## Association of plasma 25-hydroxyvitamin D with physiological performances in physically active children

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Association of plasma 25-hydroxyvitamin D with physiological performances in physically active children

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Abstract

**Background and aims:** Vitamin D is thought to regulate skeletal muscle functions and boost physical performance. The aim of this study was to assess the relationship between vitamin D and physical performance in physically active children.

**Methods:** This cross-sectional study included 125 children who practice football as leisure activity. Plasma 25-hydroxyvitamin D (25-OHD) was assessed using a chemiluminescence immunoassay method. Vitamin D inadequacy was defined as 25-OHD<20 ng/mL. Physical performance testing included measurements for muscular strength (maximal isometric contraction), jumping ability (vertical jump, standing broad jump, triple hop test), linear sprint (10-m and 20-m) and agility (9×4-m shuttle run).

**Results:** Plasma 25-OHD concentrations were positively correlated with muscular strength (r=0.539; p<0.001), vertical jump (r=0.528; p<0.001) and standing jump broad (r=0.492; p<0.001), but inversely correlated with sprint performances (r=−0.539; p<0.001). In multivariate analysis models, plasma 25-OHD concentrations were associated with each physical performance parameter independently of: age, maturity status, BMI, fat mass and protein and calcium intakes.

**Conclusions:** A low plasma 25-OHD level was associated with decreased muscular strength, agility, jumping and sprint abilities in physically active children. Vitamin D inadequacy may be an exercise performance limiting factor. Further research should verify whether correction of vitamin D deficiency will enhance physical performance.

**Keywords:** children, muscular strength, physical performance, vitamin D.
Introduction

Vitamin D has been recognized for its role in calcium and phosphate homeostasis. Accumulating evidence suggest vitamin D plays an important role in a variety of physiological functions such as cardiovascular, neuromuscular and immune functions (Holick 2007). The vitamin D receptor (VDR) has been isolated from human skeletal muscle (Bischoff et al. 2001) suggesting vitamin D may have a direct action on the muscle cell. Vitamin D deficiency has been shown to be linked to catabolic effects on muscle tissue, muscle weakness, and decreased number and size of type II muscle fibers (Glerup et al. 2000; Pfeifer et al. 2002; Sato et al. 2005). Numerous studies, though not all, have reported a correlation between vitamin D and physical performance (Close et al. 2013; Dubnov-Raz et al. 2014; Fitzgerald et al. 2015; Hamilton et al. 2013; Koundourakis et al. 2014; Wayon et al. 2014). Few studies have tested this association in untrained or in active young individuals (Foo et al. 2009; Ward et al. 2009). This study investigated whether vitamin D status is related to physical performance in physically active young boys. Physical performance may vary according to several factors including age, maturity status, body composition, and protein and calcium dietary intakes. All these potential confounders were explored in order to control for in data analysis.

Subjects and Methods

Subjects

This cross-sectional study included 125 physically active boys aged 7 to 15 years, recruited from two centers of Tunis Football Academy (Tunisia - latitude, 35° N) between January and March 2014. Participants undergo two hours of school physical activity and two sessions of football training in the academy weekly, with an average duration of football practicing for 12 months. All participants were identified as being free from current injury, and liver, intestinal, renal and bone diseases or cancer. No child was smoker or has been taking drugs
or supplements for the six months preceding the study. The study was approved by the
Institution's Ethics Committee of Rabta Hospital and informed written consent was obtained
from children’s parents or guardians.

**Experimental protocol**

Anthropometrical measurements and maturity status assessment

Standing height, seating height and body weight was measured and body mass index (BMI)
was calculated as weight per height squared (kg/m²). Triceps and subscapular skin folds
thickness were monitored with Harperden’s skinfold calipers (Baty International, West
Sussex, England). Body fat percentage was conducted using the Skinfold equation by
Slaughter et al. (1988). Biologic maturity was calculated using the equation of Mirwald et al.
(2002) that incorporates measures of body weight, standing height and seating height. This
assessment predicts the time from the peak height velocity (PHV) as a measure of maturity
offset. Maturity offset range between “-1”, “0” or “+1”, before, at the time or 1 yr after
maturity, respectively (Mirwald et al. 2002).

Physical tests

Prior to the beginning of the study, children were familiarized with the technique, equipment
and experimental procedures for each test.

*Vertical Jump test*

The vertical jump test evaluates the lower limbs strength (Bolгла et Keskula 1997). Vertical
jump height (cm) was measured using a digital vertical jump meter (Takei, 5105 Jump MD,
Tokyo, Japan). Participants were instructed to stand on the center of a rubber mat with a
special digital belt tightly fitted around waist. To avoid the negative work up 90° (lower limb)
and to optimize the reliability of the measure, children were forced with the bar below the butt
(Gheller et al. 2015). The belt was connected to the rubber plate by a cord. Before jumping,
any slack was removed from the cord, and subjects were instructed to jump vertically using a
counter movement with arm swing. The take-off was performed with both feet, with no initial steps or shuffling. Three trials of each jump were measured with a 1-minute rest period between trials and the best trial was recorded.

**Standing Long Jump test**

The standing long jump test was performed according to Eurofit Test Battery (Council of Europe 1988). A start line was determined on a non-skid floor and a tape meter was extended from the start line forward. Participants were requested to stand behind the starting line, their toes were away from behind the start line, their arms were on the front in parallel with the floor and knees were bent. Subjects were instructed to push off vigorously and jump forward as far as possible. The participant had to land with the feet together and stay upright. The distance was measured from the starting line to the point where the heel struck the ground upon completing the test. The test was repeated twice and the best score (cm) was recorded.

**Triple Hop test**

Subjects were instructed to stand in a stepping position behind the starting line with their dominant leg forward. This test was performed by starting behind the start line with only the leg in use touching the ground. The subject hopped three continuous times on the dominant leg to reach the maximal horizontal distance. Measurement was taken from the starting line to the point where the heel struck the ground upon completing the third hop (Bolgla et Keskula 1997). The test was repeated three times, and the best score (cm) for dominant leg was recorded.

**Sprint tests**

Each subject was asked to run a distance of 20-m in a straight line as fast as possible with a free standing start. Three pairs of photocells were disposed in a straight line; the first one on the starting line, the second on the line of 10-m and the third on the finish line (20-m). The sprint time is registered with photoelectric cells (Microgate SARL, Bolzano, Italy) placed at
one meter height above ground. Each subject performed two trials with 3-min of recovery between efforts. The best performance was retained for the analysis.

**Agility Test**

Agility was evaluated with the 4×9-m shuttle run test (Kibele and Behm 2009). Subjects standing behind a starting line, they started the electronic clock by passing through the first timing gate. At the end of the 9-m section, subjects were asked to step with one foot beyond a marker while reversing running direction and sprinting back to the start where the same reversal of movement direction was required. After the fourth 9-m section, the subject passed through the second timing gate to stop the electronic clock. The best time (sec) of two consecutive trials was recorded for the statistical analysis.

**Force trunk**

Maximal isometric contraction as a proxy of muscle strength (Koley et al. 2012) was assessed with a back and leg dynamometer (TKK 1858, Takei, Tokyo, Japan). Subjects stood on the dynamometer foot stand and gripped the handle in at proper height and were positioned with body erect and knees bent. They then straightened the knees and lifted the chain of the dynamometer, with the pulling force applied on the handle hands, pulling upwards as strongly as possible with the knees straight and the back at a 30° angle. Subjects completed three trials, the highest score being recorded as the measurement of maximal back strength (kg). A thirty-second rest interval was provided between each test.

**Dietary intake**

Dietary intake in children was assessed by using three-day food records (including two weekdays and one weekend day) combined with a food frequency questionnaire (FFQ), which was completed by the parents. In order to evaluate individual portion sizes, an album of photographs of Tunisian food products was used. Data on daily intake of nutrients were processed using the professional Nutri Pro7 software (CERDEN, Brussels, Belgium).
Biochemical analyses

Blood samples were collected following an overnight fast. Blood was centrifuged at 2000 g for 20 min and the plasma was frozen at - 40°C until analysis (within 3 months). Plasma 25-OHD concentrations were measured by a chemiluminescence immunoassay method using a Liaison analyzer (DiaSorin Inc., Stillwater, MN) and the respective reagents kit. The study group was divided in three subgroups by plasma 25-OHD concentration according to the most recent guidelines of The Institute of Medicine; vitamin D deficiency, <12 ng/mL; vitamin D insufficiency, 12-19.99 ng/mL; and vitamin D sufficiency, ≥20 ng/mL (Institute of Medicine 2011).

Statistical analysis

Data were analyzed using SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL). Continuous variables were tested for normality using Kolmogorov-Smirnov test. Values are expressed as mean±SD for continuous variables and as percentage for categorical variables. One way ANOVA was used to compare significant differences between the three groups that used as “Factor”. Pearson’s correlation test was used to test the relationship between variables. Independent multiple regression models were applied to test association of plasma 25-OHD concentration as response variable with each physical performance parameter while adjusting on potential confounding factors (age, maturity status, BMI, fat mass, protein and calcium intakes). The fit of logistic models were satisfactory. A two-tailed P value less than 0.05 was considered statistically significant.

Results

Main characteristics of the children according to plasma 25-OHD concentration categories are shown in Table 1. Vitamin D inadequacy was observed in 80% of children with 47.2% of children having deficiency and 32.8% of children having insufficiency. Compared to the children with vitamin D sufficiency, those with vitamin D deficiency/insufficiency showed
significantly lower protein intake, but equal calcium intake. Plasma 25-OHD concentrations were positively related to the force trunk, vertical jump height and standing jump broad height, but inversely related to 10-m sprint, 20-m sprint and shuttle run times (Figure 1). In multivariate models, plasma 25-OHD concentration was associated with each physical performance parameter independently of age, maturity status, BMI, fat mass, and protein and calcium intakes (Table 2).

**Discussion**

The study showed a very high rate of hypovitaminosis D with 80% of children having a low plasma 25-OHD concentration. It also revealed an inverse association of plasma 25-OHD with physical performance in these children. Low vitamin D status was associated with decreased force trunk, jumping ability, agility and running speed. The associations remained significant after adjusting for age, maturity status, BMI, fat mass, and protein and calcium intakes. These data suggest vitamin D has a potential role in modulating physical performance in active children. The findings of this study are in agreement with previous cross-sectional studies conducted in untrained individuals. Ward et al. (2009) reported a positive correlation of serum 25-OHD concentrations with jump height and force trunk in post-menarchal adolescent schoolgirls. They suggest lower 25-OHD levels had negative effects on physical performance and muscle contractility. Similarly, inadequate 25-OHD concentration (<20 ng/mL) were associated with reduced muscle strength in Chinese adolescent girls (Foo et al. 2009). Such association was independent of body size, level of physical activity and dietary intakes of calcium and vitamin D. The authors suggested a poor vitamin D status may compromise muscle strength. In athletes, the relationship between 25-OHD concentration and physical performance remains inconclusive. Koundourakis et al. (2014) found a positive correlation of circulating 25-OHD with muscle strength in professional soccer players. Fitzgerald et al. (2015) reported association of plasma 25-OHD with upper body strength, but
not with lower body force and power in young ice hockey players. In contrast, no association of serum 25-OHD was found with grip strength in competitive adolescent swimmers (Dubnov-Raz et al. 2014), nor with isokinetic peak torque during knee flexion and extension in elite football players (Hamilton et al. 2013). However, in the former study, players with inadequate vitamin D status showed significantly lower torque in hamstring and quadriceps muscle groups (Hamilton et al. 2013). Inter-study discrepancies may be due in part to differences in physical performance level and in vitamin D status. As suggested by Koundourakis et al. (2014), in elite athletes, training is the main factor for improvements in exercise performance, and vitamin D might play only a supportive role. Effect of vitamin D would be more noticeable in untrained or slightly trained individuals. Similarly, the role of vitamin D may be more perceptible in subjects with a low vitamin D status. The association of vitamin D with physical performance was more consistent for the groups with predominance of vitamin D inadequacy (Foo et al. 2009; Ward et al. 2009; Hamilton et al. 2013).

Pathways via which vitamin D may affect muscular strength, jumping ability and running speed are not fully understood. It has been shown that vitamin D exerts genomic and non-genomic effects on the muscle cell, enabling an increase of calcium and phosphorus transport and protein synthesis, energy-rich phosphate compounds production and type II muscle fibers readiness (Pfeifer et al. 2002; Ceglia 2008; Bartoszewska et al. 2010). Actually, muscle strength, and jumping and sprinting abilities rely extensively on type II muscle fibers (Pfifer et al. 2002; Stone et al. 2003). Vitamin D also regulates muscle cell proliferation and growth via the activation of the mitogen-activated protein kinase (MAPK) signaling pathways (Ceglia 2009; Garcia et al. 2011). In addition to a direct effect on muscle cells, vitamin D deficiency causes secondary hyperparathyroidism and secretion of proinflammatory cytokines such as TNF-alpha and interleukin-6, which may also impair muscle function (Willis et al. 2008; Willis et al. 2012; Zhang et al. 2012). This study showed low protein intake in children with
vitamin D inadequacy. Since high protein intake is critical for muscle growth and function (Desbrow et al. 2014), it could be hypothesized that muscle performance decline is linked to insufficient protein intake. Nevertheless, the association of vitamin D with physical performance remained significant while adjusting for protein intake. Despite the substantial advances made, further research is needed to clarify the metabolic pathways involved in vitamin D action on skeletal muscle cell and to understand how these cellular changes translate into clinical improvements in physical performance.

The study included a large number of children and used a variety of accurate and validated tests related to multiple facets of physical performance. Associations of vitamin D with all performance tests were consistent, coherent, biologically plausible and independent of potential confounding factors; the findings are therefore reliable. Prevalence of vitamin D inadequacy among these participants was similar to that reported in Tunisian young athletes (Sghaier-Ayadi et al. 2015), which corroborate the accuracy of the findings. Nevertheless, the cross-sectional design of the study doesn’t allow concluding to a causal relationship.

In conclusion, the study highlights an overall high prevalence of vitamin D inadequacy among physically active Tunisian children. It provides evidence of an association between vitamin D status and the physical performances. These data suggest vitamin D deficiency would be a potential physical performance limiting factor. Correction of deficiencies via appropriate and safe sunlight exposure and dietary interventions might be of importance to improve physical performance in active children with inadequate vitamin D status. Further research is needed to clarify the molecular pathways by which vitamin D may modulate physical performance.
Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Non standard abbreviations,

25-OHD, 25-hydroxyvitamin D; BMI, body mass index; PHV, peak height velocity
References


Table 1. Main clinical and nutritional characteristics of children according to vitamin D status

<table>
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<tr>
<th>Vitamin D status</th>
<th>Deficiency (n=59)</th>
<th>Insufficiency (n=41)</th>
<th>Sufficiency (n=25)</th>
<th>P</th>
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<tr>
<td>Plasma 25-OHD</td>
<td>&lt;12 ng/mL</td>
<td>12–19.99 ng/mL</td>
<td>≥20 ng/mL</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>11.4±2.03</td>
<td>11.8±2.16</td>
<td>11.0±1.87</td>
<td>0.201</td>
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<tr>
<td>Peak height velocity (years)</td>
<td>-2.83±1.52</td>
<td>-1.82±1.76</td>
<td>-2.63±1.33</td>
<td>0.005</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>18.9±4.16</td>
<td>19.7±4.29</td>
<td>19.8±4.19</td>
<td>0.565</td>
</tr>
<tr>
<td>Fat Mass (%)</td>
<td>24.0±9.24</td>
<td>25.3±8.46</td>
<td>28.6±8.08</td>
<td>0.094</td>
</tr>
<tr>
<td>Protein intake (g/day)</td>
<td>92.7±20.0</td>
<td>112±19.4</td>
<td>115±18.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>1084±245</td>
<td>1063±298</td>
<td>1089±229</td>
<td>0.895</td>
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<tr>
<td>Vitamin D intake (µg/day)</td>
<td>9.29±3.75</td>
<td>9.31±4.58</td>
<td>9.06±4.96</td>
<td>0.975</td>
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Values are expressed as mean ± SD. The significant “P” effect was calculated between the three conditions.
Table 2: Linear regression models testing the association of plasma 25-hydroxyvitamin D concentration with each physical performance parameter

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<tr>
<th>Physical test</th>
<th>β coefficient</th>
<th>SE</th>
<th>Standardized β coefficient</th>
<th>P value</th>
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<tr>
<td>Force trunk (kg)</td>
<td>0.194</td>
<td>0.037</td>
<td>0.487</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vertical jump (cm)</td>
<td>0.165</td>
<td>0.028</td>
<td>0.504</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Standing broad jump (cm)</td>
<td>0.552</td>
<td>0.116</td>
<td>0.452</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triple-hop (cm)</td>
<td>0.042</td>
<td>0.008</td>
<td>0.532</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10-m sprint (sec)</td>
<td>-6.436</td>
<td>1.026</td>
<td>-0.564</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20-m sprint (sec)</td>
<td>-3.651</td>
<td>0.912</td>
<td>-0.410</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Shuttle run (sec)</td>
<td>-4.330</td>
<td>0.805</td>
<td>-0.499</td>
<td>&lt;0.001</td>
</tr>
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SE: standard error; “*” adjustment was made for age, maturity status, body mass index, fat mass, and protein and calcium intakes.
Figures titles and legends

Figure 1. Correlations between plasma 25-hydroxyvitamin D (25-OHD) concentrations with selected physical performance tests
Figure 1. Correlations between plasma 25-hydroxyvitamin D (25-OHD) concentrations with selected physical performance tests

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