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*Original Article*

# Wintertime Vitamin D Deficiency in Male Adolescents: Effect on Parathyroid Function and Response to Vitamin $D_3$ Supplements

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**Abstract.** The first part of this study consisted of an 18 month follow-up of the vitamin D status and parathyroid function in a group of 54 French male adolescents, aged from 13 to 16 years old and all pupils of a jockey training school. During the 18 month period four samplings were made, one every 6 months. The first was during September of the first year, the second and third during March and October of the second year, and the last in March of the third year. Therefore we had two main periods: summer and winter. The summer 25-hydroxyvitamin D (25(OH)D) concentrations were higher (71.6 ± 19.9 and 52.4 ± 16.5 nmol/l) than the winter ones (20.4 ± 6.9 and 21.4 ± 6.1 nmol/l). Conversely, the winter intact parathyroid hormone (iPTH) serum levels (4.18 ± 1.18 and 4.11 ± 1.35 pmol/l) were higher than the summer ones (2.44 ± 0.82 and 2.71 ± 0.71 pmol/l). At the two winter time points the 25(OH)D concentrations were lower than 25 nmol/l (10 ng/ml) in 72% (2nd year) and 68% (3rd year) of the adolescents. In the second part of the study we tried a vitamin $D_3$ supplementation procedure designed to maintain the 25(OH)D and iPTH postsummer serum levels throughout the winter. Pairs of male adolescents matched for height, weight and Tanner pubertal stage were randomly assigned to either vitamin $D_3$ supplementation (2.5 mg, i.e., 100 000 IU) administered orally at three specific periods (end of September, November and January) or no vitamin $D_3$ treatment (control subjects). Blood was collected just before the first intake of vitamin $D_3$ and 2 months after the last intake (March). The control subjects had blood drawn at the same time points. In the vitamin $D_3$-treated subjects, the concentrations of 25 (OH)D (55.3 ± 11.5 nmol/l) and of iPTH (3.09 ± 1.16 pmol/l) in March and September (53.8 ± 12.3 nmol/l and 2.75 ± 1.26 pmol/l) were not significantly different. In the control subjects, March 25(OH)D levels (21.0 ± nmol/l were low, with values below 25 nmol/l in 78% of subjects, and iPTH concentrations (3.97 ± 1.08 pmol/l) were significantly ($p < 0.001$) higher than in September (2.91 ± 0.81 pmol/l). The constant vitamin D wintertime deficiency and wintertime rise in iPTH in adolescent French males throughout puberty has been demonstrated. In adolescents with low dairy calcium intakes, the vitamin $D_3$ treatment was sufficient to maintain 25(OH)D concentrations at their summer levels throughout winter and to prevent an excessive wintertime rise in iPTH levels.

**Keywords:** Calcidiol; Parathyroid hormone; Puberty; Vitamin D

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## Introduction

Vitamin D is essential for the normal growth and development of bone. During puberty the growth burst is concomitant with an increase in bone mass which implies an increased need for calcium and probably for vitamin D. In Western Europe very low vitamin D stores and rickets have mainly been described in Asian or in African migrant adolescents. The causes for rickets in these young people were thought to be skin pigmentation and/or low dietary vitamin D and calcium intake [1]. In these studies, rickets occurred rarely in white adolescents and a possible vitamin D deficiency in Caucasian

adolescents was overlooked. Nevertheless, in the Caucasian population achieving an optimal peak of bone mass [2] has replaced the prevention of rickets as a concern. As vitamin D status can be appraised from plasma values of 25-hydroxyvitamin D (25(OH)D), subclinical states of vitamin D deficiency or insufficiency can currently be detected.

In a previous report we measured the concentrations of 25(OH)D both at the end of the summer and 6 months later, at the end of the winter, in a group of Caucasian male adolescents [3]. In spite of the fact that, after summer, the 25(OH)D serum concentrations were high enough to suggest that the vitamin D stores were replete, they had decreased to very low levels at the end of winter. Meanwhile iPTH mean concentrations were significantly higher after winter than after summer. In the above study the adolescents were studied over a short period. We decided, therefore, to follow a larger group of Caucasian French adolescents for 18 months, so that many of them would have achieved puberty. The adolescents were studied twice a year: after summer when sun exposure is at its highest, and after winter, a period during which, in northern France, the cutaneous synthesis of vitamin D is stopped. Furthermore, a wintertime vitamin $D_3$ treatment designed to maintain the 25(OH)D serum concentrations at their summer levels throughout winter and to prevent a rise in iPTH concentrations was tested on adolescents.

## Materials and Methods

### *Subjects*

*Seasonal Variations.* Fifty-four male adolescents from a jockey training center (Le Moulin à Vent, Gouvieux, France) completed the study. The jockey training school is located in a country area in the north of Paris (latitude 49° N). The study began in the autumn at their training school enlistment. At the start of the study, they were aged from 13 years 5 months to 16 years 1 month (mean ± SD): 14 years 3 months ± 6 months). Their state of health was assessed by physical examination and measurement of 20 biochemical blood variables. Their mean (± SD) total serum calcium and serum phosphate were respectively 2.44 ± 0.09 mmol/l and 1.55 ± 0.16 mmol/l. Their daily estimated calcium intakes from dairy products was 809 ± 409 mg (175–2150 mg). They were studied for 18 months, at about 6 month intervals, after summer (either in mid-September or at the end of October) and at the end of winter (end of March). At each time point their height and weight were measured, their pubertal status was determined and blood was collected between 0600 and 0700 hours for 25(OH)D and iPTH measurement.

*Vitamin $D_3$ Treatment.* Fifty-eight male Caucasian adolescents (age from 13 years 10 months to 17 years 3 months; mean ± SD 15 years ± 10 months), all pupils in the same jockey training school, were included. They were paired according to their height, weight and Tanner pubertal stage. In each pair one subject was randomly assigned to vitamin $D_3$ supplementation while the other received no vitamin $D_3$ and served as a control. In one pair a control subject was left out of the study. The study was conducted by the end of the summer (end of September) and 6 months later, at the end of the winter (end of March). At each time point height and weight were measured and blood was collected in the morning between 0600 and 0700 hours for measurement of serum 25(OH)D, iPTH and calcium. Vitamin $D_3$ was administered three times (end of September, November and January), the interval between two doses being 2 months. At each time point the oral intake of vitamin $D_3$ was 2.5 mg, or 100 000 IU, as a phial of water-soluble oral solution (Uvédose, Laboratoires Crinex, Montrouge, France). The first blood sample was collected just before the first dose of vitamin $D_3$ and the second blood sample 2 months after the last dose.

The experimental protocols were approved by the local hospital ethics committee and informed written consent was obtained from the parents of the minors.

### *Methods*

The height and weight of each subject were measured with the subject standing barefoot and lightly clothed. All the subjects underwent a physical examination by a physician specialized in sports medicine and their pubertal stage was determined using Tanner's criteria [4]. Current calcium intake from dairy products was assessed by means of weekly dietary history. Responses were checked by a research dietitian using models of food portion sizes. The mean daily intake of calcium was analyzed using food tables established in France [5].

*Analyses.* 25(OH)D was measured by a competitive protein-binding assay after extraction and chromatographic purification (Amersham International, Amersham, UK). The within-assay variability (CV) of this method was less than 9% for low (19.5 nmol/l) and for medium (57.5 nmol/l) concentrations; the between-assay CV ranged from 7% to 10%. Serum PTH1–84 was measured by immunoradiometric assay for intact PTH (Nichols Institute, San Juan Capistrano, *C*A). The within-assay CV was less than 4% and between-assay CV less than 6%. The normal range for men ($n$ = 39), aged 23–27 years, was 1.37–5.26 pmol/l.

*Hypovitaminosis D: Definitions.* Vitamin D deficiency was defined as 25(OH)D concentrations below 25 nmol/l and vitamin D insufficiency as 25(OH)D below 50 nmol/l according to McKenna and Freaney [6].

*Statistics.* The data were expressed as mean ± SD unless otherwise stated. Within-subject values measured at two time points were analyzed by paired *t*-test. Comparisons between unpaired groups of variables were performed using an unpaired *t*-test. The statistical program used was StatView 512+ (BrainPower Inc., Calabasas, CA).

## Results

### *Seasonal Variations*

Fifty-four boys completed the 18-month follow-up; 5 subjects dropped out after 6 months for reasons unrelated to the trial design. The anthropometric data are presented in Table 1. We note that these subjects were short and thin in accordance with the criteria of enlistment in the jockey training school (height and weight between 1 and 1.5 SD below the mean; parents of small stature). Seasonal variations in 25(OH)D and iPTH are presented in Table 2. The concentrations of 25(OH)D decreased from September to March, rose again by October and declined again in March. At the two winter time points 72% and 68% of subjects had 25(OH)D concentrations below 25 nmol/l, while 1 subject only had a low value after summer.

As shown in Table 2, serum iPTH seasonal variations were in opposite phase with 25(OH)D. From September to March the serum concentrations of iPTH rose before returning to summer values in October and again to winter values in March.

**Table 1.** Anthropometric variables measured at 6 month intervals during 18 months in male adolescents

| | No. | Height (cm) | Weight (kg) | Mean Tanner stage |
|---|---|---|---|---|
| September | 54 | 151.34 ± 6.71 | 39.32 ± 4.76 | 2.79 ± 1.20 |
| March | 54 | 154.33 ± 7.18 | 41.55 ± 5.51 | 3.37 ± 1.20 |
| October | 54 | 157.46 ± 7.30 | 43.22 ± 7.99 | 3.91 ± 1.20 |
| March | 54 | 159.92 ± 6.38 | 46.13 ± 5.22 | 4.40 ± 0.94 |

Results are mean ± SD.

**Table 3.** Anthropometic variables measured in September and in March in vitamin $D_3$-treated ($n$ = 29) and untreated ($n$ = 28) male adolescents

| | Height (cm) | Weight (kg) | Mean pubertal stage |
|---|---|---|---|
| *Untreated group* | | | |
| September | 156.2 ± 7.4 | 43.7 ± 5.9 | 3.9 ± 1.1 |
| March | 159.0 ± 6.9 | 46.3 ± 5.5 | 4.2 ± 0.9 |
| *Vitamin $D_3$-treated group* | | | |
| September | 155.2 ± 8.2 | 43.4 ± 7.4 | 3.9 ± 1.3 |
| March | 157.3 ± 8.2 | 45.8 ± 8.2 | 4.1 ± 1.1 |

Results are mean ± SD.

### *Vitamin $D_3$ Treatment*

The anthropometric data of the adolescents are presented in Table 3. As a consequence of our randomization procedure those receiving vitamina $D_3$ treatment and untreated subjects did not differ in terms of height, weight or mean pubertal stage. The time courses of changes in serum 25(OH)D and iPTH are presented in Fig. 1. From September to March, non-treated subjects showed a very significant decrease in 25(OH)D ($p$ = 0.0001), with 78% of values below 25 nmol/l, and a very significant increase in iPTH ($p$ = 0.0001). Meanwhile, in the vitamin $D_3$-treated group,, both 25(OH)D and iPTH concentrations remained at the same level at the end of winter as after summer. Concentrations of 25(OH)D in September (53.7 ± 12.2 vs 61.0 ± 15.5 nmol/l) did not differ significantly between the two groups while they were significantly ($p$ = 0.0001) different in March (55.2 ± 11.5 vs 20.2 ± 0.5 nmol/l). Concentrations of iPTH did not differ significantly in September (2.81 ± 1.26 vs 2.64 ± 0.85 pmol/l) but were significantly different in March (3.02 ± 0.95 vs 4.15 ± 1.15 pmol/l; ($p$ = 0.0002) between the two groups.

## Discussion

After summer (September) mean 25(OH)D concentrations were 72 nmol/l, attesting that the vitamin D stores were replete, while after winter (March) mean serum

**Table 2.** Seasonal variations of serum concentrations of 25(OH)D and iPTH measured at 6 month intervals during 18 months in 54 male adolescents

| | 25(OH)D (nmol/l) | | iPTH (pmol/l) | |
|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range |
| September | 71.6 ± 19.9 | 40.5–120.0 | 2.44 ± 0.82 | 1.00–4.8 |
| March (1st year) | 20.4 ± 6.9 | 8.0–35.7 | 4.18 ± 1.18 | 1.73–7.18 |
| October | 52.4 ± 16.3 | 20.0–98.7 | 2.71 ± 0.71 | 1.42–4.0 |
| March (2nd years) | 21.4 ± 6.1 | 10.7–41.2 | 4.11 ± 1.35 | 1.63–7.42 |

25(OH)D, 25-hydroxyvitamin D; iPTH, intact parathyroid hormone.

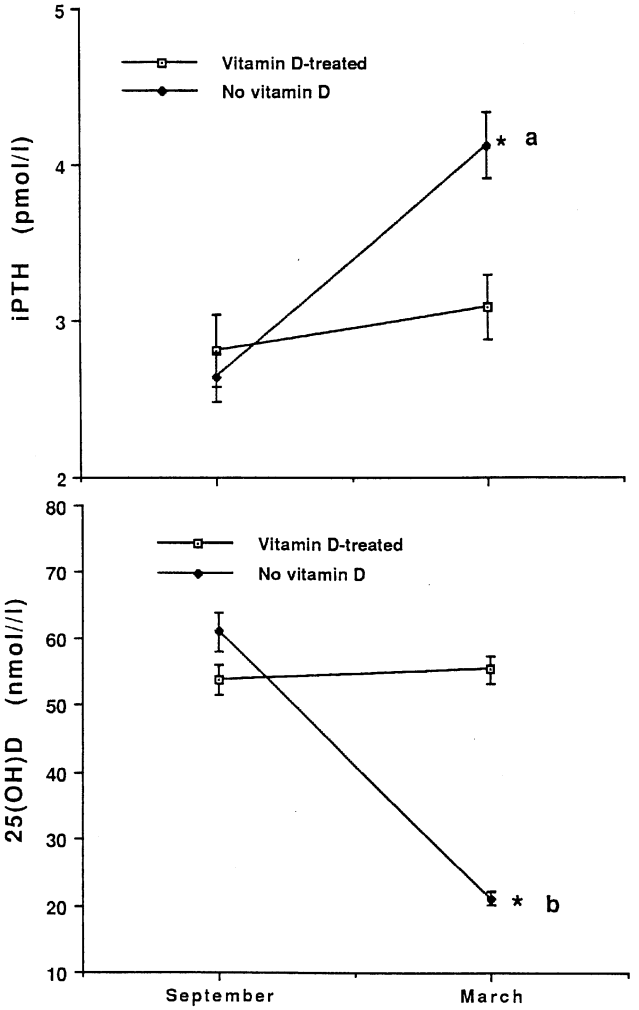


**Fig. 1.** Mean serum concentrations of 25-hydroxyvitamin D (25(OH)D; *bottom*) and intact parathyroid hormone (iPTH; *top*) measured at the end of summer (September) and of winter (March) in 29 male adolescents who received 2.5 mg (100 000 IU) of vitamin D3 at the end of September, November and January (*open symbols*) and in 28 male adolescents who did not receive vitamin D (*filled symbols*). Values are mean ± SEM. Difference between September and March values (paired *t*-test): $*p = 0.0001$. Difference between vitamin D-treated and untreated subjects (unpaired *t*-test): $^{a}p = 0.0013$; $^{b}p = 0.0001$.

25(OH)D concentrations fell to 20.2 and 21.3 nmol/l. Only a few authors have reported that, in adolescents, low serum 25(OH)D concentrations in winter can follow good summer repletion of the vitamin D stores: In 29 subjects aged 11–17 years, Ala-Houhala et al. [7] observed in the south of Finland mean 25(OH)D concentrations of 26.1 ng/ml (65.2 nmol/l) in summer and 8.3 ng/ml (20.7 nmol/l) in winter; later, in 9- to 15-year-old Finnish girls not taking vitamin D supplementation, Lehtonen-Veromaa et al. [8] found that mean serum 25(OH)D concentrations were 62.7 nmol/l in August–September and 31.2–32.9 nmol/l in February–March. These authors failed to prevent the wintertime hypovitaminosis D by 3 months of vitamin D (10 μg/day) supplementation in spite of mean calcium intakes as high as 1256–1680 mg/day. However, in the above-mentioned studies the authors did not measure serum iPTH concentrations and therefore could not establish a relationship between the low winter levels of 25(OH)D and the rise in iPTH. Furthermore, by contrast with the present study, they did not consider the pubertal stage and included prepubertal subjects. The desirable serum levels of 25(OH)D, based upon the relationship between the serum concentrations of 25(OH)D and iPTH, have been determined for elderly people [6] and middle-aged adults [9]. According to the same criterion we used in a previous study [10], a nonlinear regression analysis to determine the desirable levels of 25(OH)D in male adolescents, we found a threshold of 30 nmol/l under which iPTH levels increase abruptly. Elevated levels of iPTH during winter, concomitant with low 25(OH)D, have been observed in elderly people [11], in children [12] and in adolescents [3]. While the deleterious effects in elderly people of hypovitaminosis D-induced secondary hyperparathyroidism, as well as the benefits of vitamin D supplementation, are well documented [13], there has been, to our knowledge, no previous study demonstrating unequivocally the suppressive effect on the wintertime rise in iPTH of vitamin $D_3$ supplementation in healthy adolescents.

In growing subjects and particularly in adolescents a crucial point is to achieve the maximal peak bone mass, which is an important determinant for prevention of the future osteoporotic fracture risk. During the pubertal growth spurt the increase in intracortical bone turnover coincides with the peak incidence of lower forearm fractures [14]. Since it has been shown that, during growth, reduced rates of remodeling are associated with increased bone density [15] high levels of PTH could result in increased bone remodeling, increased bone fragility and a future increased risk of fracture.

In the present study, vitamin $D_3$ supplements were sufficient to maintain the concentrations of both 25(OH)D and PTH at their basal postsummer levels when measured 2 months after the last intake. In France the vitamin D intake from food is low (4.0 μg/day in men according to the French SUVIMAX nutritional project [9]) and at latitude 49° N sunshine irradiation is very low from October to March. We can, therefore, assume that the decrease in 25(OH)D in non-treated subjects fitted the consumption of 25(OH)D and that in vitamin $D_3$-treated adolescents the requirement for vitamin $D_3$ was provided by the treatment. It should be emphasized that these vitamin $D_3$ doses were well tolerated and did not lead to an undesirable rise in 25(OH)D concentrations and hypercalcemia, as had been demonstrated by us in a preliminary study [16] and by others too [17]. With the exception of severe vitamin D deficiency resulting in rickets and osteomalacia commonly associated with serum levels of 25(OH)D below 15 nmol/l, the indication for vitamin D supplementation during winter to adolescents living in countries where the vitamin D dietary intakes are low and sun irradiation limited to summer months is an open question. However, we can note that: (1) in our subjects whose calcium intakes are

relatively low, vitamin D-dependent active transport is predominant for calcium intestinal absorption and (2) current intakes of vitamin D during adolescence have been recognized as one of the factors affecting positively bone density in adults [18]. As a consequence, the wintertime vitamin D deficiency could add its deleterious effects to those of low dietary intakes of calcium, leading together to PTH-dependent increase in bone remodeling and disturbances in bone metabolism during the pubertal bone growth spurt. This vitamin $D_3$ treatment could represent an easily implemented option for adolescents with low dietary calcium intakes, with the hope of achieving an optimal peak bone mass.

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