Abstract: 21O

Biomechanical properties of dysferlin-deficient skeletal muscle and the impact of muscle type and age

Erin M. Lloyd[1,2], Matt S. Hepburn[3,4], Jiayue Li[3,4,5], Alireza Mowla[3,4], Yu Suk Choi[1], Yongsung Hwang[6], Connie Jackaman[2], Brendan F. Kennedy[3,4,5], Miranda D. Grounds[1]

1 Department of Anatomy, Physiology and Human Biology, School of Human Sciences, The University of Western Australia, Perth, Western Australia, Australia.
2 Curtin Medical School, Faculty of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia, Australia.
3 BRITElab, Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, The University of Western Australia, Perth, Western Australia, Australia.
4 Department of Electrical, Electronic & Computer Engineering, School of Engineering, The University of Perth, Western Australia, Australia.
5 Australian Research Council Centre for Personalised Therapeutics Technologies, Australia.
6 Soonchunhyang Institute of Medi-Bio Science, Soonchunhyang University, Cheonan-si, Chungcheongnam-do, Korea.

Skeletal muscle function is governed by the biomechanical properties and organisation of its constituent tissues including myofibres, extracellular matrix, and adipose tissue, which can be modified by the onset and progression of many disorders. For example, Duchenne muscular dystrophy, a severe childhood disease characterised by intrinsic myonecrosis, has impaired skeletal muscle function associated with altered biomechanical properties: exhibiting higher stiffness (increased elastic modulus) and increased extracellular matrix content. Similar features are also observed in response to normal 'healthy' ageing. In contrast, dysferlinopathy, a limb-girdle muscular dystrophy caused by a genetic deficiency of the membrane-associated protein dysferlin, manifests post-growth with progressive muscle weakness attributed, in part, to the replacement of myofibres with 'soft' adipocytes. However, there is limited understanding of how this dysferlin-deficient pathology, in particular adipose tissue accumulation, impacts the mechanical properties of muscle. To investigate the biomechanical properties of dysferlin-deficient muscles at different stages of disease severity, we used dysferlin-deficient BLAJ male mice, compared with wild-type (WT) C57BL/6J mice, aged 3, 10, and 24 months (n = 3 per group). We examined three muscles with varied pathology in response to dysferlin-deficiency: the *quadriceps* that has severe histopathology in later stages, the *soleus* (a model of slow-twitch muscle), and the *extensor digitorum longus* (EDL; a model of fast-twitch muscle), with these latter two not having marked histopathology. Mice were euthanised via cervical dislocation under anaesthetic (Attane isoflurane) and muscles were excised before undergoing a novel quantitative micro-elastography technique involving muscle encapsulation in gelatin methacryloyl hydrogels (Lloyd et al., 2022). The three-dimensional micro-architecture of muscles was then visualised with confocal microscopy. Results showed that dysferlin-deficiency and age reduced both the bulk elasticity (mean elastic modulus of the muscle volume) and mechanical heterogeneity (the variability of elasticity within the muscle volume) of the quadriceps and the predominantly slow-twitch soleus, but not the fast-twitch EDL muscle. The 24-month BLAJ quadriceps, compared with age-matched WT muscle, was both softer (i.e., reduced elastic modulus; -72%, $p < 0.05$) and less mechanically heterogeneous (-59%, $p < 0.05$), with substantial adipose tissue accumulation observed. Similarly, the BLAJ soleus, compared with WT, was softer (-20%, $p < 0.05$) with less variation in elasticity (-14%, $p < 0.05$). These data demonstrate the striking impact of dysferlin-deficiency on skeletal muscle biomechanical properties, which varies depending on muscle type and age. These results provide new insight into the disease-related loss of muscle contractile function in dysferlinopathy.

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