Therapeutic potential of slow muscle programming by low-frequency stimulation in dystrophic mice

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There is still no cure or effective treatment for Duchenne muscular dystrophy (DMD), a progressive and severe muscle wasting disease. In DMD and in two well-characterized murine models of the disease (*mdx* and *dko* mice), muscles are fragile, injury prone and compromised in their regenerative capacity. Fast muscle fibres are more susceptible to damage and pathological progression than slow muscle fibres and a potential therapeutic strategy is to induce fast-to-slow muscle remodelling so as to confer protection to dystrophic muscles from this damage. This could be achieved through chronic low-frequency stimulation (LFS) that contracts muscles like that in exercise and may be a suitable alternative for some patients confined to wheelchairs (Lynch, 2017). We tested the hypothesis that slow muscle programming through LFS would ameliorate the dystrophic pathology in mouse models of DMD.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). Mice were anaesthetized with ketamine/xylaine (100 mg/kg Ketamine, 10 mg/kg Xylazine, i.p.) and microelectrodes implanted in wild type (C57BL/10), *mdx* and *dko* mice to facilitate wireless stimulation of the lower hind limb muscles (10 Hz, 12 h/d, 28 d). At the conclusion of the LFS protocol adaptations in dystrophic muscles were assessed by complementary molecular, biochemical, and immunohistological analyses. *Tibialis anterior* (TA) function was also assessed in a separate cohort of anaesthetized mice (Sodium pentobarbital, 60 mg/kg, i.p.). All mice were killed by cardiac excision while anaesthetized deeply.

LFS induced a fast-to-slow remodelling in dystrophic TA muscles evident from increased SDH enzyme activity, capillary density and presence of small calibre fibres, which occurred independent of histopathologic alterations. Interestingly, in *dko* mice the lack of utrophin abrogated LFS-induced increases in muscle stem cell content (Pax7+ cells/mm^2) in dystrophic mice. Whole-genome sequencing in *extensor digitorum longus* (EDL) muscles revealed 796 and 375 differentially expressed genes by LFS in *mdx* and *dko* mice, respectively. Functional annotation revealed common biological processes (fatty acid metabolism and angiogenesis) and signalling pathways (AMPK, Ca^{2+} and insulin signalling) enriched by LFS in dystrophic muscle. Importantly, the remodelled TA muscles of *mdx* mice were less susceptible to contraction-mediated damage, indicating that LFS conferred protection from injury.

Together, these exciting findings highlight the utility of LFS to enhance our understanding of the mechanisms underlying skeletal muscle remodelling and reveal the therapeutic potential of slow muscle programming to ameliorate the pathophysiology of muscular dystrophy.

Lynch GS. (2017). In: *The Plasticity of Skeletal Muscle: From Molecular Mechanism to Clinical Applications*, ed Sakuma K. pp. 277-292. Singapore: Springer.

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