Cholesterol lowering prevents ambulatory dysfunction in muscular dystrophy

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Background: The pleiotropic, non-lipid lowering effects of statins were recently shown to attenuate muscle wasting in Muscular Dystrophy (MD). In the mdx mouse model of Duchenne MD, simvastatin ameliorated intramuscular inflammation and fibrosis, leading to improved muscle function. However, our team has shown that both mdx and dysferlin$^{-/-}$ mice, two notoriously mild models of MD, exhibit severe muscle wasting and loss of ambulation when their plasma lipid profile is altered. Hence, we hypothesized that cholesterol modulation, rather than pleiotropism, is the true mechanism of simvastatin in MD.

Methods: To test the contribution of cholesterol to MD severity and evaluate the feasibility of repurposing non-statin medications, we used two mouse models of MD (dysferlin$^{-/-}$ and mdx) with a humanized plasma lipoprotein profile; this was done by inactivating their Apolipoprotein E (ApoE) gene, a common model of atherogenesis. Mice were supplemented with a triglyceride-rich diet containing 0.2% cholesterol (TG/0.2%) to induce dyslipidemia. Cholesterol-lowering and dietary intervention spanned 2 -11 months (mo) for Dysf$^{-/-}$/ApoE$^{-/-}$, and 2-7mo for mdx/ApoE$^{-/-}$ cohorts and their appropriate controls. Mice were sacrificed under terminal anesthesia (3.5% v/v isoflurane, 2L O2). Muscle sections stained with Masson's Trichrome were used to assess fat and collagen deposition.

Results: Compared to Dysf$^{-/-}$/ApoE$^{-/-}$ mice on chow, a TG/0.2% cholesterol containing diet caused increased muscle wasting and severe fibro-fatty infiltration in triceps and *quadriceps*, leading to complete ambulatory dysfunction in 40% of mice by 11mo of age. Hypercholesterolemia induced similar detrimental effects in triceps and *gastrocnemius* muscles of mdx/ApoE$^{-/-}$ (TG/0.2%) by 7mo of age. Strikingly, the lowering of cholesterol prevented gait abnormalities and the loss of ambulation in TG/0.2% fed Dysf$^{-/-}$/ApoE$^{-/-}$ mice, and significantly reduced fibroadipogenic infiltrates in both models lacking ApoE at 11 and 7mo, respectively.

Conclusion: Our data show that hypercholesterolemia exacerbates muscle damage and the loss of ambulation in MD. Thus MD patients could benefit greatly from cholesterol lowering medications.