A slowing of relaxation in EDL muscle from the genetically obese mouse is associated with alterations in SR Ca^{2+} handling

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There is growing evidence to suggest that the isometric contractile properties of fast-twitch skeletal muscles from animal models of obesity are different from those of lean controls (Warmington *et al*., 2000; Bruton *et al*., 2002; Blazev *et al*., 2005). In the present study, we specifically investigated the relaxation kinetics of extensor digitorum longus (EDL) muscle isolated from the genetically obese (*ob/ob*) mouse and its lean counterpart. We also used a single fibre approach to more precisely address any observed differences in relaxation at the whole muscle level.

Male *ob/ob* and lean mice (18-22 weeks) were killed by halothane overdose in accordance with Victoria University AEEC procedures. EDL muscles were dissected and placed in carbogen bubbled Krebs solution at 25 °C, before being supramaximally stimulated to elicit tetanic (110 Hz) responses, as described previously (Blazev *et al*., 2005). EDL muscles not incubated in Krebs solution were used to obtain mechanically skinned fibre segments that were electrophoretically typed. Since the EDL muscles of both *ob/ob* and lean mice contain predominantly type IIB fibres (Blazev *et al*., 2005) their properties related to Ca^{2+}-sensitivity of the contractile apparatus and sarcoplasmic reticulum (SR) Ca^{2+} handling were investigated following the procedures routinely used in our laboratory (Bortolotto *et al*., 2000, 2001). All results are given as mean ± SEM.

The half relaxation time (1/2 RT) for tetanic responses was significantly slower ($p < 0.05$) in EDL muscles from *ob/ob* mouse (n = 8) as compared to lean controls (n = 8) (46.0 ± 2.3 *vs* 39.9 ± 1.3 ms). There was no difference in the Ca^{2+}-sensitivity of the contractile apparatus between IIB fibres isolated from EDL of *ob/ob* and lean mice, as determined from the pCa ($-\log_{10}[Ca^{2+}]$) giving 50% maximum force (*i.e.*, pCa_{50}: 5.77 ± 0.01, n = 14 *vs.* 5.74 ± 0.02, n = 12). A single fibre investigation of the ability of the SR to maximally sequester Ca^{2+} was carried out by normalising the area under the 30 mM caffeine-induced force response (in the presence of 0.5 mM EGTA and 0.05 mM free Mg^{2+}), following maximal SR Ca^{2+} loading at pCa 7.3, to the maximum Ca^{2+}-activated force response (F_{max}). This normalised value for EDL IIB fibres from *ob/ob* mice (175 ± 16 $\%F_{max}$.s, n = 8) was significantly smaller than that for fibres from lean mice (268 ± 37 $\%F_{max}$.s, n = 8), indicating a lower SR Ca^{2+} loading capacity at pCa 7.3 for *ob/ob* fibres. The lower SR Ca^{2+} content of these fibres was not due to differences in the rate of passive Ca^{2+} leak (% min^{-1}) from the SR between IIB *ob/ob* and lean fibres (59.6 ± 11.0, n = 5 *vs.* 59.3 ± 9.2, n = 6). Furthermore, investigation of slow/fast SR characteristics (Fryer and Stephenson, 1996; Bortolotto *et al*., 2001) by loading maximally at two different [Ca^{2+}], revealed that the ratio ($R_{6.2/7.3}$) derived from the area under the force response to 30 mM caffeine following loading at pCa 6.2 versus pCa 7.3 was similar in IIB fibres from *ob/ob* and lean mice ($R_{6.2/7.3}$: 1.50 ± 0.13, n = 7 *vs.* 1.49 ± 0.11, n = 8). Thus, functional characteristics of the SR Ca^{2+} pumps were the same in these fibres.

Taken together, the above results suggest that the density of the SR Ca^{2+} pumps (expressed per fibre volume) was significantly lower in EDL IIB fibres from *ob/ob* mice than in fibres from lean mice. This reduces the ability of the SR to sequester Ca^{2+} and return the myoplasmic [Ca^{2+}] back to ~ theoretical resting levels following muscular contraction in *ob/ob* mice as compared to the lean counterparts, and contributes to the slowing of the 1/2 RT observed in the present study for the tetanic response at the whole muscle level in *ob/ob* mice.

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