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mRNA Expression of Muscle Differentiation Markers in Wild Type and MMP-2 Knockout Mice After Functional Overload of the Plantaris Muscle

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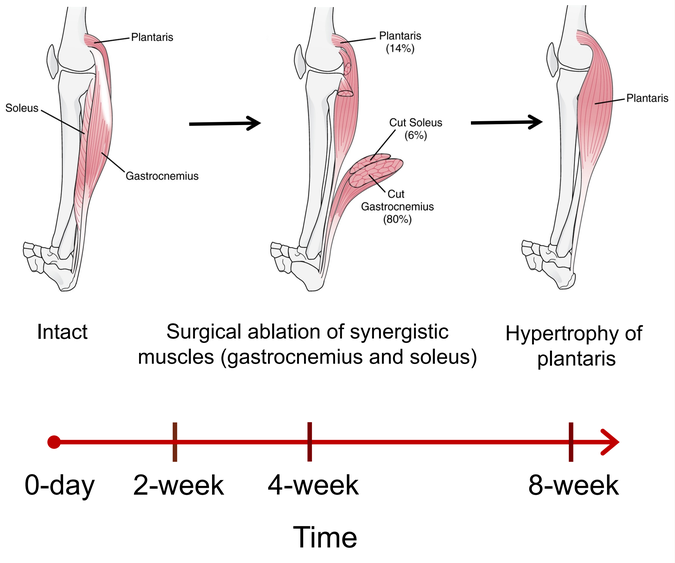
Introduction

- Skeletal muscle is highly plastic in nature and can readily adapt to injury, disease, and changes in activity; however, the mechanisms of how skeletal muscle does this remain largely unknown.
- The skeletal muscle extracellular matrix (ECM) surrounds each muscle fiber and is mostly composed of type IV collagen.
- Matrix metalloproteinases (MMPs) are enzymes important for maintaining ECM homeostasis, as they degrade and synthesize collagen, and are necessary for other cellular events after damage or injury (1).
- Matrix metalloproteinase-2 (MMP2) is a regulatory enzyme that is central in muscle growth as it cleans up ECM collagen and promotes the differentiation of satellite cells (2).
- Functional overload (FO) is a model used to induce muscle hypertrophy by surgical removal of the major synergists that perform similar functions, i.e., ankle extension (3).
- The objective of this study was to investigate the role of MMP-2 in skeletal muscle hypertrophy the plantaris muscles of adult mice after FO.
- Specifically, we examined the expression of muscle differentiation markers MyoD and myogenin using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) in the functionally overloaded plantaris muscle from wild type (WT) and MMP-2 knockout (KO) mice.
- We hypothesized that the decreased hypertrophic response to FO in MMP-2 KO mice is due to impaired ECM remodeling and impairment of protein synthesis pathways.

Materials & Methods

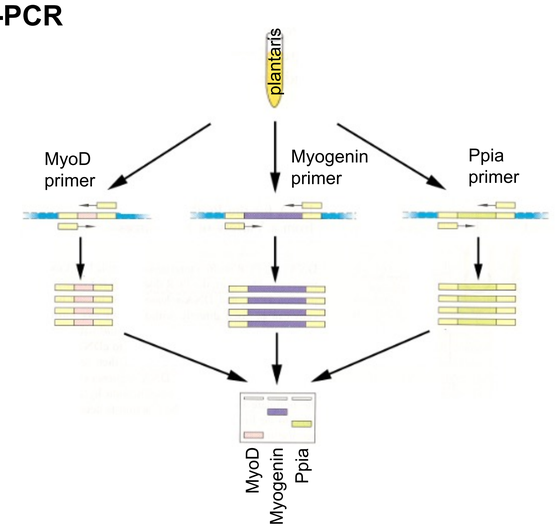
Functional Overload



- Total RNA was extracted from MMP-2 KO and WT mice at each time point after FO (n=3-6 mice per group and time point).
- cDNA was synthesized from total RNA using a First-Strand cDNA Synthesis Kit.
- Primers for each gene of interest, MyoD, Myogenin, and Ppia, a housekeeping gene for normalization, were designed and tested by PCR and further optimized for qPCR.

Materials & Methods

RT-PCR



Step 1: isolation of RNA from plantaris muscle

Step 2: reverse transcription using the First strand cDNA synthesis kit

Step 3: PCR

Step 4: analysis of RT-PCR products on a 1.8% agarose gel

Results

Agarose Gels from MyoD, Myogenin, and Ppia Primers

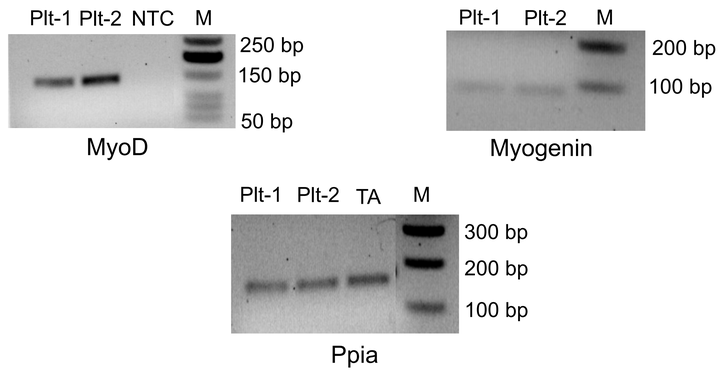


Figure 1. Agarose gels showing the expression of skeletal muscle differentiation genes MyoD (left), myogenin (right), and Ppia, a housekeeping gene (bottom), from RT-PCR experiments. All products were detected at the expected size. Plt, plantaris; TA, tibialis anterior; NTC, no template control; M, 100-bp or low molecular weight ladder.

Gradient qPCR for MyoD and Myogenin for Temperature Optimization

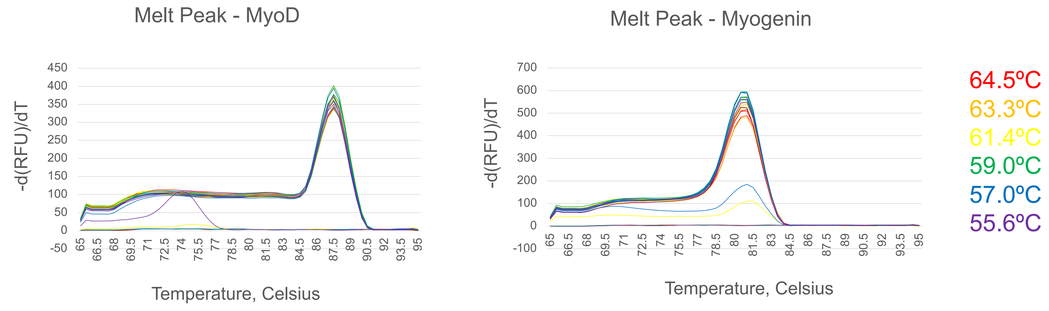


Figure 2. Gradient qPCR was performed to determine the optimum temperature for each primer pair designed from the RT-PCR experiments in Fig. 1 using cDNA from practice mouse plantaris muscles. Melt curves for MyoD (left) and myogenin (right) were generated from the annealing temperatures used shown above. Note that the melt curves for both genes are not "clean," and the shorter peaks seen in the figure are from the no template control reactions, suggesting either contamination (myogenin) or primer-dimer (MyoD, see Fig. 3). Additional experiments and analyses will be performed to evaluate those peaks.

Results

Agarose Gels from Gradient qPCR

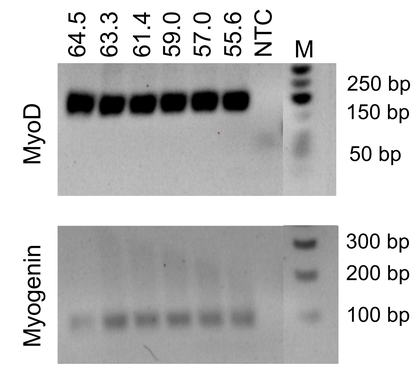


Figure 3. Agarose gels confirming the melt curves from Fig. 2. Both genes showed a single band at all temperatures of the expected size. MyoD (top) also showed a band in the no template control (NTC) that was smaller than the expected product which suggests primer-dimer. This was only observed at 55.6ºC. Myogenin (bottom) showed no product in the NTC, but will need to be repeated as there were peaks in the melt curve at 57ºC and 61.4ºC, which reactions were not run out on the gel. M, 100-bp or low molecular weight ladder.

Conclusions

- Primers for MyoD, myogenin, and Ppia showed a single band at the expected size in the mouse plantaris muscle.
- Gradient qPCR showed a single peak in the MyoD and myogenin melt curves for all temperatures tested.
- The Cq values for MyoD and myogenin ranged between 23 and 27 cycles at all temperatures (data not shown).
- The appearance of a second small peak in the melt curve for MyoD at the lowest temperature (55.6ºC) in the no template control appears to be due to primer-dimer as shown on the gel (Fig. 3).
- There were multiple peaks detected for myogenin in the no template control reactions, suggesting contamination and will need to be examined further.
- Gradient qPCR for Ppia showed significant contamination and thus was not included and will be repeated.
- Future studies include running primer efficiency experiments in order to determine the optimum annealing temperature for all primers.
- Perform qPCR experiments for MyoD and myogenin from all FO plantaris muscles from WT and MMP-2 KO mice.

References

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Acknowledgments

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