

# Effect of Vitamin D Supplementation on Testosterone Levels in Men

## Authors

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## Abstract

The male reproductive tract has been identified as a target tissue for vitamin D, and previous data suggest an association of 25-hydroxyvitamin D [25(OH)D] with testosterone levels in men. We therefore aimed to evaluate whether vitamin D supplementation influences testosterone levels in men. Healthy overweight men undergoing a weight reduction program who participated in a randomized controlled trial were analyzed for testosterone levels. The entire study included 200 nondiabetic subjects, of whom 165 participants (54 men) completed the trial. Participants received either 83 µg (3332 IU) vitamin D daily for 1 year (n=31) or placebo (n=23). Initial 25(OH)D concentrations were in the deficiency range (<50 nmol/l) and testosterone values were at the lower end of the reference range

(9.09–55.28 nmol/l for males aged 20–49 years) in both groups. Mean circulating 25(OH)D concentrations increased significantly by 53.5 nmol/l in the vitamin D group, but remained almost constant in the placebo group. Compared to baseline values, a significant increase in total testosterone levels (from 10.7±3.9 nmol/l to 13.4±4.7 nmol/l; p<0.001), bioactive testosterone (from 5.21±1.87 nmol/l to 6.25±2.01 nmol/l; p=0.001), and free testosterone levels (from 0.222±0.080 nmol/l to 0.267±0.087 nmol/l; p=0.001) were observed in the vitamin D supplemented group. By contrast, there was no significant change in any testosterone measure in the placebo group. Our results suggest that vitamin D supplementation might increase testosterone levels. Further randomized controlled trials are warranted to confirm this hypothesis.

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## Bibliography

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## Introduction

Vitamin D deficiency is currently considered an important public health problem being associated with musculoskeletal diseases, cardiovascular disease, cancer, and infectious and autoimmune diseases [1]. The vitamin D receptor (VDR), as well as key enzymes for vitamin D metabolism, are widely expressed in human tissues and cells [2]. In this context, Blomberg Jensen et al. [3] observed significant expressions of the VDR and vitamin D metabolizing enzymes in the male reproductive tract including Leydig cells of the testis. These data raised the question whether vitamin D is able to influence male reproductive hormone production. The existence of such an effect is supported by previous studies suggesting that vitamin D deficiency may contribute to reduced fertility and hypogonadism

[2,3]. These results are of particular interest because both, vitamin D deficiency and hypogonadism are associated with skeletal diseases (e.g., osteoporosis or muscle weakness) as well as extra-skeletal disorders (e.g., cardiovascular disease or obesity) [1,4,5]. Recently, some of us [6] have shown in 2299 men referred for coronary angiography that 25-hydroxyvitamin D [25(OH)D] levels are significantly associated with testosterone levels and that both hormones reveal similar seasonal variations with a peak at the end of summer. Whether there exists a causal link between vitamin D and testosterone status is, however, currently not known. Therefore, a subgroup analysis of a previously published prospective, randomized vitamin D supplementation trial was performed in overweight subjects [7]. Here, we present results on serum testosterone concentrations in the male participants of this study.

\* Both authors contributed equally to the present work.

**Table 1** Characteristics of the study groups at baseline and at the end of the study

Parameter	Placebo group		Vitamin D group		p-Value		
	Baseline	Study end	Baseline	Study end	2 vs. 4	2 vs. 3	4 vs. 5
Number	23		31		–	–	–
Age (years)	46.8±12.0		49.4±10.2		0.387	–	–
Smokers (%)	56.5		38.7		0.271	–	–
Alcohol (g/d)	20.0±19.5	14.1±15.3	17.7±15.1	15.3±13.8	0.646	0.138	0.703
25(OH)D (nmol/l)	29.7±23.7	35.5±8.1	32.5±20.0	86.4±68.8	0.659	0.215	<0.001
1,25(OH) <sub>2</sub> D (pmol/l)	77.0±25.9	97.4±32.9	96.0±39.6	127.7±94.3	0.053	0.027	0.100
PTH (pmol/l)	5.07±3.63	4.13±1.48	4.14±1.98	3.54±1.76	0.237	0.035	0.040
Body weight (kg)	105.7±14.3	99.0±13.5	109.9±16.1	104.0±17.2	0.323	<0.001	<0.001
BMI (kg/m <sup>2</sup> )	32.5±3.8	30.5±4.1	33.1±3.9	31.2±3.9	0.609	<0.001	<0.001
Albumin (mmol/l)	386±182	302±125	377±194	297±186	0.087	0.210	0.896
SHBG (mmol/l)	26.3±13.7	29.5±17.3	31.0±10.3	35.3±13.6	0.153	0.046	0.002
TT (nmol/l)	11.8±4.0	12.7±5.5	10.7±3.9	13.4±4.7	0.317	0.355	<0.001
BAT (nmol/l)	6.39±2.22	6.59±2.33	5.21±1.87	6.25±2.01	0.040	0.626	0.001
fT (nmol/l)	0.264±0.087	0.278±0.097	0.222±0.080	0.267±0.087	0.067	0.532	0.001

Data are shown as means±SD. Inter-group comparisons were performed by unpaired *t*-test and intra-group comparisons by paired *t*-test

25(OH)D: 25-hydroxyvitamin D; 1,25(OH)<sub>2</sub>D: 1,25-dihydroxyvitamin D; PTH: parathyroid hormone; BMI: body mass index; SHBG: sex-hormone binding

## Subjects and Methods

Study subjects were derived from a weight reduction program over 12 months, which included a daily supplementation of either 83 µg (3 332 IU) vitamin D or placebo as part of a double-blind randomized controlled trial [7]. Details on the study design and major outcomes of the trial have been published previously [7]. Out of 200 nondiabetic individuals (62 men) who were included in the study, 165 (54 men) completed the trial. Only male study subjects were analyzed for the present work. The number of dropouts (3 men in the placebo and 5 men in the vitamin D group) did not differ significantly between groups ( $p=0.745$ ). The original study was registered at clinicaltrials.gov as NCT004493012.

Participants were continuously recruited from December 2005 to October 2006 throughout the year. Fasting blood samples were drawn at study begin and after 1 year. Specimens were centrifuged at room temperature. Thereafter, serum aliquots were stored at  $-80^{\circ}\text{C}$  until analyses. To avoid inter-assay variations, the samples of each participant were analyzed within the same assay run. Concentrations of 25(OH)D were determined by means of a radioimmunoassay (DiaSorin, Stillwater, MN, USA) with an intra-assay CV of <7%. According to Holick [1], vitamin D deficiency is defined as a 25(OH)D level of less than 50 nmol/l, whereas a level of 52.5–72.5 nmol/l indicates a relative insufficiency, and a level of 75 nmol/l or greater indicates sufficient vitamin D. The serum concentrations of 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] were measured by a test kit provided by Immunodiagnostik (Bensheim, Germany). The serum levels of parathyroid hormone (PTH), total testosterone (TT), and sex hormone binding globulin (SHBG) were analyzed by using the Immulite 2000 system (Siemens, Munich, Germany). The reference range for total testosterone is 9.09–55.28 nmol/l for males aged 20–49 years and 6.28–26.30 nmol/l for males aged  $\geq 50$  years. The SHBG reference range for males is 13–71 nmol/l. The within-run and total coefficients of variation for SHBG and testosterone are 2.5% and 5.2%, respectively. Serum albumin was measured by using the Architect autoanalyzer (Abbott, Wiesbaden, Germany). Bioactive testosterone (BAT; reference range: 2.14–13.60 nmol/l) and free testosterone (fT; reference range: 0.090–0.580 nmol/l) were calculated according to Vermeulen et al. [8].

Baseline characteristics stratified by treatment group (vitamin D vs. placebo) are presented as means±SD for continuous variables. Intra-group comparisons (paired *t*-test) rather than inter-group comparisons were used at the end of the study because no sex-stratified randomization at baseline was performed. Statistical analyses were performed by SPSS 16.0 (SPSS Inc, Chicago, USA) and a *p*-value below 0.05 was considered statistically significant.

## Results

Baseline characteristics of the 54 male patients who completed the trial are shown in **Table 1**. At study entry, mean 25(OH)D concentrations were in the deficiency range in both groups. During follow-up, weight loss was  $5.9\pm 5.3$  kg ( $p<0.001$ ) in the vitamin D group and  $6.6\pm 5.7$  kg in the placebo group ( $p<0.001$ ), and thus similar in both groups. Circulating 25(OH)D increased by  $53.5\pm 65.3$  nmol/l to  $86.4\pm 68.8$  nmol/l in the vitamin D group ( $p<0.001$ ), but increased only nonsignificantly in the placebo group (increase by  $5.8\pm 21.3$  nmol/l to  $35.5\pm 18.0$  nmol/l;  $p=0.215$ ). PTH decreased in the placebo and vitamin D group (decrease by  $0.94\pm 3.09$ ;  $p=0.035$ , and  $0.60\pm 1.67$ ;  $p=0.040$ , respectively), whereas 1,25(OH)<sub>2</sub>D tended to increase in both groups (increase by  $20.4\pm 41.0$ ;  $p=0.027$  and  $21.7\pm 106.0$ ;  $p=0.100$ , respectively). At baseline, mean testosterone values were at the lower end of the reference range in both groups. By comparing baseline testosterone values with follow-up values in the placebo group no significant change in TT ( $11.8\pm 4.0$  nmol/l vs.  $12.7\pm 5.45$  nmol/l,  $p=0.355$ ), BAT ( $6.39\pm 2.22$  nmol/l vs.  $6.59\pm 2.33$  nmol/l,  $p=0.626$ ) or fT ( $0.264\pm 0.087$  nmol/l vs.  $0.278\pm 0.097$  nmol/l,  $p=0.532$ ) was found. In the vitamin D group, however, a significant increase in all measures of testosterone status was observed. TT increased from  $10.7\pm 3.9$  nmol/l to  $13.4\pm 4.7$  nmol/l ( $p<0.001$ ), BAT from  $5.21\pm 1.87$  nmol/l to  $6.25\pm 2.01$  nmol/l ( $p=0.001$ ) and fT from  $0.222\pm 0.080$  nmol/l to  $0.267\pm 0.087$  nmol/l ( $p=0.001$ ). In the placebo group, there were nonsignificant trends for seasonal differences in 25(OH)D and testosterone values. Compared with men recruited in the summer half-year (mid April to mid October;  $n=12$ ), men recruited in the winter half-year (mid October to mid April;  $n=11$ ) had lower values of 25(OH)D ( $21.8\pm 9.8$  nmol/l vs.  $37.4\pm 30.0$  nmol/l;

$p=0.113$ ), TT ( $11.5 \pm 4.33$  nmol/l vs.  $13.29 \pm 4.15$  nmol/l;  $p=0.336$ ), BAT ( $6.04 \pm 1.91$  nmol/l vs.  $7.37 \pm 2.58$  nmol/l;  $p=0.173$ ), and fT ( $0.255 \pm 0.078$  nmol/l vs.  $0.301 \pm 0.104$  nmol/l;  $p=0.250$ ).

In the 54 men, body mass index changes were inversely related to SHBG levels ( $r=-0.485$ ;  $p<0.001$ ), but not to other indices of testosterone status.

## Discussion

In overweight men with deficient vitamin D status a significant increase in testosterone was observed after intake of 83  $\mu$ g vitamin D daily for 1 year whereas there was no significant change in men receiving placebo. This work is, to the best of our knowledge, the first study, which specifically addresses the effect of vitamin D supplementation on androgens in men. The results of this study suggest that vitamin D supplementation might increase testosterone levels in men. Our data support several experimental and clinical findings: First, VDR knockout mice suffer from hypergonadotropic hypogonadism [2]. Second, vitamin D status is directly associated with testosterone levels in men [6]. Third, the male reproductive tract is a target tissue for vitamin D effects [3]. The nonsignificant trend for seasonal differences in both 25(OH)D and testosterone in the placebo group supports our hypothesis of a vitamin D effect on testosterone.

In our study participants, mean baseline 25(OH)D values were in the deficiency range and mean testosterone values were at the lower end of the reference range. Traditionally, low solar ultraviolet B irradiation of the skin is a major cause of vitamin D deficiency [1]. Both, vitamin D [1] and testosterone [5,9] show beneficial effects on the musculoskeletal system. From an evolutionary point of view it would make sense that an active lifestyle (leading to an adequate skin synthesis of vitamin D) also has beneficial effects on muscle function, bone health, and the male reproductive system. We are aware that no final conclusions can be drawn from our study regarding the effect of vitamin D supplementation on testosterone in men but we do believe that our work is of great importance because it provides a reasonable rationale for future studies. Besides the marked increase in 25(OH)D levels in the vitamin D group, there was also a slight (nonsignificant) increase in 25(OH)D in the placebo group during follow-up. We assume that the similar decrease in PTH and the similar trend for an increase in 1,25(OH)<sub>2</sub>D in both study groups is due to a nonlinear association of these 2 calcitropic hormones with increasing circulating 25(OH)D levels [10], with a pronounced effect at low and virtually no effect at high 25(OH)D levels. Nevertheless, the similar changes in these hor-

mones do not exclude group-specific effects on the reproductive system, since nonclassical target tissues for vitamin D largely depend on circulating 25(OH)D levels [1], which differed markedly between the vitamin D and placebo group.

Our study has both strengths and limitations. Strengths are the study design, the use of a daily vitamin D dose that was effective to increase 25(OH)D values from the deficiency range into the adequate range, and the fact that sample batching was performed to avoid inter-assay variability. One limitation is the fact that the effect of vitamin D supplementation on testosterone was not a prespecified study outcome and that we did not assess testosterone-related functions such as libido, mood, or muscle strengths. Another limitation is the relatively small number of male study participants. In addition, future studies have to clarify whether the vitamin D actions are mediated by a pituitary effect or a testicular one.

In conclusion, our study results suggest that vitamin D supplementation might increase testosterone levels in men. Further randomized controlled trials are needed to confirm this hypothesis and to evaluate whether vitamin D driven increases in testosterone levels contribute to the vitamin D effects on various health outcomes.

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