Mitochondrial Deficiency in Primary Muscle Cells from Mdx Mice

Robert Shine, Peter Dwyer, Lyndsay Olson, Jennifer Truong, Matthew Goddeeris, Effie Tozzo, Eric Bell
Mitobridge, Inc. Cambridge, MA 02138

Abstract

Duchenne muscular dystrophy (DMD) is a recessive, fatal X-linked disease that is characterized by progressive skeletal muscle wasting due to a loss of function in dystrophin, a protein that is part of a complex that bridges the cytoskeleton and extracellular matrix. The *mdx* mouse, an animal model for DMD, has a point mutation in the dystrophin gene that results in a loss of function. This study uses primary muscle satellite cell derived myoblasts and myotubes to determine differences in mitochondrial biology between the *mdx* mice and wild type (WT) control mice. Compared to cells isolated from WT mice, *mdx* cells have reductions in mitochondrial bioenergetics. Moreover, *mdx* cells have reduced levels of mitochondria which may partially explain the reduction in bioenergetics. Interestingly, the mitochondrial phenotype is apparent before dystrophin protein is increased during myogenesis.

Materials and Analysis

WT and *mdx* myoblast isolation and culture: Quadricep 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.
Mitochondrial Characterization Assays: Procedural information is contained in figure legends.
Statistical Analysis: Myoblast data generated from 3 pairs of WT/mdx mice were combined for analysis. Myotube data is from 1 pair of WT/mdx mice. Data was graphed as boxplots and statistical significance determined using GraphPad Prism. Comparisons were performed between WT and mdx in the various experimental conditions.

Results

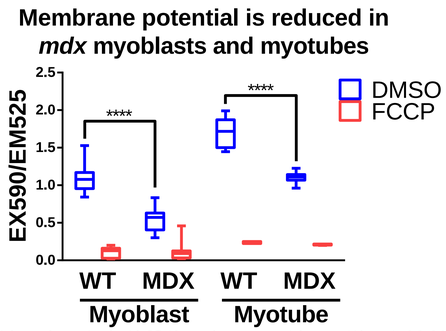

Figure 1: Membrane potential was determined with JC-10 membrane potential assay (Abcam). 3.3μM FCCP was used to ablate membrane potential as a negative control. Statistical significance between WT and mdx was determined by unpaired T-test; ****p<0.0001, myoblast n=32, myotube n=8.

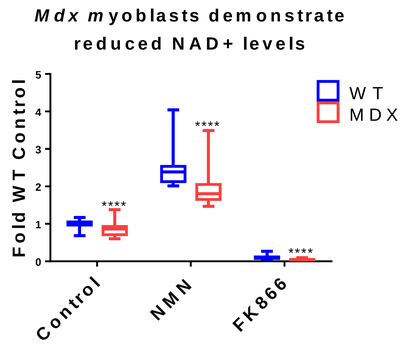

Figure 2: Myoblasts were treated for 24h with 1mM NMN or 10nM FK866. The myoblasts were then analyzed using the NAD-Glo Assay (Promega). Significant difference between WT and mdx was determined by Mann-Whitney Test; ****p<0.0001, n=44.

Results

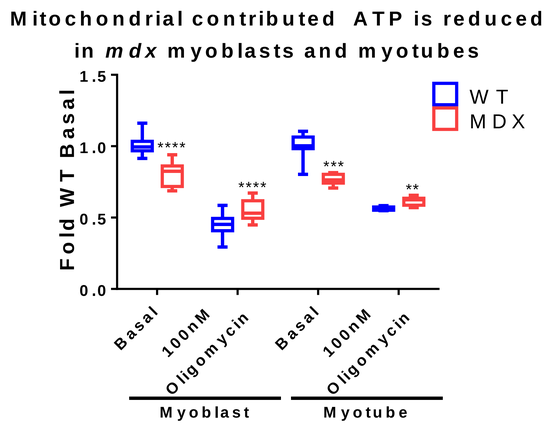

Figure 3: Cells were treated for 24h. The ATP levels were determined using the Cell TiterGlo assay (Promega). Significant difference between WT and *mdx* was determined by Mann-Whitney Test or unpaired T-Test; **p<0.01, ***p<0.001, ****p<0.0001, myoblast n=40, myotube n=8.

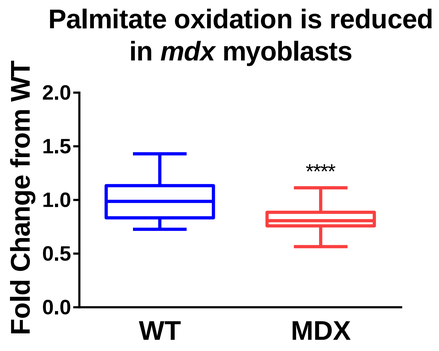

Figure 4: WT and *mdx* myoblasts were starved for 18h in low glucose media with carnitine. KHB plus BSA or BSA-palmitate was added and oxygen consumption was analyzed using a Seahorse Metabolic Analyzer. The palmitate response was determined by calculating a ratio of the FCCP-induced BSA-palmitate respiration/FCCP-induced BSA respiration. Significant difference between WT and *mdx* determined by unpaired T-test; ****p<0.0001, n=45 (WT)/43 (*mdx*).

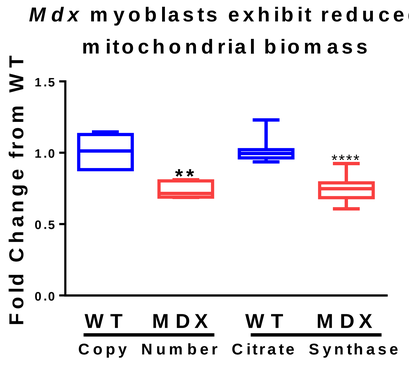

Figure 5: Mitochondrial number was determined two ways. For copy number, gDNA was extracted and analyzed by determining the ratio of 16s(mito)/HK2(nuclear). Citrate synthase was determined by using protein lysate in the Citrate Synthase Assay Kit (Sigma). Significant difference between WT and *mdx* copy number was determined with unpaired T-test; **p<0.025. Significant difference between WT and *mdx* CS was determined by Mann-Whitney test; ****p<0.0001, copy number n=5 (one pair of mice), citrate synthase n=40.

Results

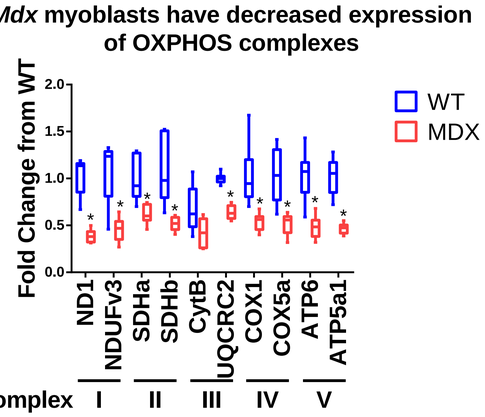

Figure 6: RNA from WT or *mdx* myoblasts was isolated using a NucleoSpin RNA kit (Macherey-Nagle) and converted to cDNA with the High Capacity cDNA Reverse Transcriptase Kit (Thermo). Gene expression was then determined using the SmartChip Real-Time PCR system (Wafergen) and analyzed with Qbase. Significant difference between WT and *mdx* were determined by unpaired T-test; *p<0.05, n=9.

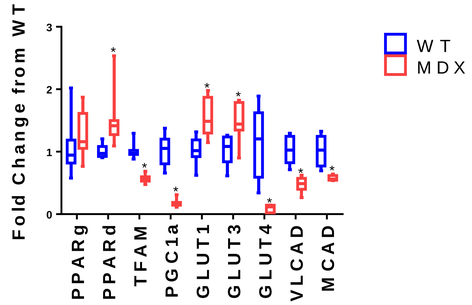

Figure 7: RNA from WT or *mdx* myoblasts was isolated using a NucleoSpin RNA kit (Macherey-Nagle) and converted to cDNA with the High Capacity cDNA Reverse Transcriptase Kit (Thermo). Gene expression was then determined using the SmartChip Real-Time PCR system (Wafergen) and analyzed with Qbase. Significant difference between WT and *mdx* were determined by unpaired T-test; *p<0.05, n=9.

Conclusions and Future Directions

It has been previously demonstrated that *mdx* muscle has mitochondrial-related deficits including decreased spare respiratory capacity[1], complex 1 mediated ATP production[2], and mitochondrial biomass[3]. However, it was unclear when these deficits manifest in the muscle and what the underlying defect(s) in mitochondrial biology might be.

We have demonstrated that the *mdx*-derived muscle cells exhibit significant mitochondrial defects and that these defects are apparent before myogenesis has completed. These defects appear to have a direct impact on cell physiology, as we observe reduced cellular ATP and inefficient fatty acid utilization. The functional deficit in mitochondria could be due to reduced mitochondrial biomass and/or a specific defect in the mitochondria present. Future experiments will focus on elucidating the mechanism for decreased functionality and assess the impact of mitochondrial deficits on the dystrophic muscle phenotype.

References

[1]Schuh RA, et al. *Am J Physiol Regul Integr Comp Physiol*. 302(6):R712-9. 2012.
[2]Rybalka E, et al. *PLoS One*. 9(12):e115763. 2014.
[3]Godin R, et al. *J. Physiol*. 590(21):5487-502. 2012.

mitobridge