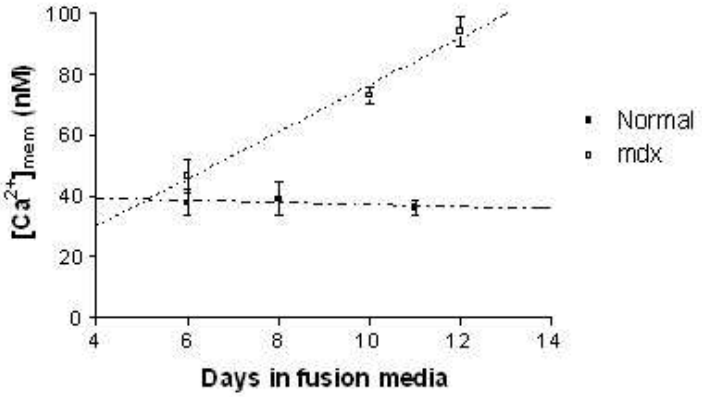
Near-membrane cytosolic [Ca^{2+}] levels and Ca^{2+} transients measured in myotubes grown from normal and dystrophic (mdx) mice using the Ca^{2+} indicator FFP-18.

A.J. Bakker[1], R. Han[1] and M.D. Grounds[2], [1]School of Biomedical & Chemical Sciences and [2]School of Anatomy & Human Biology, The University of Western Australia, Crawley, WA 6009, Australia.

Abnormal extracellular Ca^{2+} influx has been suggested to be involved in the process of muscle wasting in Duchenne muscular dystrophy. However, studies comparing the resting intracellular Ca^{2+} levels in normal and dystrophic muscle cells from patients with Duchenne muscular dystrophy and *mdx* mice have yielded contradictory findings (Gillis, 1996). Ca^{2+} indicators targeted to the inner sarcolemmal membrane have recently been reported to be more sensitive to sarcolemmal Ca^{2+} influx than standard cytosolic Ca^{2+} indicators such as fura-2 (Bruton *et al.*, 1999). In this study, we measured the resting Ca^{2+} levels and Ca^{2+} transients in myotubes grown from *mdx* and normal mice using the near-membrane Ca^{2+} indicator FFP-18.

Skeletal muscle satellite cells were isolated from the hind limbs of neonatal normal and *mdx* mice that had been killed by decapitation. Myotubes were grown on glass coverslips coated with collagen. The myotubes were loaded with the Ca^{2+} indicator by exposure to FFP-18-AM (3 μM) and 0.0125% Pluronic F-127 for 45 min at room temperature (22-23°C). Ca^{2+} measurements were made with a Cairn spectrophotometer attached to a Nikon inverted microscope equipped for epifluorescence. The myotubes were stimulated by electrical field stimulation (EFS) via two small platinum wires (single 0.2 ms pulse).



Resting near membrane [Ca^{2+}] ($[Ca^{2+}]_{mem}$) levels increased significantly during development in the *mdx* myotubes, (slope; 8.19 ± 1.47, $p<0.0001$). However, no change in $[Ca^{2+}]_{mem}$ was found in normal myotubes during development (slope; -0.40 ± 1.14, $p=0.73$). From the fitted lines, the $[Ca^{2+}]_{mem}$ in 12 days old *mdx* and normal myotubes was estimated at 93 and 36 nM respectively (Figure). Increasing the driving force for Ca^{2+} influx by raising extracellular Ca^{2+} to 18 mM, increased the steady state $[Ca^{2+}]_{mem}$ by 156.1 ± 14.2 % (to ˜ 208 nM) (n=14) in *mdx* myotubes, while in normal myotubes, the $[Ca^{2+}]_{mem}$ increased by only 28.8 ± 7.6 % (to ˜ 49 nM) (n=6), ($p=0.007$, unpaired Student's *t*-test). The half-relaxation time of EFS-induced Ca^{2+} transients was significantly increased in *mdx* (314.5 ± 36.9 ms, n=8) compared to normal myotubes (163.3 ± 28.4 ms, n=6) ($p=0.01$, unpaired t-test), which is consistent with previous studies using standard Ca^{2+} indicators.

The results of this study further support the hypothesis that increased Ca^{2+} influx results in raised intracellular levels in dystrophin-deficent skeletal muscle cells. The use of FFP-18 to measure steady state cytosolic Ca^{2+} in normal and *mdx* myotubes in the presence of raised extracellular Ca^{2+} could provide a more reliable method for detecting the altered Ca^{2+} homeostasis in dystrophic muscle cells.

Bruton J.D., Katz A. & Westerblad H. (1999) *Proceedings of the National Academy of Sciences USA,* **96**, 3281-3286.

Gillis J.M. (1996) *Acta Physiologica Scandinavica,* **156**, 397-406.