Mitochondrial ATP production rate is severely impaired in dystrophic *mdx* skeletal muscle, and is not influenced by altered calcium concentration

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Duchenne Muscular Dystrophy (DMD) is characterised by $[Ca^{2+}]$-induced hypercatabolism of skeletal muscle tissue. This is associated with reductions in high energy nucleotides of up to 50% of normal levels, which may be directly associated with elevated intracellular $[Ca^{2+}]$. We have thus examined cellular energy production by performing direct measurement of mitochondrial ATP production rate (MAPR) using substrates that mimic the major metabolic pathways in dystrophic *mdx* diaphragm, and assessed the effect of an extra-mitochondrial supraphysiological calcium spectra (0-400 nM) such to mimic "resting" dystrophic intracellular Ca^{2+} conditions in this *in vitro* setting.

Age-matched dystrophic *mdx* (n = 8) and normal control (n = 10) C57BL/10 mouse diaphragm were utilised to bioluminometrically quantify MAPR under each of carbohydrate, fat, protein, carbohydrate+fat+protein (total) and complex II metabolism. The effect of both nil and supraphysiological $[Ca^{2+}]$ on MAPR was determined for total and complex II metabolism in both diaphragm and tibialis anterior (TA) for direct comparison between diaphragm and hind limb skeletal muscle. Citrate synthase (CS) activity was also assessed. In all cases, muscles were excised from anaesthetised animals, and mitochondria were isolated via step-wise centrifugation.

MAPR was significantly depressed across all metabolic pathways except for that of complex II in *mdx* compared to control diaphragm ($p < 0.01$). The greatest depression was observed for total metabolism in which a 3-fold reduction was evident. MAPR under stimulation of isolated metabolic pathways was similarly reduced by 2.5-fold for carbohydrate, 2-fold for fat and 1.5-fold for protein metabolism. Ca^{2+} had no effect on MAPR under total or complex II metabolism at any of the concentrations assessed, in either diaphragm or TA. CS activity of *mdx* muscle was comparable to controls in both muscles, however the susceptibility of dystrophic mitochondria to mechanical damage during the mitochondrial extraction process (as determined by the ratio of CS_{after} to CS_{total} activity) was significantly greater in *mdx* compared to controls, and in diaphragm compared to TA ($p < 0.01$).

These results demonstrate severely impaired mitochondrial function of dystrophic diaphragm and hind limb muscle. This depression may be associated with physical and/or functional reductions of enzymes associated with the TCA cycle, which reduces the flow of NAD-associated electrons to the electron transport chain and hence ATP production capacity. This observation seems to be directly related to the DMD phenotype as supraphysiological $[Ca^{2+}]$ had no effect on MAPR, and dystrophic mitochondria were less resistant to mechanical damage as observed by CS analysis.