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CONTRACTING ORGANIZATION: Wright State University, Dayton, OH

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In Duchenne muscular dystrophy (DMD), loss-of-function mutations in the gene encoding dystrophin trigger skeletal muscle sarcolemma instability, causing membrane damage during muscle contraction. This leads to progressive muscle weakness and dramatic muscle degeneration that results in early mortality in affected teenagers. Most of the patients die from respiratory failure and heart failure. A primary strategy in treating DMD is to reverse the instability of muscle membranes by increasing dystrophin levels. However, there is a major obstacle in that the dystrophin gene is too large to be packaged into current gene therapy vectors. Lipin1 is a phosphatidic acid (PA) phosphatase (PAP) that catalyzes the conversion of PA to diacylglycerol, a critical step in the synthesis of glycerophospholipids. We found that using our unique muscle-specific mdx:lipin transgenic (mdx:lipin1^{Tg}) mice, increasing lipin1 expression levels attenuated inflammation infiltration, fibrosis, and necrosis in gastrocnemius of mdx mice. It also strengthened membrane integrity, and resulted in markedly improved gastrocnemius muscle contractile and eccentric force. In addition, AAV-mediated lipin1 gene delivery also attenuated gastrocnemius pathology in mdx mice. These data suggest that lipin1 could be a potential therapeutic target for the treatment of DMD.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. Introduction

Duchenne muscular dystrophy (DMD) is a progressive and devastating muscle disease, resulting from the absence of dystrophin. This leads to cell membrane instability, susceptibility to contraction-induced muscle damage, muscle cell death, and disability and early death of patients. Currently, there is no cure for DMD. Lipin1 has dual functions acting as a phosphatidic acid (PA) phosphatase (PAP) enzyme that catalyzes diacylglycerol (DAG) biosynthesis and as a transcriptional coactivator/corepressor for metabolic nuclear receptors. Our recent publication reveals that lipin1 is critical for the maintenance of membrane integrity, and lipin1 plays complementary roles in myofiber stability in dystrophic muscles. As such, we **hypothesize** that lipin1 overexpression in mdx mice is effective in suppressing the dystrophic muscle phenotype and leads to a functional improvement in skeletal muscle. We have completed studies proposed in aim 1. The **goal** of aim 2 in our State of Work (SOW) is to determine the therapeutic efficacy of AAV-mediated systemic lipin1 gene delivery, which is the main task for the second-year period.

2. Keywords

Duchenne muscular dystrophy, lipin1, phosphatidic phosphatase, diacylglycerol, membrane integrity, muscle damage

3. Accomplishments

Major goals: In the approved SOW, the **major goal** of this project for year 2 is to determine the therapeutic efficacy of AAV-mediated systemic lipin1 gene delivery. We have completed 60% of the experiments proposed in Aim 2. In addition, In the year 2 funding period, we had three publications in the *Journal of Physiology, AJP-Renal Physiology,* and *Archives of Microbiology & Immunology*, and another one has been submitted to *Acta Physiologica* and currently is under review. We are working on two more manuscripts and hope to send them out in the next two months.

Major accomplishments:

Task 2 (Aim 2) is to determine the therapeutic efficacy of AAV-mediated systemic lipin1 gene delivery.

Subtask 1: Determine the optimal dosing regimen by evaluating safety, specificity, and transduction efficiency in mdx mice using a dose-escalation study.

We recently received the AAV-lipin1 and AAV-GFP virus under the muscle-specific MHCK7 promoter from our collaborator, Dr. Scott Harper at the Ohio State University. To determine the safety and efficiency of systemic AAV-lipin1 gene therapy, three-month-old mice were injected with the AAV-lipin1 or AAV-GFP via the tail vein at two different doses of AAV, 9×10^{12} and 3×10^{13} vg/kg. One month post-injection, gastrocnemius muscles were collected. Age and gender-matched B10-WT and mdx mice injected with AAV-GFP at a dose of either 9×10^{12} or 3×10^{13} vg/kg were used as controls. The histology of mdx gastrocnemius muscles in various groups was evaluated by H&E staining as shown in Fig. 1.

We found that the mdx muscle treated with AAV-GFP at a dose of 9×10^{12} vg/kg and 3×10^{13} vg/kg had similar muscle morphology suggesting that AAV vector itself did not affect dystrophic pathology. Importantly, AAV-lipin1 treatment at doses of 9×10^{12} vg/kg and 3×10^{13} vg/kg led to a significantly reduced inflammatory cell infiltration and reduced necrosis areas in mdx muscle. Compared to the dose of 9×10^{12} vg/kg, AAV-lipin1 at a dose of 3×10^{13} vg/kg leads to an even better protective effect indicated by a dose-dependent reduction in inflammation and necrotic myofibers.

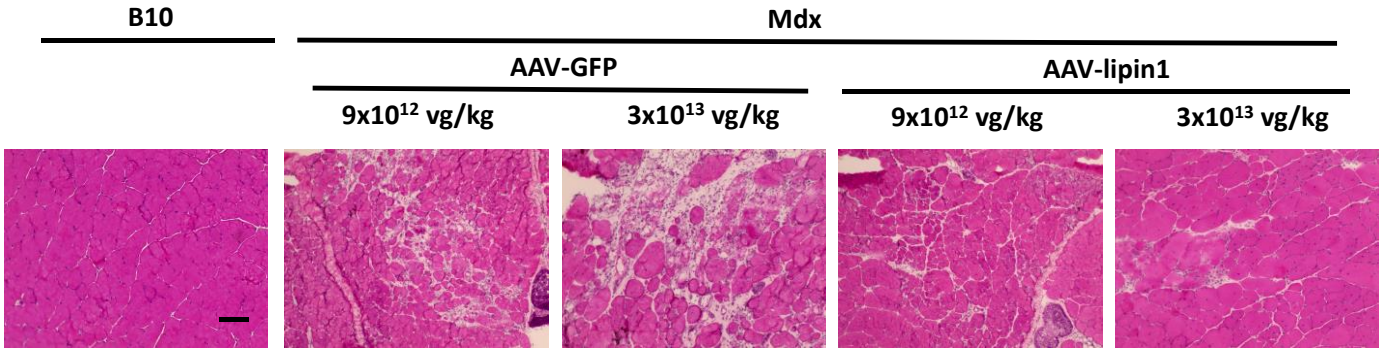

Fig 1. Systemic AAV-lipin1 gene delivery substantially attenuated dystrophic pathology in mdx mice in a dose dependent manner. Representative H&E staining of gastrocnemius muscle of mdx mice treated with AAV-lipin1 or AAV-GFP at doses of 9×10^{12} or 3×10^{13} vg/kg. B10 mice were used as positive controls. (N = 3 mice/group). Scale bar = 200μm.

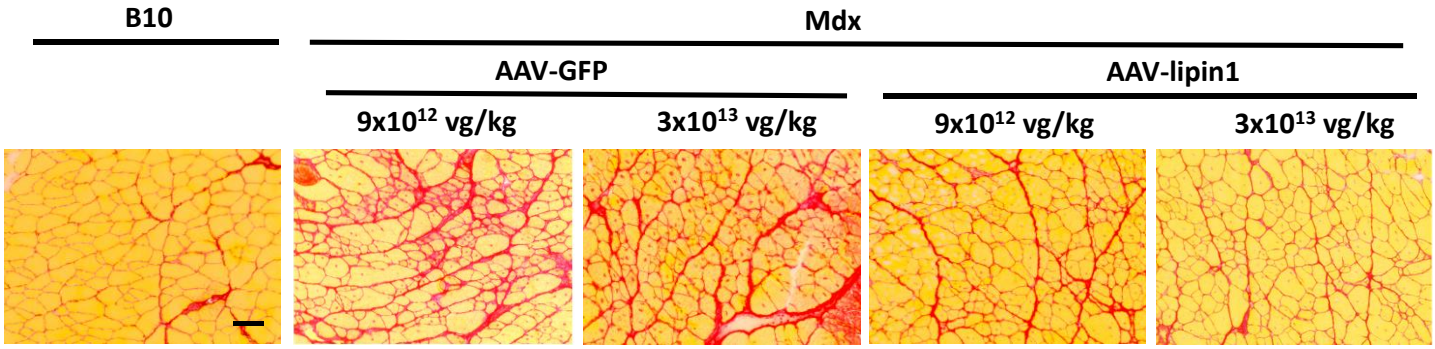

Fig 2. Systemic AAV-lipin1 gene delivery suppressed fibrosis in mdx mice in a dose dependent manner. Representative Picro Sirius red staining of gastrocnemius muscle of mdx mice treated with AAV-lipin1 or AAV-GFP at doses of 9×10^{12} or 3×10^{13} vg/kg. B10 mice were used as positive controls. (N = 3 mice/group). Scale bar = 200μm.

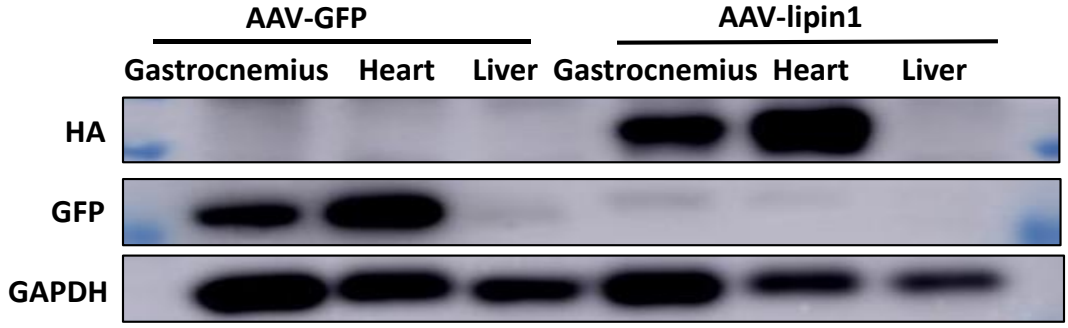

Fig 3 Western blot analysis of lipin1 in gastrocnemius of mdx mice after systemic injection of AAV-GFP or AAV-lipin1 in the dose of 3×10^{13} vg/kg to evaluate the transduction efficiency and specificity.

Skeletal muscle fibrosis leads to increased muscle stiffness, and is a structural hallmark of DMD. We also evaluated the collagen deposition in the gastrocnemius of mdx mice treated with either AAV-lipin1 or AAV-GFP at doses of 9×10^{12} or 3×10^{13} vg/kg. (Fig. 2). AAV-GFP treatment did not affect collagen deposition at doses of 9×10^{12} and 3×10^{13} vg/kg compared to mdx mice. Systemic AAV-lipin1 treatment substantially attenuated collagen deposition at both doses of 9×10^{12} and 3×10^{13} vg/kg. In

addition, gastrocnemius of mdx mice treated with AAV-lipin1 at the dose of 3×10^{13} vg/kg exhibited substantially reduced collagen deposition.

To detect transduction efficiency, lipin1 expression levels in various tissues including gastrocnemius, heart, and liver were detected by Western blotting (Fig. 3). We found that lipin1 was selectively expressed in both gastrocnemius and heart tissue. We did not detect any leakage in the liver suggesting a high transduction efficiency and tissue specificity in our study. We also evaluated the liver morphology in the treated groups, and we did not detect any morphology abnormality in AAV-lipin1 treated group compared to B10 mice suggesting that there was no lesion or liver toxicity in the treated groups. Currently, we are continuing to further evaluate the safety of the treatment.

Moreover, we also developed image analysis pipelines to quantify skeletal muscle histopathological staining for membrane damage (Fig. 4), and collagen deposition (Fig. 5). The histopathological changes in skeletal muscle tissue are central to the disease pathogenesis. Analysis of muscle histopathology is the gold standard for monitoring muscle health and disease progression. However, manual, or semi-manual quantification methods are not only immensely tedious but can be subjective. We developed four image analysis pipelines built in CellProfiler which enable users without a background in computer vision or programming to quantitatively analyze biological images. These automated tools can not only improve workflow efficiency but can provide a better understanding of the histopathological progression of muscular dystrophy.

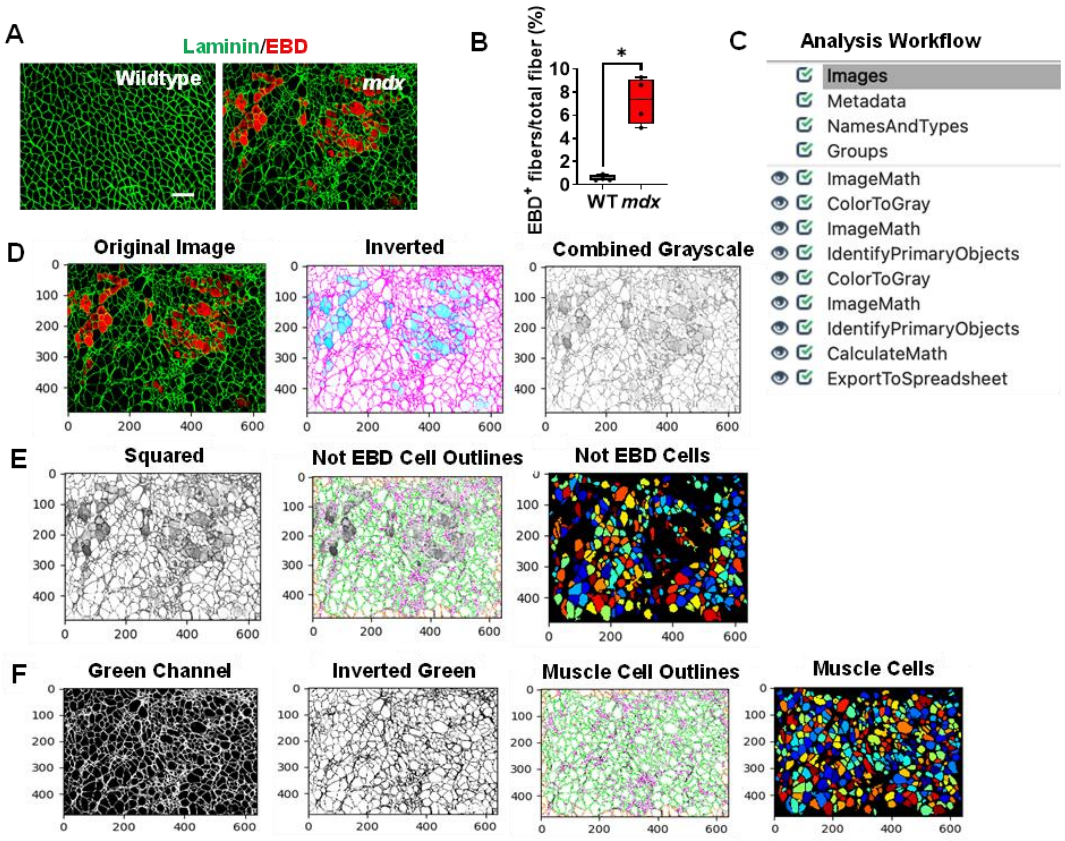

Fig. 4: Processing and quantification of EBD⁺ myofibers by CellProfiler. A) Representative images of gastrocnemius muscle cross-sections from 4-6-month-old B10 and *mdx* mice immunolabeled with laminin α1 (green), EBD (red), and DAPI (blue). Scale bar = 100μm. **B)** Quantification analysis of EBD-positive muscle fiber expressed as the percentage of the total number of muscle fibers in WT and mdx mice (n = 5 mice/group). **p<0.01. **C)** Overview of the image analysis

workflow. **D)** A sample *mdx* image was fed into the EBD quantification pipeline, which first inverts the original image. The inverted image is then converted into a grayscale image with the red and green channels combined. The pixels are then squared to mask the EBD^+ cells. The "not" EBD cell are then identified and counted. **E)** The original image is retrieved and split into separate grayscale images representing each of the color channels (red, green, and blue). **F)** The green channel grayscale image is then inverted and used for subsequent identification of all muscle cells.

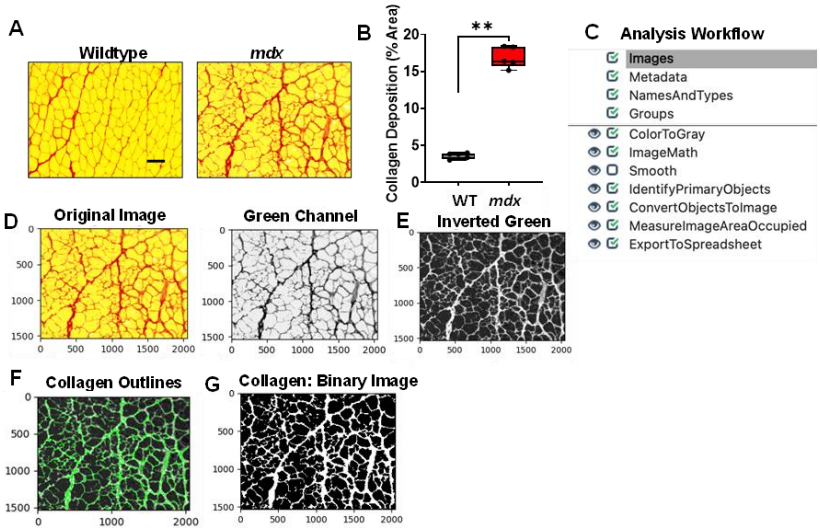

Fig. 5: Processing and quantification of collagen deposition by CellProfiler. A) Representative images of gastrocnemius muscle cross-sections from 4-6-month-old B10 and *mdx* mice stained with Picrosirius red. Scale bar = 100μm. **B)** Boxplot illustrating quantification of percent area of collagen deposition in each muscle section (n = 5 mice/group). **p<0.01. **C)** Overview of image analysis workflow. **D)** A sample *mdx* image was fed into the collagen quantification pipeline, which first converts the color image into separate grayscale images representing each of the color channels. The green channel grayscale image was selected for subsequent processing because the collagen had a prominent appearance in this channel. **E)** The green channel image was inverted and used for **F)** identification of the collagen. **G)** The identified collagen was then converted into a binary black and white image for simple quantification of collagen deposition (% area) within the muscle cross-section.

Experiments need to be done in Subtask 1: We will increase the number of mice to further evaluate safety, and transduction efficiency. Serum creatine kinase levels will be measured to evaluate the effects of lipin1 on membrane damage. Histological examinations will be conducted to investigate whether high systemic doses of AAV vectors induced any lesions in other tissues.

Subtask 2: Explore the role of lipin1 in other consequences of dystrophin deficiency (immune exposure to intracellular antigens and calcium $[Ca]^{2+}$ dynamics).

Ca^{2+} influx during action potential triggers the release of calcium from the sarcoplasmic reticulum, and results in muscle contraction. In collaboration with Dr. Andrew Voss, we utilized patch-clamp as well as two-electrode current- and voltage-clamp techniques of single dissociated flexor digitorum brevis and interosseous muscle fibers to study ion channel and whole cell function. We used intracellular Ca2+ indicator dyes, and measured the calcium flux in single myofiber of B10, mdx, and mdx: lipin1 transgenic mice. We did not see any difference in calcium release underlying during a single action potential (Fig. 6). But mdx myofiber seems to have more free calcium compared to B10 control

groups. The free calcium ions in mdx myofibers during an action potential could be removed by buffering or by extrusion. Currently, we are still collecting and analyzing data.

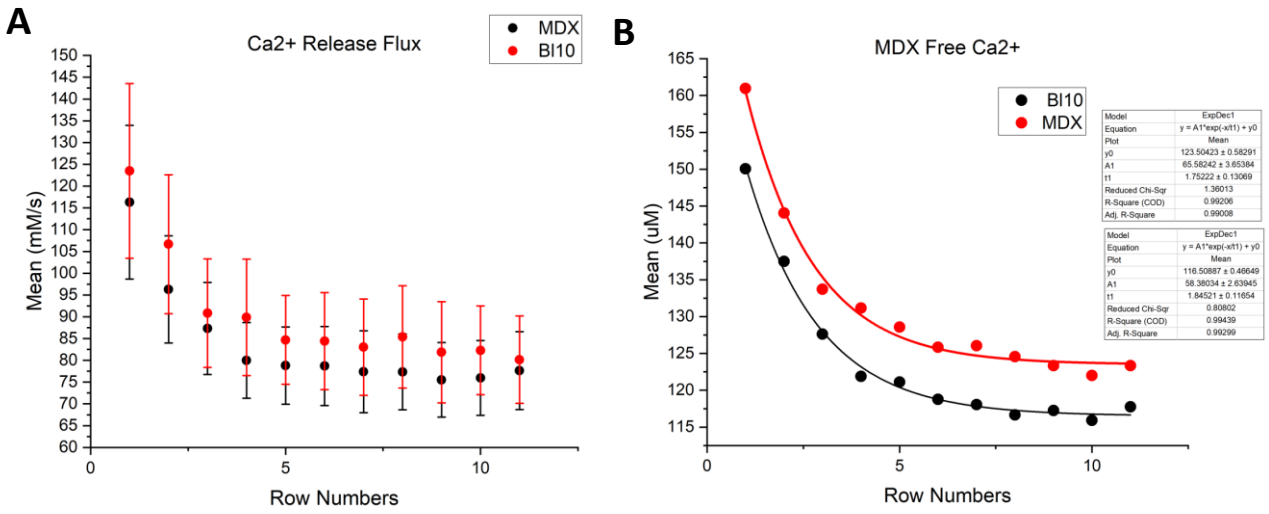

Fig 6. Calcium release evoked by single action potentials during Fura-4 loading. B) Free ca2+ in myofibers from B10 and mdx mice.

Experiments need to be done in Subtask 1: We will continue to complete calcium flux measurement in B10, mdx, and mdx:lipin1 transgenic mice.

Subtask 3: Assess the therapeutic potential of AAV-mediated lipin1 therapy in mdx mice.

To assess the therapeutic potential of AAV-mediated lipin1 therapy in mdx mice, we employed both **cell culture systems** and **animal models**. In the cell culture system**,** to elucidate the role of lipin1 in mechanisms underlying necroptosis suppression in skeletal muscle cells, we used lipin1-CRISPR

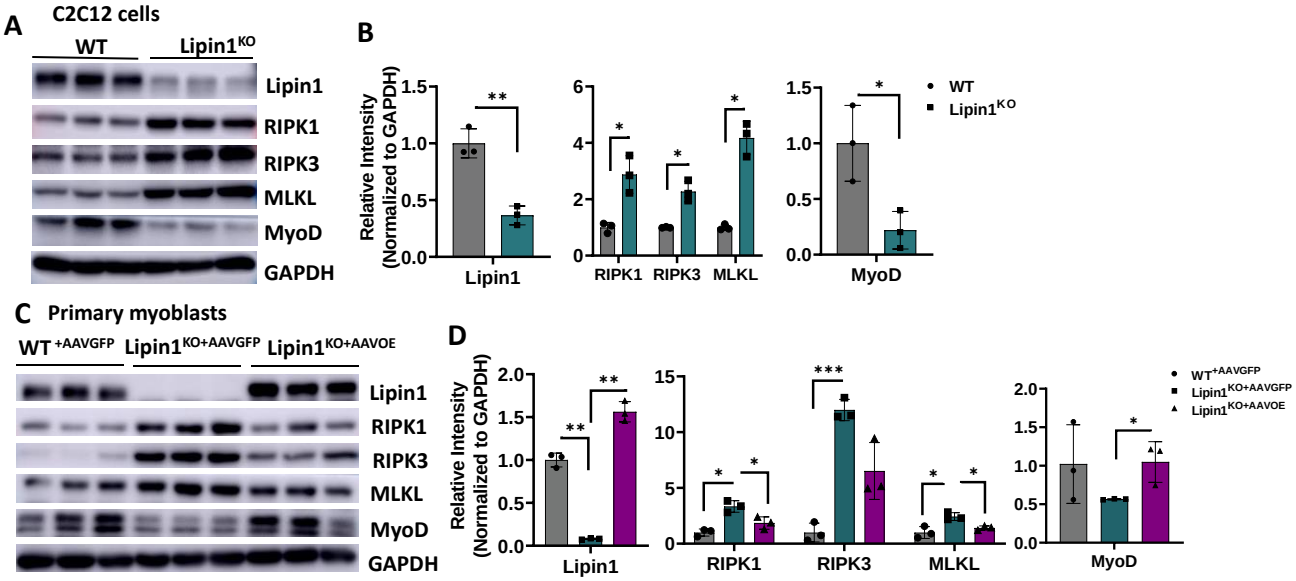

Fig 7 *In vitro* restoration of lipin1 protein suppresses necrotic cell death in lipin1-deficient myoblasts. (A) Protein expression levels and **(B)** quantification analysis of indicated markers analyzed in lipin1-CRISPR-knockout C2C12 myoblasts after the onset of differentiation for 6 days. **(C)** Protein expression levels and **(D)** quantification analysis of necroptotic makers and myogenesis markers measured by Western blotting after primary myoblasts isolated from WT and lipin1^{Myf5cKO} mice, which were infected with either AAV-lipin1 or AAV-GFP followed by differentiation for 6 days.

knockout C2C12 cell lines generated in our laboratory (Alshudukhi et al. 2018) and evaluated the role of lipin1 in regulating necroptotic and myogenesis markers. We found that knockdown of lipin1 in differentiated C2C12 myoblasts led to elevated protein expression levels of RIPK1, RIPK3, and MLKL by 287%, 227%, and 417%, respectively (Fig. 7A, 7B). In contrast, lipin1 deficiency resulted in reduced expression levels of myogenesis marker MyoD by 78%. In addition, primary myoblasts isolated from muscle-specific lipin1 KO (lipin1^{Myf5cKO}) mice were transduced with AAV9-lipin1 to induce lipin1 expression or with AAV9-GFP followed by myoblast differentiation for 6 days. Primary myoblasts from WT mice transduced with AAV9-GFP were used as positive controls. Consistent with what we observed in C2C12 cells, primary myoblasts isolated from lipin1^{Myf5cKO} mice exhibited increased RIPK1, RIPK3, and MLKL by 334%, 1200%, and 243%, respectively, compared to differentiated WT myoblast controls (Fig. 7C, 7D). In contrast, restoration of AAV9-lipin1 WT virus in differentiated lipin1^{Myf5cKO} primary myoblasts suppressed elevated RIPK1, RIPK3, and MLKL by 44%, 46% and 42%, respectively, and improved MyoD by 167% expression, suggesting that lipin1 deficiency led to upregulation of necroptosis and downregulation of myogenesis, whereas restoring lipin1 expression ameliorated muscular degenerative phenotype by suppressing necroptosis and improving myoblast differentiation.

To further identify whether lipin1 inhibits necroptosis due to stabilizing membrane integrity, we also measured CK levels in the cell culture medium (day 4) of differentiated primary myoblasts isolated from lipin1^{Myf5cKO} mice and WT controls (Fig. 8A). We found that lipin1 deficiency led to increased CK levels by 1.88-fold in the medium suggesting that lipin1 deficiency led to increased plasma membrane permeability, which may trigger enhanced necroptosis in muscle cells.

In addition, the in vitro role of lipin1 in membrane integrity was assessed by using differentiated primary myoblasts isolated from WT, lipin1^{Myf5cKO} and mdx mice. Mdx myotubes were used as positive controls (Fig. 8B). At day 6 post-differentiation, cells were washed 2 times with HBSS buffer, then incubated with 2µM of cell impermeant form of the Ca^{2+} indicator Fluo-4 for 30 minutes. After washing out of the extracellular Fluo-4 with HBSS, the intracellular Fluo-4 fluorescence was measured as an indication of increased membrane permeability caused by membrane damage. The basis of this test is that more Fluo-4 will cross a plasma membrane that is damaged. We found that lipin1-deficient myoblasts had increased membrane permeability compared to WT controls suggesting that lipin1 is important for maintaining membrane integrity and lipin1 deficiency leads to the leaky sarcolemma.

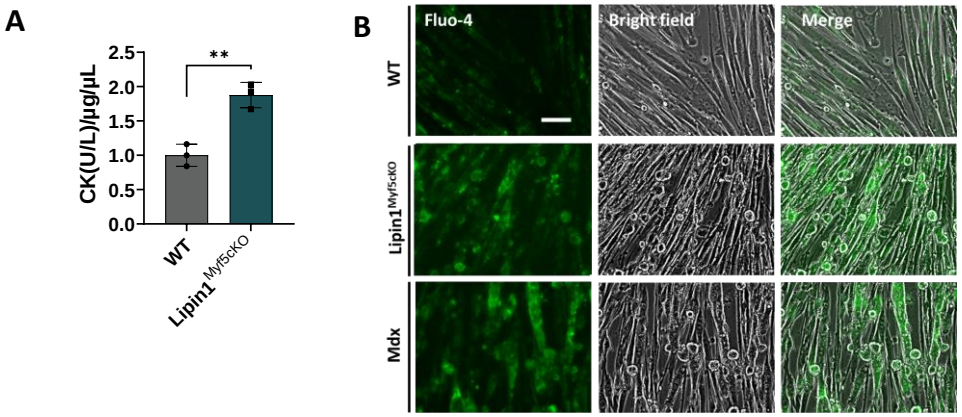

Fig 8 *In vitro* restoration of lipin1 protein suppresses necrotic cell death in lipin1-deficient myoblasts. (A) CK levels in cell culture medium of differentiated WT and lipin1-deficient primary myoblasts collected at day 4 post-differentiation treatment and normalized to total protein lysates. **(B)** Primary myoblasts from WT and lipin1^{Myf5cKO} mice were differentiated for 6 days and then treated with Fluo-4 for 30 minutes. After extensive washing with HBSS, Fluo-4 fluorescence images were acquired. Acquisition parameters were the same for all images obtained. Scale bars = 50 µm.

Experiments need to be done in Subtask 3: In animal models, to evaluate the expression of lipin1 after AAV-lipin1 injection, systemic AAV-lipin1 therapy will be performed in 1-month, 4-month, and 8-month of mdx mice at a dose of 10^{13} vg/kg. One-month post-injection, lipin1 expression levels in various tissues will be assessed by Western blot. Muscle pathology will be examined by H&E staining and Sirius red staining. Sarcolemma integrity will be assessed by EBD uptake and IgG staining, and serum creatine kinase levels. The effect of lipin1 on muscle function and physical performance will be assessed by *in vivo* gastrocnemius force measurements and a downhill treadmill.

4. Impact

Our findings suggest that lipin1 is a novel regulator to prevent dystrophic pathologies. Importantly, it is expected to be useful as a novel therapeutic target for the treatment of DMD as well as related disorders. DMD is a devastating muscle disorder and incurable disease caused by mutations in the gene that encodes the 427kDa cytoskeletal protein dystrophin. In DMD, loss-of-function mutations in the gene encoding dystrophin trigger instability of the plasma membrane in skeletal muscle, causing membrane damage during muscle contraction. This leads to progressive muscle weakness and dramatic muscle degeneration that results in early mortality in affected teenagers. Ideally, targeting the primary defect aiming to restore dystrophin could be a primary strategy for the treatment of DMD. However, the dystrophin gene is the largest gene in the mammalian genome and cannot be packaged into the current viral gene delivery vectors. Currently, there is no effective curative treatment for DMD. The current gene delivery techniques, including exon skipping, are only applicable to a subset of DMD patients with corresponding targeted mutation. Glucocorticosteroids are used to slow disease progression by facilitating the maintenance of muscle strength longer; however, they have serious side effects. Cardiomyopathy is addressed with the use of angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and/or beta blockers. Unfortunately, these treatments are only temporarily effective. An alternative or complementary target is ***urgently needed*** to improve cardiac muscle stability and integrity so that it can withstand structural stress in the absence of dystrophin.

Our data suggest that increasing the expression level of lipin1 lessened skeletal muscle degeneration and fibrosis, strengthened membrane integrity, and present promising as complementary therapy for older DMD patients. Our study is the first to investigate the role of lipin1 in maintaining myofiber stability and integrity, especially in the context of dystrophic muscle. Lipin1 gene delivery has the potential to offer treatment to all DMD patients irrespective of their dystrophin mutation. It could be an effective therapeutic target to preserve and improve the function and quality of life, and to extend the life span of military beneficiaries, and/or the American civilian who suffer from muscular dystrophy.

5. Changes/Problems

Changes in approach and reasons for change

There was no change in approach.

Actual or anticipated problems or delays and actions or plans to resolve them

We have received approval for another 12-month no-cost extension to complete the scope of work. The reasons for the delays include personnel changes and a delay in the generation of AAV-lipin1 virus. Last year the hiring process was not very smooth. We hired a research associate, Alexandra Brown, to work on Aim 2 for this project. After working with us for a very short time, she moved with

her husband to Washington DC. We plan to hire a good Research Associate who is willing to work on this project. The hiring process is ongoing. Another reason is the delayed generation of AAV-lipin1 and AAV-GFP control virus. Dr. Scott Harper's lab has had very busy lab activities recently, and the generation of AAV-lipin1 and AAV-GFP control virus was delayed. However, we received the virus in the fall of 2022. Since we received the virus, we have been working very hard on experiments proposed in Aim 2.

Even with these difficulties, the project has been going very well. We have completed Aim 1 and 50% of Aim 2. We had three publications in the past funding cycle, another one is currently under review and 2 more are in preparation. My students and I had 10 oral presentations and 5 poster presentations in total. In particular, two students from my lab received two presentation awards as shown in this report.

Changes that had a significant impact on expenditures

No.

6. Products

Publications, conference papers, and presentations

Manuscript in preparation or submitted

Manuscripts in preparation and published.

1. Brown A, Rakoczy RJ, Wyatt C, Fink B, **Ren H**. Lipin1 restoration improved diaphragm muscle morphology and respiratory function in mdx mice. In preparation.
2. Jama A, Alshudukhi AA, Burke S, Dong L, Kaumau J, Finck B, Voss A, **Ren H**. Exploring Lipin1 as a Promising Therapeutic Target for the Treatment of Duchenne Muscular Dystrophy. In preparation.
3. Ume A, Wenegieme T, Kamau J, Rockwood J, Elased D, Paul-Onyia C, **Ren H**, Lee D, William C. Transforming Growth Factor-beta Receptor Inhibition Resolves Tacrolimus-Induced Renal Fibrosis While Exacerbating Kidney Dysfunction. *Acta Physiologica* (2023) Under review.
4. Brown A, Morris B, Kamau1 JK, Alshudukhi1 AA, Jama A, Ren H. Automated Image Analysis Pipeline Development to Monitor Disease Progression in Muscular Dystrophy Using CellProfiler. *Archives of Microbiology & Immunology*. (2023) In print.
5. Ume A, Wenegieme TY, Shelby J, Paul-Onyia C, Waite III A, Kamau J, Adams D, Susuki K, Bennett E, **Ren H**, and Williams C. Tacrolimus Induces Fibroblast to Myofibroblast Transition via a TGFβ-Dependent Mechanism to Contribute to Renal Fibrosis. (2023) *Am J Physiol Renal Physiol*. (2023); 324(5): F433-F445. doi: 10.1152/ajprenal.00226.2022.
6. Jama A, Alshudukhi AA, Burke S, Dong L, Kamau JK, Voss AV, Ren H. Lipin1 plays complementary roles in myofiber stability and regeneration in dystrophic muscles. *J Physiol* (2023); 601(5): 961-978. doi: 10.1113/JP284085. PMID: 36715084.

Invited Talks

"Lipin1: a promising target for the treatment of Duchenne muscular dystrophy". (2023) Department of Biological Sciences, Georgia state university. Presenter: **Hongmei Ren**

"Lipin1: a promising target for the treatment of Duchenne muscular dystrophy". (2023) Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham. Presenter: **Hongmei Ren**

"Lipin1: a promising target for the treatment of Duchenne muscular dystrophy". (2023) Department of Biotechnical and Clinical Laboratory Sciences, University of Buffalo. Presenter: **Hongmei Ren**

"Exploring the role of lipin1 in dystrophic pathology and muscle". (2022) Department of Biological Sciences, Wright State University. Presenter: **Hongmei Ren**

"Exploring the role of lipin1 in dystrophic pathology and muscle". (2022) Department of Pharmacology and Toxicology, Wright State University. Presenter: **Hongmei Ren**

Student Oral presentation:

"Explore the potential drug treatment to elevate lipin1 expression in dystrophic muscle". (2023) COSM Festival of Research, Wright State University. Presenter: **Brooklyn Morris**

"A novel role of lipin1 in cardiac function". (2023). Brown bag seminar, Department of Biochemistry & Molecular Biology, Wright State University. Presenter: **Kamau John**

"A novel role of lipin1 in cardiac function". (2023). Biomedical Sciences, Wright State University. Presenter: **Kamau John**

"Lipin1 overexpression ameliorates the dystrophic phenotype in mdx mice by enhancing myofiber membrane integrity". (2022). Brown bag seminar, Department of Biochemistry & Molecular Biology, Wright State University. Presenter: **Alshudukhi Abdullah**

"A novel role of Lipin1 in the regulation of expression and function of nNOS." (2022). Brown bag seminar, Department of Biochemistry & Molecular Biology, Wright State University. Presenter: **Ayat Azzam**

Conferences and meeting

June 20-23, 2022 New Directions in Biology and Disease of Skeletal Muscle held in Ft. Lauderdale, FL.

April 6-7, 2022 AHA Scholars Program at Historically Black Colleges and Universities Research Symposium, Nashville.

Study sections

03/2023 Wellstone Centers Review, NINDS ZNS1 SRB

10/2022 SMEP study section, NIH - NIAMS

Poster presentation:

Covington T, Kamau J, **Ren H**. "The Role of Lipin 1 in Cardiac Function". (2023) The annual Historically Black Colleges and Universities Scholar Research Symposium in Durham, NC.

Kamau J, **Ren H**. "A novel role of lipin1 in cardiac function". (2023). COSM Festival of Research, Wright State University.

Jama A, Alshudukhi A, Burke S, Voss A, Finck B, **Ren H.** "The role of lipin1 in skeletal muscle of mdx mice". (2022). New Directions in Biology and Disease of Skeletal Muscle Conference. Ft. Lauderdale, FL.

Alshudukhi A, Jama A, **Ren H.** "Moderate exercise ameliorates myopathic phenotypes in muscle-specific lipin1 deficient mice". (2022) New Directions in Biology and Disease of Skeletal Muscle Conference. Ft. Lauderdale, FL.

Dukes K, Alkhomsi I, **Ren H**, "Generating heart specific lipin1 deficient mouse model." (2022) AHA Scholars Program at Historically Black Colleges and Universities Research Symposium, Nashville.

Student Awards:

Brooklyn Morris won 1st place undergraduate student Oral presentation award, COSM Festival of Research, (2023) Wright State University.

John Kamau won the best graduate poster presentation in the poster session of COSM Festival of Research, (2023) Wright State University.

7. Participants & Other Collaborating Organizations

Research assistant: (8-month effort)

This project was currently undertaken by our research team of graduate students and research assistants. However, two Ph.D. students graduated in April 2023. We are in the process of hiring another research assistant. This research assistant will work on the remaining subtasks of this project.

8. Special Reporting Requirements

N/A

9. Appendices

N/A