Vitamin D supplementation and testosterone concentrations in male human subjects


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Abstract

Objective
A possible association between serum 25-hydroxyvitamin D and testosterone levels has been reported; however, contradictory results have emerged.

Design
To investigate a causal link between vitamin D and testosterone status, we studied the effect of vitamin D supplementation on serum testosterone concentrations in three independent intervention studies including male patients with heart failure (study 1), male nursing home residents (study 2) and male non-western immigrants in the Netherlands (study 3).

Methods
In study 1, 92 subjects were randomized to either vitamin D (2000 IU cholecalciferol daily) or control. Blood was drawn at baseline, after 3 and 6 weeks. In study 2, 49 vitamin D deficient subjects received either vitamin D (600 IU daily) or placebo. Blood was drawn at baseline, after 8 and 16 weeks. In study 3, 43 vitamin D deficient subjects received either vitamin D (1200 IU daily) or placebo. Blood was drawn at baseline, after 8 and 16 weeks. Serum 25-hydroxyvitamin D levels were measured using LC-MS/MS or radioimmunoassay. Testosterone levels were measured using a 2nd generation immunoassay.

Results
Serum 25-hydroxyvitamin D levels significantly increased in all treatment groups (median increase of 27, 30 and 36 nmol/l in studies 1,2,3, respectively) but not in the control groups. The documented increase in 25-hydroxyvitamin D levels, however, did not affect mean testosterone concentrations at the end of the study (median increase of 0, 0.5 and 0 nmol/l in studies 1,2,3, respectively).

Conclusions
In this post-hoc analysis of three small clinical trials of limited duration in men with normal baseline testosterone concentrations, vitamin D supplementation was not associated with an increase in circulating testosterone concentrations.
Introduction

Vitamin D is well known for its role in calcium and bone metabolism and muscle function (1, 2). In spite of the importance of vitamin D, deficiency is highly prevalent worldwide. In the last years, vitamin D has been attributed a role in non-skeletal diseases such as autoimmune diseases, cancer and cardiovascular diseases (2). Recently, a potential role of vitamin D in fertility was described as well (3). With regard to male reproduction, vitamin D might be related to semen quality and androgen status. Both a high and a low vitamin D concentration might lead to an increased risk of hypogonadism in men (4). In human male reproductive tissues, the vitamin D receptor (VDR) has been observed (5, 6) and VDR knock-out mice have gonadal insufficiency, which supports this hypothesis (7).

In addition, several observational studies showed a positive association between vitamin D and testosterone concentrations (8-10), and some studies even reported a concordant seasonal variation (8, 11, 12). However, others could not establish this association between vitamin D and testosterone (4, 13) or did not observe a seasonal variation of testosterone (9, 10, 14).

Evolutionary, seasonal variation in testosterone concentrations leads to a seasonal variation in reproduction, with a peak in conception rate in summer and consequently maximum birth rate in spring (15).

The aforementioned studies addressing the possible relationship between vitamin D and testosterone were observational and therefore a causal relationship could not be established. Moreover, the reported associations between testosterone and vitamin D status are contradictory. The most crucial question therefore is whether vitamin D supplementation directly affects testosterone levels. The two previous trials studying the effect of vitamin D supplementation on testosterone concentrations show contradictory results (16, 17).

To investigate the existence of a causal link between vitamin D and testosterone, we studied the effect of vitamin D supplementation on testosterone concentrations in three independent intervention studies including males suffering from heart failure, male elderly home nursing residents, and male non-western immigrants.

Methods

Subjects

Study 1

The design of the first study is described in detail by Schroten et al (18). In short, this randomized trial was performed in 101 patients, aged 42-86 years with chronic heart failure with an estimated glomerular filtration rate (eGFR) of >60ml/min/1.73m² as calculated using the 4-point Modification of Diet in Renal Disease (MDRD) formula. The study pri...
marily investigated the effect of vitamin D supplementation on the renin-angiotensin-
aldosterone system. Patients were randomized to 2000 IU cholecalciferol daily during 6
weeks or placebo. Blood was drawn, mainly in the morning, at 0, 3 and 6 weeks after start
of supplementation.

Only male patients (n=92) were included for the current analysis.

Study 2
This randomized trial was performed in 76 male nursing home residents 71-97 years old,
and was described earlier by Chel et al (19). This study primarily investigated the efficacy
of different doses and time intervals of oral vitamin D supplementation. Blood was drawn,
in the early morning, at 0, 8 and 16 weeks after start of supplementation. The participants
received oral vitamin D3 either 600 IU per day, or 4200 IU per week, or 18,000 IU/month
or matching placebo in a daily, weekly or monthly dose. In this study, only the male pa-
tients with daily supplements were studied.

Study 3
This randomized trial was performed in the male participants of a clinical trial that pri-
marily investigated the effect of vitamin D supplementation on insulin sensitivity (20).
Forty-three male, vitamin D deficient (25OHD < 50 nmol/L), overweight (BMI ≥ 27 kg/m²),
non-western immigrants living in the Netherlands were included. Patients were 20-70
years old. Blood was drawn, in the early morning, at baseline and after 8 and 16 weeks
of supplementation with 1200 IU Vitamin D daily or placebo. All participants received
calcium 500 mg per day as calcium carbonate.

The three studies were approved by the Ethics Committee of the University Medical
Centre Groningen (study 1) and of the VU University Medical Center (study 2 and 3). In-
formed consent was obtained from all subjects participating in these studies.

Biochemical measurements
In study 1, serum 25-hydroxyvitamin D (25OHD)) was measured in the department of
laboratory medicine of the University Medical Centre Groningen using ID-LC-MS/MS, with
an inter-assay coefficient of variation (CV%) of 5.0-14%. In study 2, serum 25OHD, was
measured in the Endocrine Laboratory of the department of Clinical Chemistry of the VU
University medical center, using a radioimmunoassay (RIA) (Diasorin, Sallugia, Italy), with
an inter-assay CV% of 10% at the level of 30 nmol/L and 65 nmol/L. In study 3, serum
25OHD was measured in the same laboratory as in study 2 using an ID-LC-MS/MS as de-
scribed earlier (21, 22), with an inter-assay CV% of 9% at the level of 25 nmol/L and 6% at
the level of 63 nmol/L.
The 25OHD assays from study 1 and study 2 were compared to the 25OHD assay from study 3. This lead to the following correlation coefficient, slope, and intercept according to Passing–Bablok regression: LC-MS/MS (study 1) = 1.1 * LC-MS/MS (study 3) -3.0, with a correlation coefficient of R=0.98. RIA (study 2) = 0.89 * LC-MS/MS (study 3) +6.3, with a correlation coefficient of R=0.97.

In serum samples of these three studies, total testosterone was measured using a testosterone 2nd generation assay (Architect, Abbott Diagnostics, Abbott Park, USA) as described earlier (23, 24). Above concentrations of 4 nmol/L, this testosterone assay shows a good correlation (R= 0.97) and a mean bias of 3% compared with our LC-MS/MS method for testosterone as shown earlier (23). The inter assay CV% was 3.3% and 3.7% at a level of 5 and 13 nmol/L, respectively. The testosterone measurements were performed in the Endocrine Laboratory of the department of Clinical Chemistry in the VU University Medical Center in Amsterdam.

Statistics
Delta testosterone and delta 25OHD were calculated as the difference between the final and baseline concentrations of testosterone or 25OHD, respectively. A Mann Whitney U test was used to determine the significance of the delta 25OHD and delta testosterone between the intervention and the control group, as not all groups, also after log transformation, were normally distributed.

Spearman’s correlation was used to calculate the association between testosterone and 25OHD in the baseline samples and to calculate the association between the delta serum testosterone and delta serum 25OHD of the three studies.

P<0.05 was considered to reflect statistical significance.

We used MedCalc software version 11 (MedCalc Software BV, Oostende, Belgium) for the statistical analysis.

Results
Subjects
In the baseline samples both 25OHD and testosterone measurements were complete in 92, 49 and 42 subjects in study 1, 2 and 3, respectively. Data on age, BMI, 25OHD and testosterone concentrations in these baseline samples are shown in table 1. For some subjects not enough serum was available of all intervention samples (for numbers, see table 2). The median baseline 25OHD levels were 46.5, 27 and 27.5 nmol/L in studies 1,2 and 3, respectively. The study subjects had testosterone concentrations of 15, 11 and 13 nmol/L in study 1, 2 and 3, respectively.
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Association between vitamin D and testosterone

As the methods for measuring serum 25OHD correlated very well, all serum 25OHD concentrations from study 1 and 2 were calculated towards the 25OHD assay used in study 3, using the Passing & Bablok regression analysis shown in the method section. There was a significant correlation between baseline serum 25OHD and baseline testosterone levels of all three studies together (n=183) with a rho of 0.254, with a 95% confidence interval of 0.113-0.385, P=0.0005.

Intervention studies

All three intervention studies showed a significant increase of serum 25OHD level following vitamin D supplementation (median values 27, 30 and 36 nmol/L, in studies 1, 2 and 3, respectively) (Figure 1, the black solid and dotted lines). However, testosterone concentrations were not affected by the vitamin D supplementation as shown in table 2 and figure 1 (the median increase in testosterone was 0, 0.5 and 0 nmol/L, in study 1, 2 and 3, respectively) (the grey solid and dotted lines).

The correlation coefficients between the delta 25OHD and delta testosterone concentrations were not statistically significant in the pooled analysis (P=0.98, N=161).

Table 1. Number of subjects, the age range, BMI, 25OHD and testosterone concentration of the participating subjects in the baseline samples of each study.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Median age [range] (years)</th>
<th>Median BMI [IQR] (kg/m²)</th>
<th>Median 25OHD [IQR] (nmol/L)</th>
<th>Median testosterone [IQR] (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>49</td>
<td>82 [71 97]</td>
<td>nd</td>
<td>27 [23 31.5]</td>
<td>11 [8 15.8]</td>
</tr>
</tbody>
</table>

nd = not determined; IQR = inter quartile range

Conversion factor for 25OHD: 1 nmol/L = 0.4006 ng/mL. Conversion factor for testosterone: 1 nmol/L = 28.84 ng/dL.

Table 2. Median [interquartile range (IQR)] change in 25OHD and testosterone concentrations in the three intervention trials.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number</th>
<th>Delta 25OHD (nmol/L) (median [IQR])</th>
<th>Delta testosterone (nmol/L) (median [IQR])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44</td>
<td>-6 [-12 1]</td>
<td>0 [-2 2]</td>
</tr>
<tr>
<td>Treatment</td>
<td>42</td>
<td>27 [16 40]*</td>
<td>0 [-3 1]</td>
</tr>
<tr>
<td>Study 2</td>
<td>22</td>
<td>-2 [-8 4]</td>
<td>-1 [-3 2]</td>
</tr>
<tr>
<td>Control</td>
<td>21</td>
<td>30 [23 41]*</td>
<td>0.5 [-1 3]</td>
</tr>
<tr>
<td>Treatment</td>
<td>21</td>
<td>3 [17 10]</td>
<td>0 [-1 1]</td>
</tr>
<tr>
<td>Study 3</td>
<td>16</td>
<td>36 [25 46]*</td>
<td>0 [-2 2]</td>
</tr>
</tbody>
</table>

*P<0.0001, compared to control group

Delta is defined as final concentration minus baseline concentration. Conversion factor for 25OHD: 1 nmol/L = 0.4006 ng/mL. Conversion factor for testosterone: 1 nmol/L = 28.84 ng/dL.
Figure 1 (A-C). Effects on 25OHD and testosterone concentrations (medians are shown) upon vitamin D supplementation in the three intervention studies. In all studies only the increase in vitamin D levels in the intervention group was statistically significant. In grey the testosterone concentrations and in black the 25OHD concentrations are shown. Dotted lines represent the control group and the solid lines the intervention group. Conversion factor for 25OHD: 1 nmol/L = 0.4006 ng/mL. Conversion factor for testosterone: 1 nmol/L = 28.84 ng/dL. Study 1 is Figure 1A, study 2 is figure 1B and study 3 is figure 1C.
Discussion

In the current analysis, we investigated the effect of vitamin D supplementation on testosterone concentrations in men participating in three independent intervention studies. First, we observed a moderate but significant correlation between 25-hydroxyvitamin D and testosterone concentrations at baseline, which is in line with published data. The correlation coefficient was 0.25, so the $R^2$ is 0.06, and although statistically significant, this means only 6% of the possible variance of serum testosterone can be explained by the relation between testosterone and vitamin D status. Unfortunately, we were not able to adjust for any confounders. A correlation between 25(OH)D concentration and testosterone is not unexpected, as a healthy lifestyle is both important for 25OHD (25) and testosterone levels (26). Another confounding factor might be the concentration of the binding proteins. As vitamin D binding protein and sex hormone binding globulin concentrations are influenced by the same factors (e.g. oestrogen and insulin), these factors might have influenced the total 25OHD and total testosterone concentrations which we measured in this study in the same way.

Second, we observed that short to middle term moderate dose vitamin D supplementation caused, as expected, a clear increase in 25OHD levels, however, without any effect on testosterone levels in studies 1 and 3. In study 2, the median increase in testosterone concentration was 0.5 nmol/l, which was not statistically significant, however, might be caused by a lack of power, especially as our subjects had normal baseline testosterone concentrations. In line with our observation, Jorde et al (17) showed no effect of high dose vitamin D supplementation (20,000-40,000IU weekly) on testosterone concentrations in subjects without significant vitamin deficiency. However, our findings are in contrast with those of Pilz et al (16), who observed a significant increase in testosterone concentrations after 1 year of vitamin D supplementation (332 IU daily) within the treatment group. A possible explanation for the discrepancy could be the difference in baseline 25OHD concentrations: in the study of Pilz et al, all participants were vitamin D deficient (defined as 25OHD < 50 nmol/L) at baseline. However, a sub analysis of our data, including only subjects with 25OHD <50 nmol/L in study 1 and <30 nmol/L in study 2 and 3, did not change our conclusions, that is we did not see an effect of vitamin D supplementation on testosterone concentrations. Based upon the results of our studies, we cannot exclude an effect of vitamin D on testosterone concentrations in men with lower testosterone concentrations, as our subjects had testosterone concentrations within the reference ranges. New studies will be necessary in men with lower testosterone concentrations and are of importance as the interaction between low testosterone and low 25OHD concentrations might lead to adverse outcomes (27). Other differences between our study and that of Pilz et al are related to the study subjects receiving vitamin D supplementation (significant weight loss in Pilz’s study, patients with chronic heart failure in our study 1, and older
men in our study 2), the method used for testosterone measurements (Pilz et al. used the Immulite method which is not as accurate as the method used in the current study (23, 28)), and the time of intervention in the various studies (1 year in Pilz et al.’s study and maximum 4 months in ours). Older men might have an impaired Leydig cell response. The larger increase in 25OHD concentration in Pilz’s study might have led to an effect on testosterone concentrations; however, in the study of Jorde et al. (17), the delta 25OHD concentration was even larger (70 nmol/L) and this did not increase testosterone concentrations in their healthy male participants.

Our study has several strengths. First, we analysed data of three independent study populations with variation in age, ethnicity, and malady. These studies were performed in different centers in Netherlands. This strength adds to the robustness of our observations. Second, our studies comprised patients with relatively low baseline vitamin D levels. This is important as increasing already sufficient 25OHD concentrations would not have an effect, by analogy with the effect of vitamin D on PTH (29). Moreover, very high serum vitamin D levels might even have negative effects, leading to increased mortality or increased risk of hypogonism (4, 30). Another strength of our study is, the state-of-the-art analytical methodology to measure vitamin D and testosterone we used. Both 25OHD and testosterone are known to be difficult analytes to measure (21, 23, 31, 32). In order to be able to draw correct conclusions, measurements should be performed with accurate methods. The limitations of our study are inherent to the heterogeneity of our study subjects, and furthermore, the relative short term duration, and relative low dose of vitamin D supplementation. In addition, we did not study the free testosterone concentrations. Based upon the results of our studies, we cannot exclude an effect of vitamin D on testosterone concentrations in men with lower testosterone concentrations, as our subjects had all reasonable testosterone concentrations. Neither can we exclude an effect after a longer duration of vitamin D treatment.

In conclusion, in this post-hoc analysis of three small clinical trials, vitamin D treatment (600-2000 IU daily) for 6 weeks to 4 months does not affect the, at baseline normal, testosterone concentrations in male patients with heart failure, male nursing home residents, or male non-Western immigrants living in the Netherlands.

Funding

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Conflicts of interest

Dr. de Boer reports receiving consulting fees from Abbott and BG Medicine; speaking fees from Abbott, AstraZeneca, BG Medicine, Novartis, Pfizer, Baxter, Biomerieux, and Medicon; research support from Abbot and BG Medicine, and has ownership interest in Pectacea and scPharmaceuticals. All other authors have reported that they have no relationships relevant to the contents of this article to disclose.

References


