**Introduction** \(^{13}P\) MRS studies of skeletal muscle in patients with Duchenne muscular dystrophy (DMD, in which the sarcoglycan protein dystrophin is absent) and Becker muscular dystrophy (BMD, in which dystrophin is abnormal in structure and/or reduced in content) have consistently shown abnormalities in pH and metabolite concentration ratios. In vivo and in vitro studies have shown abnormalities of ionic regulation and homeostasis, but alterations in bioenergetics in vitro have not been proven conclusively. The mdx mouse also lacks dystrophin and has been widely studied as a model of DMD. Although it exhibits some of the pH and other metabolic markers of DMD, the mdx mouse displays little pathophysiology. This may be associated with the upregulation of utrophin, a protein closely related to dystrophin and concentrated at the neuromuscular junction. A new mouse model, the 'double knockout' (dko), which lacks both of the sarcoglycan proteins dystrophin and utrophin has recently been developed. It shows the progressive muscle wasting, cardiomyopathy and reduced life expectancy which characterise DMD. We have carried out \(^{13}P\) MRS studies on dko mouse muscle in order to assess its suitability as a model of the human disease. Here we present our initial findings of a comparison between mdx, dko and control mice.

**Methods** Three groups of animals, age 9 weeks, were used: a) 5 dko mice, b) 6 of their mdx littermates, c) 6 normal C57Bl10 controls. Mice were anaesthetised by peritoneal injection, platinum electrodes were placed around the sciatic nerve of the left hindlimb and the incision was closed with surgical thread. Knee and ankle were immobilized in a peroneal cradle and the Achilles tendon attached by thread to an isometric force transducer. Output from a strain gauge was directed to a MacLab data recorder system. Twitch tension was maximised for each experiment and a 2.2 s interpulse delay. Magnet bore air temperature was maintained at 30°C. A single 128 scan spectrum was obtained from resting muscle; subsequent spectra were all 32 scans. After collecting 2 spectra at rest, the nerve was stimulated during the collection of the next 8 spectra (9.4 min) using 30 Hz trains of 250 ms duration alternating with rest periods of 1.75 s. The stimulation was then stopped and the muscle allowed to recover during collection of the final 15 spectra (17.6 min). The electrical stimulation protocol used in these experiments was designed to approximate more closely than previous studies the exercise protocols used in human MRS studies. Data were processed using 30 Hz line broadening. Spectra were baseline corrected and peak areas analyzed using the integration program provided by Varian.

**Results and discussion** At rest, PCR/Pi (peak heights) was 20.2±1.7 in C57, lower in mdx (13.2±1.1) and lowest in dko (5.8±0.7). In human muscle the order of the ratio is: normal control >BMD >DMD, decreasing overall by about a factor of 3.\(^1\) The intracellular pH was lowest in C57 (7.10±0.02) and higher in mdx and dko at 7.13±0.02 and 7.16±0.03, respectively. The mean 0.06 pH unit increase in dko is comparable to the mean 0.09 unit increase in calf muscle in DMD.\(^1\) Force production throughout the stimulation period is shown in Fig 1. Mdx and C57 muscle behaved similarly, but in dko the tension decreased initially and remained low. The lesser degree of phosphocreatine (PCr) depletion (Fig 2A) is probably due to the lower tension generation. Neither changes in [ATP] over the entire time course of the experiment nor PCr recovery half times were significantly different in the 3 groups (results not shown). The rapid recovery of PCr would suggest that the muscle remained undamaged by the exercise regimen. During stimulation the decrease in pH was similar for mdx and dko but initially greater in C57 (Fig 2D). It is interesting to note that in BMD muscle, lactate production by glycolysis was calculated to be lower than normal later in exercise, but not in the first minute.\(^2\) After 2-3 min, the pH in C57 began to drift upward, indicating that either lactate production was diminishing or proton efflux had increased. At the end of exercise the pH in all 3 groups was similar. Recovery of pH was slow in dko but not in mdx. Slow pH recovery has been seen in mdx using a more severe stimulation protocol (continuous 10 Hz)\(^3\) and in one patient study\(^4\) but not in others,\(^1\) suggesting that such changes are protocol specific. Slow pH recovery in our experiments is unlikely to be due to low blood flow because PCr recovery, which is known to be oxidative and therefore flow dependent, is not affected (Fig 2B). It is therefore more likely to be due to a diminished rate of proton efflux.

**Fig 2. Time course of changes in PCr and pH in response to exercise.**

**Conclusions** Absence of both utrophin and dystrophin in mouse muscle results in changes in muscle performance; ionic homeostasis and concentrations of phosphorylated metabolites which are either different from or more extreme than those seen in mouse muscle deficient in dystrophin alone. Thus, the MRS findings which accompany the pathophysiological changes confirm that the dko mouse is an appropriate model for clarifying the roles of these two proteins. The metabolic response of muscle in vivo depends on the complex interaction of such factors as fibre type, contraction characteristics, blood flow, metabolic concentrations and ion fluxes across the sarcolemma. Work is continuing to allow us to interpret more fully the effects of dystrophin and utrophin on muscle metabolism and ionic homeostasis.

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**References**