The effect of dietary vitamin D manipulation on skeletal muscle function in mice

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Introduction: Vitamin D has long been known for its importance in bone health and links with falls and fractures. However, studies have also now associated low vitamin D with low muscle strength. Trials using supplementation with vitamin D in insufficiency have reported improved muscle function and resistance to fatigue. However, few studies have directly investigated the effects of altered vitamin D levels specifically on muscle function. Thus, the aim of this study was to observe any effects low and high dietary doses of vitamin D on skeletal muscle contractility and fatigue in mice.

Methods: Thirty nine female C57BL/6J mice (age 8 weeks) were supplemented *ad libitum* with one of four diets varying in vitamin D (Specialty Feeds, WA, Australia): ZERO (0 vitamin D; n = 10), LOW (1000 IU*kg^{-1}; n = 10), MEDIUM (8000 IU*kg^{-1}; n =10) and HIGH (20,000 IU*kg^{-1}; n = 9). After 4 weeks of supplementation, animals were anaesthetised with pentobarbitone sodium (60 mg*kg 1) and the extensor digitorum longus (EDL) and soleus (SOL) muscles were excised tendon to tendon and their *ex vivo* contractile and fatigue properties evaluated. Muscles were placed in a custom built organ bath containing Kreb-Henseleit Ringers's solution bubbled with carbogen (5% CO_2 in O_2) maintained at a pH of 7.4 at 30^{o}C, and attached to a sensitive force transducer at one end and an immovable pin at the other, flanked by field-stimulating platinum electrodes (Zultek Engineering, VIC, Australia). Muscles were supramaximally stimulated (15V EDL, 12V SOL) with 0.2 msec square wave pulses with a train duration of 350 and 500 msec for the EDL and SOL, respectively. Optimal length of the muscles was established, followed by force development at a range of frequencies (10 – 200Hz) until maximum tetanic force (P_o) was established. Following this, fatigue was examined by stimulating the EDL (100Hz; 1 tetanus every 4 seconds) and SOL (80Hz; 1 tetanus every 2 seconds) to elicit levels of fatigue. A single tetanic stimulation was administered at 1, 2, 5, 10, 15, 20, 30, 45 and 60 min post-fatigue to assess recovery forces. All experiments conformed to the Australian Code of Practice for the Care and use of Animal for Scientific Purposes and were approved by the Victoria University Animal Experimentation Ethics Committee (AEETH 23/11).

Results: Water intake was graduated with the ZERO dose mice drinking the most water ($P < 0.05$), and the HIGH and MEDIUM dose mice, the least water. Compared to the LOW dose group, the HIGH and MEDIUM dose group also ingested more food ($P < 0.05$). However, this made no overall difference to body mass at 12 weeks. No differences in mass due to diet were identified either in muscle mass or when expressed relative to body mass. Significantly higher forces (relative to P_o) were observed in the EDL at 10Hz in the ZERO and HIGH groups compared to LOW ($P < 0.05$). Relative and absolute tetanic forces were not different between the groups, although the SOL muscle of the HIGH dose group tended to have larger forces than the LOW group ($P < 0.07$). While there was no effect on recovery or fatigue observed in the SOL muscle, for the EDL muscle both the Zero and HIGH dose groups exhibited significantly faster recovery from fatigue than the LOW mice ($P < 0.05$) at both 20 and 30 minutes recovery. The HIGH dose group also recovered faster at 10 minutes ($P < 0.05$). Interestingly, compared to the LOW dose group, the ZERO group were also less fatigued after 1 and 2 minutes of stimulation ($P < 0.05$).

Discussion: Altering the vitamin D in the diet appeared to have no effect on the overall growth of the animals (from 8 to 12 weeks) or their skeletal muscle mass. Few effects were observed in the SOL, whereas an apparent slowing of the EDL (resulting in higher forces at a lower frequency) and a higher resistance to fatigue and faster force recovery were observed with the HIGH vitamin D diet. Paradoxically, this effect was also observed when vitamin D was completely removed from the diet (ZERO). Further work to investigate possible calcium handling alterations, fibre-type shifts or mitochondrial adaptations are required, but it is likely that different molecular mechanisms results in similar functional outcomes in the HIGH and ZERO vitamin D diets. Moreover, these results appear to be consistent with observational studies in humans demonstrating a 'U-shaped' curve of vitamin D status with frailty and falls.