

Precision Diagnostics Approach to Vitamin D Deficiency

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* Potential conflict of interest may exist. Refer to the Meeting App.

INTRODUCTION

25(OH)D: 25D
1,25(OH)₂D= 1,25D
24,25(OH)₂D= 24,25D
VitD binding protein: VDBP

Recent publications raised questions about the validity of current guidelines for assessing VitD status (1,2). To assess VitD status, one needs to understand regulation of VitD metabolism. 25D undergoes two alternative fates: 1 α -hydroxylation in the kidney, generating 1,25D (the biologically active form) or 24-hydroxylation leading to 24,25D (a biologically inactive metabolite).

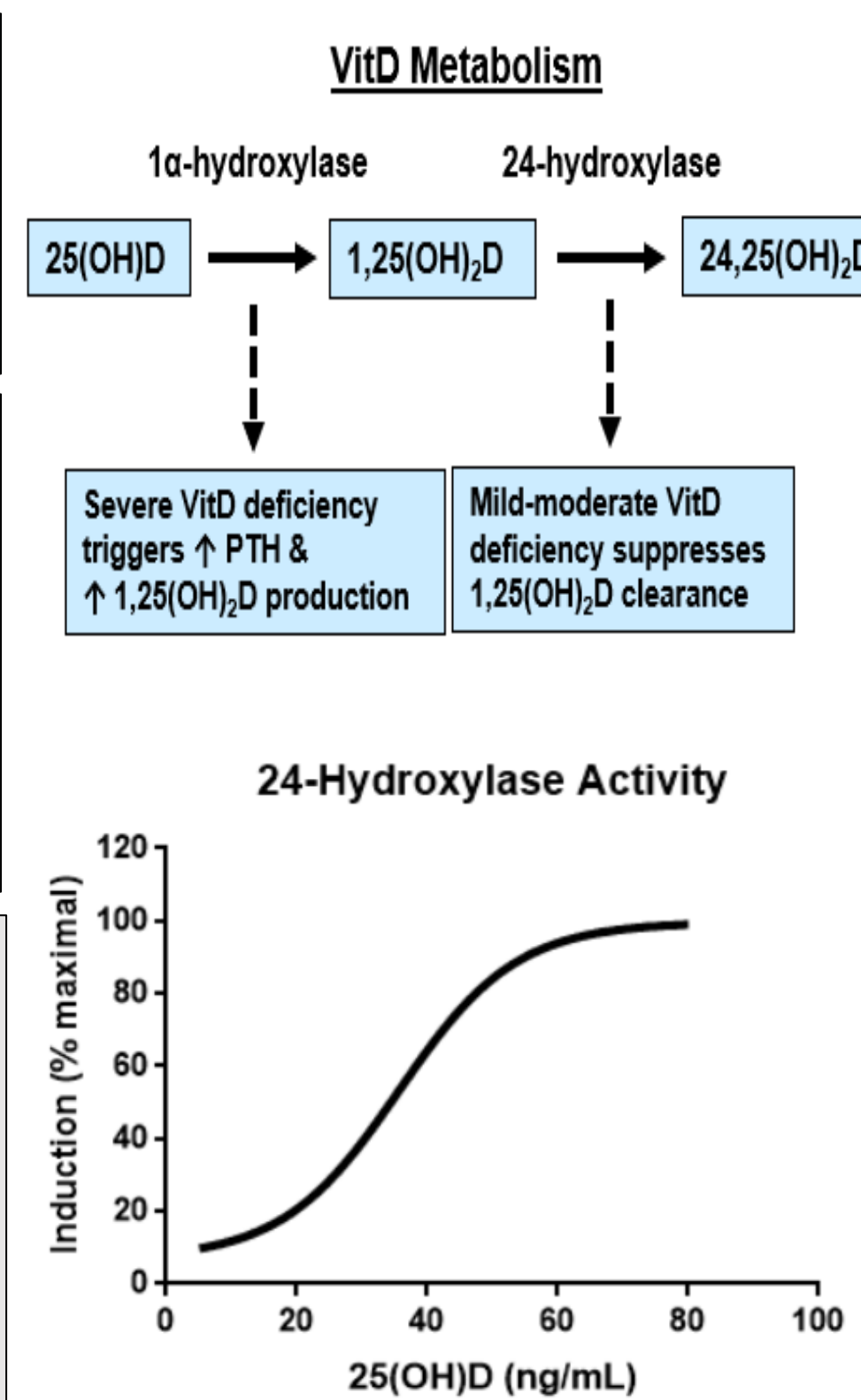
To sustain free 1,25D in a physiological range, the body can \downarrow 24-hydroxylation or \uparrow 1 α -hydroxylation through triggering secondary hyperparathyroidism.

Our novel mathematical model demonstrates that mild-moderate VitD deficiency [25D<20 ng/mL] triggers down-regulation of 24-hydroxylase to sustain 1,25D by \downarrow its degradation which we believe is the 1st line of defense when 25D becomes low.

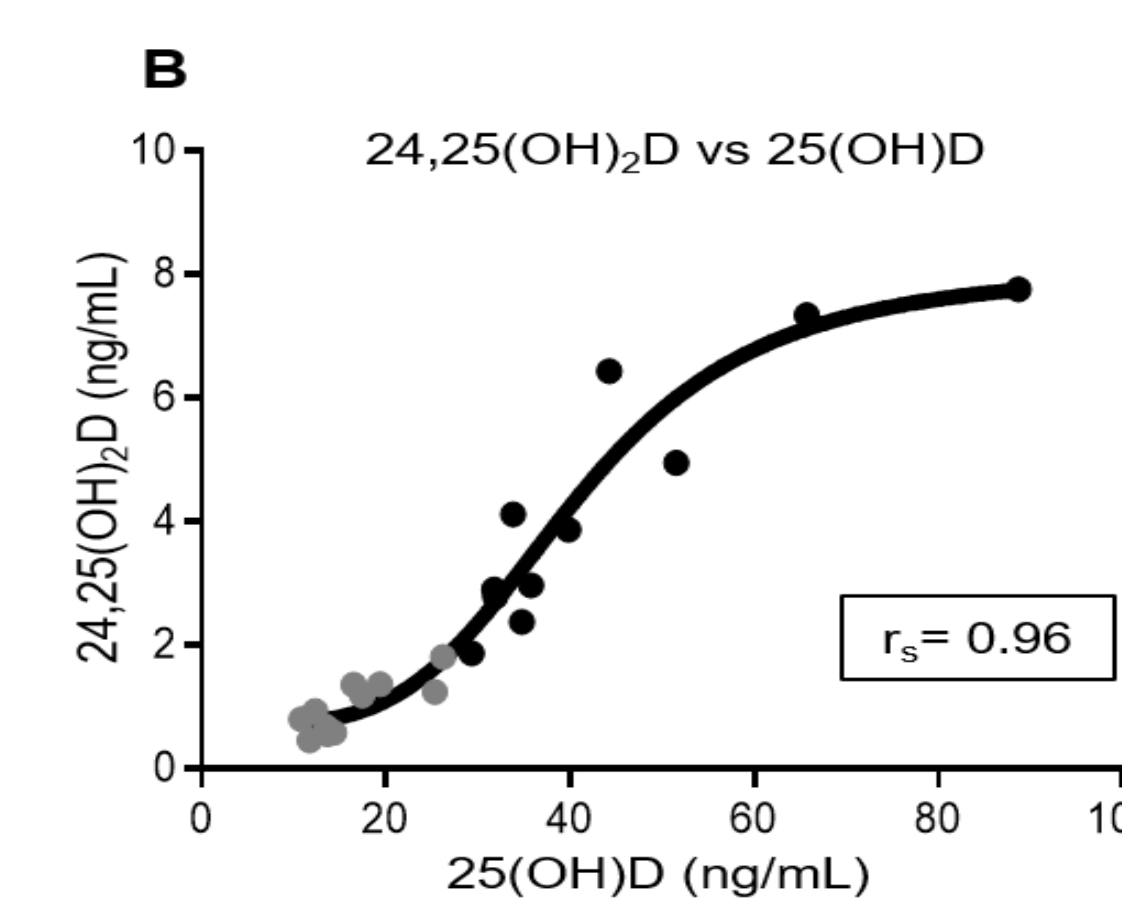
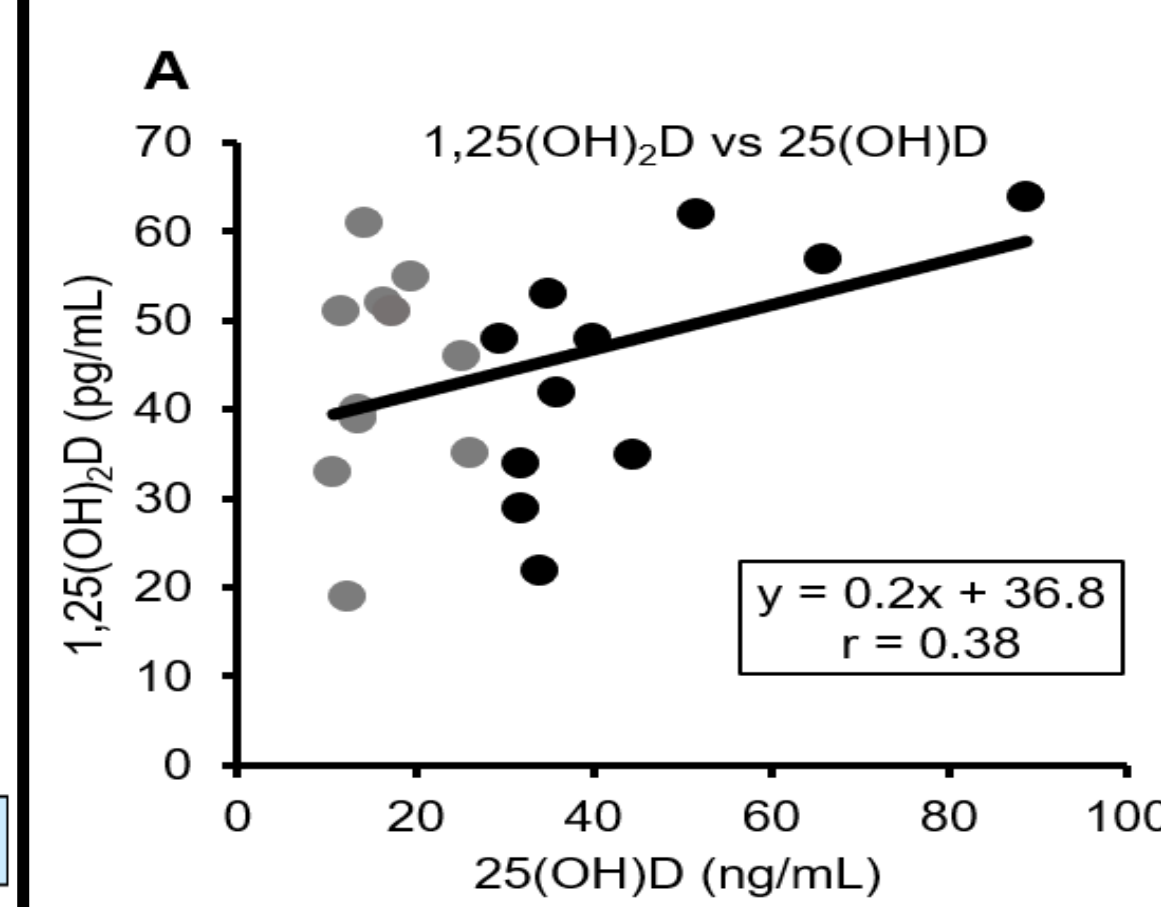
>99% of VitD metabolites are bound to VDBP. Although, the free fraction is biologically active, we measure total plasma 25D to assess VitD status. Here we discuss using VitD metabolite ratios which provide more informative indices by accounting for VDBP.

STUDY DESIGN

- We assessed the impact of VitD supplements in 11 otherwise healthy VitD deficient Amish individuals with screening total 25D < 20 ng/mL.
- Compared 25D, 1,25D, 24,25D, & PTH before versus after VitD3.
- VitD3 (50,000 IU/week) was given for 4-6 wks to achieve 25D>30 ng/mL.
- Designed a mathematical model to investigate how the body maintains 1,25D in a normal range.
- We also calculated/compared two VitD metabolite ratios in VitD deficient versus sufficient state: **25D/1,25D** and **1,25D/24,25D**. We evaluated their applicability for assessment of VitD status.



Mathematical Modeling

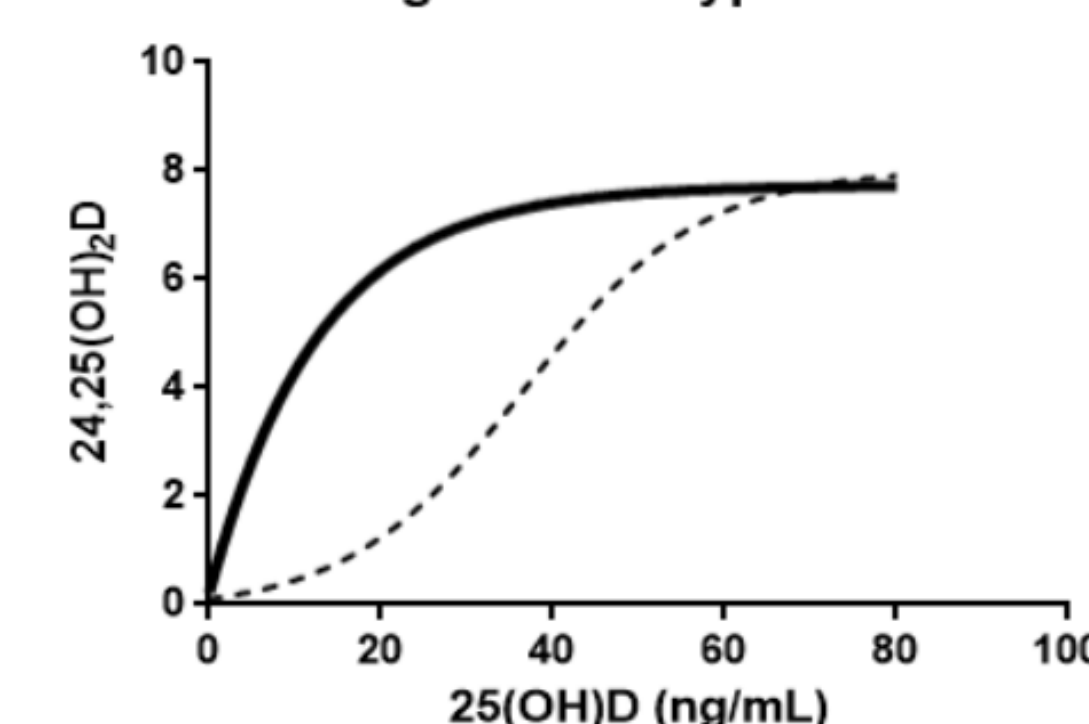


1. Poor correlation between 25D and 1,25D – consistent with previous evidence that in mild-mod VitD deficiency 1,25D is maintained in the normal range, therefore, not a useful index for assessing VitD status.

2. Correlation of 24,25D with 25D is defined by a sigmoid curve. When 25D is low, 24-hydroxylation is suppressed resulting in low levels of 24,25D. When 25D level increases, 24-hydroxylation is induced, thereby protecting against VitD toxicity.

Why sigmoid curve?

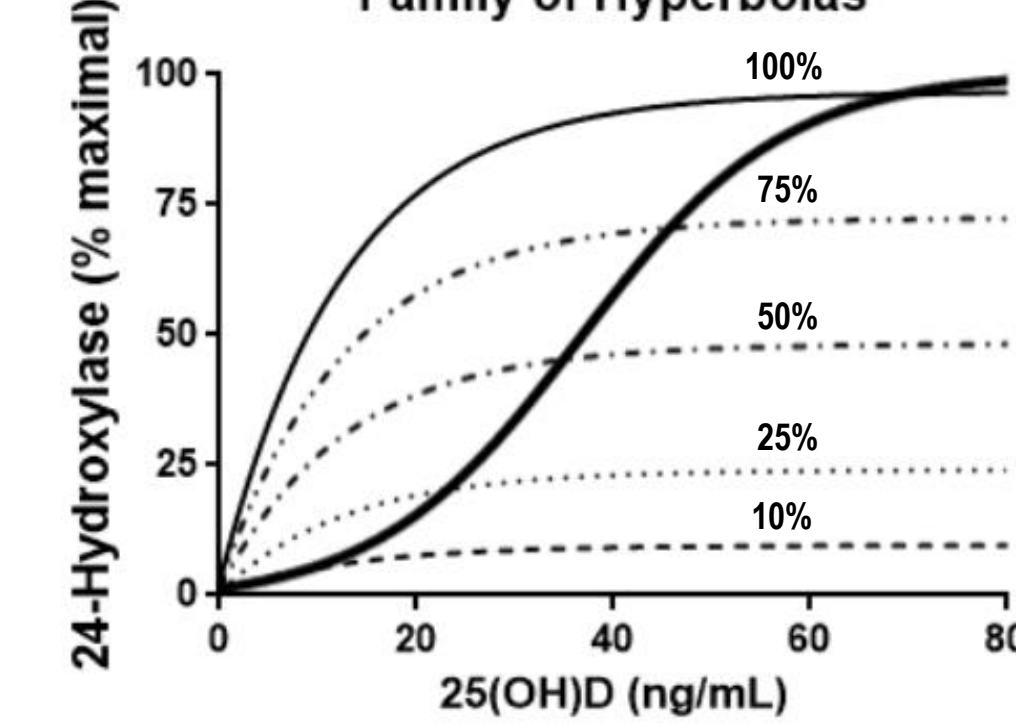
Sigmoid vs Hyperbola



3. If 24-hydroxylase followed simple Michaelis-Menten kinetics, we would have seen a hyperbola!

How to explain the sigmoidicity?

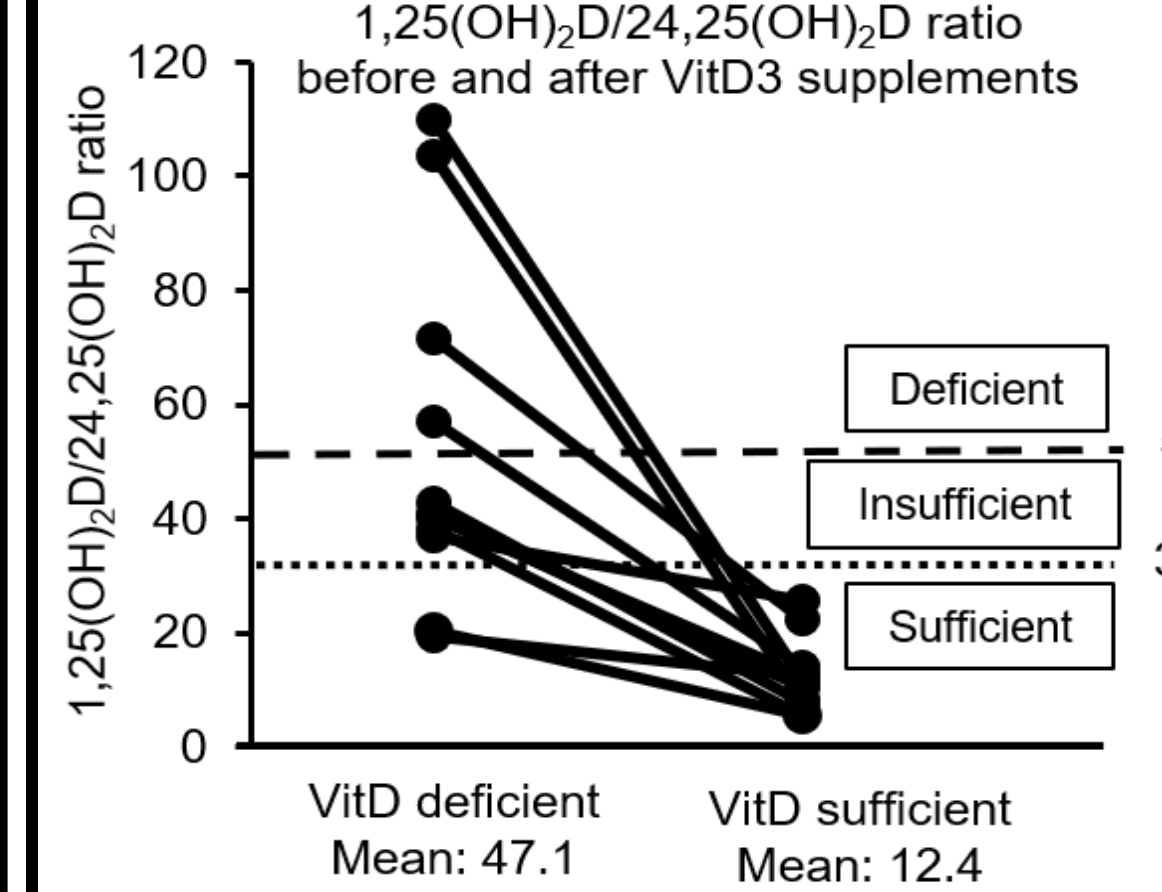
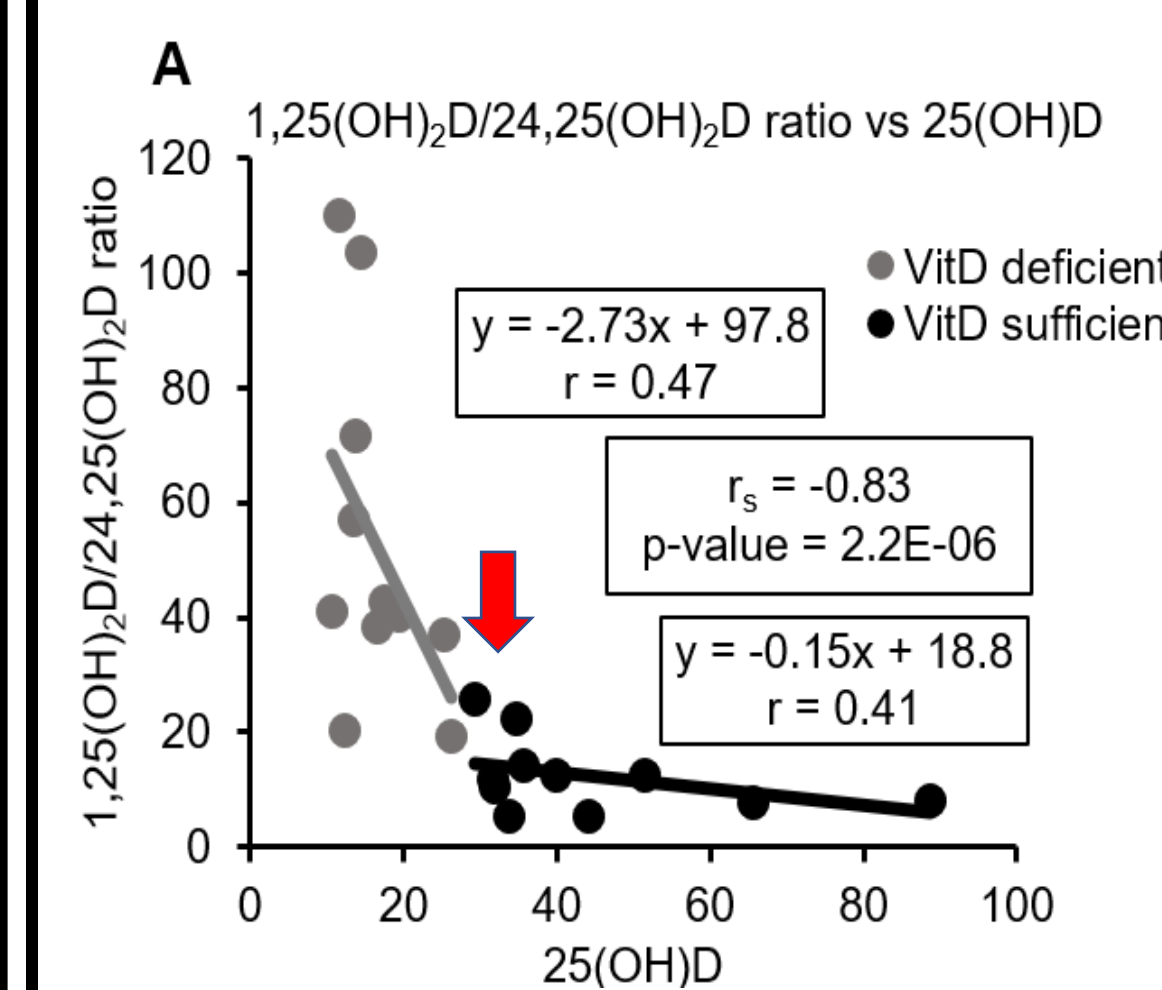
Family of Hyperbolas



4. Expression of 24-hydroxylase is regulated according to 25D levels.

RESULTS

VitD Metabolite Ratios



Baseline parameters	Subject #9	Subject #11#
25D (ng/mL)	12.3	11.7
24,25D (ng/mL)	0.9	0.5
1,25D (pg/mL)	19	51
PTH (pg/mL)	51	62
FGF23 (pg/mL)	57	50
1,25D / 24,25D	20	110
25D / 1,25D	0.65	0.23
PTH: response to VitD3	+5%	-34%

Correlation of 1,25D/24,25D ratio with 25D. Tang et al. (3) suggested this ratio to assess VitD status. \downarrow 25D is associated with an \uparrow in this ratio. There is a sharp inflection point corresponding to a 25D of ~25-30 ng/mL where the ratio \uparrow dramatically. It creates a clear demarcation which enabled Tang et al. (3) to propose a diagnostic criteria.

- Ratio ≥ 51 \rightarrow VitD deficiency
- 35-50 \rightarrow VitD insufficiency
- < 35 \rightarrow VitD sufficiency
- We Applied these cutoffs to our data and found similar results.
- We found a very close correlation between **1,25D/24,25D** & **25D/1,25D** ratio ($r_s = -0.94$; $p = 10^{-10}$).

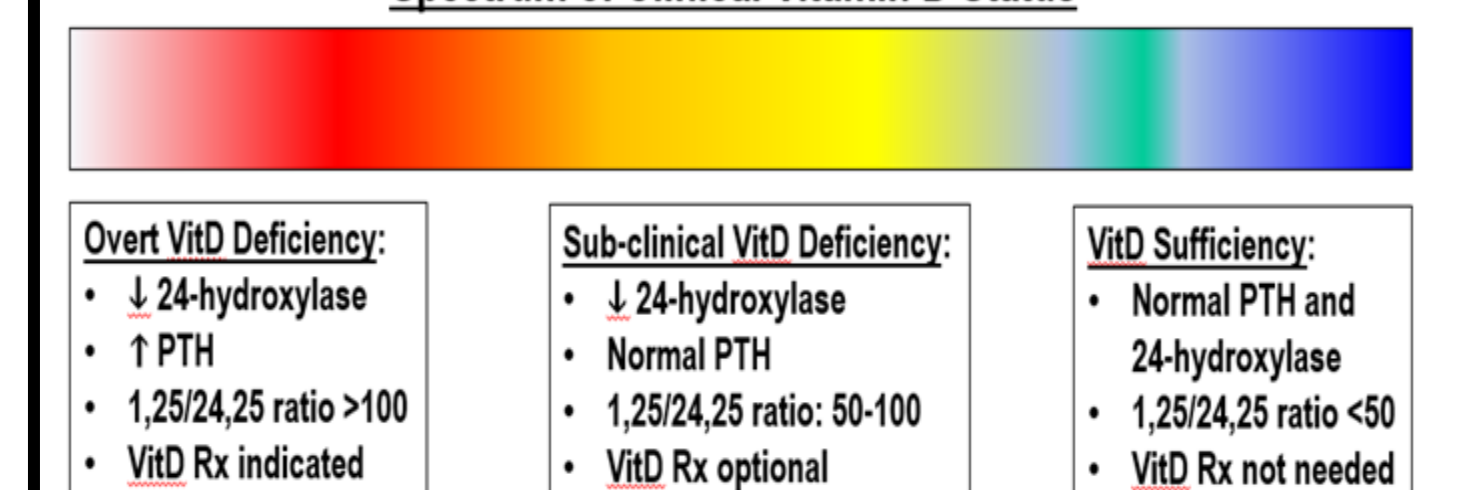
The comparison of two of subjects illustrates the potential value of **1,25D/24,25D** in assessing VitD status. Both had similar 25D levels at baseline. Endocrine Society guidelines classifies both as "VitD deficient". The **1,25D/24,25D** ratio suggests that Subject #9 is VitD sufficient while Subject #11 is severely VitD deficient. This assessment is confirmed by the observation that Subject #11 had secondary hyperparathyroidism as evidenced by the 34% suppression of PTH levels in response to VitD.

SUMMARY

Suppression of 24-hydroxylase is the 1st line of defense to maintain 1,25D levels. Secondary hyperparathyroidism is the 2nd line of defense (\uparrow 1 α -hydroxylation) and occurs in severe VitD deficiency when the 1st line is maximally deployed. **1,25D/24,25D** and **25D/1,25D** ratios provide more clinically relevant information for assessment of VitD status as compared to total plasma 25D.

An illustrative example: We compared **1,25D/24,25D** and "PTH response to VitD" in 2 subjects who were both VitD deficient by Endocrine Society definitions (25D<20 ng/mL). We think that only the one with very \uparrow **1,25D/24,25D** and a significant \downarrow in PTH after VitD, was VitD deficient. This exemplifies the value of precision diagnostics in diagnosing and treating VitD deficiency. Our conclusions are summarized below:

Spectrum of Clinical Vitamin D Status



REFERENCES

- LeBoff MS et al. Supplemental Vitamin D and Incident Fractures in Midlife and Older Adults. *N Engl J Med*. 2022;387(4):299-309.
- Cummings SR and Rosen C. VITAL Findings - A Decisive Verdict on Vitamin D Supplementation. *N Engl J Med*. 2022;387(4):368-70.
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