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

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BACKGROUND AND HYPOTHESIS

- Microgravity environments are associated with reductions in muscle strength and cardiorespiratory fitness (Cotter et al., 2015).
- These adverse effects are further exacerbated because astronauts are more likely to be vitamin D deficient due to inadequate sunlight and dietary changes (Carswell et al., 2018).
- Recent evidence suggests vitamin D stimulates muscle growth, supports optimal muscle function by regulating growth hormones, such as insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), and improves cardiorespiratory fitness (Dzik & Kaczor, 2019; Bartoszewska, Kamboj, & Patel, 2010).
- To promote overall muscle health and cardiorespiratory fitness, individuals typically perform both strength and aerobic exercise (i.e. concurrent training) (Carrithers et al., 2007).
- It is unknown as to whether vitamin D's benefits enhance adaptation to concurrent exercise compared to strength training alone.
  - The only current evidence suggests a potential to increase positive impacts of vitamin D supplementation with concurrent training (Arazi, Samadpour, & Eghbali, 2018).

***Primary* Hypothesis: Vitamin D supplementation will positively impact the ability of skeletal muscle and the cardiorespiratory system to effectively respond to concurrent training evidenced by improved contractile function, cardiorespiratory fitness and growth factor levels compared to placebo.**

METHODS

- Adult (3-4 months old), male C57 mice were divided into the following groups (n = 4-8/group):

Treatment	Activity
Placebo	Sham
	Strength (FO)
	Concurrent (FO & RW)
Vitamin D (0.5 µg/1 kg body weight)	Sham
	Strength (FO)
	Concurrent (FO & RW)

- Functional overload (FO) involves surgical removal of the soleus and gastrocnemius muscles to bilaterally overload the plantaris and mimic strength training.
- Maximal exercise tests were completed before and after 14 days of TM training 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.
- Overload mice were exposed to unlimited running wheel (RW) access or remained sedentary.
- Mice received daily vitamin D (0.5 µg/kg body weight) or placebo (saline) for 14 days following surgery.
- 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, FGF, and VEGF levels were measured in muscle homogenates with ELISA assays

PLANTARIS MASS

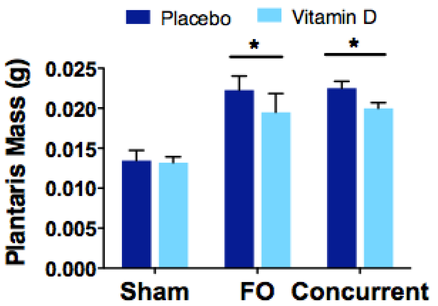


Figure 1. Functional overload (FO) & concurrent training are associated with significant muscle hypertrophy independent of vitamin D. Average plantaris mass (g) for sham, FO, and concurrent mice. *Significantly different from sham ($p<0.05$). Means ± SE.

MAXIMAL EXERCISE TEST

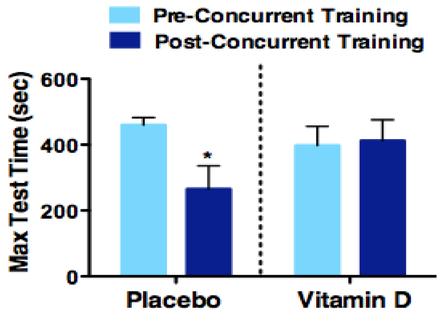


Figure 2. Concurrent training decreased exercise test time in the placebo group. Maximal test times before and after 14 days of concurrent training. *Significant change from pre to post-times ($p<0.05$). Means ± SE.

RUNNING WHEEL DISTANCE

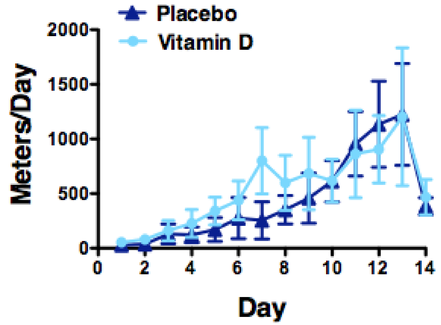
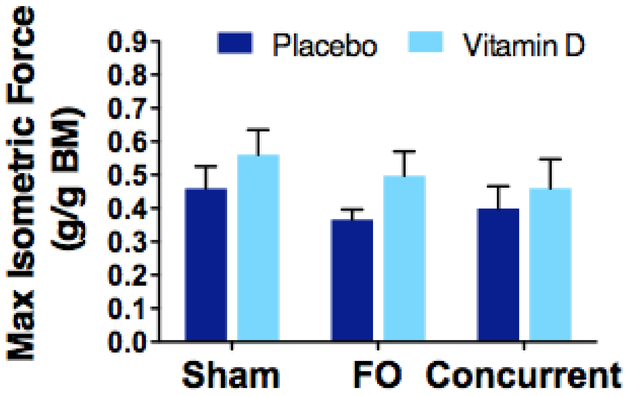
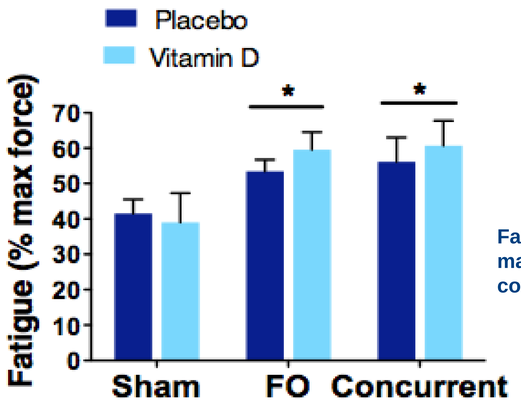


Figure 3. Vitamin D did not increase RW activity in concurrent mice. RW activity (meters/day) was recorded for 14 days. Means ± SE.

MAXIMAL ISOMETRIC FORCE & FATIGUE

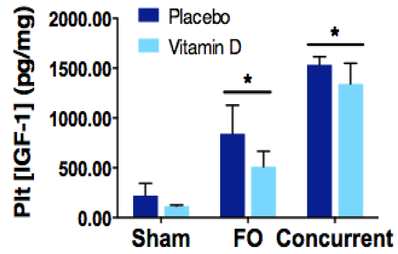


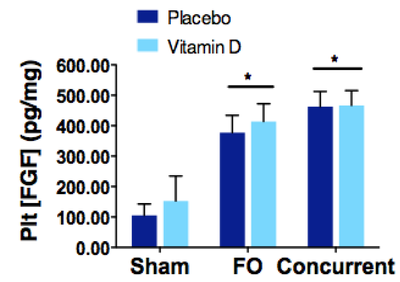


Fatigue : % max @ 10th contraction

Figure 4. Fatigue resistance was increased with FO and concurrent training independent of treatment. Maximal isometric plantarflexor force normalized to body mass (BM) and fatigue resistance after 14 days of normal cage activity (sedentary), strength, or concurrent training in mice receiving daily vitamin D or placebo. Fatigue was calculated as % max force after 10th repetition in sham, FO, and concurrent mice. *Significantly different than corresponding placebo ($p<0.05$). Means ± SE.

MUSCLE IGF-1, FGF, & VEGF





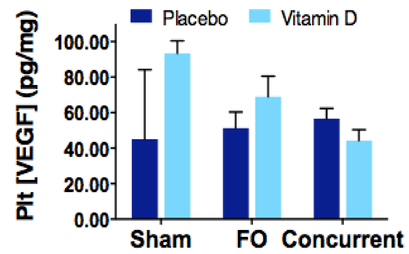


Figure 5. FO and concurrent training increases IGF-1 and FGF in the overloaded plantaris (Plt) independent of treatment. Average IGF-1, FGF, and VEGF levels (pg/mg) for sham, FO, and concurrent mice in the plantaris. *Significantly different than corresponding sham ($p<0.05$). Means ± SE.

KEY FINDINGS

- **Vitamin D positively impacted the ability of the cardiorespiratory system to respond to concurrent training based on pre and post-maximal test performance compared to placebo.**
  - Test time decreased pre to post with placebo, but was maintained with vitamin D despite similar daily running distances.
- **Significant muscle hypertrophy and increased plantaris IGF-1 and FGF levels were observed in both strength (FO) and concurrent training groups, but these adaptations were not enhanced with vitamin D supplementation.**
  - Increased IGF-1 in overloaded muscle demonstrates the direct effects of FO without systemic effects of vitamin D, growth hormones, or training type.
- **The primary contractile adaptation to FO and concurrent training was a significant increase in muscle fatigue resistance independent of vitamin D supplementation.**
  - No differences between FO and concurrent fatigue resistance suggesting the addition of aerobic exercise had minimal effect.
- **Physiological Significance: Vitamin D improved the ability of the cardiorespiratory system to positively respond to concurrent training while vitamin D in combination with FO did not significantly improve the ability of skeletal muscle to respond to a growth stimulus.**
  - No differences between strength and concurrent training in muscle mass and contractile function suggests the addition of aerobic exercise did not impair muscle hypertrophy or strength.
  - Provides a basis for future studies in humans to assess other types of dietary supplementation and exercise protocols that will provide the most effective therapy for astronauts prior, during, and post-mission to prevent muscular atrophy.

Research is supported by the NASA Iowa Space Grant under Award No. NNX16AL88H.

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