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Weekly Vitamin D₃ Supplementation Improves Aerobic Performance in Combat Sport Athletes

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Running title

Vitamin D₃ supplementation improves aerobic performance combat sports

Vitamin D₃ supplementation can affect the strength and power of an athlete, however the effect on endurance performance remains unclear. Twenty-seven recreational male combat athletes with at least 12 months experience within combat sports were recruited (age: 24±4 years, stature: 176±6 cm, weight: 77±14 kg). Participants completed baseline testing for blood haemoglobin and haematocrit, upper and lower body VO_{2peak} and upper and lower body Wingate. Following testing participants were stratified to 50000IU (D1), 80000IU (D2) or 110000IU (D3) of vitamin D₃ per week. They then completed a 6-week placebo period followed by a 6-week supplementation period. Retesting was carried out after the placebo and supplementation period. There was a significant effect for time for haemoglobin and haematocrit, upper and lower body VO_{2peak} and upper body Wingate power ($p < 0.01$) but no effect for dose of vitamin D given. Performance data was normalised to vitamin D intake and

there was a moderate effect size between D1 and D2 for lower body VO_{2peak} ($d=0.6$), upper body VO_{2peak} ($d=0.13$) and upper body average power ($d=0.75$), with a large effect size between D1 and D2 for haemoglobin ($d=1.19$), haematocrit ($d=0.93$) and upper body peak power ($d=0.95$). There was a large effect size for D1 compared to D3 for all variables ($d>0.8$). Therefore, there is no additional benefit to increasing dose above 500000IU vitamin D per week. Given the endurance adaptations from vitamin D supplementation and the importance of endurance for combat performance, recreational combat athletes should supplement at 50000IU per week for six weeks.

Keywords:

VO_{2peak} , cholecalciferol, vitamin D₃, combat sports, aerobic performance

Introduction

Vitamin D is a secosteroid hormone which affects calcium metabolism and bone health (Laird et al., 2010). Vitamin D receptors (VDRs) have been found in almost all human nucleated cells (Owens, Allison and Close., 2018). Calcitriol is the biologically active form of vitamin D and binds to VDRs, causing the expression of over 900 gene variants (Wang et al, 2005), some of which may elicit an athletic performance benefit (Dahlquist, Dieter and Koehle., 2016). Despite this, approximately 15% of the worldwide population has vitamin D inadequacy (Pfothenaur and Shubrook., 2017). Vitamin D inadequacy may result in declining training quality along with increased incidence of injury and illness (Hamilton., 2011).

Vitamin D supplementation has been shown to increase the force and power output of skeletal muscle (Ogan and Pritchett., 2013). This is possibly due to an increase in the sensitivity of calcium binding sites on the sarcoplasmic reticulum, leading to improved crossbridge cycling and muscle contraction (Ainbinder et al., 2015). Supplementation of 2000IU and 5000IU.day⁻¹ of vitamin D₃ improved muscle strength in middle aged patients

with vitamin D inadequacy who were otherwise healthy (Diamond, Wong and Golombick., 2013). Likewise, a daily dose of 5000IU vitamin D₃ has also been shown to improve 10m sprint times and vertical jump performance in professional soccer players compared to placebo (Close et al., 2013). A single bolus of 150000IU of vitamin D₃ improved hamstring and quadriceps concentric contractile ability 8 days after supplementation in judoka athletes (Wyon et al., 2016).

Although there is limited literature exploring the relationship between vitamin D supplementation and aerobic performance, recently Todd et al (2017) administered 3000IU.day⁻¹ of vitamin D₃ for 12 weeks to male and female Gaelic football players. Despite serum vitamin D levels rising in those supplementing with vitamin D₃, there was no effect on VO_{2max} or other performance variables. Jastrzebska et al (2018) supplemented 36 well trained soccer players with 5000IU.day⁻¹ of vitamin D₃ or a placebo solution while undertaking a high intensity interval (HIIT) training protocol for 8 weeks. The vitamin D group improved VO_{2max} by 20% while the placebo group improved VO_{2max} by 13%. It was suggested that vitamin D₃ supplementation elicits a significant, but moderate effect on aerobic performance in well trained soccer players. To the authors best knowledge no studies exist which explore the influence of vitamin D₃ supplementation upon the aerobic performance of combat sport athletes. Aerobic performance is one of the key elements of fitness required for successful combat sport performance yet is sparsely researched (Barley et al, 2019). Combat sports athletes are required to perform repetitive high intensity actions such as those seen during striking exchanges (Chaabene et al., 2015). In order to sustain the high physiological demands of competition, a well-developed aerobic fitness level is required (Chaabene et al., 2015).

Vitamin D inadequacy is especially prevalent amongst combat sports athletes in northern latitudes such as the United Kingdom and becomes more frequent and severe during the winter months (Magee et al., 2013). This is due to limited sun exposure from training indoors, coupled with the northern latitude reducing the effect of the sun to stimulate

exogenous vitamin D production (Ogan and Pritchett., 2013). Weight cutting practices are commonplace in combat sports at all levels with 60-80% of athletes reporting engagement in weight cutting (Barley, Chapman and Abbiss, 2019). Techniques employed include exercising in heavy clothing and dietary restriction (Barley, Chapman and Abbiss, 2019). Exercising in heavy clothing will limit exposure to the sun, lowering exogenous vitamin D production (Ogan and Pritchett, 2013).

The potential mechanisms for possible improvements in aerobic performance remain unclear. It is possible that the CYP enzymes involved in the activation of vitamin D to the biologically active 1,25-dihydroxyvitamin D₃ contain heme proteins which may affect the affinity of oxygen to bind to haemoglobin, improving oxygen transport and aerobic performance (Sugimoto and Shiro, 2012). Vitamin D may also play a role in the iron regulation of the body, promoting more iron to be biologically available for haemoglobin production (Bacchetta et al, 2014). Vitamin D also may improve mitochondria function in deficient adults (Sinha et al, 2013). This implies that potential changes in aerobic performance may be attributed to improvements in cellular respiration and improved oxygen carrying capacity.

Therefore, the purpose of this study was to evaluate if supraphysiological doses of vitamin D₃ can improve aerobic performance in male combat sports athletes in conjunction with their normal training and if so, which of the evaluated doses would be most effective. It is hypothesised that a larger dose of vitamin D will evoke greater aerobic performance benefits.

Methodology

Participants:

27 male recreational combat sport athletes who were not actively competing (MMA n=11; Brazilian Jiu-jitsu (BJJ) n=10; boxing n=5) were recruited with a minimum 1 year combat sports training who trained twice per week in their sport (mean±SD; age: 24±4 years,

stature: 176 ± 6 cm, weight: 77 ± 14 kg). Participants were excluded if they had any injury over the past six months, were lactose intolerant or recently holidayed in climates promoting high endogenous vitamin D production. Participants were informed of the study both verbally and in writing prior to giving informed consent. The study was approved by Abertay University ethics committee and completed in accordance with the Declaration of Helsinki.

Study Protocol:

A single-blind cross-over design was used with a placebo period, followed by a vitamin D intervention. Participants were blinded to supplementation throughout the protocol with the placebo period being administered first to negate the necessity of a washout period. This was done as a single dose of vitamin D may improve vitamin D status by as much as three months post-supplementation (Kearns, Alvarez and Tangpricha, 2014). Testing and supplementation was completed over the winter months (October-April) when exogenous vitamin D production would be negligible at a latitude of 57°N due to limited UVB radiation exposure (Wacker and Holick, 2013). Participants completed a familiarisation session prior to testing commencement. Participants refrained from consuming caffeine, alcohol or engaging in strenuous exercise for 24 hours and fasted for 4 hours prior to testing. Participants verbally confirmed they completed their own training weekly and did not undertake any new training stimuli.

Testing Session 1: Stature was measured using a stadiometer (SECA) and weight with bioimpedance scales (Tanita MC-780, Tokyo, Japan). To measure haematocrit and haemoglobin levels a fingerprick blood sample was obtained which was placed into a haematocrit analyser to give a reading of haematocrit and haemoglobin (Hemo Control, EKF Diagnostics, Cardiff United Kingdom).

An incremental cycling test (Monark Ergomedic 894, Vansboro, Sweden) was used to assess lower body (LB) $\text{VO}_{2\text{peak}}$ via breath by breath gas collection system (Metalyzer®3B gas analyser, Cortex, Leipzig, Germany). Heart rate (Polar Electro, Kempele, Finland) was

recorded continuously through-out the test. After a two minute rest participants cycled at 60RPM against a resistance of 1kg, with 0.5kg resistance added every three minutes until volitional exhaustion or they were unable to maintain 60RPM. VO_{2peak} was taken as the highest 10 second average and time to exhaustion (TTE) taken as the total time spent cycling.

Testing Session 2: Participants completed an incremental upper body (UB) VO_{2peak} test while connected to the breath by breath analyser (Metalyzer®3B gas analy-ser, Cortex, Leipzig, Germany). Participants knelt in front of the arm ergometer with the heels of their feet remaining in contact with their buttocks throughout the test. Heart rate was recorded continuously throughout (Polar Electro, Kempele, Finland). After a two minute rest participants cycled at 60RPM against a resistance of 1kg, with 0.2kg resistance added every three minutes until volitional exhaustion or inability to maintain 60RPM. VO_{2peak} was taken as the highest 10 second average and TTE was the total time spent cycling.

Testing Session 3: Utilising the LB ergometer, participants sprinted maximally for 30 seconds against a resistance 7.5% of their bodyweight while remaining seated. Resistance was applied once 120RPM was reached. Peak power (PP) and average power (AP) were recorded.

After a 10 minute rest, participants knelt in front of the arm ergometer as previously described and completed a 30s sprint against 5% bodyweight. Resistance was applied from the start. PP and AP were recorded. Strong verbal encouragement was provided throughout both tests.

Testing was carried out at baseline, after 6 weeks of placebo supplementation and after 6 weeks of vitamin D supplementation. Group allocation was randomised, stratified to LB

VO_{2peak} .

Supplementation Protocol: All participants were blinded to supplementation and first completed a six week placebo period, reporting to the laboratory once a week to consume three capsules totalling 300mg of maltodextrin with 300ml of Jersey full fat milk (Graham's

Gold Smooth, United Kingdom) and provided a three day food diary. After six weeks the participants then consumed either 50000IU's (D1) (n=9), 80000IU's (D2) (n=9) or 110000IU's (D3) (n=9) of vitamin D₃ with 300ml of Jersey full fat milk once a week for six weeks and continued to provide a food diary each week.

Statistical Analysis: All data are presented as mean±SD. Statistical analysis was completed using jamovi 1.0.0.0 with significance set at $P<0.05$. All performance tests and body composition were analysed with a 3X3 ANOVA with LSD post hoc analysis. Average nutritional intake across the placebo and supplement periods were evaluated using Diet Plan 7.0 with a 3X2 ANOVA with LSD post hoc analysis. Smallest worthwhile change was undertaken as outlined by Swinton et al (2018) with the change in performance normalised to total vitamin D₃ supplemented throughout the study. Cohens D effect size was calculated for each smallest worthwhile change to determine the most effective dose with effect size defined as; $d = 0.2-0.49$ representing a small effect size, $d = 0.5 -0.79$ representing a medium effect size and $d > 0.8$ representing a large effect size.

Results

VO_{2peak}: There was no significant group x time effect for LB ($P=0.292$; Table 1) or UB VO_{2peak} ($P=0.794$;Table 1) but there was a significant time effect for LB and UB VO_{2peak} ($P<0.001$; Table 1).

TTE: There was no significant group x time effect for LB ($P=0.576$) or UB TTE ($P=0.538$; Table 1) with no time effect for LB TTE ($P=0.164$; Table 1) and a significant time effect for UB TTE ($P<0.001$; Table 1).

Haemoglobin and Haematocrit: There was no significant group x time effect for either haemoglobin ($P=0.471$; Table1) or haematocrit ($P=0.648$; Table 1) but there was a significant time effect for both ($P<0.001$; Table 1). Haemoglobin and haematocrit was unchanged across the placebo period but both increased post-intervention by approximately 5-8% (Table 1).

Wingate Performance: There was no significant group x time effect for lower or upper body PP ($P=0.315$; $P=0.302$; Table 1) and AP ($P=0.454$; $P=0.726$; Table 1) but there was a significant time effect for LB PP ($P=0.004$; Table 1) and UB PP ($P<0.001$; Table 1) and AP ($P<0.001$; Table 1).

Dietary Intake: There was no significant group x time effect ($P=0.824$; Table 1) for total energy intake but there was an effect of time ($P=0.005$; Table 1). Calcium intake was higher across all groups during the intervention period ($P<0.001$; Table 1). Zinc and magnesium intake increased during the intervention period for D1 and D2 (Table 1). Vitamin D intake was similar for each group across the placebo period but increased significantly with intervention ($P<0.001$; Table 1).

VO_{2peak} smallest worthwhile change: The smallest worthwhile change for LB VO_{2peak} when normalised to total mg vitamin D₃ supplemented over the study protocol was $0.086 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{mgVitD}^{-1}$ with a moderate effect size for LB VO_{2peak} between D1 and D2 with a large effect size between the D1 and D3. ($d=0.60$ D1 v D2; $d=0.97$ D1 v D3; $d=0.23$ D2 v D3; Figure 1A). The smallest worthwhile change for UB VO_{2peak} was $0.1 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{mgVitD}^{-1}$ ($d=0.13$ D1 v D2; $d=0.5$ D1 v D3; $d=0.7$ D2 v D3; Figure 1B).

Time to exhaustion: The smallest worthwhile change for lower body TTE was $3.2 \text{ s}\cdot\text{mgVitD}^{-1}$ and for upper body TTE was $2.2 \text{ s}\cdot\text{mgVitD}^{-1}$. There was a small effect size for LB TTE between D1 and D3 ($d=0.06$ D1 v D2; $d=0.25$ D1 v D3; $d=0.20$ D2 v D3; Figure 1C). UB TTE saw a large effect size for both D1 and D2 against D3 ($d=0.16$ D1 v D2; $d=0.86$ D1 v D3; $d=1.06$ D2 v D3; Figure 1D).

Haemoglobin and haematocrit: The smallest worthwhile change for haemoglobin was $0.01 \text{ mmol}\cdot\text{l}^{-1}\cdot\text{mgVitD}^{-1}$ with a large effect size between D1 against both D2 and D3 ($d=1.19$ D1 v D2; $d=1.32$ D1 v D3; $d=0.15$ D2 v D3; Figure 1E). The smallest worthwhile change for

haematocrit was 0.05 %. mgVitD^{-1} . There was a large effect size between D1 and both the D2 and D3 groups ($d=0.93$ D1 v D2; $d=1.31$ D1 v D3; $d=0.46$ D2 v D3; Figure 1F).

Wingate peak power: The smallest worthwhile change for LB PP was $0.02 \text{ W}\cdot\text{kg}^{-1}\cdot\text{mgVitD}^{-1}$ with a small effect size between D1 and D3 seen ($d=0.03$ D1 v D2; $d=0.26$ D1 v D3; $d=0.36$ D2 v D3; Figure 2A). Smallest worthwhile change for UB PP was $0.01 \text{ W}\cdot\text{kg}^{-1}\cdot\text{mgVitD}^{-1}$ with a large effect size between D1 against D2 and D3 ($d=0.95$ D1 v D2; $d=0.83$ D1 v D3; $d=0.40$ D2 v D3; Figure 2B).

Wingate average power: The smallest worthwhile change for LB AP was $0.01 \text{ W}\cdot\text{kg}^{-1}\cdot\text{mgVitD}^{-1}$ with a small effect size for LB AP between D1 and D2 with a moderate effect size between D1 and D3 ($d=0.41$ D1 v D2; $d=0.58$ D1 v D3; $d=0.22$ D2 v D3; Figure 2C). The smallest worthwhile change for UB AP was $0.02 \text{ W}\cdot\text{kg}^{-1}\cdot\text{mgVitD}^{-1}$ with a moderate effect size between D1 and both D2 and D3 ($d=0.75$ D1 v D2; $d=0.71$ D1 v D3; $d=0.19$ D2 v D3; Figure 2D).

Discussion

The major findings of this study are that supplementation with vitamin D₃ improves LB and UB $\text{VO}_{2\text{peak}}$ with a concurrent improvement in haemoglobin concentrations and haematocrit. A small increase in LB PP was seen with D2 and all groups improved UB PP and AP (Table 1). Findings were consistent, regardless of dose. This suggests that there is no additional benefit to vitamin D supplementation above 50000IU's per week. In light of the improvements in aerobic performance, combat athletes should seek to supplement their dietary intake of vitamin D₃.

Aerobic Performance: Following 6 weeks of supplementation both LB and UB $\text{VO}_{2\text{peak}}$ were increased compared to the post-placebo period (Table 1). However, only D1 increased both LB and UB $\text{VO}_{2\text{peak}}$ compared to the pre-placebo time point (Table 1). The magnitude of change was greater than the smallest worthwhile change in all groups (Figure 1A & B), with a moderate to large effect in lower body $\text{VO}_{2\text{peak}}$ for D1 compared to the other two groups. In

D1 we see a 14-16% increase in both LB and UB VO_{2peak} and an 11-16% increase in UB in the other groups. The size of adaptation in lower body VO_{2peak} is similar to that reported in rowers following 8 weeks of supplementation at $42000IU \cdot week^{-1}$ when combined with high intensity training (Jastrzebski., 2014.) but smaller than those reported in soccer players who ingested $5000IU \cdot day^{-1}$ (Jastrzebska et al., 2018). However, it should be noted that Jastrzebski (2014) and Jastrzebska et al (2018) both reported increases in VO_{2max} and not VO_{2peak} which is an important distinction to establish between this current study and their previous work as the two terms are not interchangeable. Nevertheless, it is suggested that vitamin D_3 intake may positively benefit aerobic performance in athletes. Improvements in VO_{2peak} can come from either peripheral changes or changes in oxygen delivery (Daussin et al., 2007). Adrestani et al (2011) suggested that vitamin D_3 inadequacy may lower cardiac output and increase peripheral arterial resistance, decreasing aerobic performance. In clinical populations vitamin D_3 has been suggested to have a role in erythropoiesis (Zughaire et al., 2014). In the current study we report an increase in both haemoglobin and haematocrit regardless of dose of vitamin D (Table 1, Figure 1). When supplemented at a lower dose ($24000 IU \cdot week^{-1}$) no change in haemoglobin and a small increase in haematocrit have been reported (Mielgo-Ayuso et al., 2018) As such, it is possible that there needs to be a large excess of vitamin D_3 to switch on erythropoiesis. The improvement in VO_{2peak} may reflect, to some extent, the greater oxygen capacity associated with increased red blood cell mass. Increases in VO_{2max} of 7% has been seen in well trained men who experienced increases in haemoglobin and haematocrit via erythropoietin injection (Durussel et al., 2013).

Wingate Performance: Six weeks of vitamin D_3 supplementation saw an 8% improvement in LB PP in D2. Improvements in UB PP by 4-13% and AP by 5-12% from post-placebo testing were seen across all groups (Table 1). The magnitude of change was highest in D1 for all measures (Figure 2). The change in LB AP was less than the smallest worthwhile change for D3 (Figure 2C) and the change in UP AP was less than the smallest worthwhile change for D2 (Figure 2D). A small effect was seen for LB PP which corroborates the work

of Fitzgerald et al (2015), who demonstrated that a small correlation exists between vitamin D status and LB PP. For the first time we observed positive improvements in UB Wingate performance with vitamin D₃ supplementation. It has been suggested that vitamin D₃ insufficiency leads to an atrophy of type II fibres but not type I fibres (Hamilton., 2010). Supplementation of 4000IU.day⁻¹ of vitamin D₃ lead to a 30% increase in intramuscular VDR concentration and a 10% increase in total muscle fibre size with a greater effect on type II fibres than type I fibres in elderly females (Ceglia et al., 2013). It is not clear if supplementing with vitamin D₃ affects type II or type I fibres in a younger, healthy cohort. A correlation exists between LB and UB power measures in boxers although UB power can vary, potentially due to lower muscle mass (Giovani and Nikolaidis., 2012). As vitamin D₃ can affect type II fibres in elderly participants with a low muscle mass (Ceglia et al, 2013), it is possible that the lower muscle mass of the UB compared to the LB, coupled with the large doses of vitamin D₃ provided, may result in greater adaptation in UB Wingate performance.

Most Effective Dose: The change in performance measures when normalised to vitamin D₃ intake was consistently larger for D1 compared to both D2 and D3 and so may be considered the most effective dose evaluated to exert an ergogenic benefit (Table 1; Figure 1 and Figure 2). Owens et al (2017), states that high doses of 70000IU.week⁻¹ of vitamin D₃ was detrimental to athletic performance as excessive circulating vitamin D is converted to 24,25[OH]2D. 24,25[OH]2D then may act as a blocking molecule by binding to VDRs (Curtis et al., 2014). Other sources state that supplementing with 50000IU.week⁻¹ of vitamin D₃ are effective at correcting vitamin D insufficiency and improving strength training adaptations with aerobic training adaptations not investigated (Magee et al., 2013; Alimoradi et al., 2019). As a result, the International Olympic Committee recommend that 50000IU.week⁻¹ of vitamin D₃ supplementation be administered to athletes who decide to supplement weekly (Maughan et al., 2018).

No serum vitamin D levels were recorded during the study. Given the unreliability of assay's for measuring serum vitamin D levels (Binkley et al., 2004) and the fact that serum

levels do not reflect tissue levels of vitamin D (Ross., 2011), it was decided not to measure serum levels. However, all supplementation was observed, ensuring full compliance and weekly diet data was recorded. This means we can be confident that intake of vitamin D₃ increased performance measures. Although training was not recorded, participants verbally confirmed no changes in training. This allowed us to ascertain the effect of vitamin D supplementation with full fat milk in isolation without the addition of training stimuli. It is possible that no significant differences in aerobic performance were seen between testing sessions one and two due to martial artists and combat sports athletes engaging in similar training weekly (Toskovic, Blessing and Williford, 2004). This may lead to inadequate training overload stimuli which will result in no training adaptations due to specific genes and molecular pathways not being activated (Coffey and Hawley, 2007). Future studies should aim to evaluate the effect of training upon the measured parameters when supplemented with vitamin D. In addition, research into the effect of high dose vitamin D supplementation on trained and competitive athletes in a competition phase of training would be valuable to ascertain if training status alters the response of high dose vitamin D on aerobic performance. A significant limitation of this work was the use of a single-blind crossover design. As stated previously this was done to negate the necessity of a washout period, however it leads to the possibility that a learning effect may exist with respect to the exercise tests. However, participants were blinded to supplementation and their performance results until the study was completed which adds confidence that the obtained results are accurate to reduce the risk of participants altering their behaviours and efforts during the tests (Karanicolas et al, 2010). Nevertheless, future studies must now implement more robust protocols which assess the impact of vitamin D on the aerobic performance of combat sport athletes using randomised double-blind designs.

We demonstrate for the first time the impact of supraphysiological supplementation with vitamin D₃ on aerobic performance outcomes in combat sports. The most effective dose examined is 50000IU.week⁻¹ and there is no advantage to increasing dose on performance

measures. Given the magnitude of improvements seen in this study for both upper and lower body performance then supplementation with high dose vitamin D₃ should be recommended to recreational combat athletes. However, vitamin D toxicity, although rare, can be life threatening with symptoms including apathy, vomiting, polyuria, polydipsia, gastrointestinal cramps, elevated blood calcium and kidney damage (Marcinowska-Suchowierska et al, 2018). As such, supplementation should be undertaken under the guidance of a trained dietician. Nevertheless, it appears that six weeks of supplementation of up to 110000IU's is safe and causes no adverse effects with six weeks of 50000IU's of vitamin D supplementation recommended as optimal to reverse deficiency (Maughan et al, 2018) and convey performance benefits to recreational combat sport athletes.

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Table 1: Table of results. *= significantly different from baseline. †=significantly different from post-placebo. a= significantly different from 50000IU Group. b= significantly different from 80000IU Group. c= significantly different from 110000IU Group

	50000IU Group			80000IU Group			110000IU Group		
	Baseline	Placebo	Intervention	Baseline	Placebo	Intervention	Baseline	Placebo	Intervention
Nutritional Analysis									
Daily Energy (kcal)		1987±47 ^c	2102±357 ^{abc}		1969±272 ^c	2076±365 ^{abc}		2218±494 ^{ab}	2288 ± 446 ^{ab}
Daily Zinc (mg)		10.4±3.1	10.7±3.1 [*]		10.3±3.7	10.2±4.1		9.8±1.9	10.3±1.9 [*]
Daily PUFA (g)		12.4±4 ^b	12.7±4.2 ^b		8.7±3.5 ^{ac}	8.6±3.3 ^{ac}		13.4±5.5 ^b	13. ±5.5 ^b
Daily Calcium (mg)		786±318.3	887.1±328.9 [*]		754.8±485.5	836.5±495.2 [*]		636.8±179.3	800.2±200.3 [*]
Daily Magnesium (Mg)		244±80.3	256±81.5 [*]		216.7±74.7 ^c	211.4±72 ^c		274.7±87 ^b	286.4±86.7 ^{ab}
Weekly Vitamin D (mg)		26.1±11.1	1275.4±12.4 ^{abc}		26.4±20.3	2028.2±20.5 ^{abc}		26.4±11	2779.8±11.3 ^{ab}
Daily Iron (mg)		12.1±2.6	12±2.9 ^b		10±3.4	9.6±0.3 ^a		11±2.5	11±2.5
Haematological Analysis									

Haemoglobin (mmol.l ⁻¹)	9.5±0.6	9.6±0.7	10.3±0.6* †	10.1±0.6	10.1±0.5	10.6±0.4* † ^c	9.6±0.6	9.4±0.6	10±0.5* † ^b
Haematocrit (%)	45±3	45±3	49±3* †	48±3	48±3	51±2* † ^c	46±3	45±3	48±2* † ^b
Body Composition									
Body Fat %	16.2±6.9	16.4±6.7	17.4±7.1	17.5±7.3	17.7±6.6	16.6±6.3	17.6±16.6	17.3±6.3	17.7±6.8
Body Mass (kgs)	74±17.2	73.6±16.8	74±17.2	76.8±11.2	76.6±11.7	76.7±11.6	80.9±13.3	80.2±12.6	80.6±12.6
Performance Analysis									
LB VO _{2peak} (ml.min.kg ⁻¹)	45±6	45±7	50±5* †	46±5	43±7*	48±4* †	47±7	43±6*	47±7 †
UB VO _{2peak} (ml.min.kg ⁻¹)	35±7	35±7	39±6* †	36±6	33±6	39±2 †	37±8	34±8	39±8 †
LB TTE (s)	1090±180	1089±184	1108±298	1082±174	1050±140	1094±287	1099±279	1119±135	1226±154
UB TTE (s)	679±208	802±196	929±253*	914±361	910±312	1141±335* †	751±357	893±188*	1021±223*
LB Peak Power (W.kg ⁻¹)	10.8±1.2	11±1.1	11.6±1.5	11.4±1.9	11.3±1.8	12.2±2 †	11.7±1.7	11.3±1.7	12.1±1.2
LB Average Power (W.kg ⁻¹)	7.8±0.5	7.7±0.7	8±0.7	7.8±0.8	7.6±0.7	7.8±0.8	7.8±1	8±0.8	8.1±0.6
UB Peak Power (W.kg ⁻¹)	5.6±0.7	5.5±0.7	6.2±1 †	5±0.7	5.8±1*	6±1.1*	5±1.2	5.6±1	6.3±0.6* †
UB Average Power (W.kg ⁻¹)	4.3±0.6	4.3±0.6	4.8±0.8* †	3.6±0.7	4±1.1	4.2±1*	3.5±0.7	4±0.8*	4.5±0.5*

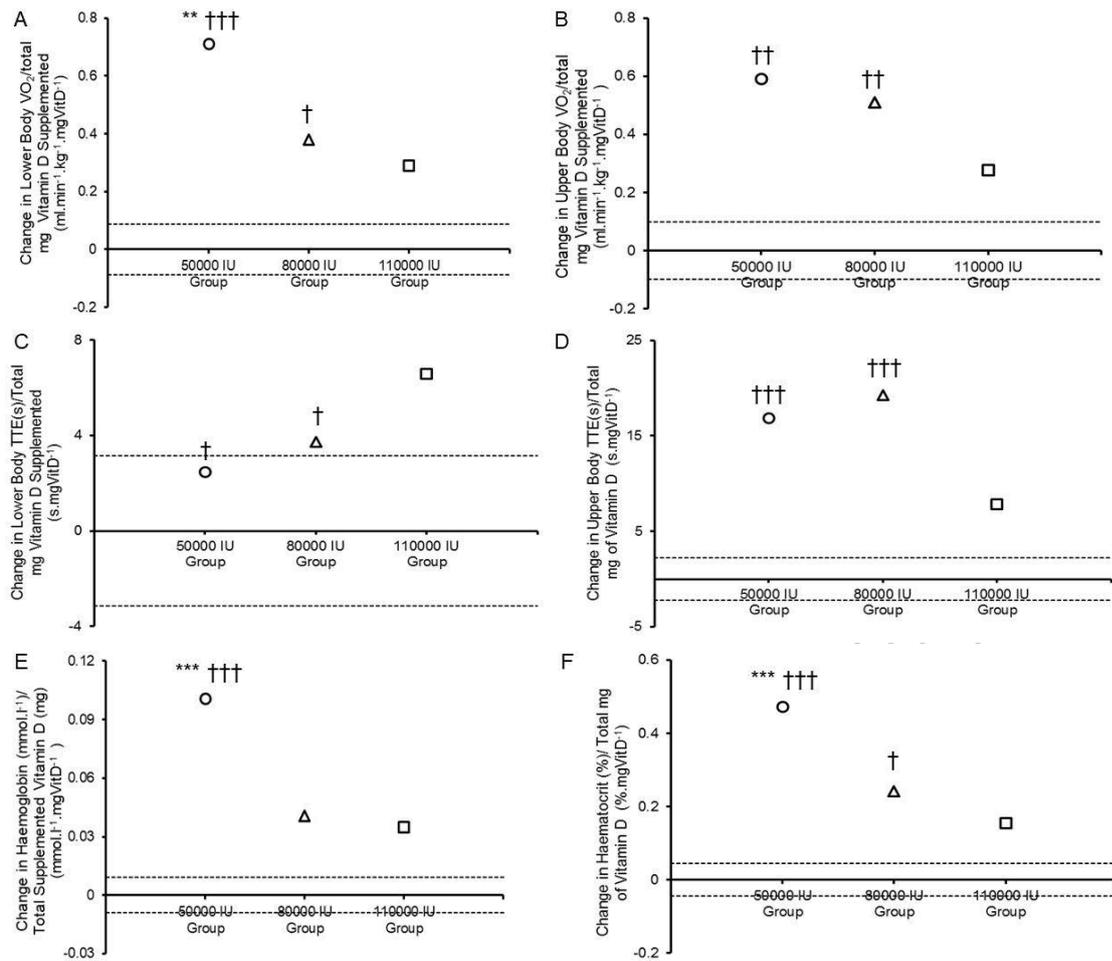


Figure 1: Change in aerobic performance normalised to total vitamin D₃ intake. Dotted line signifies smallest worthwhile change. * = small effect size between 80000IU group. ** = moderate effect size between 80000IU group. *** = large effect size between 80000IU group. † = small effect size between 110000IU group. †† = moderate effect size between 110000IU group. ††† = large effect size between 110000IU group

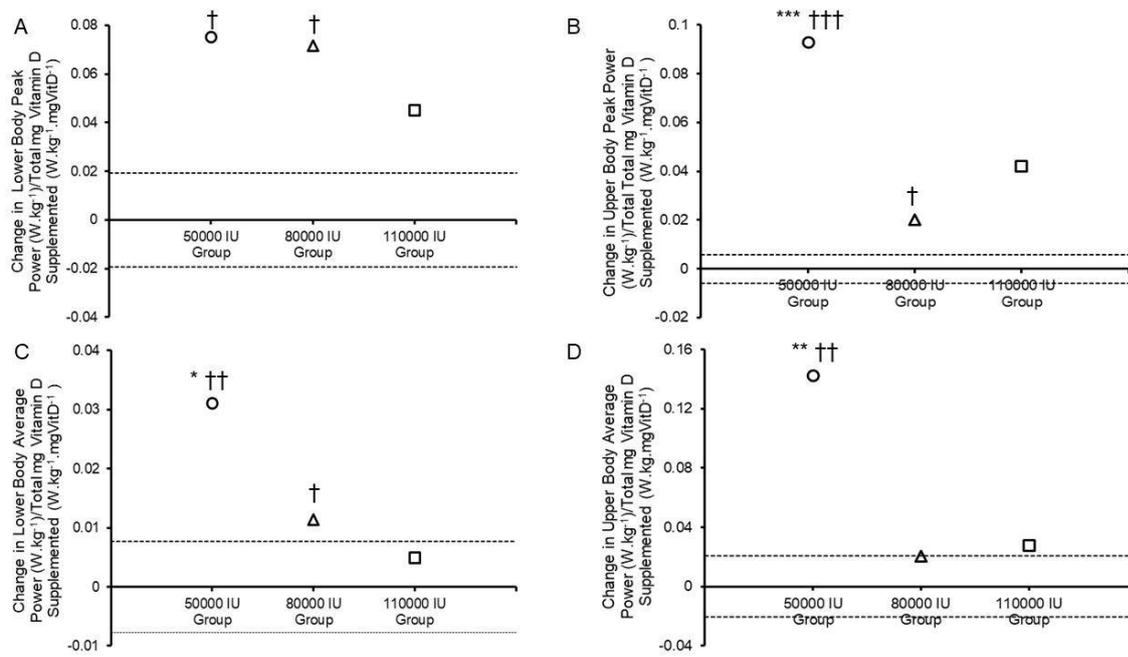


Figure 2: Change in lower and upper body wingate performance normalised to total vitamin D₃ supplemented over the intervention. Dotted line signifies smallest positive and negative worthwhile change in all figures. *= small effect size between 80000IU group. **= moderate effect size between 80000IU group. ***= large effect size between 80000IU group. †= small effect size between 110000IU group. ††= moderate effect size between 110000IU group. †††= large effect size between 110000IU group

ACCEPTED MANUSCRIPT