The contractile properties of slow and fast skeletal muscles from protease activated receptor-1 null mice

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Protease activated receptors (PARs) are G-protein-coupled receptors that are activated by proteolytic cleavage. During early skeletal muscle development, muscle fibres receive multiple motor-innervation. These multiple neural connections are eventually lost during the postnatal period through a process known as polyneuronal synapse elimination. PAR-1 receptors are thought to play a role in this process, and therefore, PAR-1 activation could affect motor unit distribution within a muscle and alter the contractile properties. The aim of this study was to compare the contractile properties of slow and fast skeletal muscles from PAR-1 null (PAR-1 KO) and control mice.

Experiments were conducted on 12-14 weeks old PAR-1 null (KO) (n=5) and wildtype (WT) littermate mice (n=4). Approval for this study was obtained from the animal ethics committee of the University of Western Australia. Mice were anaesthetized by intraperitoneal injection of pentobarbitone (40mg/kg body weight). The *soleus* (mainly slow twitch) and *extensor digitorum longus* (EDL) (fast twitch) muscles of left hind limb were surgically removed and connected to a force transducer system. The muscles were maintained in Krebs mammalian Ringer solution (pH 7.3) bubbled with Carbogen (95% O_2 and 5% CO_2) at 25°C. Twitch force characteristics, the force frequency relationship, maximum specific force (force normalized to muscle size) were determined, and the rate of fatigue and post-fatigue recovery were assessed.

The results showed that the absence of PAR-1 receptors in *soleus* muscles resulted in a significantly greater mean peak twitch force (KO: 6.2 ± 0.231 $N.cm^{-2}$, WT: 4.6 ± 0.392 $N.cm^{-2}$, $P<0.05$) and mean twitch time to peak values (KO: 57.0 ± 3.0 ms, WT: 41.0 ± 1.0 ms, $P<0.05$) and produced a significant shift of the force-frequency curve to the left. In addition, the soleus muscles from the PAR-1 null mice fatigued significantly more slowly ($P<0.05$) and exhibited a significantly faster post fatigue recovery ($P<0.05$) than muscles from control mice. There was no difference in maximum specific force between soleus muscles from PAR-1 KO and WT mice. EDL muscles from PAR-1 KO mice exhibited a small but significant decrease in the rate of fatigue compared to EDL muscles from control mice.

The results of this study indicate that the absence of PAR-1 receptors significantly alters the contractile properties of skeletal muscle, especially in muscles with a predominance of slow twitch fibres. The effects of the absence of PAR-1 receptors is consistent with a fibre type transition to a slower phenotype.