

Revised Analytical Note for NHANES 2000-2006 and NHANES III (1988-1994) 25-Hydroxyvitamin D Analysis (Revised November 2010)

Data Advisory:

The purpose of this revised note is to inform users that serum 25-hydroxyvitamin D (25(OH)D) data from NHANES 2003-2004 and 2005-2006 have been adjusted for assay drifts, as described below. In addition, this note modifies the previous analytical note about two issues that should be addressed when analyzing 25(OH)D data from NHANES 2000-2006 and NHANES III (1988-1994). First, data users should be aware that the 25(OH)D data from the NHANES 2003-2006 were most likely affected by drifts in the assay performance (method bias and imprecision) over time. These assay drifts are likely due to reagent and calibration lot changes in the reformulated DiaSorin assay and may affect comparability, and therefore the interpretability of NHANES data from 2000-2006. The 2003-2004 and 2005-2006 25(OH)D data are being adjusted to account for these assay drifts. The new 2003-2004 and 2005-2006 adjusted 25(OH)D data files replaced the unadjusted 25(OH)D files in November 2010. NCHS recommends that the adjusted data rather than the previously available unadjusted data files be used for all analyses of 25(OH)D.

Second, users are cautioned about making direct comparisons between serum 25(OH)D measurements from NHANES 2000-2006 and measurements obtained in NHANES III (1988-1994). NHANES III 25(OH)D data must be adjusted in order to make a valid comparison to the NHANES 2000-2006 survey years due to a reformulation of the DiaSorin radioimmunoassay (RIA) kit that resulted in a shift in assay results between the two time periods. Therefore, users of these various NHANES data sets are cautioned that changes in 25(OH)D results over the time

period 1988-2006 are affected by the two methodological issues described above and both should be considered when evaluating whether, and how much, differences over time are due to true changes in the vitamin D status of the US population.

Background

Measurements of serum 25(OH)D were performed as part of the nutrition biomarker component of NHANES III (1988-1994) and in the years 2000-2006 of NHANES. These 25(OH)D data are available on public use data files on the NCHS/NHANES website for NHANES III and NHANES 2001-2002, 2003-2004 and 2005-2006. The 25(OH)D data collected in 2000 are available through the NCHS Research Data Center (not available in public data sets) because of a disclosure risk of confidential information for a single-year data release. Additional variables of interest relative to 25(OH)D, such as geography, are only accessible through the NCHS Research Data Center (due to increased disclosure risk) and are subject to the analytic limitations of data used in that setting. Readers should be aware that all issues discussed below in regard to the publicly available data for 2001-2006 also apply to the controlled-access data from 2000.

Measurements of serum 25(OH)D were performed in NHANES III (1988-1994) and NHANES 2000-2006, at the National Center for Environmental Health, CDC, Atlanta, GA using the DiaSorin RIA kit (Stillwater MN). The DiaSorin assay kit had been reformulated by the manufacturer in 1998 by introducing an antibody that provided improved binding and altering the washing solution to reduce non-specific binding.

Impact of assay reformulation on serum 25(OH)D measurements from NHANES III

Steps were taken to address the changes in assay between NHANES III and NHANES

2000-2006. To assess the magnitude of assay changes that might have an impact on any observed trends in serum 25(OH)D in the population, in 2004 the CDC laboratory reanalyzed a subset of 150 banked serum samples from NHANES III using the reformulated version of the RIA assay ¹. The serum samples were selected to represent the entire distribution of serum 25(OH)D values in NHANES III. The NHANES III results as measured with the reformulated assay were regressed on the NHANES III values obtained with the original assay for these 150 specimens. The average difference between the reformulated and original RIA was -12% and is described by the following equation:

$$\text{NHANES III 25(OH)D}_{\text{reformulated RIA assay}} = (0.8429 * \text{NHANES III 25(OH)D}_{\text{original RIA assay}}) + 2.5762 \text{ nmol/L (r = 0.8966)}.$$

This adjustment equation was generated after first accounting for the assay drift occurring during 2003-2004 with the reformulated DiaSorin assay ¹. The above equation allows an approximation of NHANES III results to the level of the reformulated assay used in NHANES 2000-2006.

Impact of assay drift on serum 25(OH)D measurements from the NHANES 2000-2006

In addition to the assay reformulation, the CDC laboratory observed drifts in the serum 25(OH)D assay performance (as reflected in QC pool shifts in the mean, up or down, by up to 10%) over the period of 2003-2006. The variation in 25(OH)D values appeared to be due to method variation that probably resulted from reagent and calibration lot-to-lot variation. Two approaches for adjusting the 2003-2006 25(OH)D were tried¹: 1) a statistical adjustment model based on quality control pool data that encompassed the period of method drift, and 2) a

statistical model based on participant 25(OH)D values, transformed by the natural logarithm, and an analysis of covariance model to control for time effects of assay fluctuations. Both methods gave approximately the same statistical adjustment of the 25(OH)D data during 2003-2006. It was decided to use the model based on quality control pool data because the results should be independent of any empirical trend in the sample participant data.

The following weighted descriptive data and selected percentiles (calculated using SAS version 9 proc surveymeans) show the effect of the adjustment for 2003-2004 and 2005-2006:

Years	Adjusted	N	Mean	5 th	25 th	50 th	75 th	95 th
2003-2004	No	8294	25.3	9.9	18.3	24.2	30.6	41.4
2003-2004	Yes	8298*	24.3	9.9	17.9	23.3	29.0	38.9
2005-2006	No	8306	22.2	8.3	15.9	21.3	27.0	36.0
2005-2006	Yes	8306	23.9	8.8	17.1	23.1	28.8	38.5

*4 more participants were added for 2003-2004 that were inadvertently missed in the unadjusted data.

As seen above, the 2003-2004 25(OH)D data were adjusted to lower values and would result in higher prevalence estimates of 25(OH)D deficiency. In contrast, the 2005-2006 data were adjusted to higher values and would result in lower prevalence estimates of 25(OH)D deficiency.

Future Plans

The National Institute of Standards and Technology (NIST) has released standard

reference materials for 25(OH)D assays with certified values assigned by use of isotope dilution tandem mass spectrometry (LC-MS/MS) candidate reference measurement procedures in July 2009.² CDC intends to generate regression equations that will permit the adjustment of the 25(OH)D data from various NHANES survey years to the NIST accuracy-based standard by reanalyzing subsets of specimens from NHANES 1988-1994 and 2001-2006 using LC-MS/MS. This will improve the ability to interpret the 25(OH)D data for all types of analyses, including comparisons between NHANES 2001-2006 and NHANES III. When these equations become available, this analytical note will be updated with a revised analytical note.

References

1. Yetley EA, Pfeiffer CM, Schleicher RL, et al. NHANES monitoring of serum 25-hydroxyvitamin D: A roundtable summary. *J. Nutr.* 2010;140:2030S-2045S.
2. Phinney KW. Development of a standard reference material for vitamin D in serum. *Am J Clin Nutr* 2008 August 1;88(2):511S-512S.