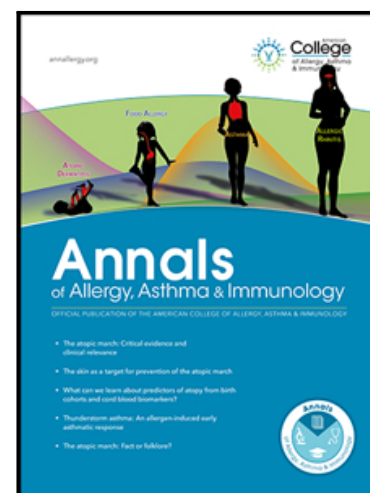


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PII: S1081-1206(18)30461-7
DOI: [10.1016/j.anai.2018.06.001](https://doi.org/10.1016/j.anai.2018.06.001)
Reference: ANAI 2578

To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 7 April 2018
Revised date: 11 May 2018
Accepted date: 5 June 2018

Please cite this article as: John J. Lima PharmD , Mario Castro MD, MPH , Tonya S. King PhD , Jason E Lang MD, MPH , Victor E. Ortega MD, PhD , Stephen P. Peters MD, PhD , Loren C. Denlinger MD, PhD , Elliot Israel M.D. , Christine A. Sorkness PharmD , Michael E. Wechsler MD MMSc , Sally E. Wenzel MD , Lewis J Smith MD , ASSOCIATION OF FREE VITAMIN D₃ CONCENTRATIONS AND ASTHMA TREATMENT FAILURES in the VIDA Trial, *Annals of Allergy, Asthma Immunology* (2018), doi: [10.1016/j.anai.2018.06.001](https://doi.org/10.1016/j.anai.2018.06.001)

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ASSOCIATION OF FREE VITAMIN D₃ CONCENTRATIONS AND ASTHMA TREATMENT**FAILURES in the VIDA Trial**

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Funding: This study was conducted with the support of grants HL098102, U10HL098096, UL1TR000150, UL1TR000430, UL1TR000050, HL098075, UL1TR001082, HL098090, HL098177, UL1TR000439, HL098098, UL1TR000448, HL098107, HL098112, HL098103, UL1TR000454, and HL098115 that were awarded by the National Heart, Lung, and Blood Institute. Ciclesonide and levalbuterol were provided without cost by Sunovion Pharmaceuticals Inc.

Word count: 3070 words; 3 figures; 4 tables

Conflicts of interest: none

TABLE OF ABBREVIATIONS

VDBP	Vitamin D binding protein
CYP2R1	Cytochrome P450 2R1
25 (OH) ₂ D ₃	1,25-dihydroxyvitamin D ₃
CYP27B1	Cytochrome P450 27B1
GC	Group-specific component
IU	International unit
C _f	Free (Unbound) concentration
C _b	Bound concentration
C _{tot}	Total concentration
f _u	Unbound fraction
f _b	Bound fraction
FEV ₁	Forced expiratory volume in 1 second

Introduction

Vitamin D (vitamin D₂ and vitamin D₃) derived from sunlight exposure, diet or supplementation are converted to vitamin D₂ (calciferol) and vitamin D₃ (cholecalciferol) (25 (OH) D₃) by vitamin D-25 hydroxylase (CYP2R1) in the liver, and subsequently converted to the biologically active 1,25-dihydroxyvitamin D₂ and 1,25-dihydroxyvitamin D₃ (25 (OH)₂ D₃) by CYP27B1 in the kidney and other tissue^{1,2}. Circulating total concentrations of vitamin D₃ but not concentrations of active 1,25-dihydroxyvitamin D₃ are used to define vitamin D status in part because of differences in total concentrations and half-life between the two metabolites¹. For the skeletal actions of vitamin D serum concentrations of vitamin D₃ ≥ 30 ng/ml defines vitamin D sufficiency whereas concentrations < 30 ng/ml are insufficient and levels < 20 ng/ml are deficient^{1,3}. Guidelines recommend higher serum concentrations for the extra-skeletal, pleiotropic actions of vitamin D in the range of 30-50 ng/ml². However, the use of serum concentrations of vitamin D₃ as a biomarker of vitamin D status has been questioned owing to uncertainties in methods of measuring total concentrations of vitamin D₃⁴.

Serum concentrations of vitamin D₃ are tightly bound (~85%) to vitamin D binding protein (VDBP) and loosely bound to albumin (~15%) with < 1% in the unbound or free form^{5,6}. The role VDBP plays in promoting bone health and in regulating extra-skeletal function is controversial. Vitamin D₃ bound to VDBP can bind to megalin, a transmembrane receptor located in the kidney and other tissue, undergo internalization through endocytosis, and be metabolized to 1,25-dihydroxyvitamin D₃ in proximal tubular cells⁷. Alternatively, the free hormone hypothesis predicts that only free concentrations of vitamin D₃ diffuse across the cell membrane and undergo metabolism to 1,25-dihydroxyvitamin D₃ which activates the vitamin D receptor^{6,8}. Consequently free

vitamin D₃ concentrations are considered better markers of vitamin D status than total serum concentrations. Indeed several recent studies report that bone density and markers of bone turnover, as well as atopy and lung function in children with asthma are better correlated with bioavailable (free vitamin D₃ + bound to albumin) or with calculated or directly measured free vitamin D₃ concentrations than to total vitamin D₃ concentrations⁹⁻¹³.

The free hormone hypothesis also predicts that inter-patient variability in VDBP concentrations influence total concentrations and the unbound fraction of vitamin D₃ in serum and have little effect on free concentrations^{6,8}. Several factors including diseases, pregnancy, obesity and genetic variation^{14,15} influence serum concentrations of VDBP and therefore contribute to the variability in serum concentrations of vitamin D₃. Variation in the GC gene encodes different VDBP isoforms: Gc1F, Gc1S and Gc2, which have different binding affinities for vitamin D¹⁶. Specific genetic variants in GC show significant differences in allele frequencies between different ancestral populations that may contribute to lower VDBP concentrations and lower total vitamin D₃ concentrations in ethnic populations with a significant African ancestry (i.e. African Americans and some Hispanic ethnic groups) compared to European Americans^{10,17,18}.

Hypovitaminosis D has been associated with increased risk of asthma, reduced lung function, increased airway responsiveness, asthma exacerbations and reduced responsiveness to glucocorticoids in children and adults with asthma¹⁹⁻²⁴. We published a randomized, double-masked, placebo-controlled multi-center trial of vitamin D₃ supplementation in vitamin D₃-insufficient participants (total serum vitamin D₃ concentrations < 30 ng/ml) with persistent asthma taking inhaled corticosteroids²⁵. The

objective of the VIDA Trial was to determine if vitamin D supplementation improves the clinical efficacy of inhaled corticosteroids. We found that vitamin D supplementation did not reduce the rates of first treatment failure or exacerbation. However, for those participants who achieved vitamin D sufficiency, i.e., serum concentrations ≥ 30 ng/ml (82%) overall rates of treatment failure and exacerbations were lower compared to placebo, thus supporting the notion that vitamin D supplementation may improve the efficacy of inhaled corticosteroids. The objective of the present VIDA analysis was to determine associations between free vitamin D₃ concentrations and treatment failure while reducing ICS in VIDA participants.

Methods

Study Design. Details of the VIDA trial design are published²⁵. Briefly, 408 vitamin D insufficient adults (serum levels < 30 ng/ml at baseline) with asthma were randomly assigned to receive either placebo (N=207) or a single oral dose of vitamin D₃, 100,000 IU followed by 4000 IU daily (Cholecalciferol, BioTech Pharmacal; N=201) added to inhaled ciclesonide, 320 µg daily and levalbuterol as needed. This initial phase of 12 weeks was followed by a reduction in the ciclesonide dose by 50% if the participant's symptoms were well-controlled. Adherence to dosing with ciclesonide and vitamin D was monitored electronically as described²⁵. Blood for vitamin D₃ determination was drawn from each participant at baseline and at 12 (visit 6), 20 (visit 8) and 28 (visit 10) weeks after baseline. The present study explored associations between free concentrations of vitamin D₃ and outcomes in 200 participants assigned to receive active vitamin D₃ supplement. Free concentrations of vitamin D₃ were not measured in participants taking placebo.

Quantification of free vitamin D₃ Concentrations and Binding to Serum Proteins. Free or unbound concentrations (C_f) of vitamin D₃ in serum were quantified by ELISA (Free 25OH Vitamin D ELISA; Future Diagnostic Solutions; DIAsource ImmunoAssays®; Belgium) <https://www.alpco.com/pdfs/38/38-KARF1991.pdf>^{26,27}. Total serum concentrations (C_{tot}) of vitamin D₃, which is the sum of C_f + the concentration bound to serum proteins (C_b), were quantified using the DiaSorin LIAISON vitamin D assay as previously described²⁵. The free fraction of vitamin D₃ (f_u) in serum was calculated as: $f_u = C_f/C_{tot}$. The bound fraction of vitamin D₃ in serum (f_b) is

$1-f_u$ and the percent of vitamin D₃ unbound and bound to serum proteins are $f_u * 100$ and $f_b * 100$, respectively. VDBP concentrations were not quantified in the VIDA study.

Outcome measures. Outcome measures in the present study included treatment failure, which was defined as previously described and included changes in peak expiratory flow, $FEV_1 < 80\%$ of baseline, increase in levalbuterol dose; addition of inhaled corticosteroid or oral or parenteral corticosteroids, and emergency department or hospitalization for asthma with corticosteroid use. Other outcomes included asthma exacerbations which were defined as failure to respond to a rescue algorithm, and $FEV_1 < 50\%$ of baseline²⁵.

Statistical Analyses. The relationship between free and total concentrations of vitamin D₃ was evaluated with the Pearson correlation coefficient and corresponding 95% confidence interval. Analysis of variance (ANOVA) was used to compare free, total and the free fraction of vitamin D₃ between racial and ethnic groups, genders, and BMI groups, as well as to compare free levels and change in free levels between those who achieved and did not achieve vitamin D₃ sufficiency as defined by change in total concentrations after 12 weeks of supplementation. The association between free concentrations of vitamin D₃ and time to first treatment failure was evaluated with Kaplan-Meier survival curves and the logrank test. An additional analysis was performed to evaluate this association allowing for multiple events per person, which was performed using Poisson regression with adjustment for clinical center, BMI, and race, and reported in terms of event rate per person-year. Time to first exacerbation and total exacerbations were analyzed according to the same approaches as treatment failures. Statistical significance was determined as $p < 0.05$.

Results

Baseline vitamin D₃ concentrations and serum protein binding, The mean (SD) C_f, C_{tot} and f_u of vitamin D₃ in serum at baseline were 4.73 (1.54) pg/ml, 19.4 (6.57) ng/ml and 2.57×10^{-4} (8.0×10^{-5}), respectively, indicating that on average 0.03% and 99.97% of vitamin D₃ respectively, were free and bound to serum proteins (VDBP and albumin). Figure 1 shows that vitamin D₃ C_f and C_{tot} are highly correlated ($r = 0.77$, 95%CI (0.71, 0.82)). Table 1 compares free vitamin D₃ concentrations and serum protein binding by race, sex and BMI. Compared to non-Hispanic White participants C_f of vitamin D₃ was lower in African Americans and Hispanic participants. The f_u of vitamin D₃ in serum varied among different races and shows that the binding of vitamin D₃ to serum proteins was higher among White and Hispanic participants compared to Black participants (Table 1). The mean (SD) vitamin D₃ C_f and f_u were higher in males compared to females: 5.08 (1.41) vs. 4.57 (1.58) pg/ml ($p=0.026$); and 2.82×10^{-4} (1.04×10^{-4}) vs. 2.46×10^{-4} (0.63×10^{-4}) ($p=0.003$). In addition to race/ethnicity and gender, body mass index influenced the C_f of vitamin D₃. The mean (SD) C_f of vitamin D₃ at baseline in participants with BMI < 25 was 5.45 (1.86) pg/ml and was higher than those with BMI > than 25, which was 4.54(1.39) pg/ml ($p = 0.004$). The inverse relationship between BMI and C_f at baseline is shown in Figure 2 (Rho = -0.3, 95% CI (-0.4, - 0.2); $p < 0.001$).

Free vitamin D₃ concentrations and binding to serum proteins after supplementation. The C_f of vitamin D₃ increased from baseline and reached a plateau

in most participants by visit 6 (12 weeks) following the administration of loading and maintenance doses of vitamin D₃ (Figure 3). The mean (SD) C_f increased from 4.73 (1.54) pg/ml at baseline to 10.4 (3.13), 11.1 (3.53) and 10.8 (3.37) pg/ml at visits 6, 8 (20 weeks) and 10 (28 weeks), respectively, representing fold-changes in C_f of 2.2 - 2.3 over baseline. We focused our analyses on V6 (12 weeks after dose administration) because C_f and the change in C_f at V6 were not different than those at V8 and V10, thus following the same approach as in the original analyses²⁵. There was substantial variability in C_f at baseline and at visits 6, 8 and 10 (Figure 3).

As previously reported 157 of 201 participants achieved sufficiency (defined as serum concentrations (C_{tot}) ≥ 30 ng/ml) following 12 weeks of vitamin D₃²⁵. Table 2 compares vitamin D₃ C_f and binding between sufficient and insufficient participants. The mean vitamin D₃ C_f at baseline and the mean change in C_f from baseline to 12 weeks of supplementation were lower in insufficient compared to sufficient participants, which suggests that insufficient participants were relatively resistant to supplementation. The binding of vitamin D₃ to serum protein was similar between insufficient and sufficient participants at baseline (Table 2). However, following 6 weeks of supplementation the vitamin D₃ percent free was 20% higher among insufficient compared to sufficient participants: 0.029% vs. 0.024% (p<0.001) (Table 2) indicating that the binding of vitamin D₃ to serum protein increased in sufficient participants during supplementation.

Association between C_f and outcomes. There were no significant associations between treatment failures or exacerbations and C_f or the change in C_f from baseline to visit 6 (see table A1 in the appendix). In order to further explore the relationship

between outcomes and vitamin D₃ levels we stratified the participants into three groups based on C_f of vitamin D₃ achieved at visit 6: low, < 9 pg/ml (N=65), medium, 9 – 12 pg/ml (N=84) and high, > 12 pg/ml (N=48). There were no significant associations between treatment outcomes and C_f (Table 3) or changes in C_f from baseline (table A1 in the appendix) in patients with asthma participating in the VIDA trial. For participants with vitamin D₃ C_f < 9, 9-12 and > 12 pg/ml: overall treatment failure rates were 0.60 (95%CI 0.46-0.78), 0.53 (95%CI 0.40-0.70) and 0.69 (95%CI 0.54-0.90)/person-year (p=0.51), respectively; and the overall exacerbation rates were 0.28 (95%CI 0.17-0.48), 0.15 (95%CI 0.08-0.30) and 0.42 (95%CI 0.27-0.66)/person-year (p=0.22). We found no significant associations between time to first treatment failure or time to first exacerbation and free levels of vitamin D₃, and found no significant associations between changes in FEV₁ and free levels or changes in free levels (table A1 in the appendix).

DISCUSSION

The primary finding of this VIDA trial analysis revealed no associations between C_f or changes in C_f and the outcomes following vitamin D₃ supplementation. Overall rates of treatment failure and exacerbation tended to decrease at C_f levels 9-12 pg/ml but increased at levels > 12 pg/ml (Table 3) and none of the changes were significant. This observation is not consistent with the VIDA finding that vitamin D supplementation improved the efficacy of inhaled corticosteroids when participants achieved levels ≥ 30 ng/ml. The inconsistency between free and total level associations with response outcomes was unexpected given the predictions of the free hormone hypothesis and the strong relationship between C_f and C_{tot} (Figure 1). Additionally our observation does not agree with those of others reporting that free levels are better associated with skeletal markers, atopy and lung function in children with asthma compared to total levels⁹⁻¹³. We conclude that free concentrations of vitamin D₃ were inferior to total concentrations in associating responsiveness to vitamin D supplementation in the VIDA trial. Our findings warrant further studies to explore the relationship between free vs. total vitamin D concentrations as biomarkers of skeletal and extra-skeletal actions vitamin D supplementation in patients with asthma.

Two additional findings are of major importance. First, baseline free vitamin D₃ concentrations differed by race, ethnicity, sex and body size (Table 1). Several studies report that C_{tot} of vitamin D₃ are lower among African Americans compared to non-Hispanic Whites despite having superior bone health^{18,28-30}, which has been referred to as a paradox²⁹. To address this paradox Powe et al.¹⁰ calculated bioavailable concentrations of vitamin D₃ (C_f + vitamin D₃ bound to albumin) from C_{tot} and VDBP

concentrations and reported that bioavailable concentrations were better related to circulating parathyroid concentrations than serum concentrations (C_{tot}) of vitamin D₃. Lower C_{tot} of vitamin D₃ in African Americans were attributed to lower VDBP concentrations as a consequence of ancestral differences in the allele frequencies of polymorphisms in the GC gene in these individuals of African descent compared to Whites. However, these results have been questioned owing to technical problems inherent in the VDBP assay used in their studies that led to overestimation of C_f and bioavailable concentrations³¹⁻³⁴. More recent studies have measured C_f directly (thereby avoiding issues related to VDBP binding) in patients and reported that C_f of vitamin D₃ are generally lower in African Americans depending on physiological or disease state^{18,26,35}. For example, directly measured C_f were identical in African American and White healthy post menopausal women¹⁸ while in patients with Type 2 Diabetes³⁵ directly measured C_f were lower in African Americans compared to Whites. The results of our study show that C_f of vitamin D₃ in African Americans with moderate asthma on ICS are lower compared to Whites at baseline (Table 1). Fitzpatrick sun-reactive skin typing and melanin index, non-laboratory techniques, can also discern vitamin D deficiency among black and white children³⁶. Additionally, our data show that vitamin D₃ C_f in Hispanics with asthma are lower compared to Whites. There is evidence that vitamin D status may contribute to the racial and ethnic disparity of the asthma burden³⁷. Future studies in this important area of research should evaluate vitamin D status by directly measuring C_f of vitamin D₃.

In addition to race and ethnicity, BMI and sex contributed to the variability in C_f of vitamin D₃ at baseline (Table 1). Previous studies have reported an inverse relationship

between C_{tot} vitamin D_3 and BMI (see (<https://www.ncbi.nlm.nih.gov/books/NBK56061/>) and C_f and obesity¹⁵. Ours is the first to report that C_f of vitamin D_3 was lower among overweight or obese participants with asthma compared to those with BMI values < 25 (Table 1; Figure 1). Lower dietary intake of vitamin D or reduced sunlight exposure probably contributed to reduced free concentrations of vitamin D_3 in overweight or obese individuals compared to non-obese individuals. Our study showed that C_f of vitamin D_3 were lower in females compared to males. Interestingly, the prevalence of asthma in adults is higher in females than males³⁸. The clinical relevance of and the mechanisms underlying the sex differences in C_f of vitamin D_3 are unknown and require further investigation.

Another major finding of our study is the apparent resistance of some participants to supplementation. We found that C_f of vitamin D_3 at baseline and at visit 6, and the change in C_f from baseline to 12 weeks were higher in VIDA participants who achieved serum concentrations ≥ 30 ng/ml compared to participants with total levels < 30 ng/ml at similar supplementation doses (Table 2). These differences suggest that participants with serum levels < 30 ng/ml were resistant to vitamin D_3 supplementation compared to participants with serum levels ≥ 30 ng/ml. The mechanisms underlying resistance are unknown and may be related to pharmacogenetics or genetic variants in vitamin D metabolic pathway genes, which alter the pharmacokinetics of C_f vitamin D_3 . For instance, several genome-wide studies have identified loci in *CYP2R1*, the gene encoding the enzyme that converts vitamin D to vitamin D_3 , and other vitamin D pathway genes that have been associated with low C_{tot} of vitamin D_3 ^{17,39-42}. Genetic loci in vitamin D_3 pathway genes provide a rationale as a determinant for therapeutic

responsiveness to supplementation therapy and the observed inter-ethnic differences in attainable concentrations. We are performing pharmacogenetic studies of biologic candidate gene variants in the vitamin D₃ metabolic pathway to identify a role for gene variation as a determinant of the inter-individual variability of C_f observed in our study.

In the present study C_f and C_{tot} were highly correlated (Figure 1, $r=0.77$), as reported in other studies^{12,13,26}, yet, outcomes were associated with C_{tot} levels > 30 ng/ml²⁵ but not with C_f levels (Table 3). The reason(s) underlying this finding is not clear. C_f is related to C_{tot} by: $C_f = f_u \cdot C_{tot}$, thus, the variation in depicted Figure 1 is related to the interpatient variability in f_u , which is determined by the concentration of VDBP (and to a much lesser extent, albumin) and its association constant for vitamin D₃. At baseline f_u values were similar among participants with C_{tot} levels < 30 ng/ml and those > 30 ng/ml (Table 2). However, after supplementation, f_u values were ~ 20% lower (higher binding to plasma proteins) in participants with C_{tot} > 30 ng/ml compared to participants with levels < 30 ng/ml (2.43×10^{-4} vs. 2.93×10^{-4}). The free hormone hypothesis predicts that reductions in f_u (increased binding) reduces C_{tot} but has no influence on C_f^{6,8}. Thus it is possible that the differences in f_u between those achieving C_{tot} < 30 ng/ml and those with levels > 30 ng/ml may have contributed to the differences in outcomes vs. C_{tot} and outcomes vs. C_f associations. Alternatively, the increased binding of vitamin D₃ in participants with C_{tot} levels > 30 ng/ml may have reflected increased VDBP and increased transport of VDBP-vitamin D₃ complex into tissue.

The determination of vitamin D status (sufficiency, insufficiency, deficiency) relies on levels of parathyroid hormone (PTH) and C_{tot} of vitamin D₃. Vitamin D sufficiency was defined when PTH levels plateaued at their nadir of ~ 30 pg/ml, which occurred at

C_{tot} of vitamin D₃ of 30-40 ng/ml¹. Sufficiency based on free concentrations of vitamin D₃ is unknown but may be estimated. For example, Walsh et al reported that the directly measured C_f of vitamin D₃ was 10.6 pmol/L (4.24 pg/ml) in their population of healthy Caucasians in the UK, while C_{tot} and PTH levels were 15-23 ng/ml and 41-42 pg/ml, respectively¹⁵. For PTH levels to decline from 40 pg/ml (insufficiency) to 30 pg/ml (sufficiency) required a doubling of C_{tot} of vitamin D₃ from 15 ng/ml to 30 ng/ml¹. Since C_f and C_{tot} are highly correlated (Figure 1) we speculate that a doubling of C_f from 4-5 pg/ml to ~9 pg/ml will reduce PTH from 40 to 30 pg/ml and achieve sufficiency. Our estimate that the C_f of vitamin D₃ of 9 pg/ml defines vitamin D sufficiency was the basis for analyzing VIDA outcomes at 3 stratifications: <9 pg/ml, 9-12 pg/ml and >12 pg/ml (table 3).

Our study has several possible limitations. First, the VIDA trial may be inadequately powered as discussed in the original publication²⁵. In support of this potential limitation, a recent meta-analysis of clinical trials of vitamin D₃ supplementation which includes the VIDA trial concluded that vitamin D₃ supplementation reduced the risk of asthma exacerbations⁴³. Second, vitamin D₃ C_f were not quantified in VIDA participants who took placebo thereby reducing our power to detect a relationship between C_f and outcomes. However, we did not detect a trend for an association between C_f and outcomes suggesting that quantifying C_f and including them in our analysis would not have influenced the outcome. Lastly, insufficient dosing of vitamin D may contribute to the failure of supplementation to alter treatment failure and exacerbation rates.

In summary, the present analysis explored associations between C_f vitamin D₃ and outcomes of the VIDA trial. We observed no associations between C_f vitamin D₃ and rates of treatment failure and exacerbations. C_f vitamin D₃ were lower in African Americans and Hispanics, in overweight participants and in females. We recommend that future studies of vitamin D supplementation in patients with asthma quantify both free and total levels of vitamin D₃.

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FIGURE LEGEND

Figure 1. Relationship between free concentrations and total serum concentrations of vitamin D₃ at baseline in VIDA participants.

Figure 2. Relationship between body mass index (BMI) and free concentrations of vitamin D₃ at baseline in VIDA participants.

Figure 3. Free vitamin D₃ concentrations at baseline and at visits 6, 8 and 10 following the administration of a loading and maintenance doses of vitamin D₃ in VIDA participants taking vitamin D₃.

FIGURES

FIGURE 1

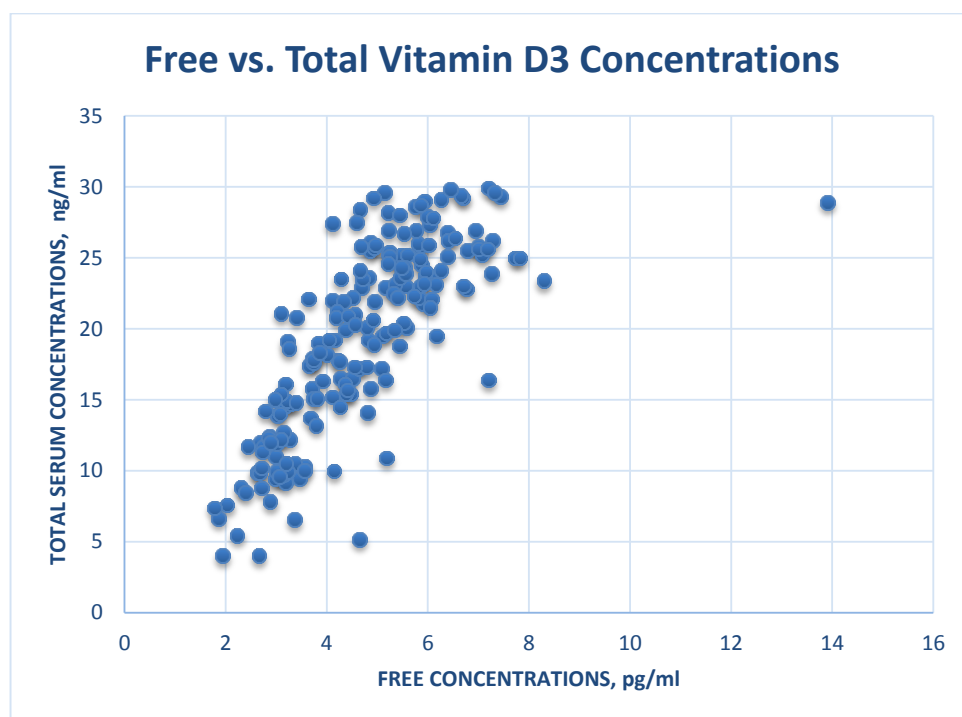


FIGURE 2

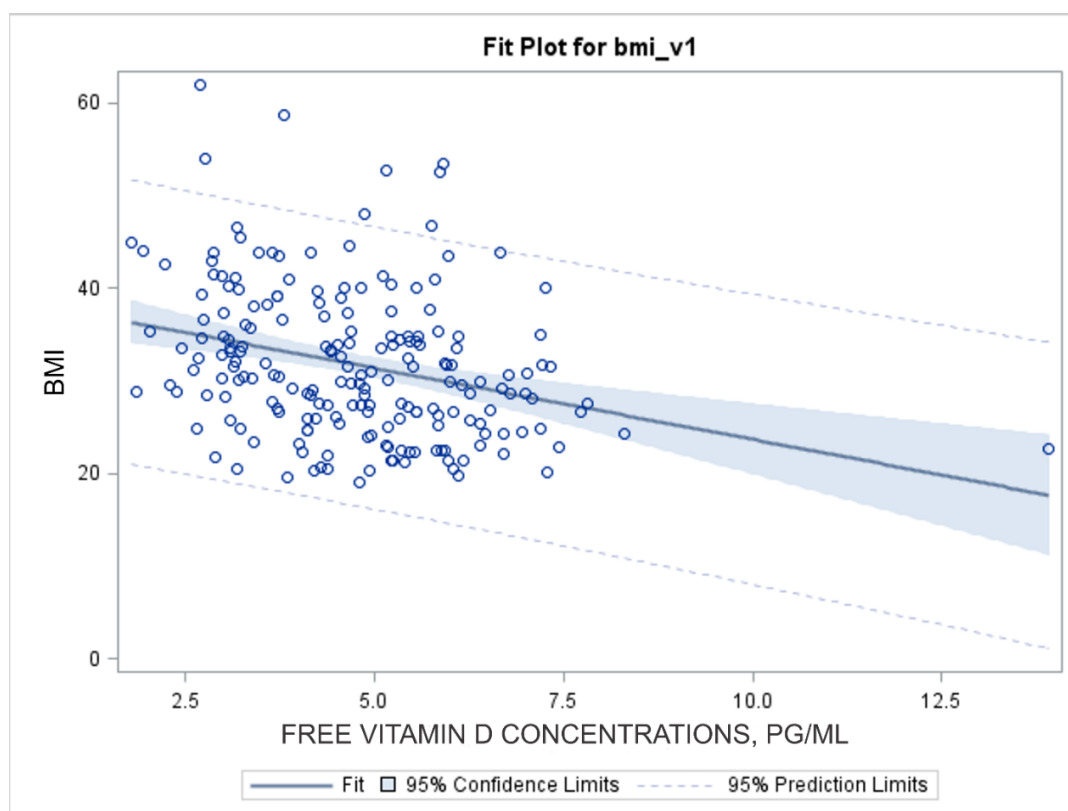


FIGURE 3

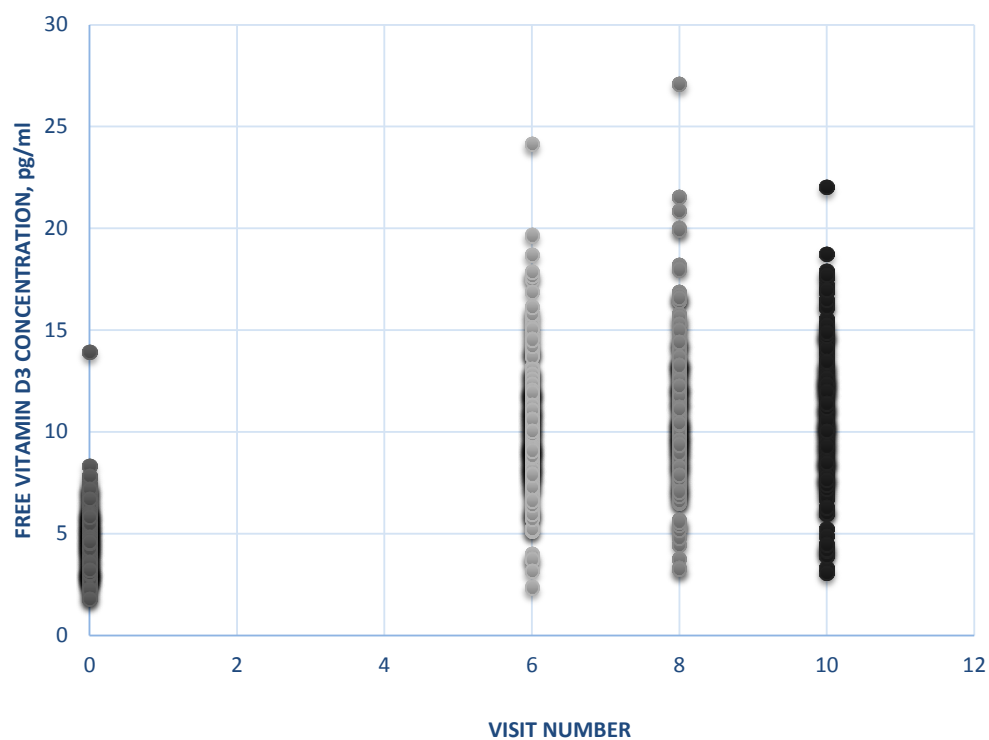


Table 1. Comparison of baseline free vitamin D₃ concentrations and binding to serum proteins by race, sex and BMI in VIDA participants.

DEMOGRAPHICS (number)	MEAN (SD)	
	C _f , pg/ml	f _u *10 ⁴
Race		
Black (64)	4.10 (1.33)	2.79 (1.14)
White (103)	5.16 (1.65)	2.49 (0.55)
Hispanic (19)	4.38 (1.11)	2.38 (0.43)
Overall p-value (Pair-wise p-values)	p<0.001 (<0.001 W vs B 0.038 W vs H)	p=0.031 (0.018 W vs B)
Sex		
Female (135)	4.57 (1.58)	2.46 (0.63)
Male (65)	5.08 (1.41)	2.82 (1.04)
p-value	0.026	0.003
BMI		
<25 (43)	5.45 (1.86)	2.55 (0.92)
>25 (157)	4.54 (1.39)	2.58 (0.77)
p-value	<0.001	0.85

Table 2. Comparison of vitamin D₃ free concentrations and binding to serum proteins in insufficient (serum concentrations < 30 ng/ml) and sufficient (serum concentrations ≥ 30 ng/ml) VIDA participants at baseline and following 12 weeks (visit 6) of vitamin D₃ supplementation.

MEAN (SD) PARAMETER	VITAMIN D STATUS		p value
	C _{tot} < 30 ng/ml (n=35)	C _{tot} ≥ 30 ng/ml (n=162)	
C _f , pg/ml baseline	4.15 (1.30)	4.85 (1.57)	0.016
f _u , baseline*10 ⁻⁴	2.78 (1.25)	2.54 (0.67)	0.11
C _f , pg/ml visit 6	7.01 (2.23)	11.1 (2.81)	<0.001
Change C _f , pg/ml visit 6	2.86 (2.01)	6.24 (2.81)	<0.001
f _u , visit 6*10 ⁻⁴	2.93 (0.59)	2.43 (0.41)	<0.001

Table 3. Association analyses of outcomes in patients with asthma participating in the VIDA trial stratified by groups of free vitamin D₃ concentrations 12 weeks (visit 6) following the beginning of supplementation.

TREATMENT OUTCOMES	VITAMIN D ₃ C _f , pg/ml (number of participants)			p value
	<9 (65)	9 – 12 (84)	>12 (48)	
Overall treatment failure rate/person-yr	0.60	0.53	0.69	0.51
Overall exacerbation rate/person-yr	0.28	0.15	0.42	0.22

Table A1. P values of associations between outcomes and predictors in participants with asthma.

Outcome	Predictor	Type	P-value*
Treatment failures	C _f	Continuous	0.51
	Change in C _f	Continuous	0.99
	Change in C _f categorical	<5 vs. 5-9 vs. ≥9	0.21
Exacerbations	C _f	Continuous	0.85
	Change in C _f	Continuous	0.85
	Change in C _f categorical	<5 vs. 5-9 vs. ≥9	0.39
Time to first treatment failure	C _f categorical	<9 vs. 9-12 vs. ≥12	0.19
Time to first exacerbation	C _f categorical	<9 vs. 9-12 vs. ≥12	0.07
Change in %predicted FEV1	C _f	Correlation (r=0.07)	0.36
	Change in C _f	Correlation (r=-0.06)	0.43

*Treatment failures and exacerbations were modeled using Poisson regression and the associations were adjusted for center, BMI and race; time to first event was evaluated using the Logrank test; associations between change in FEV1 and free Vitamin D measures were based on Pearson correlation.