

Analytical Note for NHANES 2000-2006 and NHANES III (1988-1994)

25-Hydroxyvitamin D Analysis

Data Advisory:

The purpose of this note is to inform users of serum 25-hydroxyvitamin D (25(OH)D) data from NHANES about two issues that should be addressed when analyzing these data. First, users are cautioned about making direct comparisons between serum 25-hydroxyvitamin D measurements from NHANES 2000-2006 (i.e., publicly released data for 2001-2006 and controlled-access data for 2000) 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 2000-2006 NHANES survey years due to a reformulation of the DiaSorin radioimmunoassay (RIA) kit that resulted in shifts in assay results between the two time periods. Second, data users should also be aware that the 25(OH)D data from the 2000-2006 NHANES 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. These QC drifts may affect comparability, and therefore interpretability, of the data from any combination of NHANES data from 2000-2006, even when comparisons are not being made with NHANES III. 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-hydroxyvitamin 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. 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 between 1994 and 2000 by introducing an antibody that provided improved binding. On average, 25(OH)D values from the reformulated RIA assay used in NHANES 2000-2004 were 12% lower than the original RIA assay values from NHANES III. In addition, 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%) due to changes in reagent and calibrator lots over the period of 2000-2006 have been observed in the CDC laboratory.

Preliminary steps have been taken to address the changes in assay method between NHANES III and NHANES 2000-2004. A method comparison study between NHANES III and NHANES 2000-2004 was conducted, which is described in detail in Appendix 1 that accompanied a paper published in the American Journal of Clinical Nutrition.¹ This appendix is available on the AJCN website but not in the printed version of the paper, so it is briefly

summarized here. To assess the magnitude of assay changes that might have an impact on any observed trends in serum 25(OH)D in the population, the CDC laboratory reanalyzed a subset of 150 banked serum samples from NHANES III using the reformulated version of the RIA assay. 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:

$$\text{NHANES III 25(OH)D}_{2000-2004 \text{ RIA assay}} = (0.8429 * \text{NHANES III 25(OH)D}_{1988-1994 \text{ RIA assay}}) + 2.5762 \text{ nmol/L (r = 0.8966)}.$$

This adjustment equation was generated after first accounting for the assay drifts during 2000-2004 with the reformulated DiaSorin assay.

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

The weighted mean of 25(OH)D for NHANES 2001-2006 was 59.0 nmol/L, with a range of single-year means of 52.5-66.8 nmol/L. As indicated above, the variation between single years appeared to be due to method variation (arising from method bias and imprecision) over time that results from reagent and calibration lot-to-lot variation.

An approach to address the observed methods variations of 25(OH)D over time in NHANES is currently being developed. However, because this approach will likely take many months to complete, an interim approach was used to assess the potential impact of including data from time periods with greater assay variability on means and prevalences. This approach

repeats analyses published in AJCN (Looker et al), which included the data with greater variability, by excluding the data of concern.

Results from these analyses indicated that the impact of including the data from the time period of concern was minimal on results calculated for NHANES 2000-2004. In specific, excluding the time period of concern produced means for NHANES 2000-2004 that were lower on average by -1.3 to -1.9 nmol/L (range -3.1 to +1.1 nmol/L) than means presented in the AJCN article. Percentile data from NHANES 2000-2004 were also minimally affected when excluding the time period of concern: 5th percentile values were higher on average by 0.6 nmol/L (range -1.8 to +4 nmol/L), 50th percentile values did not differ (range -2.5 to +2.4 nmol/L) and 95th percentile values were lower by -1.5 nmol/L (range -6.2 to +5.7 nmol/L). Finally, when the time period of concern was excluded, prevalence estimates for values below cut-points from NHANES 2000-2004 were slightly higher on average than the prevalences presented in the AJCN article. For example, estimates of the prevalence < 25 nmol/L were higher, on average, by 0.25 percentage points (range -0.1 to +0.6). Estimates of the prevalence < 37.5 nmol/L to < 75 nmol/L were higher, on average, by 1.5 to 2.2 percentage points (range -3.3 to +6.4).

In summary, excluding the data from the time period of concern resulted in means and percentiles for NHANES 2000-2004 that were slightly lower and prevalence estimates that were slightly higher than those originally obtained when the entire 2000-2004 dataset was used.

Analysis Recommendations:

Based on the above information and analyses, NCHS strongly urges data users to follow the following recommendation before comparing serum 25(OH)D data from NHANES III with serum 25(OH)D data from NHANES 2000-2006:

Serum 25(OH)D data from NHANES III should be adjusted using the following equation if comparisons with 25(OH)D data from the full combined 2000-2006 NHANES are being made:

$$\text{NHANES III 25(OH)D}_{2000-2004 \text{ RIA assay}} = (0.8429 * \text{NHANES III 25(OH)D}_{1988-1994 \text{ RIA assay}}) + 2.5762 \text{ nmol/L}$$

Readers should be aware that this equation allows an approximation of NHANES III results to the level of the reformulated assay used in NHANES 2000-2004. However, it is imperfect in that it cannot simultaneously adjust for drifts in assay performance that may have occurred after 2004. As a result, it should be employed with caution when making comparisons between NHANES III and the publicly available NHANES 2001-2006 25(OH)D data.

Based on the above information and analyses, NCHS strongly urges data users to follow the recommendations below when comparing serum 25(OH)D data for the 2000-2006 NHANES survey period:

1. NCHS strongly discourages analysis of, or comparisons between, any of the two-year NHANES 2001-2006 25(OH)D data due to method variability and sample design limitations. Users are discouraged from using a four-year dataset based on 2001-2004 due to a higher possibility that results may be affected significantly by assay variability.
2. NCHS recommends the use of a data set which combines all years of data (2001-2006 or 2000-2006) to provide more stable estimates of means, percentiles, and prevalence estimates for 25(OH)D. Users should note the possibility of assay variability in their results from the combined dataset as a study limitation.

3. Given the observed QC data variability, users should exercise caution when analyzing and interpreting inter-relationships between 25(OH)D and other NHANES variables using the 2000-2006 NHANES dataset. At a minimum, users should note the possibility of variability due to laboratory methods issues in their results as a study limitation. This variability may be more of an issue for population subgroup analyses.
4. Some variables of interest relative to 25(OH)D were only collected in NHANES 2003-2006 (i.e. parathyroid hormone and sun exposure variables). Interpretation of any analyses conducted on this four-year data set may not be affected in any significant way by the observed variation in 25(OH)D data during this time period, but users should be aware of, and, at a minimum, list this issue as a potential limitation of analyses and findings conducted using this four-year data set.
5. Additional variables of interest relative to 25(OH)D, geography and seasonality, 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.

Future Plans

At present, there is no gold standard method for measuring serum 25(OH)D, so that no 25(OH)D data are accuracy-based regardless of the assay used. The National Institute of Standards and Technology (NIST) will soon provide 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.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 2000-2006 using a candidate LC-MS/MS method. This will improve the ability to analyze the 25(OH)D data from NHANES 2000 and beyond for all types of analyses, including comparisons between NHANES 2000-2006 and NHANES III. When these equations become available, this analytical note will be updated with a revised analytical note.

References

1. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr* 2008 December 1;88(6):1519-27.
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.