The effect of nitrate supplementation on sarcoplasmic reticulum Ca\(^{2+}\) handling in dystrophic skeletal muscle fibres

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There is emerging evidence to suggest that dietary nitrate supplementation enhances skeletal muscle contractile performance and that nitrate may therefore have a potential therapeutic role in improving contractile function in diseased states (Hernandez et al., 2012). In the present study, we used a mechanically skinned muscle fibre preparation to investigate whether nitrate supplementation affects sarcoplasmic reticulum (SR) function in tibialis anterior (TA) muscle of the mdx mouse; a commonly used animal model of Duchenne Muscular Dystrophy.

All experiments were approved by the Victoria University Animal Ethics Experimentation Committee. Four week old male wild-type (WT) C57BL/10 and dystrophic (mdx) C57BL/10mdx mice were given 1 mM NaNO\(_3\) in drinking water for eight weeks (NITR), while non-supplemented mice were given drinking water without NaNO\(_3\). At twelve weeks of age, mice were anaesthetized via intraperitoneal injection of sodium pentobarbitone (60 mg/Kg) and the TA muscle dissected. Skinned fibre solutions and experimental protocols were similar to that described by Trinh and Lamb (2006). Because single fibres were mechanically skinned under paraffin oil they retained their endogenous SR Ca\(^{2+}\) content, which was estimated from the time-integral (area) of the force response to 30 mM caffeine (with 0.05 mM free Mg\(^{2+}\) and 0.5 mM EGTA). The SR of skinned fibre segments could then be subjected to repeated cycles in which it was loaded with Ca\(^{2+}\) at pCa (= -log\(_{10}\) [Ca\(^{2+}\)]) 6.7 (1 mM EGTA) for various times (10 – 120 s) and depleted with 30 mM caffeine, with the area of the ensuing force response indicative of the amount of Ca\(^{2+}\) sequestered by the SR. This area was normalized to the maximum Ca\(^{2+}\)-activated force (F\(_{max}\)) to allow comparisons between fibres. Passive Ca\(^{2+}\) leak out of the SR was assessed from the time-integral of the 30 mM caffeine response obtained after the SR had been loaded with Ca\(^{2+}\) for a set time and then exposed to a leak solution (0.5 mM EGTA) to prevent SR Ca\(^{2+}\) uptake. Results are reported as mean ± SEM.

There was no effect of NITR on specific force (kN/m\(^2\)) in either mdx (215.0 ± 17.9, n = 13 vs. NITR 237.9 ± 21.8, n = 12) or WT (284.5 ± 18.0, n = 10 vs. NITR 298.3 ± 17.9, n = 10) TA skinned muscle fibres. Nitrate supplementation did not alter the endogenous SR Ca\(^{2+}\) content of mdx skinned fibres (%F\(_{max}\): 120.7 ± 28.2, n = 13 vs. 100.1 ± 30.2, n = 10), but did significantly increase the endogenous SR Ca\(^{2+}\) content of WT fibres (%F\(_{max}\): 37.9 ± 12.9, n = 10 vs. NITR 253.3 ± 61.8, n = 10; \(P\) < 0.05). In mdx fibres the ability of the SR to sequester Ca\(^{2+}\) after maximal loading at pCa 6.7 was significantly lower (\(P\) < 0.05) following nitrate supplementation (%F\(_{max}\): 871 ± 66.5, n = 13 vs. NITR 627.2 ± 40.9, n = 11), while no differences were observed in WT fibres (%F\(_{max}\): 629.1 ± 112.1, n = 10 vs. NITR 637.6 ± 74, n = 10). The reduced maximum SR Ca\(^{2+}\) loading capacity observed in mdx fibres was not due to differences in passive Ca\(^{2+}\) leak from the SR (% leak: 29.9 ± 4.8, n = 13 vs. NITR 36.1 ± 6.1, n = 11), and there were no differences in leak observed in WT fibres with NITR (% leak: 24.2 ± 2.1, n = 10 vs NITR 29.3 ± 5.7, n = 8).

Thus, nitrate supplementation in mdx mice appears to decrease the capacity of the SR to maximally sequester Ca\(^{2+}\) with no effect on specific force, endogenous SR Ca\(^{2+}\) content, or SR Ca\(^{2+}\) leak.
