25-OH Vitamin D Deficiency in Inflammatory States is not a Reflection of Low Vitamin D Binding Protein Levels

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Abstract: *Background*: Vitamin D binding protein (DBP) levels can profoundly influence 25-OH vitamin D levels. An increased incidence of vitamin D deficiency exists in autoimmune diseases. We hypothesized that low 25-OH vitamin D levels were a reflection of low DBP levels due to the influence of inflammation on hepatic synthesis.

Methods: Serum and plasma samples from patients with inflammatory autoimmune diseases were evaluated for levels of DBP and 25-OH vitamin D. Inflammatory markers high-sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) were obtained from the clinical lab. Statistical significance was determined by Spearman correlation.

Results: We identified a broad range of DBP levels (74 to 614 µg/ml) across patients. However, neither DBP nor 25-OH vitamin D levels had any relationship to absolute values of hsCRP or ESR. In addition, in patients with serial samples wherein the inflammatory markers decreased by 25% or more, there was no consistent change in DBP.

Conclusions: DBP levels are unaffected by inflammation, therefore, vitamin D deficiency in autoimmune inflammatory diseases is not simply due to decreased levels of DBP.

Keywords: Vitamin D, Vitamin D binding protein, Inflammation, Autoimmune disease, Rheumatoid arthritis.

INTRODUCTION

Vitamin D deficiency (decreased levels of 25-OH vitamin D) has been reported to occur in inflammatory autoimmune diseases including inflammatory arthritis and systemic lupus erythematosus [1-4]. The nature of this association is unclear, but it has been postulated that vitamin D deficiency itself may promote the development of autoimmunity and/or inflammation [5]. An alternative possibility is that systemic inflammatory states may lead to lower vitamin D levels regardless of exposure to sunlight or vitamin D supplementation [2].

It is important to correctly identify vitamin D deficiency in order to make associations to specific diseases. Whereas it is commonly held that 25-OH vitamin D levels <30 ng/ml are considered insufficient and <20 ng/ml are considered deficient, it is clear that many with 25-OH vitamin D levels in this range do not have any alterations in serum PTH levels, suggesting that they are not truly vitamin D deficient. For instance, a study of Hawaiian surfers, who received at least 15 hours of sun exposure per week, identified 25-OH vitamin D levels ranging from 11 to 71 ng/ml [6]. Thus, serum 25-OH D levels are an imperfect predictor of a deficiency state leading to altered calcium homeostasis and secondary hyperparathyroidism.

One factor in determining the sufficiency of vitamin D status is to consider the amount of free 25-OH vitamin D available for activity. In the circulation, the majority of 25-OH vitamin D is primarily bound to vitamin D binding protein (DBP) (90%) with the remaining bound to albumin [7, 8]. This protein bound 25-OH vitamin D is not active but functions as a vitamin D reserve [9-11]. Thus, in a manner similar to thyroid hormone bound to thyroxine binding globulin, total values of 25-OH vitamin D will be dependent on the level of DBP, which may or may not reflect available unbound 25-OH vitamin D. This has been unambiguously established by Powe *et al*. who showed that lower 25-OH vitamin D levels in African American males was directly related to lower DBP levels, with no associated elevation in parathyroid hormone levels (PTH) [12].

In inflammatory states, the liver produces acute phase reactants, with a concomitant decrease in serum albumin levels due to decreased production [13]. DBP is homologous to albumin and is also produced by the liver [14]. We hypothesized that inflammation might reduce hepatic synthesis of DBP as it does albumin. In this model, serum DBP biosynthesis would decrease in inflammatory states, potentially accounting for the reported reductions in 25-OH vitamin D levels. We sought to determine if a change in the inflammatory markers high-sensitivity C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) correlated with a change in DBP, and therefore altered 25-OH vitamin D levels.

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MATERIALS AND METHODS

Patients

Study approval was obtained from the Dartmouth College Committee for the Protection of Human Subjects. Patients were recruited from the Dartmouth-Hitchcock Rheumatology Clinic following informed consent. Plasma and serum samples were collected in vacutainer tubes, aliquotted, and stored at -80° C, and clinical information was stored in a secure central database (FileMaker). Forty patients were identified with recorded hsCRP or ESR levels, of which 29 patients had serial blood collections (duplicates) with variations in levels of hsCRP, ESR, or both (as determined by the clinical lab). Thirty-five of these patients had rheumatoid arthritis (RA) while there was one patient each with systemic lupus erythematosus, psoriatic arthritis, reactive arthritis, JIA, and systemic sclerosis. No patient had a protein-losing enteropathy or nephropathy.

Measurement of 25-OH Vitamin D and Vitamin D Binding Protein

Plasma and serum samples were analyzed by mass spectrometry (Thermo-Fischer Corp, San Jose, CA, USA) for 25-OH vitamin D2 and D3 levels to determine a total 25-OH vitamin D level. The DBP value in µg/ml was determined for each sample by ELISA (R&D Systems). The standard curve for these plates had an R^2 value of 0.997 on both occasions.

Statistics

Statistical analysis was performed using STATA version 12.1 (StataCorp). Comparison between variables was performed using correlation analysis. When samples had a non-normal distribution, the values were log-transformed and correlation analysis was repeated. As there was no discernible difference between analysis of actual values and log-transformed values, Spearman correlation coefficients of the actual values and their associated p-values are reported.

RESULTS

Serum DBP levels were highly variable in these rheumatic disease patients with levels ranging from 74 to 614 µg/ml (mean of 270 µg/ml, standard deviation 131). Though there was a trend towards a relationship, DBP levels did not have a clear correlation with 25-OH vitamin D values (Figure **1**, R=0.25, p=0.09). There was also no correlation between absolute DBP value and either hsCRP (Figure **2A**, n=50, R=0.04, p=0.77) or ESR (Figure **2B**, n=50, R= -0.07, p=0.63). Similarly, there was no relationship between absolute 25-OH vitamin D levels and either hsCRP (Figure **2C**, n=25, R= -0.22, p=0.29) or ESR (Figure **2D**, n=31, R= -0.005, p=0.98).

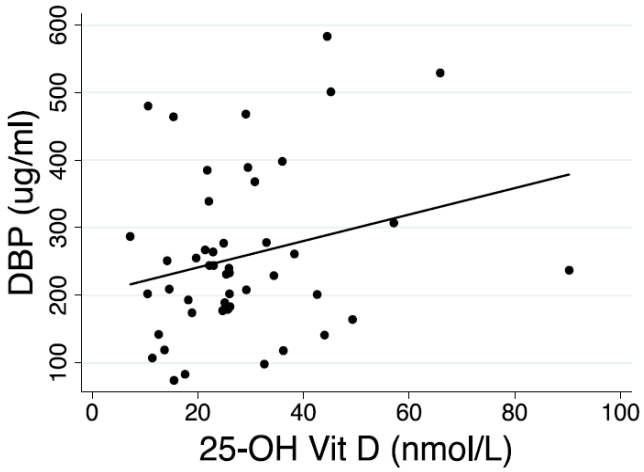

Figure 1: Vitamin D binding protein (DBP) levels in relationship to 25-OH vitamin D (n=48, R=0.25, p=0.09).

We next sought to determine if a discernible change in inflammatory state, as measured by change in either ESR or hsCRP, resulted in a measurable change in DBP levels. Twenty-nine of the 40 patients had serial samples displaying a >25% (range of 26%-98%) change in hsCRP or ESR allowing for a serial determination of the effect of systemic inflammation on DBP. The time between blood draws ranged from 1 month to 9 months (mean of 4 months). The mean decrease in hsCRP was 36.0 mg/L (range of 6.1-189.9) or 78% (50-98%) while the mean decrease in ESR was 25 mm/hr (range of 3-63) or 55% (26-83%). Despite the large mean declines in hsCRP and ESR, only a very modest decline in mean DBP was observed with a decrease of 9.4 µg/ml representing a decrease of only 2% (range includes an increase of 36% to a decrease in 29%). The percentage change in DBP in relation to percent change of hsCRP or ESR is depicted in Figure **3**.

Of the 29 patients evaluated for changes in hsCRP, ESR, or both, the majority had minimal change to DBP, with 21 having less than a 15% change in DBP level. As shown in Table **1**, among the eight patients with a >15% change in DBP, three had increases in DBP levels while decreases were observed in five. Thus, in contrast to albumin, circulating levels of DBP do not appear to be affected by inflammation.

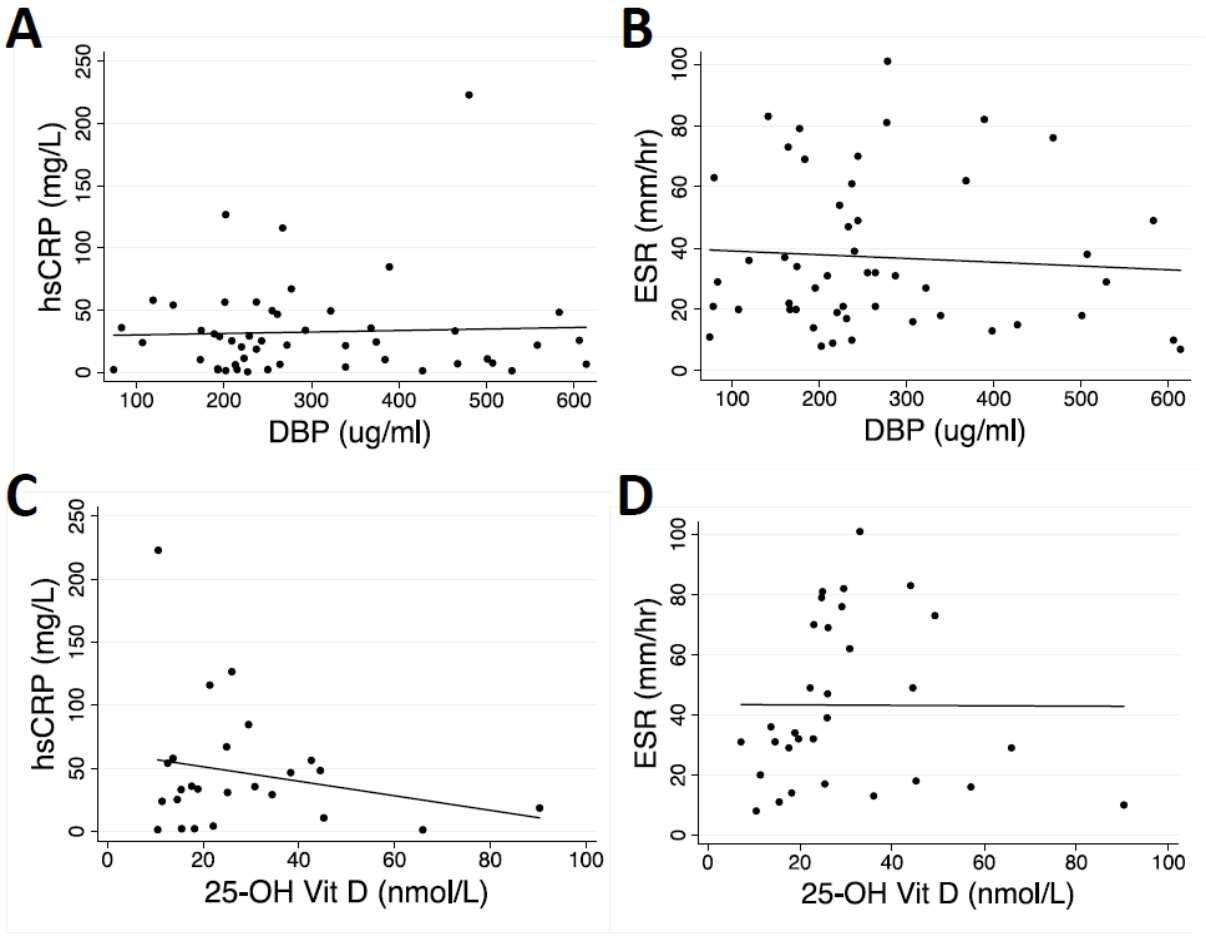

Figure 2: Vitamin D binding protein (DBP) and 25-OH vitamin D do not correlate to inflammatory states. **A**, **B**, DBP does not have a relationship to either high-sensitivity C-reactive protein (hsCRP) or erythrocyte sedimentation rate (ESR) (n=50; R=0.04, p=0.77, and R= -0.07, p=0.63, respectively). **C**, **D**, Examination of 25-OH vitamin D levels also does not identify a relationship to hsCRP or ESR (n=25, R= -0.22, p=0.29, and n=31, R= -0.005, p=0.98, respectively).

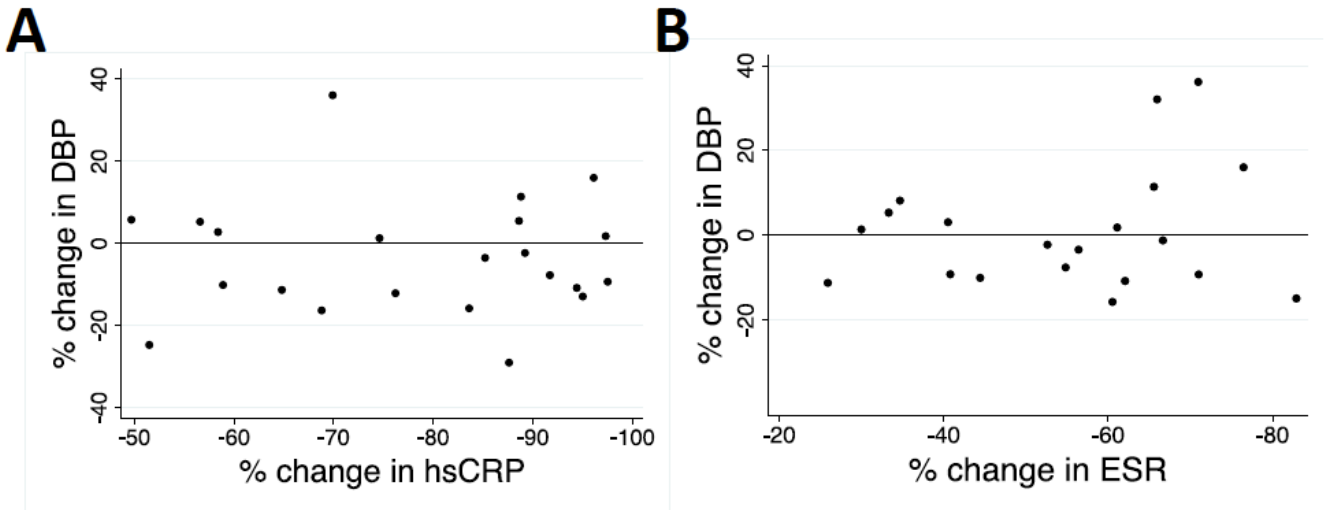

Figure 3: Vitamin D binding protein (DBP) does not change in relationship to changes in inflammatory markers. **A**, Despite marked declines in hsCRP, the DBP values changed little, with both increases and decreases in value (n=22). **B**, Similarly, considerable declines in ESR had no relationship to percent change of DBP (n=20).

Table 1: Results of Eight Patients with 15% or More Change in DBP Level

Patient	hsCRP (mg/L) High Low	ESR (mm/hr) High Low	Vitamin DBP (µg/ml)	% Change in Vitamin DBP
1	35.5 10.7	62 18	368 501	 +36.1%
2	n/a	47 16	233 307	 +32.0%
3	33.5 1.3	34 8	174 202	 +16.0%
4	n/a	76 13	468 398	 -15.0%
5	7.3 1.2	38 15	507 427	 -15.8%
6	21.8 6.8	n/a	558 467	 -16.3%
7	116 56.3	n/a	267 201	 -24.7%
8	21.8 2.7	n/a	272 193	 -29.0%

Results of eight patients whose DBP value changed by >15% from a high inflammatory state to a lower inflammatory state. Three patients had an increase in DBP as inflammation declined, and five had a decrease in DBP as inflammation declined.

DISCUSSION

We examined if variation of DBP in high and low inflammatory states could account for the finding of lower 25-OH vitamin D levels in inflammatory diseases. While DBP levels varied nearly 10-fold across patients, these levels showed no relationship to inflammatory state as measured by either hsCRP or ESR (Figure **2**) or to fluctuations in inflammatory state (Figure **3**), suggesting these were determined by other factors [15]. Importantly, our patient population is a relatively homogeneous Caucasian population with a Northern European heritage, and lacks any diseases that might lead to loss of DBP such as nephrotic syndrome.

These negative results hold greater significance in light of a recent report by Powe *et al*. [12]. These investigators identified that lower 25-OH vitamin D levels in African American males was a reflection of lower DBP levels, and did not represent a true deficiency in bioavailable vitamin D. If this 'pseudo-deficiency' of vitamin D were also true in highly inflammatory states, it would call into question most of the papers written about the association between low vitamin D status and autoimmune disease. However, our results suggest that systemic inflammation has little or no consistent effect on DBP levels.

Our results complement those found by Reid *et al*. who examined the role of inflammation in 25-OH vitamin D status in an acute inflammatory state [16]. In the setting of knee arthroplasty, these investigators measured pre and post surgical levels of CRP, 25-OH vitamin D, and DBP, and found that the CRP rose drastically and the 25-OH vitamin D levels dropped, while the DBP had only a minor decline. Whereas that study looked at acute changes in inflammation, our study looked at changes in inflammation over a longer time period in which effects on hepatic biosynthesis would be expected to emerge. We therefore conclude that DBP levels are not altered by inflammation as correlated with serum inflammatory markers. Thus, DBP and albumin levels are differentially regulated. Moreover, decreased levels of 25-OH vitamin D in inflammatory autoimmune conditions cannot be explained simply by a reduction in DBP levels.

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