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INVESTIGATING SKELETAL MUSCLE DISEASES AND THERAPEUTICS IN A DONOR-DERIVED BIOENGINEERED MUSCLE PLATFORM

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Animal models and human 2D culture models have been instrumental for investigating skeletal muscle diseases and the development of therapeutics. However in vivo models and 2D cultures are limited in their translation to clinical application. These limitations are most evident through the success of myostatin inhibitors for improving mass and function in mice studies followed by unsuccessful clinical trials in patients with sarcopenia and Duchenne Muscular Dystrophy (DMD) (3-4). Although clinical trials of myostatin inhibitors have often reported increases in lean muscle mass, efficacy endpoints of improved muscle function are typically not achieved (3-4). Additionally, studies of age-related muscle atrophy, sarcopenia, have unique barriers to translation such as age-related gene expression changes and sex related muscle aging that is not conserved between species (1-2). Due to these challenges, our team developed a donor-derived 3D skeletal muscle platform housed in standard 24-well imaging plates. This platform was then utilized to investigate retention of sex- specific characteristics in 3D muscle cultures and the effectiveness of astaxanthin (ASTX) to improve contractile signaling and function in healthy and clinically sarcopenic 3D cultures. Initial characterization and validation were carried out in samples from healthy male and female donors. Contractile function recorded through digital image correlation (DIC) analysis during electrical stimulation was similar between male and female 3D muscles, but females displayed elevated type 1 fiber proportions compared to males. Female muscle also displayed elevated levels of OPA1 and TFAM protein levels along with decreased Akt signaling. Overall, female muscle exhibited a greater reliance on mitochondrial energy utilization and reduced protein synthesis indicating retention of sex-specific characteristics in 3D culture. Samples from young female and clinically sarcopenic female donors were studied in this platform for functional differences in force production, fatigue susceptibility, and contractile protein signaling following fatigue with or without astaxanthin antioxidant therapy. Astaxanthin was assessed at 1µM, 10µM, and 25µM in young cultures and only 10µM in sarcopenic cultures due to limitations in cell quantity for 7 days. Although there were no significant effects on force outputs or fatiguability for either group, 10µM rescued P38 and Akt signaling in the sarcopenic samples restoring levels to those exhibited by young DMSO- treated controls. Additionally, 10µM and 25µM in the young cohort suppressed Akt signaling indicating astaxanthin may negatively impact this critical pathway for exercise adaptation in healthy muscle. Although astaxanthin did not improve functional performance in the clinically sarcopenic or young female 3D muscle samples, clenbuterol was evaluated as a positive control for improving performance in young muscle and showed a nearly two-fold increase in both twitch and tetanic force response in 1µM treated samples compared to DMSO controls. These findings support the utilization of this muscle platform for assessing functional differences among drug treatments and untreated donor groups.

Keywords: *3D muscle culture, bioengineered muscle, sarcopenia, muscle aging, antioxidant therapy.*

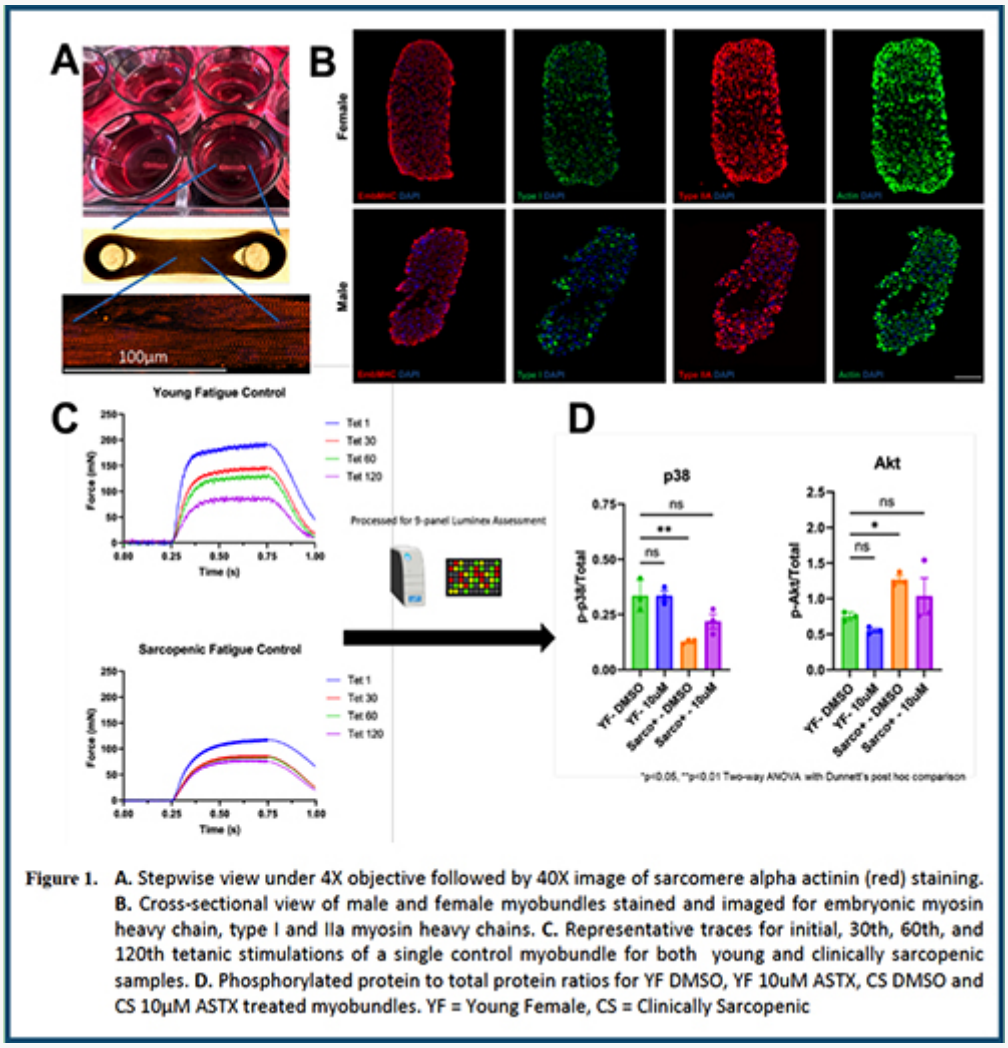

Figure 1. **A.** Stepwise view under 4X objective followed by 40X image of sarcomere alpha actinin (red) staining. **B.** Cross-sectional view of male and female myobundles stained and imaged for embryonic myosin heavy chain, type I and IIa myosin heavy chains. **C.** Representative traces for initial, 30th, 60th, and 120th tetanic stimulations of a single control myobundle for both young and clinically sarcopenic samples. **D.** Phosphorylated protein to total protein ratios for YF DMSO, YF 10uM ASTX, CS DMSO and CS 10µM ASTX treated myobundles. YF = Young Female, CS = Clinically Sarcopenic