

## Effects of vitamin D supplementation on dystrophic (mdx) muscles during damage and repair

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Duchenne Muscular Dystrophy (DMD) is a debilitating and life-ending disease characterized by repeated cycles of skeletal muscle damage and repair culminating in severe muscle wasting and weakness. As the implementation of a genetic cure remains elusive, the search for therapeutic adjuncts continues to be as important as ever. Low vitamin D (VitD) has been associated with muscle weakness and fatigue, and DMD sufferers have been reported to have low VitD (Bianchi *et al.*, 2011). Interestingly, VitD has been reported to enhance regeneration after damage of skeletal muscles (Stratos *et al.*, 2013). Thus, this study aimed to evaluate the effects of VitD supplementation on muscle strength and fatigue during damage and repair in dystrophic muscles. The *mdx* mouse, a commonly used model of DMD, was chosen due to the extensive skeletal muscle degeneration and regeneration that occurs early in its lifespan.

Animal experimentation was approved by the Victoria University Animal Ethics Experimentation Committee and performed in accordance with the Australian Code of Practice for the Care and use of Animal for Scientific Purposes. In this study, 4 week old (n=20) C57BL/10mdx (*mdx*) were provided *ad libitum* access to a standard chow (LOW) diet (containing 1000 IU.kg<sup>-1</sup> cholecalciferol) or the same diet supplemented (HIGH) with VitD (containing 20000 IU.kg<sup>-1</sup> cholecalciferol). At 8 weeks of age *mdx* mice were deeply anaesthetized via an intraperitoneal injection of sodium pentobarbitone (60mg.kg<sup>-1</sup>) and the *extensor digitorum longus* (EDL) and *soleus* muscles removed and tested for their contractile and fatigue properties. Other muscles and the heart were also removed. The EDL and *soleus* were weighed, snap frozen, sectioned and stained with haematoxylin and eosin for analysis of the extent of regeneration.

Body weights were significantly larger ( $P<0.05$ ) in the HIGH *mdx* group compared to LOW. This difference did not appear to be due to changes in muscle mass, with no differences in EDL, *soleus*, *plantaris*, *tibialis anterior*, *gastrocnemius* or heart weights. Indeed, the heart mass to body mass ratio showed a strong trend to be lower in the HIGH group ( $P=0.06$ ). Specific force was 40% greater in the EDL of the HIGH compared to the LOW group ( $16.7\pm 4.6$  v  $12.0\pm 5.1$  N.cm<sup>-2</sup>, mean $\pm$ SD, respectively;  $P=0.08$ ) with no such trend in the *soleus*. Lack of significance as a result of variability may have been due to the considerable damage and repair processes which continued to be observed in the muscles at 8 weeks of age. The level of regeneration as measured by the proportion of centralised nuclei was not different between the groups. However, since both damage and repair processes were occurring during the 4 weeks of supplementation, further work is needed to elucidate whether VitD can both lessen damage as well as enhance regeneration, and thus be a potential therapeutic adjunct.

Bianchi ML, Morandi L, Andreucci E, Via S, Frasukiewicz J & Cottafava R (2011). Low bone density and bone metabolism alterations in Duchenne muscular dystrophy: response to calcium and vitamin D treatment. *Osteoporosis Int* **22**, 529-539.

Stratos I, Li Z, Herlyn P, Behrendt AK, Mittlmeier T & Vollmar B (2013). Vitamin D increases cellular turnover and functionally restores the skeletal muscle after crush injury in rats. *Am. J Path* **182**, 895-904.