SHOULD WE MEASURE OR CALCULATE FREE 25OH VITAMIN D IN VITAMIN D RESEARCH?

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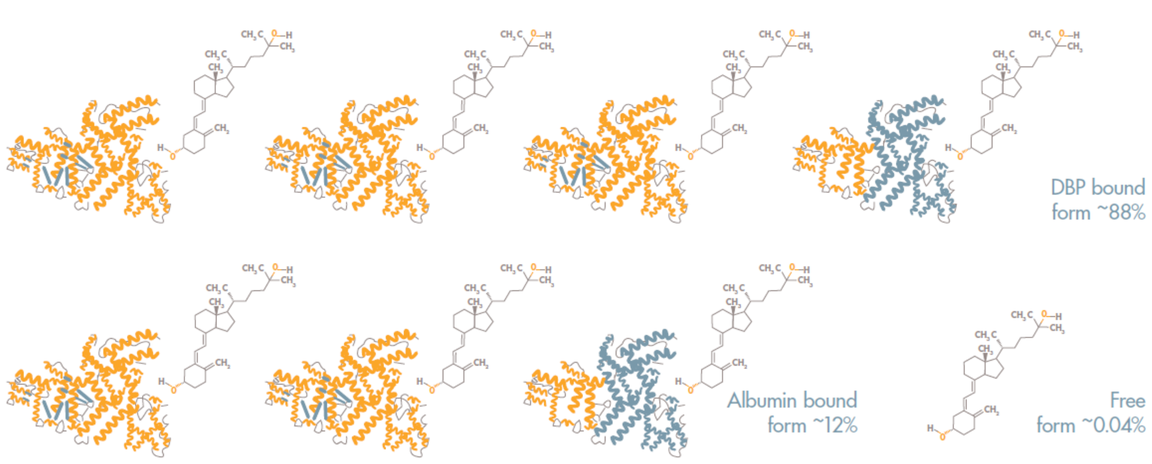
Free 25OH Vitamin D represents 0.04% of the total 25OH Vitamin D

Almost all circulating 25OH Vitamin D in serum is bound to Vitamin D Binding Protein DBP (88%) and Albumin (12 %). A very small fraction, approximately 0.04 % of the 25OH Vitamin D, circulates in the free, non-protein bound form.



Conditions affecting the free 25OH Vitamin D concentration

The concentration of DBP is not constant and can be influenced by a number of factors including

- Obesity
- Pregnancy
- The use of oral contraceptives
- Hormone replacement therapy
- Liver disease
- Renal disease
- Proteinuria
- Intensive care

In case of elevated concentration of DBP the % of free 25OH Vitamin D is decreased. In case of decreased concentration of DBP the % of free 25OH Vitamin D is increased.

- The supplementation with Vitamin D2 or D3 also affects differently the free 25OH Vitamin D levels.

Methods for the evaluation of free 25OH Vitamin D

Centrifugal ultrafiltration

- Time and labor consuming
- Uses H^3 and C^{14}
- Uses a few assumptions

Calculations

- Requires 3 assays per sample (DBP, Albumin, total 25OHD)
- Should ideally include DBP genotyping
- DBP assays are not of equal quality

Direct assay - ELISA

- Direct measurement method
- Accessible to any laboratory
- Can be automated for large cohorts

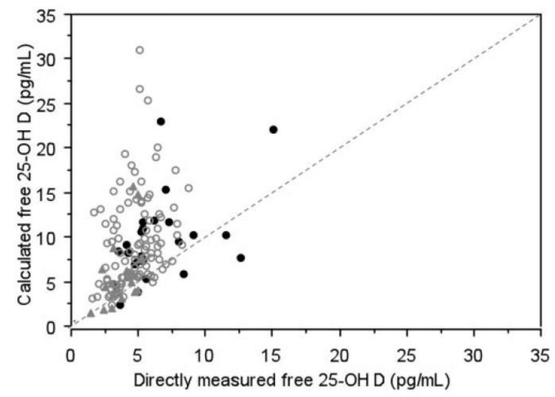
2014 – ELISA vs calculations

- Context: compare direct quantitation to calculated levels in cirrhotic patients, pregnant women (2nd and 3d trimester) and control adults.
- Methods: total 25OHD by LC-MS, Albumin by BCG and DBP by ELISA (MoAb).
- Results: calculations overestimated free 25OH D levels compared to directly measured with larger fold differences in African Americans compared to Caucasians.

Directly measured free 25OHD was correlated with iPTH and Ca, but calculated was not.

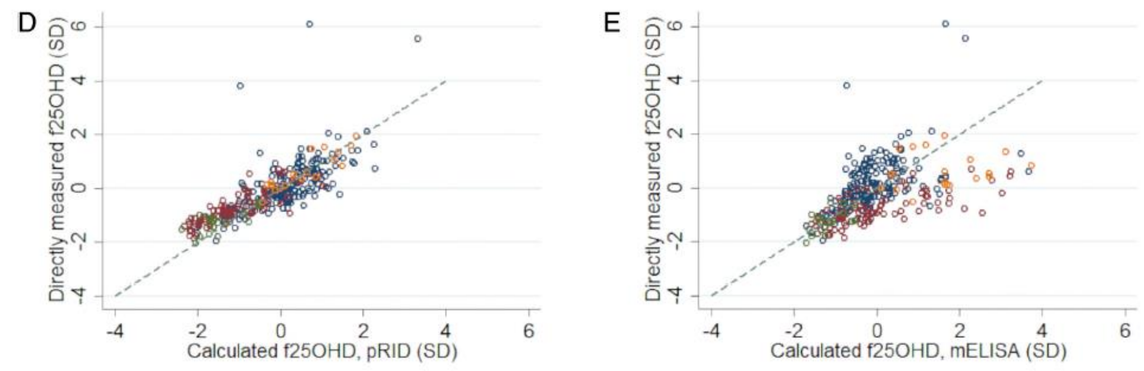
	ELISA	Calc.
iPTH	p<0.02	p = 0.46
Ca	p<0.004	p = 0.47

Schwartz J.B. *et al.* J. Clin. Endocrinol. Metab. 2014, 99(5), 1631-7.



2016 – Comparison in African Americans and Caucasians

- Context: free 25OHD was measured by ELISA and calculated amongst ethnical groups.
- Methods: total 25OHD by LC-MS, Albumin by BCG and DBP by polyclonal radial immunodiffusion (pRID) and ELISA (MoAb and PoAb).
- Results: calculated free 25OHD concentrations were higher than directly measured values. Since calculated free 25OHD derives from measures of DBP, the accuracy of DBP assays is critical but not constant.



Orwoll E.S. *et al.* J. Clin. Endocrinol. Metab. 2016, 101(5), 2226-34.

2017 – Seasonal variation of free 25OHD

- Context: seasonal variation of 25OHD was evaluated by direct measurement and by calculations.
- Methods: total 25OHD by CLIA, Albumin by Cobas and DBP by ELISA (MoAb).
- Results: the directly measured free 25OHD was significantly lower than the calculated, and directly measured showed a stronger correlation with serum iPTH (P<0.001) than calculated.

Klingberg E. *et al.* Endocr. Connect. 2017, 6(2), 111-120.

Directly measured free 25OHD is lower than calculated
Directly measured free 25OHD is better correlated to PTH and Ca

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Join the Scientific Community who talks about free Vitamin D: LinkedIn group "Free Vitamin D"

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