Journal of Physical Fitness, Medicine & Treatment in Sports
ISSN: 2577-2945

Research Article
Volume 6 Issue 1 - February 2019
DOI: 10.19080/JPFMTS.2019.06.555679

J Phy Fit Treatment & Sports

Marcello Ferrari Annual Variations In Vitamin D Levels And Exercise Capacity In Italian Amateur Cyclists

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Submission: February 10, 2019; **Published:** February 25, 2019

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Introduction

Vitamin D is a steroid hormone which plays a pivotal role in bone mineral homeostasis and is involved in the regulation of a large number of physiological processes, such as immune function, protein synthesis, inflammatory response and cell growth [1-4]. Indeed, Vitamin D is, together with testosterone, one of most important hormones for function and strength of the muscles, and it could have a significant role in the improvement of performance [5-6]. Deficiency of Vitamin D affects lower limb muscles, that are necessary in simple and frequent actions such as walking and postural balance [7]. In fact, the first symptoms of deficiency of this micronutrient could be muscle weakness, hypotonia and elongation of muscle relaxation time [8], while the relationship between Vitamin D, lung function and physical activity is not completely clear. Recently, several investigations have discovered low levels of Vitamin D in a high percentage of normal subjects and athletes, even in people living in area with extensive sunlight exposition [9]. Furthermore, some studies have shown a relationship between the serum level of 25(OH)D3 hormone and physical performance [10]. As far as the investigations in the young and the athlete are concerned, a European study performed in female adolescents has found that muscle strength is positively associated with circulating concentration of 25-hydroxy-cholecalciferol (25(OH)D) [1].

The same research found that cardiorespiratory fitness and muscle strength were positively associated with 25(OH)D serum concentration in adolescents of both sexes [1]. Furthermore, Koundourakis [11] found a relationship between 25(OH)D and maximal oxygen uptake (VO_2 max) in Greek professional football players. On the contrary, a study on football players in Qatar failed to find an association between muscle function and 25(OH)D [2]. Previous studies have examined levels and association between 25(OH)D and performance in different kinds of sports such as: football [7], swimming [3] and hockey on ice [4]. By contrast, there have been very few investigations about Vitamin D values in cyclists, and no study has considered athletes practicing this sport at our latitudes. Our study was performed in a group of male cyclists exercising in an area of Northern Italy and was aimed to measure circulating levels of 25(OH)D, Parathyroid hormone (PTH) at the beginning and the end of the sport season. We intended also to evaluate the possible association between hormone concentrations, physical performance and ventilatory efficiency.

Materials and Methods

Participants

We studied a group of 37 healthy male Caucasian amateur cyclists, living in Veneto, a region of Northern Italy. They were examined at the Unit of Sports Medicine, Department of Internal Medicine, University of Verona.

Design

The study was performed, at the beginning and at the end of the cyclist season. The first part took place in March, at the beginning of spring that in Northern Italy is linked to an increase in heat and duration of the sun during the day, while the second portion was performed in September towards the end of summer. In each experimental period, testing consisted of anthropometric measurements (i.e. height, body weight, body composition), blood testing for the assessment of Vitamin D and PTH levels, and cardiopulmonary test (CPET). During the three days before each experimental session, athletes were informed to avoid caffeine or alcohol beverages. All cyclists were familiarized with the testing protocol, as they had been previously tested with the same procedures on several occasions.

Ethics Statement

Before testing, the aim of the study and the testing procedures were explained in detail to all participants, and written informed consent was obtained. The study was performed in strict accordance with the ethical guidelines of the Helsinki Declaration and was approved by the Ethical Scientific Committee of the Sport Medicine Centre of University of Verona, Italy.

Anthropometrics Measurements and Body Composition

Participants were weighted in short-sleeved tee-shirt, shorts, and socks; stature was measured with a stadiometer to the nearest cm; BMI was calculated as body mass (kg)/stature(m^2). For body composition the bioelectrical impedance analysis was utilized, by using an Akern 101 instrument (Pontassieve, Florence, Italy). The device operates at a single frequency (50 kHz) and produces an alternating current of 800 microamperes through the body. The bioelectrical measures considered in this study are the resistance and reactance, from which fat mass (FM; kg) and fat free fass (FFM; kg) were derived using the regression formulas supplied by the manufacturer.

Lung Function Test

All cyclists underwent to lung function test with determination of the forced vital capacity (FVC; l), forced expiratory volume in the first second (FEV1; l / sec) and the ratio of Tiffeneau index (FEV1/FVC). All parameters were determined by using a portable turbine spirometer (Cosmed, Rome, Italy). The values obtained were compared with the theoretical ones using data from Quanjer [12].

Cardiopulmonary Exercise Testing

At the beginning and at the end of the season the cyclists performed an incremental maximal exercise test on a cycle ergometer with electromagnetic brake (Lode Corival). The breath by breath analysis of the expired gases and ventilation was performed by using ZAN 600 (ZAN MESSGERAETE GmbH, Oberthulva, Germany), which allows the instantaneous measurement of O_2 and CO_2 in the exhaled air. Before each test, the gas analyzer system was calibrated with two different concentrations of gas (15% O_2 and 5% CO_2, 21% O_2 and 0% CO_2). In the morning of the test, cyclists were asked to have a light breakfast and to abstain from caffeine and beverages. The protocol of the test was as follows: after a resting phase of 5 minutes, the subject underwent to an incremental ramp protocol, with an initial load of 40 watts and subsequent increments of 40 watts/minute, until exhaustion. During the test 12-lead ECG was recorded. In resting condition and during the test the following variables were recorded: Oxygen uptake (VO_2, L/min), CO_2 production (VCO_2, L/min), Ventilation (VE, L/min), Maximal heart rate (HR max). The results were expressed as VO_2max (measured value in the 10 final seconds at the highest level reached by the patient), VO_2 max/kg, and maximal Watt (Watt max, the maximum load reached at the end of the test). Also, the ventilatory equivalents for O_2 and CO_2 (VE/VO_2 and VE/VCO_2) were calculated. The VO_2max and Watt max were compared with normal values [13]. A test was considered maximal when three of the following criteria were satisfied at the time of: the plateau of VO_2 at peak exercise, the RER (VO_2/VCO_2)> 1.10, the peak heart rate ≥ 85% of the theoretical maximum frequency predicted by age and sex and reached exhaustion.

Vitamin D and PTH Measurements

25(OH)D serum concentration was measured by direct, chemiluminescent, type-competitive immunoassay (CLIA; Liaison, original assay, DiaSorin, Stillwater, MN, USA), which evaluate a range from 7- 150 ng / ml. The sensitivity of the method is 7 ng / ml. The coefficients of variation within and between assay are respectively 10.8% and 13.9%, and the normality range is between 30-80 ng / ml. PTH was measured by two-site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA); the coefficients of variation of the method are between 5.1% and 8.2%, and the normality range is between 10 -72 pg / ml.

Statistical Analysis

Results are reported as mean ± standard deviation (mean, SD) calculated by conventional procedures unless otherwise stated. The values of the different parameters measured at the beginning and at the end of the season were compared using t-tests for unpaired data. The relationships between serum hormone concentrations on one hand, and anthropometric, body composition, lung function and exercise parameters on the other, were evaluated by the Pearson coefficient. A linear regression model, considering the CPET parameters associated to Vitamin D at the univariate analysis as dependent variables, and age, FFM index, FEV_1/FVC, VO_2max/kg and Vitamin D values as determinants, was used in order to investigate the possible independent association between hormone serum levels and physical performance parameters. The level of significance was set at $p<0.05$. All calculations were performed using Microsoft Excel (Microsoft, Redmond, WA, USA) and SPSS software (version 20,0; IBM; SSPS, Chicago, IL, USA).

Results

Thirty-seven male cyclists (22-59 years, 1 smoker and 17 former smokers, BMI 24.7 ± 2.3) were studied. The anthropometric parameters did not change significantly between March and September. In lung volumes and the most relevant CPET parameters at the beginning and the end of the season are reported. No variation of FEV_1 and VC were found between March and September. On the contrary, a significant increase in VO_2 max ($p<0.001$) and Watt max ($p=0.001$) were found. Also, VE max, VE/VO_2 max and VE/VCO_2 max were significantly higher in September (all $p<0.001$). As far as Vitamin D serum concentration was concerned, we divided participants into 4 groups, considering ≥30ng/ml normal values (Group 1), and concentrations between 29 and 20 ng/ml (Group 2), 19 and 12 (Group 3) and <12 ng/ml (Group 4) indicative of insufficiency, deficiency and severe deficiency, respectively [8,9,14]. Figure 1 shows the percentage of subjects in each group in March and September. In March, the average levels of 25 (OH) D were 14.9 ± 6.5 ng/ml, and no athlete

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showed normal values. Nine athletes (24.3%) were in group 2, 12 (32.4%) in group 3, while the highest number of cyclists 16 (43.3%) were in group 4. At the same time, mean PTH circulating levels were 70.4 ± 26.7 pg/ml, and 13 athletes (35.1%) showed increased PTH concentration, i.e. PTH concentrations above the range normally considered in our laboratory (72 pg/ml) (Figure 2). In September, mean vitamin D serum levels (38.5±10.6 ng/ml) were significantly higher when compared to March (p<0.001). Over 70% of cyclists were in group 1, 7 (18.9%) in group 2, 2 (5.4%) in group 3 and none in group 4. A fairly high percentage of cyclists (16.2%), although lower than that recorded in March, presented PTH serum levels indicative of high concentration.

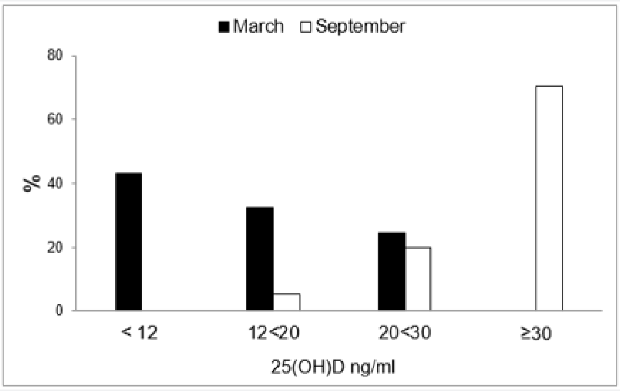

Figure 1: Values of 25(OH)D (ng/mL) in the cyclists studied, at the beginning (March, black colums) and at the end (September, white colums) of the competitive season. 25(OH)D values were divided into four groups, under 12 ng/ml, from 12 to 19 ng/ml, between 20-30 ng/mL and over 30 ng/mL.

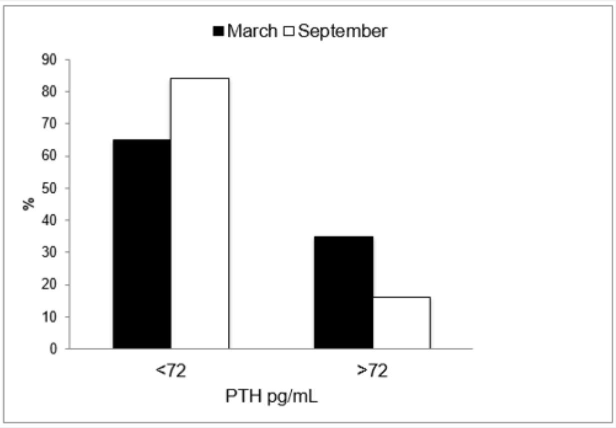

Figure 2: Values of PTH (pg/mL) in the cyclists studied, at the beginning (March, black colums) and at the end (September, white colums) of the competitive season. PTH values were divided into two groups, under 72 pg/mL and over 72 pg/mL.

Aiming at verifying whether 25(OH)D levels were associated with anthropometric parameters, we correlated hormone serum concentration with weight, height, BMI, FFM and FM in a bivariate analysis model. In March, a negative relationship was found between vitamin D and FM (r=-0.336, p=0.042) whereas no significant association was found in September. In March, no relationship was found between vitamin D serum levels and VO_2 max, Watt max, VE max, FC max and VE/VO_2 max. On the contrary, a negative relationship with VE/VCO_2 max (r=-0.363, p=0.027) and VE max (r=-0.362; p=0.028) (Figure 3) was found. No significant association between the same parameters was found in September. In a linear regression model, considering VE/VCO_2 max measured in March as dependent variable, and age, fat free mass (FFM index), airways patency (FEV_1/FVC), physical performance (VO_2max/kg) and Vitamin D values as possible determinants, only serum 25(OH)D and VO_2max/kg resulted as independent predictors (p=0.028 and p=0.020, respectively) (Tables 1-3).

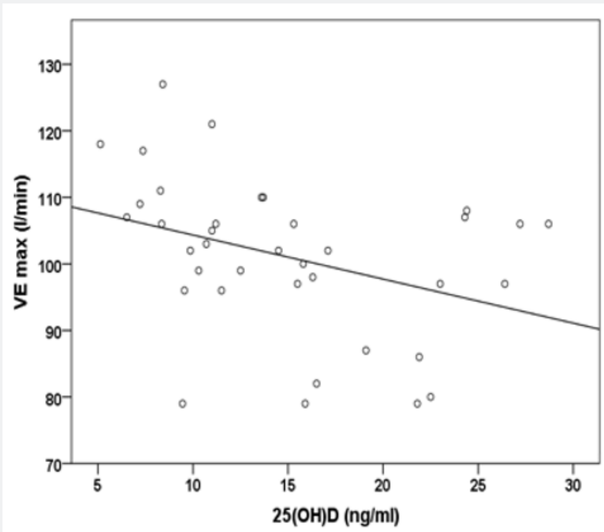

Figure 3: Relationship between 25(OH)D (ng/mL) and VEmax (l/min).

(Maximal ventilation during the cycloergometric test). R=0.362, p=0.028.

Table 1: Anthropometric data, body composition, VIT D and PTH values in 37 amateur cyclists.

Age (y)	40 ± 9	22 – 59
Height (m)	1.75 ± 6.80	1.61– 1.90
Weight (kg)	76.2 ± 8.2	61.9 – 94.2
BMI (kg/m^2)	24.7 ± 2.3	21.0 – 31.0
FM (kg)	13.7 ± 3.6	7.4- 21.6
FM index (kg/m^2)	4.5 ± 1.2	2.3 – 7.2
FFM (kg)	62.5 ± 6.3	51.9- 76.9
FFM index (kg/m^2)	20.3 ± 1.7	17.1- 23.8
Vit D (ng/ml)	14.9 ± 6.5	5.1-28.7
PTH (pg/ml)	70.4 ± 26.7	30.8-183.0

BMI (Body Mass Index); FM (Fat Mass); FM index (Fat Mass/height^2); FFM (Fat Free Mass); FFM index; (Fat Free Mass/height^2); Vit D (Vitamin D); PTH (Parathyroid hormone).

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Table 2: Values and differences in lung volumes, CPET parameters measured in March and September in 37 cyclists.

	March	September	Difference	95% CI	P
VC (l)	5.2±0.9	5.3±0.8	0.11	-0.49	0.339
FEV_1 (l)	4.5±0.9	4.5±0.8	0.04	-0.38	0.658
VO_2max (l/min)	3.8±0.4	4.2±0.3	0.39	0.22-0.56	<0.001
Watt max (Watt)	341.1±38.4	370.9±28.0	23.41	11.62-35.19	0.001
VE max (l/min)	101.1±11.8	119.8±17.7	16.41	9.53-23.29	<0.001
FR max (acts/min)	35.7±8.6	38.7±5.8	4.11	2.04-6.18	0.001
TV max (l)	2.9±0.6	3.1±0.4	0.09	-0.39	0.326
BR (l)	50.0±24.9	44.8±26.6	-7.68	-13.73	0.031
VE/VCO_2max	23.9±2.4	24.3±2.8	1.06	0.56-1.56	<0.001
VE/VO_2 max	25.7±2.9	26.9±3.2	2.11	1.22-2.97	<0.001
Slope VE/VCO_2	34.0±11.2	30.5±5.2	-5.97	-17.63	0.167
HR max (Hr/min)	174.5±9.1	173.0±8.6	-1.47	-5.68	0.289
O_2 Pulse (100ml/Hr*Kg)	29.8±4.5	32.9±3.5	3.08	1.56-4.59	0.001

VC (Vital capacity); FEV_1 (Expiratory volume.); VO_2 max (Maximum oxygen consumption); VE max (Maximal ventilation consumption); FR max (Maximal frequency respiration); TV max (Maximal tidal volume); BR (Respiratory reserve); VE/VCO_2 max (CO_2 maximal ventilatory equivalent); VE/VO_2 max (O_2 maximal ventilatory equivalent); HR max (Maximal Heart rate); O_2 Pulse (Maximal O_2 consumption to heart rate).

Table 3: Bivariate correlation between concentrations of 25 (OH) D and parameters measured at peak of CPET, in March and September.

	March		September	
	R	P	R	P
VO_2 max (l/min)	-0.156	0.356	-0.234	0.366
VO_2max/kg (ml/(kg/min)	0.066	0.71	-0.129	0.623
Watt max (Watt)	-0.166	0.325	0.015	0.954
VE max (l/min)	-0.362	0.028[a]	-0.124	0.637
FR max (acts/min)	-0.05	0.767	-0.249	0.335
TV max (l)	-0.059	0.727	0.174	0.505
BR (l)	0.281	0.092	0.209	0.436
VE/VCO_2 max	-0.363	0.027[a]	0.12	0.646
VE/VO_2 max	-0.293	0.078	0.022	0.934
Slope VE/VCO_2	0.041	0.823	-0.266	0.318
HR max	0.093	0.583	-0.132	0.613
HRR	0.01	0.912	-0.061	0.8
O_2 Pulse (100ml/Hr*Kg)	0.036	0.834	-0.088	0.738

Note: A denotes a significant difference correlation with p<0,05.

VO_2max (O_2 maximum consumption); VE max (Maximal ventilation under effort); FR max (Maximal respiratory frequency); TVmax (Maximal tidal volume); BR (Respiratory reserve); VE/VCO_2 max (CO_2 maximal ventilatory equivalent); VE/VO_2 max (O_2 maximal ventilatory equivalent); HR max (Maximal heart rate frequency);

HRR (Heart rate); O_2 Pulse (O_2 consumption to heart rate).

Discussion

The most important result in this study was the finding of low levels of 25(OH)D in all the athletes at the beginning of the sport season. Moreover, a high percentage of cyclists (43.3%) presented hormone values under 12 ng/ml, indicative of a severe deficit. Serum concentrations of Vitamin D improved significantly through the summer season, even if they remained lower than normal range in a great percentage of athletes. The detection of low levels of Vitamin D has no clear explanation and could be linked to different causes. Firstly, the deficit could be attributed to the place of residence of our athletes. All the subjects live in the Veneto, region of Northern-Eastern Italy. This area is located at 46° latitude. In winter the zenith inclination of the sun becomes more indirect in all regions over 35° latitude, and, consequently, UVB radiation is mostly absorbed or reflected by the atmosphere [15]. It is worth mentioning that the concentrations of airborne dust in this area are among the highest in the world [15]; this

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could be another reason for filtration of ultraviolet rays. Vitamin D from the diet is an important factor contributing to circulating levels of the hormone [16]. Unfortunately, we have no data on Vitamin D intake in the cyclists studied but we speculate that they would assume low quantities of the hormone, since the typical Mediterranean diet is lacking in it [9]. Moreover, the high ingestion of fibers and phytates, often observed in athletes, could also have reduced the absorption [17]. Another factor that could justify the low hormone concentrations, could be an accelerated catabolism of Vitamin D, related to intense physical exercise. In line with this hypothesis, a recent study demonstrated a decrease in circulating levels of Vitamin D after a period of combat training in military personnel [18].

There is a larger body of research into 25(OH)D deficit in the general population [6-9,19], compared to the studies that have examined this problem in athletes [20-25]. Bannert et al. [22] studying teenager gymnastics, found Vitamin D values lower than normal range in 77% of the sample. A high percentage of hypovitaminosis D was also found in Finnish athletes [23]. Bruce et al. [24] in a study performed on male athletes in the Middle-East, demonstrated that 25(OH)D deficiency is frequent even at those latitudes. In a small study conducted on seven cyclists, a 25(OH)D mean value of 32 ng/ml was found, surprisingly low for a sport performed in the open air [25]. A possible explanation for the negative relationship between circulating levels of 25(OH)D and FM ($r=-0.336$; $p=0.042$), is the fact that 25(OH)D, a lipophilic substance, is trapped in adipose tissue [26]. This hypothesis is supported by the finding that obese subjects often present circulating levels of 25(OH)D lower than normal range [27]. Taking into account the low Vitamin D levels, it was expected that a high percentage of our athletes would have secondary increased PTH concentration either in March (35.1%) or in September (16%). In these subjects, an increase in bone turnover is predictable. The increase in PTH could also affect the functioning and quality of muscles, by inducing atrophy of type II fibers by increasing catabolism of proteins, and by reducing intracellular content of phosphates, creatine-phosphate and calcium-ATPase in muscle cells [28]. Recent studies show a reduction in bone mineral density in cyclists, in comparison with sedentary controls or athletes of other sports [29-30]. Moreover, Barry & Kohrt demonstrated a decrease in bone mineral density during one year of training and competitions, in athletes practicing road cycling [31]. These authors suggested that many factors may contribute to bone mineral loss, such as down regulation of sexual hormones, together with an increase in stress hormones and pro-inflammatory cytokines [31]. We hypothesize that the reduction of bone mass in cyclists could be also due to the low levels of Vitamin D and to secondary high levels of PTH, frequent findings in our athletes.

Previous studies on athletes, mostly performed in north Europe, demonstrated the ergogenic effect of ultraviolet radiation exposure, leading to the speculation that this effect was secondary to increased Vitamin D synthesis. In contrast with this hypothesis, our study doesn't show any relationship between circulating levels of 25(OH)D and physical performance, expressed as peak VO_2 or maximal Watt. Our results also disagree with the results obtained by Koundurakis, who recently reported an association between vitamin D levels [10], muscle strenght and peak VO_2 in Greek professional football players. These contrasting results could be attributed to the different types of sport practiced by our subjects and the Greek athletes. In our study the cyclists were not professional and the lower mean values of Vitamin D in our subjects may justify the contrasting finding; the narrow range of 25(OH)D levels may make it difficult to demonstrate a relationship between the hormone and physical performance parameters. An interesting and original finding of our study is the statistically significant negative relationship between 25(OH)D serum levels and VE/VCO_2.

Our result could be related to a reduction in the sensitivity of respiratory centers to CO_2 or to lactic acid, induced by the Vitamin D. It is also possible that a reflex from muscles could influence ventilation and VE/VCO_2 ratio (32) and that Vitamin D could modulate this reflex during exercise through its positive effect on muscle strength and function. The above mentioned hypotheses are merely speculative, and neither our results, nor data from literature allow any definitive conclusions. The fact that no relationship between Vitamin D and VE/VCO_2 was found in September, could be due to the confounding effect of training. Our study has some limits. First of all, our athletes presented a narrow range of Vitamin D values, especially in March, a fact that makes it difficult to evaluate correlations with parameters of physical performance. Another limit might be the fact that we didn't collect a precise dietary information from our athletes, making it impossible to determine exactly whether the low levels of 25(OH) D were subsequent to a reduced oral intake and the increase linked to supplementation. Furthermore, training load or time spent outdoors were not quantified. Finally, the cross-sectional nature of the study doesn't allow us to establish if a cause-effect relationship between 25(OH)D levels and ventilatory parameters.

Conclusion

Hypovitaminosis D, often associated with an increase in PTH, is a frequent finding in cyclists practicing outdoor activities at our latitude, both in early spring and at the end of training season. This indicates the need for Vitamin D supplementation in this group of athletes. 25(OH)D levels do not correlate with aerobic power parameters, whereas they are inversely related to VE/VCO_2, suggesting a possible modulating effect of vitamin D on ventilation.

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