Free Vitamin D
by ELISA
Application note

INTRODUCTION

Recent studies suggest that the concentration and genotype of Vitamin D binding protein (DBP) are important factors that determine the bioavailability of 25OH Vitamin D in blood. There is accumulating data that for instance in pregnant women, chronic kidney disease, liver failure, bladder cancer and pancreatic cancer, or in hemodialysis patients the measurement of free 25OH Vitamin D in serum provides more relevant diagnostic information than total 25OH Vitamin D.

Several research groups have therefore called for a direct measurement of free 25OH Vitamin D, instead of using inaccurate calculation methods.

To measure free 25OH Vitamin D in blood, Future Diagnostics in partnership with DIAsource Immunoassays has developed a **direct ELISA assay kit**. The ELISA assay kit can be purchased in the United States through IBL-America.

This Technical Review presents the literature on free 25OH Vitamin D by ELISA, by disease fields.

1. Liver disease

2. Pregnancy

3. Renal disease

4. Intensive care / critical illness

5. Osteoporosis / bone mineral density

6. Cancer

7. Respiratory diseases

8. Obesity / Insulin

9. Miscellaneous

10. Ethnicity

1. LIVER DISEASE

- BIKLE D. (2013)

Variability in free 25(OH) vitamin D levels in clinical populations.
J. Steroid Biochem. Mol. Biol., S0960-0760.

Relationships between total and free 25(OH)D vary with clinical conditions that affect circulating protein concentrations, and may differ from predictions based on physiologic changes in circulating vitamin D binding protein and albumin. Direct measurement of free 25(OH) D warrants further evaluation to determine its clinical relevance in defining optimal vitamin D status for differing clinical conditions.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4022715/

- SCHWARTZ J.B. (2014)

A comparison of direct and calculated free 25(OH) Vitamin D levels in clinical populations.
J. Clin. Endocrinol. Metab., 99(5):1631-7.

Calculated free 25 (OH) D levels varied considerably from direct measurements of free 25 (OH) D with discrepancies greatest in data for African Americans. Differences in DBP binding affinity likely contributed to estimation errors between the races. Directly measured free 25-OHconcentrations were related to iPTH but calculated estimates were not. Current algorithms to calculate free 25-OH vitamin D may not be accurate. Further evaluation of directly measured free 25 (OH) D levels to determine its role in research and clinical management of patients is needed.

https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2013-3874

- BIKLE D. (2015)

Total 25(OH) vitamin D, free 25(OH) vitamin D and markers of bone turnover in cirrhotics with and without synthetic dysfunction.
Liver Int. 2015 Mar 11.

BACKGROUND & AIMS: Current clinical assays for total 25-hydroxy (OH) vitamin D measure vitamin D bound to vitamin D-binding protein (DBP) and albumin plus unbound ('free') D. We investigated the relationship between total and free 25(OH)D with bone metabolism markers in normal (>3.5 g/dl) vs. low (≤3.5 g/dl) albumin cirrhotics.
METHODS: Eighty-two cirrhotics underwent measurement of free and total 25(OH)D by immunoassay, DBP and markers of bone metabolism [intact parathyroid hormone (iPTH), C-telopeptide (CTX), bone-specific alkaline phosphatase (BSAP), osteocalcin, amino-terminal pro-peptide of type 1-collagen (P1NP)]. Pearson's coefficients assessed relevant associations.
RESULTS: Cirrhotics with low (n = 54) vs. normal (n = 28) albumin had lower total 25(OH)D (12.1 vs. 21.7 ng/ml), free 25(OH)D (6.2vs.8.6 pg/ml) and DBP(91.4 vs. 140.3 μg/ml) [P < 0.01 for each]. iPTH was similar in low and normal albumin groups (33 vs. 28 pg/ml; P = 0.38), although serum CTX(0.46vs.0.28 ng/ml) and BSAP(31.7 vs. 24.8 μg/L) were increased (P < 0.01). An inverse relationship was observed between total 25(OH)D and iPTH in normal (r = -0.47, P = 0.01) but not low albumin cirrhotics (r = 0.07, P = 0.62). Similar associations were seen between free 25(OH)D and iPTH(Normal: r = -0.46, P = 0.01; Low: r = -0.03, P = 0.84). BSAP, osteocalcin and P1NP were elevated above the normal range in all cirrhotics but not consistently associated with total or free 25(OH)D.
CONCLUSIONS: Cirrhotics with low vs. normal albumin have lower levels of DBP, total and free 25(OH)D. The expected relationship between total or free 25(OH)D with iPTH was observed in normal but not in low albumin cirrhotics, demonstrating that total 25(OH)D is not an accurate marker of bioactive vitamin D status in cirrhotics with synthetic dysfunction. Additional investigation into the role of vitamin D supplementation and its impact on bone mineral homoeostasis in this population is needed.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4567539/

2. PREGNANCY

- BIKLE D. (2013)

Variability in free 25(OH) vitamin D levels in clinical populations.
J. Steroid Biochem. Mol. Biol., S0960-0760.

Relationships between total and free 25(OH)D vary with clinical conditions that affect circulating protein concentrations, and may differ from predictions based on physiologic changes in circulating vitamin D binding protein and albumin. Direct measurement of free 25(OH) D warrants further evaluation to determine its clinical relevance in defining optimal vitamin D status for differing clinical conditions.

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Calculated free 25 (OH) D levels varied considerably from direct measurements of free 25 (OH) D with discrepancies greatest in data for African Americans. Differences in DBP binding affinity likely contributed to estimation errors between the races. Directly measured free 25-OHconcentrations were related to iPTH but calculated estimates were not. Current algorithms to calculate free 25-OH vitamin D may not be accurate. Further evaluation of directly measured free 25 (OH) D levels to determine its role in research and clinical management of patients is needed.

https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2013-3874

3. ETHNICITY

- ALOIA J. (2015)

Free 25(OH)D and the Vitamin D Paradox in African Americans.
J. Clin. Endocrinol. Metab. 2015 Jul 10:JC20152066.

CONTEXT: African Americans have a lower total serum 25-hydroxyvitamin D [25(OH)D] but superior bone health. This has been referred to as a "paradox". A recent publication found that free serum 25(OH)D is the same in black and white individuals. However, the study was criticized because an indirect method was used to measure free 25(OH)D. A direct method has recently been developed.
OBJECTIVE: We hypothesized that although total serum 25(OH)D is lower in African Americans, free serum 25(OH)D measured directly would not differ between races.
DESIGN: White and black healthy postmenopausal women were matched for age and BMI. Serum total 25(OH)D, PTH, 1,25(OH)2D, vitamin D binding Protein (VDBP) and bone density were measured. Measurement of free 25(OH)D was carried out using an ELISA.
SETTING: Ambulatory research unit in a teaching hospital.
OUTCOME: Cross-racial comparison of serum free 25(OH)D.
RESULTS: A propensity match resulted in selection of a total of 164 women. Total 25(OH)D was lower in black women (19.5±4.7 vs. 26.9±6.4 ng/ml) but direct measurement of free 25(OH)D revealed almost identical values (5.25±1.2 vs. 5.25±1.3 ng/ml) between races. VDBP was significantly lower in blacks when using a monoclonal based ELISA, but higher with a polyclonal based ELISA. Serum PTH, 1,25(OH)2D and bone density were higher in African Americans.
CONCLUSIONS: Free serum 25(OH)D is the same across races despite the lower total serum 25(OH)D in black women. Results comparing VDBP between races using a monoclonal vs. a polyclonal assay were discordant.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4570168/

- BOUILLON R. (2016)

Free 25-hydroxyvitamin D: impact of vitamin D binding protein assays on racial-genotypic associations.
J Clin Endocrinol Metab. 2016 May;101(5):2226-34.

Context: Total 25-hydroxyvitamin D (25OHD) is a marker of vitamin D status and is lower in African Americans than in whites. Whether this difference holds for free 25OHOD (f25OHD) is unclear, considering reported genetic-racial differences in vitamin D binding protein (DBP) used to calculate f25OHD.
Objectives: Assess racial-geographic differences in f25OHD. Understand inconsistencies in racial associations with DBP and calculated f25OHD.
Design: Cross-sectional
Setting: General community in the United States, United Kingdom, and The Gambia
Participants: Men in Osteoporotic Fractures in Men (MrOS) and Medical Research Council (MRC) studies (N=1057)
Exposures: Total 25OHD concentration, race, and DBP (GC) genotypes
Outcome: measures: Directly measured f25OHD, DBP assessed by proteomics and monoclonal and polyclonal immunoassays, and calculated f25OHD.
Results: Total 25OHD correlated strongly with directly measured f25OHD (Spearman r=0.84). Measured by monoclonal assay, mean DBP in African-ancestry subjects was 50% lower than in whites, whereas DBP measured by polyclonal DBP antibodies or proteomic methods was not lower in African-ancestry. Calculated f25OHD (using polyclonal DBP assays) correlated strongly with directly measured f25OHD (r=0.80 – 0.83). Free 25OHD, measured or calculated from polyclonal DBP assays, reflected total 25OHD concentration irrespective of race and was lower in African Americans than in US whites.

https://www.ncbi.nlm.nih.gov/pubmed/27007693

- PITTAS A.G. (2016)

Vitamin D status of black and white Americans and changes in vitamin D metabolites after varied doses of vitamin D supplementation.

Am. J. Clin. Nutr., 104(1): 205-14.

BACKGROUND: Controversy exists over the disparate circulating 25-hydroxyvitamin D [25(OH)D] concentrations between black and white Americans.
OBJECTIVE: We sought to determine whether there are differences in total and directly measured free 25(OH)D concentrations between black and white American adults and how daily supplementation with cholecalciferol changes these concentrations.
DESIGN: Cross-sectional and longitudinal analyses were conducted with the use of data from 2 placebo-controlled, randomized trials at 2 academic medical centers in the United States: CaDDM (Calcium and Vitamin D in Type 2 Diabetes) and DDM2 (Vitamin D for Established Type 2 Diabetes). A total of 208 subjects with pre- or well-controlled diabetes with a mean age of 59.1 y and mean body mass index (BMI; in kg/m(2)) of 31.6 were randomly assigned to receive daily cholecalciferol supplementation at 1 of 2 doses (2000 or 4000 IU) or a matching placebo for 16 wk. We measured serum total 25(OH)D, vitamin D-binding protein (DBP) by 2 different immunoassays (with the use of monoclonal or polyclonal antibodies), parathyroid hormone, and albumin. Free 25(OH)D concentration was directly measured and calculated.
RESULTS: Blacks had lower total 25(OH)D concentrations than whites [adjusted median: 20.3 ng/mL (95% CI: 16.2, 24.5 ng/mL) compared with 26.7 ng/mL (95% CI: 25.2, 28.1 ng/mL), respectively; P = 0.026)], and a higher proportion of blacks had total 25(OH)D concentrations <20 ng/mL (46% compared with 19%, respectively; P < 0.001). Directly measured free 25(OH)D concentrations were lower in blacks than in whites [adjusted median: 4.5 ng/mL (95% CI: 3.7, 5.4 ng/mL) compared with 5.7 ng/mL (95% CI: 5.4, 5.9 ng/mL), respectively; P = 0.044] and were strongly correlated with total 25(OH)D without an effect of race. DBP was lower in blacks when measured by the monoclonal but not the polyclonal antibody immunoassay. Cholecalciferol supplementation increased total and measured free 25(OH)D concentrations proportionally to the dose and without a difference between races.
CONCLUSIONS: The relation between free and total 25(OH)D did not vary systematically by race in this multiracial population with pre- or well-controlled diabetes. The results need to be replicated in additional cohorts before concluding that the clinical assessment of vitamin D status in blacks and whites should follow a single standard. The CaDDM and DDM2 trials were registered at clinicaltrials.gov as NCT00436475 and NCT01736865, respectively.

http://press.endocrine.org/doi/abs/10.1210/endo-meetings.2016.BCHVD.7.SAT-344

4. POST-MENOPAUSE

- ALOIA J. (2015)

Free 25(OH)D and the Vitamin D Paradox in African Americans.
J. Clin. Endocrinol. Metab. 2015 Jul 10:JC20152066.

CONTEXT: African Americans have a lower total serum 25-hydroxyvitamin D [25(OH)D] but superior bone health. This has been referred to as a "paradox". A recent publication found that free serum 25(OH)D is the same in black and white individuals. However, the study was criticized because an indirect method was used to measure free 25(OH)D. A direct method has recently been developed.
OBJECTIVE: We hypothesized that although total serum 25(OH)D is lower in African Americans, free serum 25(OH)D measured directly would not differ between races.
DESIGN: White and black healthy postmenopausal women were matched for age and BMI. Serum total 25(OH)D, PTH, 1,25(OH)2D, vitamin D binding Protein (VDBP) and bone density were measured. Measurement of free 25(OH)D was carried out using an ELISA.
SETTING: Ambulatory research unit in a teaching hospital.
OUTCOME: Cross-racial comparison of serum free 25(OH)D.
RESULTS: A propensity match resulted in selection of a total of 164 women. Total 25(OH)D was lower in black women (19.5±4.7 vs. 26.9±6.4 ng/ml) but direct measurement of free 25(OH)D revealed almost identical values (5.25±1.2 vs. 5.25±1.3 ng/ml) between races. VDBP was significantly lower in blacks when using a monoclonal based ELISA, but higher with a polyclonal based ELISA. Serum PTH, 1,25(OH)2D and bone density were higher in African Americans.
CONCLUSIONS: Free serum 25(OH)D is the same across races despite the lower total serum 25(OH)D in black women. Results comparing VDBP between races using a monoclonal vs. a polyclonal assay were discordant.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4570168/

- BINKLEY N. (2017)

DOES VITAMIN D METABOLITE MEASUREMENT HELP PREDICT 25(OH)D CHANGE FOLLOWING VITAMIN D SUPPLEMENTATION?
Endocr Pract. 2017 Apr 2;23(4):432-441.

OBJECTIVE: Variability in 25-hydroxyvitamin D (25(OH)D) change following vitamin D supplementation exists. Vitamin D metabolite measurement might assist in predicting 25(OH)D response and also contribute to defining vitamin D adequacy. This study assessed utility of vitamin D metabolite measurements to predict 25(OH)D response and explored the relationship between parathyroid hormone (PTH) and a "vitamin D composite index" comprised of the sum of serum 25(OH) D, cholecalciferol (vitamin D3) and 24,25 dihydroxyvitamin D (24,25(OH)2D).
METHODS: Sixty-two postmenopausal women were randomized to daily vitamin D3 1,800 IU or placebo for 4 months. Blood was drawn at baseline and after 1 and 4 months. Serum 25(OH)D, vitamin D3, and 24,25(OH)2D were measured by liquid chromatography tandem mass spectroscopy. Free 25(OH)D and PTH were measured by enzyme-linked immunosorbent assay. Repeated measures analysis of variance and regression analyses were performed.
RESULTS: Baseline 25(OH)D was positively correlated (P<.05) with vitamin D3, 24,25(OH)2D and free 25(OH) D. Daily vitamin D supplementation increased all metabolites (P<.001). Substantial individual variability in 25(OH) D change at 4 months was observed but was unrelated to baseline vitamin D3, 25(OH)D or 24,25(OH)2D. Only body mass index, body weight, and body fat mass was associated with 25(OH)D change at 4 months. The vitamin D composite score was associated with serum PTH, but this association was similar to that observed with 25(OH) D alone.
CONCLUSION: This study does not support measurement of vitamin D metabolites in a composite index to assist in prediction of 25(OH)D response to supplementation. Overweight individuals have less robust 25(OH) D response to supplementation, but variability precludes prediction of the result following daily supplementation.
ABBREVIATIONS: BMI = body mass index DXA = dual-energy X-ray absorptiometry LC-MS/MS = liquid chromatography tandem mass spectroscopy 25(OH)D = 25-hydroxyvitamin D 24,25(OH)2D = 24,25 dihydroxyvitamin D PTH = parathyroid hormone vitamin D3 = cholecalciferol.

http://journals.aace.com/doi/abs/10.4158/EP161517.OR?code=aace-site&journalCode=endp

5. BONES

- ALOIA J. (2015)

Free 25(OH)D and Calcium Absorption, PTH and Markers of Bone Turnover.
J. Clin. Endocrinol. Metab. 2015 Aug 27:jc20152548.

Context: It has been proposed that serum free 25(OH)D may better reflect vitamin D action than total 25(OH)D. An ELISA for serum free 25(OH)D has recently become available, permitting direct assay.
Objective: To determine if serum free 25(OH)D provides additional information in relation to calcium absorption and other biomarkers of vitamin D action compared to total serum 25(OH)D.
Setting: Ambulatory research setting in a teaching hospital.
Outcome: Serum free 25(OH)D measured in a previously performed study of varied doses of vitamin D3 (placebo, 800 IU, 2,000 IU and 4,000 IU) on calcium absorption, PTH, P1NP, and CTX. Free 25(OH)D was measured by ELISA. Calcium absorption was measured at baseline and at 10 weeks using stable dual calcium isotopes.
Results: 71 subjects completed this randomized placebo controlled trial. Baseline group mean free and total 25(OH)D varied from 4.7 1.8 pg/ml to 5.4 1.5 pg/ml and 23.7 5.9 ng/ml to 25.9 6.1 ng/ml, respectively. Participants assigned to the 4,000 IU dose arm achieved free 25(OH)D levels of 10.4 pg/ml and total 25(OH)D levels of 40.4 ng/ml. Total and free 25(OH)D were highly correlated at baseline and after increasing vitamin D dosing (r0.80 and 0.85, respectively). Free 25(OH)D closely reflected changes in total 25(OH)D. PTH was similarly correlated at baseline and follow-up with total and free 25(OH)D. Serum CTX had a moderate positive correlation with total and free 25(OH)D at follow-up. The serum 1,25(OH)2D change increased significantly with the change in 25(OH)D but not with the change in free 25(OH)D.
Conclusion: There was no advantage of measuring free over total 25(OH)D in assessing the response of calcium absorption, PTH and markers of bone turnover to vitamin D. Free 25(OH)D responded to increasing doses of vitamin D in a similar fashion to total 25(OH)D.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4702446/

- WALSH J.S. (2016)

Free 25-hydroxyvitamin D is low in obesity, but there are no adverse associations with bone health.
N. Engl. J. Med. 374(17): 1695-1696.

Background: The mechanism and clinical significance of low circulating 25-hydroxyvitamin D [25(OH)D] in obese people are unknown. Low total 25(OH)D may be due to low vitamin D–binding proteins (DBPs) or faster metabolic clearance. However, obese people have a higher bone mineral density (BMD), which suggests that low 25(OH)D may not be associated with adverse consequences for bone.
Objective: We sought to determine whether 1) vitamin D metabolism and 2) its association with bone health differ by body weight.
Design: We conducted a cross-sectional observational study of 223 normal-weight, overweight, and obese men and women aged 25–75 y in South Yorkshire, United Kingdom, in the fall and spring. A subgroup of 106 subjects was also assessed in the winter. We used novel techniques, including an immunoassay for free 25(OH)D, a stable isotope for the 25(OH)D3 half-life, and high-resolution quantitative tomography, to make a detailed assessment of vitamin D physiology and bone health.
Results: Serum total 25(OH)D was lower in obese and overweight subjects than in normal-weight subjects in the fall and spring (geometric means: 45.0 and 40.8 compared with 58.6 nmol/L, respectively; $P < 0.001$) but not in the winter. Serum 25(OH)D was inversely correlated with body mass index (BMI) in the fall and spring and in the winter. Free 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)2D] were lower in obese subjects. DBP, the DBP genotype, and the 25(OH)D3 half-life did not differ between BMI groups. Bone turnover was lower, and bone density was higher, in obese people.
Conclusions: Total and free 25(OH)D and 1,25(OH)2D are lower at higher BMI, which cannot be explained by lower DBP or the shorter half-life of 25(OH)D3. We speculate that low 25(OH)D in obesity is due to a greater pool of distribution. Lower 25(OH)D may not reflect at-risk skeletal health in obese people, and BMI should be considered when interpreting serum 25(OH)D as a marker of vitamin D status.

http://ajcn.nutrition.org/content/103/6/1465

6. CYSTIC FIBROSIS

- TANGPRICHA V. (2015)

Free 25-Hydroxyvitamin D Concentrations in Cystic Fibrosis.
Am J Med Sci 2015 Nov;350(5):374-9.

Vitamin D deficiency is common in cystic fibrosis (CF), but there is no previous data on free 25-hydroxyvitamin D (25[OH]D) in CF or in relation to healthy individuals. We assessed total serum 25(OH)D concentration by chemiluminescence and serum free 25(OH)D concentration by both direct measurement (ELISA) and calculation, using serum albumin and vitamin D binding protein (VDBP) levels in 80 subjects (28 healthy adults, 25 clinically stable adults and children with CF and 27 adults experiencing a CF exacerbation). Serum albumin and VDBP concentrations were lower in CF compared with healthy controls. Total serum 25(OH)D concentrations were positively correlated with both calculated and measured free 25(OH)D ($P < 0.001$ for both). Calculated and directly measured serum free 25(OH)D levels were positively correlated ($P < 0.001$). Serum levels of directly measured free 25(OH)D positively correlated with total 25(OH)D, suggesting that achieving sufficient total serum 25(OH)D may result in adequate free 25(OH)D levels in CF.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4629503/

7. VITAMIN D2 / D3

- HEWISON M. (2015)

Vitamin D2 vs vitamin D3: effects of total and free 25-hydroxyvitamin D on immune cells in vivo.
Endocrine Abstracts (2015) 38 P395.

Vitamin D metabolites such as 25-hydroxyvitamin D (25D) circulate bound primarily to vitamin D binding protein (DBP). However, for most extra-renal tissues 25D uptake is independent of DBP, even though the 'free' 25D fraction is very small. DBP has a lower binding affinity for 25D2 compared to 25D3. We hypothesized that this would increase serum free 25D2, with possible variations in vitamin D function. Mice were placed on diets containing equal amounts (1000 IU/kg) of vitamin D2 or D3 at week 3 of age. At week 8 mice fed D2 diet had only 25D2 in circulation (26.6 ng/ml±1.9), and mice fed D3 mice had only 25D3 (28.3 ng/ml±2.0). By contrast, measured 'free' 25D was significantly higher in D2 animals (16.8 pg/ml±0.65 vs 8.4 pg/ml±0.63, P<0.001). Parathyroid hormone showed no significant difference between D2 and D3 mice (193 pg/ml±9.0 vs 196 pg/ml±6.2). However, analysis of spleens from week 8 D2 and D3 mice showed that in female mice on D2 there was increased mRNA expression of the vitamin D-activation enzyme Cyp27b1 (2.35-fold±0.45), the monocyte-macrophages marker CD11b (1.77-fold±0.45), and the osteoclastogenesis precursor RANKL (1.80-fold±0.37) relative to female D3 mice. Conversely, another monocyte marker, CD14, showed decreased mRNA expression (0.39-fold±0.19) in D2 vs D3 mice. Flow cytometry revealed a significant increase in total CD45+ monocyte-macrophage

http://www.endocrine-abstracts.org/ea/0038/ea0038P395.htm

- HEWISON M. (2016)

Differential responses to vitamin D2 and vitamin D3 are associated with variations in free 25-hydroxyvitamin D.
Endocrinology, Jul 11:en20161139.

25-hydroxyvitamin D (25D) circulates bound primarily to serum vitamin D binding protein (DBP), with DBP showing higher binding affinity for 25D3 than 25D2. We therefore hypothesized that vitamin D2 (D2) promotes higher serum levels of unbound 25D (free 25D), with different functional responses, relative to vitamin D3 (D3). Week 3 (wk3) C56BL/6 mice were placed on diets containing either D2 or D3 alone (both 1000 IU/kg). At wk8 and wk16 D2 mice had only 25D2 in circulation (26.6±1.9 and 33.3±4.4 ng/ml), and D3 mice had only 25D3 (28.3±2.0 and 31.7±2.1 ng/ml). At wk 8 (44.5±6.4 vs. 62.4±11.6 pg/ml, p<0.05), and wk16 (78.4±12.6 vs. 95.5±11.6) D2 mice had lower serum 1,25-dihydroxyvitamin D (1,25(OH)2D) relative to D3 mice. By contrast, measured free 25D was significantly higher in D2 mice at wk8 (16.8±0.65 vs. 8.4±0.63 pg/ml, p<0.001) and wk16 (17.4±0.43 vs. 8.4±0.44, p<0.001). Two-way ANOVA of bone histomorphometry showed that wk8 D2 mice had significantly higher osteoclast surface/bone surface, eroded surface/bone surface, and mineral apposition rate compared to D3 mice. Osteoblast surface/bone surface was higher in wk8 D2 females but not wk8 D2 males. At wk16, D2 mice had significantly higher bone volume/total volume and trabecular number compared to D3 mice. Differences in bone phenotype were observed despite D2 mice reaching similar serum 25D levels and lower 1,25D levels compared to D3 mice. These data indicate that 25D2 binds less well to DBP than 25D3, with resulting higher levels of free 25D promoting differential effects on bone in mice exposed to D2 alone.

https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2016-1139

8. OBESITY

- WALSH J.S. (2016)

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Background: The mechanism and clinical significance of low circulating 25-hydroxyvitamin D [25(OH)D] in obese people are unknown. Low total 25(OH)D may be due to low vitamin D–binding proteins (DBPs) or faster metabolic clearance. However, obese people have a higher bone mineral density (BMD), which suggests that low 25(OH)D may not be associated with adverse consequences for bone.
Objective: We sought to determine whether 1) vitamin D metabolism and 2) its association with bone health differ by body weight.
Design: We conducted a cross-sectional observational study of 223 normal-weight, overweight, and obese men and women aged 25–75 y in South Yorkshire, United Kingdom, in the fall and spring. A subgroup of 106 subjects was also assessed in the winter. We used novel techniques, including an immunoassay for free 25(OH)D, a stable isotope for the 25(OH)D3 half-life, and high-resolution quantitative tomography, to make a detailed assessment of vitamin D physiology and bone health.
Results: Serum total 25(OH)D was lower in obese and overweight subjects than in normal-weight subjects in the fall and spring (geometric means: 45.0 and 40.8 compared with 58.6 nmol/L, respectively; $P < 0.001$) but not in the winter. Serum 25(OH)D was inversely correlated with body mass index (BMI) in the fall and spring and in the winter. Free 25(OH)D and 1,25-dihydroxyvitamin D [1,25(OH)2D] were lower in obese subjects. DBP, the DBP genotype, and the 25(OH)D3 half-life did not differ between BMI groups. Bone turnover was lower, and bone density was higher, in obese people.
Conclusions: Total and free 25(OH)D and 1,25(OH)2D are lower at higher BMI, which cannot be explained by lower DBP or the shorter half-life of 25(OH)D3. We speculate that low 25(OH)D in obesity is due to a greater pool of distribution. Lower 25(OH)D may not reflect at-risk skeletal health in obese people, and BMI should be considered when interpreting serum 25(OH)D as a marker of vitamin D status.

http://ajcn.nutrition.org/content/103/6/1465

9. RESPIRATORY DISEASES

- POLLARD S.L. (2016)

Measured Free 25(OH)D But Not Total 25(OH)D Is Associated With Atopy And Measures Of Pulmonary Function In Peruvian Children With Asthma.
American Thoracic Society International Conference Abstracts > A106.

http://www.atsjournals.org/doi/abs/10.1164/ajrccm-Conference.2016.193.1_MeetingAbstracts.A2764

10. MISCELLANEOUS

- SOLLID S.T. (2016)

Effects of vitamin D binding protein phenotypes and vitamin D supplementation on serum total 25(OH)D and directly measured free 25(OH)D.
Eur. J. Endocrinol. April 1, 174:445-452.

Objective: To determine the relationship between serum total 25-hydroxyvitamin D (25(OH)D), directly measured free 25(OH)D and calculated free 25(OH)D with regard to vitamin D-binding protein (DBP) phenotypes, sex, BMI, age and season, and their interrelationship to vitamin D supplementation.
Design, patients and interventions: A randomized controlled trial with 20000IU of vitamin D3 per week or placebo for 12 months was designed. A total of 472 subjects, 236 in each of the intervention groups, were included in the analyses.
Main outcome measures: Baseline serum concentrations and increases in serum total 25(OH)D, directly measured free 25(OH)D, calculated free 25(OH)D and DBP.
Results: Serum total 25(OH)D and DBP concentrations were significantly lower in subjects with the phenotype Gc2/Gc2 compared to phenotypes with the Gc1S allele, and lower in males compared to females. When using directly measured free 25(OH)D, the differences related to DBP phenotypes and sexes were clearly diminished. All calculated free 25(OH)D concentrations were overestimated compared to the directly measured free 25(OH)D. Serum parathyroid hormone showed an inverse correlation with all vitamin D parameters analyzed. The increases after 12 months of vitamin D supplementation were not significantly different for any of the vitamin D parameters regardless of DBP phenotype, sex or age. Supplementation with vitamin D did not affect serum DBP.
Conclusion: Direct measurements of free 25(OH)D reduce the differences seen in total 25(OH)D between DBP phenotype groups and sexes, probably caused by differences in DBP concentrations. With conditions affecting serum DBP concentrations, direct measurements of free 25(OH)D should be considered.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4763092/

- SCHWARTZ J.B. (2016)

Response of Vitamin D Concentration to Vitamin D3 Administration in Older Adults without Sun Exposure: A Randomized Double-Blind Trial.
Journal of the American Geriatrics Society, 64(1), 65–72.

Objectives: To determine the dose-response relationship between 25-hydroxyvitamin D (25(OH)D) and supplemental vitamin D3 in elderly nursing home residents.
Design: Randomized double-blind investigation.
Setting: Nursing home.
Participants: Of 81 women (n = 51) and men (n = 30) (mean age 87.4 ± 8) enrolled, 72 completed the study.
Intervention: Sixteen weeks of oral vitamin D3 at 800, 2,000, or 4,000 IU/d or 50,000 IU/wk.
Measurements: The main outcome was 25(OH)D concentrations (tandem mass spectrometry) after 16 weeks. Free 25(OH)D and intact parathyroid hormone (iPTH) were also analyzed. Safety monitoring of calcium and estimated glomerular filtration rate was performed, and adherence and clinical status were measured.
Results: 25(OH)D concentrations increased with dose (P < .001) and were higher with 50,000 IU/wk (P < .001) than other doses and with 4,000 IU/d than 800 or 2,000 IU/d, but 800 IU and 2,000 IU/d did not differ. One subject receiving 800 IU/d had concentrations less than 20 ng/mL. All subjects receiving more than 2000 IU/d had concentrations of 20 ng/mL and greater. Free 25(OH)D concentrations rose with total 25(OH) vitamin D. Total and free 25(OH)D were related to calcium concentrations; only free 25(OH)D was related to iPTH.
Conclusion: 25(OH)D increased linearly with 800 to 4,000 IU/d and 50,000 IU/wk of vitamin D3, without a ceiling effect. Data suggest that some elderly adults will require more than 800 IU/d of vitamin D3 to ensure adequate vitamin D levels. Changes in 25(OH)D with vitamin D3 were related to starting concentrations (greatest with the lowest concentrations and unchanged with 800 and 2,000 IU/d if 20–40 ng/mL). Relationships between serum calcium and iPTH and free 25(OH)D suggest the potential for free 25(OH)D in defining optimal 25(OH)D concentrations.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4724876/

- KLINGBERG E. (2017)

The variation in free 25-hydroxy vitamin D and vitamin D-binding protein with season and vitamin D status.
Endocr Connect. 2017 Feb;6(2):111-120.

PURPOSE:Serum 25-hydroxy vitamin D [25(OH)D] varies greatly with season at northern latitudes. The purpose of this study was to determine if the seasonal variations in serum total 25(OH)D are followed by a concomitant variation in free 25(OH)D or if the variation is damped by alterations in the binding capacity of DBP.
METHODS:Serum was collected from 540 healthy blood donors (60% men; mean age 41 ± 13 years) during 12 months and analyzed for total 25(OH)D, directly measured free 25(OH)D, vitamin D-binding protein (DBP) and albumin. Calculated free 25(OH)D was estimated.
RESULTS:The UV-B radiation during the sampling month was positively correlated with the serum levels of total 25(OH)D (r = 0.355, P < 0.001), directly measured free (r = 0.336, P < 0.001) and calculated free 25(OH)D (r = 0.275, P < 0.001), but not with DBP and albumin. The percentage of free 25(OH)D was higher during the winter months than that during the summer months (0.020 ± 0.005% vs 0.019 ± 0.004%; P = 0.007) and higher in participants with a serum 25(OH)D below 25 nmol/L than that in participants with a serum 25(OH)D above 75 nmol/L (0.031 ± 0.007% vs 0.017 ± 0.003%; P < 0.001). iPTH was correlated with directly measured free 25(OH)D (r = -0.226; P < 0.001), but only weakly with calculated free 25(OH)D (r = -0.095; P = 0.027).
CONCLUSIONS:Directly measured free serum 25(OH)D was highly correlated with total serum 25(OH)D and followed the same seasonal variation, whereas the serum concentrations of DBP and albumin were stable. The fluctuation in free 25(OH)D was only marginally damped with an increase in the percentage of free 25(OH)D during the winter months and in participants with vitamin D deficiency.

http://www.endocrineconnections.com/content/early/2017/02/08/EC-16-0078.full.pdf

- SHIEH A. (2017)

Effects of Cholecalciferol vs Calcifediol on Total and Free 25-Hydroxyvitamin D and Parathyroid Hormone.
J Clin Endocrinol Metab. 2017 Apr 1;102(4):1133-1140.

Context: Vitamin D deficiency disproportionately affects nonwhite individuals. Controversy persists over how to best restore low 25D levels, and how to best define vitamin D status [total (protein bound plus free) vs free 25D].
Objective: To assess the effects of vitamin D3 (cholecalciferol, or D3) vs 25-hydroxyvitamin D3 (calcifediol, or 25D3) on total and free 25D in a multiethnic cohort of adults, and whether change in parathyroid hormone (PTH) is more strongly associated with total vs free 25D.
Design: Sixteen-week randomized controlled trial. Biochemistries at 0, 4, 8, and 16 weeks.
Setting: Academic medical center.
Participants: Thirty-five adults ≥18 years of age with 25D levels <20 ng/mL.
Intervention: Sixty micrograms (2400 IU)/d of D3 or 20 μg/d of 25D3.
Main Outcome Measures: Total and free 25D, and PTH.
Results: Baseline total (16.2 ± 3.7 vs 17.0 ± 2.5 ng/mL; P = 0.4) and free (4.2 ± 0.8 vs 4.7 ± 1.0 pg/mL; P = 0.2) 25D were similar between D3 and 25D3 groups, respectively; 25D3 increased total (+25.5 vs +13.8 ng/mL; P = 0.001) and free (+6.6 vs +3.5 pg/mL; P = 0.03) 25D more than D3. By 4 weeks, 87.5% of 25D3 participants had total 25D levels ≥30 ng/mL, compared with 23.1% of D3 participants (P = 0.001). Change in PTH was associated with both total (P = 0.01) and free 25D (P = 0.04).
Conclusions: 25D3 increased total and free 25D levels more rapidly than D3, regardless of race/ethnicity. Free and total 25D were similarly associated with change in PTH.

https://academic.oup.com/jcem/article-abstract/102/4/1133/2982842/Effects-of-Cholecalciferol-vs-Calcifediol-on-Total?redirectedFrom=fulltext

- WANBY P. (2017)

Erythrocyte fatty acid composition does not influence levels of free, bioavailable, and total 25-hydroxy vitamin D.
Scand J Clin Lab Invest. 2017 Feb;77(1):45-52.

In vitro, mono- and polyunsaturated fatty acids (FAs) may decrease the binding affinity of vitamin D metabolites for vitamin D-binding protein, which in turn may influence their bioavailability. FAs incorporated as phospholipids in erythrocyte (ery-) cell membranes reflect dietary intake. The purpose of this study was to investigate ery-FA composition in relation to markers for vitamin D. In healthy females (age 22.6 ± 2.0 years) total 25(OH)D was measured by LC-MS/MS (n = 78), free 25(OH)D with ELISA (n = 64 of 78), and bioavailable 25(OH)D was calculated. Analysis of ery-FA composition was by gas chromatography (n = 56 of 78). A strong correlation between total 25(OH)D and free 25(OH)D was seen (r = .66, p < .001), and between total-25(OH)D and bioavailable 25(OH)D (r = .68, p < .001). No correlations between 25(OH)D fractions and specific fatty acids were found, and in particular, no associations with mono- and poly-unsaturated FA compositions. All 25(OH)D fractions were correlated with leptin (total 25(OH)D (r = -.33, p < .003); bioavailable 25(OH)D (r = -.47, p < .001); free 25(OH)D (r = -.44, p < .001). Associations were found between PTH and total 25(OH)D (r = -.35, p = .002) and weaker between bioavailable 25(OH)D (r = -.35, p = .040) and free 25(OH)D (r = -.28, p = .079). All fractions of 25(OH)D appear to correlate in a similar way to PTH, BMI and body fat (leptin). No association was found between ery-FA composition and free/bioavailable 25(OH)D. It is unlikely that FAs are a strong uncoupling factor of DBP-bound 25(OH)D.

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