Contractile characteristics of permeabilized muscle fibres from dystrophic dogs

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Duchenne muscular dystrophy (DMD) is an X-linked myopathy characterised by progressive muscular degeneration and weakness that ultimately results in premature death *via* cardiac or pulmonary failure. Like the *mdx* mouse model of DMD, the Golden Retriever muscular dystrophy (GRMD) dog model has a single mutation in the dystrophin gene. Similar to DMD patients, affected dogs with the mutant dystrophin gene have weak muscles and eventually die from the disease *via* cardiac or pulmonary failure. Although the *mdx* mouse provides a useful model for studying some aspects of the pathogenesis of muscular dystrophy, the GRMD dog provides a better model relevant to DMD (Childers *et al.*, 2005). Unfortunately, there are few GRMD dog colonies around the world making it a unique but not widely used model for understanding underlying mechanisms and investigating potential therapeutic approaches for DMD. Little information exists about many of the fundamental aspects of skeletal muscles from dystrophic compared with healthy dogs. The aim of this study was to identify the Ca^{2+}- and Sr^{2+}-activated contractile characteristics of single muscle fibres from dystrophic dogs.

Two-year old dystrophic dogs (n = 3) and control litter mates (n = 3) from a colony at the University of Ribeirão Preto were used in this experiment. Dogs were anaesthetised (Tiletamine Cloridrate 125.0 mg and Zolazepam Cloridrate 125.0 mg) and open muscle biopsies taken from the *biceps femoris* muscle. All the dogs recovered from the anaesthesia. The muscle samples were blotted on filter paper and bundles tied to a capillary tube and placed immediately in a vial containing skinning solution of the following composition (mM): potassium propionate (125), EGTA (5), ATP (2), $MgCl_2$ (2), imidazole (20) and 50 % v/v glycerol, adjusted to pH 7.1 with 4M KOH; and stored at -20°C for up to 12 weeks (Lynch *et al.*, 2000). Single permeabilized muscle fibres were isolated from the bundles, attached to a sensitive force transducer and activated by rapid immersion in buffered solutions of varying [Ca^{2+}] and [Sr^{2+}]. Based on their contractile characteristics during Ca^{2+}- and Sr^{2+}-activation, fibres were allocated into discrete populations: type I, type II and hybrid type I/II fibres. After the contractile properties had been determined, the fibre segments (volume 40nl) were placed in 10µl solubilising buffer, incubated at room temperature for 24h, boiled for 3 min and stored at –80°C, for later analysis of troponin-C isoforms by SDS-PAGE.

There was a significant (25%) decrease in specific force production of muscle fibres from dystrophic dogs (214.9 ± 17.9 kN/m^2, n = 15) compared with litter mate controls (159.6 ± 8.7 kN/m^2, n = 15). There was no difference in fibre sensitivity to Ca^{2+} [pCa50; control 6.41 ± 0.03; dystrophic 6.36 ± 0.023 (n = 15 both groups)]. A high incidence (65%) of fibres from dystrophic dogs were hybrid fibres as evident from their biphasic force-pSr relations and combination of slow and fast troponin-C isoforms (Lynch *et al.*, 1995).

Functional assessments of skinned fibres coupled with biochemical analysis of their contractile and regulatory proteins can provide important information about the progression of muscular dystrophy in the GRMD model and its relevance to DMD.

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