## The effect of a yearly dose of vitamin D on skeletal muscle function

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**Introduction:** Supplementation with vitamin D in those with a deficiency has proven successful in increasing bone mineral density, has been reported to reduce the risk of falls, and also appears to improve muscle strength and possibly resistance to fatigue. However, a study by Sanders *et al.* (2010) demonstrated that the number of falls and overall falls risk increased after a single administration of a yearly dose of vitamin D (500,000 IU). In a biochemistry subset, there appeared to be a decline in muscle strength in those with the largest percentage increase in serum 25-hydroxycholecalciferol. Whether this effect is related to a reduction in muscle force or other alterations in muscle function are unknown. Therefore, the aims of this study was to provide a similar "whole of year" dose of vitamin D to mice and investigate any alterations to muscle structure and function.

**Methods:** This study obtained approval from the Victoria University Animal Experimentation Ethics Committee (AEETH 23/11) and all experiments conformed to the Australian Code of Practice for the Care and use of Animal for Scientific Purposes. Twelve female C57BL/6J mice (age 9 weeks) were given a single intraperitoneal injection of 2,000 IU vitamin D in corn oil (YEAR) with another group injected with the vehicle as control (CON; $n$ = 7). Three weeks after injection, animals were anaesthetised with pentobarbitone sodium (60 mg.kg$^{1}$) and the extensor digitorum longus (EDL) and soleus (SOL) muscles were excised and their *ex vivo* contractile and fatigue properties evaluated in a custom built organ bath. Muscles were bathed in Kreb-Henseleit Ringer's solution maintained at a pH of 7.4 and bubbled with carbogen (5% $CO_2$ in $O_2$). One end of each muscle was attached to a sensitive force transducer whilst the other end was attached to an immovable pin, which was flanked by field-stimulation platinum electrodes (Zultek Engineering, Vic, Australia). Muscles received supramaximal stimulations of 15 V for the EDL and 12V for the SOL. Square wave pulses were 0.2 ms with a train duration of 350 and 500 ms for the EDL and SOL, respectively. Prior to conducting muscle function tests, the optimal length of each individual muscle was determined which was followed by force development at an increasing range of frequencies (10 Hz-200 Hz) until the maximal tetanic force ($P_o$) of each muscle was determined. Once $P_o$ had been established, muscles were fatigued by stimulation of the EDL at 100 Hz administered every 4 seconds whilst the SOL received a single 80 Hz tetanic stimulation every 2 seconds. Following the 3 minute fatigue run, muscles were followed during recovery phase for 60 minutes during which occasional single tetanic stimulations were administered.

**Results:** The major finding of the study was that the absolute force ($P_o$) produced from the EDL of YEAR mice was significantly lower ($P$<0.05) than the forces observed in the controls. There were no differences between groups in muscle mass or cross sectional area. The specific force ($sP_o$) of the EDL in YEAR mice tended to be reduced ($P$ <0.06) as was absolute force relative to muscle mass ($P_o$/MM) ($P$ <0.07). No differences in SOL muscle mass or force was observed between groups. Similarly, EDL and SOL both fatigued comparably in both groups. Recovery was unaffected by vitamin D in the EDL muscle, however early recovery in the SOL was impaired, with a significantly lower recovery observed at 2 minutes ($P$ < 0.05), with similar trends at 1 and 5 minutes ($P$ < 0.08).

**Discussion:** Mice that received a single yearly dose of vitamin D, compared to controls, did not differ in growth or mass of the EDL or SOL muscles. However, there does appear to be a negative impact upon fast-twitch skeletal muscle contraction. It was found that when mice were administered a single 2000 IU dose of vitamin D, the absolute force producing capabilities of the EDL were significantly reduced. This could indicate a possible shift towards slow twitch fibre characteristics, however this is not supported by the fatigue profile in the EDL, thus other possible mechanisms need to be investigated. This result could explain, in part, Sanders *et al.* (2010) finding that a single yearly administration of vitamin D in the elderly increased the number of apparent falls. Further research will be required to determine the exact mechanisms by which vitamin D exerts its effect on skeletal muscle, as well as consideration of the best supplementation regimes to address vitamin D deficiency without producing deleterious effects.

Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D & Nicolson GC (2010) *Journal of the American Medical Association* **303**, 1815-22.