Vitamin D3 supplementation combined with sprint interval training improves aerobic and anaerobic exercise performance over sprint interval training alone in recreational combat sport athletes

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La supplémentation en vitamine D3 combinée à un entraînement par intervalles de sprint améliore les performances des exercices aérobies et anaérobies par rapport à l'entraînement par intervalles de sprint seul chez les athlètes de sports de combat récréatifs

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Short title

Vitamin D_3 supplementation with SIT improves exercise performance

La supplémentation en vitamine D_3 avec SIT améliore les performances physiques

Summary

Objectives: Correcting vitamin D deficiency might improve aerobic and anaerobic exercise performance. However, it is unclear if vitamin D_3 supplementation can convey a positive ergogenic benefit to aerobic and anaerobic exercise performance when combined with sprint interval training (SIT).

Methods: 27 recreational male combat sport athletes were recruited (age: 25±5 years, stature: 178±7cm, weight: 77±10kg). Participants completed baseline haematocrit and haemoglobin testing, lower body and upper body VO_{2peak} testing and two consecutive lower and upper body Wingate tests separated by five minutes rest. Participants were randomly assigned to either the vitamin D_3 group (VITD) or placebo group (CON) and underwent 6 weeks of twice-weekly SIT and weekly supplementation (50000 IU.week^{-1}). Following the intervention, testing was repeated.

Results: Haemoglobin (P<0.001), haematocrit (P<0.001) and LB VO_{2peak} (P=0.016) increased in VITD, remaining unchanged in CON (haemoglobin P=0.981; haematocrit P=0.947, LB VO_{2peak} P=0.750). UB VO_{2peak} was unchanged in both groups (P=0.284). LB and UB time to exhaustion increased in both groups (P<0.001). LB oxygen kinetics was not affected in either group (P=0.063) with UB oxygen kinetics improved in VITD (P=0.028). LB and UB Wingate peak power improved in both groups (P<0.001). LB Wingate average power improved in both groups (P<0.001) with VITD increasing average power over CON.

Conclusion: Given the results, supplementing 50000 IU of vitamin D_3 per week for six weeks combined with six weeks of SIT may improve markers of aerobic and anaerobic performance in recreational male combat sport athletes.

Résumé

Objectifs: La correction d'une carence en vitamine D pourrait améliorer les performances des exercices aérobies et anaérobies. Cependant, il n'est pas clair si la supplémentation en vitamine D_3 peut apporter un avantage ergogénique positif aux performances des exercices aérobies et anaérobies lorsqu'elle est combinée à un entraînement par intervalles de sprint (SIT).

Méthodes: 27 athlètes masculins de sports de combat récréatifs ont été recrutés (âge: 25±5 ans, stature: 178±7cm, poids: 77±10kg). Les participants ont effectué des tests d'hématocrite et d'hémoglobine de base, des tests VO_{2peak} du bas du corps et du haut du corps et deux tests de Wingate consécutifs pour le bas et le haut du corps séparés par cinq minutes de repos. Les participants ont été répartis au hasard dans le groupe vitamine D_3 (VITD) ou dans le groupe placebo (CON) et ont subi 6 semaines de SIT deux fois par semaine et une supplémentation hebdomadaire (50000 IU.week^{-1}). Suite à l'intervention, les tests ont été répétés.

Résultats: L'hémoglobine (P<0,001), l'hématocrite (P<0.001) et LB VO_{2peak} (P=0.016) ont augmenté dans le VITD, restant inchangés dans CON (hémoglobine P=0.981; hématocrite P=0.947, LB VO2peak P=0,750). UB VO_{2peak} était inchangé dans les deux groupes (P=0.284). Le temps d'épuisement LB et UB a augmenté dans les deux groupes (P<0.001). La cinétique de l'oxygène LB n'a été affectée dans aucun des deux groupes (P=0.063) avec une cinétique de l'oxygène UB améliorée en VITD (P=0.028). La puissance de crête du Wingate LB et UB s'est améliorée dans les deux groupes (P<0.001). La puissance moyenne de LB Wingate s'est

améliorée dans les deux groupes (P<0.001) avec VITD augmentant la puissance moyenne sur CON.

Conclusion: Compte tenu des résultats, la supplémentation de 50000 IU de vitamine D_3 par semaine pendant six semaines combinée à six semaines de SIT peut améliorer les marqueurs de la performance aérobie et anaérobie chez les athlètes masculins de sports de combat récréatifs.

Keywords

Sprint interval training, vitamin D, combat sports, $VO_{2peak,}$ Wingate

Mots clés

Entraînement par intervalles sprint, vitamine D, sports de combat, VO_{2peak}, Wingate

Vitamin D_3 supplementation combined with sprint interval training improves aerobic and anaerobic exercise performance over sprint interval training alone in recreational combat sport athletes

1.0. Introduction

Combat sports involve attacking an opponent's body and are split into striking, grappling and mixed forms [1]. Although different attributes are required for each combat sport, all are characterised by intermittent periods of high intensity activity [1]. Competition formats vary, with 1-11 rounds completed lasting 2-5 minutes interspersed with rest intervals of 30s to 1 minute [2]. Strength may be a predictor of competition success in grappling sports while VO_{2max} is a predictor of success in boxing, karate, wrestling and taekwondo [1]. Therefore, aerobic performance is integral to success in most combat sport with higher aerobic capacities allowing for an increased work rate and recovery between exchanges and rounds [1].

Athletes, including combat athletes, in northern European latitudes are at an increased risk of vitamin D insufficiency/deficiency [3]. This is partly due to exogenous vitamin D production being negligible during the winter months at latitudes higher than 42°N [4] and combat athletes competing and training indoors where no synthesis of exogenous vitamin D occurs [5]. When coupled with common weight cutting practices such as dietary restriction and exercising in heavy clothing [6], vitamin D status may be further negatively impacted. Classically, vitamin D is involved in bone health with vitamin D promoting calcium absorption from the gut while also promoting bone mineralisation via enhanced osteoclast activation with vitamin D deficiency associated with the development of rickets and osteomalacia [41]. However, in recent years there is an emerging understanding that vitamin D is

associated with various bodily processes across a range of tissue, implicated in improving immune function, cell proliferation, enhanced neuroprotection and improved cardiovascular function [41]. In athletes, it has been suggested that vitamin D supplementation can improve the strength and power of skeletal muscle, improve skeletal muscle recovery and increase testosterone levels while potential improvements in aerobic exercise performance remain unclear [42]. Vitamin D deficiency in athletes has been linked to several factors which may impact performance for example an increased injury risk, impaired skeletal muscle growth, and decreased strength and power [7]. The effects of vitamin D on these various tissues are due to the interaction between the active form of vitamin D (1,25(OH)D) with vitamin D receptors (VDRs) [8]. VDRs are present in almost all nucleated cells in humans with VDR activation causing the activation of genomic pathways in a dose dependant manner with higher levels of vitamin D inducing greater physiological changes until optimal levels are reached [8]. As VDRs are present in most nucleated cells, the potential role of vitamin D in various bodily processes is wide ranging and can include an increase in type II myoblast size and proliferation [8] and a decreased risk of stress fracture due to more efficient bone mineralisation [9].

Sprint interval training (SIT) involves supramaximal exercise efforts, often at intensities ~350% VO_{2max} with work periods of $\leq$30s [14]. SIT with both 10s and 30s bouts of work has been shown to improve aerobic and anaerobic exercise performance in healthy adults [15]. SIT has improved VO_{2max} in national level karateka from 58.7 ± 3.1 to 61.4 ± 2.6 $ml.min^{-1}.kg^{-1}$ [16], collegiate taekwondo athletes from 56.1 ± 1.38 to 60.8 ± 1.58 $ml.min^{-1}.kg^{-1}$ [17] and national level wrestlers from 49.3 ± 4.4 to 52.0 ± 3.4 $ml.min^{-1}.kg^{-1}$ [18]. However, SIT twice a week consisting of two blocks of 10 x 20s maximal effort sprints with 10s recovery did not improve

lower body VO_{2peak} in national judokas from 3.62 ± 0.50 to 3.68 ± 0.80 $l.min^{-1}$. [19]. It was suggested that the four weeks of twice weekly SIT was not sufficiently long enough to induce improvements in maximal aerobic exercise performance in national level judoka [19], however four weeks of SIT has previously induced improvements in aerobic performance in combat athletes [17, 18]. However, as the work of Franchini et al was not normalised to bodyweight and measured VO_{2peak} instead of VO_{2max} [19], it is difficult to draw direct comparisons. A systematic review and meta-analysis on the use of HIIT and SIT in combat sports found that SIT increased VO_{2max} in both striking and grappling based athletes with a mean difference of 2.83 and 2.36 $L.kg^{-1}.min^{-1}$ respectively [2]. It was also noted that SIT significantly increased Wingate peak power (PP) in grapplers with a mean difference of 0.51 W but not in strikers with a mean difference of 0.61 W [2]. The lack of significance observed in this analysis of studies investigating strikers could be attributed to comparatively few studies assessing anaerobic performance in strikers which draws the validity of these findings into question [2].

Supplementation of vitamin D_3 in conjunction with SIT may have a greater performance benefit than SIT alone. 50000 IUs of vitamin D_3 supplemented once per week for six weeks improved the aerobic performance of recreational combat sport athletes, increasing VO_{2peak} from 45 ± 7 $ml.min^{-1}.kg^{-1}$ to 50 ± 5 $ml.min^{-1}.kg^{-1}$ which could be linked to a significant increase in haematocrit and haemoglobin levels by ~5-8% [10]. 8 weeks of 6000 $IU.day^{-1}$ of vitamin D_3 supplementation produced an additive effect to training in elite lightweight rowers, significantly increasing VO_{2max} from 58.7 ± 10.16 to 67.4 ± 1.42 $ml.min^{-1}.kg^{-1}$ [11]. In addition to improvements in oxygen carrying capacity, vitamin D_3 supplementation has been found to improve mitochondrial function in deficient adults, thereby improving the oxidative function of

skeletal muscle [12]. However, the link between vitamin D_3 supplementation and anaerobic performance remains unclear. Vitamin D_3 supplementation with 2000 $IU.day^{-1}$ for 12 weeks over the winter months improved Wingate performance of university students in Iran [13]. The authors suggested that vitamin D_3 supplementation may improve calcium sensitivity combined with an increase in the size and number of type II muscle fibres. However, the impact of vitamin D_3 on training adaptations remains to be elucidated.

It is still unclear if vitamin D_3 supplementation in a large weekly dose combined with SIT improves aerobic and anaerobic exercise performance in recreational combat athletes to a greater extent than SIT alone. Therefore, the purpose of this study is to evaluate the combined effect of six weeks of vitamin D_3 (50000 $IU.week^{-1}$) and SIT on aerobic and anaerobic performance in recreational combat sport athletes. It is hypothesised that SIT will improve VO_{2peak} and Wingate performance with vitamin D_3 improving VO_{2peak} and Wingate performance over SIT alone.

2.0. Methods

2.1. Participants.

27 male recreational combat athletes not actively preparing for competition (MMA n=7; BJJ n=11; boxing n=6; kickboxing n=3) were recruited. Participants engaged in sport specific skills training twice weekly with minimum of 1 year experience (mean ± SD; age: 25 ± 5 years, stature: 178 ± 7cm, weight: 77 ± 10kg). Participants were excluded if they were smokers, had any injury over the past six months, were lactose intolerant, had taken vitamin D supplementation within six months of commencement of the study or holidayed in areas where endogenous vitamin D production is high within six months of commencement of the study. Participants were informed of the

192 study both verbally and in writing prior to giving informed consent. The study was
193 approved by Abertay University ethics committee and completed in accordance with
194 the Declaration of Helsinki.

195 2.2. Study Protocol

196 A randomised doubled-blind design was used with participants assigned to either the
197 placebo group (CON) or vitamin D_3 group (VITD) stratified to baseline LB VO_{2peak}.
198 The testing and intervention was completed over winter (October-April) at a latitude
199 of 57°N. Participants were asked to refrain from consuming caffeine, alcohol or
200 engaging in strenuous exercise for 24 hours and fasted for 4 hours prior to any
201 testing session. Verbal confirmation of the continuation of twice-weekly skills training
202 with no other new training stimuli was attained. Baseline testing was completed prior
203 to six weeks of twice-weekly SIT before post-testing. Testing and training sessions
204 were separated by a minimum of 48 hours.

205 2.3. Testing session 1

206 Stature was measured using a stadiometer (SECA) with weight and body
207 composition analysed with bioelectrical impedance (Tanita MC-780, Tokyo, Japan).
208 Haematocrit and haemoglobin levels were assessed via fingerprick blood sample
209 placed into a haematocrit analyser (Hemo Control, EKF Diagnostics, Cardiff United
210 Kingdom). Fingerprick sampling to assess haematocrit and haemoglobin has a good
211 intraclass correlation when compared to the gold standard venous testing (ICC=077)
212 with a sensitivity of 86.8% to change and is therefore a valid an reliable method to
213 assess changes in haematocrit and haemoglobin [43]. An incremental cycling test
214 (Lode Excalibur Sport, Groningen, Netherlands) assessed lower body (LB) VO_{2peak}
215 with breath by breath gas analysis (Metalyzer®3B gas analyser, Cortex, Leipzig,

Germany). Heart rate (HR) was monitored throughout (Polar Electro, Kempele, Finland). Participants sat on the ergometer and rested for two minutes to obtain resting respiratory values before cycling against 60 W at a cadence of 60 RPM. 30 W resistance was added every three minutes until volitional exhaustion or participants were unable to maintain 60 RPM. VO_{2peak} was taken as the highest 10s average and time to exhaustion (TTE) taken as the total time spent cycling.

2.4. Testing session 2

An incremental upper body (UB) VO_{2peak} test was completed using an arm ergometer with participants connected to the gas analysis system (Metalyzer®3B gas analyser, Cortex, Leipzig, Germany). Kneeling in front of the arm ergometer with the heels of the feet remaining in contact with the buttocks, participants remained rested for two minutes. Participants then arm cranked at 60 RPM against a resistance of 1 kg with 0.2 kg added every three minutes until volitional exhaustion or they were unable to maintain 60 RPM. VO_{2peak} was taken as the highest 10s average and TTE was the total time spent arm cranking.

2.5. Testing session 3

Participants were seated on the ergometer (Monark Ergomedic 894, Vansboro, Sweden) before completing a Wingate test, pedalling maximally for 30s against a resistance 7.5% bodyweight. Resistance was applied once 120 RPM was reached. Upon completion, participants rested for five minutes before repeating the Wingate test a second time with peak power (PP) and average power (AP) recorded for both sprints.

2.6. Testing session 4

Participants kneeled in front of the arm ergometer before performing a maximal effort 30s UB sprint against 5% of their body weight with resistance applied from the start. Upon completion, participants remained kneeling in front of the arm ergometer for 5 minutes before repeating the test a second time with PP and AP recorded for both sprints.

2.7. Training and Supplementation Protocol

Participants were asked to be seated on the ergometer (Monark Ergomedic 894, Vansboro, Sweden) where a two minute unloaded warm up at 50 RPM was completed. Participants then completed 8 X 10s maximal effort sprints against 8% of their bodyweight with 30s rest between bouts. Upon completion of the LB SIT, participants were given a five minute rest period. Participants were then asked to kneel in front of the arm ergometer with their heels in contact with their buttocks before completing 8 X 10s maximal effort sprints against 5% of their bodyweight with 30s rest between bouts. This was completed twice-weekly for 6 weeks.

After the first training session of each week, participants consumed either 50000 IU of vitamin D_3 (5 softgel capsules) (Nu U Nutrition, Urmston, United Kingdom) or 5g of olive oil (5 softgel capsules) (Seagate, San Diego, United States) with 300ml of Jersey full fat milk (Graham's Gold Smooth, United Kingdom). Participants also provided a weekly food diary throughout this period.

2.8. Statistical Analysis

All data is presented as mean ± SD. Statistical analysis was completed using Jamovi 1.0.0.0 with significance set at $P<0.05$. Body composition and aerobic performance tests were analysed with a 2 X 2 ANOVA comparing baseline and post-intervention measures between groups with LSD post hoc analysis and anaerobic performance tests analysed with a 4 X 2 ANOVA comparing sprint 1 and 2 at baseline and post-

intervention between groups with LSD post hoc. Nutritional intake was analysed with Diet Plan 7.0 with a 6 X 2 ANOVA comparing weekly dietary data throughout the study between groups and LSD post hoc. Percentage change was calculated as the percentage difference between baseline and post-intervention for each variable for each participant before calculating a mean and standard deviation of the percentage changes. Cohens D effect sizes on percentage change from baseline to post-intervention was calculated between groups 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 [20]. The smallest worthwhile change (SWC) was calculated for each performance measure as described by Swinton et al [44].

3.0. Results

3.1. Anthropological Measures

No significant time effect for bodyweight (*P*=0.201) or group effect (*P*=0.087) with neither group expressing a change greater than the SWC of 2.0 kgs (Table 1). No significant time effect for body fat (*P*=0.166) or group effect (*P*=0.061) with neither group expressing a change greater than the SWC of 1.0% (Table 1).

3.2. VO_{2peak}

There was no significant time effect (*P*=0.116) but a significant group x time interaction for LB VO_{2peak} (*P*=0.048; Table 1). The SWC for LB VO_{2peak} was 1 $ml.min^{-1}.kg^{-1}$ with the change greater than the SWC in VITD but not CON (Table 1). There was a medium effect size between groups for percentage change from pre to post (d=0.79), with a significant increase in VITD (8.2 ± 11.9%; *P*=0.016) but no significant change in CON (-0.7 ± 10.6%; *P*=0.750). No significant time effect (*P*=0.284) and no significant group x time interaction was seen for UB VO_{2peak} (*P*=0.543; Table 1). The SWC for UB VO_{2peak} was 1 $ml.min^{-1}.kg^{-1}$ with the change

289 greater than the SWC in VITD but not CON (Table 1). There was a small effect size
290 between groups for percentage change from pre to post (d=0.48) with no significant
291 change in VITD (5.3 ± 16.1%; *P*=0.543) or CON (-1.0 ± 9.6%; *P*=0.401).

292 3.3. TTE

293 There was a significant time effect (*P*<0.001) but no significant group x time
294 interaction for LB TTE (*P*=0.433; Table 1). The SWC for LB TTE was 36s with the
295 change in both groups greater than the SWC (Table 1). A small effect size was seen
296 between groups for percentage change from pre to post (d=0.23) with a significant
297 increase in VITD (10.6 ± 10.8%; *P*=0.001) and CON (8.2 ± 9.8%; *P*=0.012). There
298 was a significant time effect (*P*<0.001) but no significant group x time interaction for
299 UB TTE (*P*=0.468; Table 1). The SWC for UB TTE was 56s with the change in both
300 groups greater than the SWC (Table 1). A trivial effect size was seen between
301 groups for percentage change from pre to post (d=0.17) with a significant increase in
302 VITD (36.6 ± 58.6%; *P*=0.002) and CON (27.1 ± 54.4%; *P*=0.003).

303 3.4. Oxygen Kinetics

304 There was no significant time effect (*P*=0.063) and no significant group x time
305 interaction for LB $\Delta VO_2/\Delta W$ (*P*=0.926; Table 1). The SWC for LB $\Delta VO_2/\Delta W$ was 0.2
306 $ml.min^{-1}$ with the change in both groups greater than the SWC (Table 1). A trivial
307 effect size was seen between groups for percentage change (d=0.12) with no
308 significant change in VITD (-3.8 ± 10.3%; *P*=0.172) or CON (-2.6 ± 10.5%; *P*=0.307)
309 There was a significant time effect (*P*=0.022) but no significant group x time
310 interaction (*P*=0.365; Table 1) for UB $\Delta VO_2/\Delta W$. The SWC for UB $\Delta VO_2/\Delta W$ was 0.7
311 $ml.min^{-1}$ with the change in both groups greater than the SWC (Table 1). A trivial
312 effect size was seen between groups for percentage change (d=0.09) with a

313 significant decrease in VITD (-8.8 ± 14.9%; *P*=0.028) but no significant change in
314 CON (-7.2 ± 20.4%; *P*=0.285).

315 3.5. Haemoglobin and haematocrit

316 There was a significant time effect (*P*=0.004) and group x time interaction for
317 haematocrit (*P*<0.001). The SWC for haematocrit was 0.7% with the change in
318 haematocrit greater than the SWC in VITD but not CON (Table 1). A large effect size
319 was seen between groups for percentage change from pre to post (d=1.97) with a
320 significant increase in VITD (12.4 ± 6.3%; *P*<0.001) and not CON (0.4 ± 5.9%;
321 *P*=0.947). There was a significant increase from baseline at week 1 in VITD to week
322 3 (8.3 ± 9.1% increase; baseline: 45 ± 4%; week 3: 48 ± 4%; *P*=0.006), remaining
323 significantly elevated from baseline for the remainder of the intervention (Figure 1a).

324 There was a significant time effect (*P*<0.001) and group x time interaction for
325 haemoglobin (*P*=0.002). The SWC for haemoglobin was 0.2 $mmol.l^{-1}$ with the change
326 in haemoglobin concentrations greater than the SWC in VITD but not CON (Table 1).
327 A large effect size was seen between groups for percentage change (d=1.94) with a
328 significant increase in VITD (12.3 ± 5.8%; *P*<0.001) and not CON (1.2 ± 5.6%;
329 *P*=0.981). There was a significant increase in haemoglobin concentrations from
330 baseline at week 1 in VITD to week 3 (5.3 ± 12.2%; baseline: 9.5 ± 0.8 $mmol^{-l}$; week
331 3: 10.0 ± 0.9 $mmol^{-1}$; *P*=0.020), remaining significantly elevated from baseline for the
332 remainder of the intervention (Figure 1b).

333 3.6. Wingate data

334 There was a significant time effect (*P*<0.001) and group x time interaction for LB
335 Wingate PP (*P*=0.043; Table 2). The SWC for sprint 1 was 0.3 $W.kg^{-1}$ and sprint 2
336 was 0.4 $W.kg^{-1}$ with both groups increasing LB Wingate PP greater than the SWC in

both sprints. This was coupled with a medium effect size seen between groups for percentage change for sprint 1 (d=0.52) and sprint 2 (d=0.62; Table 2). There was a significant increase in LB PP from pre to post in VITD for sprint 1 (21.8 ± 18.1%; *P*<0.001) and sprint 2 (24.9 ± 13.4%; *P*<0.001) and a significant increase in LB PP from pre to post in CON for sprint 1 (14.0 ± 11.1%; *P*<0.001) and sprint 2 (16.0 ± 14.4%; *P*=0.029).

There was a significant time effect (*P*<0.001) and group x time interaction for UB Wingate PP (*P*=0.015; Table 2). The SWC for sprint 1 was 0.2 $W.kg^{-1}$ and sprint 2 was 0.2 $W. kg^{-1}$ with the change greater than the SWC for both sprints in both groups (Table 2). A large effect size between groups was seen for percentage change for sprint 1 (d=0.90) and sprint 2 (d=0.99). There was a significant increase in UB Wingate PP from pre to post in VITD for sprint 1 (16.7 ± 8.7%; *P*<0.001) and sprint 2 (20.1 ± 16.0%; *P*<0.001) and a significant increase in UB Wingate PP from pre to post in CON for sprint 1 (7.5 ± 11.4%; *P*=0.04) but not sprint 2 (6.9 ± 8.3%; *P*=0.192).

There was a significant time effect (*P*<0.001) and no significant group x time interaction for LB Wingate AP (*P*=0.834; Table 2). The SWC for sprint 1 was 0.2 $W.kg^{-1}$ and sprint 2 was 0.1 $W.kg^{-1}$ with the change in both groups greater than the SWC in both sprints (Table 2). A large effect size was seen between groups for percentage change for sprint 1 (d=1.38) with a small effect size for sprint 2 (d=0.22). There was a significant increase in LB Wingate AP from pre to post in VITD for sprint 1 (13.5 ± 1.5%; *P*<0.001) and sprint 2 (8.3 ± 6.6%; *P*<0.001) and a significant increase in LB Wingate AP from pre to post in CON for sprint 1 (6.5 ± 7.0%; *P*<0.001) and sprint 2 (9.8 ± 9.5%; *P*<0.001). LB Wingate AP was significantly higher in VITD over CON post for sprint 1 (*P*=0.050) and sprint 2 (*P*=0.035).

There was a significant time effect (P=0.006) with no significant group x time interaction for UB Wingate AP (P=0.424; Table 2). The SWC for sprint 1 was 0.2 $W.kg^{-1}$ and sprint 2 was 0.2 $W.kg^{-1}$ with the change greater than the SWC for both groups during sprint 1 with the change in sprint 2 greater than the SWC in VITD and not CON (Table 2). A small effect size was seen between groups for percentage change from pre to post for sprint 1 (d=0.44) and sprint 2 (d=0.26). There was a significant increase in UB Wingate AP from pre to post in VITD for sprint 1 (9.2 ± 11.6%; P=0.016) but not sprint 2 (7.3 ± 20.3%; P=0.176) and no significant increase in UB Wingate AP in CON for sprint 1 (4.5 ± 9.9%; P=0.102) or sprint 2 (2.8 ± 14.1%; P=0.424).

3.7. Dietary Analysis

No significant time effect or group x time interaction for macronutrient or micronutrient consumption across the study (Table 3). Dietary vitamin D intake was not significantly different between groups at any point and was not changed throughout the study (Table 3). When supplementation is included VITD consumed an additional 50000IU (1250µg) of vitamin D_3 per week.

4.0. Discussion

Vitamin D_3 supplementation combined with SIT significantly improved several performance variables including; LB VO_{2peak} (Table 1), UB oxygen kinetics (Table 1), LB Wingate AP (Table 2) and UB Wingate AP (Table 2). This is coupled with improvements in haematocrit and haemoglobin from $50000IU.week^{-1}$ of vitamin D_3 supplementation (Figure 1). Given that aerobic capacity and anaerobic performance are integral to success in various combat sports [1], it is possible that 50000

385 IU.week^{-1} vitamin D_3 supplementation combined with twice-weekly SIT could improve
386 combat sport performance.

387 A significant increase in haemoglobin concentration and haematocrit was observed
388 in the VITD group but not in the CON group (Figure 1). The increase in both
389 measures peaked at week 4 of the study after two weekly doses of 50000 IU of
390 vitamin D_3, remaining elevated throughout (Figure 1). SIT has been shown to have
391 no effect on haemoglobin and haematocrit due to plasma volume increasing
392 following training [21]. These findings support our previous research where increases
393 in haemoglobin and haematocrit were seen following 6 weeks of vitamin D_3
394 supplementation at the same dose [10]. This is possibly due to vitamin D possessing
395 a role in erythropoiesis and iron recycling [22], potentially increasing haemoglobin
396 concentration due to an increase in red blood cell mass from an increased
397 haematocrit.

398 Following 6 weeks of supplementation and SIT there was a significant increase in
399 LB VO_{2peak} compared to the CON group but no significant change in UB VO_{2peak}
400 (Table 1). There was a medium effect for combination of training plus
401 supplementation to increase VO_{2peak} compared to the CON group. Previous studies
402 have shown similar findings for high intensity training combined with daily vitamin D
403 supplementation in well trained athletes [11, 23]. The magnitude of change in
404 VO_{2peak} is similar to that reported previously with only vitamin D supplementation for
405 both LB and UB [10]. This suggests a direct effect of vitamin D_3 supplementation and
406 could reflect the change in haemoglobin and haematocrit (Table 1) allowing greater
407 oxygen transport and utilisation during exercise [24].

There were no significant group x time differences in oxygen kinetics for both lower and upper body but a significant reduction in upper body oxygen kinetics with time (Table 1). A reduction in oxygen kinetics implies an improvement in exercise efficiency as less oxygen is required to complete the same volume of work [45]. The size of change in LB kinetics was similar in each group, however there was a small effect in UB kinetics with vitamin D enhancing the adaptation over 6 weeks. Both SIT and vitamin D separately have been shown to have an impact on muscle oxygenation during submaximal exercise and rest [25-26]. It is possible that the SIT protocol induced changes in capillarisation, mitochondrial biogenesis and oxidative enzyme activity [25], having a limited effect on LB $\Delta VO_2/\Delta W$. Increased mitochondrial biogenesis has been suggested to permit the same level of ATP regeneration at a lower respiratory level which when combined with increased oxidative enzyme activity can lead to improvements in oxygen kinetics [46]. With the higher type II fibre content of upper body musculature [28], the greater adaptation in upper body oxygen kinetics may reflect the impact of training and vitamin D on type IIa fibres [27]. In trained athletes, type IIa fibres possess a similar oxidative potential to type I fibres with a similar capillary density, mitochondrial content and oxidative enzyme activity [28]. Therefore, it is possible that a shift towards greater type IIa fibre content of the upper body occurs when combining SIT and vitamin D_3 supplementation, causing a significant improvement in upper body oxygen kinetics.

TTE in the LB and UB significantly increased in both groups with no difference between groups (Table 1). The magnitude of improvement was always greater in the VITD group compared to CON group with a small effect between them for lower and upper body. Incremental TTE represents a measure of muscle fatigability and can be altered by both improvements in oxidative metabolism and overall rate of anaerobic

glycolysis. Given the size of difference is similar then the change in TTE is predominantly a training effect with SIT shown to increase incremental TTE even with no change in VO_{2peak} [29]. This may reflect changes in glycogen content and glycolytic enzyme activity following SIT [30], allowing the athlete to maintain performance for longer. The small additional benefit of vitamin D supplementation on TTE may reflect the enhanced oxidative potential of the muscle and the greater improvements in the kinetics of oxygen processing, especially in type IIa fibres, leading to a longer TTE when combined with training.

As such, these results demonstrate an additional benefit of vitamin D_3 in enhancing aerobic adaptations to SIT. Within combat sports there is a strong relationship between performance level and aerobic capacity [31], and competition demands require a high aerobic capacity to sustain work rate [32]. Given the importance of aerobic performance in combat sports, these findings suggest that vitamin D supplementation with the correct training can enhance overall performance of the athletes.

There was a significant increase in LB PP for both sprints in both groups and UB PP for both sprints in VITD and sprint 1 in CON (Table 2). There was a medium effect between groups for change from baseline, with greater change in the VITD group compared to the CON group for both sprints. When vitamin D has been given at the same dose, PP increased by 7-10% [10]. This suggests that there is an additive effect of vitamin D supplementation on power generation. Following SIT, there are changes in neural excitability [33] and calcium sensitivity [34] in skeletal muscle which will allow a greater PP to be generated. In vitamin D deficiency there is a loss of calcium sensitivity in the skeletal muscle [35] therefore vitamin D supplementation could enhance the calcium sensitivity above training alone.

458 Alternatively, vitamin D has been shown to increase the PCr/Pi ratio [36] which may
459 allow a greater utilisation of PCr during contraction, which would enhance the power
460 generation following SIT, allowing greater ATP utilisation. The improvement in sprint
461 2 power may reflect better PCr resynthesis following sprint 1 due to the enhanced
462 oxidative potential of the muscle. Both SIT and vitamin D supplementation have
463 been shown to increase the rate of PCr resynthesis [12, 37]. Given the greater
464 oxidative adaptations with vitamin D then it seems likely that there is enhanced PCr
465 recovery above training alone.

466 There was a significant increase in LB AP for both sprints in both groups and a
467 significant increase in UB AP for sprint 1 in VITD only (Table 2). There was a large
468 effect between groups for change from baseline for LB sprint 1, with greater change
469 in the VITD group compared to the CON group. For UB average power and LB sprint
470 2 average power there was a small effect between groups, with a greater increase in
471 the VITD group. When vitamin D has been given at the same dose, average power
472 increased by 2.5 -10% [10], suggesting an additive effect of vitamin D
473 supplementation on average power generation. SIT has been shown to increase the
474 glycogen storage capacity of skeletal muscle while also enhancing glycolysis [38].
475 This can increase average power production due to an improvement in glycogen
476 utilisation [38]. Vitamin D supplementation may convey an additional benefit to
477 average power production via skeletal muscle remodelling towards type IIa fibres
478 following vitamin D supplementation [27]. Average power during a Wingate test has
479 been associated with the ratio of type II to type I fibre area in the quadriceps [39]. It
480 is possible that remodelling of muscle fibres towards type IIa, coupled with an
481 enhancement of skeletal muscle glycolysis, leads to an improvement in average
482 power generation .

Anaerobic exercise performance has previously been shown to be a predictor of combat sport success in various combat sports [1]. SIT and vitamin D_3 may improve anaerobic exercise performance, possibly through an improvement in bioenergetic pathways, potentially improving combat sport performance.

No serum vitamin D concentrations were measured due serum vitamin D concentration not being reflective of tissue concentration [40]. However, all training and supplementation was observed ensuring compliance with the study protocol with weekly food diaries taken so we can be confident that changes between groups are due to supplementation and not dietary changes. Future studies should aim to ascertain the effect of vitamin D_3 supplementation on the bioenergetic pathways of skeletal muscle to confirm if vitamin D_3 supplementation positively influences skeletal muscle biochemistry, inducing improvements in aerobic and anaerobic performance.

5.0. Conclusion

We demonstrate for the first time the positive impact of 50000 IU.week.$^{-1}$ vitamin D_3 supplementation in conjunction with SIT in recreational combat sport athletes. The SIT protocol induced anaerobic exercise performance benefits but no aerobic performance benefits with vitamin D_3 improving aerobic and anaerobic performance outcomes. This is possibly due to an increase in oxygen transport and delivery with an improvement in skeletal muscle bioenergetics. At present there is some controversy surrounding the correct dosage of vitamin D supplementation, however it has been suggested that an upper limit of 4000IU.day^{-1} is safe for adults with daily intakes less than 10000IU.day^{-1} unlikely to cause adverse health effects [47]. It has been suggested that doses of 50000IU.week^{-1} of vitamin D_3 is safe and sufficient to correct vitamin D deficiency in athletes [48]. Nevertheless, vitamin D toxicity is a risk

and can be life threatening. Supplementation should be completed under the guidance of a trained dietician. However, given the performance changes observed, recreational combat sport athletes should consider 6 weeks of SIT with 50000 IU.week.$^{-1}$ of vitamin D_3 supplementation to induce aerobic and anaerobic performance benefits.

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Declaration of Interest

The authors received no external funding and report no conflict of interest.

Table 1: Anthropometry and aerobic exercise performance data at baseline and post intervention for the vitamin D_3 and placebo groups.

	Vitamin D Group		Placebo Group	
	Baseline	Post	Baseline	Post
Weight (kg)	73.4 ± 8.8	73.4 ± 8.2	79.7 ± 11	80.8 ± 10.6
Body Fat (%)	14.7 ± 4.3	14.9 ± 4.0	18.2 ± 5.3	18.9 ± 5.5
LB VO_{2peak} ($ml.min^{-1}.kg^{-1}$)	45 ± 6	48 ± 7 * †	43 ± 5	43 ± 6
LB TTE (s)	1213 ± 196	1339 ± 227 *	1196 ± 176	1285 ± 161 *
LB $\Delta VO_2/\Delta W$ ($ml.min^{-1}$)	11.3 ± 1.0	10.8 ± 1.1	11.6 ± 1.2	11.2 ± 0.9
UB VO_{2peak} ($ml.min^{-1}.kg^{-1}$)	37 ± 6	40 ± 8	37 ± 5	36 ± 7
UB TTE (s)	863 ± 307	1074 ± 289 *	902 ± 262	1049 ± 199 *
UB $\Delta VO_2/\Delta W$ ($ml.min^{-1}$)	19.4 ± 4.2	16.9 ± 3.2 *	20.0 ± 3.1	18.8 ± 1.9

*= significant within group difference from baseline. †= significantly different from placebo group at same time point.

Table 2: Upper and lower body Wingate performance at baseline and post intervention for vitamin D and placebo groups

	Vitamin D Group				Placebo Group			
	Baseline		Post		Baseline		Post	
	Sprint 1	Sprint 2	Sprint 1	Sprint 2	Sprint 1	Sprint 2	Sprint 1	Sprint 2
LB Peak Power ($W.kg^{-1}$)	11.2 ± 1.0	10.8 ± 1.1	13.5 ± 1.6 *	13.4 ± 1.2 *	11.5 ± 1.9	10.7 ± 2.3	13.0 ± 2.0 *	12.3 ± 1.9 *
LB Average Power ($W.kg^{-1}$)	8.0 ± 0.6	7.5 ± 0.5	8.6 ± 0.9 * †	8.1 ± 0.8 * †	7.6 ± 0.7	6.9 ± 0.8	8.1 ± 0.6 *	7.5 ± 0.5 *
UB Peak Power ($W.kg^{-1}$)	6.0 ± 0.9	6.0 ± 1.3	6.9 ± 0.9*	7.0 ± 0.9 *	6.1 ± 1.1	6.1 ± 1.0	6.5 ± 1.1 *	6.5 ± 1.2
UB Average Power ($W.kg^{-1}$)	4.6 ± 0.7	4.7 ± 1.0	5.0 ± 0.8 *	5.0 ± 0.8	4.5 ± 0.8	4.3 ± 0.6	4.7 ± 0.9	4.4 ± 0.9

*= significant within group difference from baseline at corresponding Wingate sprint.
†= significantly different from placebo group at corresponding Wingate sprint.

566 Table 3: Dietary analysis for Vitamin D and placebo groups averaged into biweekly
567 segments. No significant differences within or between groups.

	Vitamin D Group			Placebo Group		
	Weeks 1/2	Weeks 3/4	Weeks 5/6	Weeks 1/2	Weeks 3/4	Weeks 5/6
Daily energy intake (kcal)	1961 ± 334	1975 ± 305	1975 ± 334	2221 ± 387	2185 ± 360	2180 ± 366
Daily carbohydrate (g)	208 ± 49	206 ± 35	209 ± 41	234 ± 64	223 ± 62	222 ± 58
Daily fat (g)	81 ± 23	81 ± 20	82 ± 21	91 ± 26	90 ± 29	90 ± 29
Daily protein (g)	108 ± 18	110 ± 20	110 ± 22	120 ± 28	127 ± 23	124 ± 27
Weekly vitamin D (µg)	25.2 ± 9.0	25.3 ± 8.3	23.3 ± 8.7	28.1 ± 15.8	26.5 ± 15.2	25.6 ± 15.7
Daily PUFA (g)	10 ± 5	11 ± 6	10 ± 5	13 ± 6	14 ± 6	13 ± 6
Daily magnesium (mg)	280 ± 109	290 ± 138	302 ± 129	331 ± 132	320 ± 137	323 ± 138
Daily zinc (mg)	9.2 ± 2.5	9.7 ± 3.2	9.0 ± 2.6	11.7 ± 4.1	11.4 ± 4.1	11.5 ± 4.1
Daily calcium (mg)	675 ± 255	754 ± 276	728 ± 285	852 ± 391	841 ± 441	822 ± 419
Daily iron (mg)	11.8 ± 2.5	11.9 ± 3.2	11.9 ± 2.5	12.3 ± 3.6	11.7 ± 3.6	11.8 ± 3.7

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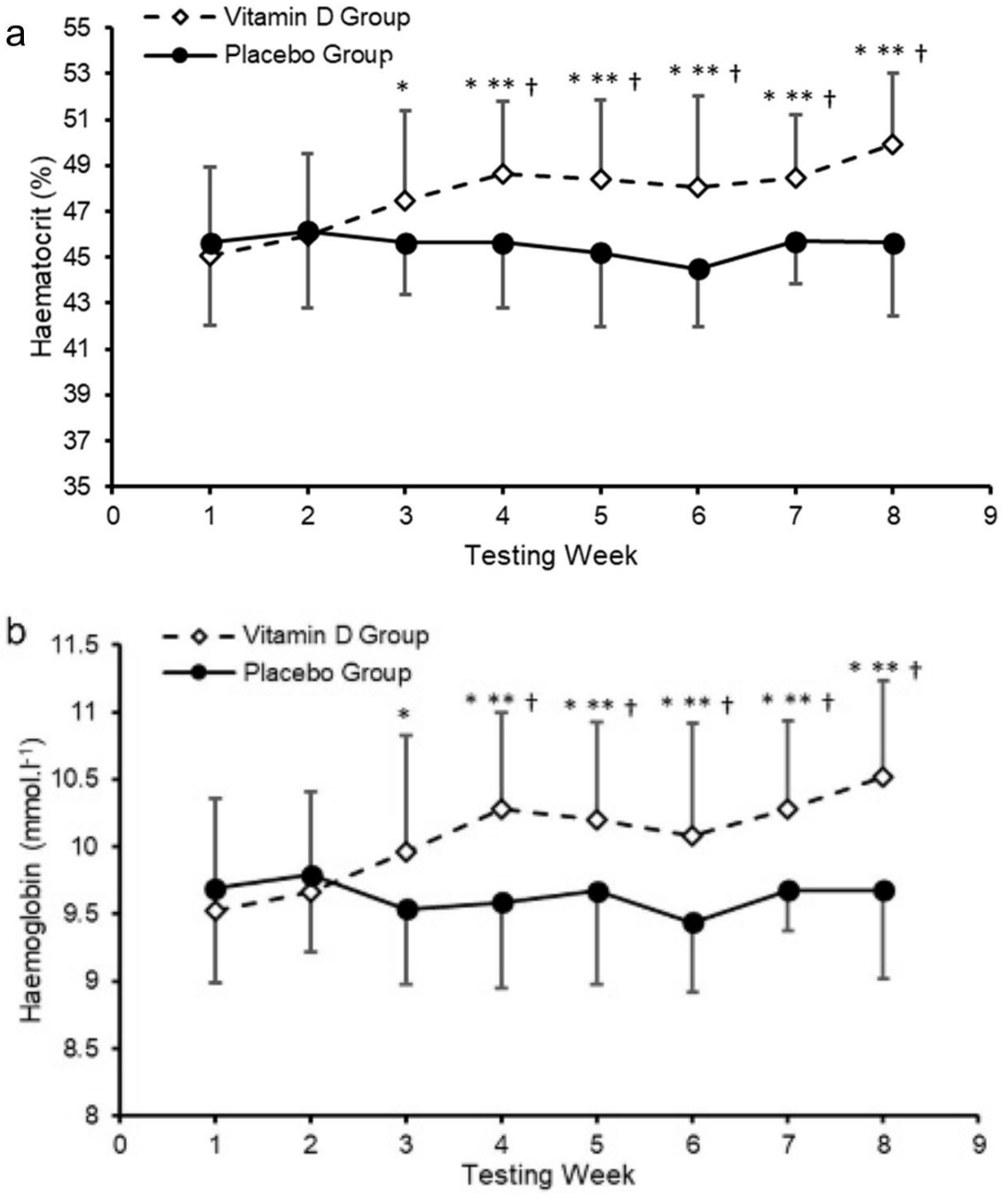

575 Figure 1: Weekly haematocrit values of vitamin D and placebo groups (figure 1a). *=
576 significantly different from baseline testing at week 1. **= significantly different from
577 week 2. †= significantly different from placebo group at the same week. Weekly
578 haemoglobin values for vitamin D and placebo groups (figure 1b). *= significantly
579 different from baseline testing at week 1. **= significantly different from week 2. †=
580 significantly different from placebo group at the same week.

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