The Human Requirement for VITAMIN D

- can be met by intake (limited number of foods or supplements) and/or by sunlight (UVB exposure).
- the substance ‘vitamin D’ is inert.
- Vitamin D is a pro-hormone. It is metabolized to 25(OH)D & eventually to 1,25(OH)\(_2\)D, a steroid hormone that generates biological responses by binding to the vitamin D receptor (VDR).
Main Dietary Sources of Vitamin D

- Fortified milk
  400 IU per quart
- Some Yogurts
  80 IU/8 oz
- Some fortified cereals
  40 to 100 IU
- Some fortified juices
  100 IU/8 oz
- Fatty fish
  (salmon, mackerel, herring, tuna)
  200-400 IU/3 oz
- Some calcium and vitamin/mineral supplements
  most often 400 IU
Prevalence of Supplement Use (NHANES 1999-2002)

Functions of Vitamin D

Sun-UVB → Skin

Intestine → Diet

Vitamin D

Ca-P homeostasis

Target cells

e.g.

Bone
Intestine
Kidney
Parathyroids

D = D2+D3

1αOHase

1,25(OH)2D

Gene expression

Endocrine functions

25OHD

VDR

1αOHase

1,25(OH)2D

25OHD

VDR

Para/autocrine functions

Gene expression

Other functions

Target cells

e.g.

Immune system
Cardiovascular system
Muscle
Cartilage
Adipose tissue
Liver
Pancreas
Brain
Lung
Skin
Breast
Ovary, testes
Uterus, placenta
Utility and validity of specific biomarkers as indicators of ‘sufficiency’ are likely to be different at each stage of life.

- Supply to target tissues (e.g. 25OHD)
- Function/biological response (e.g. PTH)
- Intermediate health endpoint (e.g. BMD, Ca absorp.)
- Stigmata of disease (e.g. fracture)

Biomarkers

- Endogenous production
  - intake
  - absorption
  - metabolism
  - excretion

Adapted from the EU-FUFOSE schema for the scientific basis for health claims on foods. Eur J Nutr 2004;43:II/3-II/6

Utility and validity of specific biomarkers as indicators of ‘sufficiency’ are likely to be different at each stage of life.
## Dietary Reference Intakes

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>AI (μg)</th>
<th>Pregnancy</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to 13</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(200 IU)</td>
<td>(200 IU)</td>
<td>(200 IU)</td>
</tr>
<tr>
<td>14–50</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(200 IU)</td>
<td>(200 IU)</td>
<td>(200 IU)</td>
</tr>
<tr>
<td>&gt; 51–70</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(400 IU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71+</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(600 IU)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vitamin D Status of the US Population?

• 25(OH)D is biomarker of exposure not status:
  – UVB radiation + intakes

• Population-based status data:
  – Dietary Reference Intake Committee of the Institute of
    Medicine on Vitamin D and calcium
  – Dietary Guidelines Advisory Committee

• 25(OH)D collected in NHANES III and continuous
  NHANES since 20000
What is NHANES?

- **National Health and Nutrition Examination Surveys**
- Conducted by the National Centers for Health Statistics (NCHS) of the CDC
- To assess the health and nutritional status of a nationally representative sample
- Data used for a variety of research and programmatic purposes
Trends in the age-adjusted prevalence of diet-related health conditions in U.S.
(adults age 20 to 74)

Data source: National Health and Nutrition Examination Surveys (NHANES); data are plotted at the midpoint of the NHANES survey period.
NHANES Mobile Exam Center
## NHANES: Serum 25OHD Samples

<table>
<thead>
<tr>
<th>Survey</th>
<th>Ages</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES III (1988-1994)</td>
<td>12+</td>
<td>18,158&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NHANES 2000</td>
<td>6+</td>
<td>4,188&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NHANES 2001-02</td>
<td>6+</td>
<td>7,807&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NHANES 2003-04</td>
<td>1+</td>
<td>8,294&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NHANES 2005-06</td>
<td>1+</td>
<td>7,402&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Looker et al., 2007 NIH conference; <sup>b</sup>Pfeiffer, Roundtable handouts, 2009
NHANES 2000-2004 serum 25(OH)D
Month and latitude of data collection

> 86%
> 75%
NHANES: Serum 25OHD Assays

- Laboratory:
  - National Center for Environmental Health (NCEH), CDC

- Methods:
  - DiaSorin RIA (25OHD$_2$ + 25OHD$_3$)
  - LC-MS/MS
    - 2007 and after

Anne C Looker, Christine M Pfeiffer, David A Lacher, Rosemary L Schleicher, Mary Frances Picciano, and Elizabeth A Yetley

ABSTRACT
Background: Changes in serum 25-hydroxyvitamin D (25(OH)D) concentrations in the US population have not been described.
Design: Serum 25(OH)D was measured with a radioimmunoassay kit in 20,289 participants in NHANES 2000–2004 and in 18,158 participants in NHANES III (1988–1994). Body mass index (BMI) was calculated from measured height and weight. Milk intake and sun protection were assessed by questionnaire. Serum concentrations were assessed by reanalyzing 150 stored serum specimens from NHANES III with the current assay.
Results: Age-adjusted mean serum 25(OH)D concentrations were 5–20 nmol/L lower in NHANES 2000–2004 than in NHANES III. After adjustment for assay shifts, age-adjusted means in NHANES 2000–2004 remained significantly lower (by 5–9 nmol/L) in most males, but not in most females. In a study subsample, adjustment for the confounding effects of assay differences changed mean serum 25(OH)D concentrations by ∼10 nmol/L, and adjustment for changes in the factors likely related to real changes in vitamin D status (ie, BMI, milk intake, and sun protection) changed mean serum 25(OH)D concentrations by 1–1.6 nmol/L.

vitamin D status. A clear understanding of changes in the vitamin D status of the US population and of factors that may have contributed to these changes is relevant in light of the considerable efforts currently underway to better define the role of this important vitamin in health (6–9).

Serum 25(OH)D concentrations were measured in the National Health and Nutrition Examination Survey (NHANES) for the first time in the third NHANES (1988–1994), and they have been part of the current continuous NHANES process since 2000. These data provide the opportunity to compare vitamin D status in representative samples of the noninstitutionalized US population who were assessed at 2 different time-points. The objectives of the present study are 1) to describe current 25(OH)D concentrations in a wide range of population subgroups, including children aged 1–11 y and pregnant women, for whom national estimates have not previously been available and 2) to compare differences in serum 25(OH)D between NHANES III and NHANES 2000–2004 before and after adjustment for assay method changes. We also performed exploratory analyses to compare the relative contributions of confounding from assay method with the combined effects of biological and behavioral factors (eg, body mass index, sun protection, and milk consumption) that may have contributed to observed differences over
Initial 25(OH)D Assay Concerns (Looker et al., 2008)

• 1988-94 vs. 2000 - 2004:
  – Reformulation (introduction of antibody to improve binding) of Diasorin RIA assay kit → shifts in assay results between these two time periods
  – Adjusted for assay drifts to compare time trends in 25(OH)D and identify contributing factors

Letter to the Editor (2009)

- Effects of drifts on population data not fully recognized until after publication

- Analyses repeated after excluding data of concern:
  - Effects were minimal
  - CDC to address methodological drift issues
Observed differences in serum 25(OH)D concentrations (NH Whites, 20-59y, NH III vs NH 03-04)


Black bars: Observed mean for NHANES III 1988-1994
Open bars: Observed mean for NHANES 2003-2004
*Calculated as NHANES III mean minus NHANES 2003-04 mean
Differences in 25(OHD) after accounting for assay difference**
(NH Whites, 20-59y, NH III vs NH 03-04)

“Brick” pattern: Predicted mean for NHANES III 1988-1994 assuming current RIA used
Clear bar: Observed mean for NHANES 2003-2004
*Calculated as NHANES III 1988-1994 minus NHANES 2003-04 mean
**Mean predicted for NHANES III 1988-1994 if current RIA was used.
Differences in 25(OH)D after accounting for assay difference and biological/behavioral factors*** (NH Whites, 20-59y, NH III vs NH 03-04)

“Brick” design: Predicted mean for NHANES III 1988-1994 assuming current RIA used
“Dot” design: Predicted mean for NHANES 2003-04 after adjusting for BMI, milk intake and sun protection changes
*Calculated as NHANES III mean minus NHANES 2003-04 mean
*** Mean predicted for NHANES III 1988-94 assuming current RIA was used and mean predicted for NHANES 2003-2004 if mean BMI, milk consumption, and sun protection applied.

Adjusted* mean serum 25OHD
Non-Hispanic whites 20-59 y, NH 03-04

*Adjusted for age and sex

Mean serum 25OHD* in males
NHANES 2000-2004 (nmol/L)

**Data for age 1-5 available from NHANES 2003-04 only

*Adjusted for season
NHANES: Mean 25(OH)D (nmol/L)*

*Not adjusted for assay drift
NHANES: % <50 nmol/L*

*Not adjusted for assay drift
25OHD (nmol/L) NHANES weighted means track well with trends seen in QC data

For methods comparison only and not for publication. Not adjusted for reformulation or assay shifts
NCHS Analytic Note (2009)

• Published data 2000 – 2004:
  – Effect of drifts in assay comparability for the serum 25(OH)D data collected in this time period not fully recognized

• Additional drifts in assay comparability for the 2005 – 2006 results

• Cautions users when assessing time trends

• **Adjustments for assay differences:**
  – Looker et al. (2008):
    • Overall mean 25(OH)D lower in 2000-2004 than in 1988-94
    • Assay changes accounted for much of the difference

• **No adjustment for assay differences:**
    • Growing epidemic of vitamin D insufficiency
  – Saintonge 2009: Vitamin D deficiency is increasing from 1988-1994 to 2006
    • Implementation of national fortification and public health strategy
Mean 25(OH)D (nmol/L) Canada vs. US.

Canadian data: Diasorin Liaison 2007-2008
US data: Diasorin RIA, NHANES 2001-2006 (unadjusted for assay drift)
10th % 25(OH)D (nmol/L) Canada vs. U.S.

Canadian data: Diasorin Liaison 2007-2008
US Data: Diasorin RIA NHANES 2001-2006 (unadjusted for assay drift)
Evidence-Based Review of Vitamin D in Relation to Bone Health

Conducted by
University of Ottawa
Evidence-Based Practice Centre

Funded by
NIH Office of Dietary Supplements &
Agency for Health Care Research and Quality

Available at
http://www.ahrq.gov/clinic/tp/vitadtp.htm
&
the ODS website
Findings from the Evidence Based Review

- Strong evidence that vitamin D supplementation reduces falls, fractures and bone loss in men and women 60+ y

- Not possible to separate the impact of vitamin D from Ca supplementation—typical amounts used were 700-800 IU Vitamin D/d and 500-1,200 mg Ca/d

- Sparse data on other subgroups i.e., younger age, and physiological stage of reproduction.

- Difficult to identify a specific blood level of 25(OH) D indicative of optimal bone health in all population subgroups
Vitamin D and Calcium: Systematic Review of Health Outcomes
Conducted to help inform the Dietary Reference intake Process
Released in July 2009
Are there risks associated with Vitamin D supplementation?
WHI Calcium Vitamin D Trial

Calcium + Vitamin D (CaD)
Primary Outcome: Hip Fracture
Secondary Outcomes: Colorectal Cancer; Other Fractures

Diet Trial
25,210 of 48,836 (52%)

Hormone
16,089 of 27,347 (59%)

Total: 68,133

Placebo - or -
CaD Group
1000 mg calcium carbonate +
400 IU vitamin D
1/2 in am: 1/2 in pm
Choice: Chewable or Swallowable Pills

CaD
36,282

at 1st (or 2nd) Annual Visit
WHI Calcium + Vitamin D Trial Results: 7 years

- **Hip fractures:** 12% decrease (not significant)
  - 21% decrease for women aged 60-80 yrs at baseline
  - 29% decrease among women who took ≥ 80% of pills
  - Improved hip bone density

- **Other Fractures (self-reported vertebral, lower arm/wrist, total)**--no differences

- **Kidney stones** significantly increased **17%** (5 per 10,000/yr)
  - Mortality reduced by 9% (*not quite significant*)
  - Gastrointestinal symptoms were similar
  - Colorectal cancer - no difference
Fortification and/or supplementation programs must be monitored for effectiveness and safety

There appear to be vulnerable groups at both ends of the spectrum
Serum 25(OH)D and All Cause Mortality

Source: Melamed et al Arch Intern Med 2008;168(15)1629-1637
Serum 25(OH)D and All Cause Mortality

NIH Vitamin D Research Initiatives

Two Evidence Based Review

Standard Reference Materials NIST
25-OH D$_2$ and D$_3$ & VITAMIN MINERAL SUPPLEMENT

Vitamin D Status
Serum 25-OH D & PTH Measures in NHANES

Analytical Methods Development
D2 & D3 in Foods & Dietary Supplements

2007 Conferences
Vitamin D & Cancer: Current Dilemmas/Future Needs
Vitamin D and Health in the 21st Century: An Update
Roundtable of Experts in Vitamin D, Laboratory Methodology and Statistics Convened

- Convened: July 27-28, 2009
- Identify issues and discuss their pros and cons
- To think constructively about next steps
- Focus on NHANES needs
  - Methods used in NHANES – Diasorin RIA and LC-MS/MS
Agenda

  - Statistical adjustments?
    - How should they be done?
    - Options – pros and cons will be delineated
  - Documentation for users
Future Challenges

- 2007 and beyond – switch from Diasorin RIA to LC-MS/MS
- Availability of SRM 972: Standard Reference material for vitamin D in human serum
- Results of Round Table will be published in the *Journal of Nutrition* —must be deposited by end of January, 2010
Measurement of 25(OH)D is Problematic

- Interlaboratory studies show poor agreement, even when using the same method
- Equivalence of assay response to 25OHD$_2$ and 25OHD$_3$
- Presence of 3-epi-25OHD of unknown origin and in variable amounts—not detected by all assays
The Vitamin D International External Quality Assessment Scheme (DEQAS)

Fig. 1. Accuracy of 250HD methods used by DEQAS participants.

ALTM = All Laboratory Trimmed means

Presence of 3-epi-25(OH)D$_3$

SRM 1950 Metabolites in Human Plasma

- Plasma pool collected from 100 individuals, half male and half female
- Healthy adults, age 40 – 50
- Racial distribution similar to that of U.S. census

LC-MS/MS analysis of 25(OH)D$_3$

Detectable in all 20 samples received from DEQAS program
SRM 972 Vitamin D in Human Serum

- Four levels, each containing 1.0 mL serum
- Certified and reference values for 25(OH)D$_2$, 25(OH)D$_3$, and 3-epi-25(OH)D$_3$
- Value assignment by isotope-dilution LC-MS and LC-MS/MS using data from NIST and CDC

- Metabolite concentrations reported in ng/mL, and nmol/L
- COA does not provide data from other analytical techniques