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Measuring Muscle Tissue Trauma in Murine and In-Vivo Models

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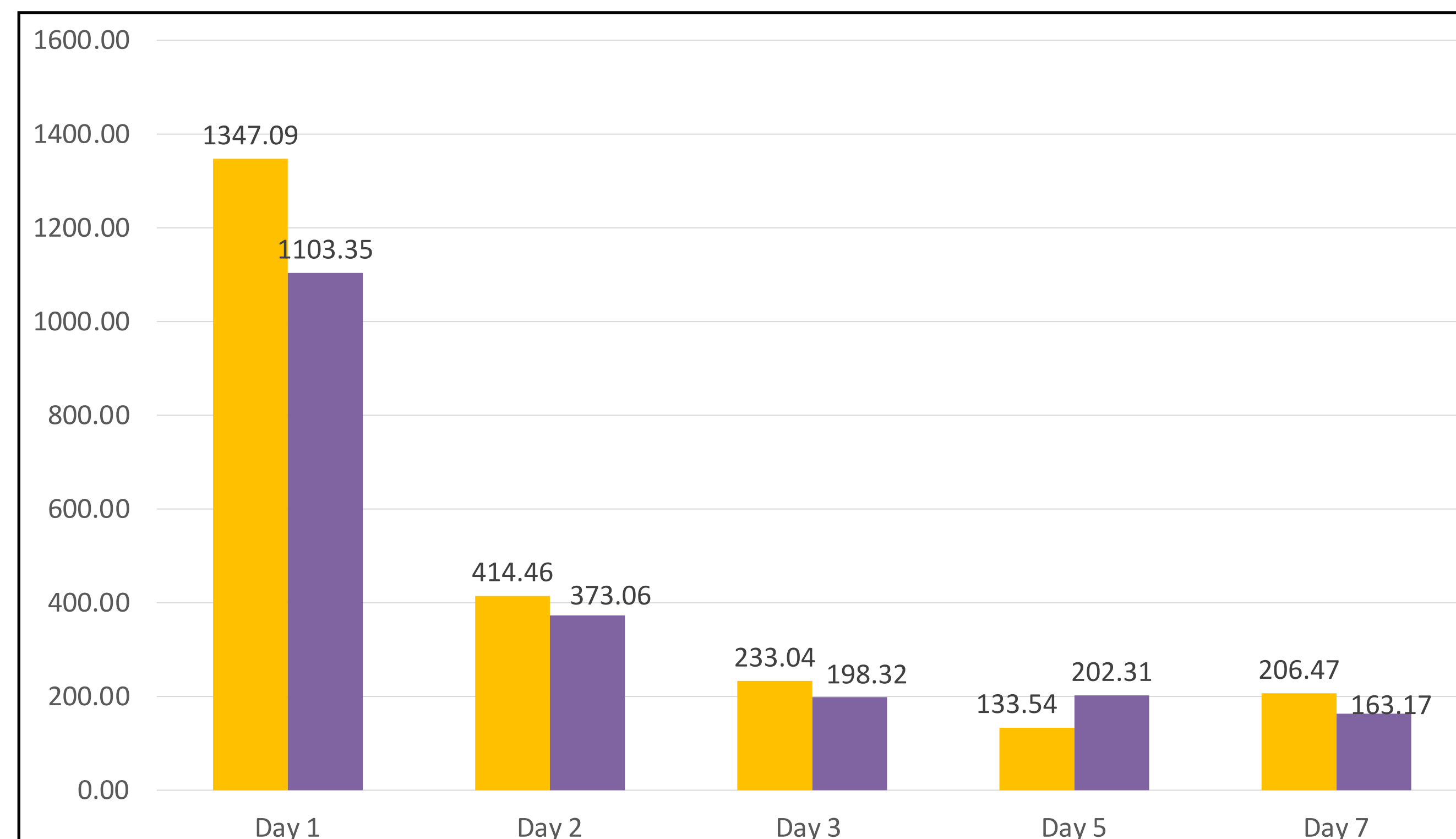
Introduction

- Extremity trauma involving bone and soft tissue injury is prone to complications such as poor tissue healing, wound dehiscence, wound infection, fracture related infection, and fracture nonunion.¹
- Many clinical outcomes are driven by the healing of the damaged muscle following extremity trauma.²
- The aim of this research is to establish methods of measuring muscle tissue trauma using discarded muscle tissue.
- These methods are first optimized in a murine model.

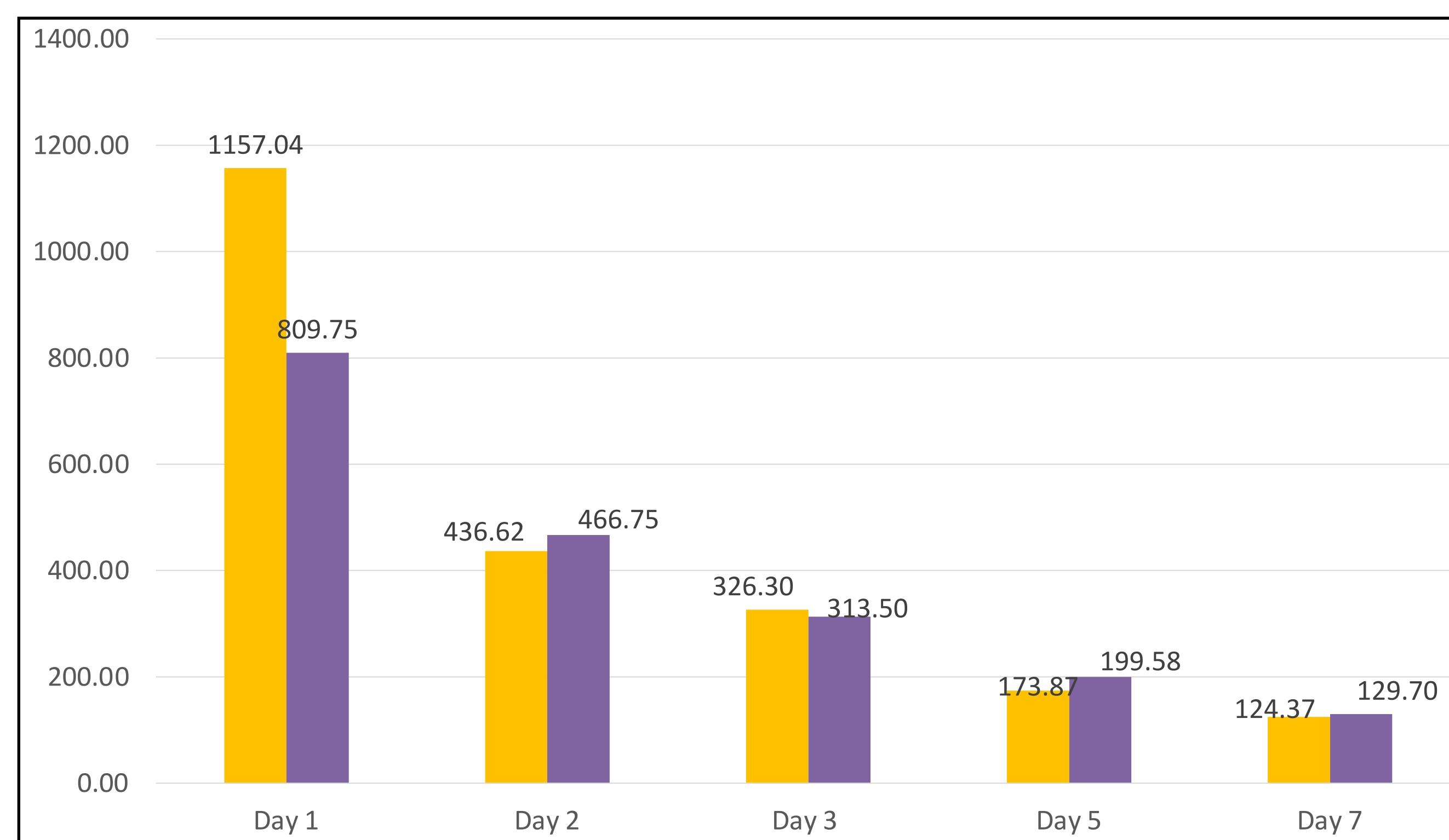
Methods

- The tibialis anterior (TA) musculature in four common lab mice (*Mus musculus*) were dissected bilaterally.
- Per mouse, one hindlimb was designated as control and one hindlimb designated as injured.
- Injury was created by clamping the muscle in 3 sections for 5 seconds each.³
- The dissected muscles were placed in A DMEM, 1% “Zonker” antibiotic, and 0.1% Ampicillin and incubated at 37°C.
- Each day for seven days, 3cc of media was removed and replaced with fresh media
- After the last day, the samples were weighed.
- Enzyme linked immunosorbent assays (ELISA) for hepatocyte growth factor (HGF) and beta-fibroblast growth factor (b-FGF) were performed to measure these muscle damage factors in the media supernatant.^{4,5}
- To identify a trend, we performed unpaired t-tests.

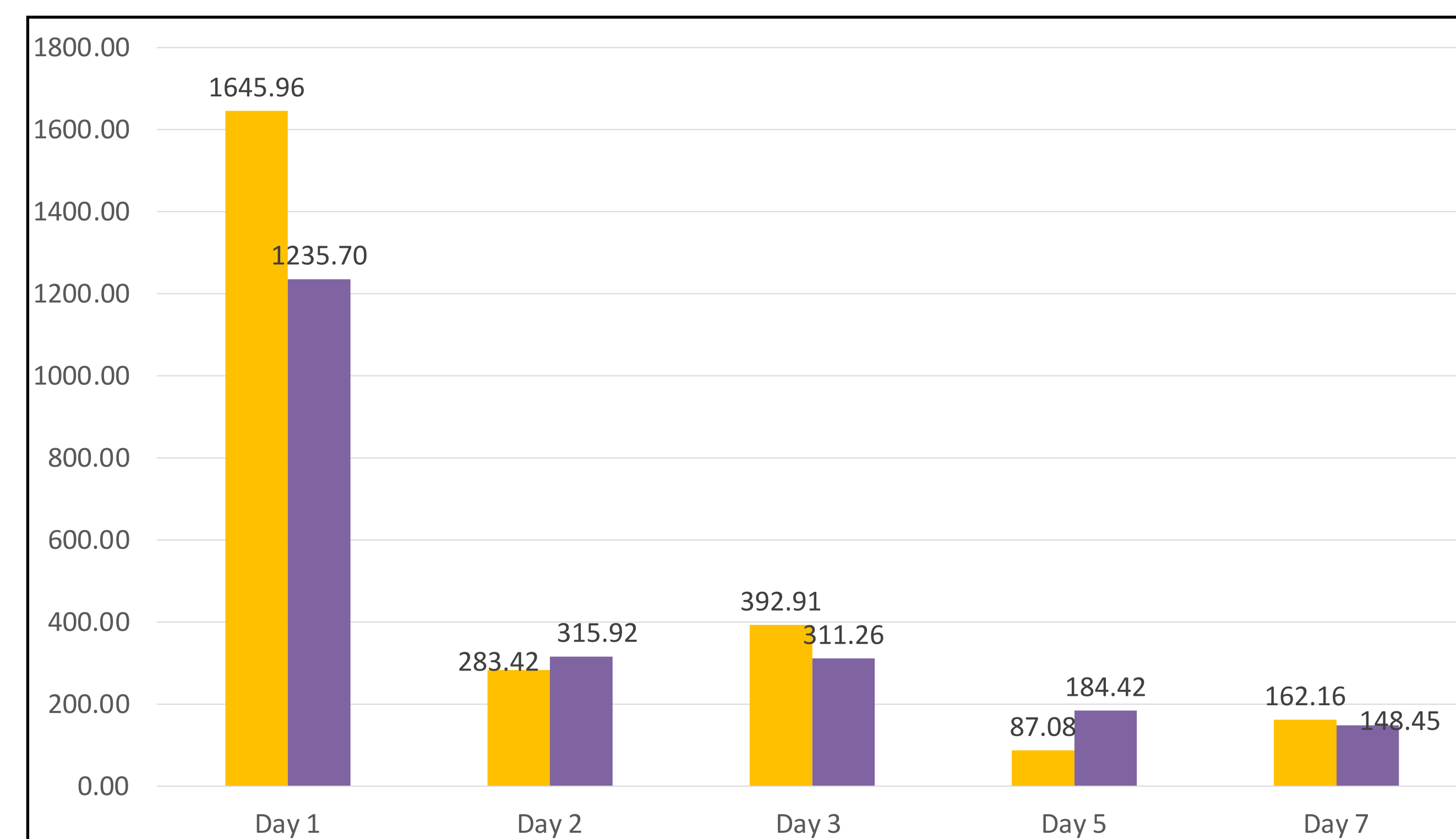
Mouse 1 Injury vs Control



Mouse 2 Injury vs Control



Mouse 3 Injury vs Control



Results

- ELISA results for b-FGF for three mice normalized to muscle sample weight are shown (left)
- There tended to be a difference in expressed b-FGF between Day 1 injury and Control samples
- When comparing Day 1 versus Day 2 for all samples, b-FGF expression had significantly declined, (1216.48+/-277.00 versus 381.71+/-71.25; p<0.0001)
- b-FGF expression for day 3-7 were similar

Conclusion

- By optimizing in vitro measurements of muscle damage, these results can be extrapolated to additional study on how damaged muscle may be recovered.
- Additionally, these methods will be used to study human muscle which is damaged by orthopaedic trauma.
- We are currently enrolling prospective human subjects and collecting discarded traumatized muscle tissue from surgical debridements.

References

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3. Rushton JL, et al. Production of consistent crush lesions of murine skeletal muscle in vivo using an electromechanical device. *J Anat*. 1997 Apr;190 (Pt 3)(Pt 3):417-22.
4. Shi H, et al. Myoprotective effects of bFGF on skeletal muscle injury in pressure-related deep tissue injury in rats. *Burns Trauma*. 2016 Aug 17;4:26.
5. Choi W, et al. Hepatocyte Growth Factor Regulates Macrophage Transition to the M2 Phenotype and Promotes Murine Skeletal Muscle Regeneration. *Front Physiol*. 2019 Jul 25;10:914.