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TITLE: Follistatin: A Potential Anabolic Treatment for Re-Innervated Muscle

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14. ABSTRACT

Follistatin is a possible anabolic treatment for denervation atrophy induced muscle weakness following prolonged denervation. Adenovirus delivery of Recombinant FS-288 DNA isoform does not induce hypertrophy in normal rodent gastrocnemius muscle. The efficacy of Adenoassociated virus delivery of FS-288 DNA or direct delivery of recombinant FS-288 protein is currently not known.

15. SUBJECT TERMS

FOLLISTATIN, ANABOLIC, DENERVATION ATROPHY, NERVE INJURY, NERVE REPAIR, MUSCLE

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. **INTRODUCTION:**
2.

Functional recovery following major peripheral nerve injuries is often suboptimal despite adherence to well accepted nerve repair principles. Though a multifaceted problem, the poor muscle functional recovery often seen following nerve regeneration is in large part due to the progressive catabolic process affecting muscle fibers called "denervation atrophy." While many researchers have approached this issue by attempting to improve axonal regeneration speed, efficiency, and accuracy (and thereby limiting the degeneration of the muscle), we have sought treatment options aimed at maximizing the potential of the muscle fibers that were able to achieve reinnervation. After experimenting with anabolic steroids (nandrolone), we determined that a more potent but safer anabolic agent would be a better option. Follistatin is a glycoprotein that both blocks the muscle inhibiting peptide myostatin and possesses remarkable independent muscle stimulating properties as well. We hypothesized that the administration of recombinant follistatin delivered to rodent muscles subjected to prolonged but temporary denervation periods (of either 3 or 6 months) would improve final muscle recovery and function. Most published studies have delivered the follistatin as recombinant DNA though some successful administration of recombinant protein has been demonstrated as well leading us to form two wings for our study—one exploring recombinant DNA administration and one exploring protein administration.

3. **KEYWORDS:**

Denervation atrophy, anabolic, follistatin, nerve injury, nerve repair, muscle

4. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

- ***Specific Aim 1:*** **Utilize an established rodent model of denervation atrophy**
- Regulatory Review and Approval Process- complete
- Testing the Protein Stability- complete
- Pilot Study (N=15; Follistatin recombinant DNA, Protein, and Alzet Pump Control Groups). Each group has 5 animals. (100% complete)
- Denervation of hind limb muscles (3 and 6 months) Twelve groups (N=12; total of 144 rodents) were divided into control (sham surgery, sham treatment), sham surgery, sham treatment, and experimental groups (denervation surgery + treatment). Experimental and sham treatment groups underwent left tibial nerve transection to denervate left gastrocnemius muscle. Control and sham surgery groups underwent exposure of the nerve without transection. - (100% complete)
- Re-innervation of hind limb muscles. (3 and 6 months) Denervation was reversed by repairing the transected tibial nerve using graft obtained from contralateral tibial nerve. Control rats underwent harvest of graft without repair. - (100% complete)

- Specific Aim 2: **Treat re-innervated muscle with Follistatin:**
  Recombinant DNA and AAV was provided by Vector BioLabs; BioVision provided the protein.
- Treatment of re-innervated hind limb muscles (3-month and 6-month groups). All rats will undergo either injection of recombinant follistatin DNA packaged in AAV (into gastrocnemius muscle) or implantation of drug delivery reservoir (with either carrier or recombinant follistatin protein + carrier)- (100% complete)

- ***Specific Aim 3:*** **Determine treatment effects utilizing strength testing, muscle morphology, electrophysiology nerve testing**
- Testing of muscle recovery/nerve regeneration (3 months). All rats underwent muscle morphology measurements, nerve conduction, and force generation studies of tibial nerve and gastrocnemius muscle. - (100% complete)
- Immunohistology staining and histology of muscle (3 months). Fiber type analysis and satellite cell quantification to be determined for all specimens. – (fiber type analysis 100% complete, satellite cell analysis pending)
- Measurement of Follistatin levels in muscle (3 months) immunoassay – (100% complete)

- Testing of muscle recovery/nerve regeneration (6 months) All rats to undergo muscle morphology measurements, nerve conduction, and force generation studies of tibial nerve and gastrocnemius muscle. - (100% complete)
- Immunohistology staining and histology of muscle (6 months) Fiber type analysis and satellite cell quantification to be determined for all specimens. - (fiber type analysis 100% complete, satellite cell analysis pending)
- Measurement of Follistatin levels in muscle (6 months) immunoassay – (100% complete)

- ***Specific Aim 4:*** **Histology (of nerve and muscle), Manuscript preparation, Presentation**
- Histology of muscle/nerve (3 and 6 months) Cross sections of muscle specimens will be stained and fiber size, axon numbers, and myelination measured. – (100% complete)
- Data Analysis (3 and 6 months) – pending
- Manuscript Preparation (3 and 6 months) – pending

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Observations: Since 2017 Annual Report, the following data analyses have been completed

Protein Treatment: (please see Appendix.2)

a. 3-month Denervation Groups:

i. ELISA testing revealed no significant differences in levels of Follistatin protein between repair groups and significantly less Follistatin protein in SHAM groups.

b. 6-month Denervation Groups:

i. Protein treatment resulted in no significant muscle mass differences in either repair or SHAM groups.

ii. Protein treatment resulted in significantly more developed muscle force in repair groups and no significant differences in SHAM groups.

iii. ELISA was unable to identify any Follistatin protein in any of these muscle samples.

Virus Treatment:

a. 3-month Denervation Groups:
Virus treatment resulted in no significant differences in Follistatin protein levels in either repair or SHAM groups (though Follistatin levels in Virus treated repair group were clearly higher, but with a large standard deviation prevented statistical significance).

b. 6-month Denervation Groups:

iv. Virus treatment resulted in significantly more muscle mass for both repair and SHAM groups.

v. Virus treatment resulted in no significant developed muscle force differences in either repair or SHAM groups (though again, there appeared to be a positive difference for the repair group).

vi. ELISA testing revealed significantly more Follistatin protein in the virus treated repair group and no significant Follistatin protein difference in SHAM groups.

Muscle Fiber Type Analysis: 3-month Denervation Groups (please see Appendix.3)

i. The Type 2B muscle fiber area of the muscles that were surgically repaired remained significantly smaller when treated with protein rather than saline.

ii. The type 2A muscle fibers were also significantly smaller in diameter and area than the muscle fibers that were treated with saline after nerve repair.

iii. In contrast, the animals treated with protein had significantly larger diameter type I muscle fibers than if they were treated with saline after nerve repair.

iv. There is significantly greater proportion of muscle fibers expressing type 2B isoforms when the muscle is treated with FS virus after sham surgery

v. In contrast, there is a significantly larger proportion of muscle fibers expressing Type I MHC when the muscle is treated with FS protein after sham surgery

Muscle Fiber Type Analysis: 6-month Denervation Groups (please see Appendix.4)

We can see that the longer denervation period led to smaller muscle fibers but also just confinement to the cages may have contributed.

The only significant differences in muscle fiber size refer to sham operated animals.

i. In Sham operated muscle, the type 2B muscle fiber area is significantly larger when treated with FS protein vs saline.
ii. There is significantly greater proportion of 2B muscle fiber types when the muscle is treated with FS protein after sham surgery.
iii. In contrast, there is significantly greater proportion of 2A muscle fiber types when the muscle is treated with FS virus after sham surgery.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Noting to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- Finalize axon count, and satellite cell data analysis
    - 3-month denervation groups (1-6):
        - Nerve Histology – Finalize analysis of axon counts
    - 6-month denervation groups (7-12):
        - Nerve Histology – Finalize analysis of axon counts
- Interpret final data analyses and Prepare Manuscript and Presentations

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

See below.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

3 Month Data:
-Only ~70% of the data was usable – the short recording distance of 20mm resulted in signal overlap between tissue artifact and nerve action potential such that the action potential was not a clear entity in some trials limiting our ability to process those nerve conduction studies.
-The only difference was a higher peak amplitude in repair+viral treatment vs repair+sham viral treatment. However, this analysis was based on 4 rats per group as the other files were not usable limiting the utility of this specific finding.

6 Month Data:
-None of the data is usable. In an attempt to correct the issue we encountered with the 3mo data, we increased the sampling frequency. The goal of this was to provide better resolution between the tissue artifact and the desired nerve action potential. Unfortunately, when the sampling frequency was increased to get better resolution at the recording electrodes, the pulses per second of the stimulus was simultaneously increased. This resulted in a double tissue artifact that completely obscured the nerve action potential, preventing us from being able to analyze any of this specific data.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  - One Abstract will be presented in American Society for Peripheral Nerve 2019 Annual Conference: (please see Appendix.6)
    Recovery of Chronically Denervated Muscle Enhanced with Follistatin Treatment

  - One Abstract will be presented in Society for Neuroscience 2018 Conference: (please see Appendix.5)
    Does time make a difference? The effect of the administration of follistatin on re-innervated skeletal muscle fiber recovery after 3 vs 6 months of denervation

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report.

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  Nothing to Report.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of

compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Jonathan Isaacs, M.D.
Project Role: PI
Nearest person month worked: 2
Contribution to project: Regulatory process, supervising the study.
Funding support: VCU salary, MCV physicians salary for clinical work, protocol no. ANG-CP-007, Cook Biotech, Inc. industry grant, Flow through funding from NIH: 1R34NS097113-01

Name: Satya Mallu, M.D.
Project Role: Co-investigator
Nearest person month worked: 4
Contribution to project: Assisted with regulatory process, performed main study surgeries.
Funding support: VCU salary, Cook Biotech, Inc.
industry grant, Flow-through funding from NIH: 1R34NS097113-01, AFSH grant

Name: Gaurangkumar Patel, B.S.
Project Role: Lab technician
Nearest person month worked: 7.5
Contribution to project: Main study, assisted with main study surgeries.
Funding support: VCU salary

Name: Mary Shall, PhD
Project Role: Co-PI
Nearest person month worked: 2.5
Contribution to project: ELISA and Muscle Fiber Type Analysis
Funding support: VCU salary

Name: Jeffery Dupree, PhD
Project Role: Co-investigator
Nearest person month worked: 0.25
Contribution to project: Pilot study
Funding support: VCU salary and NIH grants

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS:

Attached. (see appendix 1)

9. APPENDICES:

1. Quad Chart
2. Follistatin Main Study Results
3. Follistatin Histology-Muscle-3-month Group Results
4. Follistatin Histology-Muscle-6-month Group Results
5. SFN-2018 Poster
6. ASPN-2019 Abstract

Follistatin: A Potential Anabolic Treatment for Re-Innervated Muscle

Proposal #11231008

PI: Jonathan Isaacs, MD **Org: Virginia Commonwealth University** **Award Amount: $705,041**

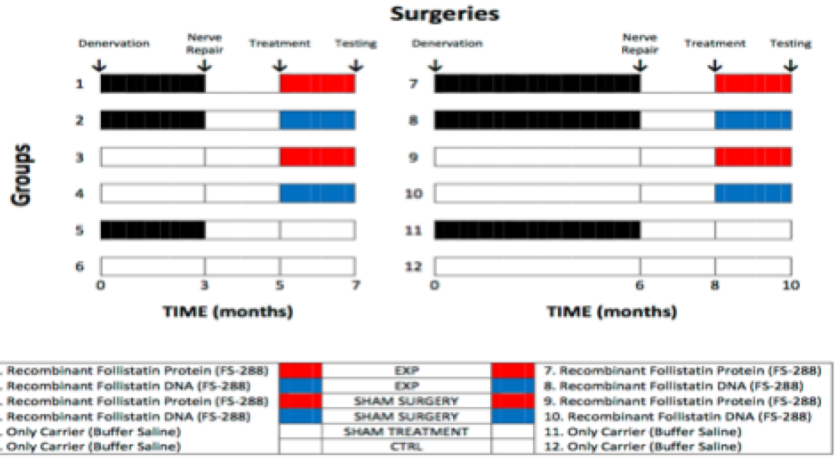
Study Aims

- To utilize an established animal model of denervation atrophy to determine if Follistatin treatment (administered either as a recombinant protein or as a recombinant DNA) will improve muscle recovery following re-innervation after prolonged periods of denervation.
- To determine Follistatin effects on nerve regeneration and intramuscular fibrosis (in re-innervated tissue).

Approach

Based on the pilot study result, rodents will undergo transection of one tibial nerve to denervate the hind limb muscles (including gastrocnemius). After a delay (of either 3 or 6 months) the nerve will be repaired and the muscles re-innervated. The re-innervated muscle will be treated with either recombinant follistatin protein (delivered thru an implantable drug delivery system) or recombinant follistatin DNA (delivered thru adeno viral vectors injected into the reinnervated gastrocnemius muscle).
After 8 weeks recovery, the effects of the follistatin treatment will be determined utilizing strength testing, muscle morphology, muscle histology, and muscle immunohistology (to determine muscle fiber type distribution and satellite, or regenerative cell, population pools). Nerve conduction testing will be performed to differentiate follistatin effects on nerve regeneration and function; muscle staining for collagen will determine effects on muscle fibrosis; and follistatin levels will be measured in treated muscle to confirm effective dosing and delivery of follistatin. Test results will be compared with sham surgery (plus FS treatment), re-innervation (without treatment), and control groups.

Follistatin Project Experiment Timeline



Note: EXP and SHAM TREATMENT undergo DENERVATION Surgeries; SHAM SURGERY and CTRL have "NO" DENERVATION Surgeries.

Timeline and Cost

Activities	CY 15	16	17	18
Regulatory Process & Pilot Project				
Denervation and re-Innervation of hind limb muscles, Treatment with FS and Testing				
Histology, Manuscript Preparation and Presentation				
Estimated Budget ($K)	**$10762**	**$390356**	**$303923**	

Updated: Richmond, VA. Sep 14, 2018

Goals/ Milestones

CY15 Goal – Utilize an established rodent model of denervation atrophy
✓ Regulatory Process – Received ACURO approved on Jan 19, 2016
CY16 Goals – Treat re-innervated muscle with Follistatin, Determine treatment effects utilizing strength testing, muscle morphology, electrophysiology nerve testing
✓ Pilot Project Surgeries Completed
✓ Denervation of hind limb muscles
CY17-18 Goals – Treat re-innervated muscle with Follistatin, Determine treatment effects utilizing strength testing, muscle morphology, electrophysiology nerve testing; Histology (of nerve and muscle), Manuscript preparation, Presentation
✓ **Reinnervation of hind limb muscles**
✓ **Synthesis of Recombinant Follistatin DNA/Protein**
✓ **Treatment with Follistatin**
✓ Testing of muscle recovery/nerve regeneration
☑ Immunohistology staining and histology of muscle
✓ Measurement of Follistatin levels in muscle
☑ Histology of muscle/nerve
D Data Analysis-**ONGOING** D
Manuscript Preparation-ONGOING

Comments/Challenges/Issues/Concerns

- Pilot Project is added after consulting with GOR. Pilot project started with Follistatin DNA (with Adeno Virus) and Control Groups. FS-DNA with AV did not show any Follistatin Protein in muscle. We worked on Follistatin DNA group with Adeno Associated Virus (AAV) vector delivery and the data analysis is complete. Some of the goals moved from CY16 to CY17 reflecting the delays occurred.

Budget Expenditure to Date
Projected Expenditure: $705,041 Actual Expenditure: $704,933

Follistatin Main Study Results

3-month Denervation Groups:

Treatment	Group #	n	Group Name	Group Description
Protein	Group 1	12	Repair + Treatment	Three-month denervation (surgery 1) followed by nerve repair (surgery 2) and then protein treatment (surgery 3) twelve weeks post-surgery 2.
	Group 3	12	SHAM + Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then protein treatment (surgery 3) twelve weeks post-surgery 2.
	Group 5a	6	Repair + SHAM Treatment	Three-month denervation (surgery 1) followed by nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.
	Group 6a	6	SHAM + SHAM Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.
Virus	Group 2	12	Repair + Treatment	Three-month denervation (surgery 1) followed by nerve repair (surgery 2) and then virus treatment (surgery 3) twelve weeks post-surgery 2.
	Group 4	12	SHAM + Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then virus treatment (surgery 3) twelve weeks post-surgery 2.
	Group 5b	6	Repair + SHAM Treatment	Three-month denervation (surgery 1) followed by nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.
	Group 6b	6	SHAM + SHAM Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.

Table 1: 3-month Denervation Experimental Groups

3-month Denervation Groups: Muscle Weight (grams):

	Protein Treatment		Virus Treatment	
	n	Value	n	Value
Repair + Treatment	11	0.64 ± 0.18	12	0.99 ± 0.37
Repair + SHAM Treatment	6	0.93 ± 0.17	6	1.08 ± 0.24
SHAM + Treatment	12	2.22 ± 0.17	12	2.43 ± 0.20
SHAM + SHAM Treatment	6	2.42 ± 0.19	6	2.20 ± 0.26

Table 2: 3-month Denervation Groups Muscle Weight Descriptive Statistics (value = average ± standard deviation, units = grams)

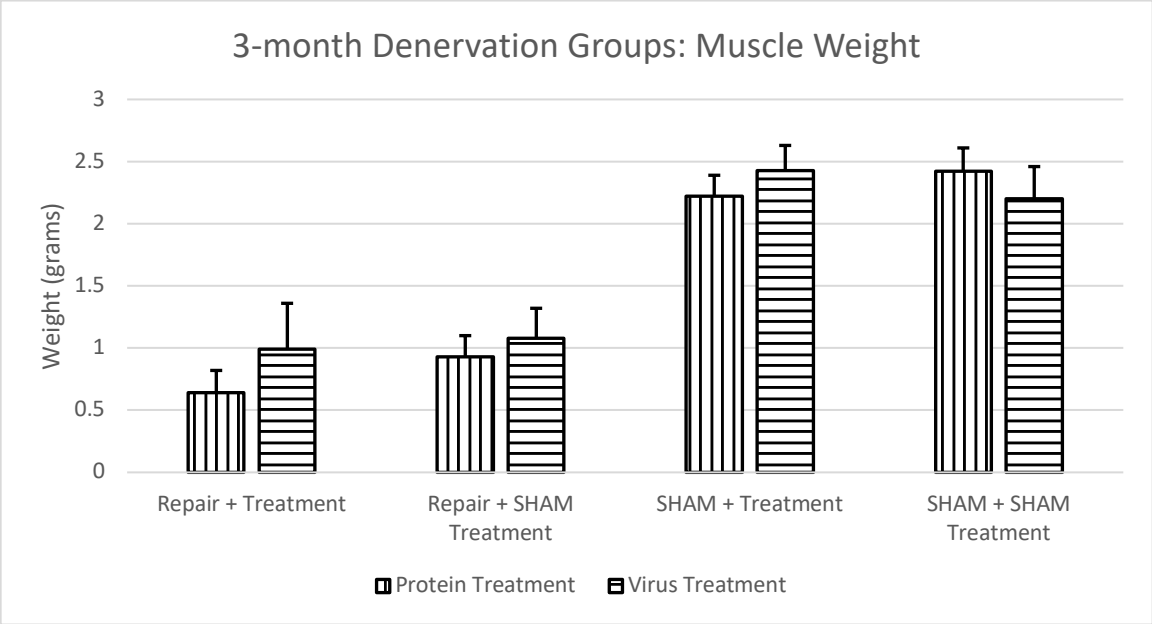


Figure 1: 3-month Denervation Groups Muscle Weight (error bars = standard deviation)

Comparison	p-value	Interpretation
Group 1 vs Group 5a	0.005	Protein treatment leads to significantly less muscle mass growth in repair groups.
Group 3 vs Group 6a	0.040	Protein treatment leads to significantly less muscle mass growth in SHAM groups.
Group 2 vs Group 5b	0.624	Virus treatment leads to no significant muscle mass difference in repair groups.
Group 4 vs Group 6b	0.059	Virus treatment leads to no significant muscle mass difference in SHAM groups.
Group 1 vs Group 2	0.009	Virus treatment leads to significant more muscle mass growth in repair group in comparison with protein treatment.
Group 3 vs Group 4	0.013	Virus treatment leads to significant more muscle mass growth in SHAM group in comparison with protein treatment.

Table 3: 3-month Denervation Groups Muscle Weight Statistical Analysis Results (student t-test used for each of the comparison)

3-month Denervation Groups: Muscle Developed Force (Newtons):

	Protein Treatment		Virus Treatment	
	n	Value	n	Value
Repair + Treatment	11	0.373 ± 0.187	12	0.495 ± 0.285
Repair + SHAM Treatment	6	0.451 ± 0.248	6	0.487 ± 0.302
SHAM + Treatment	12	0.979 ± 0.467	12	1.088 ± 0.438
SHAM + SHAM Treatment	6	1.745 ± 0.597	6	1.070 ± 0.380

Table 4: 3-month Denervation Groups Muscle Developed Force Descriptive Statistics (value = average ± standard deviation, units = Newtons)

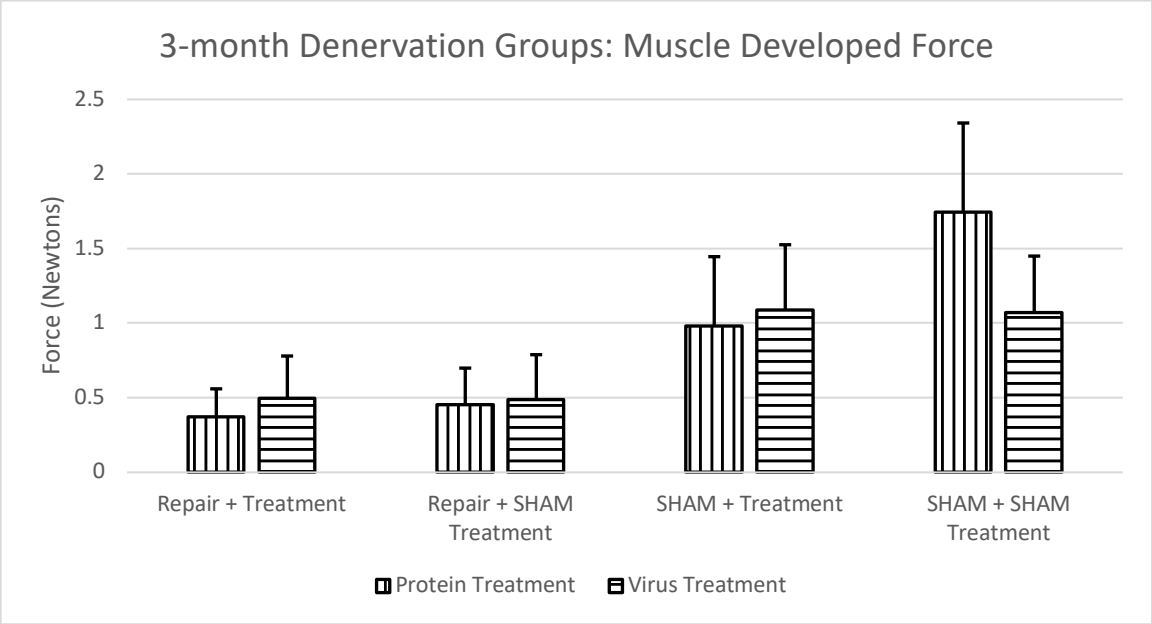


Figure 2: 3-month Denervation Groups Muscle Developed Force (error bars = standard deviation)

Comparison	p-value	Interpretation
Group 1 vs Group 5a	0.474	Protein treatment leads to no significant muscle developed force difference in repair groups.
Group 3 vs Group 6a	0.008	Protein treatment leads to significantly less muscle developed force in SHAM groups.
Group 2 vs Group 5b	0.959	Virus treatment leads to no significant muscle developed force difference in repair groups.
Group 4 vs Group 6b	0.930	Virus treatment leads to no significant muscle developed force difference in SHAM groups.
Group 1 vs Group 2	0.245	No significant difference in muscle developed force when comparing between protein and virus treatment repair groups.
Group 3 vs Group 4	0.558	No significant difference in muscle developed force when comparing between protein and virus treatment SHAM groups.

Table 5: 3-month Denervation Groups Muscle Developed Force Statistical Analysis Results (student t-test used for each of the comparison)

3-month Denervation Groups: Follistatin Protein using ELISA (pg Follistatin/mg protein):

	Protein Treatment		Virus Treatment	
	n	Value	n	Value
Repair + Treatment	11	2679 ± 2184	11	10994 ± 9789
Repair + SHAM Treatment	6	2650 ± 609	5	2897 ± 1830
SHAM + Treatment	10	1924 ± 995	11	2135 ± 737
SHAM + SHAM Treatment	6	2576 ± 715	5	1470 ± 561

Table 6: 3-month Denervation Groups Follistatin Protein using ELISA Descriptive Statistics (value = average ± standard deviation, units = pg Follistatin/mg protein)

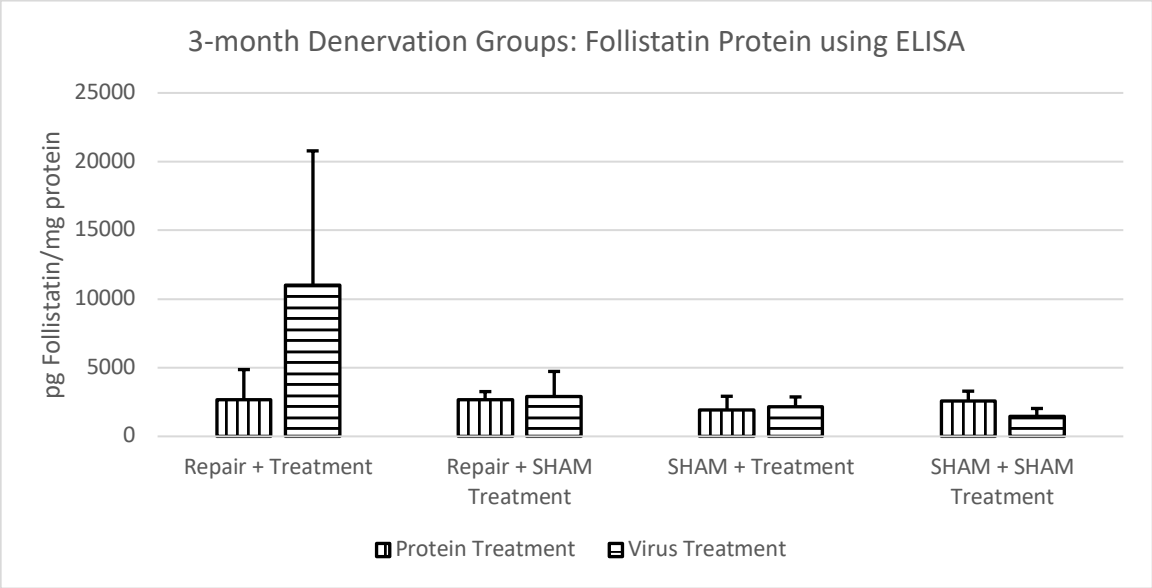


Figure 3: 3-month Denervation Groups Follistatin Protein using ELISA (error bars = standard deviation)

Comparison	p-value	Interpretation
Group 1 vs Group 5a	0.975	Protein treatment leads to no significant Follistatin protein difference in repair groups.
Group 3 vs Group 6a	0.011	Protein treatment leads to significantly less Follistatin protein in SHAM groups.
Group 2 vs Group 5b	0.093	Virus treatment leads to no significant Follistatin protein difference in repair groups.
Group 4 vs Group 6b	0.096	Virus treatment leads to no significant Follistatin protein difference in SHAM groups.
Group 1 vs Group 2	0.012	Virus treatment leads to significant more Follistatin protein in repair group in comparison with protein treatment.
Group 3 vs Group 4	0.585	No significant difference in Follistatin protein when comparing between protein and virus treatment SHAM groups.

Table 7: 3-month Denervation Groups Follistatin Protein using ELISA Statistical Analysis Results (student t-test used for each of the comparison)

6-month Denervation Groups:

Treatment	Group	n	Group Name	Group Description
Protein	Group 7	12	Repair + Treatment	Six-month denervation (surgery 1) followed by nerve repair (surgery 2) and then protein treatment (surgery 3) twelve weeks post-surgery 2.
	Group 9	12	SHAM + Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then protein treatment (surgery 3) twelve weeks post-surgery 2.
	Group 11a	6	Repair + SHAM Treatment	Six-month denervation (surgery 1) followed by nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.
	Group 12a	6	SHAM + SHAM Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.
Virus	Group 8	12	Repair + Treatment	Six-month denervation (surgery 1) followed by nerve repair (surgery 2) and then virus treatment (surgery 3) twelve weeks post-surgery 2.
	Group 10	12	SHAM + Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then virus treatment (surgery 3) twelve weeks post-surgery 2.
	Group 11b	6	Repair + SHAM Treatment	Six-month denervation (surgery 1) followed by nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.
	Group 12b	6	SHAM + SHAM Treatment	Sham denervation (surgery 1) followed by sham nerve repair (surgery 2) and then saline treatment (surgery 3) twelve weeks post-surgery 2.

Table 8: 6-month Denervation Experimental Groups

6-month Denervation Groups: Muscle Weight (grams):

	Protein Treatment		Virus Treatment	
	n	Value	n	Value
Repair + Treatment	11	1.01 ± 0.53	11	0.91 ± 0.47
Repair + SHAM Treatment	6	0.81 ± 0.33	5	0.40 ± 0.07
SHAM + Treatment	10	2.23 ± 0.12	12	2.47 ± 0.15
SHAM + SHAM Treatment	6	2.17 ± 0.49	6	2.26 ± 0.12

Table 9: 6-month Denervation Groups Muscle Weight Descriptive Statistics (value = average ± standard deviation, units = grams)

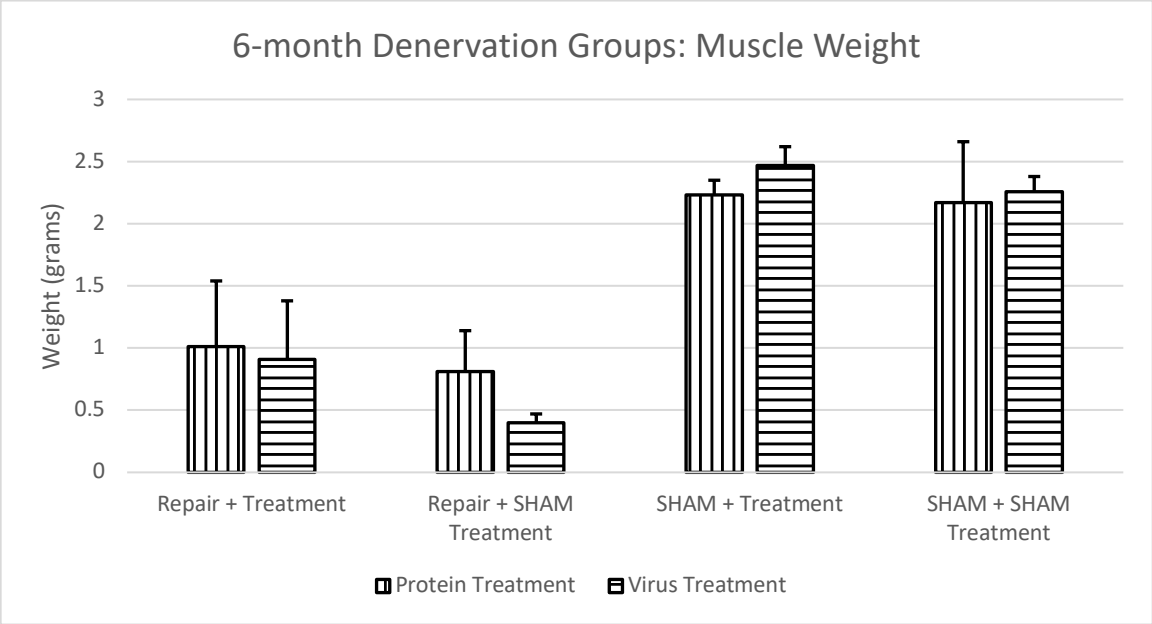


Figure 4: 6-month Denervation Groups Muscle Weight (error bars = standard deviation)

Comparison	p-value	Interpretation
Group 7 vs Group 11a	0.441	Protein treatment leads to no significant muscle mass difference in repair groups.
Group 9 vs Group 12a	0.692	Protein treatment leads to no significant muscle mass difference in SHAM groups.
Group 8 vs Group 11b	0.032	Virus treatment leads to significantly more muscle mass growth in repair groups.
Group 10 vs Group 12b	0.010	Virus treatment leads to significantly more muscle mass growth in SHAM groups.
Group 7 vs Group 8	0.659	No significant difference in muscle mass when comparing between protein and virus treatment repair groups.
Group 9 vs Group 10	0.0007	Virus treatment leads to significant more muscle mass growth in SHAM group in comparison with protein treatment.

Table 10: 6-month Denervation Groups Muscle Weight Statistical Analysis Results (student t-test used for each of the comparison)

6-month Denervation Groups: Muscle Developed Force (Newtons):

	Protein Treatment		Virus Treatment	
	n	Value	n	Value
Repair + Treatment	10	0.630 ± 0.448	10	0.328 ± 0.267
Repair + SHAM Treatment	5	0.201 ± 0.180	5	0.194 ± 0.135
SHAM + Treatment	11	1.034 ± 0.395	12	1.179 ± 0.509
SHAM + SHAM Treatment	6	1.290 ± 0.632	6	1.492 ± 0.425

Table 11: 6-month Denervation Groups Muscle Developed Force Descriptive Statistics (value = average ± standard deviation, units = Newtons)

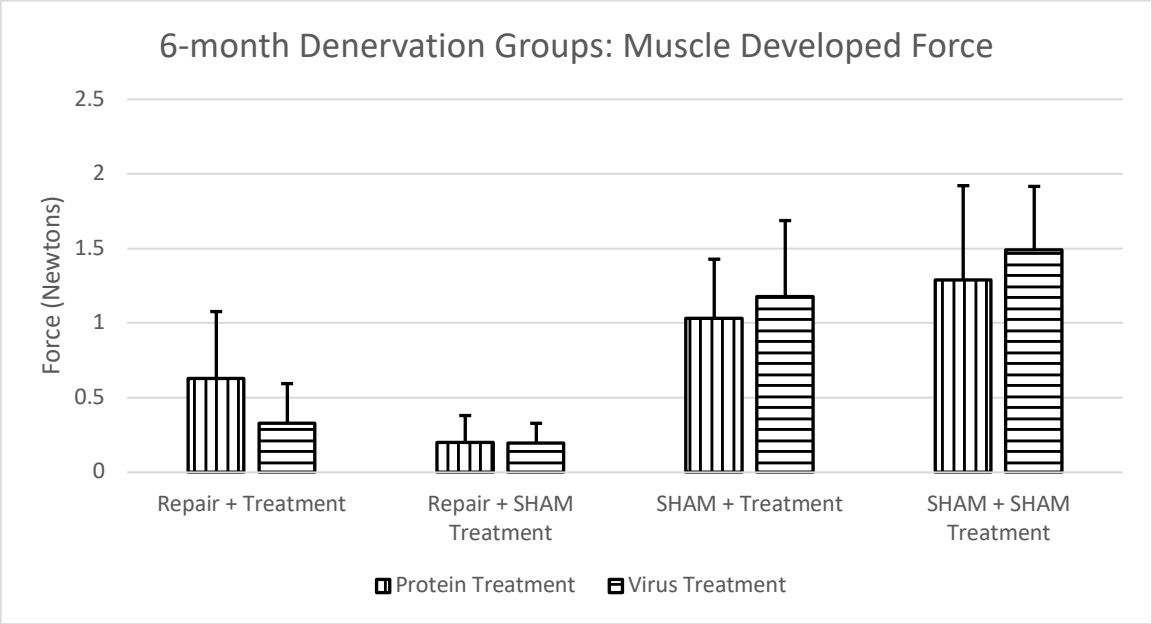


Figure 5: 6-month Denervation Groups Muscle Developed Force (error bars = standard deviation)

Comparison	p-value	Interpretation
Group 7 vs Group 11a	0.063	Protein treatment leads to significantly more muscle developed force in repair groups.
Group 9 vs Group 12a	0.316	Protein treatment leads to no significant muscle developed force in SHAM groups.
Group 8 vs Group 11b	0.315	Virus treatment leads to no significant muscle developed force difference in repair groups.
Group 10 vs Group 12b	0.215	Virus treatment leads to no significant muscle developed force difference in SHAM groups.
Group 7 vs Group 8	0.083	No significant difference in muscle developed force when comparing between protein and virus treatment repair groups.
Group 9 vs Group 10	0.457	No significant difference in muscle developed force when comparing between protein and virus treatment SHAM groups.

Table 12: 6-month Denervation Groups Muscle Developed Force Statistical Analysis Results (student t-test used for each of the comparison)

6-month Denervation Groups: Follistatin Protein using ELISA (pg Follistatin/mg protein):

	Protein Treatment		Virus Treatment	
	n	Value	n	Value
Repair + Treatment	11	0 ± 0	11	2772 ± 2762
Repair + SHAM Treatment	6	0 ± 0	6	108 ± 37
SHAM + Treatment	11	0 ± 0	10	168 ± 66
SHAM + SHAM Treatment	6	0 ± 0	6	130 ± 61

Table 13: 6-month Denervation Groups Follistatin Protein using ELISA Descriptive Statistics (value = average ± standard deviation, units = pg Follistatin/mg protein)

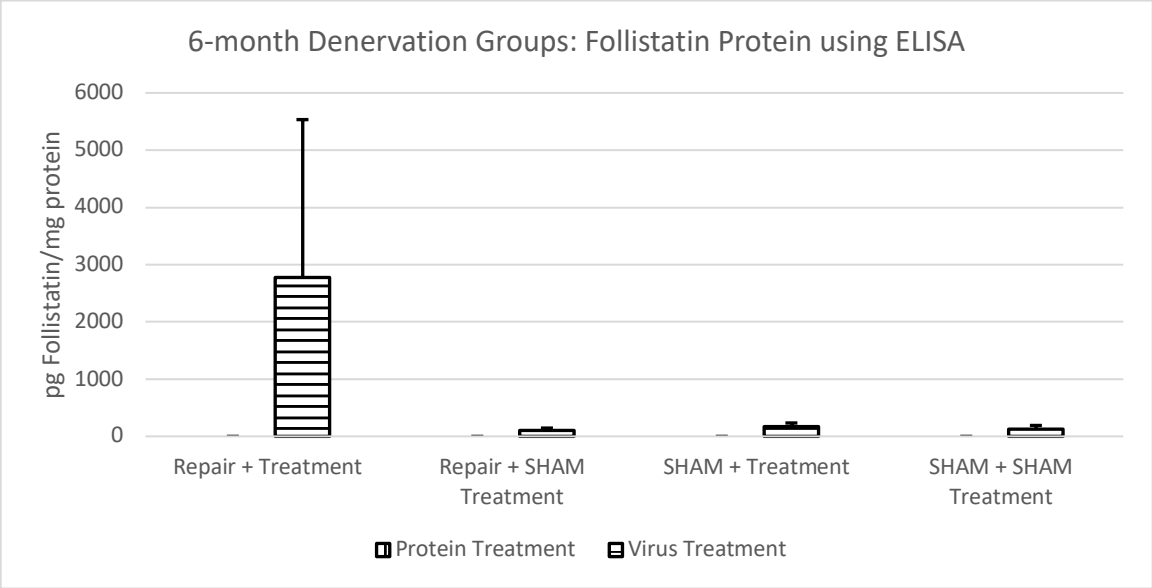


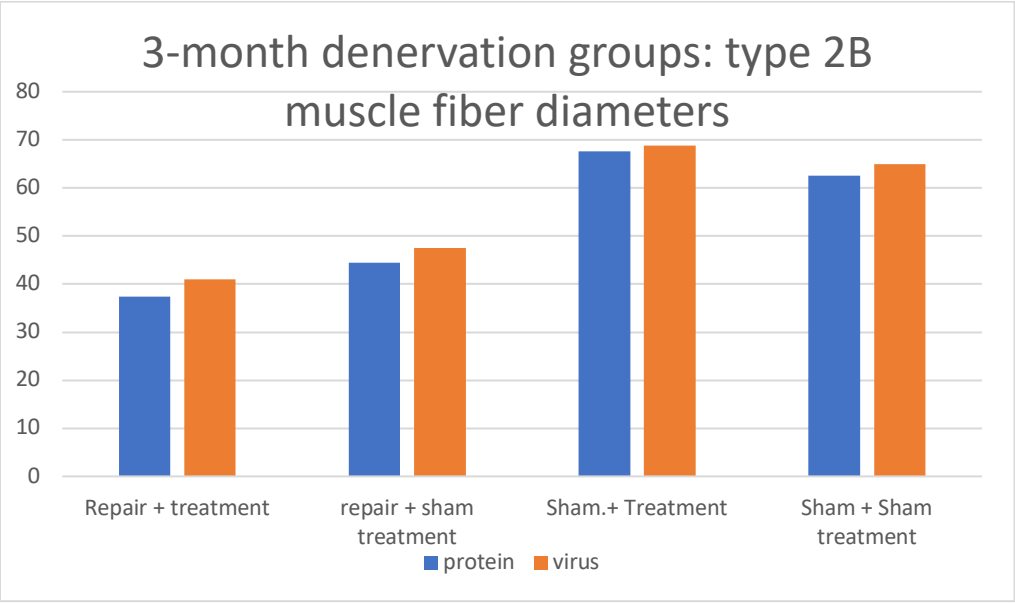
Figure 6: 6-month Denervation Groups Follistatin Protein using ELISA (error bars = standard deviation)

Comparison	p-value	Interpretation
Group 7 vs Group 11a	NA	None
Group 9 vs Group 12a	NA	None
Group 8 vs Group 11b	0.034	Virus treatment leads to significantly more Follistatin protein in repair groups.
Group 10 vs Group 12b	0.281	Virus treatment leads to no significant Follistatin protein difference in SHAM groups.
Group 7 vs Group 8	0.003	Virus treatment leads to significant more Follistatin protein in repair group in comparison with protein treatment.
Group 9 vs Group 10	0.00000007	Virus treatment leads to significant more Follistatin protein in SHAM group in comparison with protein treatment.

Table 14: 6-month Denervation Groups Follistatin Protein using ELISA Statistical Analysis Results (student t-test used for each of the comparison)

3-month denervation groups:

2B diameter

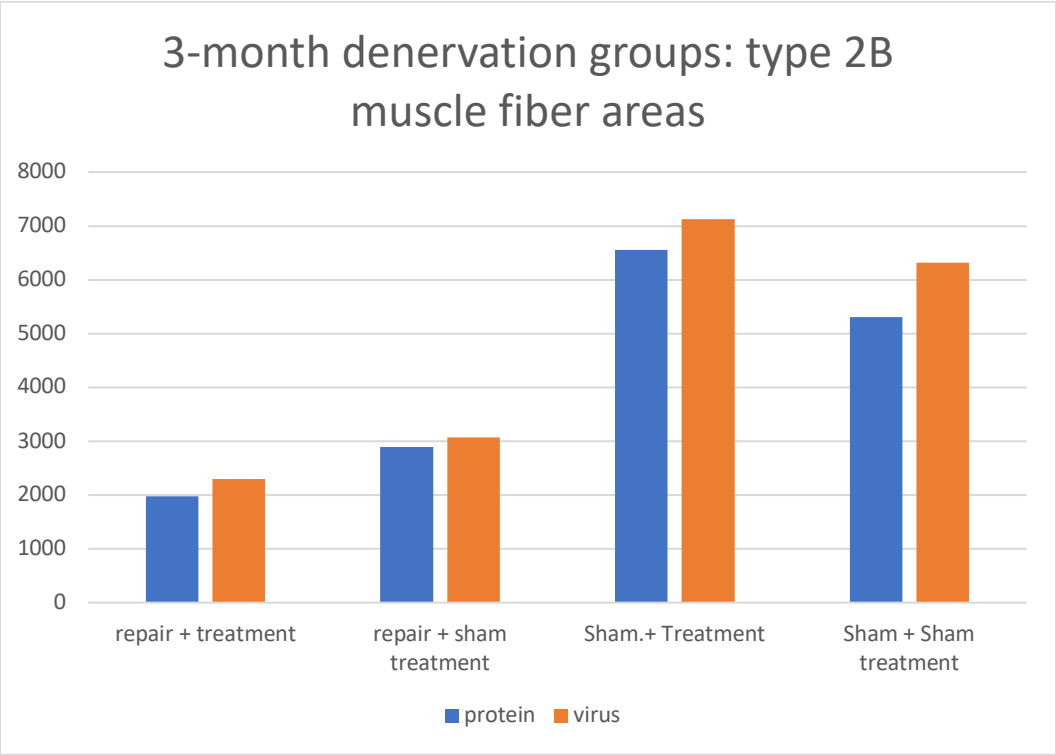


	protein treatment		virus treatment	
Repair + treatment	N	value	N	Value
Repair + Treatment	11	37.35 ± 10.75	12	40.93 ± 10.04
Repair + Sham treatment	6	44.38 ± 3.47	6	47.48 ± 9.51
Sham + treatment	12	67.22 ± 6.2	12	68.8 ± 7.2
Sham + sham treatment	6	62.6 ± 7.25	6	64.88 ± 6.45

2B diameters

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.773	Protein treatment does not enhance the 2B muscle fiber diameter of muscles that have been denervated and repaired.
Group 3 vs Group 6a	.969	Protein treatment does not enhance the 2B muscle fiber diameter of muscles that have been denervated and repaired.
Group 2 vs Group 5b	.861	Protein treatment does not enhance the 2B muscle fiber diameter of muscles that have been denervated and repaired.
Group 4 vs Group 6b	1.0	Virus treatment has no effect on the 2B muscle fiber diameter that have had only sham denervation
Group 1 vs Group 2	.978	There is no difference in 2B muscle fiber diameter when treated with protein vs virus.
Group 3 vs Group 4	1.0	There is no difference in 2B muscle fiber diameter when treated with protein vs virus even if there has only been sham surgery

3-month denervation groups:
2B areas

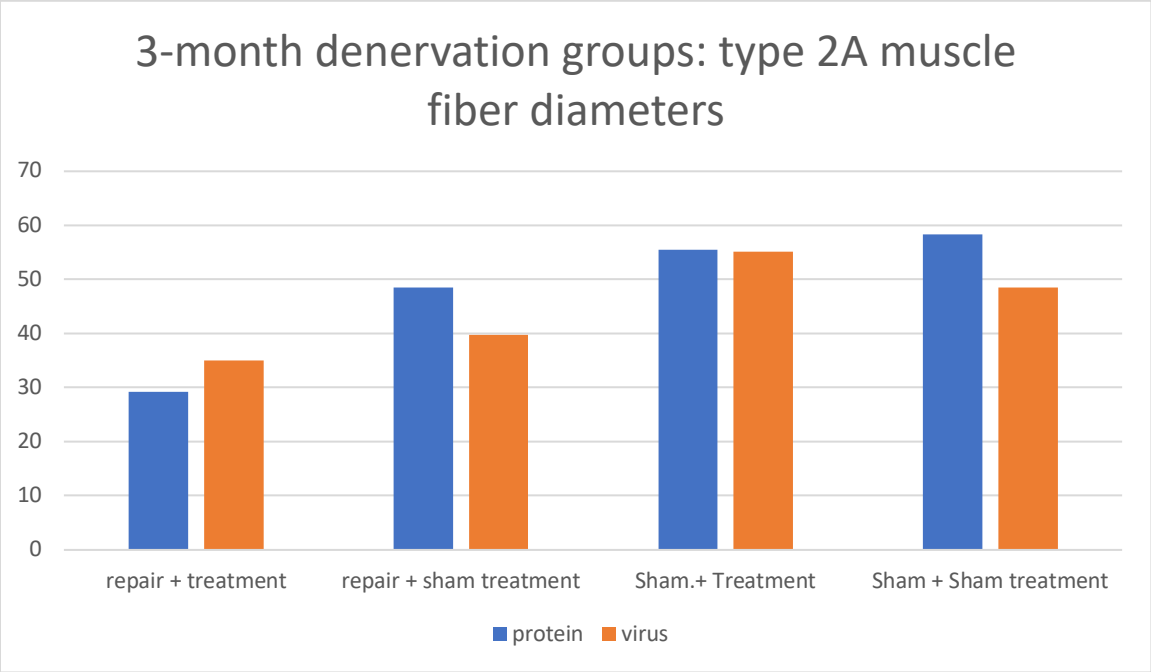


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	1979.49 ± 1008.91	11	2295.8 ± 1042.97
Repair + Sham treatment	6	2891 ± 659.23	5	3066.0 ± 1226.21
Sham + treatment	12	6556.66 ± 1153.42	11	7124.74 ± 1339.29
Sham + sham treatment	6	5309.57 ± 1720.33	5	6316.48 ± 815.16

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.0001	The Type 2B muscle fiber area of the muscles that were surgically repaired remained significantly smaller when treated with protein.
Group 3 vs Group 6a	.996	There is no difference of Type 2B muscle fiber area
Group 2 vs Group 5b	.926	There is no difference of Type 2B muscle fiber area
Group 4 vs Group 6b	.738	There is no difference of Type 2B muscle fiber area
Group 1 vs Group 2	.610	There is no difference of Type 2B muscle fiber area
Group 3 vs Group 4	1.0	There is no difference of Type 2B muscle fiber area

3-month denervation groups:

2A diameter

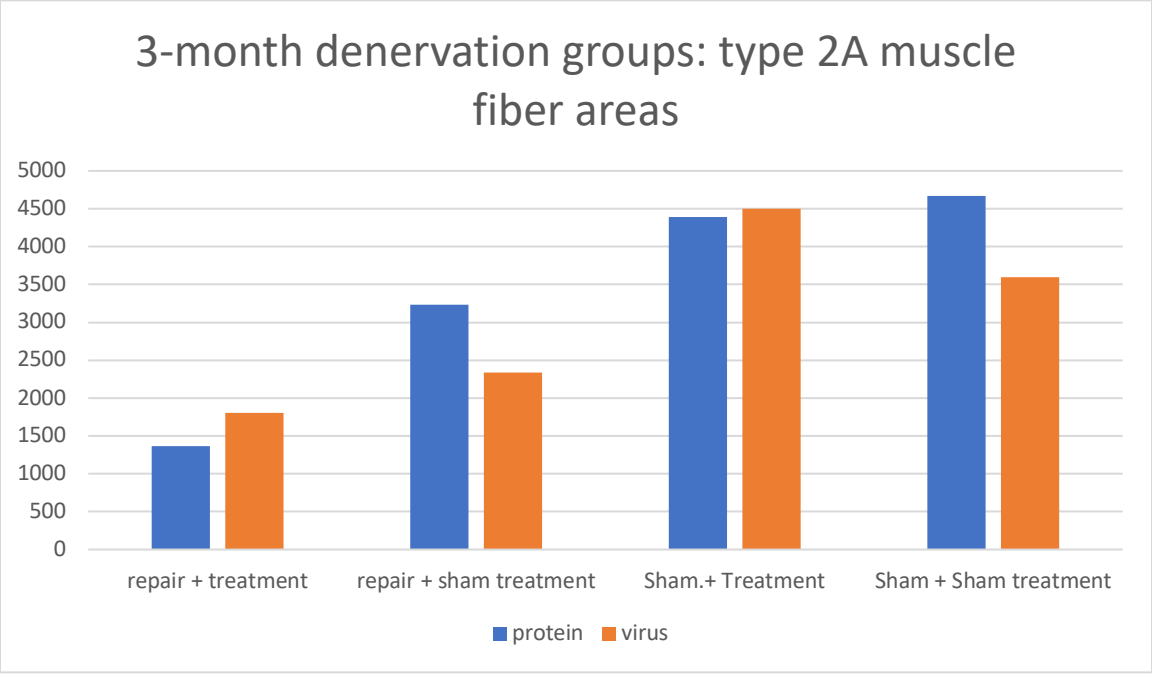


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	29.24 ± 7.48	11	35.04 ± 9.4
Repair + Sham treatment	6	48.54 ± 6.0	5	39.67 ± 8.0
Sham + treatment	12	55.54 ± 5.81	11	55.13 ± 6.74
Sham + sham treatment	6	58.32 ± 3.71	5	48.54 ± 12.52

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.0001	The type 2A muscle fibers were significantly smaller in diameter than the muscle fibers that were treated with saline after nerve repair.
Group 3 vs Group 6a	.996	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 2 vs Group 5b	.926	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 4 vs Group 6b	.738	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 1 vs Group 2	.610	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 3 vs Group 4	1.0	There is no statistical difference of type 2A muscle fiber diameters between groups

3-month denervation groups:

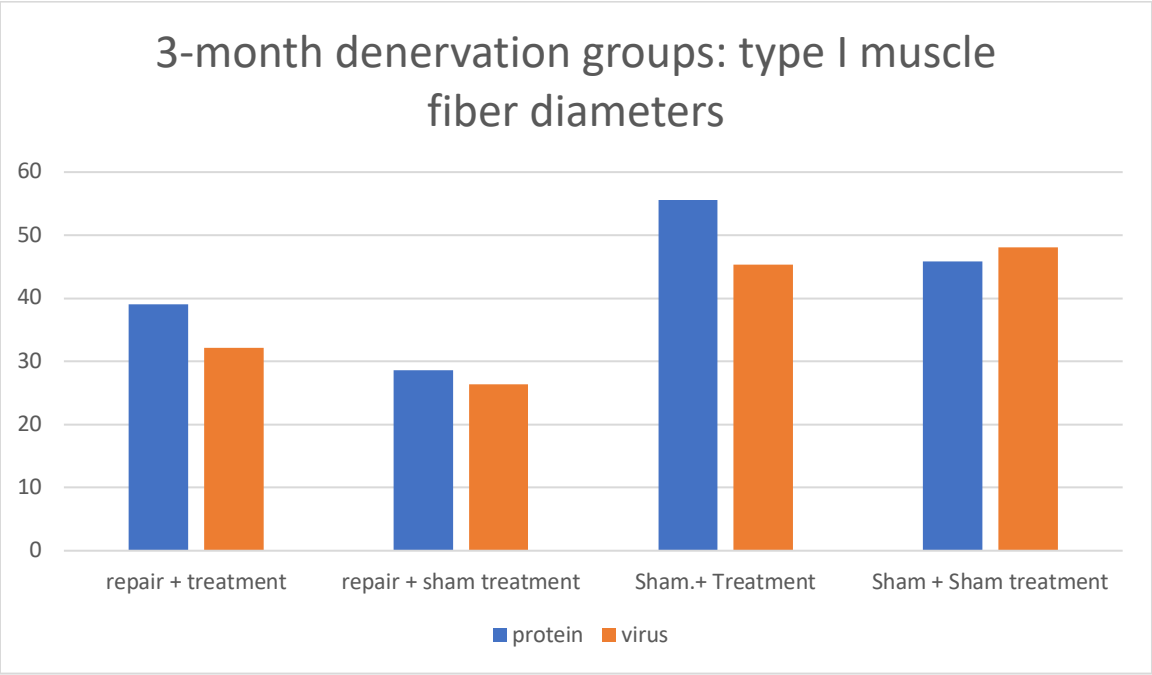
2A area



	protein treatment		virus treatment	
	n	Value	n	value
Repair + Treatment	11	1369.21 ± 560.3	11	1807.84 ± 938.82
Repair + Sham treatment	6	3237.23 ± 779.11	5	2335.35 ± 872.82
Sham + treatment	12	4389.45 ± 646.85	11	4499.88 ± 912.16
Sham + sham treatment	6	4673.85 ± 403.98	5	3594.77 ± 1328.5

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.001	The type 2A muscle fibers are significantly smaller in area than the muscles that were treated with saline after nerve repair
Group 3 vs Group 6a	.997	There is no difference in the areas of type 2A muscle fibers between groups
Group 2 vs Group 5b	.900	There is no difference in the areas of type 2A muscle fibers between groups
Group 4 vs Group 6b	.441	There is no statistically significant difference in the areas of type 2A muscle fibers between groups
Group 1 vs Group 2	.901	There is no difference in the areas of type 2A muscle fibers between groups
Group 3 vs Group 4	1.0	There is no difference in the areas of type 2A muscle fibers between groups

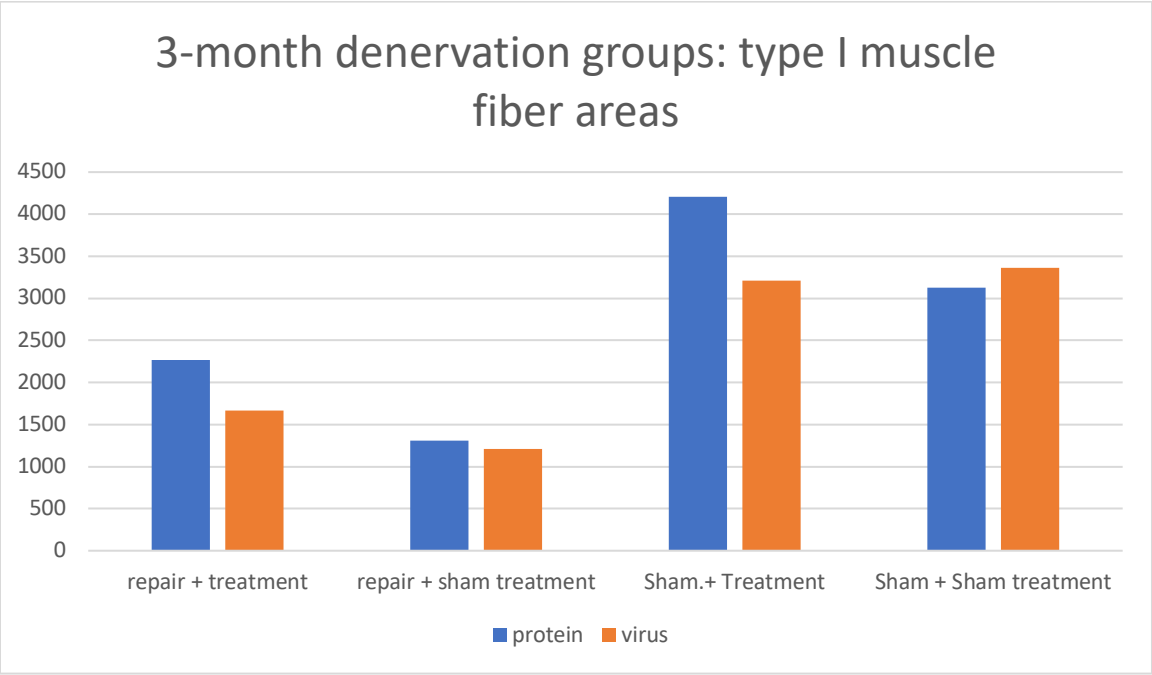
3-month denervation groups:
I diameter



	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	37.42 ± 10.07	11	37.41 ± 5.03
Repair + Sham treatment	6	48.8 ± 8.45	5	41.85 ± 7.3
Sham + treatment	12	54.53 ± 6.41	11	54.39 ± 5.0
Sham + sham treatment	6	57.9 ± 5.2	5	51.34 ± 10.29

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.05	The animals treated with protein had significantly larger diameter type I muscle fibers after denervation
Group 3 vs Group 6a	.983	The difference in muscle fiber diameter is not significantly different
Group 2 vs Group 5b	.934	The difference in muscle fiber diameter is not significantly different
Group 4 vs Group 6b	.993	The difference in muscle fiber diameter is not significantly different
Group 1 vs Group 2	1.0	The difference in muscle fiber diameter is not significantly different
Group 3 vs Group 4	1.0	The difference in muscle fiber diameter is not significantly different

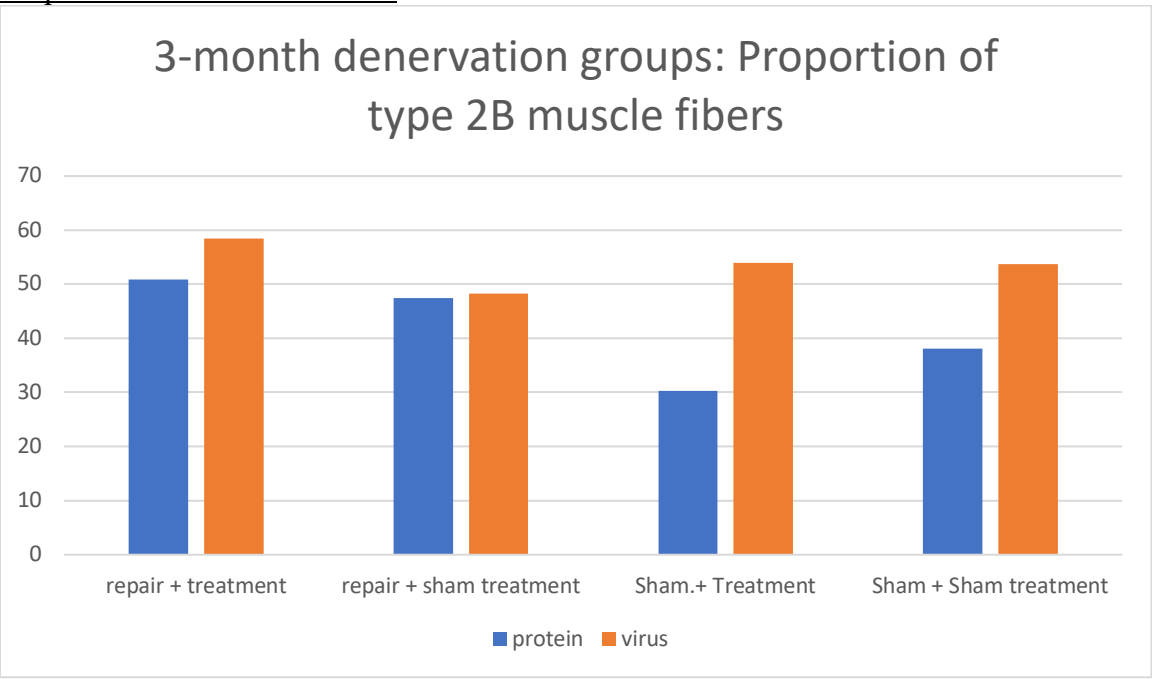
3-month denervation groups:
I area



	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	2298.89 ± 772.61	11	2019.07 ± 602.36
Repair + Sham treatment	6	3222.52 ± 1068.88	5	2501.93 ± 743.56
Sham + treatment	12	4335.33 ± 726.24	11	4225.34 ± 521.33
Sham + sham treatment	6	4434.22 ± 750.21	5	4202.2 ± 1646.11

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.355	The difference in muscle fiber diameter is not significantly different though there is a tendency toward larger fibers if treated with protein
Group 3 vs Group 6a	1.0	The difference in muscle fiber diameter is not significantly different
Group 2 vs Group 5b	.945	The difference in muscle fiber diameter is not significantly different
Group 4 vs Group 6b	1.0	The difference in muscle fiber diameter is not significantly different
Group 1 vs Group 2	.933	The difference in muscle fiber diameter is not significantly different
Group 3 vs Group 4	1.0	The difference in muscle fiber diameter is not significantly different

3-month denervation groups:
Proportion of 2B muscle fibers

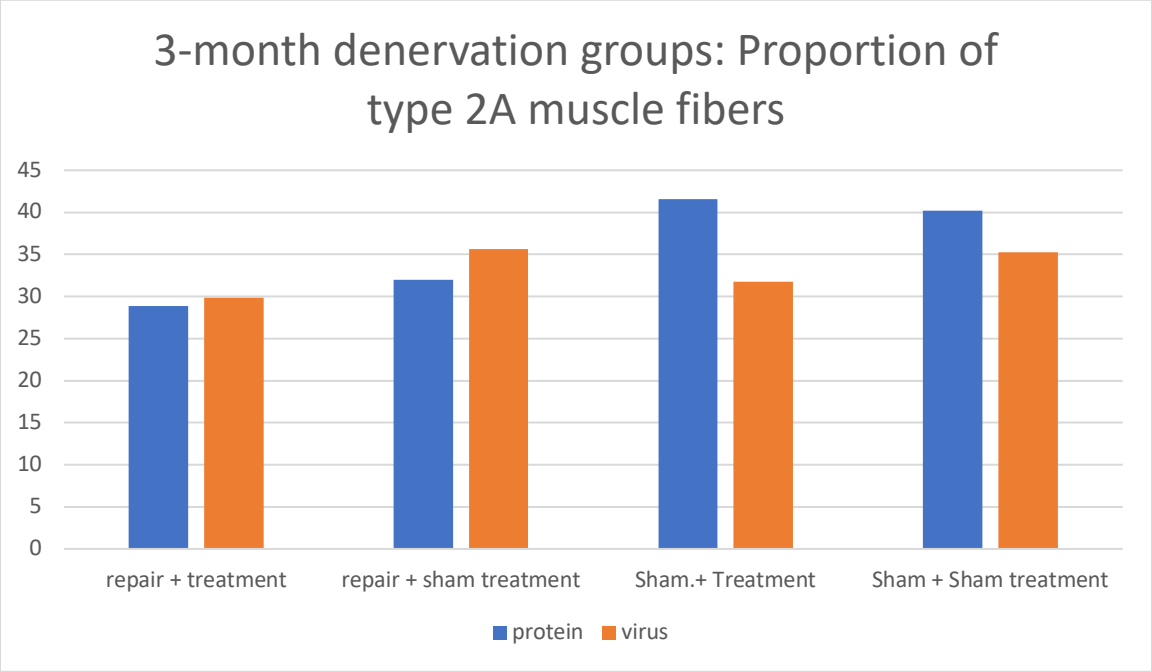


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	50.84 ± 9.05	11	58.42 ± 11.33
Repair + Sham treatment	6	47.4 ± 8.34	5	48.27 ± 5.44
Sham + treatment	12	30.33 ± 13.71	11	53.98 ± 10.45
Sham + sham treatment	6	38.06 ± 12.6	5	53.68 ± 13.65

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.999	There is no significant difference in the proportion of type 2B muscle fibers
Group 3 vs Group 6a	.855	There is no difference in the proportion of type 2B muscle fibers
Group 2 vs Group 5b	.600	There is no significant difference in the proportion of type 2B muscle fibers
Group 4 vs Group 6b	1.0	There is no difference in the proportion of type 2B muscle fibers
Group 1 vs Group 2	.749	There is no difference in the proportion of type 2B muscle fibers
Group 3 vs Group 4	.0001	There is significantly greater proportion of 2B muscle fiber types when the muscle is treated with FS virus after sham surgery

Proportion of 2A muscle fibers

3-month denervation groups:

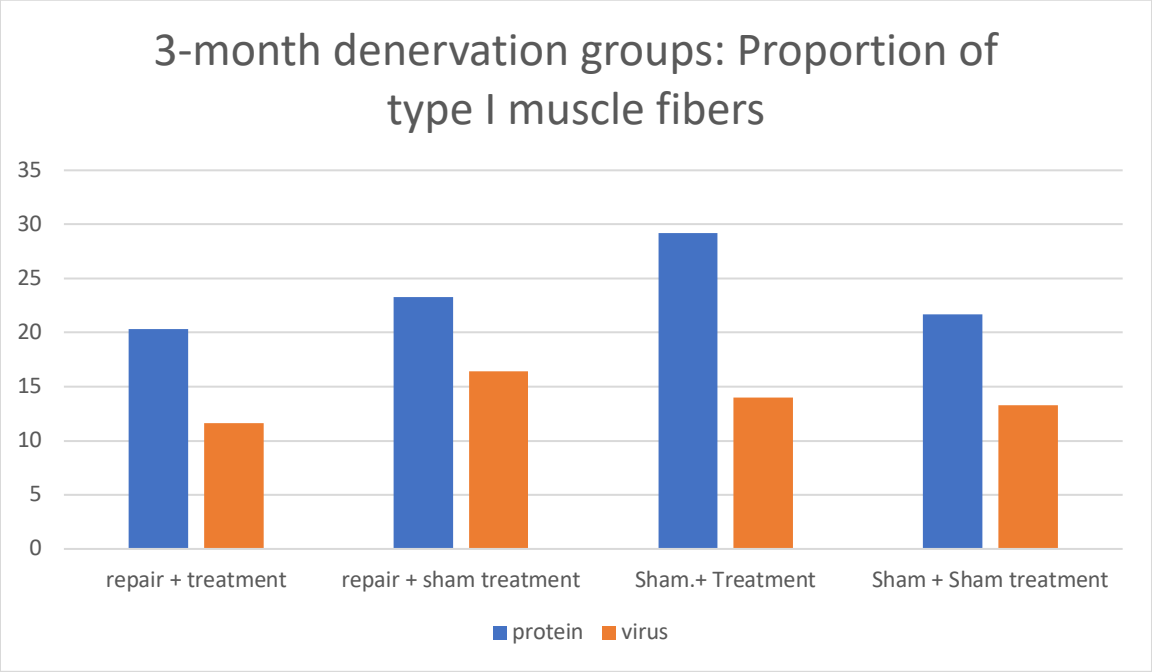


	protein treatment		virus treatment	
	N	value	n	value
Repair + Treatment	11	28.88 ± 7.62	11	29.89 ± 8.04
Repair + Sham treatment	6	31.99 ± 12.34	5	35.65 ± 9.33
Sham + treatment	12	41.56 ± 7.0	11	31.74 ± 9.48
Sham + sham treatment	6	40.23 ± 11.95	5	35.25 ± 10.33

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.998	There is no significant difference in the proportion of type 2A muscle fibers
Group 3 vs Group 6a	1.0	There is no difference in the proportion of type 2A muscle fibers
Group 2 vs Group 5b	.91	There is no significant difference in the proportion of type 2A muscle fibers
Group 4 vs Group 6b	.996	There is no difference in the proportion of type 2B muscle fibers
Group 1 vs Group 2	1.0	There is no difference in the proportion of type 2B muscle fibers
Group 3 vs Group 4	.186	There is a tendency toward a greater proportion of 2A muscle fiber types when the muscle is treated with FS protein after sham surgery

Proportion of type I muscle fibers

3-month denervation groups:



	protein treatment		virus treatment	
	N	value	n	value
Repair + Treatment	11	20.32 ± 6.29	11	11.63 ± 5.57
Repair + Sham treatment	6	23.27 ± 18.73	5	16.44 ± 4.14
Sham + treatment	12	29.2 ± 16.77	11	13.98 ± 8.11
Sham + sham treatment	6	21.68 ± 11.41	5	13.27 ± 5.91

Comparison	**p-value**	**Interpretation**
Group 1 vs Group 5a	.998	There is no significant difference in the proportion of type I muscle fibers
Group 3 vs Group 6a	.8610	There is no difference in the proportion of type I muscle fibers
Group 2 vs Group 5b	.986	There is no difference in the proportion of type I muscle fibers
Group 4 vs Group 6b	1.0	There is no difference in the proportion of type I muscle fibers
Group 1 vs Group 2	.578	There is no significant difference in the proportion of type I muscle fibers though there tends to be more type I when the muscle is treated with protein.
Group 3 vs Group 4	.028	There is a significantly larger proportion of muscle fibers expressing Type I MHC when the muscle is treated with FS protein after sham surgery

3-month denervation groups:

Conclusions:

The significant differences at three months:

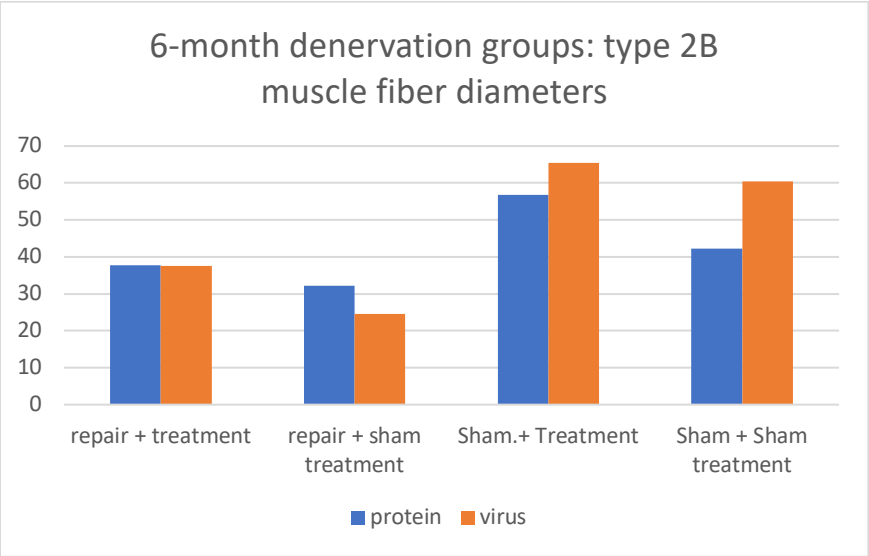
1. The Type 2B muscle fiber area of the muscles that were surgically repaired remained significantly smaller when treated with protein rather than saline.
2. The type 2A muscle fibers were also significantly smaller in diameter and area than the muscle fibers that were treated with saline after nerve repair.
3. In contrast, the animals treated with protein had significantly larger diameter type I muscle fibers than if they were treated with saline after nerve repair.
4. There is significantly greater proportion of muscle fibers expressing type 2B isoforms when the muscle is treated with FS virus after sham surgery
5. In contrast, there is a significantly larger proportion of muscle fibers expressing Type I MHC when the muscle is treated with FS protein after sham surgery

Tendencies:

1. There is a tendency that the virus treatment (rather than saline) leads to larger 2B muscle fiber diameter of muscles that have been denervated and repaired
2. The animals treated with protein (rather than saline) had significantly larger diameter type I muscle fibers after denervation.
3. There is a tendency toward a greater proportion of 2A muscle fiber types when the muscle is treated with FS protein after sham surgery
4. There is no significant difference in the proportion of repaired type I muscle fibers when denervated though there tends to be more type I when the muscle is treated with protein.

6-month denervation groups:

2B diameter



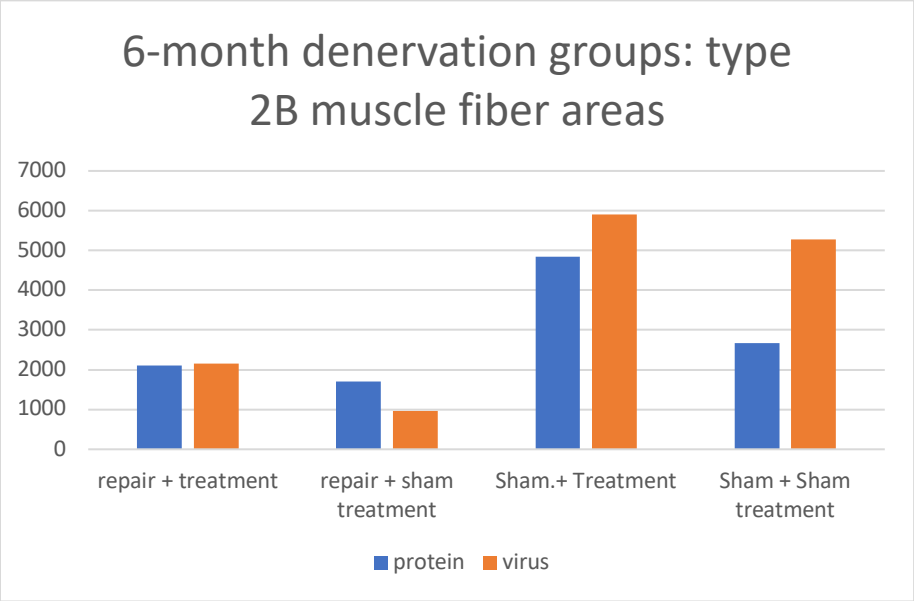
	protein treatment		virus treatment	
Repair + treatment	N	value	N	Value
Repair + Treatment	11	37.74 ± 12.12	12	37.47 ± 13.60
Repair + Sham treatment	6	32.24 ± 11.60	6	24.48 ± 12.29
Sham + treatment	12	56.76 ± 8.51	12	65.4 ± 10.07
Sham + sham treatment	6	42.27 ± 2.91	6	60.42 ± 6.08

2B diameters

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.969	Protein treatment does not enhance the 2B muscle fiber diameter of muscles that have been denervated and repaired.
Group 8 vs Group 11b	.251	There was a tendency that the FS virus treatment (rather than saline) led to larger 2B muscle fiber diameter of muscles that have been denervated and repaired.
Group 9 vs Group 12a	.140	After sham denervation, there was a tendency that protein treatment led to larger 2B muscle fiber diameter of muscles than muscles that had saline injected.
Group 10 vs Group 12b	.981	Virus treatment has no effect on the 2B muscle fiber diameter that have had only sham denervation
Group 7 vs Group 8	1.0	There is no difference in 2B muscle fiber diameter when treated with protein vs virus.
Group 9 vs Group 10	.520	The difference in muscle fiber diameter is not significantly different.

6-month denervation groups:

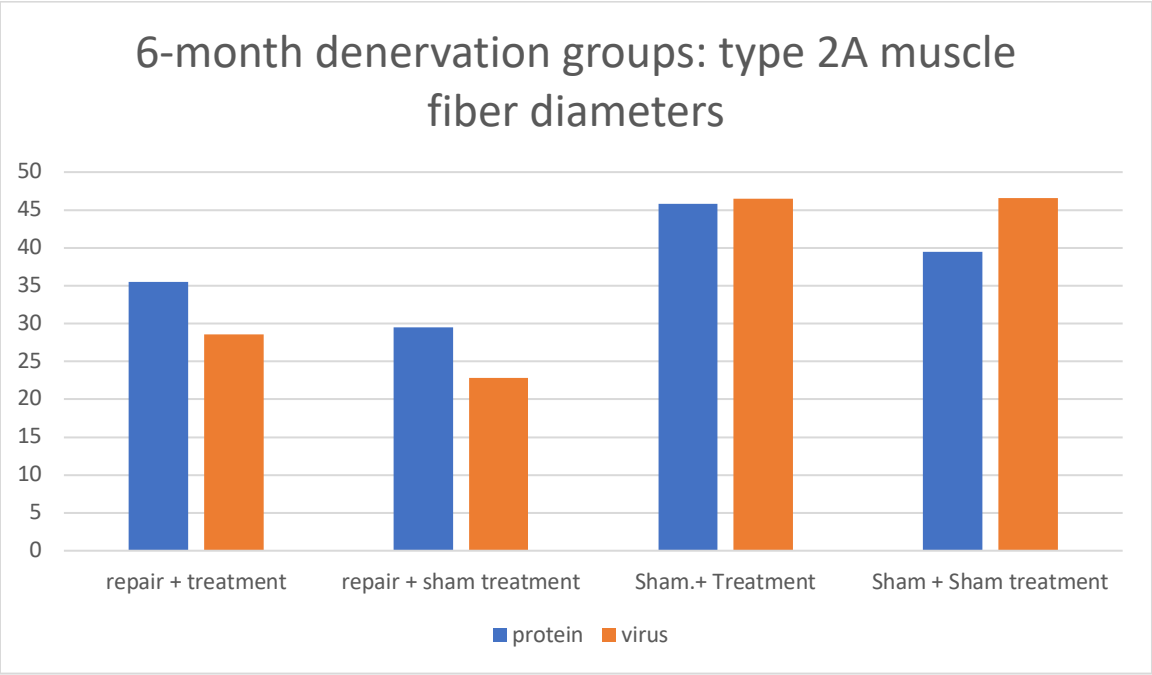
2B areas



	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	2106.1 ± 1120.75	11	2148.88 ± 1456.93
Repair + Sham treatment	6	1702.41 ± 1010.38	5	971.18 ± 1049.17
Sham + treatment	12	4836.35 ± 1322.75	11	5909.49 ± 1680.28
Sham + sham treatment	6	2666.76 ± 326.57	5	5280.64 ± 1075.16

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.988	There is no difference of Type 2B muscle fiber area if treated with FS protein
Group 9 vs Group 12a	.028	In Sham operated muscle, the type 2B muscle fiber area is significantly larger when treated with FS protein rather than saline.
Group 8 vs Group 11b	.611	There is no difference of Type 2B muscle fiber area
Group 10 vs Group 12b	.975	There is no difference of Type 2B muscle fiber area
Group 7 vs Group 8	.993	There is no difference of Type 2B muscle fiber area
Group 9 vs Group 10	.480	There is no difference of Type 2B muscle fiber area

6-month denervation groups:
2A diameter

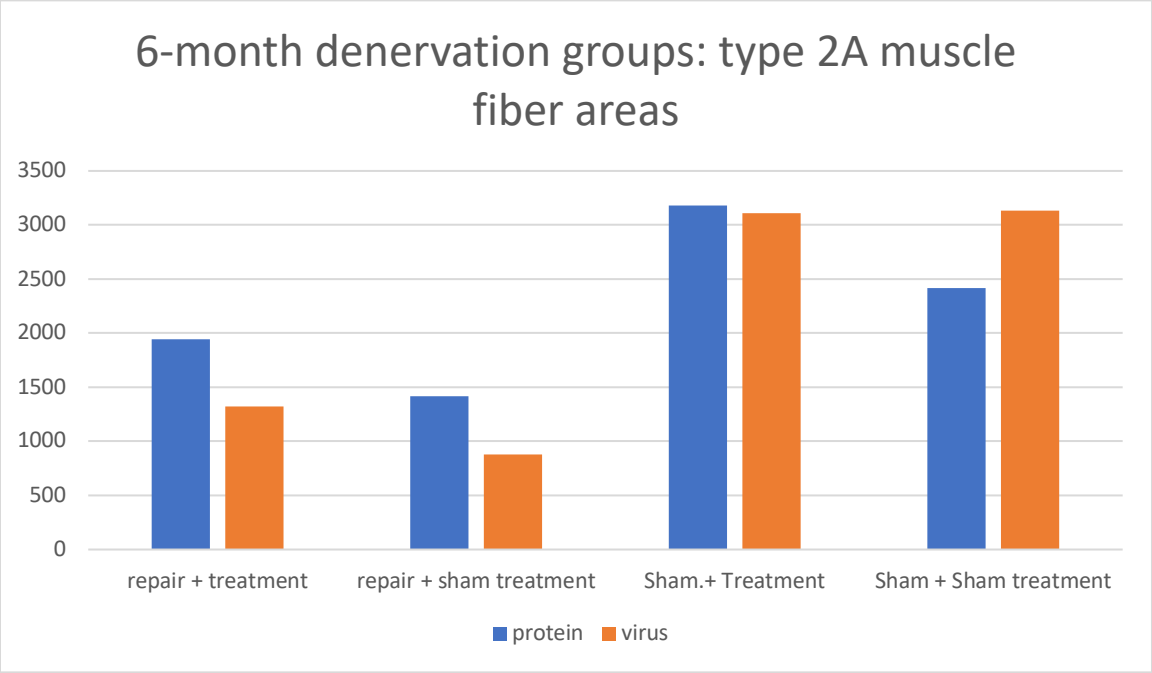


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	35.55 ± 12.1	11	28.65 ± 9.4
Repair + Sham treatment	6	29.47 ± 7.89	5	22.85 ± 6.37
Sham + treatment	12	45.79 ± 12.1	11	46.46 ± 5.33
Sham + sham treatment	6	39.54 ± 3.95	5	46.62 ± 6.74

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.799	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 9 vs Group 12a	.766	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 8 vs Group 11b	.835	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 10 vs Group 12b	1.0	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 7 vs Group 8	.463	There is no statistical difference of type 2A muscle fiber diameters between groups
Group 9 vs Group 10	1.0	There is no statistical difference of type 2A muscle fiber diameters between groups

6-month denervation groups:

2A area

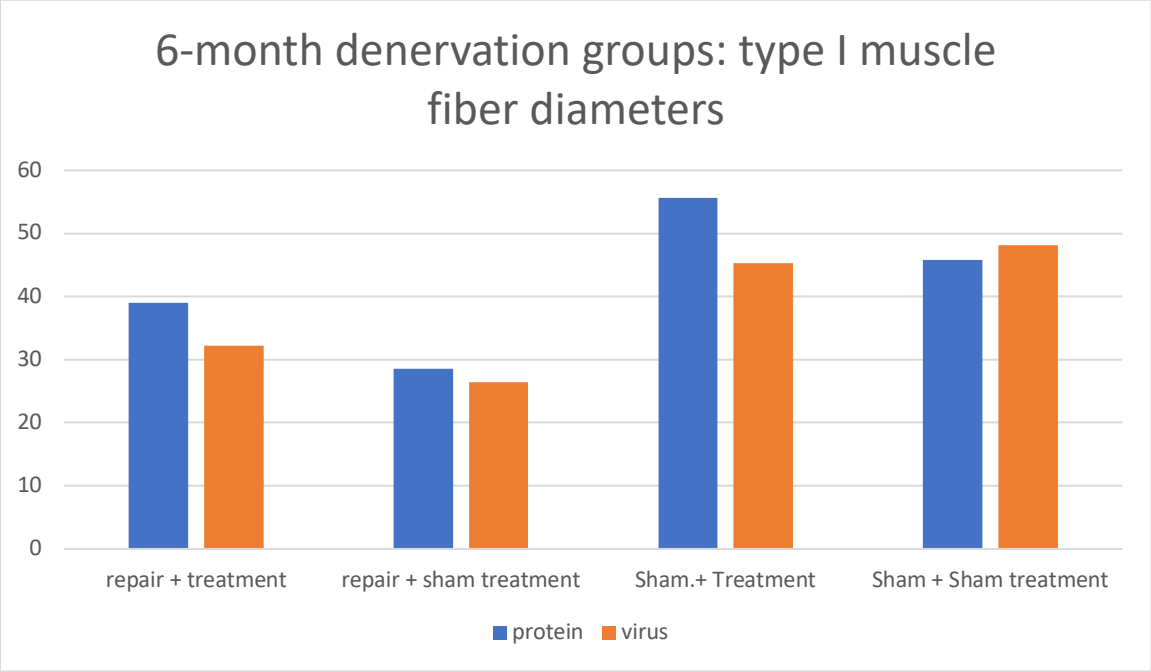


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	1942.72 ± 1200.2	11	1323.4 ± 713.62
Repair + Sham treatment	6	1419.89 ± 590.36	5	881.17 ±489.15
Sham + treatment	12	3178.64 ± 675.59	11	3109.89 ± 582.37
Sham + sham treatment	6	2419.09 ±435.49	5	3133.63 ± 740.93

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.867	There is no statistically significant difference in the areas of type 2A muscle fibers between groups
Group 9 vs Group 12a	.495	There is no statistically significant difference in the areas of type 2A muscle fibers between groups though the protein tended to enhance the area.
Group 8 vs Group 11b	.940	There is no statistically significant difference in the areas of type 2A muscle fibers between groups
Group 10 vs Group 12b	1.0	There is no statistically significant difference in the areas of type 2A muscle fibers between groups
Group 7 vs Group 8	.534	There is no statistically significant difference in the areas of type 2A muscle fibers between groups
Group 9 vs Group 10	1.0	There is no statistically significant difference in the areas of type 2A muscle fibers between groups

I diameter

6-month denervation groups:

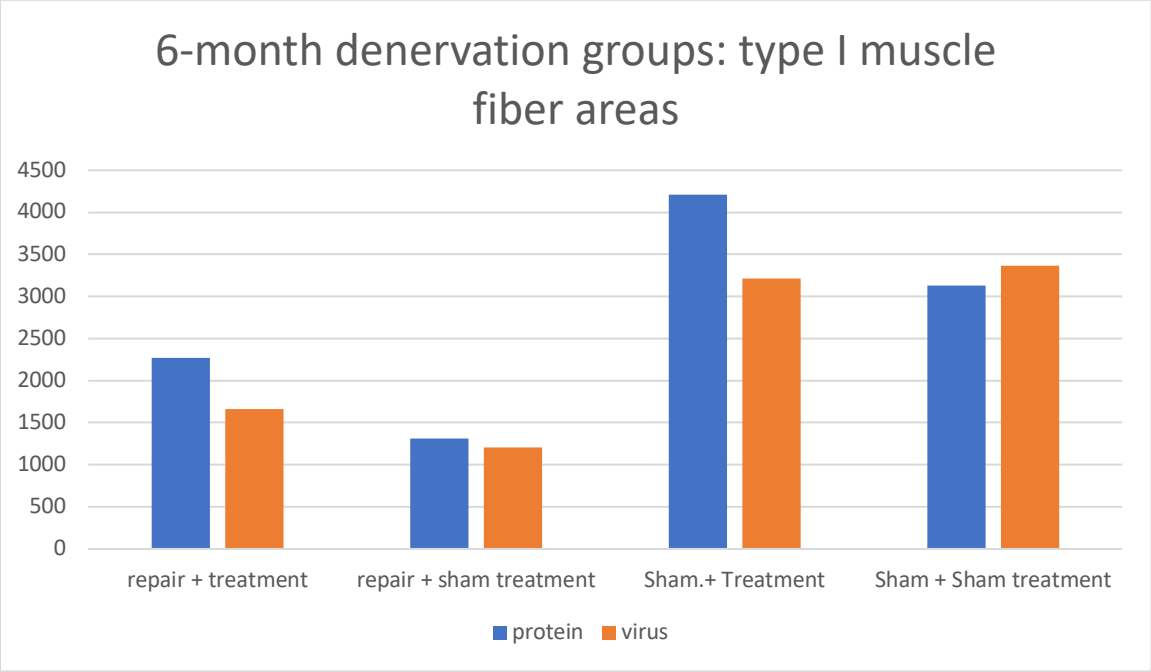


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	39.02 ± 14.16	11	32.2 ± 10.17
Repair + Sham treatment	6	28.58 ± 12.21	5	26.35 ± 9.35
Sham + treatment	12	55.63 ± 9.94	11	45.26 ± 3.75
Sham + sham treatment	6	45.82 ± 4.17	5	48.06 ± 3.79

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.389	The difference in muscle fiber diameter is not significantly different though the tendency is larger type I muscle fibers when treated with protein.
Group 9 vs Group 12a	.469	The difference in muscle fiber diameter is not significantly different
Group 8 vs Group 11b	.926	The difference in muscle fiber diameter is not significantly different
Group 10 vs Group 12b	.999	The difference in muscle fiber diameter is not significantly different
Group 7 vs Group 8	.766	The difference in muscle fiber diameter is not significantly different
Group 9 vs Group 10	.172	There is a tendency toward larger type 1 muscle fiber in sham operated muscle when using protein treatment.

I area

6-month denervation groups:

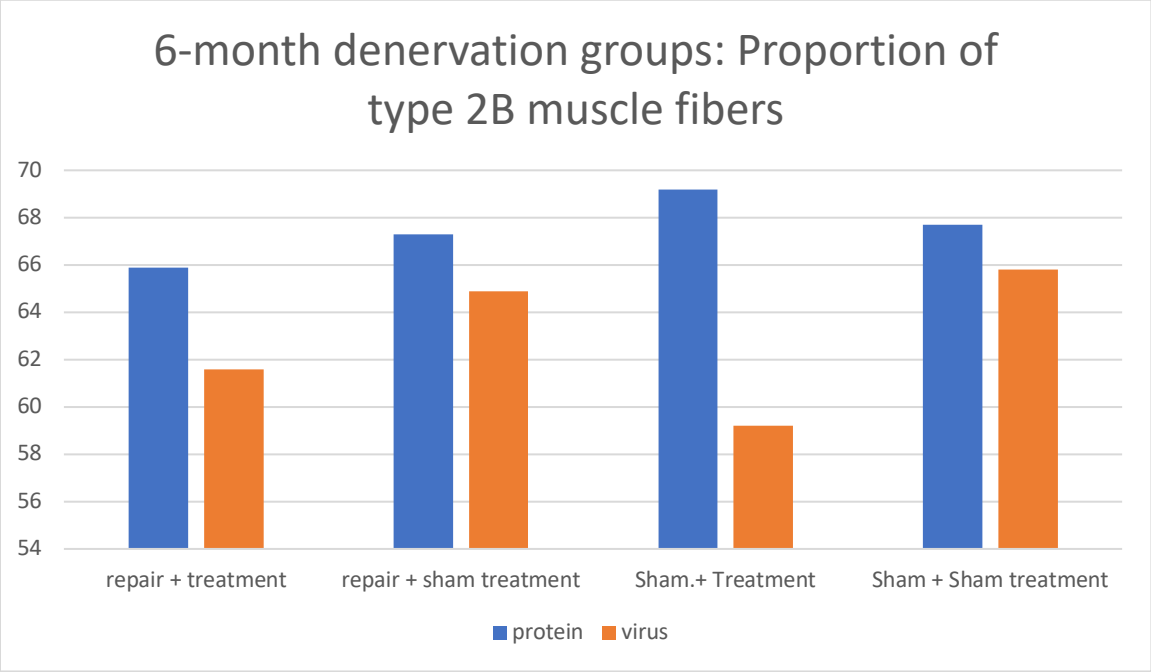


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	2268.92 ± 1410.07	11	1663.84 ± 927.58
Repair + Sham treatment	6	1312.04 ± 953.18	5	1206.44 ± 815.15
Sham + treatment	12	4207.26 ± 1112.04	11	3213.39 ± 633.86
Sham + sham treatment	6	3130.18 ±426.87	5	3364.22 ± 621.66

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.511	The difference in muscle fiber diameter is not significantly different
Group 9 vs Group 12a	.357	The difference in muscle fiber diameter is not significantly different
Group 8 vs Group 11b	.981	The difference in muscle fiber diameter is not significantly different
Group 10 vs Group 12b	1.0	The difference in muscle fiber diameter is not significantly different
Group 7 vs Group 8	.814	The difference in muscle fiber diameter is not significantly different
Group 9 vs Group 10	.221	There is a tendency toward a difference between a larger type 1 muscle fiber in sham operated muscle when using virus treatment.

Proportion of 2B muscle fibers

6-month denervation groups:

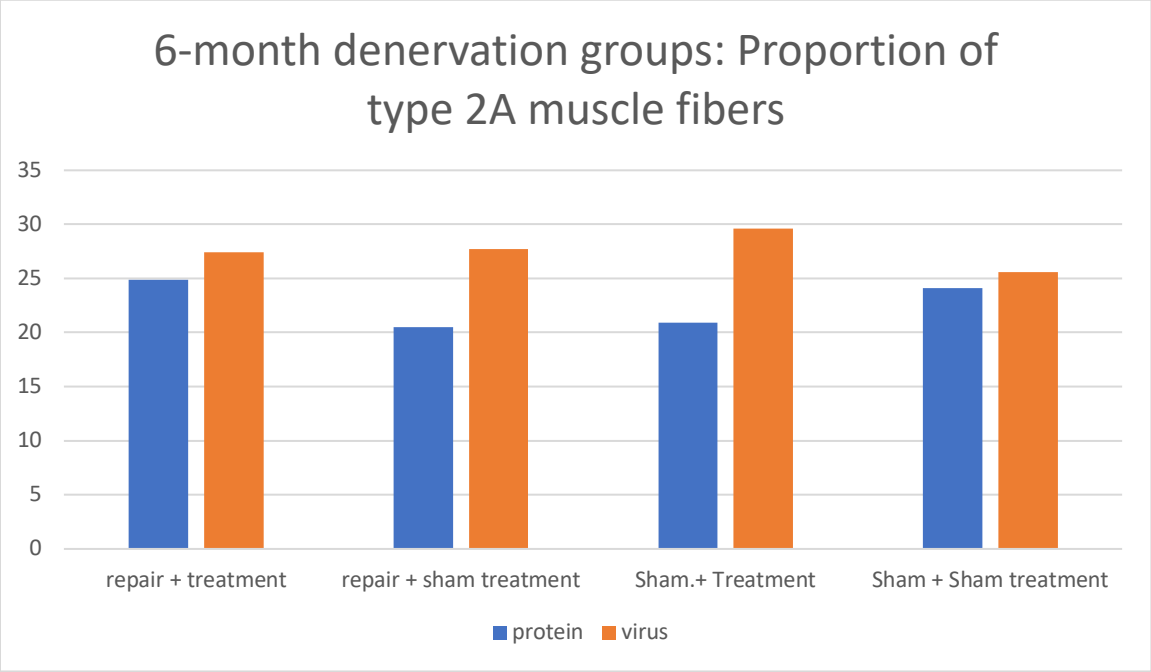


	protein treatment		virus treatment	
	n	value	n	value
Repair + Treatment	11	65.87 ± 3.76	11	61.55 ± 8.27
Repair + Sham treatment	6	67.3 ± 2.25	5	64.88 ± 3.42
Sham + treatment	12	69.2 ± 5.23	11	59.18 ± 7.33
Sham + sham treatment	6	67.72 ± 2.89	5	65.82 ± 1.79

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	1.0	There is no difference in the proportion of type 2B muscle fibers
Group 9 vs Group 12a	.999	There is no difference in the proportion of type 2B muscle fibers
Group 8 vs Group 11b	.930	There is no difference in the proportion of type 2B muscle fibers
Group 10 vs Group 12b	.250	There is no significant difference in the proportion of type 2B muscle fibers
Group 7 vs Group 8	.591	There is no difference in the proportion of type 2B muscle fibers
Group 9 vs Group 10	.001	There is significantly greater proportion of type 2B muscle fiber types when the muscle is treated with FS protein after sham surgery

Proportion of 2A muscle fibers

6-month denervation groups:

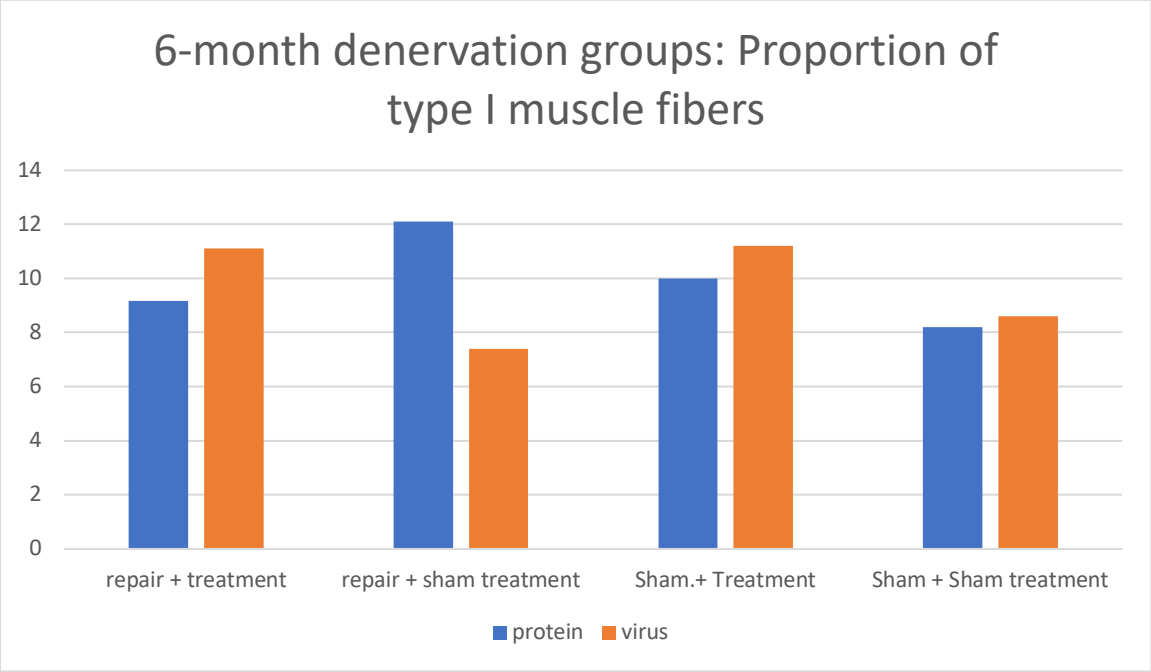


	protein treatment	virus treatment		
	N	value	n	value
Repair + Treatment	11	24.94 ±4.74	11	27.37 ± 4.6
Repair + Sham treatment	6	20.47 ± 3.46	5	27.75 ± 4.15
Sham + treatment	12	20.86 ± 3.23	11	29.64 ± 7.0
Sham + sham treatment	6	24.1 ± 3.27	5	25.62 ± 1.28

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.545	There is no difference in the proportion of type 2A muscle fibers
Group 9 vs Group 12a	.857	There is no difference in the proportion of type 2A muscle fibers
Group 8 vs Group 11b	1.0	There is no difference in the proportion of type 2A muscle fibers
Group 10 vs Group 12b	.654	There is no difference in the proportion of type 2A muscle fibers
Group 7 vs Group 8	.915	There is no difference in the proportion of type 2A muscle fibers
Group 9 vs Group 10	.001	There is significantly greater proportion of 2A muscle fiber types when the muscle is treated with FS virus after sham surgery

Proportion of type I muscle fibers

6-month denervation groups:



	protein treatment		virus treatment	
	N	value	n	value
Repair + Treatment	11	9.18 ± 2.9	11	11.07 ± 5.04
Repair + Sham treatment	6	12.12 ± 2.19	5	7.37 ± 2.06
Sham + treatment	12	9.95 ± 3.27	11	11.18 ± 4.15
Sham + sham treatment	6	8.18 ± 4.35	5	8.57 ± 1.48

Comparison	**p-value**	**Interpretation**
Group 7 vs Group 11a	.741	There is no difference in the proportion of type I muscle fibers
Group 9 vs Group 12a	.977	There is no difference in the proportion of type I muscle fibers
Group 8 vs Group 11b	.467	There is no difference in the proportion of type I muscle fibers
Group 10 vs Group 12b	.828	There is no difference in the proportion of type I muscle fibers
Group 7 vs Group 8	.918	There is no difference in the proportion of type I muscle fibers
Group 9 vs Group 10	.991	There is no difference in the proportion of type I muscle fibers

6-month denervation groups:

Conclusions:

We can see that the longer denervation period led to smaller muscle fibers but also just confinement to the cages may have contributed.

The only significant differences in muscle fiber size refer to sham operated animals.

1. In Sham operated muscle, the type 2B muscle fiber area is significantly larger when treated with FS protein vs saline.
2. There is significantly greater proportion of 2B muscle fiber types when the muscle is treated with FS protein after sham surgery.
3. In contrast, there is significantly greater proportion of 2A muscle fiber types when the muscle is treated with FS virus after sham surgery.

Tendencies

1. There is a tendency that the virus treatment (rather than saline) leads to larger 2B muscle fiber diameter of muscles that have been denervated and repaired.
2. After sham denervation, there was a tendency that protein treatment led to larger 2B muscle fiber diameter and larger type I muscle fiber diameters than muscles that had saline injected.

Does time make a difference? The effect of the administration of follistatin on re-innervated skeletal muscle fiber recovery after 3 vs 6 months of denervation

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Abstract:
The objective is to evaluate the effect of Follistatin on the recovery of skeletal muscle strength and skeletal muscle fiber diameter after different periods of denervation time and re-innervation.
Rationale: Functional recovery following traumatic peripheral nerve injury is often suboptimal despite appropriate treatment. Due to the slow rate of axonal regeneration (1-3 mm/d), the target muscle may undergo significant atrophy before the axon attempts reinnervation. Follistatin influences muscle regeneration at several levels including directly inhibiting myostatin, a signal transduction protein that regulates muscle mass by inhibiting muscle regeneration. As a result, Follistatin stimulates muscle fiber hypertrophy and hyperplasia in normal animal models.
Methods: Transection of the tibial nerve in the hindlimb of Sprague-Dawley rats, followed by delayed (3 or 6 month) repair (utilizing microsurgical nerve suturing with nerve graft) induced partial recovery of the muscle with mild or moderate residual strength deficits due to irreversible atrophy. Recombinant protein and recombinant DNA were synthesized (and the DNA packaged in adeno-associated viral vectors) in the Virginia Commonwealth University (VCU) Biological Macromolecule Core Facility. The Follistatin protein was delivered, after reinnervation, to the gastrocnemius muscle utilizing an Alzet (Cupertino, CA, USA) implantable drug delivery system. Treatment effects on the muscle were evaluated by cryosectioning the muscles after evaluation of muscle force. Muscle fiber types identified with Immunohistochemistry allowed differential evaluation of three primary muscle fiber types.
Results: The muscles of the animals that were denervated 3 months and repaired, followed by Follistatin treatment, exerted about the same force as experimental animals without Follistatin treatment and had about the same size muscle fibers. Muscles that were denervated 6 months before reinnervation and treatment with Follistatin protein developed significantly more force than denervated animals without treatment. The muscle fibers expressing type IIa and IIb myosin heavy chains were larger in diameter in the experimental animals that were treated with either type of Follistatin than control animals that received no Follistatin.
Conclusions: In this rodent model of delayed peripheral nerve repair, we found that Follistatin, administered 1 month after reinnervation had a greater facilitating effect after 6 months than 3 months of denervation on the recovery of type IIa and IIb muscle fibers and muscle force.
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Recovery of Chronically Denervated Muscle Enhanced with Follistatin Treatment

Introduction: Poor functional recovery following major peripheral nerve injury is largely due to the progressive catabolic process (denervation atrophy) affecting muscle fibers. Follistatin is a glycoprotein that blocks the muscle growth inhibiting peptide myostatin and also possesses remarkable independent muscle stimulating properties. We hypothesized that the administration of follistatin to rodent muscles subjected to prolonged but temporary denervation (3 or 6 months) would improve final muscle recovery and function.

Materials and methods:
One hundred forty-four (three-month old female) Sprague-Dawley rats were divided into 8 groups comprising animal muscles with or without temporary denervation and subsequent repair (3 or 6 months) and with or without follistatin treatment (delivered via Adenovirus viral vector containing recombinant DNA or direct delivery of recombinant protein via subcutaneous osmotic pumps).

After final recovery, muscle weight and force were recorded and statistically compared between groups with an alpha level of .05.

Results:
For 3-month denervation groups, there was no improvement in muscle weight or force generation following either protein or recombinant DNA treatment. For 6-month group, virus administration resulted in higher final muscle weights in both denervation and sham denervation groups. Protein treatment resulted in greater muscle force generation in the denervation group.

Conclusion: Six month chronically denervated muscle showed modest improvements in muscle mass and strength recovery following follistatin treatment. The effect was not consistent and further study will be necessary to elucidate any future role of this novel treatment strategy.