The contractile properties of slow and fast skeletal muscle from dysferlin deficient mice

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Dysferlinopathies are a clinically heterogeneous subgroup of the muscular dystrophies caused by a deficiency of the membrane associated protein dysferlin. Disease onset typically occurs post growth in early adulthood and is characterized by skeletal muscle wasting, muscle weakness, increased fatigability, and inflammation (Amato & Brown Jr, 2011). Dysferlin is highly expressed in skeletal muscle and appears to have a role in calcium (Ca^{2+}) handling (Klinge *et al.*, 2010), however, it is not understood how dysferlin deficiency contributes to the disease pathology. Therefore, to aid in elucidating the mechanisms underlying the dysferlinopathy pathology, this study examined the impact of dysferlin deficiency on the *ex vivo* contractile properties of slow and fast twitch skeletal muscle, from aged mice.

Experiments were performed on male C57BL6 wild type control (WT) mice and dysferlin deficient ($dysf^{-/-}$) BLAJ mice, aged 10 months old (an age when histopathology is manifested in some BLAJ muscles). Mice were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.) and *soleus* and *extensor digitorum longus* (EDL) muscles were surgically removed and mounted in a force transducer system. Muscles were maintained in mammalian Krebs Ringer solution (pH 7.3) at 25°C and bubbled with Carbogen (5% CO_2 in O_2). Twitch force characteristics, the force-frequency relationship, maximum specific force, and fatigue and post-fatigue recovery were compared in *soleus* and EDL muscles from WT and BLAJ mice. All values are expressed as means ± SEM.

In the *soleus* muscle (for WT compared with BLAJ), dysferlin deficiency resulted in a 21% decrease in the twitch time to peak (TTP) (WT: 39.07 ± 2.64 ms; BLAJ: 30.93 ± 0.83 ms, $P<0.05$), and a 20% decrease in the half relaxation time (½RT, WT: 54.96 ± 2.78 ms; BLAJ: 44.22 ± 2.15 ms, $P<0.05$). The $dysf^{-/-}$ *soleus* also exhibited a significantly slower recovery in force after fatigue (*e.g.* At 5 min post fatigue: WT: 79 ± 4 $\%P_i$; BLAJ: 66 ± 3 $\%P_i$, $P<0.05$). However, the maximum specific force (Po), peak twitch force (Pt) and fatigue of the *soleus* were unaffected by the absence of dysferlin. In the $dysf^{-/-}$ (BLAJ) EDL, there was a 25% increase in the maximum rate of force development (WT: 1148.22 ± 96.68 g/s; BLAJ: 1431.10 ± 76.11 g/s, $P<0.05$), although no significant differences in Po, Pt, TTP, ½RT, fatigue and post fatigue recovery were found.

These findings indicate that dysferlin deficiency significantly alters the contractile properties of slow twitch skeletal muscle (predominantly oxidative metabolism), with lesser effects on fast twitch myofibres from older mice (aged 10 months). Findings suggest some involvement of disturbed Ca^{2+} handling affecting excitation contraction coupling. These results provide new insight into the loss of function that manifests in later life in dysferlin deficient muscles.

Amato AA & Brown Jr RH. (2011) Dysferlinopathies. *Handb Clin Neurol* **101**, 111-118.

Klinge L, Harris J, Sewry C, Charlton R, Anderson L, Laval S, Chiu Y-H, Hornsey M, Straub V, Barresi R, Lochmüller H & Bushby K. (2010) Dysferlin associates with the developing T-tubule system in rodent and human skeletal muscle. *Muscle Nerve* **41**, 166-173.