Increased fatigue resistance in EDL muscle of the obese mouse is associated with an increase in the proportion of hybrid IIB+IID fibres

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Fatigue resistance is an important indicator of the functional status of a muscle. Current data on the fatigue characteristics of the extensor digitorum longus (EDL) muscle from the genetically obese (*ob/ob*) mouse, a commonly used animal model of type 2 diabetes, are limited and inconsistent. Of the two studies carried out to date on this muscle, one shows an increased fatigue resistance in the obese animal (Warmington *et al.*, 2000) while the other shows no difference between the obese animal and its lean control (Bruton *et al.*, 2002). Therefore, in the present study we re-examined the fatigue characteristics of EDL muscles from *ob/ob* and lean mice. We also determined, using a single fibre approach, the fibre type composition of the two muscles as this parameter is closely related to muscle fatigability.

Male *ob/ob* and lean mice (18-22 weeks, C57BL strain) were killed by halothane overdose in accordance with Victoria University AEEC procedures, and muscle dissection was carried out as described in Bortolotto *et al.* (2000). Isometric contractions in EDL muscle were elicited at optimal length *via* supramaximal pulses (13 V cm^{-1}; 0.2 ms duration) in carbogen bubbled Krebs solution (Pedersen *et al.*, 2003) with 10 mmol l^{-1} glucose and 10 μmol l^{-1} tubocurarin, at 25 ± 1°C. Force-frequency responses were determined using stimulation trains of 500 ms and train frequencies of 1-110 Hz, with a 3 min rest period between stimuli. Fatigue resistance was evaluated using a fatigue protocol similar to that described in Chin & Allen (1997), and consisted of repeated maximum tetanic stimulation (110 Hz, 350 ms train duration) at decreasing time intervals (4 s, 3 s, 2.5 s; each for total 2 min) until the force declined to 30% of the initial force (P_0). This protocol was repeated following a 60 min rest period. Contralateral EDL muscles were employed for electrophoretic analyses of myosin heavy chain isoform (MHC_i) composition in whole muscle homogenates and single muscle fibres using a modified version of the Talmadge & Roy (1993) SDS-PAGE protocol.

In comparison to EDL muscle from lean mice (n=8), EDL muscle from *ob/ob* mice (n=8) displayed an increased resistance to the first fatigue bout (time to 30% P_0: 164.4 ± 6.2 s *vs* 146.1 ± 2.8 s; P<0.05) and greater recovery of peak force between fatigue bouts. Type IIB was the predominant fibre type in randomly dissected single fibres from EDL muscle of *ob/ob* (78.9%, n=57) and lean (95.1%, n=61) mice. However, the fibre population from *ob/ob* mice contained a greater proportion of hybrid fibres (21.1% *vs* 4.9%) co-expressing MHCIIb and MHCIId isoforms (i.e. hybrid IIB+IID fibres). Consistent with this result, EDL muscle (n=6) from *ob/ob* mice contained a smaller proportion of MHCIIb (52.4% *vs* 65.7%) and larger proportions of MHCIId (31.9% *vs* 25.7%) and MHCIIa (15.7% *vs* 8.6%) isoforms. This shift in the MHC_i composition of EDL muscle from *ob/ob* mice towards a slower profile was also reflected in the force-frequency relationship at suboptimal frequencies (greater % force relative to maximum force at 30 Hz and 50 Hz in obese muscle) and a prolonged twitch half-relaxation rate (72.4 ± 6.0 ms in obese *vs* 49.2 ± 3.4 ms in lean; P<0.05).

The shift towards slower fibre types and the increased fatigue resistance observed in the present study for EDL muscle from the *ob/ob* mouse may be part of an adaptive response to the obese/diabetic condition, whereby the physiological role of the EDL muscle changes from a muscle enabling rapid movement to a muscle enabling better maintenance of posture under conditions of increased body weight.

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