Precision diagnostic approaches for assessing clinical vitamin D status

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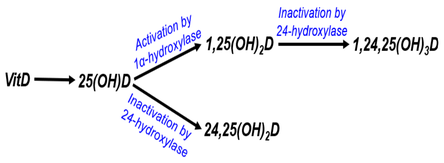
BACKGROUND

Because of the importance of VitD in calcium balance and bone health, the body has evolved effective homeostatic mechanisms to maintain $1,25(OH)_2D$ – the biologically active form of VitD – in a narrow physiological range.

Two enzymes play major roles in VitD metabolism:

- 24-hydroxylase degrades 25(OH)D and $1,25(OH)_2D$.
- 1α-hydroxylase activates 25(OH)D.



OBJECTIVE

We designed a mathematical model to investigate regulation of VitD metabolism with a focus on the role of 24-hydroxylase enzyme in maintaining $1,25(OH)_2D$ level within a narrow physiological range despite wide variation in availability of VitD. We compared alternative indices of VitD status with the currently used index (25(OH)D) with regard to their ability to predict modeled 24-hydroxylase activity and secondary hyperparathyroidism.

METHODS

Participants: 11 otherwise healthy VitD deficient [25(OH)D≤20 ng/mL] participants were studied before and after VitD3 supplementation

Intervention: VitD3 capsules (50,000 IU) once/twice a week based on BMI for 4-6 wks.

Measured endpoints:
25(OH)D, $1,25(OH)_2D$, $24,25(OH)_2D$, PTH, and FGF23

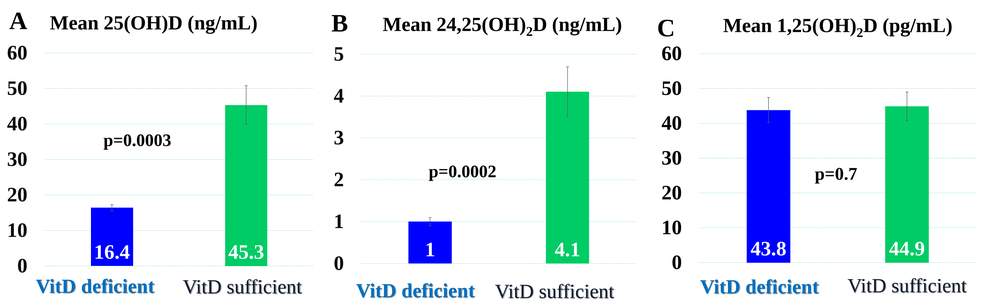
We calculated 3 ratios of VitD metabolites:

- $1,25(OH)_2D$ / $24,25(OH)_2D$
- 25(OH)D / $1,25(OH)_2D$
- $24,25(OH)_2D$ / 25(OH)D

We compared the performance of several VitD metabolite ratios with 25(OH)D in predicting modeled 24-hydroxylase activity.
We also compared three of our participants who had similar baseline 25(OH)D levels and were judged to be VitD deficient based on the Endocrine Society's proposed cutoffs. We applied insights from our mathematical model to differentiate them with regard to VitD status.

Statistical analysis: Two-tailed paired Student's t-test was used; p<0.05 was selected as the criterion for statistical significance.

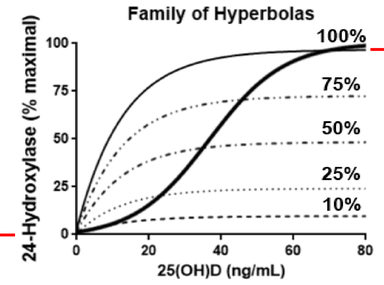
Fig 1. Effect of VitD3 supplementation on VitD metabolites



VitD3 supplementation
* Increased mean 25(OH)D by 2.7-fold **(A)**
* Increased mean $24,25(OH)_2D$ by 4.3-fold **(B)**
* Did not change mean plasma levels of $1,25(OH)_2D$ **(C)**

Fig 2. VitD deficiency suppresses 24-hydroxylase activity

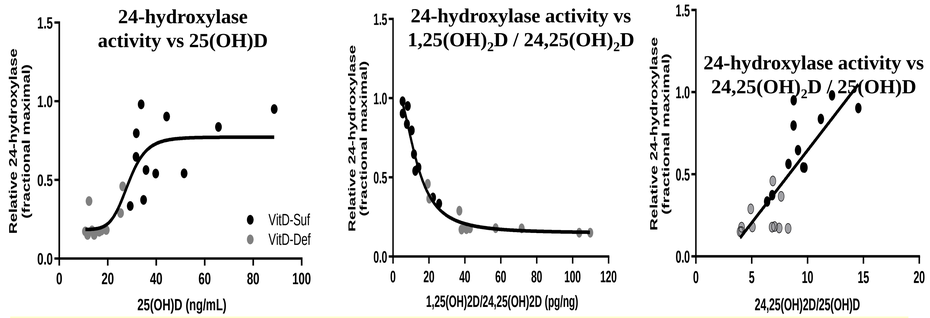
24-hydroxylase achieves a minimum when 25(OH)D<10-20 ng/mL.



24-hydroxylase activity achieves a maximum when 25(OH)D> 50 ng/mL

Solid curve illustrates modeled 24-hydroxylase activity as a function of 25(OH)D levels. The family of hyperbolas illustrate % maximal activity for 24-hydroxylase. The point where the curve crosses a hyperbola corresponds to the amount of 24-hydroxylase activity at that level of 25(OH)D.

Fig 3. Correlation of modeled 24-hydroxylase activity with indices of VitD status



Modeled 24-hydroxylase activity is better correlated with the $1,25(OH)_2D/24,25(OH)_2D$ ratio than with total plasma 25(OH)D levels or the $24,25(OH)_2D/25(OH)D$ ratio.

Three research participants exemplifying impact of precision diagnostics strategy for diagnosis of VitD deficiency. This Table summarizes selected laboratory data and calculated VitD metabolite ratios for participant #1, who exhibited the highest $1,25(OH)_2D/24,25(OH)_2D$ ratio in our clinical trial. Participants #2 and #3 were selected as matches for participant #1 based on having similar baseline total plasma 25(OH)D.

	Patient #1	Patient #2	Patient #3
Baseline 25(OH)D	11.7 ng/mL (29nmol/L)	10.8 ng/mL (27 nmol/L)	12.3 ng/mL (30.7 nmol/L)
Baseline $24,25(OH)_2D$	0.5 ng/mL (1.3 nmol/L)	0.8 ng/mL (2 nmol/L)	0.9 ng/mL (2.3 nmol/L)
Baseline $1,25(OH)_2D$	51 pg/mL (127 pmol/L)	33 pg/mL (82 pmol/L)	19 pg/mL (47 pmol/L)
Baseline iPTH	62 pg/mL (6.6 pmol/L)	29 pg/mL (3.1 pmol/L)	51 pg/mL (5.4 pmol/L)
Baseline iFGF23	50 pg/mL	60 pg/mL	57 pg/mL
[$1,25(OH)_2D$] / [$24,25(OH)_2D$]	110 pg/ng	41 pg/ng	20 pg/ng
[25(OH)D] / [$1,25(OH)_2D$]	0.23 ng/pg	0.33 ng/pg	0.65 ng/pg
[$24,25(OH)_2D$] / [25(OH)D]	0.040	0.075	0.076
PTH: response to VitD3 (Δ%)	-34%	+3%	+5%
VitD status classification using total plasma 25(OH)D (The Endocrine Society's guideline)	VitD deficient	VitD deficient	VitD deficient
VitD status classification using [$1,25(OH)_2D$] / [$24,25(OH)_2D$] ratio	Overt VitD deficiency	Subclinical VitD deficiency	VitD Sufficiency
% Maximal 24-hydroxylase activity	15%	18%	37%

All three participants had similar baseline total plasma 25(OH)D levels.

Participant #1 had high $1,25(OH)_2D/24,25(OH)_2D$ ratio (>100), which has been suggested to be predictive of secondary hyperparathyroidism.

Participant #1 showed a significant decrease in PTH in response to VitD3 supplementation, which suggests baseline secondary hyperparathyroidism.

ABBREVIATIONS

- Vitamin D: VitD
- Vitamin D binding proteins: VDBP
- 25-hydroxyvitamin D: 25(OH)D
- 1,25-dihydroxyvitamin D: $1,25(OH)_2D$
- 24,25-dihydroxyvitamin D: $24,25(OH)_2D$

CONCLUSIONS

- The body has 2 defense mechanisms to maintain $1,25(OH)_2D$ in a physiological range despite variability in VitD availability.
- The 1st line of defense is triggered at early stages of VitD deficiency and preserves VitD from degradation by suppressing 24-hydroxylase.
- In more severe VitD deficiency, the 1st line may be insufficient. Therefore, the **2nd line of defense** – secondary hyperparathyroidism – is triggered to upregulate 1α-hydroxylation and increase $1,25(OH)_2D$ production.

- The level of 24-hydroxylase activity provides a sensitive assessment of whether the body has perceived a deficiency of VitD and has triggered homeostatic mechanisms. Thus, VitD metabolite ratios that include $24,25(OH)_2D$ levels offer insights into 24-hydroxylase regulation and the body's perception of VitD status.
- >99% of all VitD metabolites are bound to albumin and VDBP in the circulation. Also, there is a ~ 3-fold inter-individual variation in the VDBP level in the population. Thus, VitD metabolite ratios have been suggested as an indirect way to account for the impact of VDBP to assess VitD status.

- When we compared the performance of several VitD metabolite ratios with 25(OH)D (the currently recommended index to assess VitD status) with respect to their ability to predict modeled 24-hydroxylase activity, the modeled 24-hydroxylase activity was best correlated with the $1,25(OH)_2D/24,25(OH)_2D$ ratio.
- When we compared three participants who had similar baseline total plasma 25(OH)D levels (10.8-12.3 ng/mL), one had very high $1,25(OH)_2D/24,25(OH)_2D$ ratio and that participant showed a significant decrease in PTH after VitD3 supplementation. This demonstrates the value of the $1,25(OH)_2D/24,25(OH)_2D$ ratio in reflecting the regulation of 24-hydroxylase activity and also predicting secondary hyperparathyroidism.