Calcium Homeostasis and Vitamin D: *What Are Vitamin D Tests Actually Measuring?*

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Outline:

- Background, deficiency
- How much is enough?
- What should we be measuring?
- How do methods compare to each other?
- Measurement issues
- Standardization efforts

VITAMIN D: BACKGROUND

Calcium Homeostasis:

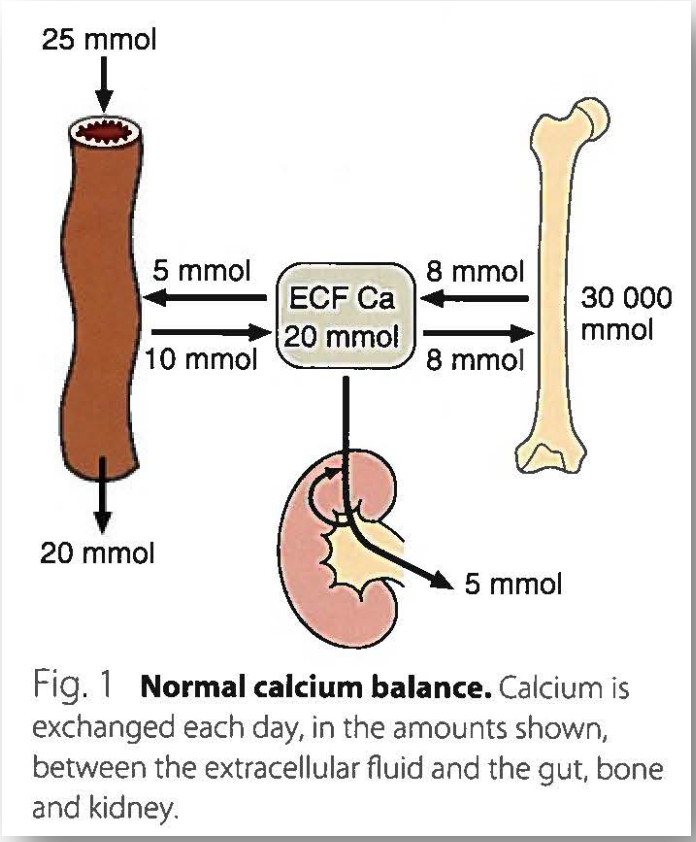
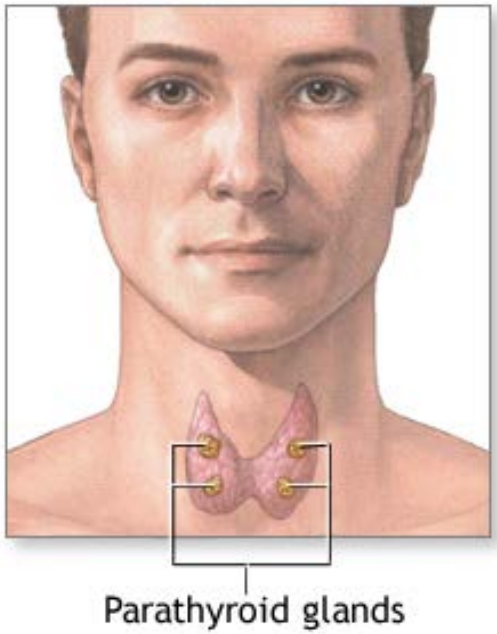


Fig. 1 **Normal calcium balance.** Calcium is exchanged each day, in the amounts shown, between the extracellular fluid and the gut, bone and kidney.

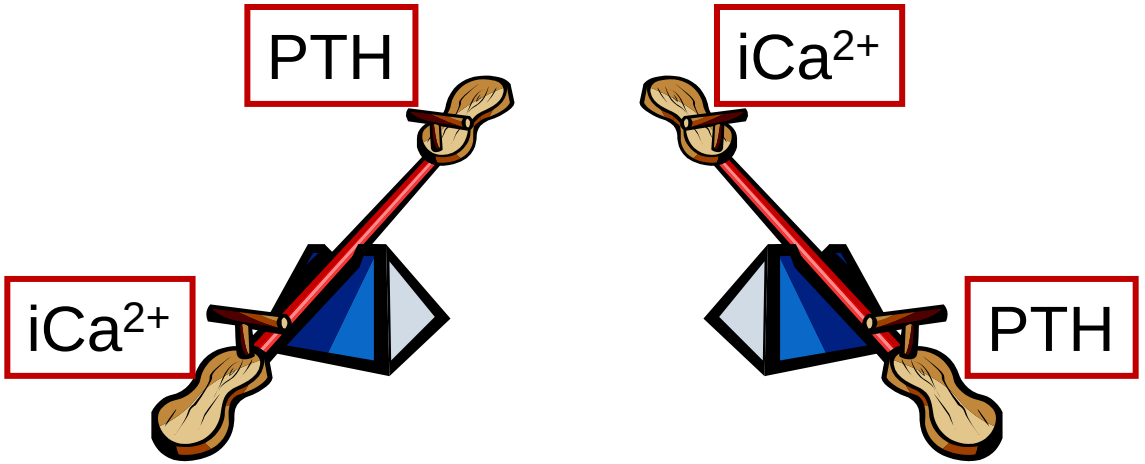
- Dynamic process…not static
- 99% stored in bone
- Remaining 1%:
  - Slowly exchanged
  - Rapidly exchanged
    - 50% = ionized (bioactive)
    - 40% = protein-bound (albumin, globulins)
    - 10% = anion-bound (bicarb, phos, citrate, lactate)
  - Ionized and anion-bound forms pass through glomerulus

Gaw et al., Clinical Biochemistry, ©2008

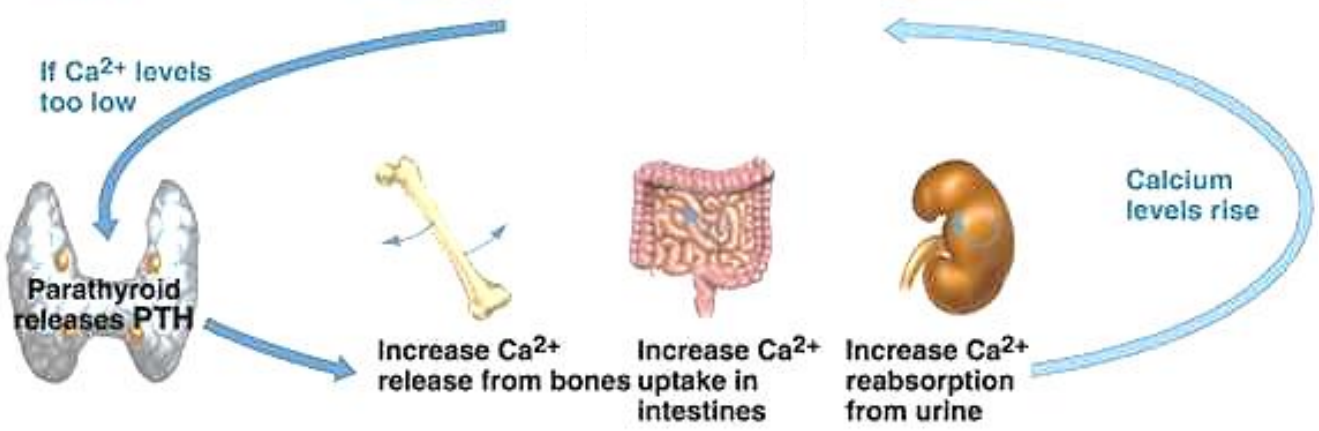
Calcium Sensing: Parathyroid Glands



- Predominant hormone in calcium homeostasis:
  Parathyroid Hormone (PTH)
- Calcium sensing receptors (CaSR)

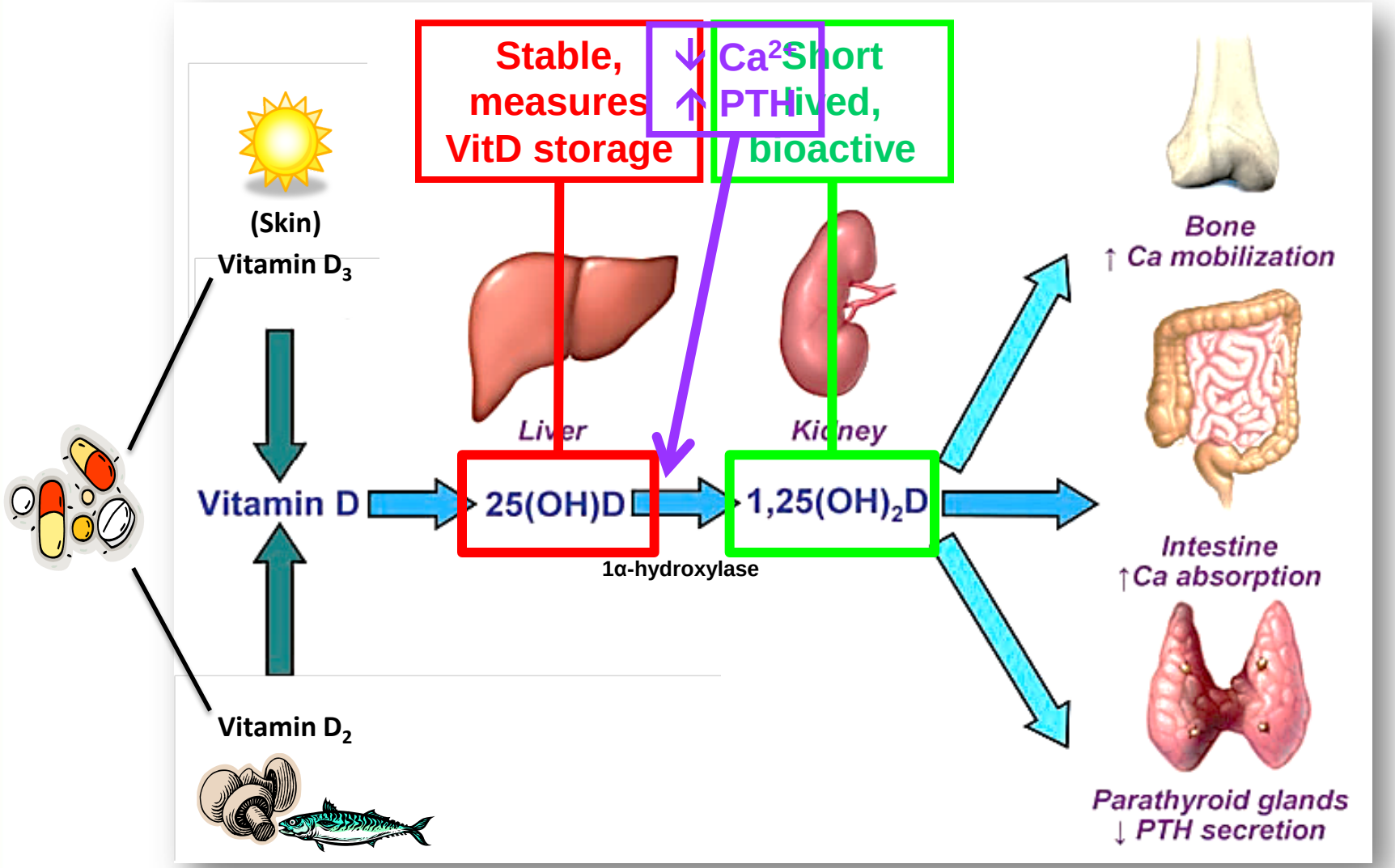


Endocrine Control of Ca^{2+} Homeostasis:



http://biology.clc.uc.edu/fankhauser/Labs/Anatomy_&_Physiology/A&P202/202_lecture_notes/calcium_regulation.jpg

Vitamin D's Role in Calcium Homeostasis:



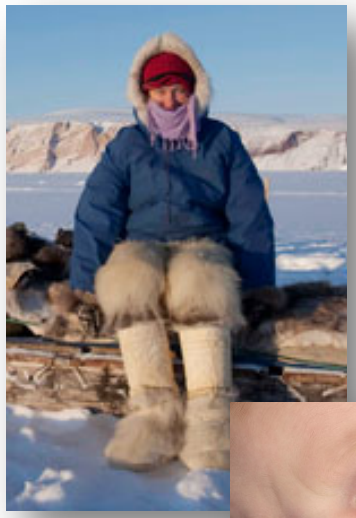
Modified from: Thacher TD and Clarke BL, Mayo Clin Proc 2011;86:50-60

Causes of Vitamin D Deficiency:

- Deficient intake or absorption
  - Dietary (few sources), malnutrition
  - Inadequate sunlight exposure
  - Malabsorption
  - Gastrectomy
  - Small bowel disease
  - Pancreatic insufficiency
- Defective 25-hydroxylation
  - Alcoholic, biliary cirrhosis
  - Anticonvulsants
- Loss of vitamin D binding protein(VDBP)
  - Nephrotic syndrome
- Practical matters
  - Increased use of sunscreen
  - Increased indoor activities
  - Geography
  - Seasonality

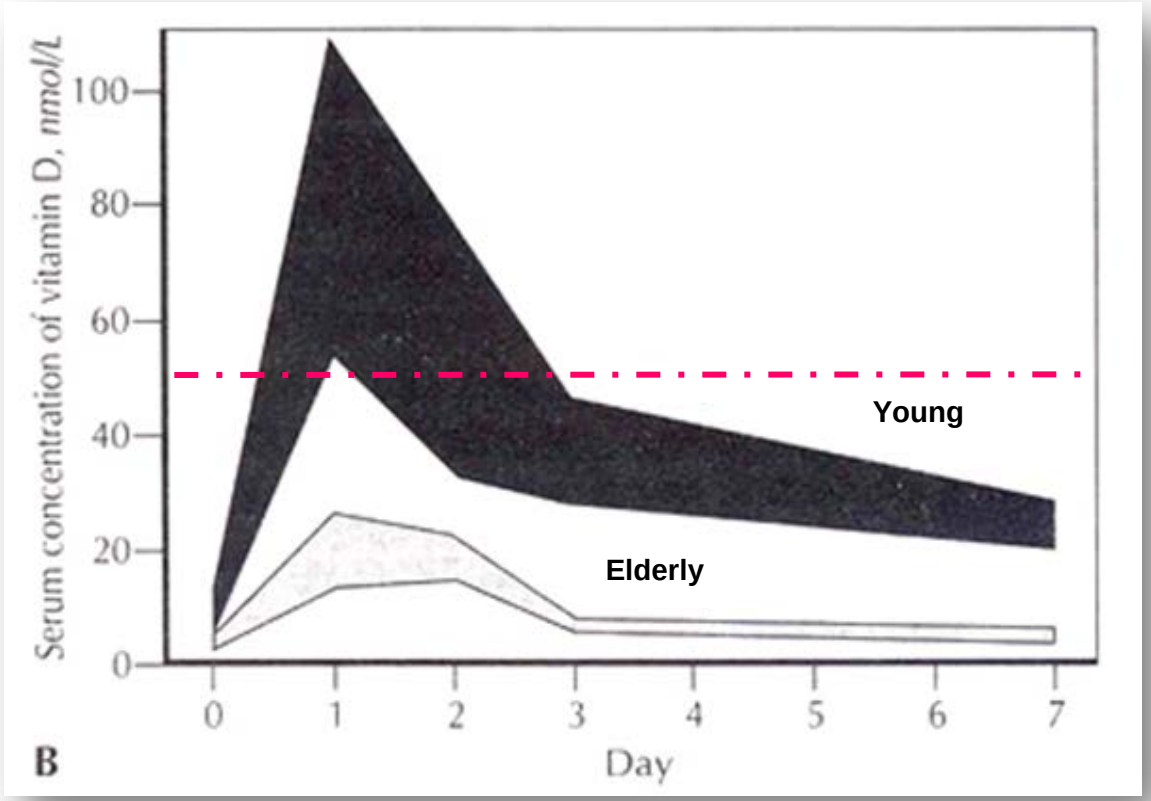
Who is at risk for Vitamin D deficiency?

- Females
- Elderly
- People of color
- 37th parallel
- Hospitalized, indoors
- Covered
- Infants, especially breast fed
- Obese



Effect of Age on Vitamin D:

- Vitamin D levels in response to whole-body exposure to simulated sunlight:



Holick, MF Curr Opin Endo Diab 2002;9:87-98

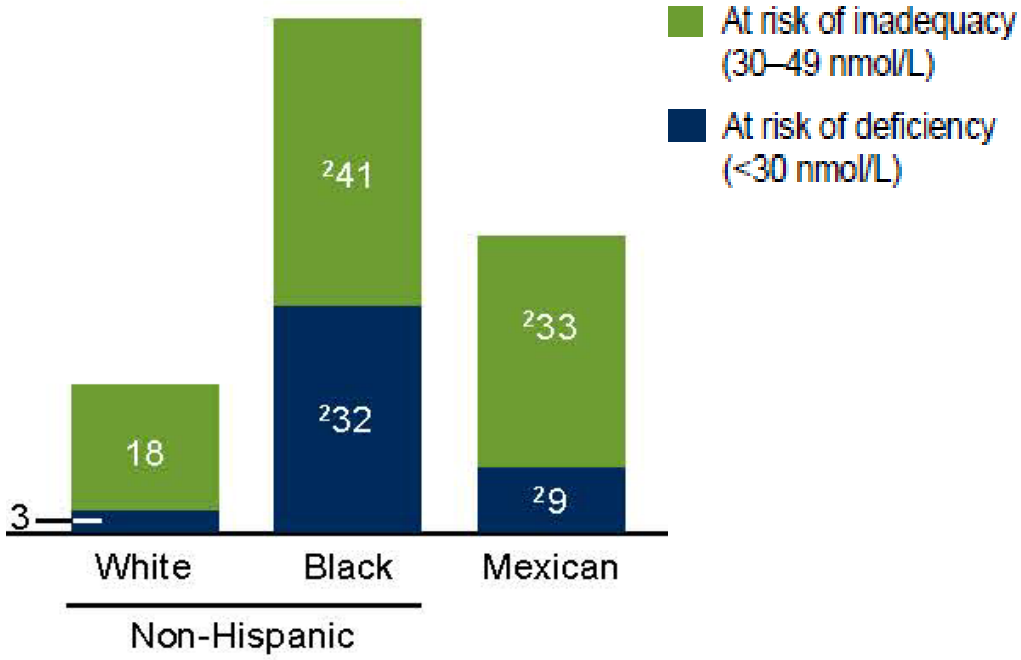
Effect of Ethnicity on Vitamin D:

NCHS Data Brief ■ No. 59 ■ March 2011

Vitamin D Status: United States, 2001–2006

Anne C. Looker, Ph.D.; Clifford L. Johnson, M.P.H.; David A. Lacher, M.D.; Christine M. Pfeiffer, Ph.D.; Rosemary L. Schleicher, Ph.D.; and Christopher T. Sempos, Ph.D.

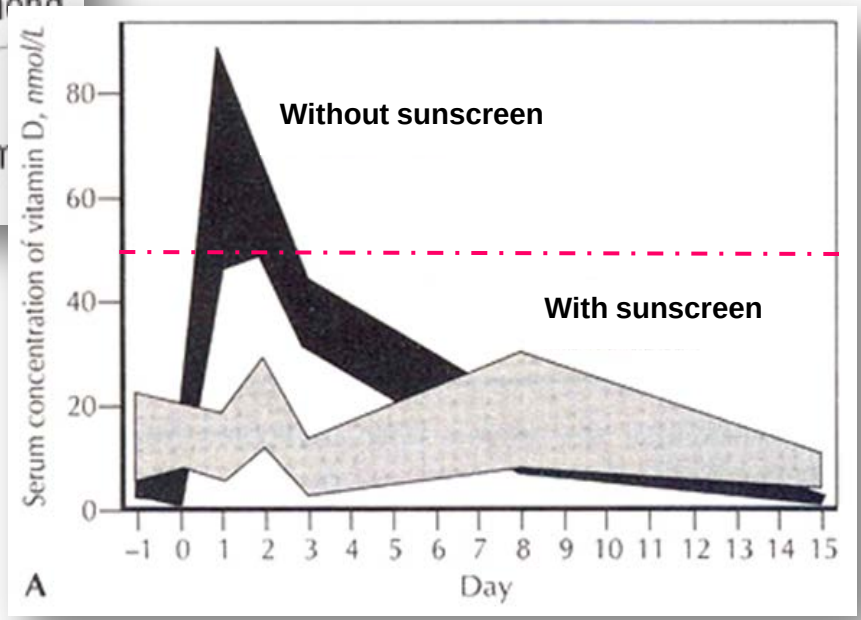
Figure 4. Age- and season-adjusted prevalence at risk of deficiency and inadequacy among persons aged 1 year and over: United States, 2001–2006



[2] $p < 0.05$ compared with non-Hispanic white persons.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey (NHANES); data for ages 1–5 years from NHANES 2003–2006.

Effect of UV on Vitamin D:



www.studentpulse.com

Holick, MF Curr Opin Endo Diab 2002;9:87-98

TABLE 2. Indications for 25(OH)D measurement (candidates for screening)

- Rickets
- Osteomalacia
- Osteoporosis
- Chronic kidney disease
- Hepatic failure
- Malabsorption syndromes
  - Cystic fibrosis
  - Inflammatory bowel disease
  - Crohn's disease
  - Bariatric surgery
  - Radiation enteritis
- Hyperparathyroidism
- Medications
  - Antiseizure medications
  - Glucocorticoids
  - AIDS medications
  - Antifungals, *e.g.* ketoconazole
  - Cholestyramine
- African-American and Hispanic children and adults
- Pregnant and lactating women
- Older adults with history of falls
- Older adults with history of nontraumatic fractures
- Obese children and adults (BMI > 30 kg/m^2)
- Granuloma-forming disorders
  - Sarcoidosis
  - Tuberculosis
  - Histoplasmosis
  - Coccidiomycosis
  - Berylliosis
- Some lymphomas

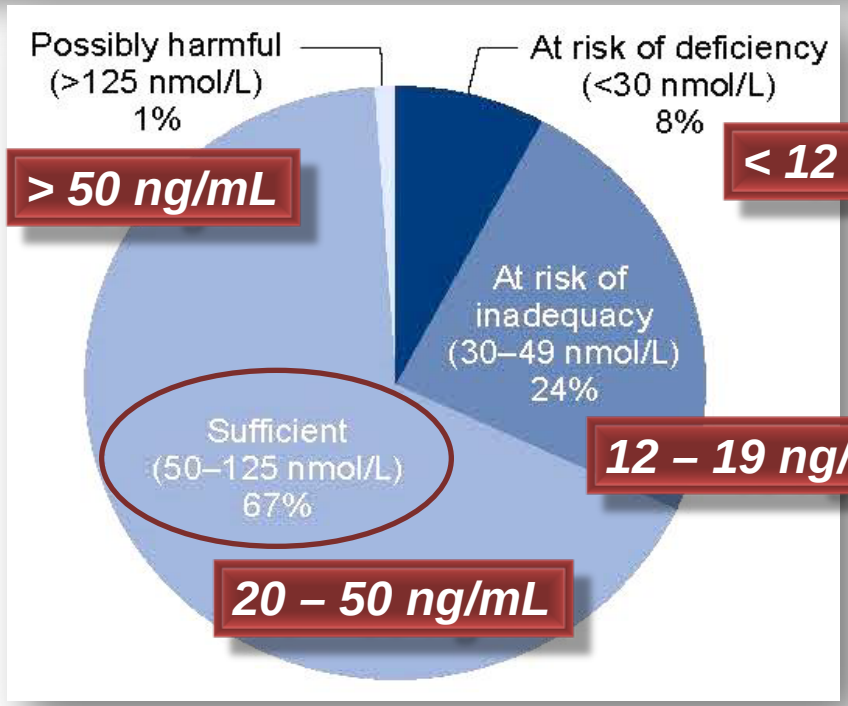
J Clin Endocrinol Metab 2011;96:1911-30

Deficiency Statistics: United States

NCHS Data Brief ■ No. 59 ■ March 2011

Vitamin D Status: United States, 2001–2006

Anne C. Looker, Ph.D.; Clifford L. Johnson, M.P.H.; David A. Lacher, M.D.; Christine M. Pfeiffer, Ph.D.; Rosemary L. Schleicher, Ph.D.; and Christopher T. Sempos, Ph.D.



> 50 ng/mL

< 12 ng/mL

12 – 19 ng/mL

20 – 50 ng/mL

Benefits Associated With Vitamin D:

- Lower cardiovascular mortality
- Reduced risk of:
  - Autoimmune diseases
    - DM, MS, allergy, asthma
  - Cardiovascular disorders
  - Infectious diseases
  - Cancers
  - Renal disease (in African Americans)
  - Mental illness
- Granuloma-forming disorders
- Lower mortality rate
- *…and many more*

Important:

Only skeletal effects have been positively associated with vitamin D concentrations.

Endocr Rev 2012;33:456-92

Endocrine Reviews, June 2012, 33(3):456–492

The Nonskeletal Effects of Vitamin D: An Endocrine Society Scientific Statement

Clifford J. Rosen, John S. Adams, Daniel D. Bikle, Dennis M. Black, Marie B. Demay, JoAnn E. Manson, M. Hassan Murad, and Christopher S. Kovacs

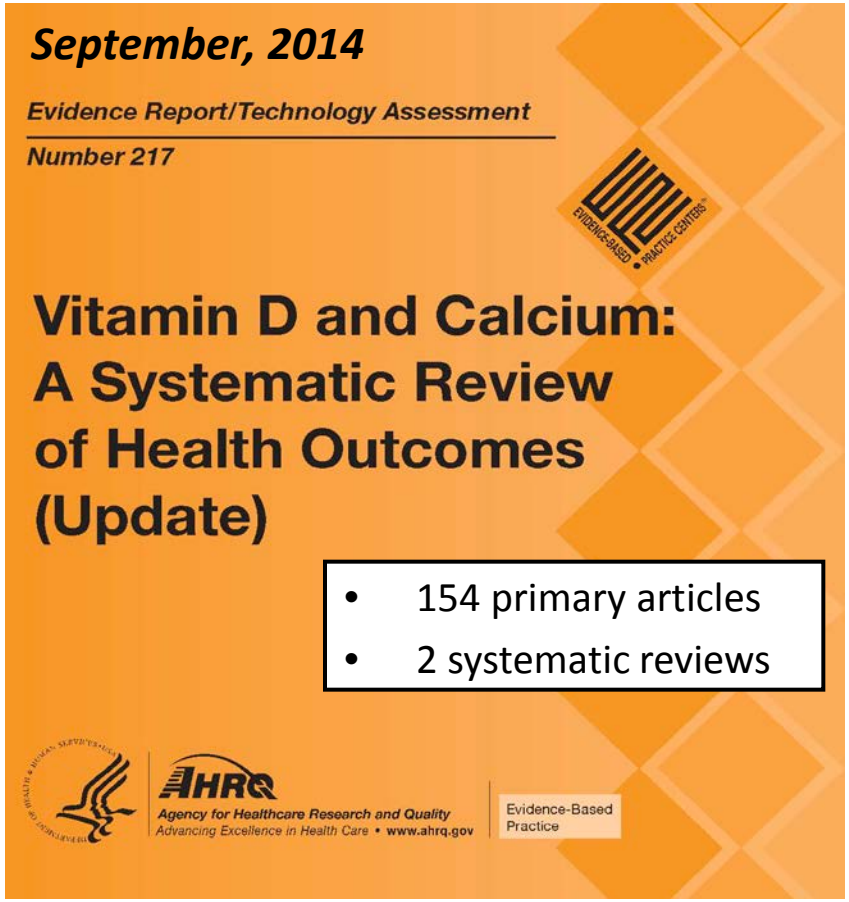
In summary, not surprisingly there remains a persistent need for large randomized controlled trials and dose-response data to test the effects of vitamin D on chronic disease outcomes including autoimmunity, obesity, diabetes mellitus, hypertension, and heart disease. The VITAL trial, as noted above, could help determine whether higher doses of vitamin D (*i.e.*, 2000 IU/d) will reduce the risk of osteoporosis, cancer, and CVD. Similarly, a very large, placebo-controlled, randomized trial of vitamin D, 4000 IU/d, to prevent the onset of type 2 diabetes mellitus in prediabetics is currently in the planning stage. Any potential benefit of high-dose vitamin D supplementation on maternal or fetal outcomes will also await larger trials. Notwithstanding, large-scale clinical trials of a single nutrient may not fully answer the many questions inherent in vitamin D actions. Thus, the role of vitamin D supplementation in the prevention and treatment of chronic nonskeletal diseases remains to be determined.

Vitamin D Benefits: Recent Statements

- "Vitamin D Deficiency: Screening"
  - Draft recommendation statement
  - Public comment period ended 7/21/2014

"The USPSTF concludes that current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency."

Vitamin D Benefits: Recent Statements



…"inconsistent evidence regarding the effect of vitamin D alone or in combination with calcium on most health outcomes"

Findings are "inconsistent across studies for bone health; breast, colorectal, and prostate cancer; cardiovascular disease and mortality; immune function; and pregnancy-related outcomes."

Vitamin D and calcium supplementation may have "positive effects on bone mineral density and bone mineral content."

Risks of Excessive Vitamin D:

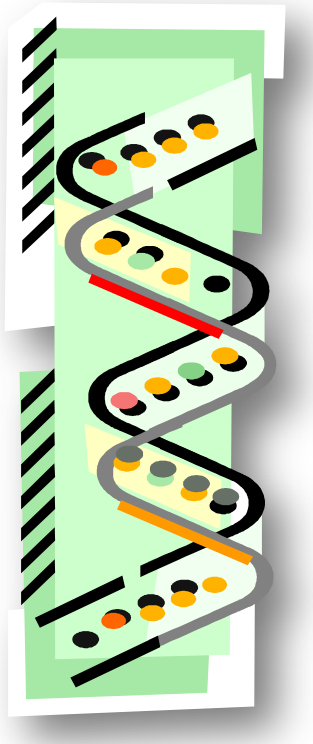
- Sunshine can provide up to 10,000 IU/day
- 100 IU/day raises serum concentration of 25(OH)D by 1 ng/mL
- Serum 25(OH)D is safe up to 100 ng/mL and perhaps 200 or 300 ng/mL
  - Toxic levels are reported at 150 ng/mL
- 10,000 IU/day Tolerable Upper Intake Level (no toxicity observed up to 5 months)
- Increased risk of kidney stones with excess calcium intake (1000 mg)
- IOM states > 4000 IU/day increases risk for harm
- Little evidence from existing trials that excess vitamin D intake is harmful

Vitamin D toxicosis is extremely rare.

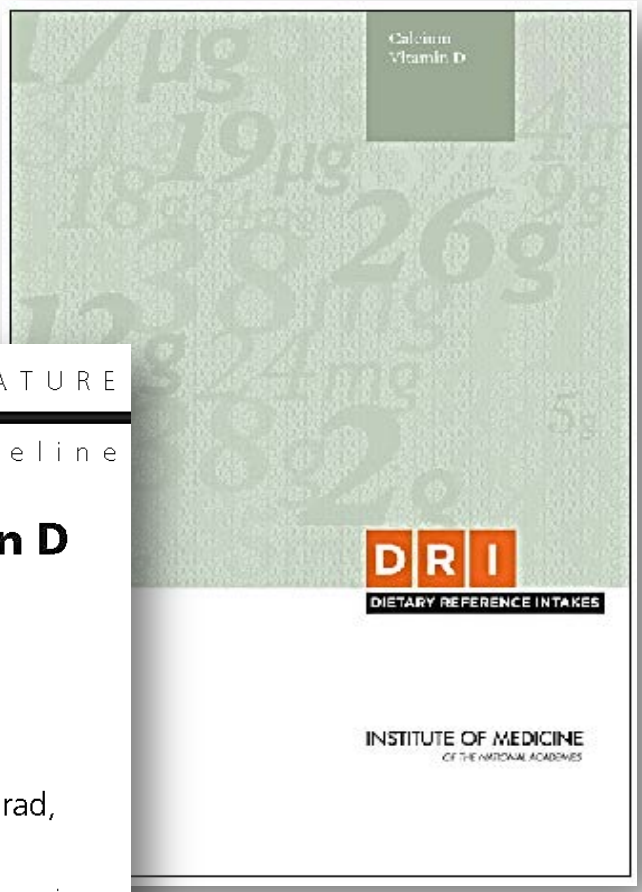
Am J Clin Nutr 2007;85:6-18; NEJM;57:266-81;
Evid Rep Technol Assess (Full Rep). 2007;158:1-235

VITAMIN D: HOW MUCH IS ENOUGH?

Why is this so difficult?



- Can't we just measure 120 healthy adults, create a histogram and determine the mean ± 2SD…like any other reference range?
- Not that easy…
  - What parallel do you live on?
  - Is it summer or winter?
  - Are your subjects lifeguards or office workers?
  - "normal" vs. "optimal"
- *Note: We use decision limits, not reference intervals, to describe Vitamin D concentrations.*



SPECIAL FEATURE

Clinical Practice Guideline

Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline

Michael F. Holick, Neil C. Binkley, Heike A. Bischoff-Ferrari, Catherine M. Gordon, David A. Hanley, Robert P. Heaney, M. Hassan Murad, and Connie M. Weaver

Boston University School of Medicine (M.F.H.), Boston, Massachusetts 02118; University of Wisconsin (N.C.B.), Madison, Wisconsin 53706; University Hospital Zurich (H.A.B.-F.), CH-8091 Zurich, Switzerland; Children's Hospital Boston (C.M.G.), Boston, Massachusetts 02115; University of Calgary Faculty of Medicine (D.A.H.), Calgary, Alberta, Canada T2N 1N4; Creighton University (R.P.H.), Omaha, Nebraska 68178; Mayo Clinic (M.H.M.), Rochester, Minnesota 55905; and Purdue University (C.M.W.), West Lafayette, Indiana 47907

IOM (Institute of Medicine), 2011. Washington, DC: The National Academies Press; J Clin Endocrinol Metab 2011;96:1911-30

Vitamin D: Recommended Ranges

Clinical Condition	Institute of Medicine (IOM) (General population)	Endocrine Society (Population at risk for ficiency)
Deficiency	<12 ng/mL (<30 nm	ng/mL nmol/L)
Insufficiency	/L)	21-29 ng/mL (52.5 - 72.5 nmol/L)
Sufficienc	>20 ng/mL (>50 nmol/L)	>30 ng/mL (75 nmol/L)
Upper limit	50 ng/mL (125 nmol/L)	100 ng/mL (250 nmol/L)

No consensus.

Sources: J Clin Endocrinol Metab. 2011 Oct;96(10):2987-96; J Clin Endocrinol Metab. 2011 Oct;96(10):3065-66; J Clin Endocrinol Metab. 2011 Jul;96(7):1911-30; IOM (Institute of Medicine), 2011. Washington, DC: The National Academies Press

Vitamin D: What to Measure?

What form should be measured?

- Circulating serum 25(OH) vitamin D – best available indicator of cutaneous synthesis (sunlight, skin) and total intake (food, supplements)
  - Remember: 25(OH)D = 25(OH)D_2 + 25(OH)D_3
    - Major circulating form, long half-life
    - Measures storage, not function
- Because of the widespread use of both vitamin D_2 and vitamin D_3 supplements, assays should measure 25(OH) vitamin D_2 and 25(OH) vitamin D_3 equally

Adapted from: IOM (Institute of Medicine), 2011. *Dietary Reference Intakes for Calcium and Vitamin D.* Washington, DC: The National Academies Press.

What should NOT be measured?

- Parent vitamin D
  - Precursor
  - Protein bound
  - Water insoluble
  - Lower circulating concentrations
- $1,25(OH)_2$ vitamin D
  - Bioactive form
  - Short half-life

Remember:

25(OH) vitamin D concentrations correlate best with clinical signs of vitamin D deficiency

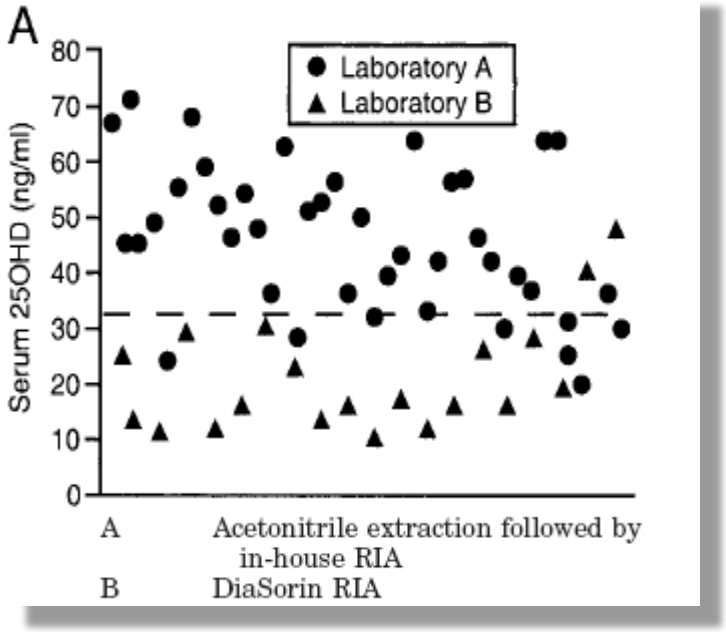
Clarifying Nomenclature:

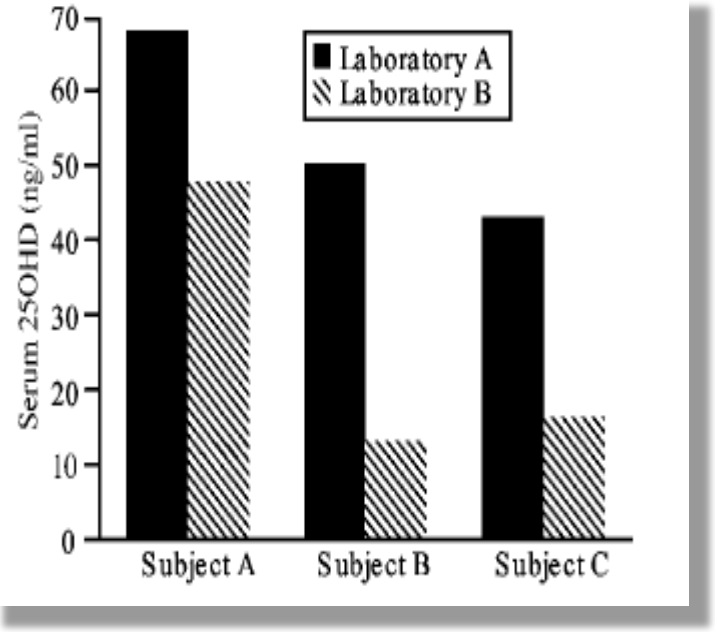
When someone says…	This is being measured…
"Vitamin D_2"	$25(OH)D_2$
"Vitamin D_3"	$25(OH)D_3$
"Total Vitamin D"	$25(OH)D_2 + 25(OH)D_3$

Not the "parent" vitamin D prohormone…

Vitamin D: How do different methods compare with each other?

Biases Between Methods: A Historical Problem





- 59 postmenopausal women (similar age, race, geographic residence, bone mass)
- 62 measurements, 2 laboratories, 2 assays
- No overlap between groups (90% vs. 17% insufficiency)

Binkley N, et al. JCEM 2004;89:3152-7

Total 25(OH)D

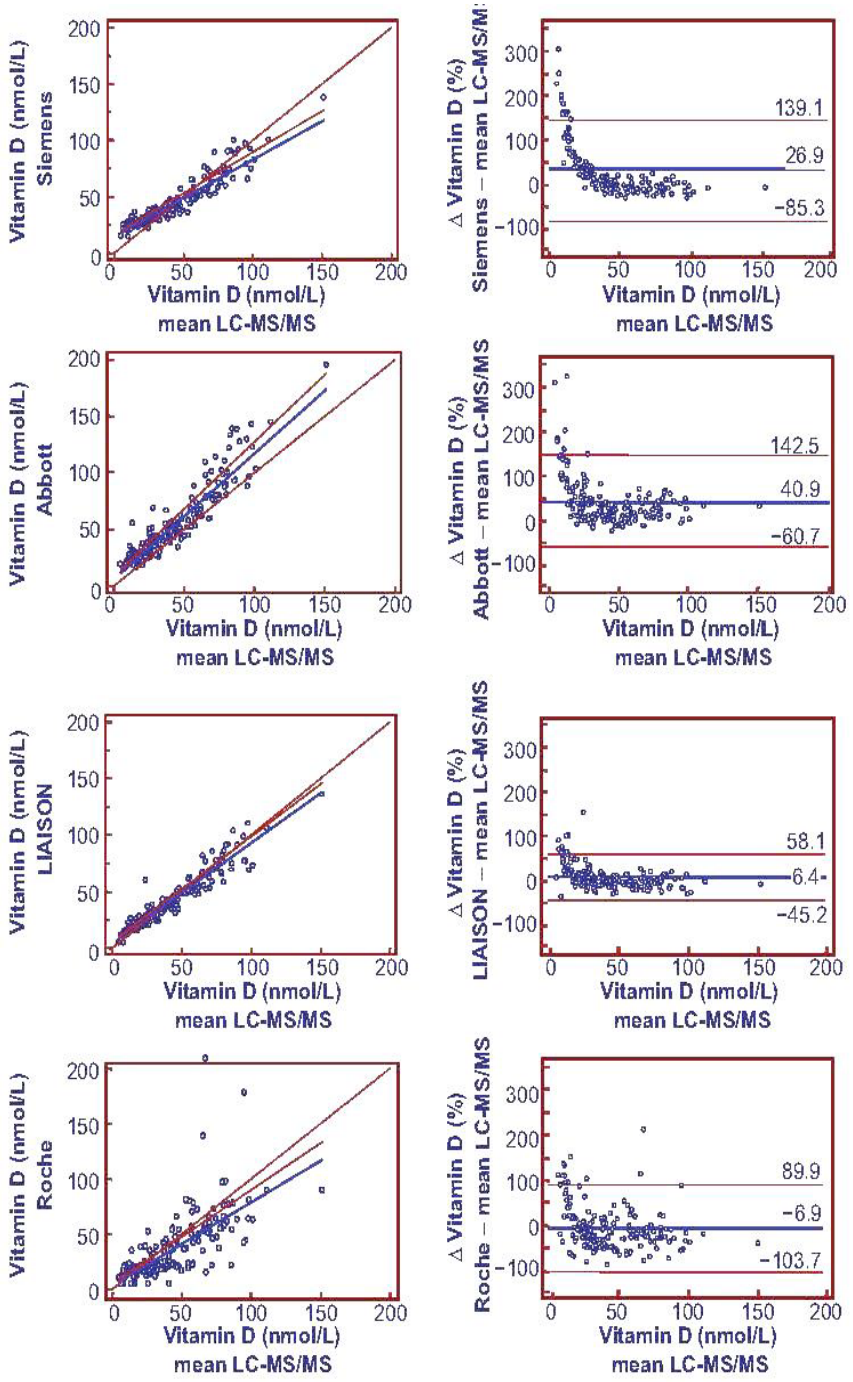
$25(OH)D_2$, $25(OH)D_3$

	Supplier	Strengths	Limitations[a]
Automated immunoassay			
Liaison Total	Diasorin	Extensively used Technically simple High throughput	Susceptible to matrix effects Possible between lot variability in product
iSYS	IDS	Technically simple High throughput	Susceptible to matrix effects Under-recovers 25-hydroxyvitamin D2 Possible between lot variability in product
Elecys	Roche	Technically simple High throughput	Susceptible to matrix effects Only detects 25-hydroxyvitamin D3 Possible between lot variability in product
Direct detection methods			
HPLC	Usually developed or adapted 'in-house'	Solvent or solid phase extraction followed by and interferences Process can be automated or semi-automated Separate simultaneous measurement of 25OHD2 and 25OHD3 User able to control standardisation Low reagent costs	Requires specialised staff Some procedures require large sample volume. Lower sample throughput and relatively longer turnaround time compared to immunoassay Possible interference from C3-25OHD epimer
LC/MS/MS	Usually developed or adapted 'in-house'	Solvent or solid phase extraction followed by chromatography minimises matrix effects and interferences Process can be automated or semi-automated User able to control standardisation Separate simultaneous measurement of 25OHD2 and 25OHD3 Highly accurate and precise when properly validated	Equipment is expensive Requires specialised staff Lower sample throughput and relatively longer turnaround time compared to immunoassay Susceptible to ion suppression interference Possible interference from C3-25OHD epimer

Adapted from: Wallace AM, et al. Steroids 2010;75:477-88

Method Comparisons: Automated Immunoassays

- Many new commercial assays
- Differences between methods
- Both positive and negative biases



Adapted from: Farrell et al., Clin Chem 2012;58:531

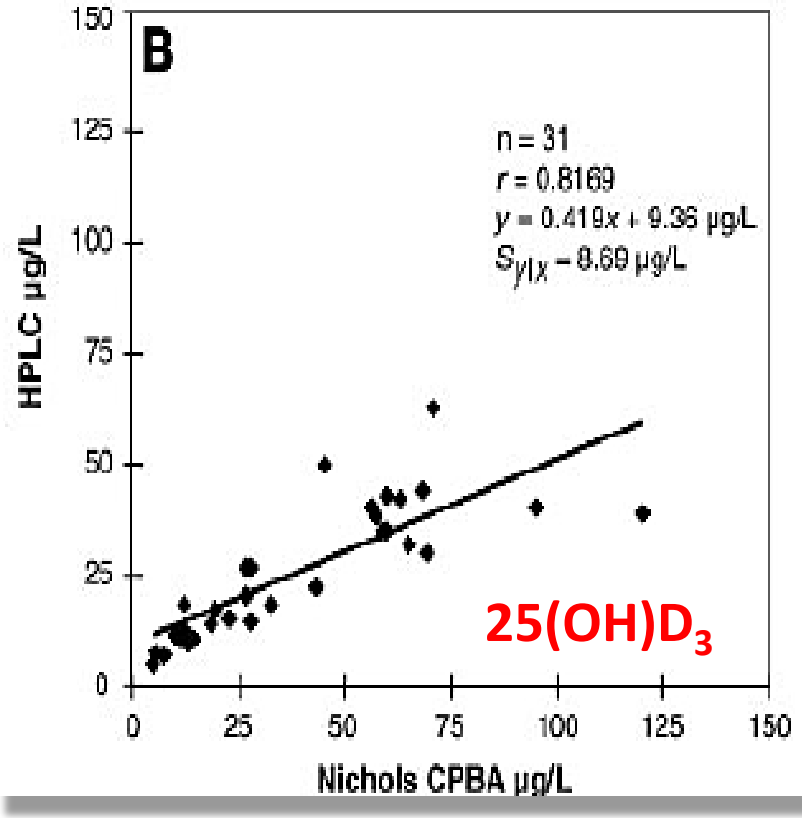
VITAMIN D: MEASUREMENT ISSUES

Why are there differences between vitamin D methods?

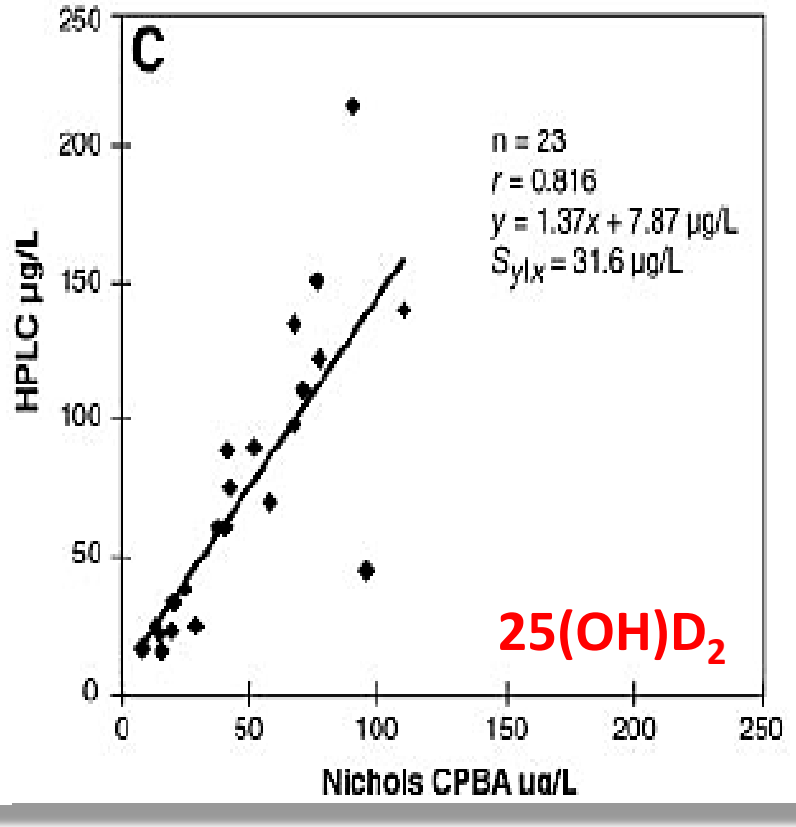
- Non-equimolar detection of 25(OH) vitamin D_2 and D_3
- Vitamin D binding protein and proprietary methods of release (hydrophobic)
- Cross-reactivity with metabolites, including 3-epi-25(OH) vitamin D_3
- Heterophilic antibody interferences
- Differences in standardization – historical lack of international standard

Non-equimolar Detection of D_2 and D_3: Comparison to an HPLC Method

Over-estimates



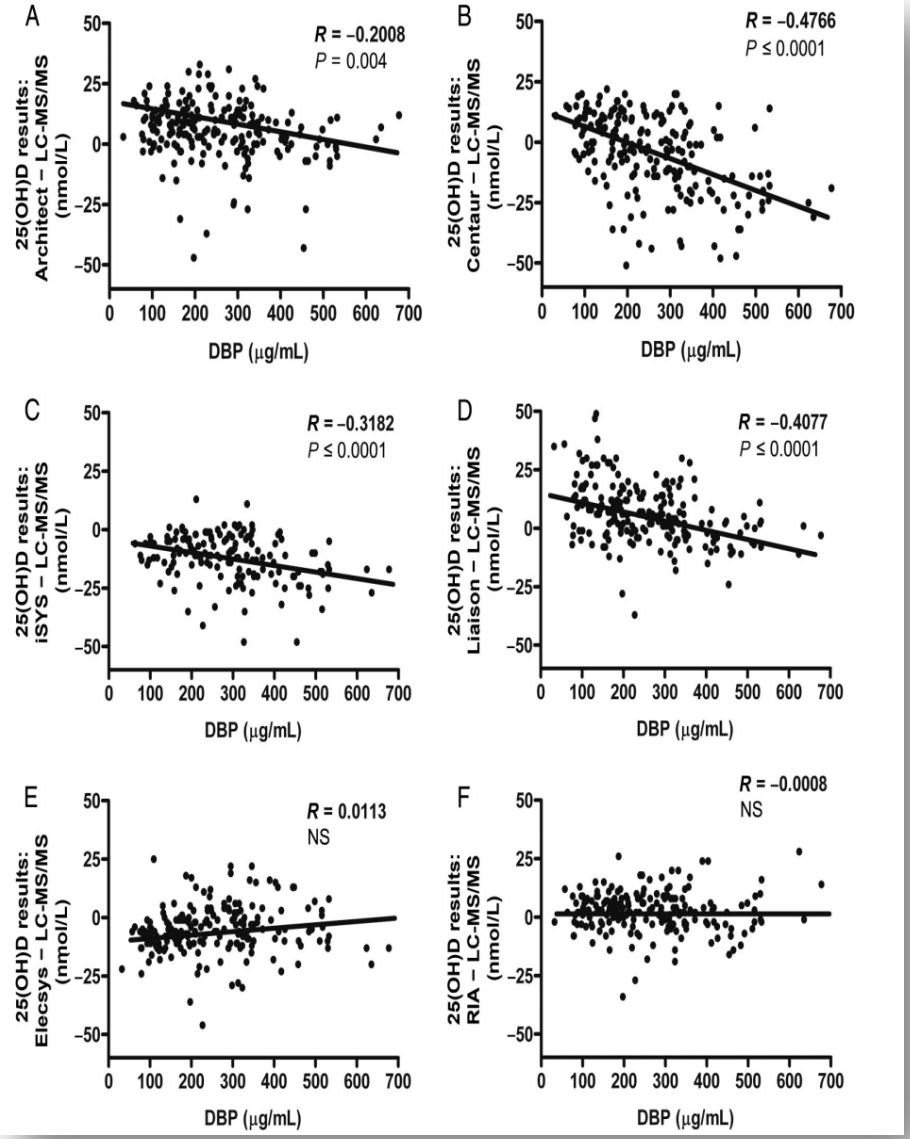
Under-estimates



Lensmeyer GL, et al. Clin Chem 2006;52:1120-6

Vitamin D Binding Protein (DBP):

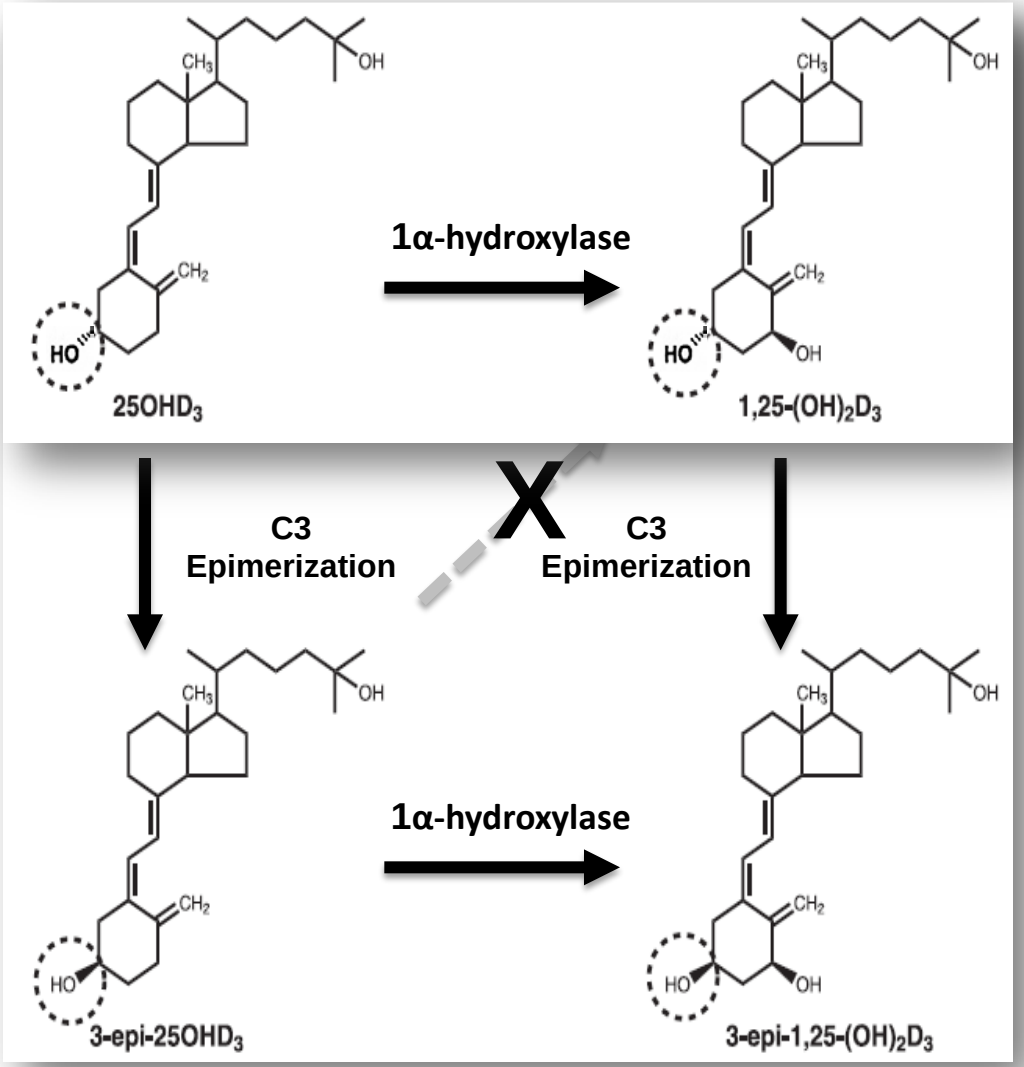
- Tightly binds 25(OH) vitamin D
- Release prior to testing
- Relationship between DBP and deviation from MS
- Elevated DBP:
  - Pregnancy
- Decreased DBP:
  - ICU patients
- Ethnic differences[1]



[1]Powe CE et al. NEJM 2013;369:1991

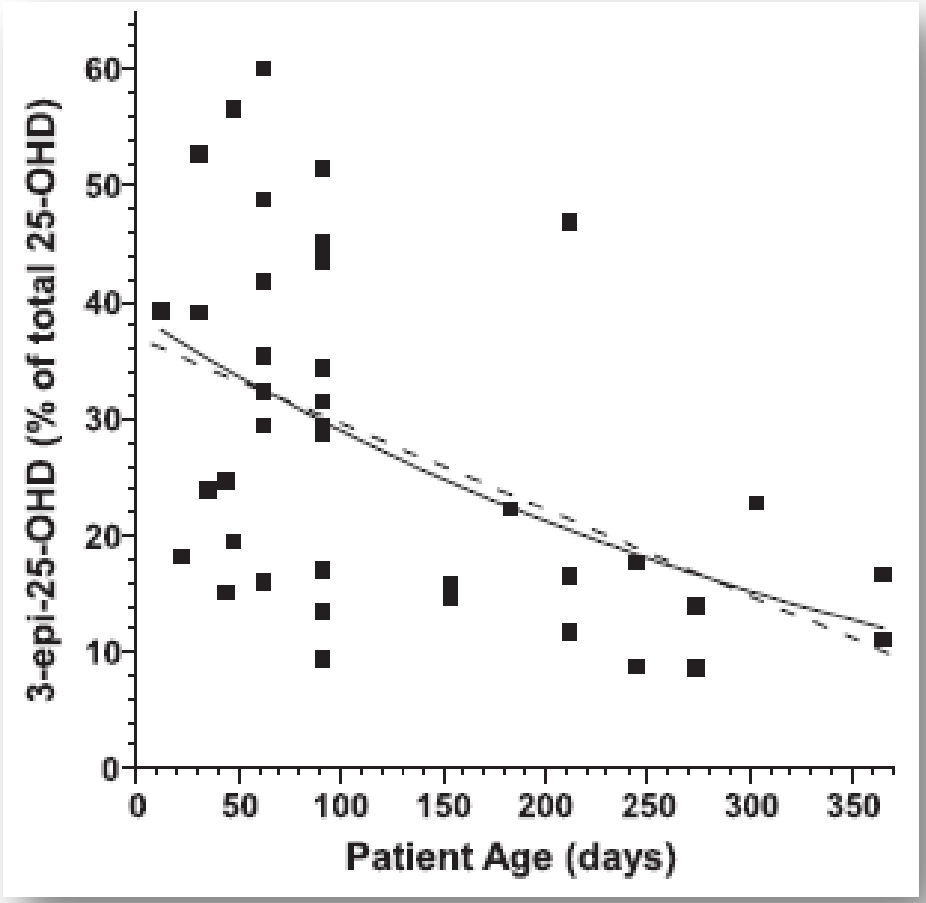
Heijboer AC et al. Clin Chem 2012;58:543-8

Vitamin D Metabolite: C3-epimer



Adapted from: Singh RJ, et al. JCEM 2006;91:3055-61

C3 Epimer as a Function of Age:

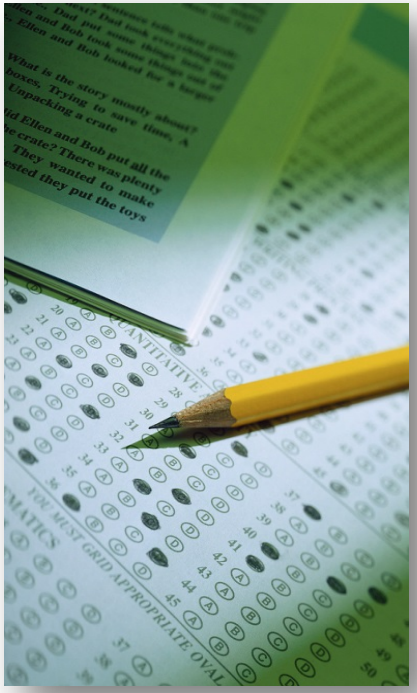


Singh RJ, et al. JCEM 2006;91:3055-61

- 3-epi-25(OH)D_3 concentrations
  - 15 – 41% of infant samples
  - 2.5 – 17% of adult samples
    - CCLM 2011;49:253-6
  - Present in 99% of samples from patients neonate to >80 yrs
  - ≤ 3 ng/mL in 92% of samples
    - JCEM 2012;97:163-8

Vitamin D: Proficiency Testing Programs

- Goal: Ensure reliability of 25(OH) vitamin D measurements…regardless of the assay used



- College of American Pathologists (CAP)
  - Accuracy Based Vitamin D (ABVD) survey
- Vitamin D External Quality Assessment Scheme (DEQAS)
- NIST-NIH Vitamin D Metabolites Quality Assurance Program (VitDQAP)
- Quality Management Program-Laboratory Services (QMP-LS)

Proficiency Testing Programs: College of American Pathologists (CAP)

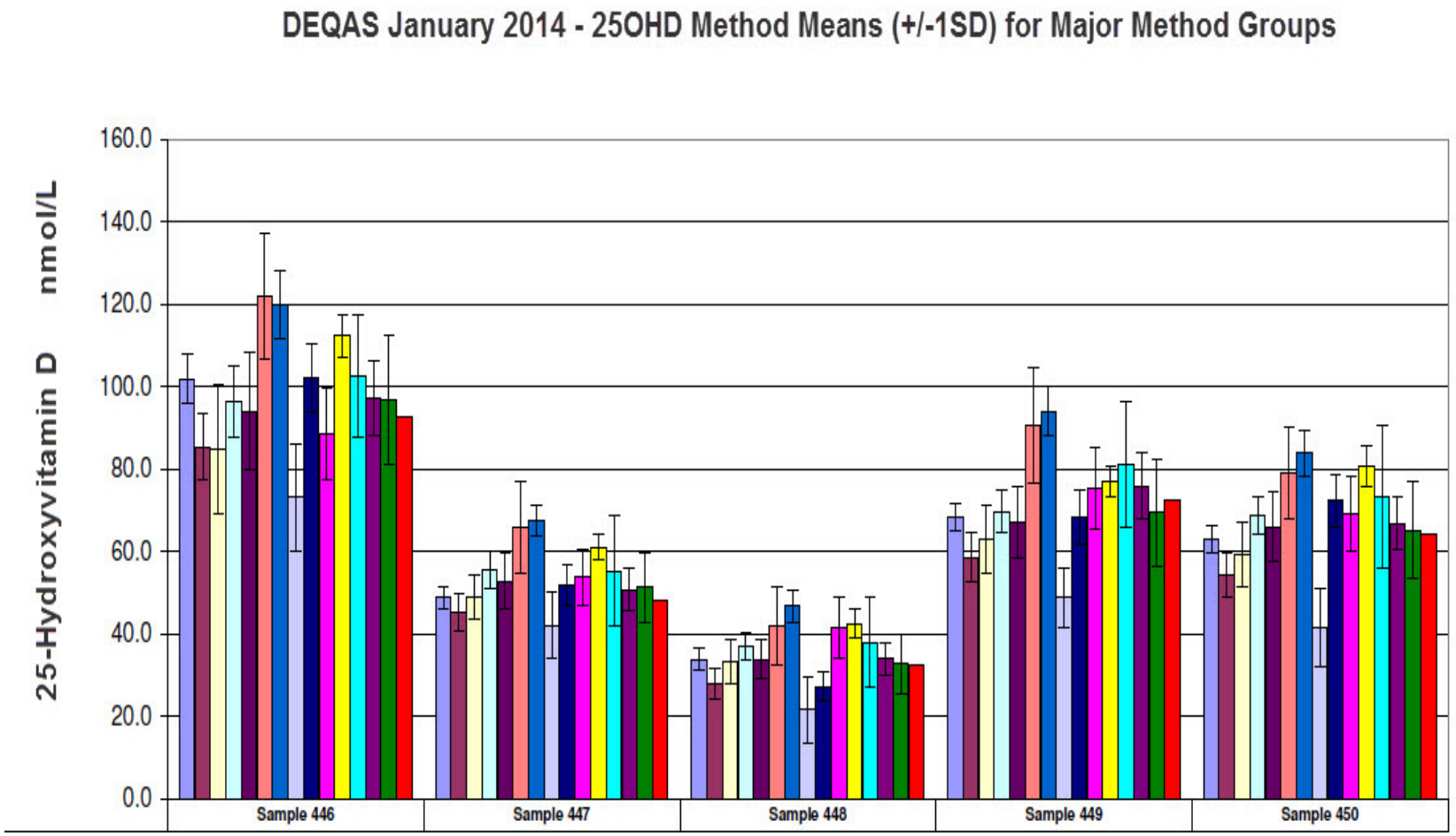
- Accuracy-Based Vitamin D (ABVD) survey
  - Pooled, fresh frozen serum samples
    - Supplemented with oral vitamin D_2
  - Target values: CDC LC-MS/MS method
    - Traceable to NIST and University of Ghent reference methods
  - Acceptance criteria = ± 25% of target value

CAP Accuracy Based Vitamin D (ABVD) Survey A: April, 2014

	25 (OH) Vitamin D (ng/mL) - Target			
Sample	Total	D_2	D_3	D_3-Epimer
ABVD-07	59.18	0.49	58.69	5.6
ABVD-08	14.52	4.42	10.10	0.9
ABVD-09	18.99	1.11	17.88	2.1

- Improved agreement over non-commutable materials
- Variation among manufacturers
- Under-recovery of 25(OH) vitamin D_2
- Over-recovery in presence of C3-epimer

Vitamin D External Quality Assessment Scheme: DEQAS



Vitamin D: Standardization Among Methods

Toward Vitamin D Standardization:

- "A standardized laboratory measurement is one that is accurate and comparable over time, location and laboratory procedure." --NIH, VDSP
- Milestones:
  - Reference method procedure
    - NIST, University of Ghent
  - NIST Standard Reference Material (SRM 972 and 2972)
  - CDC Vitamin D Standardization Program (VDSP)
    - Vitamin D Certification Program
  - CDC Hormone Standardization Program (HoSt)

Summary:

- 25(OH) vitamin D concentrations correlate best with clinical signs of vitamin D deficiency
- Extra-skeletal benefits of vitamin D are not well substantiated
- There is no consensus on optimal recommended serum 25(OH) vitamin D concentrations
- Vitamin D methods should ideally measure D_2 and D_3 metabolites equally
- Separating D_2 and D_3 concentrations may aid in monitoring therapy
- Standardization of methods is necessary
  - Differences still exist among methods

Questions?

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