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The Vitamin D Metabolite Ratio (VMR) is a Biomarker of Vitamin D Status That is Not Affected by Acute Changes in Vitamin D Binding Protein

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BACKGROUND: 25-hydroxyvitamin D[25(OH)D] may be a poor marker of vitamin D status due to variability in levels of vitamin D binding protein (VDBP). The vitamin D metabolite ratio (VMR) is the ratio of 24,25-dihydroxyvitamin D[24,25$(OH)_2D_3$] to 25$(OH)D_3$ and has been postulated to reflect vitamin D sufficiency independent of variability in VDBP. Therapeutic plasma exchange (TPE) is a procedure that removes plasma, including VDBP, and may lower bound vitamin D metabolite concentrations. Effects of TPE on the VMR are unknown.

METHODS: We measured 25(OH)D, free 25(OH)D, 1,25-dihydroxyvitamin D[1,25$(OH)_2D$], 24,25$(OH)_2D_3$, and VDBP in persons undergoing TPE, before and after treatment. We used paired *t*-tests to assess changes in these biomarkers during a TPE procedure.

RESULTS: Study participants (n = 45) had a mean age of 55 ± 16 years; 67% were female; and 76% were white. Compared to pretreatment concentrations, TPE caused a significant decrease in total VDBP by 65% (95%CI 60,70%), as well as all the vitamin D metabolites—25(OH)D by 66% (60%,74%), free 25(OH)D by 31% (24%,39%), 24,25$(OH)_2D_3$ by 66% (55%,78%) and 1,25$(OH)_2D$ by 68% (60%,76%). In contrast, there was no significant change in the VMR before and after a single TPE treatment, with an observed mean 7% (−3%, 17%) change in VMR.

CONCLUSIONS: Changes in VDBP concentration across TPE parallel changes in 25(OH)D, 1,25$(OH)_2D$, and 24,25$(OH)_2D_3$, suggesting that concentrations of these metabolites reflect underlying VDBP concentrations. The VMR is stable across a TPE session despite a 65% reduction in VDBP. These findings suggest that the VMR is a marker of vitamin D status independent of VDBP levels.

Introduction

Vitamin D is an essential pro-hormone that aids in calcium metabolism including intestinal calcium absorption and bone mineralization (1). Vitamin D deficiency is prevalent in the USA and can lead to poor health outcomes including osteomalacia, rickets, and fractures (2). To curb these adverse outcomes, many individuals take vitamin D supplements, often based on m/05/23 at 15:01 "2484"easurements of serum 25-hydroxyvitamin D [25(OH)D] the inactive precursor of active vitamin D, 1,25-dihidroxyvitamin D [1,25$(OH)_2D$ or calcitriol]. Vitamin D supplementation in the United States has increased substantially over the past few decades from <1% of the population in 1999 to about 20% in 2014 (3). However, it remains unclear whether 25(OH)D concentrations accurately reflect vitamin D status and bone health (4, 5, 6, 7). Given the clinical importance of administering the appropriate dose of vitamin D as well as the high financial burden of measuring vitamin D levels and administering supplementation to patients, it is critical that we understand the parameters that may affect 25(OH)D concentrations.

The total 25(OH)D concentration measured as the standard clinical marker of vitamin D adequacy includes 3 forms of 25(OH)D: free (1%), albumin-bound (14%), and bound to vitamin D binding protein (VDBP) (85%). Importantly, VDBP-bound vitamin D is not bioavailable (8). Mounting evidence suggests that there are substantial differences in VDBP concentrations between individuals and that there is genetic variability in the binding affinity of vitamin D to VDBP (9, 10, 11). Thus, with a total 25(OH)D concentration, it is challenging to determine what percentage

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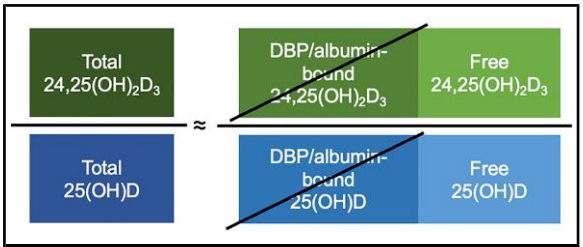

Fig. 1. The VMR is hypothetically independent of VDBP concentrations. The effects of VDBP on 25(OH)D (in the denominator) and $24,25(OH)_2D_3$ (in the numerator) hypothetically cancel out in the VMR.

of total 25(OH)D is truly bioavailable in an individual (12).

Recent studies suggest that the ratio of $24,25(OH)_2D$ to 25(OH)D (vitamin D metabolite ratio or VMR) is an alternative marker of vitamin D status and bone health (13, 14, 15, 16, 17). As a homeostatic mechanism to prevent tissue vitamin D toxicity, increased binding of $1,25(OH)_2D$ to the vitamin D receptor (VDR) stimulates the catabolism of 25(OH)D to $24,25(OH)_2D$ (18). Therefore, an increase in VDR stimulation leads to an increase in the ratio of $24,25(OH)_2D$ to 25(OH)D. As both $24,25(OH)_2D$ and 25(OH)D are VDBP-bound, it has been postulated that the VMR may provide an indicator of vitamin D adequacy independent of VDBP levels (Fig. 1) (19). To our knowledge, no interventional study has been conducted to investigate the effects of directly changing VDBP concentrations on 25(OH)D or the VMR.

Therapeutic plasma exchange (TPE) is a procedure in which blood is extracted from the patient, separated into its components, and returned to the patient without the plasma. TPE is typically performed for the purpose of removing antibodies contained in the plasma as part of treatments for various autoimmune conditions. The removed plasma is typically exchanged for albumin, or more rarely, transfused plasma. In the process, other proteins such as VDBP are also removed from the patient's blood, and not replaced (unless plasma is transfused). One study among 11 patients who underwent 5 plasma exchange treatments demonstrated a decrease in total 25(OH)D and VDBP, however, no assessment of $24,25(OH)_2D$ or the VMR was reported (20). If, as hypothesized, the VMR is not impacted by VDBP concentrations, then the VMR may not change during TPE despite marked reductions in VDBP and 25(OH)D.

In this study, we evaluated the effect of TPE on vitamin D metabolites, as well as the VMR, among persons treated with chronic TPE at the University of California, San Diego Apheresis Unit. We hypothesized that TPE would lower all vitamin D metabolites, as well as VDBP, but would not affect free vitamin D or VMR levels.

Methods

STUDY POPULATION

Participants were recruited over the course of 3 months from July to September of 2020 from the Apheresis Unit at the University of California San Diego. All 52 patients undergoing TPE during the recruitment period were approached, and 45 patients were recruited. Of these patients, 43 were enrolled during a course of TPE (>3 treatments) and 2 were undergoing their first TPE treatment. Participants were required to be at least 18 years old and be undergoing acute or chronic TPE. Participants undergoing other apheresis procedures including cytapheresis or red blood cell exchange were excluded. The study was approved by the institutional review board at the University of California San Diego and informed consent was obtained from all participants.

Blood samples were collected immediately before and after a single TPE treatment. All patients had their plasma volume entirely replaced by albumin except for one who was replaced with half albumin and half plasma. In all patients, blood was drawn from the same intravenous line that was being used for the TPE procedure. Samples were stored at −80°C until sent for testing, as the concentration of vitamin D metabolites remains stable under these conditions for extended periods of time (21, 22).

OUTCOME VARIABLES

Our primary outcomes were the changes in vitamin D metabolite concentrations, the VMR, and VDBP with TPE. Vitamin D metabolites including 25(OH)D, $24,25(OH)_2D_3$, and $1,25(OH)_2D$ were quantified using immunoaffinity enrichment and LC-MS/MS (14). We have been unable to detect the presence of $24,25(OH)_2D_2$, so this metabolite was not included in analyses. Free 25(OH)D was measured using a DIAsource ELISA assay. VDBP concentration and phenotype were determined simultaneously via LC-MS/MS (23). Details of these assays have been described elsewhere previously (19). The VMR was calculated by dividing serum $24,25(OH)_2D_3$ by serum $25(OH)D_3$ and multiplying by 100 (13).

OTHER MEASUREMENTS

Participant characteristics (including age, gender, race, medication use, comorbidities including osteopenia, osteoporosis, fractures, kidney, or liver disease) as well as laboratory measurements were extracted from the medical records and confirmed by interviewing the study participants. Use of medications and medical history were additionally confirmed by self-report.

Table 1. Baseline characteristics of TPE patients (N = 45).

Demographics	
Age (years)	55 ± 16
Female, n (%)	30 (67%)
Race, n (%)	
White	34 (76)
Asian	3 (7)
Black	2 (4)
Other	6 (13)
Body mass index	25.5 ± 5.6
Treatment parameters	
Number of treatments [IQR]	122 [34, 409]
Length of treatment (min)	106 ± 23
Days between treatments	6 ± 5
Plasma removed (mL)	3055 ± 637
Fluid replaced (mL)	3463 ± 688
Comorbidities, n (%)	
Hypertension	14 (31)
Diabetes	14 (31)
Osteopenia/osteoporosis	5 (11)
Bone fracture in past 5 years	18 (40)
CKD	8 (18)
End-stage kidney disease	1 (2)
Liver disease	1 (2)
Supplementation	
Ergocalciferol, n (%)	14 (31)
Daily IU [IQR]	0 [0, 7143]
Cholecalciferol, n (%)	32 (71)
Daily IU [IQR]	2000 [0, 5000]
No vitamin D supp., n (%)	5 (11)

IU, International Units.

Hypertension was defined as prescription of an antihypertensive medication. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (GFR) of <60 mL/min/1.73 m^2 at baseline. Liver disease was defined as ongoing review by a hepatologist and a diagnosis of a liver-related disorder. A new start TPE patient was defined as any patient who had not received TPE within 1 year prior to their current procedure.

STATISTICAL ANALYSIS

Baseline characteristics are described using means and standard deviations. Concentrations of vitamin D metabolites as well as VDBP are described using medians and interquartile ranges. We used paired *t*-tests to assess the unadjusted change in each variable before and after TPE. We then developed an adjusted model using multiple linear regression to assess whether any of these changes were attenuated by confounding variables. We adjusted for age, sex, race, TPE indication, duration, time since last treatment, plasma volume exchanged, number of units of fresh frozen plasma transfused, time in days since last treatment, number of total prior treatments, BMI, estimated GFR, presence of liver disease, and VDBP genotype. Secondary outcomes included percent change in metabolites in relation to the percentage changes in VDBP.

All analyses were conducted in Stata SE v.15.1. Statistical significance was determined by P values <0.05 for all analyses.

Results

We recruited 45 participants to this study, with baseline characteristics described in Table 1. The mean age was 55 ± 16 years; 67% were female; 76% were white; 40% had been diagnosed with osteopenia or osteoporosis; 18% had at least one bone fracture within the previous 5 years, and 8 patients had CKD while one had end-stage kidney disease. The most common indication for TPE was multiple sclerosis (47%), followed by myasthenia gravis (18%) and autoimmune autonomic neuropathy (13%). The median (IQR) number of TPE procedures prior to sample collection was 122 (34, 409), with the duration of each TPE procedure lasting on average 106 ± 23 minutes. The baseline median (IQR) concentration of 25(OH)D was 43.5 ng/mL (32.6, 56.8) [108.6 nmol/L (81.4, 141.8)], free 25(OH)D was 12.2 pg/mL (8.98, 16.7) [15.87 pmol/L (11.68, 21.7)], $24,25(OH)_2D_3$ was 1.63 ng/mL (1.03, 2.41) [3.91 nmol/L (2.47, 5.78)], $1,25(OH)_2D$ was 47.8 pg/mL (38.6, 60.5) [114.72 pmol/L (92.6,145.2)], VMR was 4.3 (ng/mL)/(ng/mL) (3.5, 5.8) [equivalent in (nmol/L)/(nmol/L)], and VDBP was 217.2 μg/mL (198.7, 253.7).

In unadjusted models, TPE caused a significant decrease in VDBP [65% (60%, 70%)] in addition to all the vitamin D metabolites that bind to VDBP including total 25(OH)D [66%, (60, 74%)], free 25(OH)D [31%, (24, 39%)], $24,25(OH)_2D_3$ [66%, (55, 78%)], $1,25(OH)_2D$ [68%, (60, 76%)] ($P < 0.001$ for all). There was no significant change in VMR before and after a single TPE treatment, with a mean 7% (−3%, 17%) increase in VMR ($P = 0.16$) (Fig. 2). In fully adjusted models, none of the covariates in any of the models were significantly associated with the changes in each of the metabolites or VDBP.

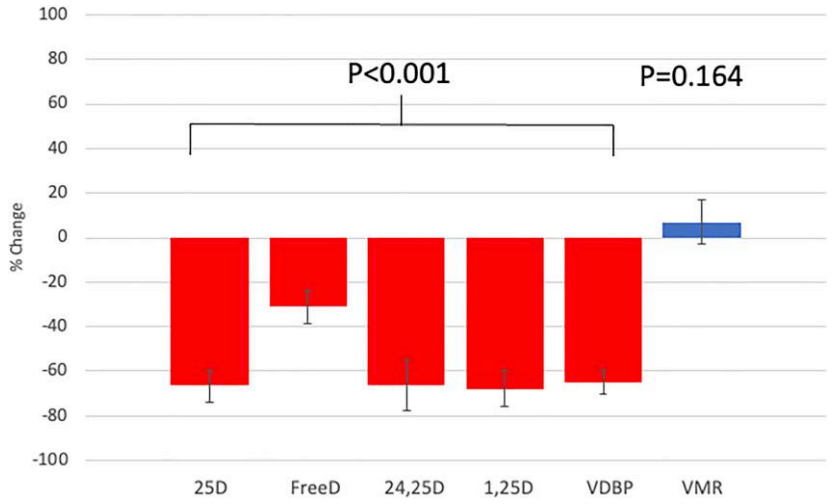

Fig. 2. Changes in vitamin D metabolites, VDBP, and VMR during TPE (N = 45). Acute reductions in VDBP are associated with similar reductions in all vitamin D metabolites but not the VMR. Abbreviations: 25D, 25-hydroxyvitamin D; Free D, Free 25-hydroxyvitamin D; 24,25D$_3$, 24,25-dihydroxyvitamin D$_3$; 1,25D, 1,25-dihydroxyvitamin D.

Table 2. Association of changes in VDBP with changes in vitamin D metabolites in 45 persons undergoing TPE.

	25D		Free D		24,25D$_3$		1,25D		VMR	
	% Change per 10% change in VDBP (95% CI)	*P*	% Change per 10% change in VDBP (95% CI)	*P*	% Change per 10% change in VDBP (95% CI)	*P*	% Change per 10% change in VDBP (95% CI)	*P*	% Change per 10% change in VDBP (95% CI)	*P*
Decline	6 (3, 9)	<0.001	6 (−7, 18)	0.337	6 (3, 10)	0.002	9 (5, 12)	<0.001	−3 (−37, 30)	0.838

Abbreviations: 25D, 25-hydroxyvitamin D; Free D, Free 25-hydroxyvitamin D; 24,25D3, 24,25-dihydroxyvitamin D$_3$; 1,25D, 1,25-dihydroxyvitamin D.

In companion analyses, we evaluated the relationship of the change in VDBP with the changes in each metabolite and the VMR. In fully adjusted models, a 10% decrease in VDBP during TPE was associated with a 6% (3%, 9%), 6% (3%, 10%), and 9% (5%, 12%) decrease in 25(OH)D, 24,25(OH)$_2$D$_3$, and 1,25(OH)$_2$D, respectively (Table 2). The change in VDBP was not associated with changes in free vitamin D or the VMR, during TPE.

Discussion

We and others have hypothesized that the VMR is a superior marker of vitamin D status and bone health, compared to 25(OH)D alone, because it may be less influenced by VDBP (19). In this study, we demonstrate for the first time the impact of modifying the level of VDBP on vitamin D metabolite concentrations and the VMR in adults treated with TPE. Changes in VDBP concentration across TPE paralleled changes in 25(OH)D, 24,25(OH)$_2$D$_3$, and 1,25(OH)$_2$D suggesting that concentrations of these metabolites may reflect underlying VDBP concentrations. The lack of change in VMR across TPE despite a substantial reduction in VDBP demonstrate that the VMR is independent of VDBP concentration.

There are several lines of evidence that support the hypothesis that the VMR is a superior marker of vitamin

D status compared to total 25(OH)D levels. First, as previously hypothesized and newly confirmed here, the VMR is independent of VDBP. In a prior observational study, we evaluated the association of vitamin D metabolites and the VMR with VDBP in older adults in the Health Aging and Body Composition Study and found that each metabolite was strongly associated with VDBP concentrations while the VMR was not associated with VDBP (19). Our work extends these observational findings, by evaluating these associations in the setting of TPE, which markedly reduces VDBP. Second, since the metabolism of 25(OH)D to 24,25(OH)D is dependent on VDR activity, the ratio of these metabolites as represented by the VMR may better reflect tissue level VDR activity (15). This has led some to suggest defining vitamin D sufficiency as having detectable $24,25(OH)_2D_3$ concentrations (24); notably, in this analysis we were able to detect $24,25(OH)_2D_3$ concentrations in all participants. Third, we and others have shown that a lower VMR is significantly associated with changes in BMD, fractures, progression of kidney disease, and all-cause mortality in older adults and in persons with CKD (13, 15, 16), whereas no association between 25(OH)D and these outcomes was observed.

Whereas the focus of our study was on intraindividual change in 25(OH)D and the VMR, we additionally hypothesized that free 25(OH)D concentrations would not be affected by TPE. Contrary to our hypothesis, we found free 25(OH)D concentrations were also decreased during TPE, albeit with a smaller reduction than that observed with the other vitamin D metabolites. The reason for this reduction is unclear and we are unable to rule out matrix effects, as previously demonstrated for patients on hemodialysis (25). However, it is notable that patients undergoing TPE have plasma removed and primarily albumin infused, generally in greater volume than the total volume removed, to prevent hypotension. Thus, it is possible that infused albumin may bind vitamin D metabolites and affect free 25(OH)D levels. Additionally, citrate was used for anticoagulation, and a calcium infusion was used to prevent resultant hypocalcemia. These may have affected vitamin D metabolism during TPE and could have led to small changes in free 25(OH)D.

The strengths of this study include repeated measurements before and after TPE, as well as the use of LC-MS for accurate and precise vitamin D measurements. Whereas prior studies evaluating the relationship between vitamin D metabolites, VDBP, and VMR have been observational, we leveraged a medical intervention that markedly influences VDBP to determine concurrent effects on vitamin D parameters and the VMR. (19)

There are still several limitations to this study. The study population was relatively small with minimal racial diversity. This study does not have an external control group; however, given the nature of our intervention; we believe pretreatment levels serve as a strong internal control for each participant. Finally, we could not evaluate fat and liver stores of vitamin D that are unlikely to be acutely affected by TPE and likely impact recovery of vitamin D metabolites.

In summary, marked acute reductions in VDBP during TPE parallel reductions in 25(OH)D, 1,25(OH)2D, and 24,25(OH)2D3 suggesting that levels of these metabolites are largely influenced by VDBP levels. Despite marked reductions in VDBP, there was no significant change in VMR across TPE. Overall, these findings suggest that the heretofore hypothesized advantage of the VMR due to lack of dependence on VDBP concentrations appears to hold true, and may be one reason why the VMR appears to be more strongly associated with bone-related outcomes than 25(OH)D.

Nonstandard Abbreviations: 25(OH)D, 25-hydroxyvitamin D; VDBP, vitamin D binding protein; VMR, vitamin D metabolite ratio; 24,25(OH)2D, 24,25-dihydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D; VDR, vitamin D receptor; CKD, chronic kidney disease; GFR, glomerular filtration rate; TPE, therapeutic plasma exchange.

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