A single mega dose of vitamin D3 improves selected physical variables in vitamin D insufficient young amateur soccer players: a randomized controlled trial

Ikram Bezrati[1,2], PhD, Mohamed Kacem Ben Fradj[1], PhD, Raouf Hammami[2,*], PhD, Nejmeddine Ouerghi[1,3,*], PhD, Johnny Padulo[4,5], PhD, Moncef Feki[1,#], MD.

(1) University of Tunis El Manar, Faculty of Medicine of Tunis, LR99ES11, Rabta Hospital, Laboratory of Biochemistry, 1007 Tunis, Tunisia,

(2) National Center of Medicine and Sciences in Sports, Tunisian Research Laboratory "Sport Performance Optimization", 2010 Tunis, Tunisia,

(3) University of Jendouba, High Institute of Sports and Physical Education of Kef, Research Unit Sportive Performance and Physical Rehabilitation, Kef, Tunisia

(4) University Campus, Novedrate, Italy

(5) University of Split, Faculty of Kinesiology, Split, Croatia

* These authors contributed equally to this work.

#, Correspondence to Moncef Feki, Laboratory of Biochemistry, Rabta Hospital, 1007 Jebbari, Tunis, Tunisia. Tel/fax, + 216 71 561 912; e-mail, monssef.feki@gmail.com

Abstract

This randomized controlled trial aimed to test whether vitamin D (VD) supplementation affects measures of physical performance in VD-insufficient mildly trained children. Thirty-six recreationally soccer player boys were randomly assigned to single dose (200,000 IU) of VD3 (n=19) or placebo (n=17). Plasma 25-hydroxyvitamin D (25-OHD) was assessed and measures of physical performance (i.e. vertical and standing broad jumps, triple hop, 10-m and 30-m sprints, shuttle run) were performed before and 12 weeks after the loading dose. Mixed ANCOVA models were performed and effect size was estimated by partial eta squared (η_p^2). Baseline 25-OHD and physical variables were equivalent in the two groups. Twelve weeks after VD loading, plasma 25-OHD increased and physical variables improved in VD group, only. There was a significant interaction effects for group by time for vertical jump ($F=14.9$, $p=0.001$, $\eta_p^2=0.394$), triple hop jump ($F=24.2$, $p<0.001$, $\eta_p^2=0.513$), 10-m ($F=4.46$, $p=0.046$, $\eta_p^2=0.162$) and 30-m ($F=6.56$, $p=0.017$, $\eta_p^2=0.222$) sprints, and shuttle run ($F=13.4$, $p=0.001$, $\eta_p^2=0.369$). In conclusion**,** a single bolus of VD3 resulted in significant improvements in jumping ability, agility, and running speed in VD-insufficient mildly trained children. The findings suggest that correcting VD deficit might be beneficial for physical performance.

Novelty bullets

- A mega dose of vitamin D3 improves jumping ability, agility, and running speed in VD-insufficient mildly trained children
- Effect of vitamin D on measures of physical performance is noticeable three months after the loading dose

Key words

calcitriol, child, dietary supplement, physical performance, sports, vitamin

Résumé

L'étude visait à tester l'effet de la supplémentation en vitamine D (VD) sur la performance physique d'enfants insuffisants en VD et de faible aptitude physique. Trente six garçons pratiquant le football comme activité récréative ont été randomisés à une méga-dose (200 000 UI) de VD3 (n=19) ou un placebo (n=17). La 25-hydroxy-vitamine D plasmatique (25-OHD) a été dosée et des mesures physiques ont été effectuées avant et 12 semaines après la charge en VD. Des modèles d'ANCOVA mixte ont été appliqués et l'ampleur de l'effet a été estimée par l'indice êta carré (ηp^2). La 25-OHD et les variables physiques de base étaient comparables dans les 2 groupes. En post charge, la 25-OHD était plus élevée et les variables physiques ont été améliorées dans le groupe supplémenté. Un effet d'interaction groupe-temps a été observé pour le saut vertical ($p=0,001$; $\eta p^2=0,394$), le triple saut ($p<0,001$; $\eta p^2=0,513$), la course de 10-m ($p=0,046$; $\eta p^2=0,162$) et de 30-m ($p=0,017$; $\eta p^2=0,222$) et la course navette ($p=0,001$; $\eta p^2=0,369$). En conclusion, une dose de charge en VD3 a entraîné une amélioration du saut, de la vitesse et de l'agilité chez ces enfants. Ces résultats suggèrent que la correction d'un déficit en VD serait bénéfique pour la performance physique.

Nouveautés

• Une méga dose de vitamine D3 améliore le saut, l'agilité et la vitesse chez l'enfant insuffisant en vitamine D ayant un faible niveau d'aptitude physique

• L'effet de la vitamine D sur les mesures de la performance physique est perceptible trois mois après la dose de charge

Mots clés

calcitriol, enfant, performance physique, sport, supplément alimentaire, vitamine.

Introduction

Vitamin D (VD) is considered as a key factor in establishing musculoskeletal health during childhood and adolescence (Saggese et al. 2015). Being involved in the regulation of transcription, gene expression and metabolism in skeletal muscle (Ceglia 2008; Boland 2011), VD may play a role in muscle function and could affect measures of physical performance (Hamilton et al. 2013; Koundourakis et al. 2014; Dahlquist et al. 2015). Evidence from transversal studies suggests that poor VD status negatively affects musculoskeletal health and physical performance (El Hajj Fuleihan et al. 2006; Ward et al. 2009, Close et al. 2013a; Hamilton et al. 2013, Koundourakis et al. 2014). In earlier studies, we showed that hypovitaminosis D is common (Bezrati et al. 2016a) and associated with reduced force trunk, jumping ability, agility and running speed (Bezrati et al. 2016b) in Tunisian young amateur soccer players. A number of trials tested effect of VD supplementation on physical performance, leading to inconsistent findings (Close et al. 2013a; Close et al. 2013b; Barker et al. 2013; Wyon et al. 2014; Shanely et al. 2014; Owens et al. 2014; Wyon et al. 2015; Dubnov-Raz et al. 2015; Jastrzebska et al. 2016; Todd et al. 2017; Jastrzebska et al. 2018; Fairbairn et al. 2018, Jung et al. 2018). Discrepancies are likely due to considerable variation between studies in modalities of supplementation, characteristics of participants, and the measures of physical performance investigated. Actually, physical performance in an individual depends on various muscle parameters, namely strength, speed, power, endurance, and balance, which could be more or less influenced by VD. Since VD affect type II fast-twitch fibers (van der Meijden et al. 2016), which are essential for explosive exercise, it would mainly impacts muscle power. Almost all previous trials were conducted in adults or youths, generally highly trained athletes, but no study has been conducted in children. We hypothesized that the impact of VD on physical performance, if any, would be more noticeable when VD is provided at high doses for individuals with poor VD status and low physical aptitude, who have a great potential for physical progress. We therefore designed an interventional study in order to test whether a single mega-dose of vitamin D3 (VD3) have an impact on muscle power measures (i.e. jumping, sprint, agility) in VD-insufficient mildly trained children.

Methods

Study design and setting

A double-blind randomized, placebo-controlled trial was conducted among children attending two football academy centers. The study took place in Tunis city (latitude 36° N), where the sun shines almost all year round. It was conducted during winter, when sunshine time is the shortest (around 10 hours) and sun's rays are the weakest of the year. During this season, children’s sun exposure was feeble, even in sunny days, since they are wearing clothes covering the body and limbs.

Subjects

Children attending the two centers were invited to participate in the study. Criteria for eligibility to be enrolled were male gender, age from 8 to 15 years, plasma 25-OHD<20 ng/mL and parental approval. Children with acute or chronic disease, those under therapy or taking supplements, and those who refused blood collection were not included. An independent investigator allocated participants to a VD or placebo group using a 1:1 allocation. This investigator blinded the supplements and dispensed to the participants either VD3 (one vial of 1 ml of solution containing 200 000 IU VD3, Boucharra Ricordatti) or placebo (one vial of 1 ml of distilled water for intramuscular injection). Supplements were prepared in numbered cups containing 150 ml of packaged orange juice identical in their appearance. Participants and researchers were also blinded to the allocation until the completion of the study. Medical history, eating practices and daily tasks were collected from each participant jointly with one parent/guardian, allowing estimation of nutritional intake and physical activity level. Anthropometric, physical and biological evaluations were performed at baseline and 12 weeks post-intervention. A sample of urine was collected 7 days post-loading for urinary calcium and creatinine analysis. Children sustained their usual tasks, eating practices and physical activities during the study. Participants in both groups underwent the same physical program with two 40-min football-training sessions in academy and one session of school-based physical education, weekly. The protocol was approved by the Ethical Committee of Rabta Hospital and informed written consent was obtained from all children's parents or guardians. Of 166 eligible participants, 40 boys met inclusion criteria, and were admitted into the study. Only 36 participants completed the study; four children (one in the VD group and three in the placebo group) dropped out due to personal reasons (Figure 1).

Height and body mass were measured and body mass index (BMI) was calculated. Participants were divided according to the WHO child growth standards for BMI in three groups; normal-weight group (BMI≤85th percentile), overweight group (85th percentile<BMI<97th percentile) and obese group (BMI>97th percentile) (WHO, 2012). Triceps and subscapular skinfolds were measured using Harpenden's skinfold calipers (Baty International, West Sussex, England), and body fat percentage was calculated using the equation by Slaughter et al. (1988). Fat mass excess was considered as being fat percent above 26%. Biological maturity was assessed by incorporating anthropometric variables (weight, standing height, and sitting height) and peak height velocity (PHV) was calculated (Mirwald et al. 2002). The assessment predicts the time from peak height velocity as a measure of maturity offset. Maturity offset values of "–1", "0", and "+1" represent one year before, at the time of, and one year after maturity, respectively. A three-day food record (including two week days and one week end day) was collected in the presence of one parent

and nutrient intake was processed using NutriPro7 software (CERDEN, Brussels, Belgium). Fasting venous blood samples were collected in heparinized tubes. After centrifugation at 2000 g for 20 min, plasma was frozen at - 40°C until analysis (within 3 months). Plasma 25-OHD concentrations were assessed by a chemiluminescence immunoassay method using a Liaison analyzer (DiaSorin Inc., Stillwater, MN) and the respective reagents kit. Urinary calcium and creatinine were analyzed using conventional methods on an Architect C8000 analyzer and the respective reagents kits (Abbott Diagnostics, Abbott Park, Il).

Physical performance was investigated by a battery of physical tests, which were supervised by two experienced investigators (IB and RH), and the children's coaches. Jumping ability was assessed by vertical jump, standing broad jump, and triple-hop tests. Running speed was evaluated by sprint times for 10 m and 30 m, and agility was evaluated with the 4×9-m shuttle run test. Prior to the beginning of the study, all participants were familiarized with physical tests. Before assessments, participants performed a warm-up session that included light jogging and stretching for ten minutes. Physical measures were performed using techniques, experimental procedures and equipment as previously described (Bezrati et al. 2016b).

Statistical analysis

Statistical analysis was performed using SPSS 18.0 software for windows (SPSS Inc, Chicago, Il). Between-group comparison of variables at baseline was conducted using t-tests for independent samples. Two-way mixed ANCOVA models were applied (with time as within-subjects factor and supplementation as between-subjects factor) to examine the interaction effect for group by time for physical variables. Covariates were age, total energy and VD dietary intakes (continuous variables), and BMI category (normal-weight/overweight/obesity), fat mass class (normal/excess) and PHV class (before/at/after). If a significant interaction was detected, dependent sample t-tests between pre- and post-values in each group were applied. Partial eta-squared (η_p^2) was used to estimate the effect size (Cohen 1973). Effect size was considered small, medium, or large for a η_p^2 value of 0.01, 0.06 and 0.14, respectively (Sink and Mvududu 2010). A p-value <0.05 based on two-sided calculation was considered significant.

Results

At inclusion, no differences were found between VD and placebo groups for age, BMI, body fat, biological maturity, total energy and VD intakes, and plasma 25-OHD concentration (Table 1). As well, baseline physical variables did not differ between the two groups (Table 2). Twelve weeks after the loading, plasma 25-OHD concentrations increased in both groups. However, post-loading values were significantly higher in the VD group (28.6±5.34 ng/mL *vs.* 19.3±4.87 ng/mL, p<0.001). Individual changes in 25-OHD concentrations between pre- and post-test in the two groups are

shown in Figure 2. There was a significant interaction effect for group by time for plasma 25-OHD ($F=34$, $p<0.001$, $\eta_p^2=0.355$). Physical variables, except standing broad jump, significantly improved after the supplementation in VD group, only. Significant interaction effects for group by time were found for vertical jump, triple hop test, 10-m and 30-m sprints, and shuttle run test (Table 2). No other interaction effect for group by time was found. Magnitudes of change in physical variables in both groups are shown in Figure 3. No adverse effects were reported, and post-loading urinary calcium:creatinine ratio was within the normal range (<0.15) in all the participants.

Discussion

Consistent with our hypothesis, VD supplementation resulted in improvements in almost all physical variables investigated. As expected, plasma 25-OHD levels increased in VD group, which was associated with the improvement in physical variables. These findings suggest that correcting VD deficit in mildly trained children could positively affect physical performance.

Data from cross-sectional studies suggest a beneficial role of VD in athletic performance (Ward et al. 2009; Hamilton et al. 2013; Koundourakis et al. 2014; Bezrati et al. 2016). However, interventional studies that tested effect of VD supplementation in athletes/active persons are inconclusive. The studies showed either positive (Close et al. 2013a; Jastrzebska et al. 2014; Jastrzebska et al. 2018; Wyon et al. 2014; Wyon et al. 2016), null (Close et al. 2013b; Owens et al. 2014; Dubnov-Raz et al. 2015; Jastrzebska et al. 2016; Todd et al. 2017) and mixed effects (Fairbairn et al. 2018; Jung et al. 2018). Methodological issues such as inappropriate sample size, confounders, differences in the form (VD2/VD3), dose, frequency (daily/weekly/single dose), and duration of VD supplementation, as well as in measures and instruments employed for physical assessment could explain such discrepancies. Characteristics of participants including age, initial VD status and fitness level may also have influenced outcomes. Positive effects seem more likely to occur in populations with poor VD status (Close et al. 2013a; Wyon et al. 2014; Jastrzebska et al. 2018; Jung et al. 2018). However, a null effect was also observed in such populations (Owens et al. 2014; Dubnov-Raz et al. 2015; Jastrzebska et al. 2016). On the other hand, the potential for a dietary supplement to improve physical performance would be greater in untrained individuals than highly trained athletes since the latter have well developed muscle strength and superior physical performance. Even so, VD supplementation in highly trained athletes resulted in both positive (Close et al. 2013a; Wyon et al. 2014; Wyon 2015), null (Close 2013b; Niemann 2013; Dubnov-Raz et al. 2015; Jastrzebska et al. 2016; Todd et al. 2017) and mixed (Fairbairn et al. 2018; Jung 2018) effects on physical variables. As well, in mildly trained individuals, VD supplementation resulted in either positive (Barker et al. 2013) and null (Owens et al. 2014) effects. According to the form of VD, two trials using VD2 supplements (Niemann et al. 2013; Shanley et al. 2014) resulted

in no significant effect on physical variables, which corroborate the assumption that VD2 is less active than VD3 (Armas et al. 2004). It should however be noted that in these two studies, duration of supplementation was short (6 weeks) and cumulated doses were low (25 000 and 160 000 IU). In most previous studies, supplements were provided, daily or weekly, in the form of VD3 at a dose of 3000 to 5000 IU per day for 4 to 16 weeks, with a cumulated dose ranging from 140 000 IU to 840 000 IU. Yet, VD3 supplementation resulted in both positive and null effects, and no clear relationship was established between cumulative dose of VD and physical measures. Positive effects were observed with doses of 168 000 IU (Barker et al. 2013) and 280 000 IU (Close 2013a; Jastrzebska et al. 2018), and null effect was found with doses of 140 000 IU (Close et al. 2013b) as well as with doses of 840 000 IU (Owens et al. 2014). Finally, mixed results (positive effect for some variables and null effect for others) were observed with doses of 140 000 IU (Jung et al. 2018) and 300 000 IU (Fairbairn et al. 2018). Only one previous trial applied a single bolus of VD3 (150 000 IU) and demonstrated a beneficial acute effect (one week post-loading) on muscle strength in highly trained athletes (Wyon et al. 2015). Considering the diversity of muscle parameters measured, no consensus has emerged that one parameter improves more than others in athletes/active individuals. Actually, VD supplementation resulted in both positive and null effects on muscle strength, force, jumping ability, as well as aerobic fitness. Table 3 summarizes data of randomized trials testing effects of VD supplements on physical performance in athletes/active subjects. Inconsistency in literature suggests that if VD has an impact on physical performance, its effect should be modest and not decisive. Response to VD supplementation may depend on a combination of interrelated factors among which baseline VD status, level of fitness of participants and cumulated dose administrated as well as the physical variable measured could be of importance. The present study was conducted in individuals characterized by poor VD status, young age, low physical performance, and a high potential for physical progress. Supplements were provided as a single bolus of VD3. This method was shown to be safe and efficient to achieve and maintain adequate VD status over months (Mallet et al. 2010; Shepherd et al. 2015). It also has the advantage of avoiding incorrect dosage and poor compliance, which could likely occur in children in a sustained low-dose regimen. Participants were seemingly physically active since they are engaged in sports. However, they practiced football as a leisure activity and their practice was generally recent, irregular and far from intense. Apart from two to three sessions of training in the academy and school, the children engaged in few other forms of physical activity. They spent most of their leisure activities watching TV or playing with digital devices and could, therefore, be considered sedentary or mildly active children. The study was conducted during winter, when sun exposure and endogenous VD synthesis are low, which is supposed to make supplementation more efficient.

However, by the end of follow-up (during February), temperatures were warmer and sunrays were intense, which could explain why plasma 25-OHD concentrations also increased in placebo group.

The role of VD in muscle function has long attracted attention. VD deficiency was closely associated with muscle atrophy of type II fast-twitch fibers, resulting in reduced muscle function, and VD supplementation improved muscle strength and power in VD deficient animals and humans (Endo et al. 2003; Al-Said et al. 2009; Sinha et al. 2013). A number of molecular mechanisms by which VD may control muscle cell have been revealed. Effects may be direct or indirect, rapid or delayed, involving VDR-dependent or independent mechanisms, and genomic or non-genomic pathways. At molecular level, VD controls expression of multiple proteins involved in calcium signaling and handling as well as phosphate-dependent metabolic processes including ATP and creatine phosphate synthesis in muscle cells (Berchtold et al. 2000; Schubert and DeLuca 2010). In that way, VD triggers contraction and relaxation, maintains structural integrity, regulates energy metabolism, and influences the development and differentiation of the muscle. VD also exerts direct effects on muscle cell via the regulation of expression of contractile proteins and myogenic transcription and growth factors (Endo et al. 2003; Garcia et al. 2013), thus influencing muscle development, plasticity and strength, as well as regeneration and neovascularization after injury. VD also controls muscle cell metabolism by releasing arachidonic acid from the cell membrane of muscle cell (de Boland and Boland 1993), influencing caveolin-I, a scaffolding protein within the membrane (Huhtakangas et al. 2004), increasing expression of insulin receptors (Zhou et al. 2008), and enhancing oxygen consumption and mitochondrial biogenesis in skeletal muscle cell (Sinha et al. 2013; Ryan et al. 2016). There is increasing evidence that VD interconnects muscle and bone. The fact that VD deficiency and abnormal VD signaling result in concomitant defects in the musculoskeletal system suggests an integrated role of VD in muscle/bone health. Potential mechanisms may relate to effects of VD in the expression of myokines and osteokines such as osteocalcin, sclerostin, IL-6, FGF23 and myostatin (Dawson-Hughes et al. 2014). Pojednic and Ceglia (2014) showed that VD decreases the expression of myostatin, a negative regulator of muscle mass (Lee and McPherron 2001) while stimulating the formation of follistatin and IGF-2, which positively regulate muscle mass (Barbé et al. 2015). The roles of VD in muscle function have been the subject of excellent revues (Ceglia 2008; Bartoszewska et al. 2010; Girgis et al. 2013; Girgis et al. 2014; Gunton et al. 2015). While several mechanisms have been revealed, further investigations are needed to fully elucidate the mechanisms and pathways involved in VD muscle cell control. At macroscopic level, VD supplementation resulted in an increase in muscle fiber size (Ceglia et al. 2013; Girgis et al. 2014) and the proportion and size of type II fibers (Al-Said et al. 2009; van der Meijden et al. 2016). These fast-twitch fibers are closely associated with ability to

perform explosive exercises such as sprints, jumps, rapid changes of movement (Koundourakis et al, 2016), which are investigated in the present study.

Biological maturity and body composition, as well as energy and VD intakes could be confounding factors for physical performance in children and adolescents. It is unlikely that these factors influenced our results since they did not differ between VD and placebo groups; the majority of children were far from maturity. Moreover, positive effects of VD supplementation on physical variables are found while adjusting for these factors. A role of training in the differences between the groups is also unlikely since participants in both groups underwent the same training program and developed similar physical tasks.

In this study, we investigated multiple facets of physical performance using validated physical tests. Conclusions derive from a multivariate analysis adjusting on key potential confounders for physical performance. No power analysis was performed prior to the study. However, effect size was large for almost all physical variables investigated, which corroborates the accuracy of the findings. The study has limitations that should be acknowledged. Physical measures were achieved 12 weeks post-loading and no evaluation was completed earlier after the loading. While plasma 25-OHD peaks after 2 to 4 weeks after the bolus, more time may be required for tissue stores to be refilled. A single mega dose of VD3 was shown to maintain optimal circulating 25-OHD over few months in children (Mallet et al. 2010; Shepherd et al. 2015). Also, since VD mainly acts through genomic pathways, effects would require some time to be perceptible. Finally, we had to space the two blood sampling to ensure adherence of the children and parents to the study. Actually, the study looked for the medium-term effects rather than the acute effects of VD. Biological maturity was assessed based on estimated age from PHV, but not on Tanner stage. Level of physical activity was estimated by the means of questionnaire, which is not as precise as when determined using an accelerometer. Body fat was evaluated based on skinfold measure, which is less accurate than DEXA and MRI. These methods are efficient, but are costly and less available.

In conclusion, we demonstrated beneficial effects of a bolus of VD3 on selected physical variables in VD-deficient mildly trained children during the winter months. Considering the potential role of VD in musculoskeletal function and in health, resolution of VD deficit may have an impact on improving physical performance. Although the study focused on children practicing sport as a leisure activity and not athletes, we suggest that returning active people/athletes to adequate VD status could optimize physical performance. Further research is required to determine adequate serum 25-OHD levels and optimal supplementation methods in athletes.

Acknowledgements

The authors thank the children enrolled and the children's parents and coaches for their contribution

Funding

The study was supported by The Ministry of Higher Education and Scientific Research of Tunisia

Conflict of Interest

The authors have no conflicts of interest to report.

References

Al-Said, Y.A., Al-Rached, H.S., Al-Qahtani, H.A., Jan, M.M. 2009. Severe proximal myopathy with remarkable recovery after vitamin D treatment. Can. J. Neurol. Sci. **36**(3):336–339.

Armas, L.A., Hollis, B.W., and Heaney, R.P. 2004. Vitamin D2 is much less effective than vitamin D3 in humans. J. Clin. Endocrinol. Metab. **89**(11):5387-5391.doi: 10.1210/jc.2004-0360.

Barbé, C., Kalista, S., Loumaye, A., Ritvos, O., Lause, P., Ferracin, B., Thissen, J.P. 2015. Role of IGF-I in follistatin-induced skeletal muscle hypertrophy. Am. J. Physiol. Endocrinol. Metab. **309**(6):E557-E567. doi: 10.1152/ajpendo.00098.2015.

Barker, T., Schneider, E.D., Dixon, B.M., Henriksen, V.T., and Weaver, L.K. 2013. Supplemental vitamin D enhances the recovery in peak isometric force shortly after intense exercise. Nutr. Metab. (Lond). **10**(1):69. doi: 10.1186/1743-7075-10-69.

Bartoszewska, M., Kamboj, M., and Patel, D.R. 2010.Vitamin D, muscle function, and exercise performance. Pediatr. Clin. North. Am. **57**(3):849-861. doi: 10.1016/j.pcl.2010.03.008.

Berchtold, M.W., Brinkmeier, H., Müntener, M. 2000. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol. Rev. **80**(3):1215-1265.

Bezrati, I., Ben Fradj, M.K., Ouerghi, N., Feki, M., Chaouachi, A., and Kaabachi, N. 2016. Vitamin D inadequacy is widespread in Tunisian active boys and is related to diet but not to adiposity or insulin resistance. Libyan J. Med. 22;11:31258. doi: 10.3402/ljm.v11.31258.

Bezrati, I., Hammami, R., Ben Fradj, M.K., Martone, D., Padulo, J., Feki, M., et al. 2016. Association of plasma 25-hydroxyvitamin D with physical performance in physically active children. Appl. Physiol. Nutr. Metab. **41**(11):1124-1128. doi: 10.1139/apnm-2016-0097.

Boland, R.L. 2011. VDR activation of intracellular signalling pathways in skeletal muscle. Mol. Cell. Endocrinol. **347**(1-2):11-16. doi: 10.1016/j.mce.2011.05.021.

Buitrago, C., Pardo, V.G., and Boland, R. 2013. Role of VDR in 1α,25-dihydroxyvitamin D3-dependent non-genomic activation of MAPKs, Src and Akt in skeletal muscle cells. J. Steroid. Biochem. Mol. Biol. **136**:125-130. doi: 10.1016/j.jsbmb.2013.02.013.

Ceglia, L. 2008. Vitamin D and skeletal muscle tissue and function. Mol. Aspects Med. **29**(6):407-414. doi: 10.1016/j.mam.2008.07.002.

Ceglia, L., Niramitmahapanya, S., da Silva Morais, M., Rivas, D.A., Harris, S.S., Bischoff-Ferrari, H., et al. 2013. A randomized study on the effect of vitamin D_3 supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. J. Clin. Endocrinol. Metab. **98**(12):E1927-E1935. doi: 10.1210/jc.2013-2820.

Close, G.L., Leckey, J., Patterson, M., Bradley, W., Owens, D.J., Fraser, W.D. et al. 2013. The effects of vitamin D(3) supplementation on serum total 25(OH)D concentration and physical performance: a randomised dose-response study. Br. J. Sports Med. **47**(11):692-696. doi: 10.1136/bjsports-2012-091735.

Close, G.L., Russell, J., Cobley, J.N., Owens, D.J., Wilson, G., Gregson, W. et al. 2013. Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. J. Sports Sci. **31**(4):344-353. doi: 10.1080/02640414.2012.733822.

Cohen, J. 1973. Eta-squared and partial eta-squared in fixed factor ANOVA designs. Educ. Psychol. Meas. **33**:107–112.

Dahlquist, D.T., Dieter, B.P., and Koehle, M.S. 2015. Plausible ergogenic effects of vitamin D on athletic performance and recovery. J. Int. Soc. Sports Nutr.**19**;12:33. doi: 10.1186/s12970-015-0093-8.

Dawson-Hughes, B., Harris, S.S., Ceglia, L., Palermo, N.J. 2014. Effect of supplemental vitamin D and calcium on serum sclerostin levels. Eur. J. Endocrinol. **170**(4):645–650. doi: 10.1530/EJE-13-0862.

de Boland, A.R. and Boland, R.L. 1993. 1,25-dihydroxyvitamin D-3 induces arachidonate mobilization in embryonic chick myoblasts. Biochim. Biophys. Acta **1179**(1):98–104.

Dubnov-Raz, G., Livne, N., Raz, R., Rogel, D., Cohen, A.H., and Constantini, N.W. 2014. Vitamin D concentrations and physical performance in competitive adolescent swimmers. Pediatr. Exerc. Sci. **26**(1):64-70. doi: 10.1123/pes.2013-0034.

El-Hajj Fuleihan, G., Nabulsi, M., Tamim, H., Maalouf, J., Salamoun, M., Khalife, H., et al. 2006. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J. Clin. Endocrinol. Metab. **91**(2):405-512.

Endo, I., Inoue, D., Mitsui, T., Umaki, Y., Akaike, M., Yoshizawa, T., et al. 2003. Deletion of vitamin D receptor gene in mice results in abnormal skeletal muscle development with deregulated expression of myoregulatory transcription factors. Endocrinology **144**(12):5138–5144.

Fairbairn, K.A., Ceelen, I.J.M., Skeaff, C.M., Cameron, C.M., and Perry, T.L. 2018. Vitamin D3 Supplementation Does Not Improve Sprint Performance in Professional Rugby Players: A Randomized, Placebo-Controlled, Double-Blind Intervention Study. Int. J. Sport. Nutr. Exerc. Metab. **28**(1):1-9.doi: 10.1123/ijsnem.2017-0157.

Garcia, L.A., Ferrini, M.G., Norris, K.C., Artaza, J.N. 2013. 1,25(OH)(2)vitamin D(3) enhances myogenic differentiation by modulating the expression of key angiogenic growth factors and angiogenic inhibitors in C(2)C(12) skeletal muscle cells. J. Steroid Biochem. Mol. Biol. **133**:1–11. doi: 10.1016/j.jsbmb.2012.09.004.

Girgis, C.M., Clifton-Bligh, R.J., Hamrick, M.W., Holick, M.F., Gunton, J.E. 2013. The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocr. Rev. **34**(1):33-83. doi: 10.1210/er.2012-1012.

Girgis, C.M., Clifton-Bligh, R.J., Mokbel, N., Cheng, K., Gunton, J.E. 2014. Vitamin D signaling regulates proliferation, differentiation and myotube size in C2C12 skeletal muscle cells. Endocrinology **155**(2):347–357. doi: 10.1210/en.2013-1205.

Gunton, J.E., Girgis, C.M., Baldock, P.A., Lips, P. 2015. Bone muscle interactions and vitamin D. Bone **80**:89-94. doi: 10.1016/j.bone.2015.02.029.

Hamilton, B., Whiteley, R., Farooq, A., and Chalabi, H. 2014. Vitamin D concentration in 342 professional football players and association with lower limb isokinetic function. J. Sci. Med. Sport. **17**(1):139-143. doi: 10.1016/j.jsams.2013.03.006.

Huhtakangas, J.A., Olivera, C.J., Bishop, J.E., Zanello, L.P., Norman, A.W. 2004. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 alpha,25(OH)2-vitamin D3 in vivo and in vitro. Mol. Endocrinol. **18**(11):2660–2671.

Jastrzębska, M., Kaczmarczyk, M., and Jastrzębski, Z. 2016. Effect of vitamin D supplementation on training adaptation in well-trained soccer players. J. Strength Cond. Res. **30**(9):2648-2655. doi: 10.1519/JSC.0000000000001337.

Jastrzębska, M., Kaczmarczyk, M., Michalczyk, M., Radzimiński, Ł., Stępień, P., Jastrzębska, J., et al. 2018. Can supplementation of vitamin D improve aerobic capacity in well trained youth soccer players? J. Hum. Kinet. **61**:63-72. doi: 10.2478/hukin-2018-0033.

Jung, H.C., Seo, M.W., Lee, S., Jung, S.W., and Song, J.K. 2018. Correcting vitamin D insufficiency improves some, but not all aspects of physical performance during winter training in Taekwondo athletes. Int. J. Sport. Nutr. Exerc. Metab. **28**(6):635-643. doi: 10.1123/ijsnem.2017-0412.

Koundourakis, N.E., Androulakis, N.E., Malliaraki, N., and Margioris, A.N. 2014.Vitamin D and exercise performance in professional soccer players. PLoS ONE, **9**(7):e101659.

Koundourakis, N.E., Avgoustinaki, P.D., Malliaraki, N., Margioris, A.N. 2016. Muscular effects of vitamin D in young athletes and non-athletes and in the elderly. Hormones (Athens) **15**(4):471-488. doi: 10.14310/horm.2002.1705.

Lee, S.J. and McPherron, A.C. 2001. Regulation of myostatin activity and muscle growth. Proc. Natl. Acad. Sci. U S A. **98**(16):9306-9311.

Mallet, E., Philippe, F., Castanet, M., and Basuyau, J.P. 2010. Administration of a single winter oral dose of 200,000 IU of vitamin D3 in adolescents in Normandy: evaluation of the safety and vitamin D status obtained. Arch. Pediatr. **17**(7):1042-1046. doi: 10.1016/j.arcped.2010.04.013.

Mirwald, R.L., Baxter-Jones, A.D., Bailey, D.A., and Beunen, G.P. 2002. An assessment of maturity from anthropometric measurements. Med. Sci. Sports Exerc. **34**(4):689–694. doi:10.1097/00005768-200204000-00020.

Nieman, D.C., Gillitt, N.D., Shanely, R.A., Dew, D., Meaney, M.P., and Luo, B. 2013. Vitamin D2 supplementation amplifies eccentric exercise-induced muscle damage in NASCAR pit crew athletes. Nutrients **6**(1):63-75. doi: 10.3390/nu6010063.

Owens, D.J., Webber, D., Impey, S.G., Tang, J., Donovan, T.F., Fraser, W.D., et al. 2014. Vitamin D supplementation does not improve human skeletal muscle contractile properties in insufficient young males. Eur. J. Appl. Physiol. **114**(6):1309-1320. doi: 10.1007/s00421-014-2865-2.

Pojednic, R.M. and Ceglia, L. 2014. The emerging biomolecular role of vitamin D in skeletal muscle. Exerc. Sport. Sci. Rev. **42**(2):76-81. doi: 10.1249/JES.0000000000000013.

Ryan, Z.C., Craig, T.A., Folmes, C.D., Wang, X., Lanza, I.R., Schaible, N.S., et al. 2016. 1α,25-Dihydroxyvitamin D3 Regulates Mitochondrial Oxygen Consumption and Dynamics in Human Skeletal Muscle Cells. J. Biol. Chem. **291**(3):1514-1528. doi: 10.1074/jbc.M115.684399.

Saggese, G., Vierucci, F., Boot, A.M., Czech-Kowalska, J., Weber, G., Camargo, C.A., et al. 2015. Vitamin D in childhood and adolescence: an expert position statement. Eur. J. Pediatr. **174**(5):565-576. doi: 10.1007/s00431-015-2524-6.

Schubert, L. and DeLuca, H.F. 2010. Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. Arch. Biochem. Biophys. **500**(2):157–161. doi: 10.1016/j.abb.2010.05.029.

Shanely, R.A., Nieman, D.C., Knab, A.M., Gillitt, N.D., Meaney, M.P., Jin, F., et al. 2014. Influence of vitamin D mushroom powder supplementation on exercise-induced muscle damage in vitamin D insufficient high school athletes. J. Sports Sci. **32**(7):670-679. doi: 10.1080/02640414.2013.847279.

Shepherd, D., Day, A.S., Leach, S.T., Lopez, R., Messenger, R., Woodhead, H.J., et al. 2015. Single high-dose oral vitamin d3 therapy (Stoss): A solution to vitamin d deficiency in children with inflammatory bowel disease? J. Pediatr. Gastroenterol. Nutr. **61**(4):411-414. doi: 10.1097/MPG.0000000000000823.

Sinha, A., Hollingsworth, K.G., Ball, S., Cheetham, T. 2013. Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. J. Clin. Endocrinol. Metab. **98**(3), E509–E513. doi: 10.1210/jc.2012-3592.

Sink, C.A. and Mvududu, N.H. Statistical power, sampling, and effect sizes: Three keys to research relevancy. counselling outcome research and evaluation. Downloaded from: https://journals.sagepub.com (Accessed October 15, 2018)

Slaughter,M.H., Lohman,T.G., Boileau,R.A., Horswill,C.A., Stillman,R.G., Vanloan, M.D., et al. 1988. Skinfold equation for estimation of body fatness in children and youth. Hum. Biol. **60**:709-723.

Todd, J.J., McSorley, E.M., Pourshahidi, L.K., Madigan, S.M., Laird, E., Healy, M., et al. 2017. Vitamin D3 supplementation using an oral spray solution resolves deficiency but has no effect on VO2 max in Gaelic footballers: results from a randomised, double-blind, placebo-controlled trial. Eur. J. Nutr. **56**(4):1577-1587. doi: 10.1007/s00394-016-1202-4.

van der Meijden, K., Bravenboer, N., Dirks, N.F., Heijboer, A.C., den Heijer, M., de Wit, G.M., et al. 2016. Effects of 1,25(OH)2 D3 and 25(OH)D3 on C2C12 myoblast proliferation, differentiation, and myotube hypertrophy. J. Cell. Physiol. **231**(11):2517-2528. doi: 10.1002/jcp.25388.

Ward, K.A., Das, G., Berry, J.L., Roberts, S.A., Rawer, R., Adams, J.E., and Mughal, Z. 2009.Vitamin D status and muscle function in post-menarchal adolescent girls. J. Clin. Endocrinol. Metab. **94**(2):559–563.doi: 10.1210/jc.2008-1284.

World Health Organization. Global strategy on diet, physical activity and health: childhood overweight and obesity. http://www.who.int/dietphysicalactivity/childhood/en/(2012). (Accessed February 06, 2019).

Wyon, M.A., Koutedakis, Y., Wolman, R., Nevill, A.M., and Allen, N. 2014. The influence of winter vitamin D supplementation on muscle function and injury occurrence in elite ballet dancers: a controlled study. J. Sci. Med. Sport. **17**(1):8-12. doi: 10.1016/j.jsams.2013.03.007.

Wyon, M.A., Wolman, R., Nevill, A.M., Cloak, R., Metsios, G.S., Gould, D., et al. 2015. Acute effects of vitamin D3 supplementation on muscle strength in judoka athletes: A randomized placebo-controlled, double-blind trial. Clin. J. Sport. Med. **26**(4):279-284. doi: 10.1097/JSM.0000000000000264.

Zhou, Q.G., Hou, F.F., Guo, Z.J. Liang, M., Wang, G.B., Zhang, X. 2008. 1,25-dihydroxyvitamin D improved the free fatty-acid-induced insulin resistance in cultured C2C12 cells. Diabetes Metab. Res. Rev. **24**(6):459-464. doi: 10.1002/dmrr.873.

Table 1. Baseline characteristics of study participants in vitamin D and placebo groups

		Placebo group (n=17)	Vitamin D group (n=19)	P*
Age, years		10.8±12.2	10.7±2.15	0.840
Body mass index, kg/m^2		18.7±5.01	18.8±4.33	0.774
Fat mass, %		20.9±9.62	21.1±7.02	0.668
Peak height velocity, years		- 2.87±1.72	- 2.61±1.99	0.673
Body mass index, kg/m^2	≤ 85th percentile	47.1	42.1	0.750
	85th-97th percentile	29.4	42.1	
	> 97th percentile	23.5	15.8	
Fat mass,%	Normal	70.6	68.4	0.588
	Exceeded	29.4	31.6	
Biological maturity (PHV)[a]	-1	76.5	78.9	0.984
	0	17.6	15.8	
	+1	5.9	5.3	
Total energy intake, Kcal/day		2135±341	2086±292	0.671
Vitamin D intake, µg/day		4.34±1.31	4.45±0.85	0.324
25- hydroxyvitamin D, ng/mL		12.1±3.93	12.4±3.49	0.801

Values are means ± SD or percent; PHV, peak height velocity, [a], "-1", "0", and "+1" represent one year before, at the time of, and one year after maturity, respectively; *, comparisons were performed using t tests for independent samples or chi-squared tests.

Table 2. Physical variables at inclusion (PRE) and 12 weeks after loading (POST) in vitamin D and placebo groups

	Placebo group (n=17)		Vitamin D group (n=19)		Interaction (time*group) [b]	
	PRE	POST [a]	PRE	POST [a]	F	η_p^2
Vertical jump, cm	11.5±14.0	11.4±12.3	11.4±13.9	11.9±10.1*	14.9[##]	0.394
Standing broad jump, cm	158±3.39	156±3.06	156±2.66	154±2.31	0.03	-
Triple-Hop, cm	400±58.8	396±52.1	398±51.8	419±53.0*	24.2[##]	0.513
10-m sprint, sec	2.44±0.48	2.39±0.46	2.45±0.48	2.23±0.40*	4.50[#]	0.162
30-m sprint, sec	5.17±0.30	5.15±0.32	5.21±0.34	5.09±0.38*	6.56[#]	0.222
Shuttle run, sec	6.58±0.59	6.54±0.61	6.59±0.67	6.44±0.66*	13.4[##]	0.369

Data are expressed as mean ± SD; η_p^2, partial eta squared; [a], comparison to respective pre-test value using paired t test (*, $p<0.01$); [b], interaction was tested using two-way mixed ANCOVA models adjusting on age, total energy and VD dietary intakes, and BMI, fat mass and PHV classes ([#], $p<0.05$; [##], $p<0.01$)

Table 3. Randomized controlled trials on the effect of vitamin D supplementation on physical performance in athletes/physically active subjects

Fist author, year	Participants	Age, years	25-OHD, ng/mL	Dosage VD3, Duration	Effect on measures of physical performance
Close 2013	10 UK soccer players	18.0±5.0	70%, <20	5000 IU/d, 8 wks	Increase in 10-m sprint time and vertical jump height
Close 2013b	30 club-level athletes	21.3±1.3	57%, <20	20 000 IU/wk, 12 wks 40 000 IU/wk, 12 wks	No significant changes in bench press and leg press and vertical jump height
Barker 2013	28 active adult males	30.5±5.5	50% <30	4000 IU/day, 6 wks	Increase in muscle strength recovery post-exercise
Niemann 2013	28 NASCAR pit crew athletes	38.8±1.9	40.7±2.10	3800 IU VD2/day, 6 wks	No significant effect on leg-back and hand grip strength, body weight bench press to exhaustion, vertical jump and 30-s Wingate test
Wyon 2014	24 elite ballet dancers	28.8±5.13	75%, <30	2000 IU/day ,16 wks	Increase in isometric strength and vertical jump
Owens 2014	29 healthy young males	22.7 ±3.0	16±6.8	10000 IU/day, 12 wks	No significant changes in isokinetic muscle force
Shanely 2014	50 young male athletes	16.2 ±0.2	All, < 30	600 IU VD2/day, 6 wks	No significant changes in vertical jump height and leg/back strength
Wyon 2015	22 judoka athletes	29 6±10.6	ND	150 000 IU, Single dose	Positive effects on isokinetic muscle force (evaluation occurred after 8 days from VD bolus)
Dubnov-Raz 2015	53 young trained swimmers	ND	All, < 30	2000 IU/day, 12 wks	No significant changes in swimming performance at several speeds, arm-grip strength, and one-legged balance
Jastrzebska 2016	42 trained soccer players	17.5±0.6	ND	5000 IU/day, 8 wks	No significant change in 5, 10, 20, and 30 m running speed, squat jump and countermovement jump heights
Todd 2017	42 Gaelic footballers	20.0±2.0	72%, < 20	3000 IU/day, 12 wks	No change in VO2max, vertical jump height, and handgrip strength
Jastrzebska 2018	36 trained soccer players	17.5±0.6	All, < 20	5000 IU/day, 8 wks	Moderate improvement inVO2max
Fairbairn 2018	57 professional rugby men	21.0 ±2.8	All, > 30	50 000 IU/15 day, 12 wks	Increase in weighted reverse-grip chin up, but no significant changes in 10-m and 30-m-sprint times, intermittent exercise recovery test performance, and predicted bench pull and bench press
Jung 2018	35 taekwondo athletes	19-22	All, < 20	5000 IU/d, 4 wks	Increase in peak power and isokinetic knee extension, but no changes in countermovement jump test, agility test, and 20-m sprint

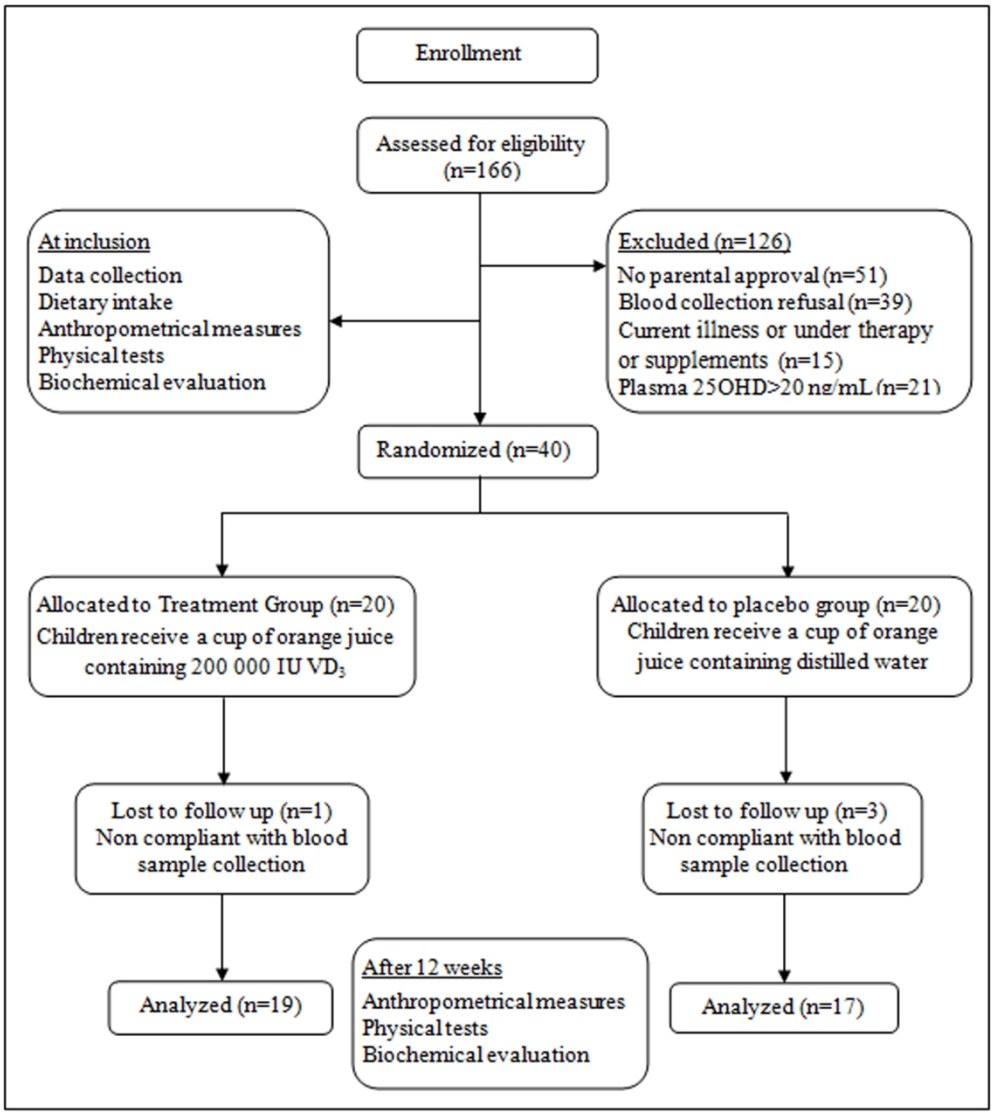
ND, not determined; VD, vitamin D

Figure captions

Figure 1. Study participants flow chart

Figure 2. Individual changes in 25-hydoxyvitamin D concentrations between pre- and post-tests in vitamin D and placebo groups

Figure 3. Comparative change between pre- and post-tests in selected physical variables in vitamin D and placebo groups



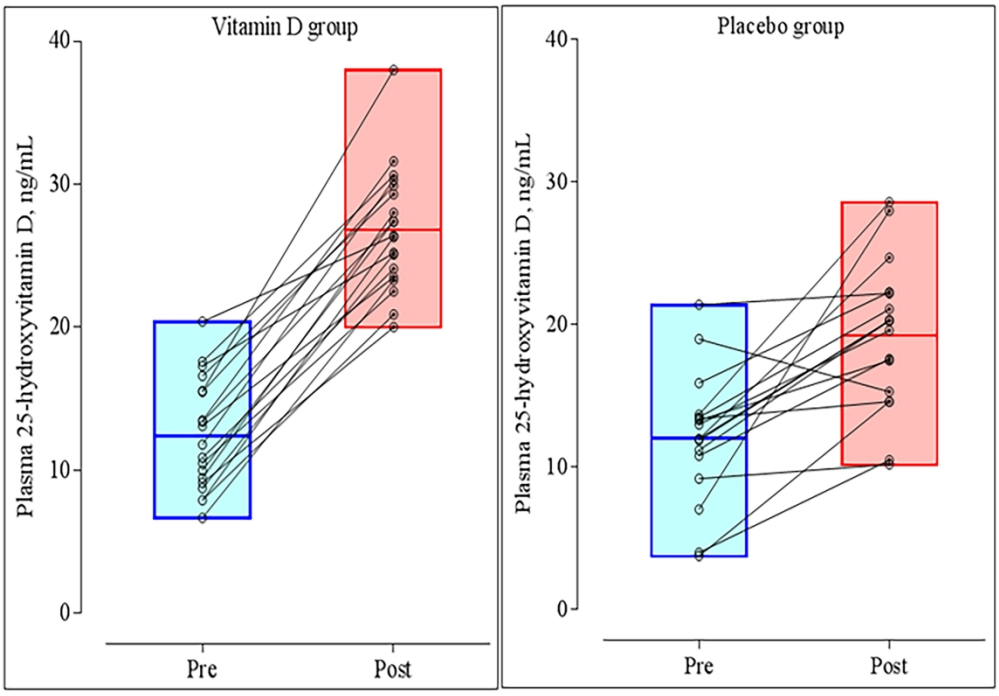


Figure 2. Individual change in 25-hydoxyvitamin D concentrations between pre- and post-tests in vitamin D and placebo groups

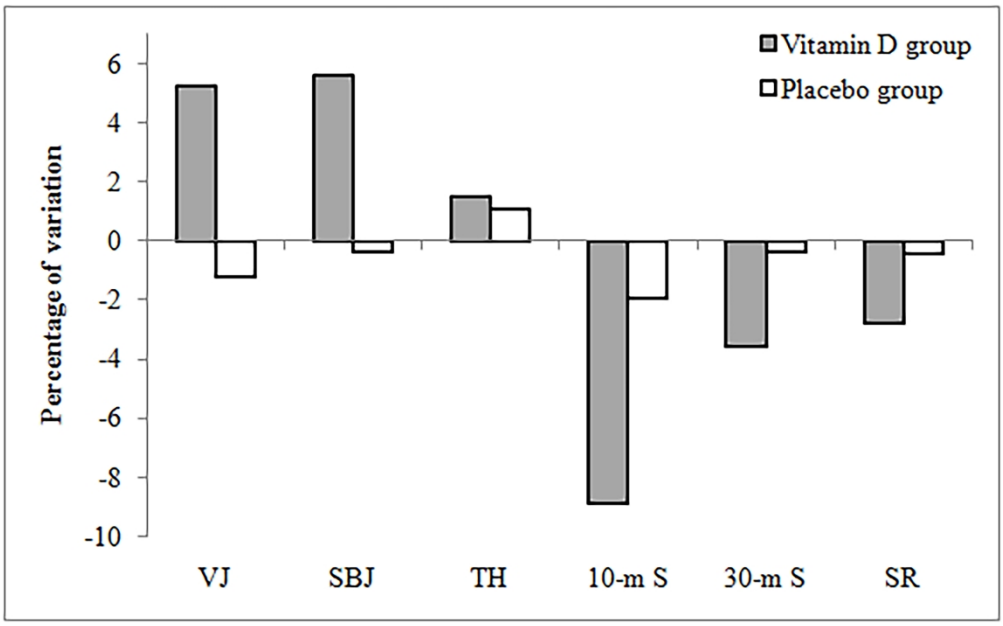


Figure 3. Comparative change between pre- and post-tests in selected physical variables in vitamin D and placebo groups