Summer 2021

Quantification of Skeletal Muscle Synthesis Pathway Proteins in Wild Type and MMP-2 Knockout Mice After Functional Overload of the Plantaris Muscle

Naia N. Kenney
University of Puget Sound

Jung Kim
University of Puget Sound

Quantification of Skeletal Muscle Synthesis Pathway Proteins in Wild Type and MMP-2 Knockout Mice After Functional Overload of the Plantaris Muscle

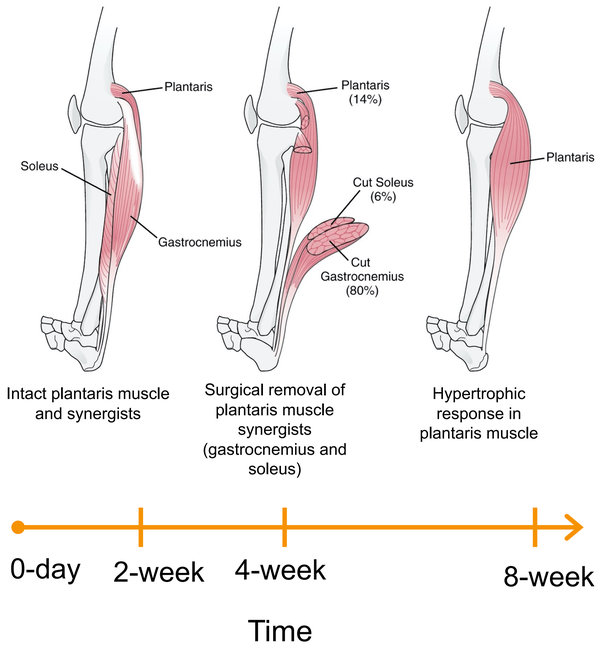
Naia Kenney, Gary E. McCall, PhD, FACSM, and Jung A. Kim, PhD
Department of Exercise Science, 1500 N Warner, Tacoma, WA 98416

Introduction

- The most abundant tissue in the human body, skeletal muscle, is highly plastic allowing it to adapt to changing functional needs including exercise and injury (1).
- Functional overload (FO) is a surgical procedure involving the removal of a muscle, which forces the surrounding muscles to compensate. This procedure results in significant hypertrophy in the remaining muscle (1).
- Previous studies highlight the role of the extracellular matrix (ECM) in skeletal muscle plasticity. The skeletal muscle ECM is primarily composed of proteoglycans and collagens. The ECM's ability to regenerate in response to injury, exercise, or disease is integral to muscles adaptability (2).
- ECM homeostasis is largely maintained by matrix metalloproteinases (MMPs), enzymes that are responsible for degrading extracellular proteins.
- Specifically, MMP-2 is believed to be linked to the structural remodeling of the ECM during periods of growth and repair.
- Disruption of ECM synthesis and MMP activity has been shown to affect skeletal muscle regeneration, indicating the importance of ECM homeostasis in controlling cellular behavior (3).
- The purpose of this study was to determine the effects of MMP-2 knockout (KO) on protein synthesis and degradation pathways after 0-day, 2-, 4-, and 8- weeks FO in adult mice.
- We hypothesized that the decreased hypertrophic response to FO in MMP-2 KO mice is due to impaired ECM remodeling, and subsequent protein synthesis.

Methods

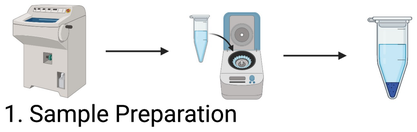
Functional Overload Model



- Plantaris muscle synergists, gastrocnemius and soleus, were removed in both wild type (WT) and MMP-2 KO female mice (n=3-6 mice for each group at each time point).
- Total protein from plantaris muscles were extracted and concentration determined using the *DC* Protein Assay (Biorad)

Methods

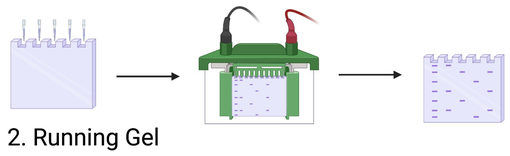
Western Blot Procedure



1. Sample Preparation

Cut cross sections of mouse plantaris muscle using cryostat and homogenized in lysis buffer

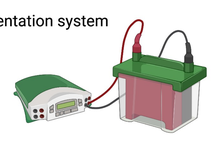
Centrifuged and removed supernatant containing pure protein



2. Running Gel

40 µg of protein sample was loaded onto gradient SDS-PAGE gel, the gel was then run for ~40 minutes at 150V

The gel was imaged using the gel documentation system (Li-Cor Odyssey)



3. Transfer gel

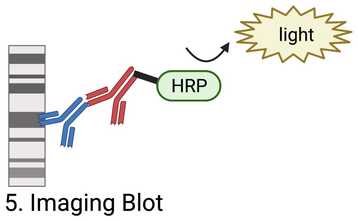
Transferred gel onto polyvinylidene difluoride (PVDF) membrane for two hours at 30V

4. Antibody Incubation

Blocked blot for 2 hours using 5% BSA in TBST

Probed blot with primary antibody of interest (AKT, mTOR, IGF1, or S6K1) overnight

Washed blot in TBST and incubated with horseradish peroxidase-conjugated secondary antibodies



5. Imaging Blot

Signal intensity was determined using Li-Cor Odyssey

Results

Gel and Blot Image for Akt and pAkt using different protein concentrations

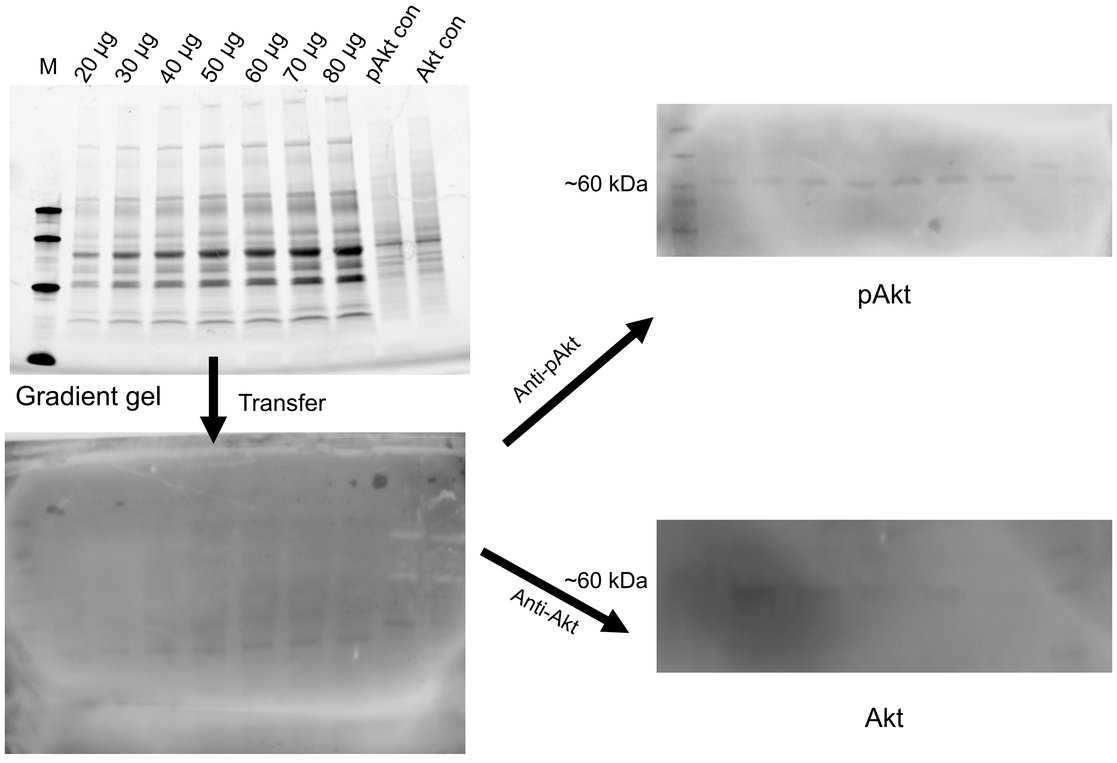


Figure 1. Gradient gel (top left) showing total proteins from mouse plantaris muscle at different loading amounts. Image of the blot after transfer (bottom left) of the gel. The images on the right display pAkt and Akt protein bands at 60 kDa after primary and secondary antibody incubation from the membrane on the left. M, WesternC protein ladder.

Results

Dynamic Linear Curve for pAkt and Akt

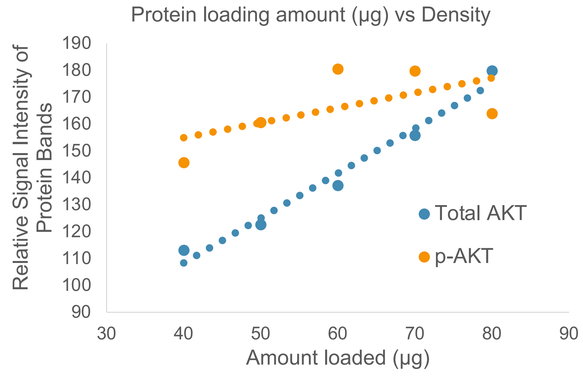


Figure 2. Relative densities of pAkt and Akt protein bands plotted against sample concentration. The linear curve reveals the appropriate sample loading concentration for each antibody. The linear ranges indicate that 40-60 µg is an appropriate loading amount for pAkt and Akt.

Conclusions

- Using stain-free gradient gels, we determined that 10% stain-free gels produce the same results and is optimal for our proteins of interest.
- We optimized the gel running time (40 min) and voltage (150V), as well as transfer time (2 hrs) and voltage (30V).
- Based on the dynamic linear curves for total Akt, phosphorylated Akt, and total p70Sk (data not shown), 40 µg is an appropriate gel loading amount for determining protein expression.

Future Directions

- Continue to optimize western blot parameters for mTOR and IGF-1 using the dynamic linear curve to determine the appropriate loading amount.
- We will determine the expression of active (phosphorylated) vs total protein expression of each gene of interest (Akt, mTOR, IGF-1, p70S6k) to compare differences between WT and MMP-2 KO mice at each of the different time points after FO.
- Perform western blots from WT and MMP-2 KO samples for all proteins of interest.

References

(1) Baldwin, K.M., et al., *Biochemical properties of overloaded fast-twitch skeletal muscle.* J Appl Physiol Respir Environ Exerc Physiol, 1982. **52**(2): p. 467-72.
(2) Gillies, A.R. and R.L. Lieber, *Structure and function of the skeletal muscle extracellular matrix.* Muscle Nerve, 2011. **44**(3): p. 318-31.
(3) Zhang, Q., et al., *Matrix metalloproteinase-2 plays a critical role in overload induced skeletal muscle hypertrophy.* Muscle Ligaments Tendons J, 2014. **4**(4): p. 446-54.

Acknowledgments

- This research was made possible thanks to the Caputo Summer Science Research Fund and the University of Puget Sound Math and Science Research Program.
- A special thank you to Professor Jung Kim for her ongoing guidance and support .
- This research was also supported through funding by the University Enrichment Committee

PRINTED BY PRINT & COPY SERVICES AT UNIVERSITY OF PUGET SOUND | PUGETSOUND.EDU/PCS