effect on males more than females.

It is likely that the impairment in vasodilation in female mice seen in this study would be further exacerbated by the loss of estrogen following follicular loss in a future study. It is clear that estrogen plays an important role in PAOD etiology; the risk for reduction of vasodilation

becomes apparent at the age of menopause in women, more than a decade later than in men, suggesting a protective role of estrogen in cardiovascular disease etiology [20]. Postmenopausal women have higher levels of oxidative stress, insulin resistance, and inflammation when compared with men of a similar age, which may contribute to IC [20]. Additionally, chronic estrogen treatment preserves endothelium-dependent vasodilation in the peripheral vasculature of both ovariectomized rodents and post-menopausal women [20]. We saw more impaired vasodilation in female mice than in male mice in immature vessels, and female mice used in this study had intact ovaries. The loss of estrogen would likely more severely impair vasodilation in female mice. Clearly, sex differences in PAOD etiology should be carefully considered when considering novel disease treatments.

The primary limitations of this study were that young and healthy mice were used which may not accurately represent co-morbidities commonly presented in patients with PAOD, measurement variation may exist in vessel diameters, the activity of individual mice in the exercise-trained groups cannot be determined due to housing limitations, and isolation housing may have decreased collateral remodeling.

Our model of cardiovascular disease that utilizes young and healthy mice is limited, because vasculature is less reactive when co-morbidities are present, and co-morbidities are often present in patients presenting peripheral arterial occlusive disease [52]. The remodeled collaterals in this study are likely more robust and functional than they would be in mice with co-morbidities such as diabetes, hypertension, or post-menopause [52]. A clinically accurate representation of vessel reactivity and development could come from a diseased model of diabetes or hypertension developed through diet adjustment and/or genotyping.

Understanding the differences in mechanisms by evaluating functional vasodilation in a diseased model would be advantageous in evaluating the efficacy of naturally remodeled collaterals.

Although all vessel diameters within an anatomical position were averaged (i.e. 1st order ischemic tree), the average may not have accurately portrayed vessel diameters due to

measurement variation, and certain methods could be implemented in future experiments to further minimize this variation. Each vessel diameter was measured three times and the average was recorded, and each diameter measurement was slightly different, within half a micron for smaller vessels (ACCs), and within two microns for larger vessels (PECs, ischemic tree, and stem). This may have either increased or decreased average vessel diameters. Hand-measurement is subjective, and a more controlled automated algorithm would be useful to eliminate measurement variability. Automatic vessel diameter measurement algorithms exist, such as an Unsupervised Linear Discriminant Analysis Diameter Measurement, which requires a user to identify the region of interest, then automatically separates the vessel from the background to obtain an accurate diameter measurement [53], or Rapid Analysis of Vessel Elements, which uses black and white pixels to differentiate between vessels and background, and counts pixels to make vessel diameter measurements [54]. These algorithms are very accurate and consistently performed better than most other techniques, and could be very useful to eliminate measurement error in future studies [53,54]. Additionally, blood flow and oxygen saturation levels could be measured using a technique called phosphorescence quenching in which oxygen quenches phosphor when oxygen molecules collide with phosphor and emits fluorescence [55]. Phosphorescence quenching could be used to map the oxygen pressure in the vasculature in vivo to determine if there is a correlation between blood flow and vasodilation [55].

Another limitation in this study is that the activity of individual mice in the exercise-trained groups cannot be determined. Because of this, we estimated the distance run by each animal as the total distance run for the entire cage divided by the number of mice in the cage. If one mouse ran often and prevented the three others from running as much, the impact of exercise on functional vasodilation would not be accurately portrayed in the data. It is possible that if all four mice ran equally, we would see more of an impact on the data than we saw in this study, because the effects of exercise would be distributed amongst four data points rather than concentrated in one. In the future, this could be solved by adding a unique RFID tag to each mouse with a monitor on the exercise wheel, so rotations could be counted for each mouse individually. In addition, the inclusion of the running wheel tended to create aggression among

the animals, more commonly in male cages, as mice tended to appear possessive over the running wheel. For example, mice would knock a cage mate off the wheel to use it for themselves, and commonly we observed one mouse, possibly the dominant mouse, running while all others slept. If one mouse controlled the wheel and ran more than others, the potential effects of exercise on vasodilation would be maximized compared to the other mice. Aggression-induced stress may also have delayed vessel maturation during the recovery from chronic ischemia in sedentary mice [56]. Mice were group-housed with their original cage mates as much as possible following surgery, even if surgeries were 1 day apart. When surgeries were 1 day apart, existing mice became aggressive when vulnerable, recently-sedated mice were reintroduced into the cage. In cases of extreme aggression, the dominant mouse was isolation housed for the duration of recovery. Mice are social animals that do not respond well to isolation housing, which induces depression-like symptoms and signs of psychological stress [56]. In addition to psychological and behavioral responses, isolation housing affects the cardiovascular system, for example it increases blood pressure and heart rate in rats [57]. Therefore, it is possible that isolation housing decreased collateral remodeling in the current study, in the three mice that were isolation housed. Isolation housed mice had smaller collaterals than group-housed, however, since functional vasodilation was assessed on day-7 in all isolation housed mice, and vasodilation is significantly impaired at day-7 in ACCs and the ischemic zone, it is difficult to say if isolation housing had any effect on functional vasodilation.

In conclusion, and despite these potential limitations, functional vasodilation is impaired in male BALB/c mice and female BALB/c mice in ACCs, PECs, the ischemic tree, and the stem, 7 days following spinotrapezius feed artery ligation, and restored by day-21. This supports the hypothesis that impaired vasodilation may be involved in the pathophysiology of PAOD. Additionally, vasodilation in ACCs was more impaired in female mice than in males, and exercise training appears to enhance collateral reactivity, more in ACCs in males than in females, suggesting that its therapeutic benefits are linked not only to structural adaptation but also to vessel functionality. Therefore, future research is required to determine the cause of sex differences in exercise therapy to treat peripheral arterial occlusive disease.

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Appendices

Appendix A: Surgery and Experimental Protocols

Date _____

Spinotrapezius Ligation

Initials _____

Mouse Information

DOB: _____

Sex: _____

Tag: _____

Genotype/strain: _____

Cage: _____

Weight: _____

Materials

Instruments

- ____ 1. Standard Pattern Forceps (1)
- ____ 2. Iris Scissors (1)
- ____ 3. S & T (2)
- ____ 4. 5/45 (1)
- ____ 5. Dumont #7's (1)
- ____ 6. Microdissection scissors (1)
- ____ 7. Needle Holders (1)

Pre-sterilize in autoclave

- ____ 8. Cotton gauze (4)
- ____ 9. Cotton swabs (4)
- ____ 10. 6.0 silk suture (2x1 in)
- ____ 11. Surgical Drape

Obtained in surgery suite

- ____ 12. Petri dish w/ sterile saline
- ____ 13. Gloves
- ____ 14. Sterile 7-0 prolene suture
- ____ 15. FST heat pad w/ rectal probe
- ____ 16. heat pad
- ____ 17. Recovery bin and weigh boat
- ____ 18. Depilatory cream
- ____ 19. non-sterile cotton swabs
- ____ 20. non-sterile cotton gauze
- ____ 21. Isolation mask & cap
- ____ 22. Analgesic (buprenorphine)

Surgery Preparation

- ____ 23. Spray surgery area with Nolvasan
- ____ 24. Weigh animal in weight boat
- ____ 25. Place animal in anesthesia box
- ____ 26. Open the oxygen cylinder and set anesthesia-machine flow meter to $\sim 3 \text{ l} \cdot \text{min}^{-1}$
- ____ 27. Anesthetize animal w/ 5% isoflurane
- ____ 28. Reduce flow rate to $0.5\text{-}1.0 \text{ l} \cdot \text{min}^{-1}$ and the isoflurane to 1-3%
- ____ 29. Lay animal prone with nose in nose-cone
- ____ 30. Remove hair on anterior dorsal aspect of the animal with clippers and depilatory cream.
- ____ 31. Apply veterinary ointment to eyes to avoid drying during the procedure
- ____ 32. Give the animal subcutaneous injection of buprenorphine
- ____ 33. Lay animal prone on circulating heat pad (w/ 4x4 on top) w/ nose in nose-cone
- ____ 34. Insert rectal probe and set thermo-controller to 35°C

- ____ 35. Insert rectal probe and set thermo-controller to 35 ° C
- ____ 36. Adjust focus, lighting, and center mouse
- ____ 37. Don isolation mask, bonnet, and scrubs
- ____ 38. Open sterile surgical instrument pack
- ____ 39. Open sterile pack and dump in surgical instrument field
- ____ 40. Place sterile petri dish in surgical field and fill with sterile saline
- ____ 41. Don sterile gloves
- ____ 42. Transfer the content of sterile pack to surgical instrument sterile field (leave 1 4x4 on bench for non-sterile hands)

Surgery

- ____ 43. Make 5mm incision parallel to spine at intersection of fat pad and muscle
- ____ 44. Dissect skin overlying the spinotrapezius muscle
- ____ 45. Search for lateral feed artery vein pair jumping from the underlying fat pad to the spinotrapezius
- ____ 46. Blunt dissect fat pad to reveal arteriole-venule pair, using lidocaine as a vasodilator to aid in visualization
- ____ 47. Blunt dissect arteriole away from venule
- ____ 48. Pass a separated strand from 6-0 silk suture below the arteriole and tie off
- ____ 49. Repeat again about 1mm above previous ligature
- ____ 50. Cut arteriole between the two ligatures with micro dissection scissors
- ____ 51. Suture incision with 7-0 prolene suture
- ____ 52. Repeat for contralateral side to perform sham, but do not tie off ligature

Post-Surgical

- ____ 53. Give the animal a subcutaneous injection of buprenorphine
- ____ 54. Place the animal in the recovery bin, on a blue bench cover, above a heat pad and allow to recover
- ____ 55. Turn the flow meter down to 0, turn off the isoflurane, and close the oxygen cylinder
- ____ 56. Indicate surgery on cage card.

Notes

Date _____

Functional Vasodilation

Initials _____

Mouse Information

DOB: _____

Sex: _____

Tag: _____

Genotype/strain: _____

Cage: _____

Post-Experiment

____ 30. Cervical dislocation to euthanize animal

____ 31. Clean instruments and apply IPA

Materials

- ____ 1. forceps (2)
- ____ 2. fine forceps (2)
- ____ 3. ultrafine forceps (1)
- ____ 4. fine scissors (1)
- ____ 5. plastic wrap
- ____ 6. non-sterile cotton swabs
- ____ 7. non-sterile cotton gauze
- ____ 8. electrodes (black and red)
- ____ 9. veterinary ointment

Notes _____

Surgery preparation

- ____ 10. Weigh animal in weight boat
- ____ 11. Place animal in anesthesia box
- ____ 12. Open the oxygen cylinder and set anesthesia-machine flow meter to $\sim 3 \text{ l} \cdot \text{min}^{-1}$
- ____ 13. Anesthetize animal w/ 5% isoflurane
- ____ 14. Reduce flow rate to $0.5\text{--}1.0 \text{ l} \cdot \text{min}^{-1}$ and the isoflurane to 1-3%
- ____ 15. Lay animal supine with nose in nose-cone
- ____ 16. Lay animal supine on preparation bench in nose-cone
- ____ 17. Use trimming clippers & depilatory cream to remove hair on the dorsal aspect of the animal
- ____ 18. Transfer mouse to stage on FST heat pad
- ____ 19. Insert rectal probe and set thermo-controller to 35°C

Measurement preparation

- ____ 20. Make an incision on the dorsal aspect of the animal
- ____ 21. Extend the incision to expose the spinotrapezius muscle
- ____ 22. Blunt dissect the subcutaneous connective tissue to maximize surgical exposure
- ____ 23. Place black and red electrodes on medial aspect of spinotrapezius and secure
- ____ 24. Open Labchart and apply 1 Hz, $500 \mu\text{s}$, 1 mA stimulus as a test to optimize electrode placement
- ____ 25. Place plastic wrap over muscle
- ____ 26. Allow 30 minutes for vessels to equilibrate
- ____ 27. Stimulate at 8 Hz, $500 \mu\text{s}$, 1 mA for 90 seconds
- ____ 28. Immediately capture images
- ____ 29. Repeat procedure on contralateral side

Functional Vasodilation Experimental Setup:

1. Dissecting microscope
2. Heat pad
3. Olympus BXFM intravital microscope
4. Retiga 2000R mono camera (Qimaging)

Electrode Placement on the Spinothrapezius for Functional Vasodilation:

Appendix B: Sample Raw Images

ACCs Day-7 Operated

ACCs Day-7 Sham

ACCs Day-21 Operated

ACCs Day-21 Sham

ACCs Day-7 Exercise Operated

ACCs Day-7 Exercise Sham

PECs Re-entry Day-7 Operated

PECs Re-entry Day-7 Sham

PECs Midzone Day-7 Operated

PECs Midzone Day-7 Sham

PECs Stem Day-7 Operated

PECs Stem Day-7 Sham

PECs Re-entry Day-21 Operated

PECs Re-entry Day-21 Sham

PECs Midzone Day-21 Operated

PECs Midzone Day-21 Sham

PECs Stem Day-21 Operated

PECs Stem Day-21 Sham

Ischemic Tree 1st Order Day-7 Operated

Ischemic Tree 1st Order Day-7 Sham

Ischemic Tree 2nd Order Day-7 Operated

Ischemic Tree 2nd Order Day-7 Sham

Ischemic Tree 3rd Order Day-7 Operated

Ischemic Tree 3rd Order Day-7 Sham

Ischemic Tree 1st Order Day-21 Operated

Ischemic Tree 1st Order Day-21 Sham

Ischemic Tree 2nd Order Day-21 Operated

Ischemic Tree 2nd Order Day-21 Sham

Ischemic Tree 3rd Order Day-21 Operated

Ischemic Tree 3rd Order Day-21 Sham

Ischemic Tree 1st Order Day-7 Exercise Operated

Ischemic Tree 1st Order Day-7 Exercise Sham

Ischemic Tree 2nd Order Day-7 Exercise Operated

Ischemic Tree 2nd Order Day-7 Exercise Sham

Ischemic Tree 3rd Order Day-7 Exercise Operated

Ischemic Tree 3rd Order Day-7 Exercise Sham

Stem 1st Order Day-7 Operated

Stem 1st Order Day-7 Sham

Stem 2nd Order Day-7 Operated

Stem 2nd Order Day-7 Sham

Stem 3rd Order Day-7 Operated

Stem 3rd Order Day-7 Sham

Stem 1st Order Day-21 Operated

Stem 1st Order Day-21 Sham

Stem 2nd Order Day-21 Operated

Stem 2nd Order Day-21 Sham

Stem 3rd Order Day-21 Operated

Stem 3rd Order Day-21 Sham

Stem 1st Order Day-7 Exercise Operated

Stem 1st Order Day-7 Exercise Sham

Stem 2nd Order Day-7 Exercise Operated

Stem 2nd Order Day-7 Exercise Sham

Stem 3rd Order Day-7 Exercise Operated

Stem 3rd Order Day-7 Exercise Sham

Appendix C: Raw Data

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Female	Day-7	ACC	Operated	Resting	6.071
Female	Day-7	ACC	Operated	Dilated	6.382
Female	Day-7	ACC	Sham	Resting	5.817
Female	Day-7	ACC	Sham	Dilated	11.00
Male	Day-7	ACC	Operated	Resting	7.908
Male	Day-7	ACC	Operated	Dilated	8.662
Male	Day-7	ACC	Sham	Resting	7.407
Male	Day-7	ACC	Sham	Dilated	13.68
Female	Day-7 Exercise	ACC	Operated	Resting	6.399
Female	Day-7 Exercise	ACC	Operated	Dilated	7.585
Female	Day-7 Exercise	ACC	Sham	Resting	6.582
Female	Day-7 Exercise	ACC	Sham	Dilated	12.16
Male	Day-7 Exercise	ACC	Operated	Resting	7.325
Male	Day-7 Exercise	ACC	Operated	Dilated	9.396
Male	Day-7 Exercise	ACC	Sham	Resting	7.206
Male	Day-7 Exercise	ACC	Sham	Dilated	14.31
Female	Day-21	ACC	Operated	Resting	5.122
Female	Day-21	ACC	Operated	Dilated	10.22
Female	Day-21	ACC	Sham	Resting	5.961
Female	Day-21	ACC	Sham	Dilated	11.34
Male	Day-21	ACC	Operated	Resting	6.391
Male	Day-21	ACC	Operated	Dilated	11.92
Male	Day-21	ACC	Sham	Resting	6.888
Male	Day-21	ACC	Sham	Dilated	12.42
Female	Day-7	PEC - Re-entry	Operated	Resting	39.22
Female	Day-7	PEC - Re-entry	Operated	Dilated	60.07
Female	Day-7	PEC - Re-entry	Sham	Resting	26.43
Female	Day-7	PEC - Re-entry	Sham	Dilated	49.80
Female	Day-7	PEC - Midzone	Operated	Resting	27.53
Female	Day-7	PEC - Midzone	Operated	Dilated	40.86
Female	Day-7	PEC - Midzone	Sham	Resting	22.55
Female	Day-7	PEC - Midzone	Sham	Dilated	44.01
Female	Day-7	PEC - Stem	Operated	Resting	26.73
Female	Day-7	PEC - Stem	Operated	Dilated	39.96
Female	Day-7	PEC - Stem	Sham	Resting	17.32

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Female	Day-7	PEC - Stem	Sham	Dilated	33.67
Male	Day-7	PEC - Re-entry	Operated	Resting	39.09
Male	Day-7	PEC - Re-entry	Operated	Dilated	60.16
Male	Day-7	PEC - Re-entry	Sham	Resting	33.99
Male	Day-7	PEC - Re-entry	Sham	Dilated	61.28
Male	Day-7	PEC - Midzone	Operated	Resting	36.26
Male	Day-7	PEC - Midzone	Operated	Dilated	50.63
Male	Day-7	PEC - Midzone	Sham	Resting	23.23
Male	Day-7	PEC - Midzone	Sham	Dilated	47.37
Male	Day-7	PEC - Stem	Operated	Resting	34.01
Male	Day-7	PEC - Stem	Operated	Dilated	55.58
Male	Day-7	PEC - Stem	Sham	Resting	20.89
Male	Day-7	PEC - Stem	Sham	Dilated	41.28
Female	Day-21	PEC - Re-entry	Operated	Resting	26.84
Female	Day-21	PEC - Re-entry	Operated	Dilated	49.71
Female	Day-21	PEC - Re-entry	Sham	Resting	29.23
Female	Day-21	PEC - Re-entry	Sham	Dilated	64.67
Female	Day-21	PEC - Midzone	Operated	Resting	18.90
Female	Day-21	PEC - Midzone	Operated	Dilated	41.34
Female	Day-21	PEC - Midzone	Sham	Resting	22.59
Female	Day-21	PEC - Midzone	Sham	Dilated	51.21
Female	Day-21	PEC - Stem	Operated	Resting	17.99
Female	Day-21	PEC - Stem	Operated	Dilated	47.72
Female	Day-21	PEC - Stem	Sham	Resting	17.27
Female	Day-21	PEC - Stem	Sham	Dilated	39.45
Male	Day-21	PEC - Re-entry	Operated	Resting	31.00
Male	Day-21	PEC - Re-entry	Operated	Dilated	62.87
Male	Day-21	PEC - Re-entry	Sham	Resting	31.30
Male	Day-21	PEC - Re-entry	Sham	Dilated	58.39
Male	Day-21	PEC - Midzone	Operated	Resting	22.41
Male	Day-21	PEC - Midzone	Operated	Dilated	53.11
Male	Day-21	PEC - Midzone	Sham	Resting	28.15
Male	Day-21	PEC - Midzone	Sham	Dilated	53.03
Male	Day-21	PEC - Stem	Operated	Resting	21.44
Male	Day-21	PEC - Stem	Operated	Dilated	52.26

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Male	Day-21	PEC - Stem	Sham	Resting	22.09
Male	Day-21	PEC - Stem	Sham	Dilated	42.85
Female	Day-7	Ischemic Tree - 1st Order	Operated	Resting	45.71
Female	Day-7	Ischemic Tree - 1st Order	Operated	Dilated	54.34
Female	Day-7	Ischemic Tree - 1st Order	Sham	Resting	26.43
Female	Day-7	Ischemic Tree - 1st Order	Sham	Dilated	49.80
Female	Day-7	Ischemic Tree - 2nd Order	Operated	Resting	31.96
Female	Day-7	Ischemic Tree - 2nd Order	Operated	Dilated	35.16
Female	Day-7	Ischemic Tree - 2nd Order	Sham	Resting	22.55
Female	Day-7	Ischemic Tree - 2nd Order	Sham	Dilated	44.01
Female	Day-7	Ischemic Tree - 3rd Order	Operated	Resting	25.44
Female	Day-7	Ischemic Tree - 3rd Order	Operated	Dilated	31.97
Female	Day-7	Ischemic Tree - 3rd Order	Sham	Resting	17.32
Female	Day-7	Ischemic Tree - 3rd Order	Sham	Dilated	33.67
Male	Day-7	Ischemic Tree - 1st Order	Operated	Resting	47.67
Male	Day-7	Ischemic Tree - 1st Order	Operated	Dilated	52.72
Male	Day-7	Ischemic Tree - 1st Order	Sham	Resting	33.99
Male	Day-7	Ischemic Tree - 1st Order	Sham	Dilated	61.28
Male	Day-7	Ischemic Tree - 2nd Order	Operated	Resting	40.46
Male	Day-7	Ischemic Tree - 2nd Order	Operated	Dilated	45.10
Male	Day-7	Ischemic Tree - 2nd Order	Sham	Resting	23.23
Male	Day-7	Ischemic Tree - 2nd Order	Sham	Dilated	47.37
Male	Day-7	Ischemic Tree - 3rd Order	Operated	Resting	23.83
Male	Day-7	Ischemic Tree - 3rd Order	Operated	Dilated	28.41
Male	Day-7	Ischemic Tree - 3rd Order	Sham	Resting	20.89
Male	Day-7	Ischemic Tree - 3rd Order	Sham	Dilated	41.28
Female	Day-21	Ischemic Tree - 1st Order	Operated	Resting	26.95
Female	Day-21	Ischemic Tree - 1st Order	Operated	Dilated	60.10
Female	Day-21	Ischemic Tree - 1st Order	Sham	Resting	29.23
Female	Day-21	Ischemic Tree - 1st Order	Sham	Dilated	64.67
Female	Day-21	Ischemic Tree - 2nd Order	Operated	Resting	20.12
Female	Day-21	Ischemic Tree - 2nd Order	Operated	Dilated	44.54
Female	Day-21	Ischemic Tree - 2nd Order	Sham	Resting	22.59
Female	Day-21	Ischemic Tree - 2nd Order	Sham	Dilated	51.21

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Female	Day-21	Ischemic Tree - 3rd Order	Operated	Resting	20.11
Female	Day-21	Ischemic Tree - 3rd Order	Operated	Dilated	44.54
Female	Day-21	Ischemic Tree - 3rd Order	Sham	Resting	17.27
Female	Day-21	Ischemic Tree - 3rd Order	Sham	Dilated	39.45
Male	Day-21	Ischemic Tree - 1st Order	Operated	Resting	39.64
Male	Day-21	Ischemic Tree - 1st Order	Operated	Dilated	78.54
Male	Day-21	Ischemic Tree - 1st Order	Sham	Resting	31.30
Male	Day-21	Ischemic Tree - 1st Order	Sham	Dilated	58.39
Male	Day-21	Ischemic Tree - 2nd Order	Operated	Resting	30.12
Male	Day-21	Ischemic Tree - 2nd Order	Operated	Dilated	61.35
Male	Day-21	Ischemic Tree - 2nd Order	Sham	Resting	28.15
Male	Day-21	Ischemic Tree - 2nd Order	Sham	Dilated	53.03
Male	Day-21	Ischemic Tree - 3rd Order	Operated	Resting	22.27
Male	Day-21	Ischemic Tree - 3rd Order	Operated	Dilated	50.33
Male	Day-21	Ischemic Tree - 3rd Order	Sham	Resting	22.09
Male	Day-21	Ischemic Tree - 3rd Order	Sham	Dilated	42.85
Female	Day-7 Exercise	Ischemic Tree - 1st Order	Operated	Resting	51.04
Female	Day-7 Exercise	Ischemic Tree - 1st Order	Operated	Dilated	56.90
Female	Day-7 Exercise	Ischemic Tree - 1st Order	Sham	Resting	34.64
Female	Day-7 Exercise	Ischemic Tree - 1st Order	Sham	Dilated	56.66
Female	Day-7 Exercise	Ischemic Tree - 2nd Order	Operated	Resting	37.16
Female	Day-7 Exercise	Ischemic Tree - 2nd Order	Operated	Dilated	44.03
Female	Day-7 Exercise	Ischemic Tree - 2nd Order	Sham	Resting	21.41
Female	Day-7 Exercise	Ischemic Tree - 2nd Order	Sham	Dilated	38.54
Female	Day-7 Exercise	Ischemic Tree - 3rd Order	Operated	Resting	29.31
Female	Day-7 Exercise	Ischemic Tree - 3rd Order	Operated	Dilated	35.23
Female	Day-7 Exercise	Ischemic Tree - 3rd Order	Sham	Resting	15.50
Female	Day-7 Exercise	Ischemic Tree - 3rd Order	Sham	Dilated	28.12
Male	Day-7 Exercise	Ischemic Tree - 1st Order	Operated	Resting	56.80
Male	Day-7 Exercise	Ischemic Tree - 1st Order	Operated	Dilated	66.73
Male	Day-7 Exercise	Ischemic Tree - 1st Order	Sham	Resting	34.65
Male	Day-7 Exercise	Ischemic Tree - 1st Order	Sham	Dilated	63.14
Male	Day-7 Exercise	Ischemic Tree - 2nd Order	Operated	Resting	41.83
Male	Day-7 Exercise	Ischemic Tree - 2nd Order	Operated	Dilated	46.83
Male	Day-7 Exercise	Ischemic Tree - 2nd Order	Sham	Resting	24.48

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Male	Day-7 Exercise	Ischemic Tree - 2nd Order	Sham	Dilated	49.31
Male	Day-7 Exercise	Ischemic Tree - 3rd Order	Operated	Resting	26.45
Male	Day-7 Exercise	Ischemic Tree - 3rd Order	Operated	Dilated	33.67
Male	Day-7 Exercise	Ischemic Tree - 3rd Order	Sham	Resting	16.38
Male	Day-7 Exercise	Ischemic Tree - 3rd Order	Sham	Dilated	32.00
Female	Day-7	Stem - 1st Order	Operated	Resting	42.20
Female	Day-7	Stem - 1st Order	Operated	Dilated	80.86
Female	Day-7	Stem - 1st Order	Sham	Resting	26.43
Female	Day-7	Stem - 1st Order	Sham	Dilated	49.80
Female	Day-7	Stem - 2nd Order	Operated	Resting	26.72
Female	Day-7	Stem - 2nd Order	Operated	Dilated	56.34
Female	Day-7	Stem - 2nd Order	Sham	Resting	22.55
Female	Day-7	Stem - 2nd Order	Sham	Dilated	44.01
Female	Day-7	Stem - 3rd Order	Operated	Resting	20.09
Female	Day-7	Stem - 3rd Order	Operated	Dilated	125.0
Female	Day-7	Stem - 3rd Order	Sham	Resting	17.32
Female	Day-7	Stem - 3rd Order	Sham	Dilated	33.67
Male	Day-7	Stem - 1st Order	Operated	Resting	49.22
Male	Day-7	Stem - 1st Order	Operated	Dilated	80.35
Male	Day-7	Stem - 1st Order	Sham	Resting	33.99
Male	Day-7	Stem - 1st Order	Sham	Dilated	61.28
Male	Day-7	Stem - 2nd Order	Operated	Resting	50.57
Male	Day-7	Stem - 2nd Order	Operated	Dilated	74.00
Male	Day-7	Stem - 2nd Order	Sham	Resting	23.23
Male	Day-7	Stem - 2nd Order	Sham	Dilated	47.37
Male	Day-7	Stem - 3rd Order	Operated	Resting	32.95
Male	Day-7	Stem - 3rd Order	Operated	Dilated	44.60
Male	Day-7	Stem - 3rd Order	Sham	Resting	20.89
Male	Day-7	Stem - 3rd Order	Sham	Dilated	41.28
Female	Day-21	Stem - 1st Order	Operated	Resting	34.56
Female	Day-21	Stem - 1st Order	Operated	Dilated	74.12
Female	Day-21	Stem - 1st Order	Sham	Resting	29.23
Female	Day-21	Stem - 1st Order	Sham	Dilated	64.67
Female	Day-21	Stem - 2nd Order	Operated	Resting	22.22
Female	Day-21	Stem - 2nd Order	Operated	Dilated	56.90

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Female	Day-21	Stem - 2nd Order	Sham	Resting	22.59
Female	Day-21	Stem - 2nd Order	Sham	Dilated	51.21
Female	Day-21	Stem - 3rd Order	Operated	Resting	17.73
Female	Day-21	Stem - 3rd Order	Operated	Dilated	41.07
Female	Day-21	Stem - 3rd Order	Sham	Resting	17.27
Female	Day-21	Stem - 3rd Order	Sham	Dilated	39.45
Male	Day-21	Stem - 1st Order	Operated	Resting	39.53
Male	Day-21	Stem - 1st Order	Operated	Dilated	82.69
Male	Day-21	Stem - 1st Order	Sham	Resting	31.30
Male	Day-21	Stem - 1st Order	Sham	Dilated	58.39
Male	Day-21	Stem - 2nd Order	Operated	Resting	25.77
Male	Day-21	Stem - 2nd Order	Operated	Dilated	62.11
Male	Day-21	Stem - 2nd Order	Sham	Resting	28.15
Male	Day-21	Stem - 2nd Order	Sham	Dilated	53.03
Male	Day-21	Stem - 3rd Order	Operated	Resting	21.70
Male	Day-21	Stem - 3rd Order	Operated	Dilated	41.02
Male	Day-21	Stem - 3rd Order	Sham	Resting	22.09
Male	Day-21	Stem - 3rd Order	Sham	Dilated	42.85
Female	Day-7 Exercise	Stem - 1st Order	Operated	Resting	45.03
Female	Day-7 Exercise	Stem - 1st Order	Operated	Dilated	88.35
Female	Day-7 Exercise	Stem - 1st Order	Sham	Resting	34.64
Female	Day-7 Exercise	Stem - 1st Order	Sham	Dilated	56.66
Female	Day-7 Exercise	Stem - 2nd Order	Operated	Resting	35.24
Female	Day-7 Exercise	Stem - 2nd Order	Operated	Dilated	74.22
Female	Day-7 Exercise	Stem - 2nd Order	Sham	Resting	21.41
Female	Day-7 Exercise	Stem - 2nd Order	Sham	Dilated	38.54
Female	Day-7 Exercise	Stem - 3rd Order	Operated	Resting	21.37
Female	Day-7 Exercise	Stem - 3rd Order	Operated	Dilated	49.40
Female	Day-7 Exercise	Stem - 3rd Order	Sham	Resting	15.50
Female	Day-7 Exercise	Stem - 3rd Order	Sham	Dilated	28.12
Male	Day-7 Exercise	Stem - 1st Order	Operated	Resting	44.81
Male	Day-7 Exercise	Stem - 1st Order	Operated	Dilated	85.92
Male	Day-7 Exercise	Stem - 1st Order	Sham	Resting	24.48
Male	Day-7 Exercise	Stem - 1st Order	Sham	Dilated	49.31
Male	Day-7 Exercise	Stem - 2nd Order	Operated	Resting	31.97

Sex	Time Point	Anatomical Location	Limb	Rest or Dilated?	Mean Diameter
Male	Day-7 Exercise	Stem - 2nd Order	Operated	Dilated	67.30
Male	Day-7 Exercise	Stem - 2nd Order	Sham	Resting	24.48
Male	Day-7 Exercise	Stem - 2nd Order	Sham	Dilated	49.31
Male	Day-7 Exercise	Stem - 3rd Order	Operated	Resting	23.73
Male	Day-7 Exercise	Stem - 3rd Order	Operated	Dilated	46.00
Male	Day-7 Exercise	Stem - 3rd Order	Sham	Resting	16.38
Male	Day-7 Exercise	Stem - 3rd Order	Sham	Dilated	31.99

Appendix D: Statistics

Shapiro Wilk Normality Test: Resting Diameters:

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7	ACC	Operated	0.2861	Yes
Female	Day-7	ACC	Sham	0.5828	Yes
Male	Day-7	ACC	Operated	0.0539	Yes
Male	Day-7	ACC	Sham	0.4571	Yes
Female	Day-21	ACC	Operated	0.4785	Yes
Female	Day-21	ACC	Sham	0.8173	Yes
Male	Day-21	ACC	Operated	0.4980	Yes
Male	Day-21	ACC	Sham	0.2628	Yes
Female	Day-7 Exercise	ACC	Operated	0.3439	Yes
Female	Day-7 Exercise	ACC	Sham	0.5776	Yes
Male	Day-7 Exercise	ACC	Operated	0.1443	Yes
Male	Day-7 Exercise	ACC	Sham	0.0190	No
Female	Day-7	PEC - Reentry	Operated	0.7717	Yes
Female	Day-7	PEC - Midzone	Operated	0.0934	Yes
Female	Day-7	PEC - Stem	Operated	0.6579	Yes
Female	Day-7	1st Order	Sham	0.3558	Yes
Female	Day-7	2nd Order	Sham	0.3516	Yes
Female	Day-7	3rd Order	Sham	0.4605	Yes
Male	Day-7	PEC - Reentry	Operated	0.9340	Yes
Male	Day-7	PEC - Midzone	Operated	0.1234	Yes
Male	Day-7	PEC - Stem	Operated	0.2847	Yes
Male	Day-7	1st Order	Sham	0.6698	Yes
Male	Day-7	2nd Order	Sham	0.7995	Yes
Male	Day-7	3rd Order	Sham	0.1399	Yes
Female	Day-21	PEC - Reentry	Operated	0.1621	Yes
Female	Day-21	PEC - Midzone	Operated	0.1104	Yes
Female	Day-21	PEC - Stem	Operated	0.7354	Yes
Female	Day-21	Reentry	Sham	0.3558	Yes
Female	Day-21	Midzone	Sham	0.3516	Yes
Female	Day-21	Stem	Sham	0.4605	Yes
Male	Day-21	PEC - Reentry	Operated	0.0234	No
Male	Day-21	PEC - Midzone	Operated	0.0581	Yes
Male	Day-21	PEC - Stem	Operated	0.2634	Yes
Male	Day-21	Reentry	Sham	0.9204	Yes
Male	Day-21	Midzone	Sham	0.8076	Yes
Male	Day-21	Stem	Sham	0.7947	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7	Ischemic Tree 1st Order	Operated	0.4936	Yes
Female	Day-7	Ischemic Tree 2nd Order	Operated	0.5285	Yes
Female	Day-7	Ischemic Tree 3rd Order	Operated	0.7280	Yes
Male	Day-7	Ischemic Tree 1st Order	Operated	0.2643	Yes
Male	Day-7	Ischemic Tree 2nd Order	Operated	0.4820	Yes
Male	Day-7	Ischemic Tree 3rd Order	Operated	0.8122	Yes
Female	Day-21	Ischemic Tree 1st Order	Operated	0.2876	Yes
Female	Day-21	Ischemic Tree 2nd Order	Operated	0.3094	Yes
Female	Day-21	Ischemic Tree 3rd Order	Operated	0.6806	Yes
Male	Day-21	Ischemic Tree 1st Order	Operated	0.6918	Yes
Male	Day-21	Ischemic Tree 2nd Order	Operated	0.6944	Yes
Male	Day-21	Ischemic Tree 3rd Order	Operated	0.0633	Yes
Female	Day-7 Exercise	Ischemic Tree 1st Order	Operated	0.4550	Yes
Female	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	0.2600	Yes
Female	Day-7 Exercise	Ischemic Tree 3rd Order	Operated	0.4518	Yes
Male	Day-7 Exercise	Ischemic Tree 1st Order	Operated	0.0514	Yes
Male	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	0.7095	Yes
Male	Day-7 Exercise	Ischemic Tree 3rd Order	Operated	0.6555	Yes
Female	Day-7	Stem 1st Order	Operated	0.7601	Yes
Female	Day-7	Stem 2nd Order	Operated	0.9521	Yes
Female	Day-7	Stem 3rd Order	Operated	0.9949	Yes
Male	Day-7	Stem 1st Order	Operated	0.1296	Yes
Male	Day-7	Stem 2nd Order	Operated	0.0027	No
Male	Day-7	Stem 3rd Order	Operated	0.4668	Yes
Female	Day-21	Stem 1st Order	Operated	1.0000	Yes
Female	Day-21	Stem 2nd Order	Operated	1.0000	Yes
Female	Day-21	Stem 3rd Order	Operated	1.0000	Yes
Male	Day-21	Stem 1st Order	Operated	0.2829	Yes
Male	Day-21	Stem 2nd Order	Operated	0.0170	No
Male	Day-21	Stem 3rd Order	Operated	0.3274	Yes
Female	Day-7 Exercise	Stem 1st Order	Operated	0.7757	Yes
Female	Day-7 Exercise	Stem 2nd Order	Operated	0.2188	Yes
Female	Day-7 Exercise	Stem 3rd Order	Operated	0.1919	Yes
Male	Day-7 Exercise	Stem 1st Order	Operated	0.1230	Yes
Male	Day-7 Exercise	Stem 2nd Order	Operated	0.5477	Yes
Male	Day-7 Exercise	Stem 3rd Order	Operated	0.3352	Yes
Female	Day-7 Exercise	1st Order	Sham	0.5333	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7 Exercise	3rd Order	Sham	0.0087	No
Male	Day-7 Exercise	1st Order	Sham	0.8549	Yes
Male	Day-7 Exercise	2nd Order	Sham	0.3629	Yes
Male	Day-7 Exercise	3rd Order	Sham	0.1317	Yes

Levene Test and Wilcoxon test: Resting diameters:

Group 1	Group 2	Levene P-value	Equal variances?	Wilcoxon P-value	Different Means?
ACC_M_D7Ex_Op	ACC_M_D7Ex_Shram	0.8775	Yes	0.8463	No
ACC_F_D7Ex_Shram	ACC_M_D7Ex_Shram	0.6964	Yes	0.0821	No
ACC_M_D7Ex_Shram	ACC_M_D7_Shram	0.3837	Yes	0.5197	No
PEC_M_D21_Reentry_Op	PEC_M_D21_Reentry_Shram	0.3601	Yes	0.7469	No
PEC_M_D21_Reentry_Op	PEC_M_D21_Midzone_Op	0.9429	Yes	0.0169	Yes
PEC_M_D21_Reentry_Op	PEC_M_D21_Stem_Op	0.6890	Yes	0.0358	Yes
PEC_F_D21_Reentry_Op	PEC_M_D21_Reentry_Op	0.1294	Yes	0.8973	No
PEC_M_D7_Reentry_Op	PEC_M_D21_Reentry_Op	0.4354	Yes	0.0552	Yes
Isch_F_D7Ex_3rdOrder_Op	Isch_F_D7Ex_3rdOrder_Shram	0.7937	Yes	0.0200	Yes
Stem_M_D7_2ndOrder_Op	Stem_M_D7_2ndOrder_Shram	0.0158	No	0.0369	Yes
Stem_M_D7_1stOrder_Op	Stem_M_D7_2ndOrder_Op	0.9463	Yes	0.6625	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7_3rdOrder_Op	0.1957	Yes	0.3827	No
Stem_F_D7_2ndOrder_Op	Stem_M_D7_2ndOrderOp	0.0895	Yes	0.0227	Yes
Stem_M_D21_2ndOrder_Op	Stem_M_D21_2ndOrder_Shram	0.6766	Yes	0.4113	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_2ndOrder_Op	0.0598	Yes	0.0662	No
Stem_M_D21_2ndOrder_Op	Stem_M_D21_3rdOrder_Op	0.5841	Yes	0.7133	No
Stem_F_D21_2ndOrder_Op	Stem_M_D21_2ndOrder_Op	0.3841	Yes	1.0000	No
Stem_M_D7_2ndOrder_Op	Stem_M_D21_2ndOrder_Op	0.8424	Yes	0.7453	No
Stem_F_D7Ex_3rdOrder_Op	Stem_F_D7Ex_3rdOrder_Shram	0.0560	Yes	0.0122	Yes
Stem_M_D7_2ndOrder_Op	Stem_M_D7Ex_2ndOrder_Op	0.0311	Yes	0.0525	No
F_D7_3rdOrder_Shram	F_D7Ex_3rdOrder_Shram	0.0419	No	0.5498	No

F-test and ANOVA: Resting diameters:

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
ACC_F_D7_Op	ACC_F_D7_Sham	0.6462	Yes	0.5614	No
ACC_M_D7_Op	ACC_M_D7_Sham	0.3181	Yes	0.3468	No
ACC_F_D7_Op	ACC_M_D7_Op	0.2715	Yes	0.0012	Yes
ACC_F_D7_Sham	ACC_M_D7_Sham	0.6655	Yes	0.0027	Yes
ACC_F_D21_Op	ACC_F_D21_Sham	0.7756	Yes	0.0960	No
ACC_M_D21_Op	ACC_M_D21_Sham	0.2612	Yes	0.4624	No
ACC_F_D21_Op	ACC_M_D21_Op	0.0791	Yes	0.0400	Yes
ACC_F_D21_Sham	ACC_M_D21_Sham	0.8896	Yes	0.1158	No
ACC_F_D7Ex_Op	ACC_F_D7Ex_Sham	0.3902	Yes	0.8123	No
ACC_F_D7Ex_Op	ACC_M_D7Ex_Op	0.3186	Yes	0.1705	No
ACC_F_D7_Op	ACC_F_D21_Op	0.6678	Yes	0.0213	Yes
ACC_F_D7_Sham	ACC_F_D21_Sham	0.8896	Yes	0.1158	No
ACC_M_D7_Op	ACC_M_D21_Op	0.4271	Yes	0.0483	Yes
ACC_M_D7_Sham	ACC_M_D21_Sham	0.4260	Yes	0.2888	No
ACC_F_D7Ex_Op	ACC_F_D7_Op	0.0636	Yes	0.5233	No
ACC_F_D7Ex_Sham	ACC_F_d7_Sham	0.8318	Yes	0.1719	No
ACC_M_D7Ex_Op	ACC_M_D7_Op	0.7472	Yes	0.3188	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Reentry_Sham	0.0898	Yes	0.1758	No
PEC_F_D7_Midzone_Op	PEC_F_D7_Midzone_Sham	0.3773	Yes	0.3218	No
PEC_F_D7_Stem_Op	PEC_F_D7_Stem_Sham	0.3789	Yes	0.0283	Yes
PEC_F_D7_Midzone_Op	PEC_F_D7_Stem_Op	0.5776	Yes	0.7970	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Reentry_Sham	0.8189	Yes	0.4251	No
PEC_M_D7_Midzone_Op	PEC_M_D7_Midzone_Sham	0.3122	Yes	0.0140	Yes
PEC_M_D7_Stem_Op	PEC_M_D7_Stem_Sham	0.2348	Yes	0.0827	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Midzone_Op	0.8385	Yes	0.6149	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Stem_Op	0.4824	Yes	0.4957	No
PEC_M_D7_Midzone_Op	PEC_M_D7_Stem_Op	0.3691	Yes	0.7524	No
PEC_F_D7_Reentry_Op	PEC_M_D7_Reentry_Op	0.1486	Yes	0.9895	No
PEC_F_D7_Midzone_Op	PEC_M_D7_Midzone_Op	0.4350	Yes	0.1007	No
PEC_F_D7_Stem_Op	PEC_M_D7_Stem_Op	0.0523	Yes	0.3213	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Reentry_Sham	0.1401	Yes	0.6401	No
PEC_F_D21_Midzone_Op	PEC_F_D21_Midzone_Sham	0.8427	Yes	0.3837	No
PEC_F_D21_Stem_Op	PEC_F_D21_Stem_Sham	0.1928	Yes	0.8486	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Midzone_Op	0.0880	Yes	0.0144	Yes
PEC_F_D21_Reentry_Op	PEC_F_D21_Stem_Op	0.2471	Yes	0.0542	No

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
PEC_F_D21_Midzone_Op	PEC_F_D21_Stem_Op	0.8530	Yes	0.8653	No
PEC_M_D21_Midzone_Op	PEC_M_D21_Midzone_Sham	0.6604	Yes	0.1418	No
PEC_M_D21_Stem_Op	PEC_M_D21_Stem_Sham	0.4820	Yes	0.8729	No
PEC_M_D21_Midzone_Op	PEC_M_D21_Stem_Op	0.4716	Yes	0.8003	No
PEC_F_D21_Midzone_Op	PEC_M_D21_Midzone_Op	0.9782	Yes	0.4093	No
PEC_F_D21_Stem_Op	PEC_M_D21_Stem_Op	0.4786	Yes	0.4452	No
PEC_F_D7_Reentry_Op	PEC_F_D21_Reentry_Op	0.3918	Yes	0.3827	No
PEC_F_D7_Midzone_Op	PEC_F_D21_Midzone_Op	0.5884	Yes	0.0745	No
PEC_F_D7_Stem_Op	PEC_F_D21_Stem_Op	0.2595	Yes	0.0811	No
PEC_M_D7_Midzone_Op	PEC_M_D21_Midzone_Op	0.7669	Yes	0.0085	Yes
PEC_M_D7_Stem_Op	PEC_M_D21_Stem_Op	0.0857	Yes	0.0800	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7_2ndOrder_Sham	1.0000	Yes	0.0895	No
Isch_F_D7_3rdOrder_Op	Isch_F_D7_3rdOrder_Sham	0.2792	Yes	0.0732	No
Isch_F_D7_1stOrder_Op	Isch_F_D7_2ndOrder_Op	0.0722	Yes	0.2788	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7_3rdOrder_Op	0.1512	Yes	0.2507	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_1stOrder_Sham	0.7839	Yes	0.0932	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7_2ndOrder_Sham	0.8468	Yes	0.0016	Yes
Isch_M_D7_3rdOrder_Op	Isch_M_D7_3rdOrder_Sham	0.2462	Yes	0.6844	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_2ndOrder_Op	0.3808	Yes	0.2152	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_3rdOrder_Op	0.5040	Yes	0.0533	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7_3rdOrder_Op	0.1468	Yes	0.1027	No
Isch_F_D7_1stOrder_Op	Isch_M_D7_1stOrder_Op	0.1751	Yes	0.9018	No
Isch_F_D7_2ndOrder_Op	Isch_M_D7_2ndOrderOp	0.3356	Yes	0.1580	No
Isch_F_D7_3rdOrder_Op	Isch_M_D7_3rdOrder_Op	0.0639	Yes	0.8451	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_1stOrder_Sham	0.2391	Yes	0.5977	No
Isch_F_D21_2ndOrder_Op	Isch_F_D21_2ndOrder_Sham	0.9059	Yes	0.5681	No
Isch_F_D21_3rdOrder_Op	Isch_F_D21_3rdOrder_Sham	0.2430	Yes	0.5116	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_2ndOrder_Op	0.7717	Yes	0.2065	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_3rdOrder_Op	0.8487	Yes	0.2580	No
Isch_F_D21_2ndOrder_Op	Isch_F_D21_3rdOrder_Op	0.9198	Yes	0.9315	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_1stOrder_Sham	0.4696	Yes	0.1166	No
Isch_M_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Sham	0.6712	Yes	0.6052	No
Isch_M_D21_3rdOrder_Op	Isch_M_D21_3rdOrder_Sham	0.6105	Yes	0.9619	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_2ndOrder_Op	0.1245	Yes	0.0011	Yes
Isch_M_D21_1stOrder_Op	Isch_M_D21_3rdOrder_Op	0.1516	Yes	0.0030	Yes
Isch_M_D21_2ndOrder_Op	Isch_M_D21_3rdOrder_Op	0.6160	Yes	0.0455	Yes
Isch_F_D21_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.6531	Yes	0.0755	No
Isch_F_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.9321	Yes	0.0485	Yes

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
Isch_F_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.9321	Yes	0.0485	Yes
Isch_F_D21_3rdOrder_Op	Isch_M_D21_3rdOrder_Op	0.6976	Yes	0.5477	No
Isch_F_D7_1stOrder_Op	Isch_F_D21_1stOrder_Op	0.1020	Yes	0.2032	No
Isch_F_D7_2ndOrder_Op	Isch_F_D21_2ndOrder_Op	0.6981	Yes	0.0548	No
Isch_F_D7_3rdOrder_Op	Isch_F_D21_3rdOrder_Op	0.2339	Yes	0.2253	No
Isch_M_D7_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.7247	Yes	0.2884	No
Isch_M_D7_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.4433	Yes	0.0512	No
Isch_M_D7_3rdOrder_Op	Isch_M_D21_3rdOrder_Op	0.1030	Yes	0.7926	No
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_1stOrder_Shram	0.3454	Yes	0.0002	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_F_D7Ex_2ndOrder_Shram	0.7960	Yes	0.0002	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_2ndOrder_Op	0.7761	Yes	0.0006	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.3800	Yes	0.0001	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.4985	Yes	0.0114	Yes
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_1stOrder_Shram	1.0000	Yes	0.0001	Yes
Isch_M_D7Ex_2ndOrder_Op	Isch_M_D7Ex_2ndOrder_Shram	0.3736	Yes	0.0001	Yes
Isch_M_D7Ex_3rdOrder_Op	Isch_M_D7Ex_3rdOrder_Shram	0.1072	Yes	0.0073	Yes
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_2ndOrder_Op	0.6046	Yes	0.0002	Yes
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.9601	Yes	0.0001	Yes
Isch_M_D7Ex_2ndOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.5858	Yes	0.0003	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.6794	Yes	0.1069	No
Isch_F_D7Ex_2ndOrder_Op	Isch_M_D7Ex_2ndOrderOp	0.7837	Yes	0.0920	No
Isch_F_D7Ex_3rdOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.2136	Yes	0.4076	No
Isch_F_D7_1stOrder_Op	Isch_F_D7Ex_1stOrder_Op	0.0097	No	0.6383	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7Ex_2ndOrder_Op	0.1592	Yes	0.2159	No
Isch_F_D7_3rdOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.9319	Yes	0.1069	No
Isch_M_D7_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.5506	Yes	0.0746	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7Ex_2ndOrder_Op	0.8328	Yes	0.6754	No
Isch_M_D7_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.1429	Yes	0.6836	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_1stOrder_Shram	0.6040	Yes	0.0176	Yes
Stem_F_D7_2ndOrder_Op	Stem_F_D7_2ndOrder_Shram	0.8589	Yes	0.3666	No
Stem_F_D7_3rdOrder_Op	Stem_F_D7_3rdOrder_Shram	0.7368	Yes	0.4338	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_2ndOrder_Op	0.4229	Yes	0.0149	Yes
Stem_F_D7_1stOrder_Op	Stem_F_D7_3rdOrder_Op	0.1134	Yes	0.0015	Yes
Stem_F_D7_2ndOrder_Op	Stem_F_D7_3rdOrder_Op	0.3637	Yes	0.1110	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_1stOrder_Shram	0.0907	Yes	0.2899	No
Stem_M_D7_3rdOrder_Op	Stem_M_D7_3rdOrder_Shram	0.5449	Yes	0.0765	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_3rdOrder_Op	0.2018	Yes	0.3880	No

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
Stem_F_D7_1stOrder_Op	Stem_M_D7_1stOrder_Op	0.0951	Yes	0.5899	No
Stem_F_D7_3rdOrder_Op	Stem_M_D7_3rdOrder_Op	0.2592	Yes	0.0287	Yes
Stem_F_D21_1stOrder_Op	Stem_F_D21_1stOrder_Shram	0.0520	Yes	0.1491	No
Stem_F_D21_2ndOrder_Op	Stem_F_D21_2ndOrder_Shram	0.6556	Yes	0.9427	No
Stem_F_D21_3rdOrder_Op	Stem_F_D21_3rdOrder_Shram	1.0000	Yes	0.8734	No
Stem_F_D21_1stOrder_Op	Stem_F_D21_2ndOrder_Op	0.0641	Yes	0.0262	Yes
Stem_F_D21_1stOrder_Op	Stem_F_D21_3rdOrder_Op	0.0611	Yes	0.0157	Yes
Stem_F_D21_2ndOrder_Op	Stem_F_D21_3rdOrder_Op	0.9690	Yes	0.2673	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_1stOrder_Shram	0.2386	Yes	0.2171	No
Stem_M_D21_3rdOrder_Op	Stem_M_D21_3rdOrder_Shram	0.5200	Yes	0.9298	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_3rdOrder_Op	0.1160	Yes	0.0496	Yes
Stem_F_D21_3rdOrder_Op	Stem_M_D21_3rdOrder_Op	0.8567	Yes	0.3560	No
Stem_F_D7_2ndOrder_Op	Stem_F_D21_2ndOrder_Op	0.5367	Yes	0.4781	No
Stem_F_D7_3rdOrder_Op	Stem_F_D21_3rdOrder_Op	0.8258	Yes	0.5773	No
Stem_M_D7_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.2812	Yes	0.5619	No
Stem_M_D7_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.3040	Yes	0.0867	No
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_1stOrder_Shram	0.4491	Yes	0.0020	Yes
Stem_F_D7Ex_2ndOrder_Op	Stem_F_D7Ex_2ndOrder_Shram	0.6730	Yes	0.0034	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_2ndOrder_Op	0.5550	Yes	0.0171	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.8196	Yes	0.0001	Yes
Stem_F_D7Ex_2ndOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.7148	Yes	0.0036	Yes
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_1stOrder_Shram	0.4581	Yes	0.0516	No
Stem_M_D7Ex_2ndOrder_Op	Stem_M_D7Ex_2ndOrder_Shram	0.1476	Yes	0.0368	Yes
Stem_M_D7Ex_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Shram	0.4326	Yes	0.0028	Yes
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_2ndOrder_Op	0.4430	Yes	0.0068	Yes
Stem_M_D7Ex_2ndOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.1704	Yes	0.0082	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.1800	Yes	0.9611	No
Stem_F_D7Ex_2ndOrder_Op	Stem_M_D7Ex_2ndOrderOp	0.8844	Yes	0.3834	No
Stem_F_D7Ex_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.5320	Yes	0.3456	No
Stem_F_D7_1stOrder_Op	Stem_F_D7Ex_1stOrder_Op	0.0839	Yes	0.6127	No
Stem_F_D7_2ndOrder_Op	Stem_F_D7Ex_2ndOrder_Op	0.5767	Yes	0.0719	No
Stem_F_D7_3rdOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.9166	Yes	0.6871	No
Stem_M_D7_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.0524	Yes	0.5916	No
Stem_M_D7_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.0613	Yes	0.0350	Yes
F_D7_1stOrder_Shram	M_D7_1stOrder_Shram	0.7668	Yes	0.2053	No
F_D7_1stOrder_Shram	F_D21_1stOrder_Shram	0.1549	Yes	0.5394	No
M_D7_1stOrder_Shram	M_D21_1stOrder_Shram	0.5862	Yes	0.6102	No

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
ACC_F_D7_Sham	ACC_F_D21_Sham	0.8318	Yes	0.7784	No
ACC_F_D7_Sham	ACC_F_D7Ex_Sham	0.8318	Yes	0.1719	No
ACC_M_D7_sham	ACC_M_D21_Sham	0.4260	Yes	0.2888	No
ACC_M_d7_Sham	ACC_M_D7Ex_sham	0.7136	Yes	0.6485	No
ACC_F_D7_Sham	ACC_M_d7_Sham	0.6655	Yes	0.0027	Yes
ACC_F_D7Ex_Sham	ACC_M_D7Ex_sham	0.8072	Yes	0.2698	No
ACC_F_D21_Sham	ACC_M_D21_Sham	0.8896	Yes	0.1158	No
F_D7_2ndOrder_Sham	M_D7_2ndOrder_Sham	0.2516	Yes	0.8766	No
F_D7_2ndOrder_Sham	F_D21_2ndOrder_Sham	0.5291	Yes	0.9928	No
M_D7_2ndOrder_Sham	M_D21_2ndOrder_Sham	0.6637	Yes	0.1654	No
F_D7_2ndOrder_Sham	F_D7Ex_2ndOrder_Sham	0.2602	Yes	0.7962	No
F_D7_3rdOrder_Sham	M_D7_3rdOrder_Sham	0.8175	Yes	0.4068	No
F_D7_3rdOrder_Sham	F_D21_3rdOrder_Sham	1.0000	Yes	1.0000	No
M_D7_3rdOrder_Sham	M_D21_3rdOrder_Sham	0.9166	Yes	0.7949	No
M_D7_2ndOrder_Sham	M_D7Ex_2ndOrder_Sham	0.4124	Yes	0.6272	No
M_D7_3rdOrder_Sham	M_D7Ex_3rdOrder_Sham	0.0750	Yes	0.2028	No

F-test and Welch (Resting diameters):

Group 1	Group 2	F-test value	Equal variances?	ANOVA P-value	Different means?
PEC_F_D7_Reentry_Op	PEC_F_D7_Midzone_Op	0.0424	No	0.3376	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Stem_Op	0.0155	No	0.3084	No
Isch_F_D7_1stOrder_Op	Isch_F_D7_1stOrder_Sham	0.0485	No	0.3065	No
Isch_F_D7_1stOrder_Op	Isch_F_D7_3rdOrder_Op	0.0186	No	0.2898	No
Stem_F_D21_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.0155	No	0.5217	No
Stem_F_D7_1stOrder_Op	Stem_F_D21_1stOrder_Op	0.0194	No	0.1593	No
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.0410	No	0.0004	Yes
F_D7_1stOrder_Sham	F_D7Ex_1stOrder_Sham	0.0422	No	0.0573	No

Shapiro Wilk Normality Test: Dilated diameters:

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7	ACC	Operated	0.2932	Yes
Female	Day-7	ACC	Sham	0.3895	Yes
Male	Day-7	ACC	Operated	0.0150	No
Male	Day-7	ACC	Sham	0.2816	Yes
Female	Day-21	ACC	Operated	0.0036	No
Female	Day-21	ACC	Sham	0.6560	Yes
Male	Day-21	ACC	Operated	0.2231	Yes
Male	Day-21	ACC	Sham	0.3968	Yes
Female	Day-7 Exercise	ACC	Operated	0.2397	Yes
Female	Day-7 Exercise	ACC	Sham	0.7427	Yes
Male	Day-7 Exercise	ACC	Operated	0.7550	Yes
Male	Day-7 Exercise	ACC	Sham	0.3598	Yes
Female	Day-7	PEC - Reentry	Operated	0.8427	Yes
Female	Day-7	PEC - Midzone	Operated	0.9666	Yes
Female	Day-7	PEC - Stem	Operated	0.7669	Yes
Female	Day-7	Reentry	Sham	0.4935	Yes
Female	Day-7	Midzone	Sham	0.8464	Yes
Female	Day-7	Stem	Sham	0.4508	Yes
Male	Day-7	PEC - Reentry	Operated	0.8319	Yes
Male	Day-7	PEC - Midzone	Operated	0.3638	Yes
Male	Day-7	PEC - Stem	Operated	0.9473	Yes
Male	Day-7	Reentry	Sham	0.5286	Yes
Male	Day-7	Midzone	Sham	0.1288	Yes
Male	Day-7	Stem	Sham	0.0870	Yes
Female	Day-21	PEC - Reentry	Operated	0.1143	Yes
Female	Day-21	PEC - Midzone	Operated	0.2684	Yes
Female	Day-21	PEC - Stem	Operated	0.6723	Yes
Female	Day-21	Reentry	Sham	0.4935	Yes
Female	Day-21	Midzone	Sham	0.8464	Yes
Female	Day-21	Stem	Sham	0.4508	Yes
Male	Day-21	PEC - Reentry	Operated	0.3750	Yes
Male	Day-21	PEC - Midzone	Operated	0.7027	Yes
Male	Day-21	PEC - Stem	Operated	0.0922	Yes
Male	Day-21	Reentry	Sham	0.7621	Yes
Male	Day-21	Midzone	Sham	0.4791	Yes
Male	Day-21	Stem	Sham	0.8988	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7	Ischemic Tree 1st Order	Operated	0.2750	Yes
Female	Day-7	Ischemic Tree 2nd Order	Operated	0.1384	Yes
Female	Day-7	Ischemic Tree 3rd Order	Operated	0.1210	Yes
Male	Day-7	Ischemic Tree 1st Order	Operated	0.2830	Yes
Male	Day-7	Ischemic Tree 2nd Order	Operated	0.7815	Yes
Male	Day-7	Ischemic Tree 3rd Order	Operated	0.8345	Yes
Female	Day-21	Ischemic Tree 1st Order	Operated	0.0698	Yes
Female	Day-21	Ischemic Tree 2nd Order	Operated	0.6904	Yes
Female	Day-21	Ischemic Tree 3rd Order	Operated	0.4297	Yes
Male	Day-21	Ischemic Tree 1st Order	Operated	0.6371	Yes
Male	Day-21	Ischemic Tree 2nd Order	Operated	0.1022	Yes
Male	Day-21	Ischemic Tree 3rd Order	Operated	0.4124	Yes
Female	Day-7 Exercise	Ischemic Tree 1st Order	Operated	0.7217	Yes
Female	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	0.9740	Yes
Female	Day-7 Exercise	Ischemic Tree 3rd Order	Operated	0.9588	Yes
Male	Day-7 Exercise	Ischemic Tree 1st Order	Operated	0.1194	Yes
Male	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	0.2260	Yes
Male	Day-7 Exercise	Ischemic Tree 3rd Order	Operated	0.8511	Yes
Female	Day-7	Stem 1st Order	Operated	0.6215	Yes
Female	Day-7	Stem 2nd Order	Operated	0.2857	Yes
Female	Day-7	Stem 3rd Order	Operated	0.0787	Yes
Male	Day-7	Stem 1st Order	Operated	0.6875	Yes
Male	Day-7	Stem 2nd Order	Operated	0.5488	Yes
Male	Day-7	Stem 3rd Order	Operated	0.0473	No
Female	Day-21	Stem 1st Order	Operated	1.0000	Yes
Female	Day-21	Stem 2nd Order	Operated	1.0000	Yes
Female	Day-21	Stem 3rd Order	Operated	1.0000	Yes
Male	Day-21	Stem 1st Order	Operated	0.7299	Yes
Male	Day-21	Stem 2nd Order	Operated	0.6404	Yes
Male	Day-21	Stem 3rd Order	Operated	0.2509	Yes
Female	Day-7 Exercise	Stem 1st Order	Operated	0.0921	Yes
Female	Day-7 Exercise	Stem 2nd Order	Operated	0.7121	Yes
Female	Day-7 Exercise	Stem 3rd Order	Operated	0.2504	Yes
Male	Day-7 Exercise	Stem 1st Order	Operated	0.1580	Yes
Male	Day-7 Exercise	Stem 2nd Order	Operated	0.1366	Yes
Male	Day-7 Exercise	Stem 3rd Order	Operated	0.3255	Yes
Female	Day-7 Exercise	1st Order	Sham	0.7211	Yes
Female	Day-7 Exercise	2nd Order	Sham	0.1305	Yes
Female	Day-7 Exercise	3rd Order	Sham	0.0397	No

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Male	Day-7 Exercise	1st Order	Sham	0.0085	No
Male	Day-7 Exercise	2nd order	Sham	0.6633	Yes
Male	Day-7 Exercise	3rd Order	Sham	0.7104	Yes

Levene's test for equal variances and Wilcoxon test: Dilated diameters:

Group 1	Group 2	Levene P-value	Equal Variances?	Wilcoxon P-value	Different Means?
ACC_M_D7_Op	ACC_M_D7_Sham	0.5493	Yes	0.0034	Yes
ACC_F_D7_Op	ACC_M_D7_Op	0.0157	No	0.0120	Yes
ACC_F_D21_Op	ACC_F_D21_Sham	0.9449	Yes	0.1749	No
ACC_F_D21_Op	ACC_M_D21_Op	0.1408	Yes	0.1701	No
ACC_F_D7_Op	ACC_F_D21_Op	0.2044	Yes	0.0004	Yes
ACC_M_D7_Op	ACC_M_D21_Op	0.1018	Yes	0.0027	Yes
ACC_M_D7Ex_Op	ACC_M_D7_Op	0.7796	Yes	0.4772	No
Isch_F_D7Ex_3rdOrder_Op	Isch_F_D7Ex_3rdOrder_Sham	0.2694	Yes	0.1113	No
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_1stOrder_Sham	0.6392	Yes	0.1939	No
Stem_M_D7_3rdOrder_Op	Stem_M_D7_3rdOrder_Sham	0.6511	Yes	1.0000	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_3rdOrder_Op	0.4020	Yes	0.0809	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7_3rdOrder_Op	0.3630	Yes	0.0809	No
Stem_F_D7_3rdOrder_Op	Stem_M_D7_3rdOrder_Op	0.8432	Yes	0.8973	No
Stem_F_D7Ex_3rdOrder_Op	Stem_F_D7Ex_3rdOrder_Sham	0.0688	Yes	0.0122	Yes
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_1stOrder_Sham	0.7972	Yes	0.0149	Yes
Stem_M_D7_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.1022	Yes	0.9185	No
F_D7_3rdOrder_Sham	F_D7Ex_3rdOrder_Sham	0.1002	Yes	0.2963	No
M_D7Ex_1stOrder_Sham	M_D7_1stOrder_Sham	0.7462	Yes	0.8345	No

F-test and ANOVA (Dilated diameters):

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
ACC_F_D7_Op	ACC_F_D7_Sham	0.1152	Yes	0.0001	Yes
ACC_F_D7_Sham	ACC_M_D7_Sham	0.5683	Yes	0.0021	Yes
ACC_M_D21_Op	ACC_M_D21_Sham	0.3721	Yes	0.6769	No
ACC_F_D21_Sham	ACC_M_D21_Sham	0.8617	Yes	0.3200	No
ACC_F_D7Ex_Op	ACC_F_D7Ex_Sham	0.9283	Yes	0.0011	Yes
ACC_M_D7Ex_Op	ACC_M_D7Ex_Sham	0.5534	Yes	0.0001	Yes
ACC_F_D7Ex_Op	ACC_M_D7Ex_Op	0.5070	Yes	0.0439	Yes
ACC_F_D7Ex_Sham	ACC_M_D7Ex_Sham	0.9580	Yes	0.0653	No
ACC_F_D7_Sham	ACC_F_D21_Sham	0.6284	Yes	0.7040	No
ACC_M_D7_Sham	ACC_M_D21_Sham	0.2156	Yes	0.1596	No
ACC_F_D7Ex_Sham	ACC_F_D7_Sham	0.7223	Yes	0.2219	No
ACC_M_D7Ex_Sham	ACC_M_D7_Sham	0.3269	Yes	0.4448	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Reentry_Sham	0.1752	Yes	0.4589	No
PEC_F_D7_Midzone_Op	PEC_F_D7_Midzone_Sham	0.9584	Yes	0.7237	No
PEC_F_D7_Stem_Op	PEC_F_D7_Stem_Sham	0.7675	Yes	0.3764	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Midzone_Op	0.2134	Yes	0.2758	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Stem_Op	0.0773	Yes	0.2366	No
PEC_F_D7_Midzone_Op	PEC_F_D7_Stem_Op	0.5342	Yes	0.9124	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Reentry_Sham	0.1093	Yes	0.8497	No
PEC_M_D7_Midzone_Op	PEC_M_D7_Midzone_Sham	0.7240	Yes	0.5527	No
PEC_M_D7_Stem_Op	PEC_M_D7_Stem_Sham	0.5572	Yes	0.1271	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Midzone_Op	0.4128	Yes	0.0441	Yes
PEC_M_D7_Midzone_Op	PEC_M_D7_Stem_Op	0.2023	Yes	0.5318	No
PEC_F_D7_Midzone_Op	PEC_M_D7_Midzone_Op	0.3189	Yes	0.1966	No
PEC_F_D7_Stem_Op	PEC_M_D7_Stem_Op	0.3925	Yes	0.1120	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Reentry_Sham	0.1559	Yes	0.1651	No
PEC_F_D21_Midzone_Op	PEC_F_D21_Midzone_Sham	0.2585	Yes	0.3733	No
PEC_F_D21_Stem_Op	PEC_F_D21_Stem_Sham	0.0902	Yes	0.4521	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Midzone_Op	0.9462	Yes	0.6056	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Stem_Op	0.8734	Yes	0.9110	No
PEC_F_D21_Midzone_Op	PEC_F_D21_Stem_Op	1.0000	Yes	0.7030	No
PEC_M_D21_Reentry_Op	PEC_M_D21_Reentry_Sham	0.7355	Yes	0.5660	No
PEC_M_D21_Midzone_Op	PEC_M_D21_Midzone_Sham	0.4622	Yes	0.9900	No
PEC_M_D21_Stem_Op	PEC_M_D21_Stem_Sham	0.9156	Yes	0.2755	No
PEC_M_D21_Reentry_Op	PEC_M_D21_Midzone_Op	0.9235	Yes	0.1933	No
PEC_M_D21_Reentry_Op	PEC_M_D21_Stem_Op	0.9169	Yes	0.2055	No

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
PEC_M_D21_Midzone_Op	PEC_M_D21_Stem_Op	1.0000	Yes	0.9133	No
PEC_F_D21_Reentry_Op	PEC_M_D21_Reentry_Op	0.3963	Yes	0.2478	No
PEC_F_D21_Midzone_Op	PEC_M_D21_Midzone_Op	0.2443	Yes	0.2509	No
PEC_F_D21_Stem_Op	PEC_M_D21_Stem_Op	0.3557	Yes	0.7184	No
PEC_F_D7_Reentry_Op	PEC_F_D21_Reentry_Op	0.6221	Yes	0.6202	No
PEC_F_D7_Midzone_Op	PEC_F_D21_Midzone_Op	0.4141	Yes	0.9701	No
PEC_F_D7_Stem_Op	PEC_F_D21_Stem_Op	0.1743	Yes	0.5352	No
PEC_M_D7_Reentry_Op	PEC_M_D21_Reentry_Op	0.0859	Yes	0.6642	No
PEC_M_D7_Midzone_Op	PEC_M_D21_Midzone_Op	0.2776	Yes	0.7164	No
PEC_M_D7_Reentry_Op	PEC_M_D21_Reentry_Op	0.7808	Yes	0.7207	No
Isch_F_D7_1stOrder_Op	Isch_F_D7_1stOrder_Shram	0.4435	Yes	0.7143	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7_2ndOrder_Shram	0.2897	Yes	0.2399	No
Isch_F_D7_3rdOrder_Op	Isch_F_D7_3rdOrder_Shram	0.5055	Yes	0.8130	No
Isch_F_D7_1stOrder_Op	Isch_F_D7_2ndOrder_Op	0.0909	Yes	0.1134	No
Isch_F_D7_1stOrder_Op	Isch_F_D7_3rdOrder_Op	0.1415	Yes	0.1615	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7_3rdOrder_Op	0.7811	Yes	0.5768	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_1stOrder_Shram	0.4491	Yes	0.2998	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7_2ndOrder_Shram	0.4628	Yes	0.7076	No
Isch_M_D7_3rdOrder_Op	Isch_M_D7_3rdOrder_Shram	0.4866	Yes	0.2198	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_2ndOrder_Op	0.7537	Yes	0.1672	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_3rdOrder_Op	0.2637	Yes	0.0688	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7_3rdOrder_Op	0.1682	Yes	0.1562	No
Isch_F_D7_1stOrder_Op	Isch_M_D7_1stOrder_Op	0.1492	Yes	0.9074	No
Isch_F_D7_2ndOrder_Op	Isch_M_D7_2ndOrderOp	0.5635	Yes	0.1041	No
Isch_F_D7_3rdOrder_Op	Isch_M_D7_3rdOrder_Op	0.2508	Yes	0.7347	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_1stOrder_Shram	0.2101	Yes	0.6093	No
Isch_F_D21_2ndOrdere_Op	Isch_F_D21_2ndOrder_Shram	0.7773	Yes	0.4559	No
Isch_F_D21_3rdOrder_Op	Isch_F_D21_3rdOrder_Shram	0.4180	Yes	0.7781	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_2ndOrder_Op	0.7375	Yes	0.2340	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_3rdOrder_Op	0.7264	Yes	0.1705	No
Isch_F_D21_2ndOrder_Op	Isch_F_D21_3rdOrder_Op	0.9173	Yes	0.7796	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_1stOrder_Shram	0.3609	Yes	0.0530	No
Isch_M_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Shram	0.3341	Yes	0.0844	No
Isch_M_D21_3rdOrder_Op	Isch_M_D21_3rdOrder_Shram	0.7710	Yes	0.2964	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_2ndOrder_Op	0.7247	Yes	0.0001	Yes
Isch_M_D21_1stOrder_Op	Isch_M_D21_3rdOrder_Op	0.1304	Yes	0.0095	Yes
Isch_M_D21_2ndOrder_Op	Isch_M_D21_3rdOrder_Op	0.1872	Yes	0.0408	Yes

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
Isch_F_D21_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.7391	Yes	0.1774	No
Isch_F_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.0869	Yes	0.0208	Yes
Isch_F_D21_3rdOrder_Op	Isch_M_D21_3rdOrder_Op	0.7172	Yes	0.2945	No
Isch_F_D7_1stOrder_Op	Isch_F_D21_1stOrder_Op	0.6528	Yes	0.7078	No
Isch_F_D7_2ndOrder_Op	Isch_F_D21_2ndOrder_Op	0.3055	Yes	0.2454	No
Isch_F_D7_3rdOrder_Op	Isch_F_D21_3rdOrder_Op	0.3878	Yes	0.2920	No
Isch_M_D7_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.1561	Yes	0.0832	No
Isch_M_D7_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.8382	Yes	0.0040	Yes
Isch_M_D7_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.4190	Yes	0.0341	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_1stOrder_Sham	0.4593	Yes	0.9477	No
Isch_F_D7Ex_2ndOrder_Op	Isch_F_D7Ex_2ndOrder_Sham	0.1697	Yes	0.0855	No
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_2ndOrder_Op	0.4458	Yes	0.0003	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.3767	Yes	0.0001	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.7802	Yes	0.0016	Yes
Isch_M_D7Ex_2ndOrder_Op	Isch_M_D7Ex_2ndOrder_Sham	0.7941	Yes	0.3693	No
Isch_M_D7Ex_3rdOrder_Op	Isch_M_D7Ex_3rdOrder_Sham	0.3001	Yes	0.6855	No
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_2ndOrder_Op	0.3390	Yes	0.0001	Yes
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.6449	Yes	0.0001	Yes
Isch_M_D7Ex_2ndOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.1720	Yes	0.0030	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.4838	Yes	0.0160	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_M_D7Ex_2ndOrderOp	0.4863	Yes	0.2151	No
Isch_F_D7Ex_3rdOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.0891	Yes	0.7188	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7Ex_2ndOrder_Op	0.0622	Yes	0.0331	Yes
Isch_F_D7_3rdOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.1931	Yes	0.3644	No
Isch_M_D7_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	1.0000	Yes	0.0136	Yes
Isch_M_D7_2ndOrder_Op	Isch_M_D7Ex_2ndOrder_Op	0.7111	Yes	0.5888	No
Isch_M_D7_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.1836	Yes	0.5136	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_1stOrder_Sham	0.8767	Yes	0.0051	Yes
Stem_F_D7_2ndOrder_Op	Stem_F_D7_2ndOrder_Sham	0.8097	Yes	0.1489	No
Stem_F_D7_3rdOrder_Op	Stem_F_D7_3rdOrder_Sham	0.8511	Yes	0.1685	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_2ndOrder_Op	0.8914	Yes	0.0191	Yes
Stem_F_D7_1stOrder_Op	Stem_F_D7_3rdOrder_Op	0.5029	Yes	0.0013	Yes
Stem_F_D7_2ndOrder_Op	Stem_F_D7_3rdOrder_Op	0.5729	Yes	0.1469	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_1stOrder_Sham	0.2114	Yes	0.1755	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7_2ndOrder_Sham	0.1057	Yes	0.0562	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_2ndOrder_Op	0.9698	Yes	0.7607	No
Stem_F_D7_1stOrder_Op	Stem_M_D7_1stOrder_Op	0.4236	Yes	0.9711	No

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
Stem_F_D7_2ndOrder_Op	Stem_M_D7_2ndOrderOp	0.3764	Yes	0.1919	No
Stem_F_D21_1stOrder_Op	Stem_F_D21_1stOrder_Shram	0.4425	Yes	0.1969	No
Stem_F_D21_2ndOrdere_Op	Stem_F_D21_2ndOrder_Shram	0.3303	Yes	0.5708	No
Stem_F_D21_3rdOrder_Op	Stem_F_D21_3rdOrder_Shram	0.3124	Yes	0.8463	No
Stem_F_D21_1stOrder_Op	Stem_F_D21_2ndOrder_Op	0.9382	Yes	0.0229	Yes
Stem_F_D21_1stOrder_Op	Stem_F_D21_3rdOrder_Op	0.2291	Yes	0.0801	No
Stem_F_D21_2ndOrder_Op	Stem_F_D21_3rdOrder_Op	0.2519	Yes	0.2541	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_1stOrder_Shram	0.2804	Yes	0.0527	No
Stem_M_D21_2ndOrder_Op	Stem_M_D21_2ndOrder_Shram	0.3059	Yes	0.2635	No
Stem_M_D21_3rdOrder_Op	Stem_M_D21_3rdOrder_Shram	0.6496	Yes	0.8136	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_2ndOrder_Op	0.4321	Yes	0.1603	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_3rdOrder_Op	0.1509	Yes	0.0171	Yes
Stem_M_D21_2ndOrder_Op	Stem_M_D21_3rdOrder_Op	0.4079	Yes	0.0491	Yes
Stem_F_D21_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.1554	Yes	0.6566	No
Stem_F_D21_2ndOrder_Op	Stem_M_D21_2ndOrder_Op	0.2655	Yes	0.6749	No
Stem_F_D21_3rdOrder_Op	Stem_M_D21_3rdOrder_Op	0.4546	Yes	0.9964	No
Stem_F_D7_1stOrder_Op	Stem_F_D21_1stOrder_Op	0.2310	Yes	0.6042	No
Stem_F_D7_2ndOrder_Op	Stem_F_D21_2ndOrder_Op	0.2693	Yes	0.9634	No
Stem_F_D7_3rdOrder_Op	Stem_F_D21_3rdOrder_Op	0.6013	Yes	0.7764	No
Stem_M_D7_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.9112	Yes	0.9032	No
Stem_M_D7_2ndOrder_Op	Stem_M_D21_2ndOrder_Op	0.4399	Yes	0.4148	No
Stem_M_D7_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.4885	Yes	0.6972	No
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_1stOrder_Shram	0.8157	Yes	0.0001	Yes
Stem_F_D7Ex_2ndOrder_Op	Stem_F_D7Ex_2ndOrder_Shram	0.3654	Yes	0.0001	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_2ndOrder_Op	0.3289	Yes	0.0262	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.3402	Yes	0.0001	Yes
Stem_F_D7Ex_2ndOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.9810	Yes	0.0043	Yes
Stem_M_D7Ex_2ndOrder_Op	Stem_M_D7Ex_2ndOrder_Shram	0.8426	Yes	0.0001	Yes
Stem_M_D7Ex_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Shram	0.3791	Yes	0.0028	Yes
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_2ndOrder_Op	0.2048	Yes	0.0003	Yes
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.5704	Yes	0.0001	Yes
Stem_M_D7Ex_2ndOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.4609	Yes	0.0001	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.4213	Yes	0.6128	No
Stem_F_D7Ex_2ndOrder_Op	Stem_M_D7Ex_2ndOrderOp	0.1520	Yes	0.1304	No
Stem_F_D7Ex_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.4482	Yes	0.4886	No
Stem_F_D7_1stOrder_Op	Stem_F_D7Ex_1stOrder_Op	0.0681	Yes	0.3628	No
Stem_F_D7_2ndOrder_Op	Stem_F_D7Ex_2ndOrder_Op	0.4023	Yes	0.0501	No

Group 1	Group 2	F-test	Equal variances?	ANOVA P-value	Different means?
Stem_F_D7_3rdOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.7266	Yes	0.4482	No
Stem_M_D7_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.0524	Yes	0.5916	No
F_D7_1stOrder_Shram	M_D7_1stOrder_Shram	0.6242	Yes	0.1977	No
F_D7_1stOrder_Shram	F_D21_1stOrder_Shram	0.2515	Yes	0.0829	No
M_D7_1stOrder_Shram	M_D21_1stOrder_Shram	0.6881	Yes	0.7224	No
F_D7_1stOrder_Shram	F_D7Ex_1stOrder_Shram	0.1225	Yes	0.3810	No
ACC_F_D7_Shram	ACC_F_D21_Shram	0.6284	Yes	0.7040	No
ACC_F_D7_Shram	ACC_F_D7Ex_Shram	0.7223	Yes	0.2219	No
ACC_M_D7_shram	ACC_M_D21_Shram	0.2156	Yes	0.1596	No
ACC_M_d7_Shram	ACC_M_D7Ex_shram	0.3269	Yes	0.6282	No
ACC_F_D7_Shram	ACC_M_d7_Shram	0.5683	Yes	0.0021	Yes
ACC_F_D7Ex_Shram	ACC_M_D7Ex_shram	0.9580	Yes	0.0653	No
ACC_F_D21_Shram	ACC_M_D21_Shram	0.8617	Yes	0.3200	No
F_D7_2ndOrder_Shram	M_D7_2ndOrder_Shram	0.4067	Yes	0.6527	No
F_D7_2ndOrder_Shram	F_D21_2ndOrder_Shram	0.8215	Yes	0.358	No
M_D7_2ndOrder_Shram	M_D21_2ndOrder_Shram	0.9548	Yes	0.3421	No
F_D7_2ndOrder_Shram	F_D7Ex_2ndOrder_Shram	0.1266	Yes	0.4408	No
F_D7_3rdOrder_Shram	M_D7_3rdOrder_Shram	0.9530	Yes	0.3015	No
F_D7_3rdOrder_Shram	F_D21_3rdOrder_Shram	1.0000	Yes	1.0000	No
M_D7_3rdOrder_Shram	M_D21_3rdOrder_Shram	0.8364	Yes	0.8386	No
M_D7_2ndOrder_Shram	M_D7Ex_2ndOrder_Shram	0.2395	Yes	0.6822	No
M_D7_3rdOrder_Shram	M_D7Ex_3rdOrder_Shram	0.1117	Yes	0.1205	No

F-test and Welch test (Dilated diameters):

Group 1	Group 2	F-test Value	Equal Variances?	Welch P-value	Different Means?
ACC_F_D7Ex_Op	ACC_F_D7_Op	0.0343	No	0.1336	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Stem_Op	0.0477	No	0.5492	No
PEC_F_D7_Reentry_Op	PEC_M_D7_Reentry_Op	0.0045	No	0.9955	No
Isch_F_D7_1stOrder_Op	Isch_F_D7Ex_1stOrder_Op	0.0121	No	0.8583	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7Ex_2ndOrder_Op	0.0031	No	0.6708	No

Shapiro Wilk Normality Test: Percent Change:

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7	ACC	Operated	0.5210	Yes
Female	Day-7	ACC	Sham	0.1019	Yes
Male	Day-7	ACC	Operated	0.7404	Yes
Male	Day-7	ACC	Sham	0.7651	Yes
Female	Day-21	ACC	Operated	0.5110	Yes
Female	Day-21	ACC	Sham	0.5188	Yes
Male	Day-21	ACC	Operated	0.0019	No
Male	Day-21	ACC	Sham	0.0759	Yes
Female	Day-7 Exercise	ACC	Operated	0.9445	Yes
Female	Day-7 Exercise	ACC	Sham	0.1733	Yes
Male	Day-7 Exercise	ACC	Operated	0.3229	Yes
Male	Day-7 Exercise	ACC	Sham	0.9862	Yes
Female	Day-7	PEC - Reentry	Operated	0.7190	Yes
Female	Day-7	PEC - Midzone	Operated	0.1037	Yes
Female	Day-7	PEC - Stem	Operated	0.2907	Yes
Female	Day-7	Reentry	Sham	0.9243	Yes
Female	Day-7	Midzone	Sham	0.1466	Yes
Female	Day-7	Stem	Sham	0.0561	Yes
Male	Day-7	PEC - Reentry	Operated	0.4068	Yes
Male	Day-7	PEC - Midzone	Operated	0.3201	Yes
Male	Day-7	PEC - Stem	Operated	0.8715	Yes
Male	Day-7	Reentry	Sham	0.9945	Yes
Male	Day-7	Midzone	Sham	0.2980	Yes
Male	Day-7	Stem	Sham	0.7803	Yes
Female	Day-21	PEC - Reentry	Operated	0.0310	No
Female	Day-21	PEC - Midzone	Operated	0.0349	No
Female	Day-21	PEC - Stem	Operated	0.0570	Yes
Female	Day-21	Reentry	Sham	0.1922	Yes
Female	Day-21	Midzone	Sham	0.0723	Yes
Female	Day-21	Stem	Sham	0.7704	Yes
Male	Day-21	PEC - Reentry	Operated	0.5209	Yes
Male	Day-21	PEC - Midzone	Operated	0.3160	Yes
Male	Day-21	PEC - Stem	Operated	0.8202	Yes
Male	Day-21	Reentry	Sham	0.1181	Yes
Male	Day-21	Midzone	Sham	0.6888	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Male	Day-21	Stem	Sham	0.5651	Yes
Female	Day-7	Ischemic Tree 1st Order	Operated	0.4469	Yes
Female	Day-7	Ischemic Tree 2nd Order	Operated	0.1700	Yes
Female	Day-7	Ischemic Tree 3rd Order	Operated	0.7816	Yes
Male	Day-7	Ischemic Tree 1st Order	Operated	0.1564	Yes
Male	Day-7	Ischemic Tree 2nd Order	Operated	0.0908	Yes
Male	Day-7	Ischemic Tree 3rd Order	Operated	0.7751	Yes
Female	Day-21	Ischemic Tree 1st Order	Operated	0.7050	Yes
Female	Day-21	Ischemic Tree 2nd Order	Operated	0.6638	Yes
Female	Day-21	Ischemic Tree 3rd Order	Operated	0.3010	Yes
Male	Day-21	Ischemic Tree 1st Order	Operated	0.2608	Yes
Male	Day-21	Ischemic Tree 2nd Order	Operated	0.2168	Yes
Male	Day-21	Ischemic Tree 3rd Order	Operated	0.0523	Yes
Female	Day-7 Exercise	Ischemic Tree 1st Order	Operated	0.8815	Yes
Female	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	0.1187	Yes
Female	Day-7 Exercise	Ischemic Tree 3rd Order	Operated	0.4086	Yes
Male	Day-7 Exercise	Ischemic Tree 1st Order	Operated	0.1483	Yes
Male	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	0.7819	Yes
Male	Day-7 Exercise	Ischemic Tree 3rd Order	Operated	0.0140	No
Female	Day-7	Stem 1st Order	Operated	0.9861	Yes
Female	Day-7	Stem 2nd Order	Operated	0.0539	Yes
Female	Day-7	Stem 3rd Order	Operated	0.2082	Yes
Male	Day-7	Stem 1st Order	Operated	0.6503	Yes
Male	Day-7	Stem 2nd Order	Operated	0.7445	Yes
Male	Day-7	Stem 3rd Order	Operated	0.2556	Yes
Female	Day-21	Stem 1st Order	Operated	1.0000	Yes
Female	Day-21	Stem 2nd Order	Operated	1.0000	Yes
Female	Day-21	Stem 3rd Order	Operated	1.0000	Yes
Male	Day-21	Stem 1st Order	Operated	0.7182	Yes
Male	Day-21	Stem 2nd Order	Operated	0.9124	Yes
Male	Day-21	Stem 3rd Order	Operated	0.6468	Yes
Female	Day-7 Exercise	Stem 1st Order	Operated	0.0851	Yes
Female	Day-7 Exercise	Stem 2nd Order	Operated	0.0631	Yes
Female	Day-7 Exercise	Stem 3rd Order	Operated	0.8075	Yes
Male	Day-7 Exercise	Stem 1st Order	Operated	0.6713	Yes
Male	Day-7 Exercise	Stem 2nd Order	Operated	0.9946	Yes
Male	Day-7 Exercise	Stem 3rd Order	Operated	0.2051	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Normal?
Female	Day-7 Exercise	Reentry	Sham	0.8470	Yes
Female	Day-7 Exercise	Midzone	Sham	0.0538	Yes
Female	Day-7 Exercise	Stem	Sham	0.1346	Yes
Male	Day-7 Exercise	Reentry	Sham	0.8971	Yes
Male	Day-7 Exercise	Midzone	Sham	0.0399	No
Male	Day-7 Exercise	Stem	Sham	0.6196	Yes

Levene's test for equal variances and Wilcoxon test (Percent Change):

Group 1	Group 2	Levene P-value	Equal variances?	Wilcoxon P-value	Different Means?
ACC_M_D21_Op	ACC_M_D21_Sham	0.5626	Yes	0.2594	No
ACC_F_D21_Op	ACC_M_D21_Op	0.6354	Yes	0.0235	Yes
ACC_M_D7_Op	ACC_M_D21_Op	0.1442	Yes	0.0005	Yes
PEC_F_D21_Reentry_Op	PEC_F_D21_Reentry_Sham	0.4801	Yes	0.0369	Yes
PEC_F_D21_Midzone_Op	PEC_F_D21_Midzone_Sham	0.8943	Yes	0.1709	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Midzone_Op	0.2557	Yes	0.1360	No
PEC_F_D21_Reentry_Op	PEC_F_D21_Stem_Op	0.1093	Yes	0.0809	No
PEC_F_D21_Midzone_Op	PEC_F_D21_Stem_Op	0.5157	Yes	0.2330	No
PEC_F_D21_Reentry_Op	PEC_M_D21_Reentry_Op	0.3951	Yes	0.0933	No
PEC_F_D21_Midzone_Op	PEC_M_D21_Midzone_Op	0.2955	Yes	0.1243	No
PEC_F_D7_Reentry_Op	PEC_F_D21_Reentry_Op	0.1590	Yes	0.0518	Yes
PEC_F_D7_Midzone_Op	PEC_F_D21_Midzone_Op	0.6298	Yes	0.0373	Yes
Isch_M_D7Ex_2ndOrder_Op	Isch_M_D7Ex_2ndOrder_Sham	0.0607	Yes	0.0058	Yes
Isch_M_D7Ex_3rdOrder_Op	Isch_M_D7Ex_3rdOrder_Sham	0.5010	Yes	0.0081	Yes
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.0278	No	0.0383	Yes
Isch_M_D7Ex_2ndOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.1215	Yes	0.0034	Yes
Isch_F_D7Ex_3rdOrder_Op	Isch_M_D7Ex_3rdOrder_Op	0.2689	Yes	0.7491	No
Stem_M_D7Ex_2ndOrder_Op	Stem_M_D7Ex_2ndOrder_Sham	0.3767	Yes	0.4208	No
M_D7_2ndOrder_Sham	M_D7Ex_2ndOrder_Sham	0.4170	Yes	0.5309	No

F-test and ANOVA (Percent change):

Group 1	Group 2	F-test	Equal Variances?	ANOVA P-value	Different means?
ACC_F_D7_Sham	ACC_M_D7_Sham	0.3260	Yes	0.5956	No
ACC_F_D21_Op	ACC_F_D21_Sham	0.5131	Yes	0.2270	No
ACC_F_D21_Sham	ACC_M_D21_Sham	0.4624	Yes	0.1764	No
ACC_F_D7Ex_Op	ACC_F_D7Ex_Sham	0.1990	Yes	0.0001	Yes
ACC_F_D7Ex_Op	ACC_M_D7Ex_Op	0.3073	Yes	0.0421	Yes
ACC_F_D7Ex_Sham	ACC_M_D7Ex_Sham	0.1016	Yes	0.2927	No
ACC_F_D7_Sham	ACC_F_D21_Sham	0.1512	Yes	0.9713	No
ACC_M_D7_Sham	ACC_M_D21_Sham	0.8488	Yes	0.5257	No
ACC_F_D7Ex_Sham	ACC_F_d7_Sham	0.2858	Yes	0.6127	No
ACC_M_D7Ex_Op	ACC_M_D7_Op	0.0986	Yes	0.0008	Yes
ACC_M_D7Ex_Sham	ACC_M_D7_Sham	0.0911	Yes	0.2382	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Reentry_Sham	0.8533	Yes	0.0232	Yes
PEC_F_D7_Midzone_Op	PEC_F_D7_Midzone_Sham	0.7398	Yes	0.0083	Yes
PEC_F_D7_Stem_Op	PEC_F_D7_Stem_Sham	0.8444	Yes	0.0101	Yes
PEC_F_D7_Reentry_Op	PEC_F_D7_Midzone_Op	0.5580	Yes	0.6160	No
PEC_F_D7_Reentry_Op	PEC_F_D7_Stem_Op	0.9974	Yes	0.6413	No
PEC_F_D7_Midzone_Op	PEC_F_D7_Stem_Op	0.5559	Yes	0.8968	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Reentry_Sham	0.4580	Yes	0.1368	No
PEC_M_D7_Midzone_Op	PEC_M_D7_Midzone_Sham	0.3131	Yes	0.0013	Yes
PEC_M_D7_Reentry_Op	PEC_M_D7_Midzone_Op	0.7823	Yes	0.3605	No
PEC_M_D7_Reentry_Op	PEC_M_D7_Stem_Op	0.5049	Yes	0.5983	No
PEC_M_D7_Midzone_Op	PEC_M_D7_Stem_Op	0.3512	Yes	0.2171	No
PEC_F_D7_Reentry_Op	PEC_M_D7_Reentry_Op	0.5129	Yes	0.8223	No
PEC_F_D7_Midzone_Op	PEC_M_D7_Midzone_Op	0.8281	Yes	0.8331	No
PEC_F_D7_Stem_Op	PEC_M_D7_Stem_Op	0.2288	Yes	0.3444	No
PEC_F_D21_Stem_Op	PEC_F_D21_Stem_Sham	0.2508	Yes	0.0339	Yes
PEC_M_D21_Reentry_Op	PEC_M_D21_Reentry_Sham	0.4170	Yes	0.0374	Yes
PEC_M_D21_Stem_Op	PEC_M_D21_Stem_Sham	0.1204	Yes	0.0119	Yes
PEC_M_D21_Reentry_Op	PEC_M_D21_Stem_Op	0.1107	Yes	0.0149	Yes
PEC_M_D21_Midzone_Op	PEC_M_D21_Stem_Op	0.4214	Yes	0.9719	No
PEC_F_D21_Stem_Op	PEC_M_D21_Stem_Op	0.7040	Yes	0.4934	No
PEC_F_D7_Stem_Op	PEC_F_D21_Stem_Op	0.7698	Yes	0.0008	Yes
PEC_M_D7_Reentry_Op	PEC_M_D21_Reentry_Op	0.1488	Yes	0.0097	Yes
PEC_M_D7_Midzone_Op	PEC_M_D21_Midzone_Op	0.2190	Yes	0.0012	Yes
PEC_M_D7_Stem_Op	PEC_M_D21_Stem_Op	0.6052	Yes	0.0131	Yes
Isch_F_D7_1stOrder_Op	Isch_F_D7_1stOrder_Sham	0.5161	Yes	0.0057	Yes

Group 1	Group 2	F-test	Equal Variances?	ANOVA P-value	Different means?
Isch_F_D7_2ndOrder_Op	Isch_F_D7_2ndOrder_Sham	0.1040	Yes	0.0001	Yes
Isch_F_D7_3rdOrder_Op	Isch_F_D7_3rdOrder_Sham	0.6732	Yes	0.0025	Yes
Isch_F_D7_1stOrder_Op	Isch_F_D7_3rdOrder_Op	0.4087	Yes	0.8311	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7_3rdOrder_Op	0.4560	Yes	0.1565	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_1stOrder_Sham	0.1427	Yes	0.0008	Yes
Isch_M_D7_3rdOrder_Op	Isch_M_D7_3rdOrder_Sham	0.5282	Yes	0.0001	Yes
Isch_M_D7_1stOrder_Op	Isch_M_D7_2ndOrder_Op	0.3224	Yes	0.9549	No
Isch_M_D7_1stOrder_Op	Isch_M_D7_3rdOrder_Op	0.2010	Yes	0.5508	No
Isch_F_D7_1stOrder_Op	Isch_M_D7_1stOrder_Op	0.0647	Yes	0.3466	No
Isch_F_D7_2ndOrder_Op	Isch_M_D7_2ndOrderOp	0.1052	Yes	0.9722	No
Isch_F_D7_3rdOrder_Op	Isch_M_D7_3rdOrder_Op	0.9233	Yes	0.5765	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_1stOrder_Sham	0.6015	Yes	0.8573	No
Isch_F_D21_2ndOrder_Op	Isch_F_D21_2ndOrder_Sham	0.3773	Yes	0.7637	No
Isch_F_D21_3rdOrder_Op	Isch_F_D21_3rdOrder_Sham	0.0935	Yes	0.3349	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_2ndOrder_Op	0.6426	Yes	0.9381	No
Isch_F_D21_1stOrder_Op	Isch_F_D21_3rdOrder_Op	0.7482	Yes	0.6086	No
Isch_F_D21_2ndOrder_Op	Isch_F_D21_3rdOrder_Op	0.7482	Yes	0.6086	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_1stOrder_Sham	0.4603	Yes	0.6047	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_3rdOrder_Op	0.5491	Yes	0.5633	No
Isch_M_D21_2ndOrder_Op	Isch_M_D21_3rdOrder_Op	0.3201	Yes	0.1600	No
Isch_F_D21_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.6697	Yes	0.4003	No
Isch_F_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.8962	Yes	0.1868	No
Isch_F_D21_3rdOrder_Op	Isch_M_D21_3rdOrder_Op	0.2980	Yes	0.5454	No
Isch_F_D7_1stOrder_Op	Isch_F_D21_1stOrder_Op	0.6883	Yes	0.5786	No
Isch_F_D7_2ndOrder_Op	Isch_F_D21_2ndOrder_Op	0.8089	Yes	0.0058	Yes
Isch_F_D7_3rdOrder_Op	Isch_F_D21_3rdOrder_Op	0.2282	Yes	0.0001	Yes
Isch_M_D7_1stOrder_Op	Isch_M_D21_1stOrder_Op	0.3736	Yes	0.0108	Yes
Isch_M_D7_2ndOrder_Op	Isch_M_D21_2ndOrder_Op	0.0626	Yes	0.0011	Yes
Isch_F_D7Ex_1stOrder_Op	Isch_F_D7Ex_1stOrder_Sham	0.2243	Yes	0.0036	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_F_D7Ex_2ndOrder_Sham	0.1202	Yes	0.0001	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.9024	Yes	0.7909	No
Isch_M_D7Ex_1stOrder_Op	Isch_M_D7Ex_2ndOrder_Op	0.5347	Yes	0.0891	No

Group 1	Group 2	F-test	Equal Variances?	ANOVA P-value	Different means?
Isch_F_D7Ex_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.1732	Yes	0.0233	Yes
Isch_F_D7Ex_2ndOrder_Op	Isch_M_D7Ex_2ndOrderOp	0.5181	Yes	0.1072	No
Isch_F_D7_2ndOrder_Op	Isch_F_D7Ex_2ndOrder_Op	0.6366	Yes	0.1723	No
Isch_F_D7_3rdOrder_Op	Isch_F_D7Ex_3rdOrder_Op	0.2646	Yes	0.6066	No
Isch_M_D7_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.6767	Yes	0.0870	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7Ex_2ndOrder_Op	0.2681	Yes	0.8050	No
Isch_M_D7_1stOrder_Op	Isch_M_D7Ex_1stOrder_Op	0.3032	Yes	0.2726	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_1stOrder_Sham	0.1856	Yes	0.7362	No
Stem_F_D7_2ndOrder_Op	Stem_F_D7_2ndOrder_Sham	0.0719	Yes	0.4432	No
Stem_F_D7_3rdOrder_Op	Stem_F_D7_3rdOrder_Sham	0.0852	Yes	0.3265	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_2ndOrder_Op	0.5121	Yes	0.4614	No
Stem_F_D7_1stOrder_Op	Stem_F_D7_3rdOrder_Op	0.4479	Yes	0.3528	No
Stem_F_D7_2ndOrder_Op	Stem_F_D7_3rdOrder_Op	0.8908	Yes	0.8204	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_1stOrder_Sham	0.1679	Yes	0.7614	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7_2ndOrder_Sham	0.7332	Yes	0.0019	Yes
Stem_M_D7_3rdOrder_Op	Stem_M_D7_3rdOrder_Sham	0.0617	Yes	0.0078	Yes
Stem_M_D7_1stOrder_Op	Stem_M_D7_2ndOrder_Op	0.2562	Yes	0.3045	No
Stem_M_D7_1stOrder_Op	Stem_M_D7_3rdOrder_Op	0.8118	Yes	0.2634	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7_3rdOrder_Op	0.3539	Yes	0.6641	No
Stem_F_D7_1stOrder_Op	Stem_M_D7_1stOrder_Op	0.7755	Yes	0.5208	No
Stem_F_D7_2ndOrder_Op	Stem_M_D7_2ndOrderOp	0.1714	Yes	0.0632	No
Stem_F_D7_3rdOrder_Op	Stem_M_D7_3rdOrder_Op	0.5994	Yes	0.0459	Yes
Stem_F_D21_1stOrder_Op	Stem_F_D21_1stOrder_Sham	0.5987	Yes	0.2586	No
Stem_F_D21_2ndOrdere_Op	Stem_F_D21_2ndOrder_Sham	0.8588	Yes	0.3354	No
Stem_F_D21_3rdOrder_Op	Stem_F_D21_3rdOrder_Sham	0.0518	Yes	0.9805	No
Stem_F_D21_1stOrder_Op	Stem_F_D21_2ndOrder_Op	0.4780	Yes	0.1132	No
Stem_F_D21_1stOrder_Op	Stem_F_D21_3rdOrder_Op	0.2617	Yes	0.6760	No
Stem_F_D21_2ndOrder_Op	Stem_F_D21_3rdOrder_Op	0.6195	Yes	0.4519	No
Stem_M_D21_3rdOrder_Op	Stem_M_D21_3rdOrder_Sham	0.6062	Yes	0.4109	No
Stem_M_D21_1stOrder_Op	Stem_M_D21_2ndOrder_Op	0.9048	Yes	0.2732	No
Stem_F_D21_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.3229	Yes	0.9910	No
Stem_F_D21_2ndOrder_Op	Stem_M_D21_2ndOrder_Op	0.7255	Yes	0.7197	No
Stem_F_D7_1stOrder_Op	Stem_F_D21_1stOrder_Op	0.3100	Yes	0.5861	No
Stem_F_D7_2ndOrder_Op	Stem_F_D21_2ndOrder_Op	0.5700	Yes	0.3648	No

Group 1	Group 2	F-test	Equal Variances?	ANOVA P-value	Different means?
Stem_F_D7_3rdOrder_Op	Stem_F_D21_3rdOrder_Op	0.9568	Yes	0.9449	No
Stem_M_D7_1stOrder_Op	Stem_M_D21_1stOrder_Op	0.7679	Yes	0.2818	No
Stem_M_D7_2ndOrder_Op	Stem_M_D21_2ndOrder_Op	0.2868	Yes	0.0080	Yes
Stem_M_D7_3rdOrder_Op	Stem_M_D21_3rdOrder_Op	0.0584	Yes	0.0324	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_1stOrder_Sham	0.6405	Yes	0.0043	Yes
Stem_F_D7Ex_2ndOrder_Op	Stem_F_D7Ex_2ndOrder_Sham	0.9224	Yes	0.0297	Yes
Stem_F_D7Ex_3rdOrder_Op	Stem_F_D7Ex_3rdOrder_Sham	0.6733	Yes	0.0276	Yes
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_2ndOrder_Op	0.7187	Yes	0.1924	No
Stem_F_D7Ex_1stOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.3175	Yes	0.0268	Yes
Stem_F_D7Ex_2ndOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.5130	Yes	0.1725	No
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_1stOrder_Sham	0.9931	Yes	0.4486	No
Stem_M_D7Ex_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Sham	0.1044	Yes	0.9769	No
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_2ndOrder_Op	0.4251	Yes	0.2202	No
Stem_M_D7Ex_1stOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.5847	Yes	0.9665	No
Stem_M_D7Ex_2ndOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.7866	Yes	0.2524	No
Stem_F_D7Ex_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.3464	Yes	0.9078	No
Stem_F_D7Ex_2ndOrder_Op	Stem_M_D7Ex_2ndOrderOp	0.2236	Yes	0.8197	No
Stem_F_D7Ex_3rdOrder_Op	Stem_M_D7Ex_3rdOrder_Op	0.7388	Yes	0.0501	No
Stem_F_D7_1stOrder_Op	Stem_F_D7Ex_1stOrder_Op	0.0828	Yes	0.9884	No
Stem_F_D7_2ndOrder_Op	Stem_F_D7Ex_2ndOrder_Op	0.0533	Yes	0.8271	No
Stem_F_D7_3rdOrder_Op	Stem_F_D7Ex_3rdOrder_Op	0.1511	Yes	0.7719	No
Stem_M_D7_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.2589	Yes	0.4244	No
Stem_M_D7_2ndOrder_Op	Stem_M_D7Ex_2ndOrder_Op	0.3812	Yes	0.0107	Yes
Stem_M_D7_1stOrder_Op	Stem_M_D7Ex_1stOrder_Op	0.6851	Yes	0.0233	Yes
F_D7_1stOrder_Sham	M_D7_1stOrder_Sham	0.8247	Yes	0.6041	No
F_D7_1stOrder_Sham	F_D21_1stOrder_Sham	0.8688	Yes	0.0324	Yes
M_D7_1stOrder_Sham	M_D21_1stOrder_Sham	0.1332	Yes	0.7570	No
F_D7_1stOrder_Sham	F_D7Ex_1stOrder_Sham	0.2387	Yes	0.0293	Yes
ACC_F_D7_Sham	ACC_F_D21_Sham	0.1512	Yes	0.9713	No
ACC_F_D7_Sham	ACC_F_D7Ex_Sham	0.2858	Yes	0.6127	No
ACC_M_D7_sham	ACC_M_D21_Sham	0.8488	Yes	0.5257	No
ACC_M_d7_Sham	ACC_M_D7Ex_sham	0.0911	Yes	0.2382	No
ACC_F_D7_Sham	ACC_M_d7_Sham	0.3260	Yes	0.5956	No
ACC_F_D7Ex_Sham	ACC_M_D7Ex_sham	0.1016	Yes	0.2927	No
ACC_F_D21_Sham	ACC_M_D21_Sham	0.4624	Yes	0.1764	No
F_D7_2ndOrder_Sham	M_D7_2ndOrder_Sham	0.3249	Yes	0.7211	No

Group 1	Group 2	F-test	Equal Variances?	ANOVA P-value	Different means?
F_D7_2ndOrder_Shram	F_D21_2ndOrder_Shram	0.4293	Yes	0.0960	No
M_D7_2ndOrder_Shram	M_D21_2ndOrder_Shram	1.0000	Yes	1.0000	No
F_D7_2ndOrder_Shram	F_D7Ex_2ndOrder_Shram	0.5300	Yes	0.1991	No
F_D7_3rdOrder_Shram	M_D7_3rdOrder_Shram	0.2463	Yes	0.7437	No
F_D7_3rdOrder_Shram	F_D21_3rdOrder_Shram	0.2370	Yes	0.0198	Yes
M_D7_3rdOrder_Shram	M_D21_3rdOrder_Shram	0.8129	Yes	0.5585	No
M_D7_3rdOrder_Shram	M_D7Ex_3rdOrder_Shram	0.8281	Yes	0.5563	No

F-test and Welch (Percent Change):

Group 1	Group 2	F-test	Equal Variances?	Welch P-value	Different means?
ACC_F_D7_Op	ACC_F_D7_Shram	0.0001	No	0.0001	Yes
ACC_M_D7_Op	ACC_M_D7_Shram	0.0302	No	0.0001	Yes
ACC_F_D7_Op	ACC_M_D7_Op	0.0005	No	0.0732	No
ACC_M_D7Ex_Op	ACC_M_D7Ex_Shram	0.0151	No	0.0010	Yes
ACC_F_D7_Op	ACC_F_D21_Op	0.0001	No	0.0001	Yes
ACC_F_D7Ex_Op	ACC_F_D7_Op	0.0001	No	0.0013	Yes
PEC_M_D7_Stem_Op	PEC_M_D7_Stem_Shram	0.0302	No	0.1931	No
PEC_M_D21_Midzone_Op	PEC_M_D21_Midzone_Shram	0.0007	No	0.0150	Yes
PEC_M_D21_Reentry_Op	PEC_M_D21_Midzone_Op	0.0156	No	0.0350	Yes
Isch_F_D7_1stOrder_Op	Isch_F_D7_2ndOrder_Op	0.0723	No	0.3926	No
Isch_M_D7_2ndOrder_Op	Isch_M_D7_2ndOrder_Shram	0.0514	No	0.0001	Yes
Isch_M_D7_2ndOrder_Op	Isch_M_D7_3rdOrder_Op	0.0420	No	0.5763	No
Isch_M_D21_2ndOrder_Op	Isch_M_D21_2ndOrder_Shram	0.0123	No	0.1938	No
Isch_M_D21_3rdOrder_Op	Isch_M_D21_3rdOrder_Shram	0.0024	No	0.0974	No
Isch_M_D21_1stOrder_Op	Isch_M_D21_2ndOrder_Op	0.0304	No	0.5498	No
Isch_M_D7_3rdOrder_Op	Isch_M_D21_3rdOrder_Op	0.0075	No	0.0004	Yes
Stem_M_D21_1stOrder_Op	Stem_M_D21_1stOrder_Shram	0.0057	No	0.2421	No
Stem_M_D21_2ndOrder_Op	Stem_M_D21_2ndOrder_Shram	0.0020	No	0.0365	Yes
Stem_M_D21_1stOrder_Op	Stem_M_D21_3rdOrder_Op	0.0444	No	0.5098	No
Stem_M_D21_2ndOrder_Op	Stem_M_D21_3rdOrder_Op	0.0358	No	0.0343	Yes
Stem_F_D21_3rdOrder_Op	Stem_M_D21_3rdOrder_Op	0.0489	No	0.3870	No

Paired t-test: Resting vs. Dilated Diameters:

Sex	Time Point	Anatomical Location	Limb	P-value	Different diameters?
Female	Day-7	ACC	Operated	1.078E-07	Yes
Male	Day-7	ACC	Operated	3.822E-03	Yes
Female	Day-21	ACC	Operated	2.130E-06	Yes
Male	Day-21	ACC	Operated	4.728E-10	Yes
Female	Day-7 Exercise	ACC	Operated	3.167E-04	Yes
Male	Day-7 Exercise	ACC	Operated	3.870E-05	Yes
Female	Day-7	PEC - Reentry	Operated	1.519E-02	Yes
Female	Day-7	PEC - Midzone	Operated	3.173E-02	Yes
Female	Day-7	PEC - Stem	Operated	1.146E-02	Yes
Female	Day-21	PEC - Reentry	Operated	2.629E-02	Yes
Female	Day-21	PEC - Midzone	Operated	1.590E-02	Yes
Female	Day-21	PEC - Stem	Operated	3.540E-02	Yes
Male	Day-7	PEC - Reentry	Operated	2.167E-03	Yes
Male	Day-7	PEC - Midzone	Operated	5.815E-03	Yes
Male	Day-7	PEC - Stem	Operated	6.895E-03	Yes
Male	Day-21	PEC - Reentry	Operated	8.218E-05	Yes
Male	Day-21	PEC - Midzone	Operated	1.452E-05	Yes
Male	Day-21	PEC - Stem	Operated	9.993E-04	Yes
Female	Day-7	Ischemic Tree 1st Order	Operated	3.096E-02	Yes
Female	Day-7	Ischemic Tree 2nd Order	Operated	2.675E-02	Yes
Female	Day-7	Ischemic Order 3rd Order	Operated	5.252E-02	No
Female	Day-21	Ischemic Tree 1st Order	Operated	3.640E-03	Yes
Female	Day-21	Ischemic Tree 2nd Order	Operated	4.230E-03	Yes
Female	Day-21	Ischemic Order 3rd Order	Operated	1.640E-02	Yes
Female	Day-7 Exercise	Ischemic Tree 1st Order	Operated	5.578E-05	Yes
Female	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	2.740E-04	Yes
Female	Day-7 Exercise	Ischemic Order 3rd Order	Operated	3.568E-03	Yes
Male	Day-7	Ischemic Tree 1st Order	Operated	1.321E-02	Yes
Male	Day-7	Ischemic Tree 2nd Order	Operated	1.240E-02	Yes
Male	Day-7	Ischemic Order 3rd Order	Operated	8.638E-02	No
Male	Day-21	Ischemic Tree 1st Order	Operated	1.200E-04	Yes
Male	Day-21	Ischemic Tree 2nd Order	Operated	4.292E-07	Yes
Male	Day-21	Ischemic Order 3rd Order	Operated	6.890E-05	Yes
Male	Day-7 Exercise	Ischemic Tree 1st Order	Operated	1.416E-05	Yes
Male	Day-7 Exercise	Ischemic Tree 2nd Order	Operated	7.125E-04	Yes
Male	Day-7 Exercise	Ischemic Order 3rd Order	Operated	7.334E-04	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Different diameters?
Female	Day-7	Stem 1st Order	Operated	4.419E-04	Yes
Female	Day-7	Stem 2nd Order	Operated	2.611E-04	Yes
Female	Day-7	Stem 3rd Order	Operated	9.409E-04	Yes
Female	Day-21	Stem 1st Order	Operated	1.500E-02	Yes
Female	Day-21	Stem 2nd Order	Operated	6.240E-04	Yes
Female	Day-21	Stem 3rd Order	Operated	1.010E-01	No
Female	Day-7 Exercise	Stem 1st Order	Operated	2.095E-05	Yes
Female	Day-7 Exercise	Stem 2nd Order	Operated	7.583E-05	Yes
Female	Day-7 Exercise	Stem 3rd Order	Operated	3.390E-04	Yes
Male	Day-7	Stem 1st Order	Operated	1.135E-02	Yes
Male	Day-7	Stem 2nd Order	Operated	2.372E-02	Yes
Male	Day-7	Stem 3rd Order	Operated	9.478E-02	No
Male	Day-21	Stem 1st Order	Operated	4.090E-03	Yes
Male	Day-21	Stem 2nd Order	Operated	5.600E-04	Yes
Male	Day-21	Stem 3rd Order	Operated	1.750E-03	Yes
Male	Day-7 Exercise	Stem 1st Order	Operated	2.850E-07	Yes
Male	Day-7 Exercise	Stem 2nd Order	Operated	2.073E-07	Yes
Male	Day-7 Exercise	Stem 3rd Order	Operated	4.410E-06	Yes
Female	Day-7	ACC	Sham	4.578E-06	Yes
Male	Day-7	ACC	Sham	3.534E-07	Yes
Female	Day-21	ACC	Sham	1.770E-05	Yes
Male	Day-21	ACC	Sham	6.418E-06	Yes
Female	Day-7 Exercise	ACC	Sham	8.198E-05	Yes
Male	Day-7 Exercise	ACC	Sham	7.536E-05	Yes
Female	Day-7	1st Order	Sham	8.650E-05	Yes
Female	Day-7	2nd Order	Sham	4.426E-05	Yes
Female	Day-7	3rd Order	Sham	1.022E-03	Yes
Male	Day-7	1st Order	Sham	3.511E-06	Yes
Male	Day-7	2nd Order	Sham	2.060E-04	Yes
Male	Day-7	3rd Order	Sham	2.129E-04	Yes
Female	Day-21	1st Order	Sham	7.669E-05	Yes
Female	Day-21	2nd Order	Sham	1.490E-04	Yes
Female	Day-21	3rd Order	Sham	2.323E-04	Yes
Male	Day-21	1st Order	Sham	5.768E-06	Yes
Male	Day-21	2nd Order	Sham	7.339E-06	Yes
Male	Day-21	3rd Order	Sham	5.365E-04	Yes
Female	Day-7 Exercise	1st Order	Sham	2.430E-04	Yes

Sex	Time Point	Anatomical Location	Limb	P-value	Different diameters?
Female	Day-7 Exercise	2nd Order	Sham	2.315E-05	Yes
Female	Day-7 Exercise	3rd Order	Sham	1.692E-03	Yes
Male	Day-7 Exercise	1st Order	Sham	2.934E-04	Yes
Male	Day-7 Exercise	2nd Order	Sham	1.379E-04	Yes
Male	Day-7 Exercise	3rd Order	Sham	7.627E-05	Yes