Effects of Vitamin D Supplementation on Muscular and Cardiorespiratory Adaptations to Endurance Training in Mice

[1]Zachary Conroy, [2]McKenzie Temperly, and [2]Kimberly Huey (Mentor)

Drake University College of Arts & Sciences[1], College of Pharmacy & Health Sciences[2]

BACKGROUND AND HYPOTHESIS

- Reductions in muscle strength and cardiorespiratory fitness have been associated with microgravity environments (Cotter et al., 2015).
- Astronauts have an increased likelihood of a vitamin D deficiency due to inadequate sunlight and diet changes, exacerbating adverse effects of microgravity (Carswell et al., 2018).
- Recent evidence has suggested vitamin D stimulates muscle growth and supports optimal muscle function by regulating growth hormones, such as IGF-1 and VEGF (Dzik & Kaczor, 2019).
- Vitamin D has been proposed to improve cardiorespiratory fitness (Bartoszewska, Kamboj, & Patel, 2010).
  - Vitamin D levels have been positively associated with endurance exercise performance (Carswell et al., 2018).
  - Vitamin D supplementation has been shown to improve physical activity rates in deficient individuals (Karefylakis et al., 2018).

***Primary* Hypothesis: Vitamin D supplementation will positively impact the ability of the cardiorespiratory and muscular systems to effectively respond to endurance training as evidenced by increased running wheel distance, maximal treadmill test time, and muscle endurance and strength.**

METHODS

- Adult (3-4 months old), male C57 mice were divided into the following groups (n = 2-6/group):

Treatment	Activity
Placebo	Sedentary
	Running Wheel (RW)
Vitamin D (0.5 µg/1 kg body weight)	Sedentary
	Running Wheel (RW)

- Maximal exercise tests were completed before and after 14 days of RW access or normal cage activity in sedentary groups.
  - Following a 5 min. warm-up, mice ran at 12 m/min and 5% grade and speed was increased 2 m/min every 2 min. up to 18 m/min. At 22 min. speed was increased 2 m/min every 2 min. until volitional fatigue.
- Mice had unlimited running wheel (RW) access or remained sedentary for 14 days.
- Mice received daily vitamin D (0.5 µg/kg body weight) or placebo (saline) for 14 days.
- After 14 days, maximal isometric plantarflexor force and fatigue were measured in anesthetized mice with a dual-mode footplate system.
  - 10 contractions were evoked every 5 seconds by sciatic nerve stimulation.
  - Fatigue was calculated as the decline in force over 10 contractions relative to maximal force.
- IGF-1, IL-6, and VEGF levels were measured in muscle homogenates with ELISA assays.

MUSCLE MASS

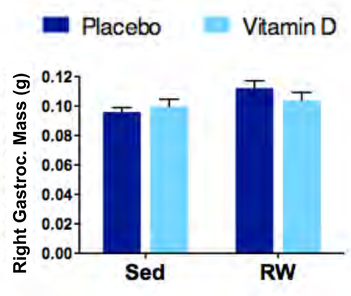

Figure 1. Vitamin D did not increase muscle mass independent of activity level. Average right gastrocnemius mass (g) for Sedentary and RW mice. Means ± SE.

MAXIMAL EXERCISE TEST

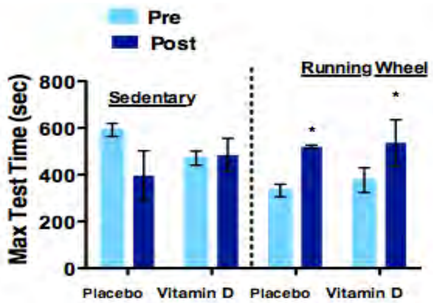

Figure 2. Running wheel access increased max exercise test time independent of treatment. Maximal test times (sec) before and after 14 days of RW access or normal cage activity (Sedentary). *Significant change from pre to post-times ($p<0.05$). Means ± SE.

RUNNING WHEEL DISTANCE

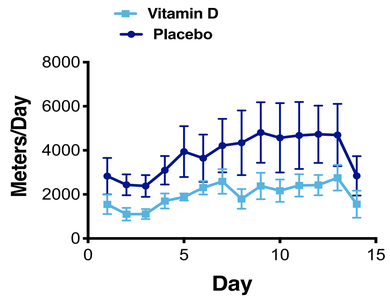

Figure 3. Vitamin D decreased RW activity in mice. RW activity (meters/day) was recorded for 14 days. Significant difference observed ($p<0.05$). Means ± SE.

MAXIMAL ISOMETRIC FORCE & FATIGUE

Figure 4. Vitamin D and/or activity level did not increase maximal isometric force or fatigue resistance. Maximal isometric plantarflexor force normalized to body mass (BM) and fatigue resistance after 14 days of normal cage activity (Sedentary) or RW access in mice receiving daily Vitamin D or placebo. Fatigue was calculated as % max force after 10th contraction. Means ± SE.

KEY FINDINGS

- **Vitamin D did not positively impact the ability of the cardiorespiratory system to effectively respond to endurance training, as vitamin D decreased RW activity, and increases in max exercise test time were not enhanced with vitamin D supplementation.**
- **Vitamin D supplementation was associated with reductions in IL-6 levels independent of treatment and reductions in VEGF levels in sedentary mice. Running wheel activity was also associated with reductions in VEGF level in placebo mice.**
- **Neither vitamin D supplementation nor endurance training impacted muscle mass, maximal isometric force, or fatigue resistance.**
  - No differences between any groups, suggesting that neither aerobic exercise nor vitamin D supplementation affected muscle function.
- **Physiological Significance: Vitamin D did not improve the ability of skeletal muscle or cardiorespiratory system to positively respond to endurance training.** Observed differences between sedentary and RW groups were independent of treatment indicating vitamin D supplementation did not enhance cardiorespiratory function.
  - Provides a basis for future studies in humans to assess various types of exercise and dietary supplementation that will provide the most effective therapy for astronauts prior, during, and post-mission to prevent muscular atrophy and deficiencies in cardiorespiratory function.

MUSCLE IGF-1, IL-6, & VEGF

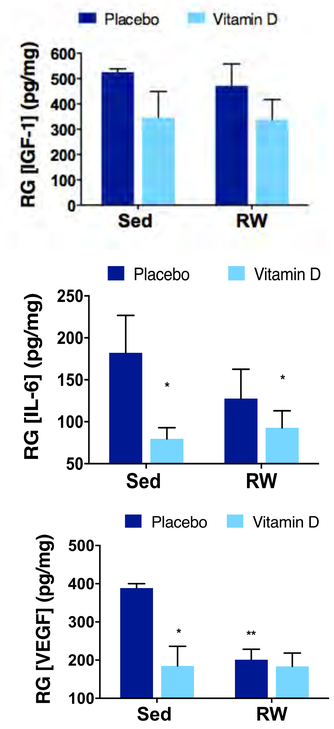

Figure 5. Vitamin D decreased muscle IL-6 independent of activity level and muscle VEGF in sedentary mice. Average IGF-1, IL-6, and VEGF levels (pg/mg) for sedentary and RW mice in the right gastrocnemius. *Significantly different than corresponding placebo ($p<0.05$). **Significantly different than corresponding sedentary ($p<0.05$) Means ± SE.

References

Bartoszewska, M., Kamboj, M., & Patel, D. R. (2010). Vitamin D, muscle function, and exercise performance. Pediatric Clinics, 57(3), 849-861. doi:10.1016/j.pcl.2010.03.008

Carswell, A. T., Oliver, S. J., Wentz, L. M., Kashi, D. S., Roberts, R., Tang, J. C., ... & Fraser, W. D. (2018). Influence of vitamin D supplementation by sunlight or oral D3 on exercise performance. Medicine and Science in Sports and Exercise, 50(12), 2555-2564. 10.1249/MSS.0000000000001721

Cotter, J. A., Yu, A., Haddad, F., Kreitenberg, A., Baker, M. J., Tesch, P. A., ... Adams, G. R. (2015). Concurrent exercise on a gravity-independent device during simulated microgravity. Medicine and Science in Sports and Exercise, 47(5), 990–1000. doi:10.1249/MSS.0000000000000483

Dzik, K. P., & Kaczor, J. J. (2019). Mechanisms of vitamin D on skeletal muscle function: oxidative stress, energy metabolism and anabolic state. European Journal of Applied Physiology, 119(4), 825–839. https://doi.org/10.1007/s00421-019-04104-x

Karefylakis, C., Särnblad, S., Ariander, A., Ehlersson, G., Rask, E., & Rask, P. (2018). Effect of vitamin D supplementation on body composition and cardiorespiratory fitness in overweight men — A randomized controlled trial. *Endocrine*, 61(3), 388-397. https://doi.org/10.1007/s12020-018-1665-6

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