Adenylosuccinic acid therapy attenuates skeletal muscle histopathology in the dystrophin-deficient *mdx* mouse

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Introduction: Duchenne Muscular Dystrophy (DMD) is a debilitating and fatal skeletal muscle wasting disease that arises from the ablation of the cytoskeletal protein dystrophin, which renders muscle fibres more permeable to extracellular calcium (Ca^{2+}) influx, and chronic Ca^{2+}-induced damage. As repair mechanisms fail to match degenerative and inflammatory activity, functional muscle is replaced with non-functional connective, fibrotic and fatty tissue and normal function is lost. Dystrophic muscle is also characterized by mitochondrial and metabolic impairments leading to a severe reduction in resting ATP content (Cole *et al.* 2002). It is likely that the inability to produce sufficient ATP is key to the failure to match regenerative and degenerative activity. Thus, increasing the metabogenic potential of dystrophin-deficient muscle could be an effective treatment avenue to ameliorate Ca^{2+}-induced degeneration, promote regenerative capacity and delay loss of function. Adenylosuccinic Acid (ASA) is a purine nucleotide cycle metabolite that promotes adenine nucleotide recovery and oxidative anaplerosis during metabolic stress. The aim of our study was to determine the efficacy of dietary ASA supplementation to stimulate metabolism and buffer skeletal muscle wasting in the *mdx* mouse model of DMD.

Methods: Animal experimentation was approved by the Victoria University Animal Ethics Experimentation Committee and performed in accordance with the Australian Code of Practice for the Care and use of Animal for Scientific Purposes. Four week old male C57BL/10 (CON) and C57BL/10*mdx* (*mdx*) mice were randomly assigned into four groups: CON unsupplemented (CON UNSUPP), CON supplemented (CON ASA), *mdx* UNSUPP and *mdx* ASA. Mice in the ASA groups received *ad libitum* access to RO water supplemented with ASA (3000µg/mL) while unsupplemented groups received RO water only. Following an 8 week supplementation period, mice were deeply anaesthetized by an intraperitoneal injection of sodium pentobarbitone ($60mg.kg^{-1}$) and the *flexor digitorum brevis* (FDB), *extensor digitorum longus* (EDL), *soleus* (SOL) and *tibialis anterior* (TA) excised. Both basal- and contraction-induced glucose uptake (GU) was assessed in the EDL and SOL. The FDB was dissociated into single fibres and mitochondrial function (oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)) was assessed using the XF24 Analyser (Seahorse Bioscience) to determine the metabolic potential and bioenergetics health index. Mitochondrial density and mitochondrial superoxide production was also assessed *via* MitoTracker probes and MitoSOX, respectively. To assess muscle architecture, TA was stained with H&E and fibre size, centronucleation and damaged area (measured as areas infiltrated by nuclei) was determined *via* histological analysis.

Results: Overall, GU (both basal- and contraction-induced) was significantly less in *mdx* EDL and SOL ($P<0.01$) compared to healthy controls, with ASA having no effect in either strain ($p>0.05$). The oxidative metabolic potential – a measure of the oxidative flexibility during metabolic stress - was significantly reduced in *mdx* compared to CON ($P<0.01$) but was not improved by ASA ($P>0.05$). In contrast, the anaerobic metabolic potential – a measure of glycolytic flexibility during metabolic stress – was significantly higher in dystrophin-deficient FDB fibres ($P<0.01$), but again, was not effected by ASA treatment. Overall, the bioenergetic health index was significantly reduced in *mdx* compared to healthy control fibres ($P<0.01$) and was not improved with ASA ($P>0.05$). This was despite ASA significantly increasing mitochondrial density ($P<0.05$) and decreasing mitochondrial superoxide production ($P<0.05$) in *mdx* FDB fibres. Histologically, ASA attenuated the hallmark pathological signs of dystrophin-deficient myopathy, including compensatory hypertrophy and active degenerative area of mdx TA (both $P<0.05$).

Conclusions: Our data suggests that ASA provides protection to dystrophin-deficient mdx skeletal muscle against psuedohypertophy and degeneration – two histological hallmarks of DMD – but not *via* metabogenesis. We speculate that ASA might be a genetic modifier of utrophin expression.

Cole MA, Rafael JA, Taylor DJ, Lodi R, Davies KE, Styles P. (2002). A quantitative study of bioenergetics in skeletal muscle lacking utrophin and dystrophin. *Neuromuscul Disord* **12:** 247-57.