Comparison of contractile characteristics of permeabilized muscle fibres from the golden retriever muscular dystrophy (GRMD) dog and the *mdx* dystrophic mouse

H.Q. Lim,[1] C. van der Poel,[1] J.E. Church,[1] J.C.S. Bizario,[2] M.C.R. Costa,[2] M.L. Pinto[2] and G.S. Lynch,[1] [1]Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia and [2]Associação de Amigos dos Portadores de Distrofia Muscular, University of Ribeirão Preto, São Paulo, Brasil.

Duchenne muscular dystrophy (DMD) is a severe X-linked progressive muscle disease caused by the absence of dystrophin. A number of animal models are available to investigate underlying mechanisms and identify potential therapies for DMD. Although the most commonly used animal model of DMD, the *mdx* mouse, carries the same genetic mutation of the dystrophin gene, it exhibits a considerably milder phenotype (Lynch, 2004). The golden retriever muscular dystrophy (GRMD) dog represents an appealing animal model for DMD as it not only shares the mutation in the dystrophin gene but also exhibits a similar phenotype (Childers *et al*. 2002). Few GRMD dog colonies exist worldwide, making it a unique but not widely used model. Little information exists about many of the fundamental aspects of skeletal muscles from dystrophic compared with healthy dogs. The aim was to identify Ca^{2+}- and Sr^{2+}-activated contractile characteristics of single muscle fibres from dystrophic dogs and to compare these with fibres from *mdx* dystrophic mice.

GRMD dogs (n = 3) and littermate controls (n = 3) were housed in the University of Ribeirâo Preto Sao Paulo, Brazil. Dogs were anaesthetized (Tiletamine Cloridrate 125.0 mg and Zolazepam Cloridrate 125.0 mg) and open muscle biopsies taken from the biceps femoris muscle. All dogs recovered from the anaesthesia. The muscle samples were 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 4 M KOH; and stored at -20°C for up to 12 weeks (Lynch *et al.* 2000). C57BL/10 and *mdx* mice were killed by cardiac excision while anaesthetized (sodium pentobarbital, 60 mg/kg, *i.p.*) and the extensor digitorum longus (EDL) and soleus muscles were surgically excised and immersed in skinning solution for preparation of permeabilized muscle fibres. Individual fibres were isolated, attached to a sensitive force recording device, and immersed in a series of solutions with increasing $[Ca^{2+}]$ and $[Sr^{2+}]$. After the contractile properties had been determined, the fibre segments were stored for later analysis of contractile and regulatory protein isoforms by SDS-PAGE.

There was a significant decrease in maximum Ca^{2+}-induced force by GRMD dog fibres (120 ± 1 kN/m^2, n = 62), compared with littermate controls (134 ± 2 kN/m^2, n = 67), however, maximum Ca^{2+}-induced force was not different between EDL or soleus muscle fibres from *mdx* and C57BL/10 mice. There was a significant decrease in the Hill coefficient (n_H) of the force-pCa relationship for GRMD dogs, compared with littermate controls but n_H was not different between fibres from *mdx* and C57BL/10 mice. Fibre sensitivity to Ca^{2+} (pCa_{50}) was not different between GRMD dogs (5.95 ± 0.02, n = 55) and littermate controls (5.99 ± 0.03, n = 54) nor was it different between *mdx* and WT mice. These findings highlight differences between animal models for DMD and that a lack of dystrophin can result in variable phenotypes between species.

Childers MK, Okamura CS, Bogan DJ, Bogan JR, Petroski GF, McDonald K, Kornegay JN. (2002). *Archives of Physical Medicine and Rehabilitation* , **83:** 1572-8.

Lynch GS. (2004). *Clinical and Experimental Pharmacology and Physiology*, **31:** 557-61.

Lynch GS, Rafael JA, Chamberlain JS, Faulkner JA. (2000) *American Journal of Physiology*, **279:** C1290-4.

Supported by the Muscular Dystrophy Association (USA)