Transcriptional Profiling following Repeated Optogenetic Activation of Skeletal Muscle in Young Mice

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Disclosures: NONE.

INTRODUCTION: Exercise is an important factor for maintaining health across the lifespan. In adults, exercise-induced molecular regulation of skeletal muscle has been well-studied (1). However, it is unclear how muscle adapts to acute and repeated activity during skeletal growth. With a rise in participation in sports by adolescents in recent years, a better understanding of muscle adaptation during growth is needed. In this study, we aimed to identify how transcriptional profiles of skeletal muscle change following repeated muscle contractions in young mice without the concomitant effect of systemic metabolic changes associated with exercise. To accomplish this aim, we used optogenetic stimulation to induce pulsed contractions of the triceps surae muscles in 3-week-old mice to mimic daily exercise bouts and compared transcriptional profiles after 5 and 12 days using RNA sequencing.

METHODS: The Unit for Laboratory Animal Medicine at the University of Michigan approved all animal procedures. Right triceps surae muscles of 3-week-old Acta1-Cre; Ai32 homozygous male mice were stimulated daily with blue light (455 nm) at 10 Hz (70 ms on-/30 ms offtime) for 20 minutes using a custom-built setup (2). Stimulations were repeated for 5 or 12 days (n = 3/time point). Contralateral limb served as non-stimulated internal control. Mice were euthanized three hours after the last stimulation session and triceps surae muscles were snap-frozen in liquid nitrogen and stored in -80 ºC until RNA isolation. Samples were pulverized in TRIzol, using a tissue homogenizer, and total RNA was isolated using PureLink RNA Mini Kit with on-column DNA digestion (Invitrogen). RNA Integrity Number (RIN) was tested using a bioanalyzer (RIN > 9.0 for all samples). Poly-A mRNA library preparation and Next-Generation sequencing were performed on the Illumina NovaSeq Shared platform, and Snakemake pipeline (3) was followed for quality control and sequence alignment. *Data Analysis:* Differential expression (DE) of genes was determined from the count matrix with a paired design in DESeq2 in R//Bioconductor (4). A threshold of 1.25-fold change was applied within the Wald tests of significance. p-values were corrected for multiple testing using the Benjamini and Hochberg method, and significance was set at p-adjusted < 0.05. For gene ontology (GO) biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, DE genes lists were input separately for each time point into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (5,6) and analyzed using the functional annotation tool ($p < 0.01$ and $p < 0.05$ for GO and KEGG, respectively).

RESULTS: After 5 days of repeated stimulation, muscles from stimulated limb had 326 up-regulated and 110 down-regulated DE genes compared to non-stimulated contralateral muscles (Fig. 1B). Transcriptional activation reduced after 12 days of stimulation, with 56 downregulated and 116 up-regulated genes (Fig. 1D). 99 DE genes were shared between the two time points. Of the total 21 KEGG pathway analyses included in this study, less activation was observed after 12 days of stimulation (Fig. 2). Adipocytokine signaling and glycolysis/ gluconeogenesis pathways were enriched at both time points. PPAR signaling and insulin resistance pathways were also enriched by stimulation. Enrichment was observed in several immune system-related pathways, such as B cell receptor and chemokine signaling. Signaling pathways, e.g., Jak-STAT, MAPK, and TNF were also enriched. After 5 days of stimulation, there was a large transcriptional response in immune related genes and pathways which diminished after 12 days. Enriched biological processes included common pathways related to exercise-induced changes (Table 1).

DISCUSSION: This study explored the transcriptional changes in skeletal muscle following repeated optogenetic stimulation in young mice. Optogenetic stimulation uses blue light exposure through the skin to non-invasively and locally contract the skeletal muscle (2), while minimizing systemic (organism-scale) metabolic adaptation. It provides a controlled experimental approach for paired (intraanimal) comparisons that cannot be performed using traditional exercise methods (e.g., treadmill running). In this study, we observed differential expression in genetic programs of interest, such as myogenic (*Ankdr2, Cspr3, MyoD1*), anti-myogenic (*Tead4, Tnfrsf12a*), adipogenic (*Adipoq, Cebpa, Lpl,* etc.), inflammatory (*Il1r1*), and fibrotic (*Lox*) genes over the stimulation period. Expression of these genes followed similar trends to previous work studying muscle transcriptional responses after tenotomy (7). Future studies will focus on the transcriptional profile in aged mice following optogenetic stimulation as well as comparing optogenetic transcriptional responses to that of "gold standard" exercise regimens (e.g., treadmill running) and injury (e.g., denervation).

SIGNIFICANCE/CLINICAL RELEVANCE: Repeated training of skeletal muscle elicit differential transcriptional responses over time, and these findings in mice can inform our understanding of exercise-induced muscle adaptation. Findings of this study highlight the differential transcriptional response of skeletal muscle following repeated bouts of exercise-mimicking muscle contractions.

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ACKNOWLEDGEMENTS: NSF CAREER Award 1944448 (MLK); NIH R01AR079367 (MLK); next-generation sequencing was carried out in the Advanced Genomics Core at the University of Michigan. Thanks to Zachary Tata for help with mouse husbandry.

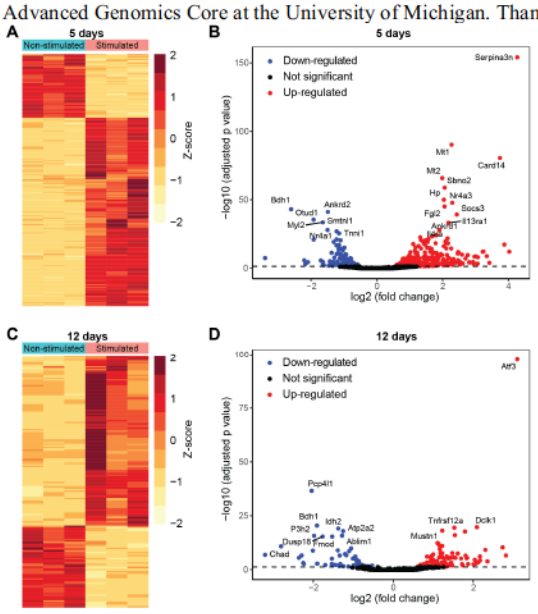

Fig. 1: RNAseq of muscles revealed changes in gene expression following optogenetic stimulation. A, C) Heat maps of DE genes at 5 and 12-day post stimulation, respectively. B, D) Volcano plots, with top 20 significant DE genes labeled at 5 and 12-day post stimulation, respectively.

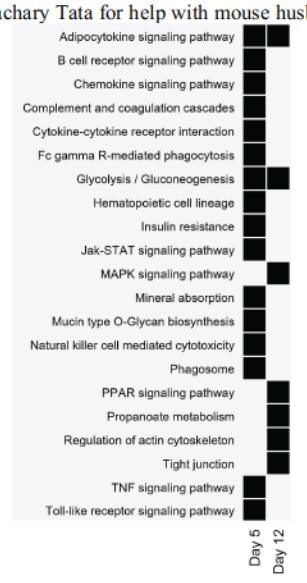

Fig. 2: KEGG enrichment after filtering out disease/ tissue specific pathways. 12 days of optogenetic stimulation showed fewer enriched pathways.

Table 1: Enriched biological processes

5 days

Biological process	Genes
Skeletal muscle contraction	*Tnni1, Myh8, Tcap, etc.*
Positive regulation of angiogenesis	*Hmox1, Fgf1, Tnfrsf1a, etc.*
Skeletal muscle cell differentiation	*Fos, Atf3, Myod1, etc.*
Positive regulation of TNF production	*Ccl2, Cd14, Tnfrsf1a, etc.*
Transition between fast and slow fiber	*Tnnt1, Tnni1, Myh7, etc.*

12 days

Biological process	Genes
Skeletal muscle cell differentiation	*Ankrd1, Sox11, Atf3, etc.*
Response to hypoxia	*Kcnma1, Pak1, Lep, etc.*
Negative regulation of cell proliferation	*Cebpa, Rgcc, Fgfr3, etc.*