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Effect of Amputation on Muscle Structure Properties in a Rabbit Model

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To the Graduate Council:

I am submitting herewith a thesis written by Roy Caleb Stubbs entitled "Effect of Amputation on Muscle Structure Properties in a Rabbit Model." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biomedical Engineering.

Dustin, L, Crouch, Major Professor

We have read this thesis and recommend its acceptance:

Dustin L. Crouch, Jeff Reinbolt, David A. Anderson, Joshua T. Weinhandl

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Effect of Amputation on Muscle Structure Properties in a Rabbit Model

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ABSTRACT

After amputation, muscles in the residual limb are detached from their insertion points and no longer span the missing joints. Our objective was to quantify the effect of amputation-induced disuse on residual muscle structure, an indirect indicator of muscle force-generating capacity. One hind paw was surgically removed at the ankle joint of ten rabbits. At two weeks (n=5) and 4 weeks (n=5) post-amputation and for select muscles (gastrocnemius, soleus, tibialis cranialis, extensor digitorum, and flexor digitorum superficialis), we measured and computed several muscle structure properties. Additionally, we qualitatively assessed the muscle fiber appearance of histological samples at each timepoint. At 2 weeks post-amputation, most muscle parameters were comparable between the residual and contralateral intact limbs. At 4 weeks post-amputation, there was a non-significant but general trend of degeneration in all the residual muscles. Degeneration, by structure and histological appearance, was most pronounced in the flexor digitorum longus and soleus muscles. The observed muscle structure changes following amputation were similar to those reported for other disuse conditions such as tenotomy and joint immobilization. Our findings are clinically relevant because muscle degeneration could impair motor function of both the residual limb and emergent muscle-attached limb prostheses.

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ABBREVIATIONS

LG – lateral gastrocnemius

MG – medial gastrocnemius

SOL – soleus

TC – tibialis cranialis

ED – extensor digitorum

FD – flexor digitorum longus

CHAPTER ONE
INTRODUCTION

Acquired (i.e., traumatic or surgical) amputation severely disrupts many organ systems. For example, muscles and tendons are severed and released from their distal insertion points so that they no longer cross the missing joints. Despite the disruption, muscles in the residual limb appear to retain some useful, coordinated contractile function[1] that is exploited for controlling multi-functional myoelectric prostheses[2,3]. Such contractile function may be leveraged more directly by previous[1-3] and emergent[4,5] methods that involve physically attaching residual muscles to prosthetic limbs. Multi-articular residual muscles may also still cross and contribute to movement at one or more residual joints.

Though residual muscles support prosthetic and residual limb function, their structural integrity following amputation is unknown. Muscle structure is considered an indirect indicator of muscle force-generating capacity[6-8]. Following amputation, it is expected that residual muscles retract and essentially become immobilized in a shortened position. Residual muscles that cross an intact residual joint will still experience some forces and length changes, though they are likely altered from those of the intact limb. Other conditions of muscle disuse or decreased use, such as tenotomy[4] and limb immobilization[5], lead to degenerative changes in muscle structure such as decreased muscle mass (i.e., atrophy), optimal fiber length (*i.e.*, fewer sarcomeres in series), and fiber cross-sectional area. Such muscle structure changes are expected to reduce both the range of lengths over which a muscle can produce active force and the maximal force a muscle can produce[6-8].

The objective of our study was to quantify muscle structure in select residual muscles (gastrocnemius, soleus, tibialis cranialis, extensor digitorum, and flexor digitorum superficialis) following unilateral hind-paw amputation. For evaluation of the effect of amputation independent of muscle retraction (shortening) after tendon release, the muscles were held approximately at their pre-amputation *in situ* lengths by suturing their

tendons to the distal end of the tibia. We hypothesized that muscle structure property values of the select muscles would be lower, or more degenerated, in the residual limb than in the intact contralateral limb.

CHAPTER TWO

METHODS

All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee. 10 healthy, 13-week-old New Zealand white rabbits underwent unilateral (right) hind paw disarticulation of the tibiotarsal (ankle) joint. Once the rabbits were anesthetized, the skin surrounding and about 2-3 cm distal to the ankle was retracted.

The insertion tendons of the triceps surae muscle group (i.e., the Achilles tendon), consisting of the lateral and medial gastrocnemius and soleus muscles, and the tibialis cranialis muscle were sutured to the distal end of the tibia to hold the muscles approximately at their pre-amputation lengths. These specific muscle-tendons were selected because of their known contribution to ankle plantarflexion and dorsiflexion, respectively; these muscles will be attached to a jointed, implanted foot-ankle prosthesis that we are currently developing and testing[4,5]. At the level of the ankle, a hemostat was clamped to the insertion tendons of the triceps surae muscle group (i.e. the Achilles tendon) and the tibialis cranialis muscle to limit their proximal retraction. The insertion tendons were cut distal to the hemostat or, for unclamped muscles, at the level of the ankle. The remaining tissues connecting the tibia and fibula to the foot were cut and the hind paw was removed. The retracted skin was replaced and sutured together over the end of the shank.

Immediately after surgery, the limb was bandaged up to the base of the knee (without immobilizing the knee) for at least 2 weeks until the incision healed. The bandage was changed at least once every three days. First, silver sulfadiazine (SSD) topical cream was applied over the incision to prevent infection. The limb was then bandaged using, from inner to outer layers, non-adherent dressing (Telfa, Covidien), undercast padding, elastic bandage wrap, elastic tape (ELASTIKON, Johnson & Johnson), and bandage tape to protect the incision site. We administered an analgesic of either buprenorphine (0.03 mg/kg) subcutaneously or hydromorphone (0.2 mg/kg) intramuscularly every 6 hours for at least 72 hours post-surgery; antibiotics (enrofloxacin 5 mg/kg diluted) either subcutaneously or orally every 12 hours for at least 7 days post-surgery; and an anti-inflammatory drug (meloxicam 0.6 mg/kg) either subcutaneously or orally every 24 hours for at least 7 days post-surgery. Rabbits were given 15 minutes of pen time every day starting the first or second day post-surgery; after bandages were removed, pen time was increased to 30-45 minutes a day. All rabbits also received daily enrichment in the form of music, audiobooks, treats, fresh greens, chewing blocks, and toys.

The rabbits were divided into two groups. Rabbits in each group were euthanized at either 2 weeks (2W) or 4 weeks (4W) after amputation by anesthetic barbiturate overdose. The residual and contralateral intact hindlimbs were detached at the hip and fixed in formalin for at least 3 days with the knee in a neutral posture. After fixation, limbs were transferred to either phosphate-buffered saline (3 samples in 2W group) or ethanol (remaining samples).

The lateral gastrocnemius (LG), medial gastrocnemius (MG), soleus (SO), tibialis cranialis (TC), extensor digitorum (ED), and flexor digitorum superficialis (FDS) muscle-tendon units were dissected from the bone. The tendons were removed from the muscles approximately at the muscle-tendon junctions. After blotting muscles dry with a paper towel, we measured muscle length using a digital caliper and mass using a digital scale. We measured muscle fiber length along the direction of the muscle fibers at the mid-belly of each muscle. Sarcomere lengths were measured by laser diffraction[6] using a He-Ne laser (HNLS008L, Thorlabs); samples of fibers were taken from the mid-belly of the muscle and placed on a glass microscope slide. Sarcomere length was calculated using the following equation:

$$SL = \frac{n\lambda}{(sin\left(tan^{-1}\left(\frac{x_n}{h}\right)\right))} \qquad \text{Equation 1.}$$

where x_n is the measured width between the bands (mm), n is the order of the band measured, h is the distance between the slide and the projection screen, and λ is the wavelength of the laser. Muscle fiber pennation angle was measured by overlaying a goniometer[15] along the middle of the muscle belly. The physiological cross-sectional area ($PCSA$) was calculated from the measured muscle mass and fiber length using the following formula:

$$PCSA = \frac{(muscle\ mass) * cos\ (\theta)}{(fiber\ length) * 1.054} \qquad \text{Equation 2.}$$

where 1.054 is the reported density of skeletal muscle (g/cm^3)[6]. Optimal fiber length, also called optimal fiber length (OFL), was calculated using the formula:

$$OFL = MFL * (\frac{OSL}{MSL}) \qquad \text{Equation 3.}$$

where MFL is the measured fiber length, OSL is the optimal sarcomere length (≈2.2μm)[6], and MSL is the measured sarcomere length. Optimal fiber length is proportional to the range of muscle lengths over which the muscle can produce active force[7,8], and PCSA is proportional to the maximum force a muscle can produce[9]. For each muscle structure property, we computed the percent difference ($PD\%$) in group means, $\underline{x}$, between sides as:

$$PD\% = \left(\frac{\left|\underline{x}_{residual}\right|}{\underline{x}_{intact}}\right) \times 100 \qquad \text{Equation 4.}$$

At the muscle mid-belly, muscles were sectioned perpendicular to the long axis of the fibers for histology and stained with standard H&E stain. All images of the stained muscle fibers were collected using a microscope (MU1403, AmScope) at 20x magnification and converted to digital images using a digital camera.

CHAPTER THREE

STATISTICAL ANALYSIS

We used a mixed-effects ANOVA model with the fixed effects “muscle”, “timepoint”, and “limb” (residual vs contralateral intact) and their interaction terms, with the random effects as rabbit, the rabbit- timepoint, and the rabbit-timepoint-limb. The dependent variables in each iteration of the mixed-effect ANOVA model were muscle mass, muscle length, pennation angle, fiber length, sarcomere length, OFL, and PCSA. The Shapiro-Wilk and Anderson-Darling tests and QQ normality plots were performed to evaluate the normality of ANOVA residuals. The residuals were not normally distributed for two of the dependent variables, fiber length and optimal fiber length; therefore, the values of these variables were logarithmically transformed. The least square means was computed and separated with the Tukey's HSD (honestly significant difference) test. All statistical assumptions regarding normality and equality of variances were met after making the logarithmic transformations. $p<0.05$ was considered significant. All statistical analyses were performed with statistical analysis software (JMP Pro version 16, SAS).

CHAPTER FOUR

RESULTS AND DISCUSSION

Results

Muscle mass was not statistically significantly different between limbs for any muscle at timepoint 2W. At 4W, there was a significant difference between limbs for FD (PD%=56.5±16.6, p=0.028). A large between-limb difference was also observed for SO at 4W, but the difference was not statistically significant (PD%=34.62±55.98 p=.2).

Muscle length was comparable between the intact and residual limbs. However, there was a consistent trend of lesser muscle length in the residual limb at timepoint 4W.

Pennation angle was not significantly different between limbs for any muscle or timepoint. There was a large but non-significant difference in pennation angle between limbs for LG at timepoint 4W (PD%=162.34±15.92, p=0.76).

At timepoint 4W, PCSA of FD was significantly smaller in the residual limb than in the contralateral intact limb (PD%=52.02±29.53, p=0.017). PCSA was smaller, but not significantly so, in the residual limb for SO and ED at 2W and for SO and FD at 4W.

There was no significant difference in measured or optimal fiber lengths between limbs for any muscle and timepoint. Measured fiber lengths of FD showed an increase and the greatest change in fiber length in comparison to the intact (p=.71). There was a trend of decreasing measured fiber lengths from timepoints 2W to 4W for the LG, MG, SO, TC, and ED muscles, which follows the trend of muscle length noted above. Mean values of measured and optimal fiber lengths were similar to one another for all muscles and timepoints.

Sarcomere length didn't show any statistically significant changes, but there were observable changes that occurred at both the 2 and 4 week timepoints. After 2 weeks, there were increases in the sarcomere length of the residual LG, SO, and FD in comparison to the intact muscles, in contrast the residual MG, TC, and ED showed decreases in sarcomere length compared to the intact muscles. After 4 weeks, there were greater increases in the sarcomere length of the residual LG, MG, SO (p=.17), and FD in comparison to the intact muscles, in contrast the residual TC, and ED showed decreases in sarcomere length compared to the intact muscles.

Qualitatively, there were no apparent bilateral differences in muscle fiber diameter, shape, and pigmentation in the glycolytic muscles such as the lateral gastrocnemius in the rabbits euthanized 2-weeks post surgery (Figure 8). However, in the rabbits euthanized after 4 weeks, there was adipocyte formation, nucleation, a small drop in fiber size, and the fibers were more pale in comparison to the intact fibers. The oxidative muscles such

as the residual soleus in both the 2 and 4 week groups appeared degenerated compared to the intact soleus (Figure 9), with smaller, paler fibers (indicating lower myoglobin content), more nuclei at the muscle fiber periphery, and adipocytes within the epimysium.

Discussion

In previous studies muscle structure degeneration has been well characterized for other decreased-use conditions such as tenotomy[16] and immobilization[17]. In our study, after 2 weeks we observed the largest bilateral differences were in the soleus and flexor digitorum superficialis, while not significant, they showed a large general trend of degeneration. After 4 weeks a general trend of degeneration was noticeable across all measured muscles, but still the largest bilateral differences occurred in the soleus and flexor digitorum superficialis.

Residual muscle degeneration has significant functional implications for people with amputation. For example, muscle degeneration degrades voluntary muscle activation[10], which could impair amputees' function with myoelectric prostheses. Even residual muscles that crossed the knee (e.g. gastrocnemius and tibialis cranialis) were degenerated, which would likely impair residual joint function in patients.
The soleus exhibited the most severe observable degeneration of all the select muscles, with a few possible explanations. For one, the soleus did not cross the residual knee like some other residual muscles such as the gastrocnemius. A second possible explanation is the difference in fiber type composition among muscles. The soleus is primarily(98-100%)[18] made of type I (slow oxidative) muscle fibers[11], whereas other residual muscles such as the gastrocnemius[12], extensor digitorum longus(93%)[13] and tibialis cranialis(96%)[13] are primarily composed of type II (fast glycolytic) muscle fibers. A previous *in vivo* study involving NZW rabbits reported similar rapid, substantial degeneration of the soleus muscle two weeks after Achilles tenotomy[11]. Other studies have shown that severe injury and disuse can cause type 1 fibers to transition into type 2 fibers due to their high metabolic cost, however type 2 muscle fibers only transition between type 2a and type 2b except for when undergoing chronic electrical stimulation[20]. Thus, muscles primarily comprised of type 1 fibers are more prone to change than muscles comprised primarily of type 2 fibers.

The flexor digitorum longus was the only muscle to display statistically significant changes out of the select muscles. Similar to the soleus, the flexor digitorum longus does not cross the knee. In addition, while the fiber type composition of the flexor digitorum in NZW rabbits has not been previously classified, in rats the flexor digitorum muscles are classified as primarily type 2[22], which contrasts the type 1 fiber type composition of the soleus. In a biomechanics study of NZW rabbits, the flexor digitorum longus was determined to be the hindlimb muscle primarily responsible for stabilizing the ankle

during the stance phase of hopping by bearing the majority of the load[21]. Due to the importance of this muscle in the stability of the ankle, it's possible that upon disarticulation, the flexor digitorum was more impacted than muscles such as the gastrocnemius, soleus, and tibialis cranialis which play a lesser role in the stability of the ankle during stance phase.

We sutured the insertion tendons of the tibialis cranialis and triceps surae (soleus and gastrocnemius) muscles to the distal end of the tibia so that they would remain at their approximate pre-amputation *in situ* length. Previous immobilization studies showed that muscles fixed at longer, rather than shorter, lengths experience less degeneration[5]. Surgeons often manipulate the residual muscles in various ways to shape the residual limb for prosthesis sockets[14]; suturing residual muscles to bone is one such manipulation, but muscles may not stretched back to their pre-amputation in situ length in such cases. Muscles that are not manipulated are left untethered at their distal ends and, thus, remain at a chronically shortened length. In a future study we will rigorously test our hypothesis that suturing residual muscles at their pre-amputation *in situ* length preserves residual muscle structure.

Despite suturing the tendons to bone, muscles in the residual limb were shorter, though not significantly so, in the residual limb than in the contralateral intact limb at 4 weeks post-amputation. One possible explanation for this is that the lower optimal fiber length (i.e., fewer sarcomeres in series), increased passive tension in the muscles relative to the tendon, causing the muscle to shorten. Possibly, our suturing technique did not prevent all muscle retraction at the time of surgery. Another possibility is that continued growth of the rabbit in the weeks following surgery resulted in an increased muscle length in the intact limb while the muscle lengths in the residual limb did not change. While not significant there was a trend which showed that the average intact muscle at 4 weeks had a greater muscle length than the average intact muscle at 2 weeks. We did not measure muscle or tendon lengths at the time of surgery or before dissection (though the muscles were fixed *in situ*), which could help determine when the length changes occurred; we will make such measurements in future studies.

In comparison to the changes in muscle 4 weeks post-surgery, muscle fiber length and optimal fiber length followed almost the exact same trend. After 2 weeks there was no noticeable change in muscle or optimal fiber length between the intact and residual muscles, but after 4 weeks, the residual fiber and optimal fiber lengths were lower than the intact. A couple of things to note that could contribute to these results are that there were greater weight differences between the 4 week rabbits immediately post-surgery to when they were euthanized in comparison to the 2 week rabbits. The data also shows that the muscle lengths, fiber lengths, and optimal fiber lengths of the residual muscles after 4 weeks are slightly smaller than those of the intact and residual muscles at 2 weeks, but the rabbits in the 2 weeks study on average had higher masses at surgery as well as at euthanasia which could influence the comparative results and indicate that the differences at 4 weeks were due to the post-surgical growth of the rabbits.

Though none of the changes were statistically significant, there were large bilateral differences in mean pennation angles for the gastrocnemius and extensor digitorum muscles that differed from our expectations. For a given muscle length, when a muscle atrophies (i.e., loses mass), its diameter decreases. As this happens, we would expect the pennation angle of the muscle fibers to also decrease. Our observations, in contrast, showed that the pennation angle of the gastrocnemius and extensor digitorum muscles at 4 weeks post-amputation was greater on the residual side despite having a lesser mean mass (bilateral mass difference was not significant). This could have been due to the fact that the same muscles were also shorter on the residual side, which would tend to increase the pennation angle. Since bilateral differences in pennation angle were not statistically significant, the larger pennation on the residual side could have been due to chance; this is supported by the fact that pennation angle was highly variable among rabbits in each group and our study sample size was small.

Since physiological cross-sectional area (PCSA) was calculated using the measured pennation angles, some PCSA results were also unexpected. For example, atrophy would be expected to decrease PCSA. However, at 4 weeks post-amputation, PCSA of the gastrocnemius and extensor digitorum muscles was not statistically or qualitatively different between limbs despite a large but non-significant bilateral difference in muscle mass.

There was a trend of greater sarcomere lengths in all residual muscles except extensor digitorum at both 2 and 4 weeks post-amputation. Coincidentally, measured muscle fiber lengths were also shorter on the residual side, though not significantly so, in the same muscles at 4 weeks post-amputation. Therefore, one potential explanation for greater sarcomere lengths is that there was a reduction in the number of sarcomeres in series post-amputation, causing the remaining sarcomeres to elongate. It is hypothesized that, when muscle fibers are chronically shortened, sarcomeres are shorter than their optimal length; this stimulates the removal sarcomeres in series so that the remaining ones elongate toward optimal length. In another study that involved tenotomy of the extensor digitorum muscle in NZW rabbits[16], sarcomeres were also longer than normal at 21 days post-surgery.

Our pilot study had several limitations. We used the contralateral intact limb as the experimental control; the rabbits' increased reliance on the intact limb post-amputation may have caused confounding muscle changes (e.g. hypertrophy) in the contralateral intact limb. Variabilities in surgical technique such as not anchoring the tendons at a consistent, determined length at surgery could have resulted in varied muscle and fiber lengths. We had a small sample size of 10 unique rabbits, we tried to keep our methodologies as consistent as possible, however each rabbit and their muscles were unique in their own ways. The differences in the rabbits and the development of their muscles sometimes led to a single data point of a structural property in some cases creating a larger standard deviation or the difference between a comparison being statistically significant or not. A larger sample size would have helped to eliminate the

noise and create a more consistent data set, less impacted by the high biological variability of individuals. The small sample size could also be the reason that a logarithmic transformation had to be performed for muscle fiber length and optimal fiber length to achieve normality. There was a large time gap in between when G06, G07 and G08 were dissected and originally measured, the muscles from these rabbits were fixed in a saline buffer and some were not in a condition to be re-measured, reducing the sample size of the 2 week group. The extensor digitorum and flexor digitorum longus were not collected for G06 and were harvested from G07, G08, and G18 by a different lab member than the one who dissected and harvested the rest of the muscles, which could lead to some inconsistencies in the 2 week groups measurements. The time gap and any changes in the enrichment protocol between the rabbits could have also impacted their rehabilitation process and therefore impacted the adaptation of the muscles. All intact muscles were re-measured for consistency.

CHAPTER FIVE

CONCLUSIONS

In conclusion, our data indicated that, in rabbits with hindlimb ankle disarticulation, muscle structure in the soleus and flexor digitorum longus can degenerate after only two weeks of amputation, as well as in the rest of measured muscles after 4 weeks. Post-amputation muscle degeneration may have meaningful adverse effects on prosthetic and residual limb function. Specifically, our team is currently developing and testing a muscle-driven endoprosthesis (MDE) that is physically attached to and articulated by residual muscles. Such a device could enable realistic anatomical musculoskeletal reconstruction as well as more realistic sensorimotor function in comparison to externally worn prostheses. Understanding the changes in muscle structural integrity over time can inform MDE implantation timing and subsequent rehabilitation strategies. Our results motivate additional research to quantify the extent and rate of post-amputation muscle degeneration, identify factors that determine a muscle's susceptibility to degeneration, and identify practical surgical approaches to preserve muscle structure.

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APPENDIX

Figure Legends

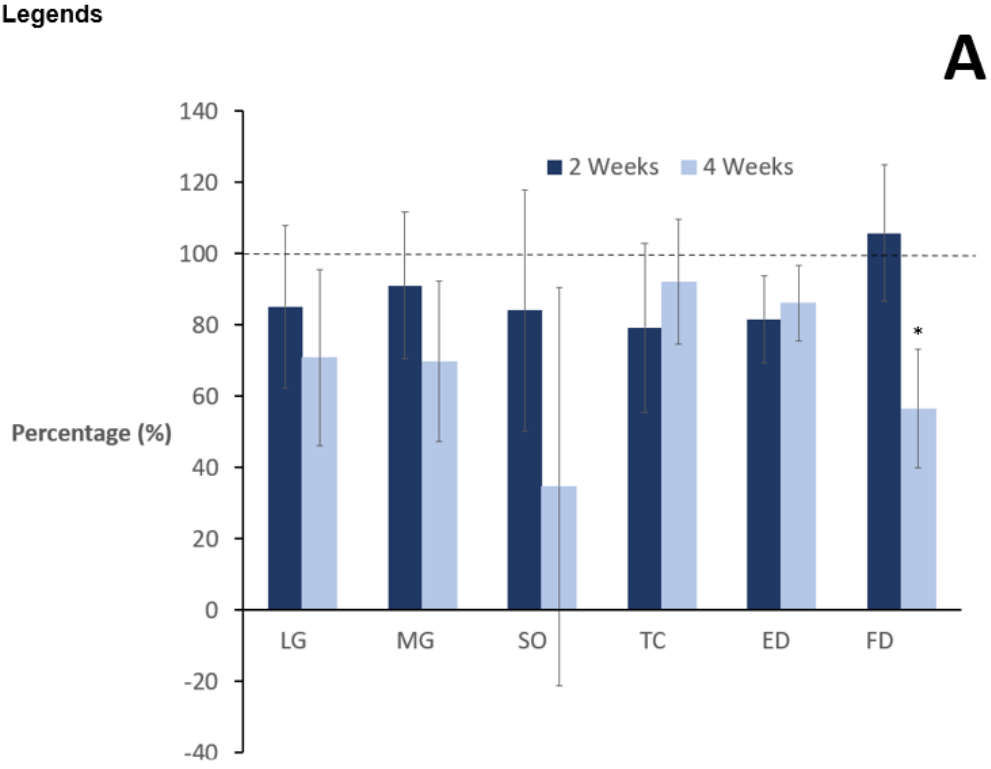

Figure 1.A: Percent difference comparison of muscle mass between the intact and residual limbs after 2 and 4 weeks post-surgery.

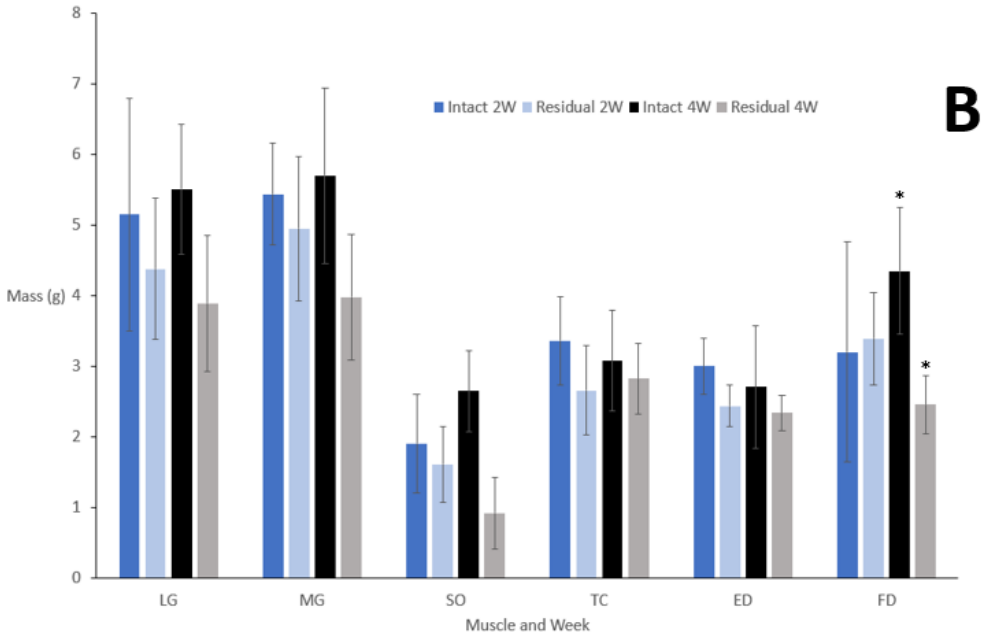

Figure 1 continued: B. Raw data value comparison of intact and residual muscle masses.

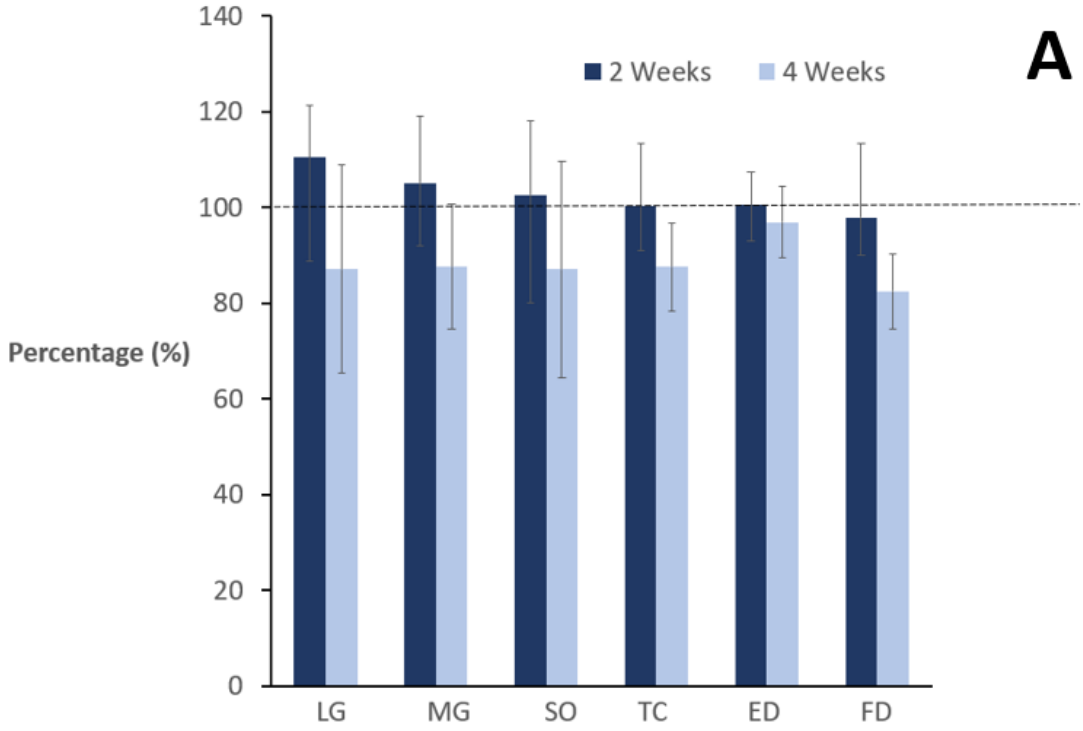

Figure 2.A: Percent difference comparison of muscle length between the intact and residual limbs after 2 and 4 weeks post-surgery.

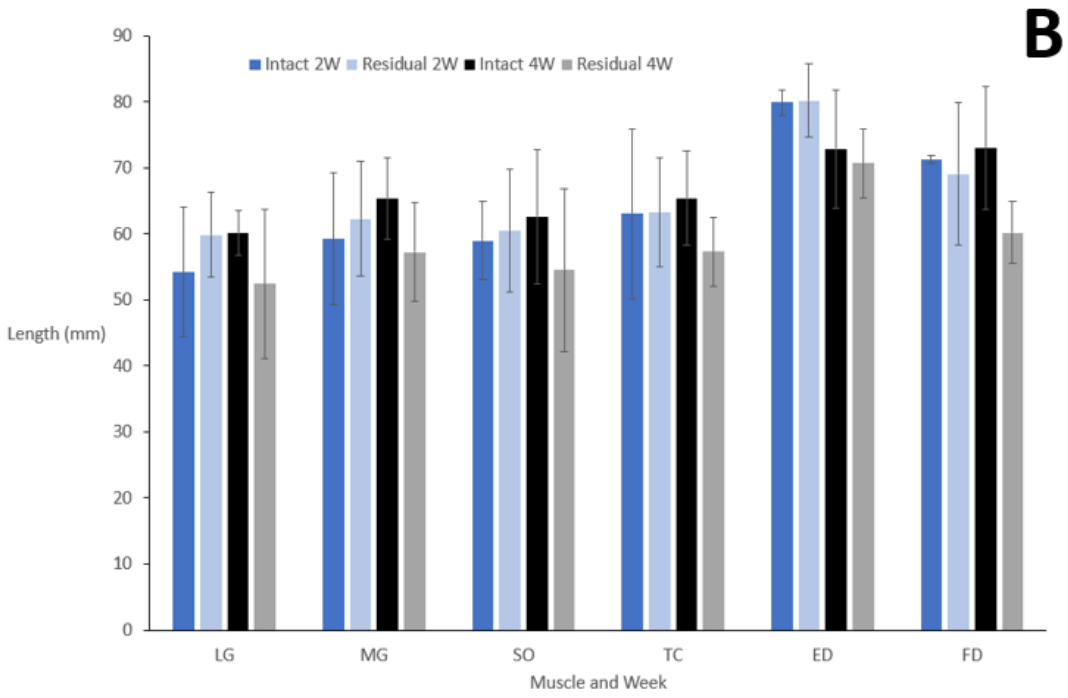

Figure 2.B: Raw data value comparison of intact and residual muscle lengths.

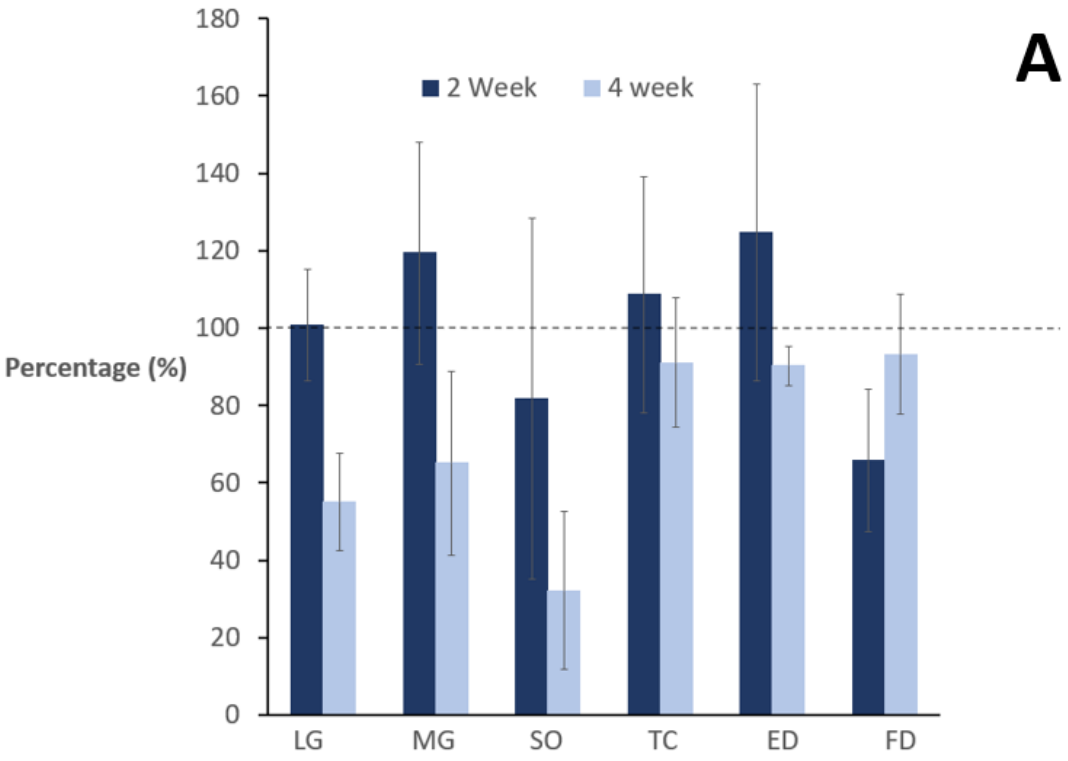

Figure 3.A: Percent difference comparison of fiber length between the intact and residual limbs after 2 and 4 weeks post-surgery.

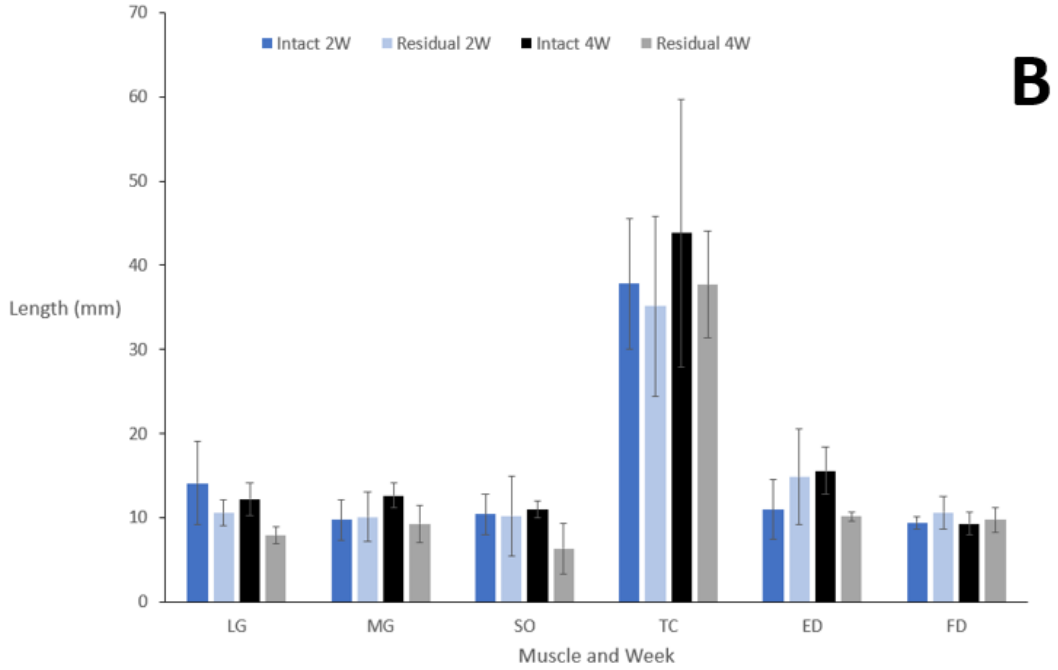

Figure 3.B: Raw data value comparison of intact and residual muscle fiber lengths.

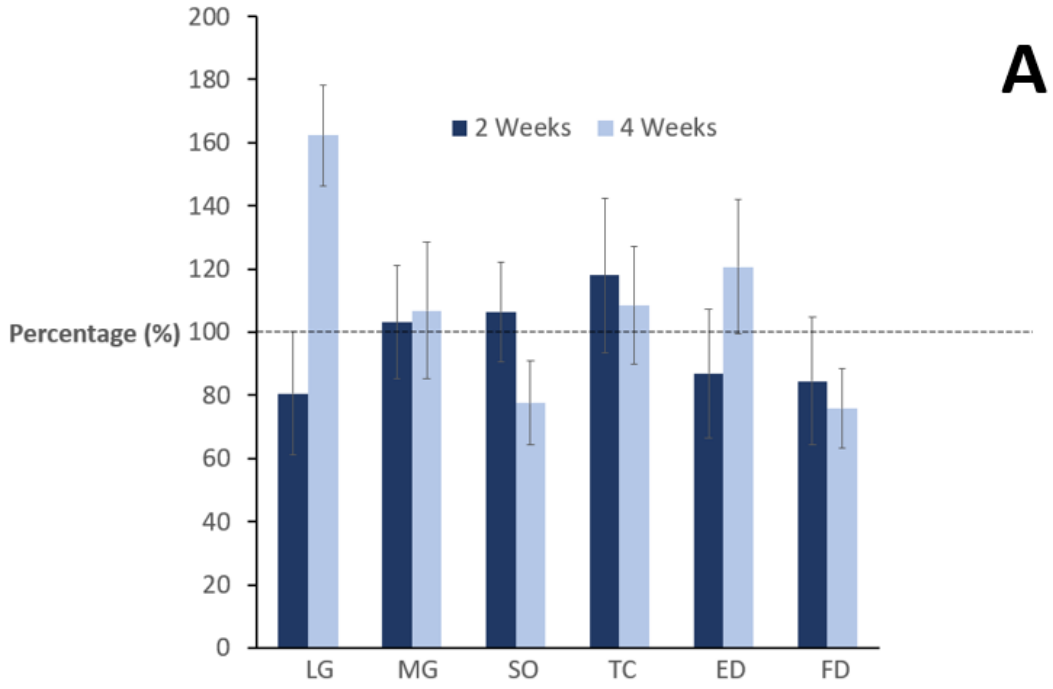

Figure 4.A: Percent difference comparison of pennation angle between the intact and residual limbs after 2 and 4 weeks post-surgery.

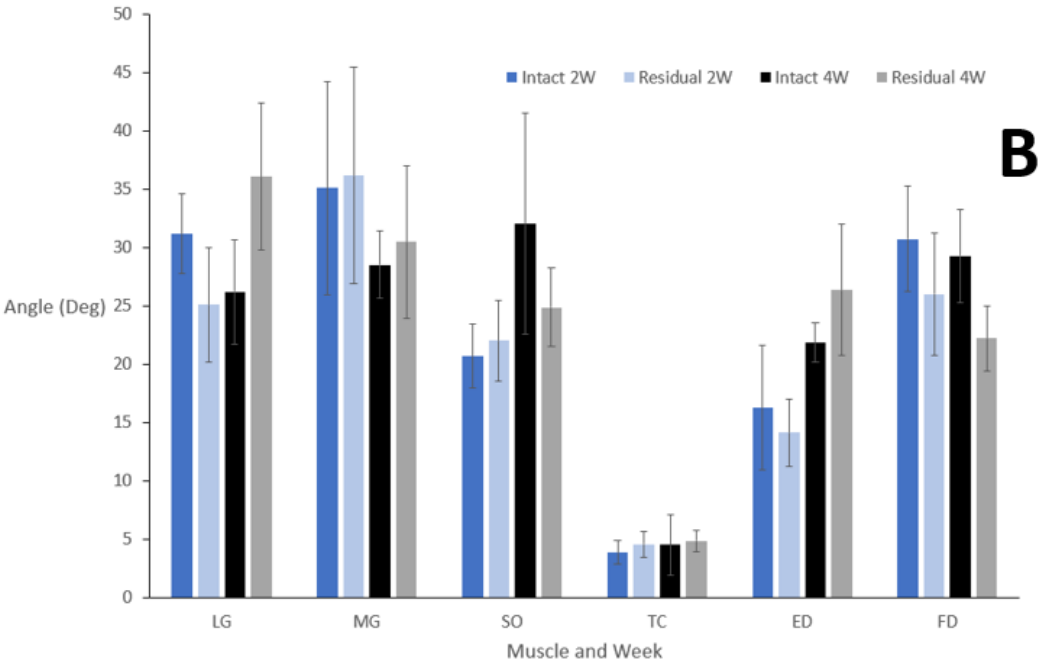

Figure 4.B: Raw data value comparison of intact and residual pennation angles.

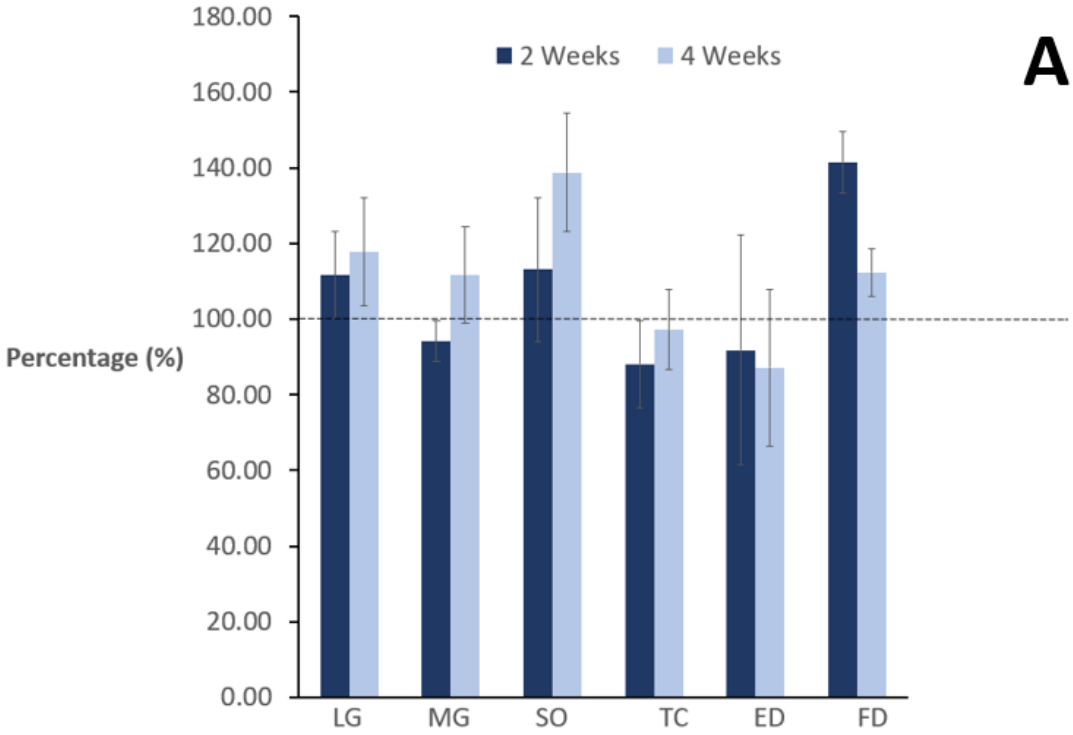

Figure 5.A: Percent difference comparison of sarcomere length between the intact and residual limbs after 2 and 4 weeks post-surgery.

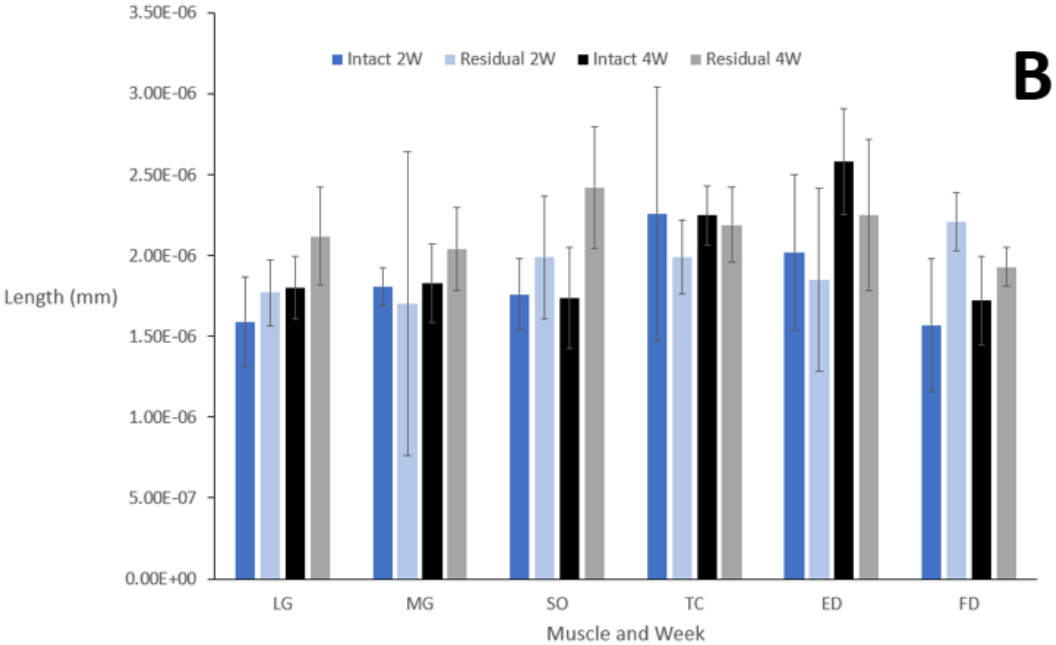

Figure 5.B: Raw data value comparison of intact and residual sarcomere lengths.

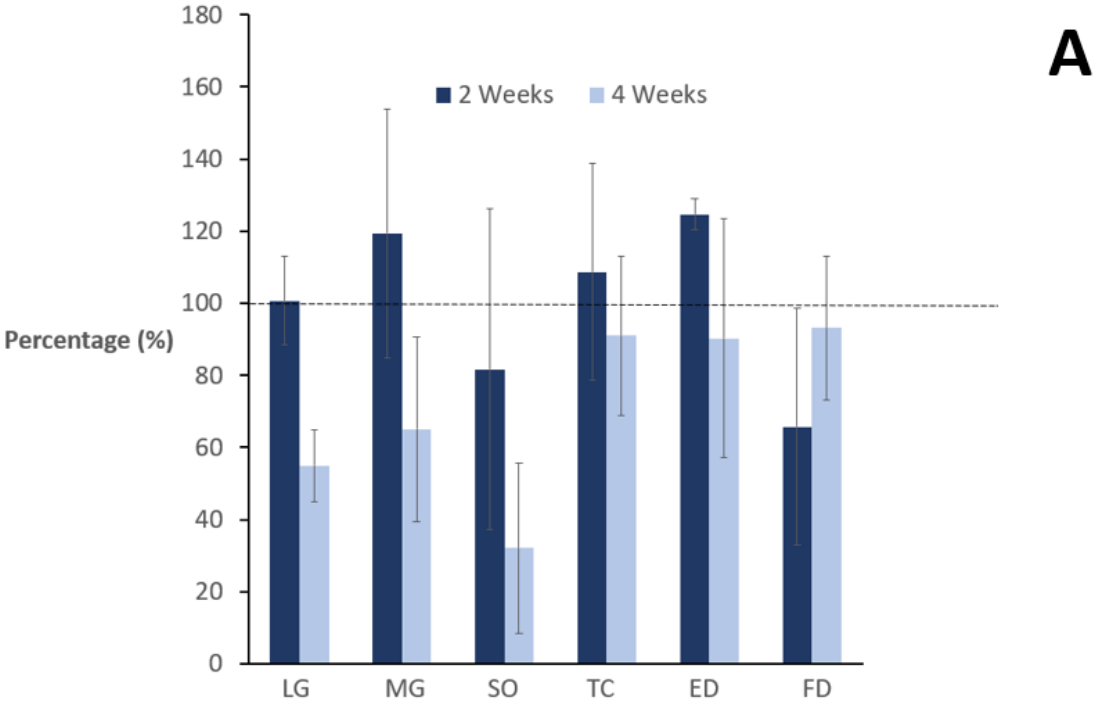

Figure 6.A: Percent difference comparison of optimal fiber length (OFL) between the intact and residual limbs after 2 and 4 weeks post-surgery.

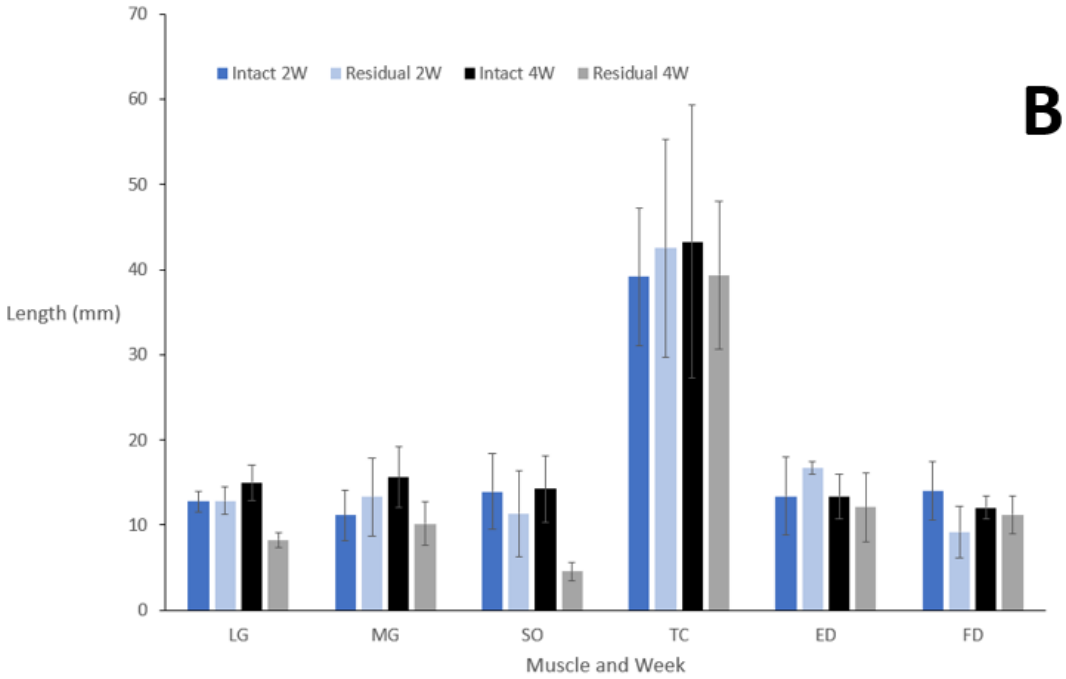

Figure 6.B: Raw data value comparison of intact and residual optimal fiber lengths.

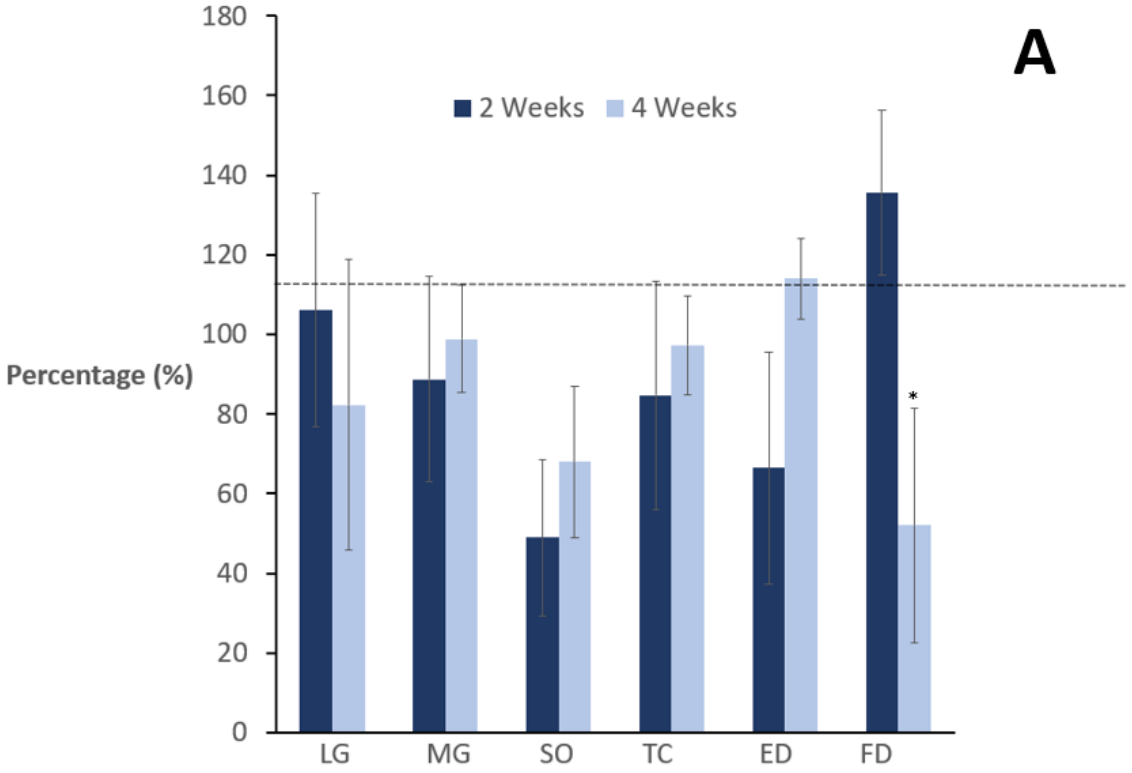

Figure 7.A: Percent difference comparison of physiological cross-sectional area (PCSA) between the intact and residual limbs after 2 and 4 weeks post-surgery.

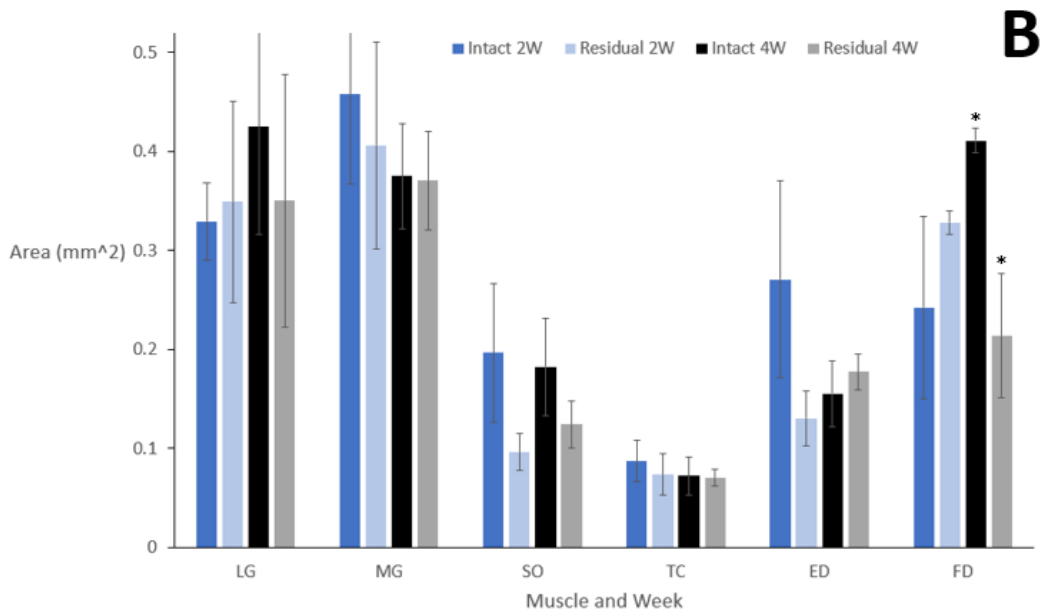

Figure 7.B: Raw data value comparison of intact and residual PCSA's

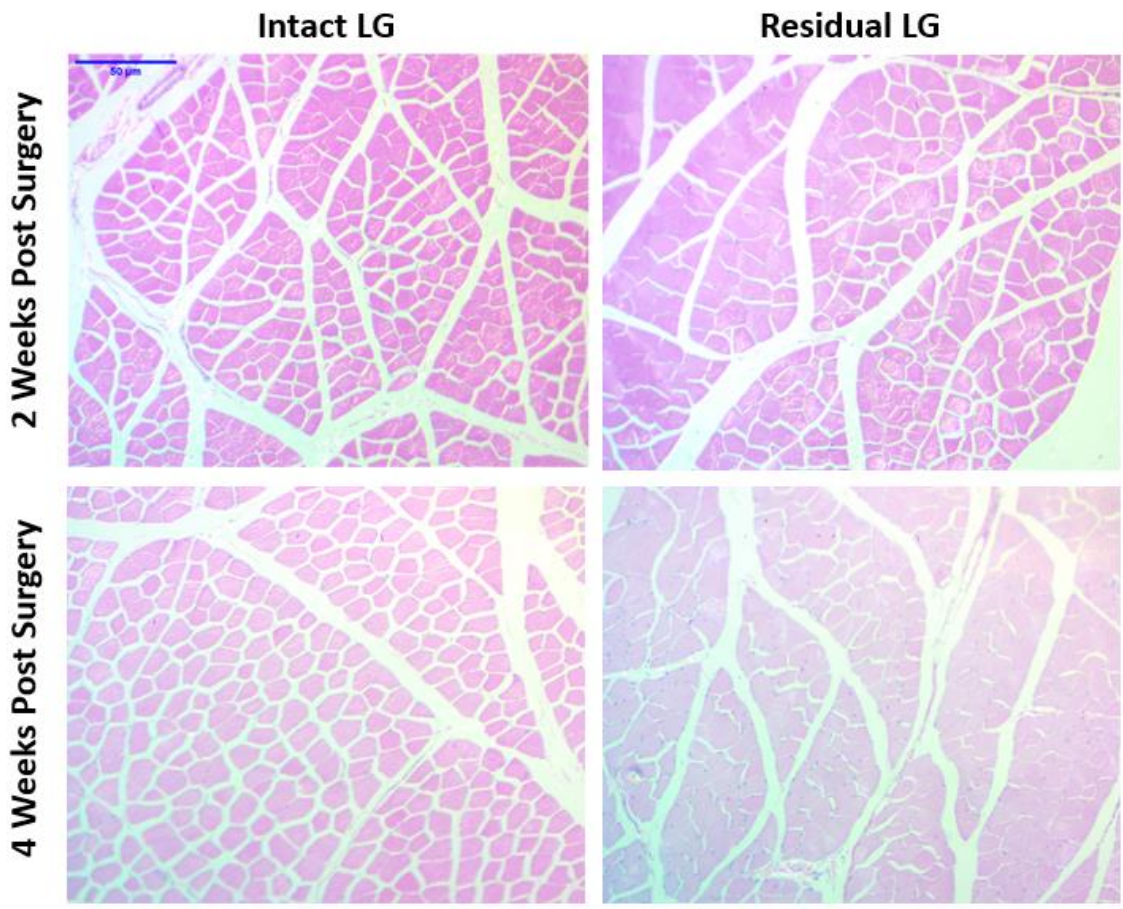

Figure 8.A: Histological sections of a glycolytic muscle (LG) of interest from rabbits G18 (2 week) and G24 (4 week). After 2 weeks there were no significant differences, but after 4 weeks the muscle fibers were smaller, paler, and more nucleated than the intact lateral gastrocnemius (LG), adipocytes were also present. H&E stain, 20x magnification. Blue bar = 50 μm.

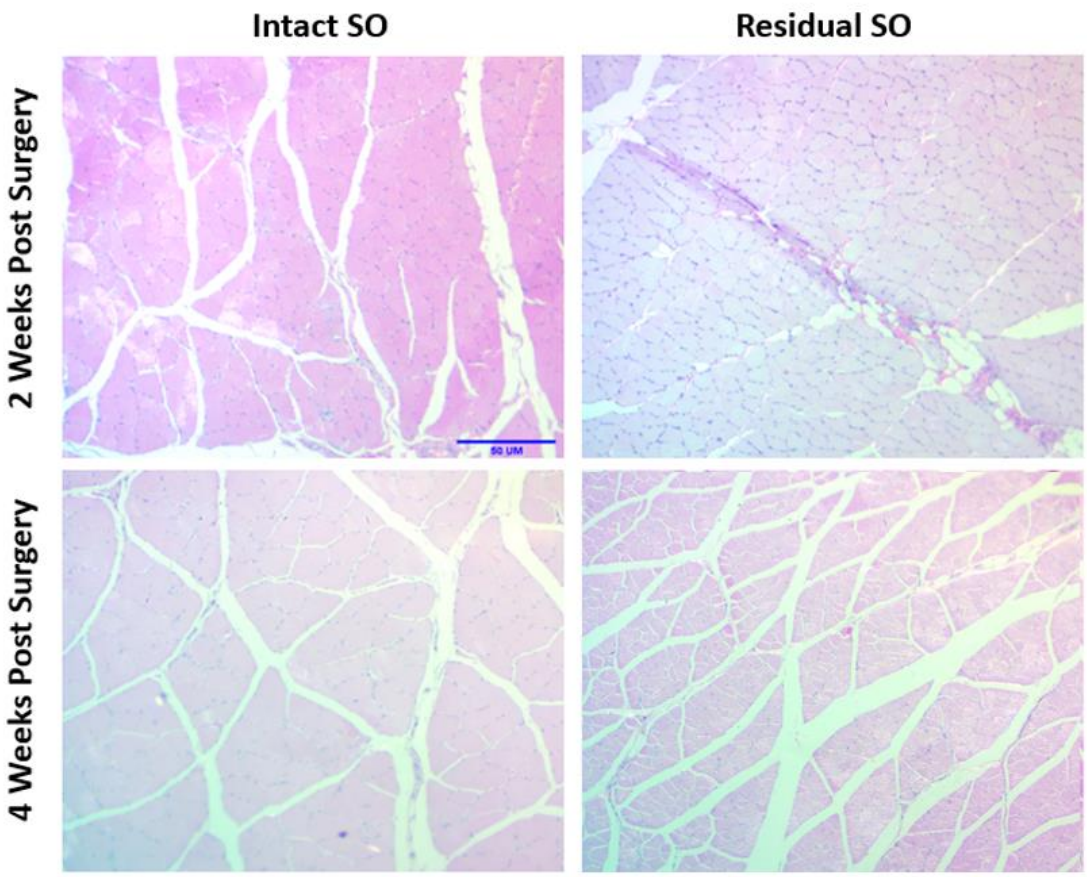

Figure 9.A: Histological sections of an oxidative muscle (SO) of interest from rabbits G18 (2 week) and G24 (4 week). After 2 and 4 weeks the muscle fibers were smaller, paler (2 week), and more nucleated than the intact soleus (SO), adipocytes were also present. H&E stain, 20x magnification. Blue bar = 50 μm.

VITA

Roy Caleb Stubbs was born August 24th, 1995. He grew up as a runner and an actor, living in multiple states such as Kentucky, Massachusetts, West Virginia, and Texas before moving to Tennessee to pursue engineering. During his time as an engineering student at Lipscomb University, Roy served as a student leader within the mechanical engineering department, being class representative freshman and sophomore years, while serving as vice president then president junior and senior years. He was ran cross country and track and field for the school, was an all-conference athlete in academics, a member of the honors college, worked as both an athletic tutor and as a dog runner, was responsible for STEM outreach and student engagement for the mechanical engineering department, and graduated with a Bachelors of Science in Mechanical Engineering with a Minor in Applied Mathematics. After college Roy worked as an associate quality engineer for a year before enrolling as a graduate student at the University of Tennessee Knoxville, where under Dr. Dustin Crouch in the Upper Limb Assist Lab, he researched muscle driven endoprostheses, artificial tendons, and the effects of amputation on muscle structural properties. He also invested a large deal of time into working with research animals and learning more about animal sciences in the hopes that one day he could combine the different fields that he'd worked in to assist both animals and humans. Roy is currently aspiring to work as a biomedical engineer in Nashville Tennessee after graduation.