The effects of vitamin D supplementation and exercise enrichment on *in vivo* analysis of physical activity behaviour, exercise capacity and metabolism

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Introduction: Vitamin D (VitD) plays a major role in maintaining muscle integrity and function. While supplementation with VitD in deficient individuals is beneficial to muscle strength and fatigue, whether increasing VitD above normal levels has any additive benefits on muscle function is unknown. The aims of this study were to investigate the effect of VitD supplementation on body composition, voluntary running performance, and muscle contractile function and fatigue in sedentary and physically active mice.

Methods: 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, 8 week old (n=48) C57BL/10 were provided *ad libitum* access to a standard chow diet (containing 1500 IU.kg⁻¹ cholecalciferol) or the same diet supplemented with VitD (containing 20000 IU.kg⁻¹ cholecalciferol; VITD). To investigate the effects of VitD supplementation in combination with exercise, each dietary group was further separated into sedentary (SED) and exercise-enriched (voluntary access to running wheel; EXER) intervention groups for 8 weeks. After the VitD and exercise enrichment period, *in vivo* body composition (EchoMRI) and 24h metabolic analysis (Promethion metabolic cage system) was conducted.

Results: There was no effect of VitD on running distance during 8-weeks of exercise enrichment, however, EXER_VITD mice ran faster (P < 0.05). Exercise enriched mice had significantly higher lean mass and lower fat mass compared to sedentary animals (P < 0.0001). No differences in running performance were observed during 24h of metabolic screening between the exercise-enriched groups; however they did average faster speeds and longer distances than the sedentary mice (P < 0.0001). Interestingly, VitD decreased non-wheel activity distances in both sedentary and exercise-enriched groups (P < 0.05). There were no effects of VitD on substrate utilisation and energy expenditure during different activity levels; however the exercise-enriched mice demonstrated a greater shift towards carbohydrate metabolism compared to the sedentary mice (P < 0.0001).

Conclusions: In this setting, there were no differences observed in voluntary running wheel behaviour between mice with and without prior wheel exposure. However, whilst VitD did not affect exercise capacities within groups, the exercise-enriched mice were more efficient on the wheel running faster average speeds and covering further distances during 24h of screening. Interestingly, increasing VitD above normal decreased general 'non-wheel' activity measures with and without exercise training. This suggests that VitD may play a role in modulating pathways responsible for explorative/recovery/lounging behaviours regardless of exercise status.