

PPAR δ modulation partially restores mitochondrial defects resulting from dystrophin loss of function

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Abstract

Duchenne muscular dystrophy (DMD) is a recessive, fatal X-linked disease that is characterized by progressive skeletal muscle wasting due to absence of dystrophin, which is an essential protein that bridges the inner cytoskeleton and extra-cellular matrix. The *mdx* mouse has a point mutation in the dystrophin gene that results in a similar muscle wasting observed in DMD patients. It has been previously demonstrated that *mdx* muscle has mitochondrial-related deficits including decreased spare respiratory capacity¹, complex 1 mediated ATP production², and mitochondrial biomass³. However, it was unclear when these deficits manifest in the muscle and what the underlying defect(s) in mitochondrial biology might be.

This study set out to characterize the mitochondria in primary muscle satellite cell derived myoblasts and myotubes from *mdx* mice and wild type control mice. Compared to background-matched healthy cells isolated from wild type mouse muscle, *mdx* derived cells have reduced mitochondrial bioenergetics. Moreover, *mdx* derived cells show reduced levels of mitochondria, which may partially explain the reduction in bioenergetics. These *mdx* cells also have increased levels of the nuclear hormone receptor PPAR δ without increased levels of putative target genes such as *Angptl4* and *Pdk4*. Here, we demonstrate that a novel PPAR δ modulator improves mitochondrial function in the *mdx* model, which supports the notion that modulating PPAR δ may be therapeutically beneficial in DMD patients.

Materials and Analysis

Wild type (WT) and *mdx* myoblast isolation and culture: Quadriceps and gastrocnemius muscles from a single mouse were pooled and subjected to a mechanical/collagenase digestion. Isolated myoblasts were cultured on Matrigel-coated cultureware in DMEM/F-12 with 10% FBS and 20ng/mL bFGF. Myoblast homogeneity was determined by a co-staining for vimentin and desmin (data not shown).

Myotube differentiation: Confluent myoblasts were changed to DMEM + 2%FBS + insulin-selenium-transferrin for 5 days with the assay performed Day 6.

Steady state ATP measurement: Cells were treated with DMSO (basal) or 100nM oligomycin for 24h. Steady state ATP was then measured using the CellTiter-Glo assay (Promega).

Fatty Acid Oxidation: Myoblasts were starved for 18h in low glucose media with carnitine. KHB plus BSA or BSA-palmitate was added and oxygen consumption was analyzed using a Seahorse Metabolic Analyzer. The palmitate response was determined by calculating a ratio of the FCCP-induced BSA-palmitate respiration/FCCP-induced BSA respiration.

Gene expression: RNA was isolated using a NucleoSpin RNA kit (Macherey-Nagel) and converted to cDNA with the High Capacity cDNA Reverse Transcriptase Kit (Thermo). Gene expression was then determined using the SmartChip Real-Time PCR system (Wafergen) and analyzed with Qbase (BioGazelle).

Statistical analysis: Data was graphed as boxplots and statistical significance determined using GraphPad Prism. Comparisons were performed between WT and *mdx* in the various experimental conditions. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

Results

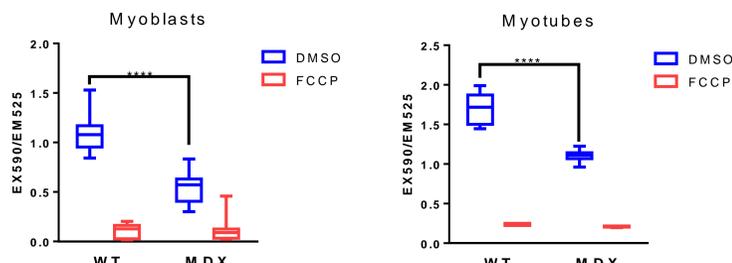


Figure 1- A lower aggregate/monomer JC10 signal in *mdx* myoblasts and tubes demonstrates a lower membrane potential

Results

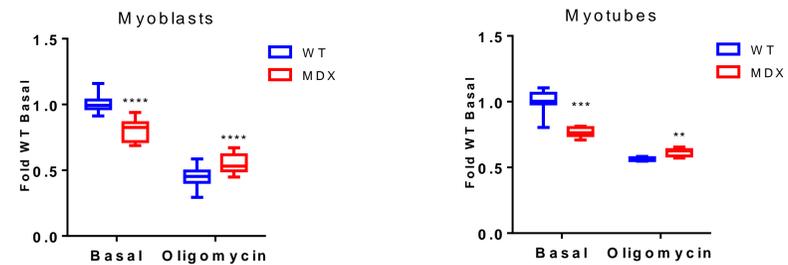


Figure 2- *mdx* myoblasts and myotubes have lower steady state levels of ATP and are less sensitive to oligomycin treatment indicating decreased mitochondrial ATP production.

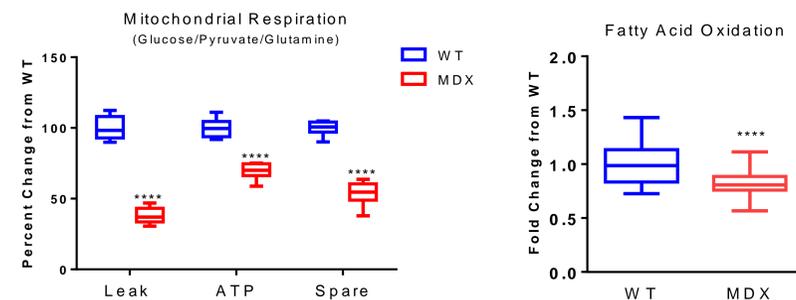


Figure 3- Oxidation of multiple substrates is decreased in the context of dystrophin mutation.

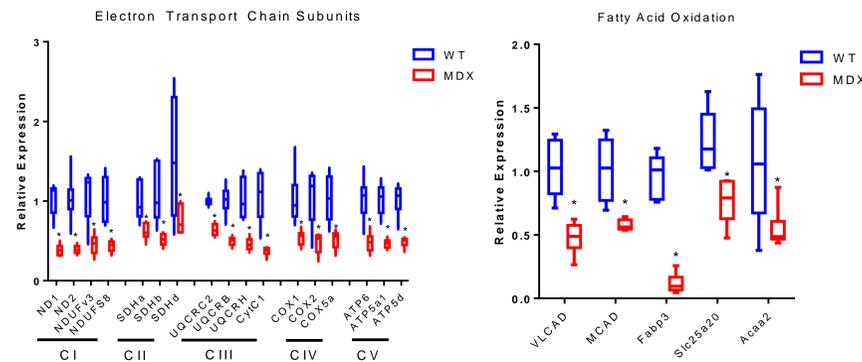


Figure 4- *mdx* myoblasts exhibit lower decreased expression of electron transport chain subunits and fatty acid oxidation genes.

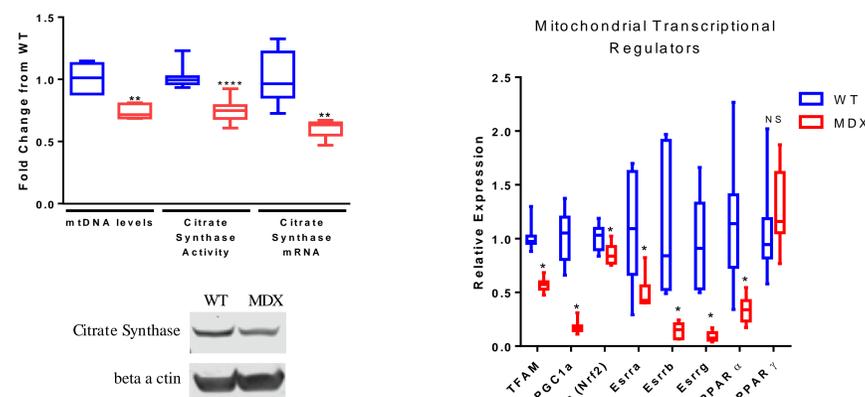


Figure 5- Loss of dystrophin function decreases mitochondrial abundance

Results

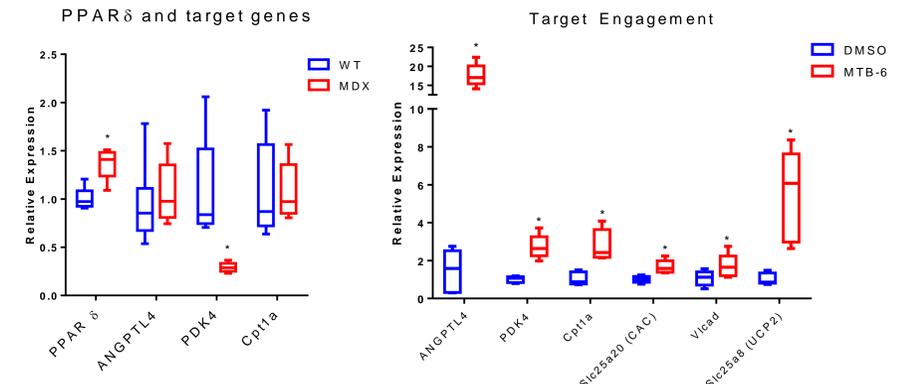


Figure 6- Expression of PPAR δ is increased in *mdx* myoblasts without increased levels of putative target genes; treatment with a PPAR δ modulator increases PPAR δ -target gene expression.

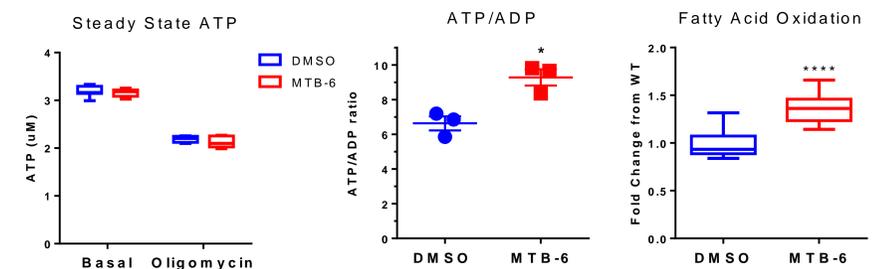


Figure 7- Treatment of *mdx* myoblasts with the PPAR δ modulator MTB-6 improves mitochondrial defects.

Conclusions and Future Directions

We have demonstrated that the *mdx* derived muscle cells exhibit significant deficit in mitochondrial function, which are apparent in myoblasts, before myogenesis has completed. These defects present as decreased membrane potential, reduced cellular ATP, and inefficient oxidation of various substrates. Mitochondrial abundance is decreased in *mdx* derived muscle cells as are transcripts encoding mitochondrial electron transport chain subunits, fatty acid oxidation genes, and master regulators of mitochondrial biogenesis. The functional deficit in mitochondrial output could be due to reduced mitochondrial biomass and/or a specific defect in the mitochondria present.

PPAR δ expression in *mdx* derived muscle cells is increased; however, the putative target genes that are indicative of activity are not increased. Treatment of *mdx* myoblasts with a novel PPAR δ modulator, MTB-6, increases the ATP/ADP ratio and fatty acid oxidation. Future experiments will focus on elucidating the mechanism for decreased functionality and assess the impact of mitochondrial deficits on the dystrophic muscle isolated from mice and humans.

References

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