Mitochondrial profiling of immortalised myoblasts from a Duchenne Muscular Dystrophy patient

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Introduction: Mitochondria are increasingly implicated in a variety of debilitating diseases including the fatal X-linked neuromuscular disease, Duchenne Muscular Dystrophy (DMD) (Timpani *et al.*, 2015). We and others have documented mitochondrial dysfunction, morphological anomalies and oxidative stress in skeletal muscle from the *mdx* mouse model of DMD (Rybalka *et al.*, 2014; Timpani *et al.*, 2015; Timpani *et al.*, 2016). In this study, we aimed to establish the mitochondrial profile of human DMD myoblasts and the therapeutic potential of a metabolic (Krebs cycle) stimulant to normalize any dysfunctions.

Methods: Using immortalised myoblasts derived from the *fascia lata* of a 10-year-old DMD patient (DMD) and the paraspinal muscles of a 12-year-old healthy child (CON), we have generated a mitochondrial profile including respiratory function (extracellular flux analysis), pool density and viability (MitoTracker probes) and reactive oxygen species (ROS) production (MitoSOX).

Results: DMD myoblasts had a higher basal mitochondrial respiration than CON myoblasts. However, oligomycin-sensitive phosphorylating respiration was depressed by ~60% while leak respiration was increased 5-fold compared to CON, demonstrating significant mitochondrial uncoupling. Since inducible uncoupling is linked to electron slip at Complexes I and III and consequently ROS production, we next investigated mitochondrial O_2^-content which was ~30% higher in DMD myoblasts. This corresponded with a 2-fold higher non-mitochondrial O_2 consumption. Metabolic stimulation actually enhanced these dysfunctions. Perhaps to overcome the reduced capacity for ATP production, the mitochondrial density of DMD myoblasts was ~20% higher than CON myoblasts albeit viability was ~40% lower.

Conclusions: Our data highlight significant mitochondrial dysfunction of DMD myoblasts whereby metabolic substrates are oxidised to generate ROS rather than ATP. Targeting these features of the mitochondria could be therapeutically beneficial for the treatment of DMD patients.

Rybalka E, Timpani CA, Cooke MB, Williams AD & Hayes A. (2014) Defects in mitochondrial ATP synthesis in dystrophin-deficient Mdx skeletal muscles may be caused by complex I insufficiency. *PLoS ONE,* **9**(12):e115763. doi: 0.1371/journal.pone.0115763.

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