# The Rat Model for Traumatic Volumetric Muscle Loss

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The use of Army medical and/or other Army records in the preparation of this material is acknowledged, but is not to be construed as implying official Department of the Army approval of the conclusions presented.

**Abstract:** Background: Soft tissue injuries of extremities comprise the largest number of wounds received by United States military personnel in war-time activities and make up the largest percentage of hospital care cost and disability outcome among total battlefield injuries. Current surgical procedures are not able to repair massive volumetric muscle loss (VML) with functional and cosmetic recovery. Tissue engineering and regenerative medicine (TE/RM) may offer the best solutions to this problem. However, there are currently no standard models to test TE/RM solutions for VML. The aim of this study was to develop a critical size defect of VML in a rat model. Critical size is defined as the amount of muscle that must be removed that does not allow regeneration of muscle function. Characterization of the injury was done by assessing in vivo and in situ muscle function. Methods: In order to perform in vivo muscle function testing, five rats were first implanted with electrode cuff on the right common peroneal nerve. One month later, a defect to the right Tibialis Anterior (TA) was induced by removing twenty percent of the total weight of the muscle. In-vivo isometric muscle function testing was performed weekly for 8 weeks, and in-situ muscle function testing for both injured and normal legs were performed at tenth week. Results: The in-vivo tetanic force was reduced 30% at week 2 and back to 10% reduction at week 8 compared to the baseline. At week 10 of injury, the wet weight, absolute isometric tetanic force and isokinetic peak power output of injured TA were significantly reduced by 18%, 25% and 31% of normal leg. Conclusion: This study provides a rat model for a critical size defect of traumatic VML. This model should provide a useful platform to develop and test TE/RM approaches for the repair of VML.

## Background

- Soft tissue injuries of extremities comprise the largest number of battlefield injuries

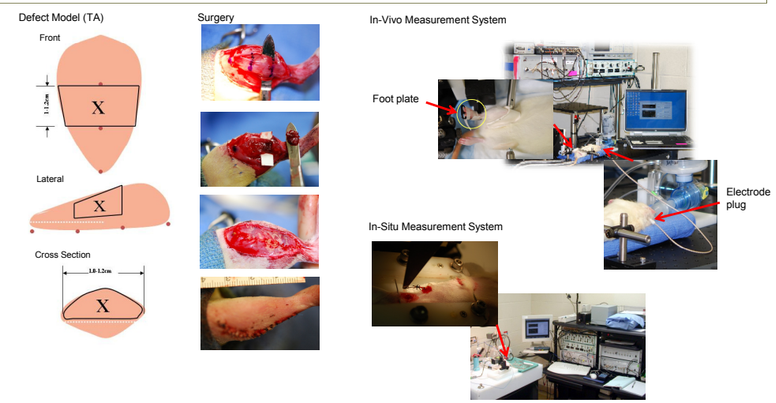
  Owens, B.D., et al. J Orthop Trauma, 2007; Mabry, R.L., et al. . J Trauma, 2000; Zouris, J.M., et al., Mil Med, 2006.
- These injuries often involve the physical loss of large amounts of skeletal muscle (volumetric muscle loss (VML))
- Current surgical approaches are not satisfactory
- Tissue engineering and regenerative medicine (TE/RM) approaches offer possible solutions
- An animal model is needed to serve as a testing platform for TE/RM

## Objective

- To develop a critical size defect of VML in a rat model

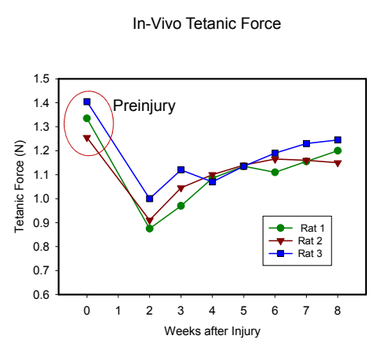
## Methods and Measurements

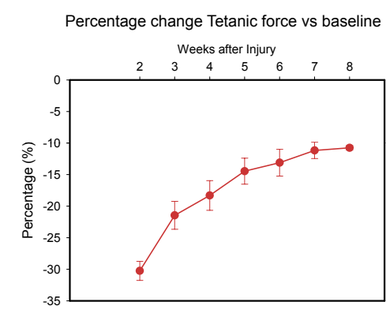
- Five inbred Lewis male rats at weight about 400 grams.
- Nerve cuff implantation: under anesthesia, the electrode cuff was implanted around common peroneal nerve of right leg. The wires of nerve cuff was buried under the skin and connected to the mini-plug which was fixed at the posterior of the neck.
- The muscle contractile properties were measured using a dual-mode servo muscle lever system. A personal computer loaded with Labview® software and a National Instruments A/D board controlled the muscle lever and stimulator, recorded all signal, and performed real-time analysis of the force and length signals.
- In-vivo muscle function testing was performed weekly starting 3 weeks after nerve cuff implantation. The isometric tetanic force was measured at frequency of150 Hz. The rats with sign of nerve injury were excluded from the study.
- Critical size of muscle defect: the surgery (n=5) was performed at least four weeks after nerve cuff implantation, when the rats have the stable results from in-vivo muscle function testing. Under anesthesia, muscle tissue about 20% of TA weight (the TA weight was predicted based on the body weight) was excised at middle portion of TA. The TA including the defect area was closed by the fascia.
- The in-vivo muscle function testing of injured leg was performed once a week starting at third week after injury. Three rats were followed for eight weeks.
- At week ten, in-situ muscle function tests of all five rats were performed at both injured leg (right) and contra lateral normal leg (left), including isometric tetanic force, and isokinetic peak force (at shortening velocity from 10 mm/sec. to 70 mm/sec.).
- After muscle function tests, the rats were euthanized and entire TA was isolated for wet weight measurement.
- The data are expressed by mean ± standard error of mean. The statistic analysis was performed using student *t* test, and $p<0.05$ was accepted for significant difference.



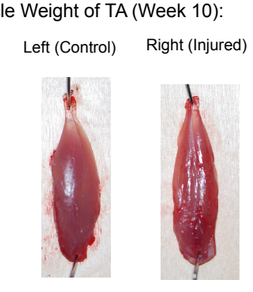

## Results

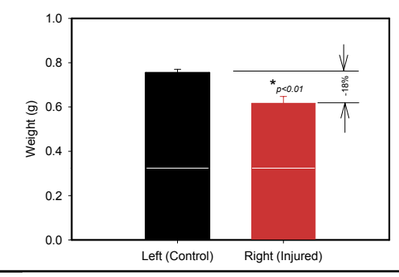
In-Vivo Peak Force:



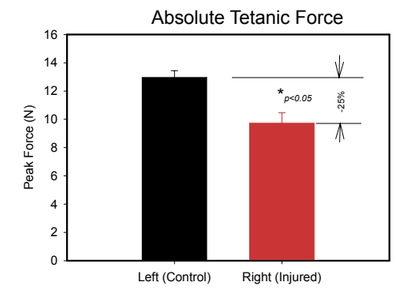



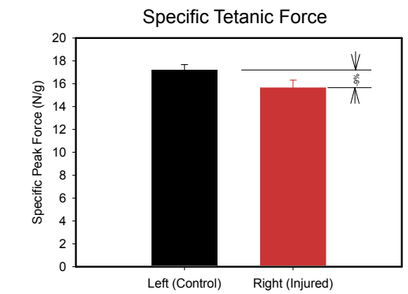

Muscle Weight of TA (Week 10):



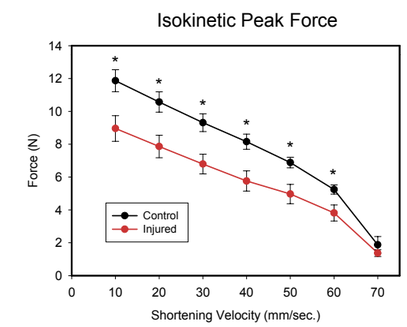



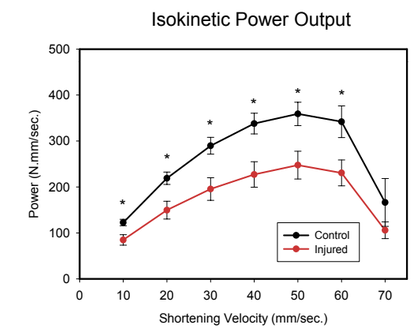

In-Situ (Week 10): Isometric Contraction:




In-Situ (Week 10): Isokinetic Contraction:




## Summary

- Muscle function could be followed in-vivo for more than eight weeks using the rat model with nerve cuff implantation. The in-vivo tetanic force was reduced 30% at week 2 and 10% at week 8 compared to the baseline.
- TA with 20% of volumetric muscle loss was thinner, and the muscle weight was significantly lower than the TA at contralateral leg after ten weeks of injury.
- At week 10 of injury, the injured TA had 25% reduction of absolute tetanic force by isometric stimulation, which was significantly lower than the normal leg, however, no significant difference of specific tetanic force was found between the injured and normal leg.
- In isokinetic contraction, the peak force and power output of injured TA were significantly lower than the TA of contralateral normal leg from 10 mm/sec to 60 mm/sec of shortening velocity. The peak power output of TA was occurred at 50 mm/sec, where the injured TA was 31% reduction from the normal TA.

## Conclusions

- This study provides a rat model for traumatic volumetric muscle loss with critical size of muscle defect at the muscle of TA, where the muscle function and volume is not able to recover spontaneously after injury.
- The model will provide a useful platform for the development and testing of TE/RM approaches to the treatment of VML
- Our in vivo muscle function testing method will allow us to assess TE/RM approaches with a minimal number of animals

**Acknowledgment:**

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