Vitamin D binding protein is not affected by high-dose vitamin D supplementation: a post hoc analysis of a randomised, placebo-controlled study

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Abstract

Objectives: Vitamin D binding protein (VDBP) is the main transporter of 25-hydroxyvitamin D3 (25-OHD) in the circulation. The aim of this study was to investigate if VDBP is affected by high dose vitamin D supplementation and if VDBP-levels correlate with free 25-OHD. Correlation between free 25-OHD measured with ELISA and total 25-OHD in the circulation was also analysed. Plasma samples from a randomized, controlled trial in which persistent MRSA-carriers were randomized to treatment with vitamin D, 4000 IE/day, (n = 27) or placebo (n = 32) for 12 months were used. Plasma from baseline and after 6 months of treatment were analysed for VDBP, 25-OHD and free 25-OHD.

Results: VDBP levels were not affected by vitamin D treatment, although the 25-OHD levels increased significantly in the vitamin D treated subjects. There was a strong correlation between 25-OHD and free 25-OHD (r² = 0.68, p < 0.0001), while there was no correlation between VDBP and free 25-OHD. Thus, our data shows that VDBP are not affected by vitamin D supplementation and the levels of VDBP are not associated with the free fraction of 25-OHD. Since there was a strong correlation between free 25-OHD and total 25-OHD it appears to be sufficient to measure only total 25-OHD.

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Keywords: Vitamin D, MRSA, Vitamin D binding protein, Free 25-hydroxyvitamin D

Introduction

25-Hydroxyvitamin D₃ (25-OHD) is considered as the best marker of vitamin D status in the human body [1]. Nearly all circulating 25-OHD are bound to proteins and it is only the limited fraction of free 25-OHD that has biological effects in target cells after conversion to the active 1,25-dihydroxyvitamin D. Vitamin D binding protein (VDBP), is the main transporter for 25-OHD in the circulation, transporting approximately 85% of total 25-OHD. A smaller proportion of 25-OHD, 10–15%, is transported by albumin [2]. VDBP, also known as GC-globulin, is synthesized in the liver and has numerous physiological roles, including immune-modulation, binding of fatty acids and regulation of bone development [3]. Thus, VDBP has many other functions than being a transporter and less than 5% of the binding sites on VDBP are occupied by 25-OHD [3].

Given that only the free 25-OHD is biologically active, it has been suggested that the levels of VDBP would determine the effects of vitamin D in the human body by regulating the levels of the free fraction of 25-OHD [4]. This hypothesis was the basis for a highly cited study that demonstrated that Afro-Americans had lower levels of VDBP—but similar levels of free 25OHD—than Caucasians [5]. However, other studies have failed to show...
ethnical differences in VDBP-levels [6]. The discrepancy between various studies was recently explained and found to be caused by the use of different kits for VDBP-analysis. In fact, VDBP-kits that used a monoclonal antibody failed to detect different variants of the highly polymorphic VDBP-protein, which could be detected by ELISA-kits using polyclonal sera [7].

Previously, free 25-OHD was determined by the use of a formula based on 25-OHD, VDBP and albumin-levels [8]. Recently, a commercially available ELISA was developed to measure the free fraction of 25-OHD in plasma directly, which has replaced the indirect calculation method [9].

We had access to samples from a previous study where vitamin D was given to eradicate carriage of methicillin resistant Staphylococcus aureus (MRSA). The rationale behind this study was that (i) vitamin D induces antimicrobial peptides with anti-staphylococcal activity; (ii) MRSA-carriers have lower levels of 25OHD than non-carriers and (iii) vitamin D supplementation previously has been shown to reduce carriage rates of S. aureus. Vitamin D supplementation did not affect carriage rate of MRSA in this study [10]. However, the role of VDBP and free 25-OHD in the context of a vitamin D supplementation study has not been well described before. Thus, using these samples, we set out to study how VDBP-levels were affected by vitamin D supplementation. We also investigated whether there was a correlation between free 25-OHD and VDBP in plasma as well as between free 25-OHD and total 25-OHD.

Main text

Method

The D-STAPH study was a double blind, randomised and placebo-controlled trial performed during 2014–2015 [10]. The aim of the study was to investigate if vitamin D treatment could eradicate methicillin resistant S. aureus (MRSA) in persistent MRSA-carriers. Persistent MRSA-carriers were given the study drug (4000 IU vitamin D or placebo daily) during a 12 months period and were followed closely during this period with visits every 3 months. No effects of vitamin D supplementation on MRSA-carriage could be observed in this trial [10].

Plasma samples from baseline and 6 months were analysed in the current project. There were samples available from 27 vitamin D treated subjects and 32 placebo treated subjects. Measurements of total 25-OHD were made with chemiluminescence immunoassay (CLIA) on a LIAISON-instrument (DiaSorin Inc, Stillwater, MN, USA,) with a detectable range of 7.5–175 nmol/L, CV 2–5% at the Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden. Vitamin D binding protein was measured with ELISA, using polyclonal anti-VDBP antibodies according to the manufacturer’s protocol (Immundiagnostik AG, Bensheim, Germany). Free 25-OHD was measured with quantitative two-step ELISA immunoassay (Future Diagnostics, DiASource, Wijc, The Netherlands) [9].

Statistical analysis

Statistical analyses were performed using Graph Pad Prism vs 6.0. When comparing 25-OHD levels between the different groups, student's t-test was used since the data showed Gaussian distribution. Correlation between 25-OHD levels and free 25-OHD and between free 25-OHD and VDBP levels was determined with linear regression.

Results

The levels of VDBP were similar at baseline in both groups and were not affected over time or by high dose vitamin D supplementation for 6 months (Fig. 1a). Vitamin D supplementation resulted in significantly increased levels of 25-OHD as expected, p < 0.001 (Fig. 1b), while the levels in the placebo-group did not increase significantly. Free 25-OHD increased in a similar way in the vitamin D group (Fig. 1c). However, also in the placebo group a slight, but statistically significant, increase of free 25-OHD was observed (Fig. 1c).

There was a strong correlation between 25-OHD levels and free 25-OHD, linear regression showed $r^2=0.68$, p < 0.0001 (Fig. 2a). In contrast, no significant correlation between free 25-OHD and VDBP levels was observed (Fig. 2b).

There was no significant correlation between the ratio free 25-OHD/total 25-OHD and VDBP, linear regression showed $r^2=0.01$, p = 0.31 (data not shown).

There was no correlation between VDBP levels and MRSA-carriage after 6 months (data not shown). Albumin-levels at baseline were within the normal range 33–48 g/L, mean 37 g/L, median 39 g/L, and did not change during the study.

Discussion

Here we show that VDBP-levels in plasma are not affected by high-dose vitamin D supplementation for 6 months. In addition, our findings suggest that VDBP-levels do not correlate with the levels of free 25-OHD in the circulation. This is in contrast to some previous reports also using direct methods to measure the free
fraction [11–13]. However, taking into account that less than 5% of the binding sites on VDBP are occupied by 25-OHD [3], it is reasonable to suggest that VDBP levels only have a minor impact on the levels of free 25-OHD. Notably, albumin-levels were within the normal range in all participants and remained unchanged during the study. Unexpectedly, the levels of free vitamin D showed a slight increase in the placebo-group. At this point, we have no explanation for this observation.

Since there was a strong correlation between 25-OHD levels and free 25-OHD, it may be sufficient to measure only total 25-OHD in order to obtain information on the vitamin D status of individual patients in vitamin D supplementation studies.

However, it should be noted that the patients in this study were generally healthy and recruited based on their status as persistent MRSA-carriers. In an early study on patients with liver disease, it was shown that the ratio between free and total vitamin D correlated with VDBP-levels or albumin, whereas there was no correlation between the specific levels of free or total vitamin D with VDBP or albumin [11]. Therefore, there could still be a role for analyses of free 25-OHD in vitamin D supplementation trials, especially in populations with altered VDBP-levels, such as cirrhotic, obese and pregnant individuals [14]. Today, there are accurate and simple ELISA methods available on the market for analyses of free 25-OHD.

A recent cross-sectional study assessed free 25-OHD in a large cohort and found that cirrhotic patients had lower VDBP-levels and higher fraction of free 25-OHD-levels. Reciprocally, pregnant women had higher VDBP-levels but—somewhat unexpected—normal levels of free 25-OHD [14]. Combined, these recent results suggest that the relations between VDBP, total 25-OHD and free 25-OHD are complex and that further studies are needed to determine the role of free 25-OHD analyses in health and disease.

**Limitations**

The most important limitation is the relatively small study cohort (vitamin D n = 27; placebo n = 32), which could have hidden minor associations in the material. However, if there was a true linear correlation between VDBP and free 25-OHD, as suggested by other researchers, we would have expected at least a trend towards correlation, but this was not the case. As expected, there was a strong correlation between total 25-OHD and free 25-OHD. A final limitation was the lack of information of different VDBP haplotypes,
which could have affected the results. However, since a polyclonal antibody was used in the ELISA assay the risk for this potential problem is limited.

**Abbreviations**

25-OHD: 25-hydroxyvitamin D; MRSA: methicillin resistant Staphylococcus aureus; VDBP: vitamin D binding protein.

**Authors’ contributions**

LBB and PB designed the study, performed the original clinical study and wrote the first draft of the manuscript. ET and LE developed the methods and performed the lab-analysis of VDBP and free 25-OHD. All authors analysed the data and contributed to the final version of the manuscript. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The dataset is available from the corresponding author on reasonable request.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

The study was approved by the local Ethical Committee at Karolinska Institutet (2014/476-31/4) and was performed in accordance with the declaration of Helsinki. The study was also approved by the Swedish Medical Product Agency (EudraCT 2014-000149-53). Written informed consent was obtained from all study participants before inclusion.

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**References**

1. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–81.
2. Yousefzadeh P, Shapses SA, Wang X. Vitamin D binding protein impact on 25-hydroxyvitamin D levels under different physiologic and pathologic conditions. Int J Endocrinol. 2014;2014:981581.
3. Speeckaert MM, Speeckaert R, van Geel N, Delanghe JR. Vitamin D binding protein: a multifunctional protein of clinical importance. Adv Clin Chem. 2014;63:1–57.
4. Malmstroem S, Rejnmark L, Imboden JB, Shoback DM, Bikle DD. Current assays to determine free 25-hydroxyvitamin D in serum. J AOAC Int. 2017;100(5):1322–7.
5. Powe CE, Evans MK, Wengen J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369(21):1991–2000.
6. Yao S, Hong CC, Bandera EV, Zhu Q, Liu S, Cheng TD, et al. Demographic, lifestyle, and genetic determinants of circulating concentrations of 25-hydroxyvitamin D and vitamin D binding protein in African American and European American women. Am J Clin Nutr. 2017;105(6):1362–71.
7. Hoofnagle AN, Eckfeldt JH, Lutsey PL. Vitamin D-binding protein concentrations quantified by mass spectrometry. N Engl J Med. 2015;373(15):1480–2.
8. Bikle DD, Gee E, Halloran B, Kozlowski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab. 1986;63(4):954–9.
9. Heureux N, Lindhout E, Swinkels L. A direct assay for measuring free 25-hydroxyvitamin D. J AOAC Int. 2017;100(5):1318–22.
10. Bjorkhem-Bergman L, Missalidis C, Karlsson-Vaik, J Tamminen A, Bostrom L, Bottai M, et al. Vitamin D supplementation to persistent carriers of MRSA—a randomized and placebo—controlled clinical trial. Eur J Clin Microbiol Infect Dis. 2018. https://doi.org/10.1007/s10096-018-3306-7.
11. Bikle DD, Halloran BP, Gee E, Ryzen E, Haddad JG. Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels. J Clin Invest. 1986;78(3):748–52.

12. Lee MJ, Kearns MD, Smith EM, Hao L, Ziegler TR, Alvarez JA, et al. Free 25-hydroxyvitamin D concentrations in cystic fibrosis. Am J Med Sci. 2015;350(5):374–9.

13. Lima JJ, Castro M, King TS, Lang JE, Ortega VE, Peters SP, et al. Association of free vitamin D3 concentrations and asthma treatment failures in the vida trial. Ann Allergy Asthma Immunol. 2018. https://doi.org/10.1016/j.anai.2018.06.001.

14. Schwartz JB, Gallagher C, Jorde R, Berg V, Walsh J, Eastell R, et al. Determination of free 25(OH)D concentrations and their relationships to total 25(OH)D in multiple clinical populations. J Clin Endocrinol Metab. 2018. https://doi.org/10.1210/jc.2018-00295.