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Serum 25-hydroxyvitamin D status of the US population: 1988–1994 versus 2000–2004[1]

Anne C. Looker[1], **Christine M. Pfeiffer**[2], **David A. Lacher**[1], **Rosemary L. Schleicher**[2], **Mary Frances Picciano**[3], and **Elizabeth A. Yetley**[3]

[1] From the National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville MD

[2] National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA

[3] Office of Dietary Supplements, National Institutes of Health, Bethesda MD

Abstract

Background—Changes in serum 25-hydroxyvitamin D (25OHD) concentrations in the US population have not been described.

Objective—Use data from the National Health and Nutrition Examination Surveys (NHANES) to compare serum 25OHD concentrations in the US population in 2000–2004 versus 1988–1994, and to identify contributing factors.

Design—Serum 25OHD was measured with a radioimmunoassay kit in 20,289 participants in NHANES 2000–2004 and 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. Assay differences were assessed by re-analyzing 150 stored sera specimens from NHANES III with the current assay.

Results—Age-adjusted mean serum 25OHD concentrations were significantly lower by 5–20 nmol/L in NHANES 2000–2004 than in NHANES III. After accounting 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, accounting for the confounding effects of assay differences changed mean serum 25OHD by ~10 nmol/L, while accounting for changes in the factors likely related to real changes in vitamin D status (BMI, milk intake, and sun protection) changed means by 1–1.6 nmol/L.

[1]The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, the National Institutes of Health, or the Department of Health and Human Services.

Corresponding author: Anne C. Looker, Ph.D. Room 4310, National Center for Health Statistics, 3311 Belcrest Rd, Hyattsville, MD 20782, 301/458-4352 (phone), 301/458-4029 (fax), acl1@cdc.gov.

The authors' responsibilities were as follows: a) the study concept and design and the collection of data: all authors; b) the statistical data analysis: ACL, CMP, and RLS; c) the interpretation of data: all authors; d) the writing of the manuscript draft: ACL, CMP; e) critical revision of the manuscript: all authors; and f) contributions to the final manuscript: all authors. None of the authors had any personal or financial conflict of interest.

Conclusions—Overall, mean serum 25OHD was lower in 2000–2004 than 1988–1994. Assay changes unrelated to changes in vitamin D status accounted for much of the difference in most population groups. In an adult subgroup, combined changes in BMI, milk intake and sun protection appeared to contribute to a real decline in vitamin D status.

Keywords

Serum 25-hydroxyvitamin D; Vitamin D status; NHANES

INTRODUCTION

Interest in vitamin D status is high given its potential links with an increasing number of diseases and conditions (1). The vitamin D status of the population has not been assessed in a representative sample of the current US population; the most recent published national estimates are based on data that are over a decade old (2). Since that time, there have been trends in other factors in the population that could potentially affect vitamin D status. For example, the prevalence of overweight increased in the US population in the past decade (3, 4). Body fat is inversely related to serum 25-hydroxyvitamin D (25OHD) (5), but it is not known whether the increased prevalence of overweight has been accompanied by a decline in vitamin D status. A clear understanding of changes in the vitamin D status of the US population and factors that may have contributed to these changes is relevant in light of considerable efforts currently underway to better define the role of this important vitamin in health (6–9).

Serum 25OHD concentrations were measured in the National Health and Nutrition Examination Survey (NHANES) for the first time in the third NHANES (1988–1994), and have been part of the current continuous NHANES since 2000. These data provide the opportunity to compare vitamin D status in representative samples of the non-institutionalized US population that were assessed at two different time points. The objectives of this study are to: a) describe current 25(OH)D concentrations for a wide range of population subgroups including children ages 1–11 years and pregnant women for whom national estimates have not been previously available, and b) compare differences in serum 25OHD between NHANES III and NHANES 2000–2004 before and after adjusting for assay method changes.. We also performed exploratory analyses to assess the relative contributions of confounding from assay method vs. the combined effects of biological and behavioral factors (e.g., BMI, sun protection, and milk consumption) that may have contributed to observed differences over time. This evaluation was limited to a single population subgroup for whom data on these factors were available. Identifying the contribution of confounding factors is essential to avoid erroneous conclusions about observed changes in the population's vitamin D status, since changes in confounding factors are unrelated to changes in vitamin D status over time. Identifying biological and behavioral factors that contribute to a real change in population status can provide essential information for subsequent discussions of how the population status change can best be addressed.

METHODS

Subjects

Vitamin D status was assessed using data from the NHANES, which is conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), to assess the health and nutritional status of a large representative sample of the non-institutionalized, civilian US population. In NHANES III, a nationally representative sample was obtained in two 3-year cycles between 1988–1994, while in NHANES 2000–2004, a nationally representative sample was collected in each year. Although a representative sample is collected annually, data are released in 2-year periods starting with 1999 to protect

confidentiality and increase statistical reliability. Serum 25OHD data from NHANES 2000 are available through the NCHS Research Data Center only. All procedures in each NHANES were approved by the NCHS Institutional Review Board, and written informed consent was obtained from all subjects (10,11).

In each NHANES, data were collected via household interviews and direct standardized physical examinations conducted in specially equipped mobile examination centers (10,11). Because the mobile examination centers can be adversely affected by weather, data are collected in northern latitudes in summer and in southern latitudes in winter. This created a season-latitude structure in both surveys in which approximately ≥75% of the data collected between November and March came from latitudes < 35 degrees North, and ≥86% of the data collected between April-October came from latitudes ≥ 35 degrees North.

NHANES III and NHANES 2000–2004 were designed to provide reliable estimates for three race/ethnic groups: non-Hispanic whites, non-Hispanic blacks, and Mexican Americans. Race and ethnicity were self-reported by the participants. Because race/ethnic groups are not evenly distributed geographically across the US, the season-latitude aspect of the survey affects race/ethnic comparisons (2). In both surveys, non-Hispanic whites were significantly more likely to have been examined in April-October and to be living at higher latitudes than either non-Hispanic blacks or Mexican Americans ($p<0.05$). Furthermore, the race/ethnic composition of the US population has changed since the 1990's due to an increase in the Hispanic population (12). This may explain why race/ethnicity differed significantly between surveys for the sample examined between November-March (Supplemental Table S1). There were significantly more Mexican Americans and persons of other races (and concomitantly fewer non-Hispanic whites and non-Hispanic blacks) examined in these months in NHANES 2000–2004 than in NHANES III.

The present study used serum 25OHD measurements from 20,289 individuals from NHANES 2000–2004 (11,995 individuals ages 6 years and older from NHANES 2000–2002, and 8294 individuals ages 1 year and older from NHANES 2003–2004). The analytic sample size for NHANES 2000–2004 represents 69% of the individuals that were originally selected for the survey, and 90% of those examined in the survey. Serum 25OHD measurements from 18,158 individuals ages 12 years and older in NHANES III were used in the present study, which represents 67% of those originally selected for the survey, and 92% of those examined in the survey.

The description of the observed difference in serum 25OHD in the population is based on data collected in NHANES III (1988–1994) and NHANES 2000–2004. However, data from the current NHANES that were used to assess potential explanatory factors for the observed serum 25OHD difference had to be limited to NHANES 2003–2004 because sun protection data were not collected in NHANES prior to 2003. Information on sun protection also was not collected in NHANES III, so data from the 1992 National Health Interview Survey (NHIS) were used to assess sun protection in the population at the time of NHANES III. The NHIS obtains a nationally representative sample annually and is conducted via household interview (13). The items on sun protection were part of the Cancer Control Supplement administered to 12,035 adults ages 18 years and older in 1992. The response rate to this supplement was 87% (13). All procedures in the 1992 NHIS were approved by the NCHS Institutional Review Board, and written informed consent was obtained from all subjects. For the present study, the age range of the 1992 NHIS sample was limited to age 20–59 years, in order to be comparable to the age range in NHANES 2003–2004 with sun protection data. Sun protective data were available for 8697 adults, which represent 99% of the Cancer Control Supplement participants in this age range.

Variables

Serum 25OHD measurements were performed in both NHANES surveys at the National Center for Environmental Health, CDC, using a radioimmunoassay (RIA) kit (DiaSorin, Stillwater MN) (11,14). Based on quality control pools that passed specification limits, the inter-assay coefficient of variation (CV) was 15–25% for lower values (20–62 nmol/L) and 14–18% for higher values (86–143 nmol/L) during NHANES III, and 8.3–11% for lower values (24–58 nmol/L) and 10% for higher values (102–112 nmol/L) during NHANES 2000–2004. Long-term participation of this laboratory in the U.K. DEQAS international vitamin D proficiency testing program (www.deqas.org) has shown that the 25OHD measurements met performance targets. However, small shifts in the serum 25OHD assay performance due to changes in reagent and calibrator lots over a period of years have been observed in the CDC laboratory. The kit manufacturer also reformulated the kit in the late 1990's by introducing an antibody that improved binding. To assess whether these changes in the assay contributed to observed differences in population serum 25OHD data from NHANES, the CDC laboratory reanalyzed a subset of 150 banked sera samples (stored at −70°C) from NHANES III with the current version of the RIA assay over a three-month period in 2004. This study is described in detail in Supplemental Appendix 1.

Pregnancy status in NHANES 2000–2004 was based on a positive urinary pregnancy test or self-reported pregnancy.

Data for the following vitamin-D-related variables from NHANES III and NHANES 2003–2004 were used when analyzing trends in vitamin D status: body mass index (BMI), season and latitude of blood collection, dietary calcium intake, frequency and type of milk consumption, vitamin-mineral supplement (VMS) use in the past month, and physical activity level. BMI was calculated as body weight (kilograms) divided by height squared (meters^2). Body weight was measured using an electronic load cell scale, and standing height was measured with a fixed stadiometer (10,11). Latitude and season were based on the geographical location and month of blood specimen collection. Dietary calcium intake from food was based on a single 24 hour recall. Milk intake was based on self-reported frequency of consumption in the past month. The questions on milk intake differed slightly between the two surveys. In NHANES 2003–2004 respondents were instructed not to include milk used in cooking in their responses, whereas NHANES III respondents did not receive that instruction. In addition, milk consumption was coded as times per month consumed in NHANES III. To be comparable with milk consumption in NHANES 2003–2004, responses from NHANES III were recoded as "never/rarely" (0–3 times/month) or "sometimes/often" (≥4 times/month). Type of milk was based on the fat content (whole, 2%, 1%, or skim/nonfat milk) of the milk self-reported as usually consumed. VMS use was based on self-reported use of any type of supplement in the past month. Physical activity was based on self-assessment of usual activity compared to others of the same age and sex. Responses were coded as "more", "same" and "less".

Data on sun protection from the 1992 NHIS and from NHANES 2003–2004 were also used in the analysis of vitamin D status trends. In both surveys, respondents were asked whether they practiced the following behaviors if they were outside for more than 1 hour on a sunny day: stay in the shade, wear protective clothing (long sleeves, hat with brim), or use sunscreen. Response categories differed between the surveys, so responses to NHANES 2003–2004 items were recoded to be comparable to the 1992 NHIS by combining "always" and "most of the time" to represent "very likely", "sometimes" to represent "somewhat likely", and "rarely" and "never" to represent "unlikely". Individuals who reported not going out into the sun in NHANES 2003–2004 were excluded (n=40), since there was no comparable response category in the 1992 NHIS. A single sun protection variable was created by assigning the highest frequency reported for any of the three behaviors. For example, a respondent who reported

being “very likely” to stay in the shade was coded as “very likely” to practice sun protection overall even if they did not report wearing protective clothing or using sunscreen.

Data analyses

Sample weights were used when calculating point estimates in all analyses. Analyses were performed using SUDAAN 9.01 (15). Data collected in the contiguous US (25–47° N) from both surveys were included in the analyses. The distribution of race/ethnicity by season of blood collection was compared between NHANES III and NHANES 2000–2004 using chi-square analyses. Means, selected percentiles and prevalence with serum 25OHD below selected thresholds for NHANES 2000–2004 were calculated by age, sex, and race/ethnicity. Adjusted means by selected characteristics (age, sex, race/ethnicity, season of blood collection and, for women of child-bearing age, pregnancy status) were also calculated for NHANES 2000–2004 using multiple linear regression. The mean for each characteristic was adjusted for all the other characteristics in the model. When multiple comparisons of means between groups were made, a Bonferoni correction was used.

Changes in serum 25OHD in the population age 12 years and older between NHANES III and NHANES 2000–2004 were examined by calculating age-standardized means by sex and season of blood collection for each survey period for the total population and by race/ethnicity where possible. Data were limited to age 12 years and older because serum 25OHD was not measured for younger individuals in NHANES III. Means were calculated separately by season and sex to avoid confounding due to differences in the race/ethnic composition of the sample by season between surveys, and because an interaction was also found between sex and survey. Means were age-standardized to 2000 US Census population estimates. Regression models that included NHANES survey and age were used to test the significance of observed differences. These models were run separately by sex, season, and race/ethnicity. When multiple comparisons were made, a Bonferoni correction was used. Because the proportion of nonwhites in the sample examined in November-March differed significantly between surveys, the sample used in the comparison for this season was limited to non-Hispanic whites.

Age-adjusted serum 25OHD means were compared between NHANES III and NHANES 2000–2004 before and after accounting for assay differences detected in the comparison study described in Supplemental Appendix 1. The NHANES III values were predicted using the following equation: NHANES III $25OHD_{\text{corrected 2004 RIA assay}}$ = (0.8429* NHANES III $25OHD_{\text{1988–1994 RIA assay}}$) + 2.5762 nmol/L.

The analyses to identify and assess the relative contribution of potential explanatory factors for the observed difference in serum 25OHD between surveys were limited to a single subpopulation group because data for some relevant factors were not available for all groups from both surveys. In specific, the analyses were limited to a subsample of non-Hispanic whites who were examined in April-October to avoid confounding due to differences in the race/ethnic composition of the sample by season between surveys. We chose April-October because the observed difference in serum 25OHD between surveys was greater for the sample examined for this season than for November-March. We limited the comparison to non-Hispanic whites because the sample size for April-October was greatest for this race/ethnic group. Data from the current NHANES were limited to 2003–2004 because sun protection data were only available for these survey years. The analyses were focused on age 20–59 because the sun protection data in NHANES 2003–2004 were only collected for this age group. Data were analyzed separately by sex due to the interaction of sex and survey noted previously. Mean age of this subsample differed between surveys (mean = 38.1 vs 40.3 years in NHANES III vs NHANES 2003–2004, respectively; $p < 0.05$), so means were age-standardized to the 2000 Census.

Using this subsample, a two step modeling approach was sequentially employed: 1) regression to predict serum 25OHD means after accounting for the confounding effect of assay differences (NHANES III only), and 2) regression to predict serum 25OHD means after accounting for changes in biological and behavioral factors (NHANES 2003–2004 only).

The first step in the analyses of potential explanatory factors was to assess the contribution of assay differences. This was accomplished by calculating predicted mean age-standardized serum 25OHD for 10,472 non-Hispanic white adults aged 20–59 from NHANES III assuming the current assay had been used. The predicted mean was calculated by applying the previously-described regression equation from the assay comparison study to the serum 25OHD data for this NHANES III subsample.

The second step was to assess the potential contribution of changes in vitamin-D-related biological and behavioral factors in this population subgroup between surveys. We first identified factors that changed between 1988–1994 and 2003–2004 in a manner consistent with the observed serum 25OHD changes. Specifically, we used linear or logistic regression to compare means or percentages of selected vitamin-D related factors between NHANES III or the 1992 NHIS and NHANES 2003–2004 for non-Hispanic whites ages 20–59 years.

Next, regression equations to assess the impact of the variables identified in the preceding step on serum 25OHD data from NHANES 2003–2004 were created for this subgroup. Regression equations to predict serum 25OHD using age, latitude, physical activity, VMS use, BMI, milk intake and sun protection were developed separately by season and sex due to the interactions noted previously. These regression equations were then used to predict mean age-standardized serum 25OHD for NHANES 2003–2004 assuming the factors that had been identified earlier as having changed had not changed since the mid-1990's. This was done by substituting means or percentages for these variables from the mid-1990's (either from NHANES III or the 1992 NHIS) into the equation and then calculating the adjusted mean serum 25OHD.

RESULTS

Adjusted mean serum 25OHD values in 2000–2004 by selected characteristics are shown for a wide range of US population groups in Table 1. More detailed information about the unadjusted serum 25OHD distribution by age, sex, and race/ethnicity for NHANES 2000–2004 is shown in supplemental Table S2 (means, medians and selected percentiles) and supplemental Tables S3 and S4 (prevalence with serum 25OHD below selected cutoff values). After adjusting for sex, race/ethnicity, and season, mean serum 25OHD differed significantly by age, being highest in children age 1–5 years and then significantly lower in each succeeding age category. The adjusted mean serum 25OHD was significantly higher in males than in females, and in those whose blood was drawn in April-October compared to those whose blood was drawn in November-March. Non-Hispanic whites had the highest adjusted mean serum 25OHD concentration, followed by Mexican Americans, and then non-Hispanic blacks. Pregnant females had a significantly higher adjusted mean serum 25OHD than those who were not pregnant.

Differences in age-standardized mean serum 25OHD from NHANES III versus NHANES 2000–2004 are shown in Figure 1 for individuals ages 12 years and older. Data were combined for the two three-year phases comprising NHANES III (1988–1991 and 1991–1994) and for the three survey periods comprising NHANES 2000–2004 (2000, 2001–2002, and 2003–2004) because means did not differ significantly within these time periods. Age-standardized means based on observed serum 25OHD concentrations were significantly higher (by 12–20 nmol/L in males and 5–13 nmol/L in females, depending on season, $p<0.0003$) in NHANES III than in NHANES 2000–2004 in all groups examined. Accounting for assay differences by

predicting means for NHANES III assuming the current assay had been used reduced, but did not completely remove, the differences between surveys. The age-standardized predicted means for NHANES III accounting for assay differences remained significantly higher in all male groups except one (Mexican Americans examined in April-October). In contrast, the age standardized predicted means from NHANES III were only significantly higher in one group of females (non-Hispanic blacks examined in April-October) **(**Figure 1). Differences between the predicted means after accounting for assay differences from NHANES III versus observed means from NHANES 2000–2004 ranged from 5–9 nmol/L in males and 0.7–6.1 nmol/L in females. These differences likely represent a real difference in serum 25OHD status between surveys.

Results of the subgroup analyses to identify vitamin-D-related biological and behavioral factors that had changed significantly between NHANES III and NHANES 2003–2004 among 20–59 year old non-Hispanic whites are shown in Tables 2 and 3. Of the variables examined, BMI, milk consumption, and sun protection differed significantly between surveys in a direction that was consistent with a decrease in serum 25OHD. BMI and sun protection increased significantly and milk consumption decreased significantly between NHANES III and NHANES 2003–2004. As shown in Figure 2, higher BMI, lower milk intake, and more frequent sun protection were associated with significantly lower serum 25OHD values. VMS use and dietary calcium also changed significantly between NHANES III and NHANES 2003–2004, but the direction of the change was not consistent with a decrease in serum 25OHD, so these variables were not used in subsequent modeling.

Results of the multiple step analyses to explore the relative contribution of confounding factors due to changes in the serum 25OHD assay versus BMI, milk intake, and sun protection to the observed difference in serum 25OHD concentrations between surveys are shown in Figure 3A–C for NHANES III and NHANES 2003–2004 by sex among the subgroup of non-Hispanic whites ages 20–59 years who were examined between April and October. The observed age-standardized means were approximately 10–18 nmol/L higher in NHANES III than in NHANES 2003–2004 in this population subgroup (Figure 3A). The difference between surveys was reduced by 10.3–11.2 nmol/L, depending on sex, by accounting for assay differences using predicted age-standardized means for NHANES III values: the predicted age-standardized means for NHANES III assuming the current assay had been used were only 7.1nmol/L higher than the observed age-standardized mean for males from NHANES 2003–2004, and did not differ between surveys in females. **(**Figure 3B). This remaining difference in males likely represents a real difference in serum 25OHD status between NHANES III and NHANES 2003–2004 for this subsample.

Results of modeling to assess biological and behavioral factors that may have contributed to a true decline in serum 25OHD in status between surveys in the non-Hispanic white adult subgroup are shown in Figure 3C. Adjusting the NHANES 2003–2004 serum 25OHD values for changes in mean BMI, milk consumption rates, or sun protection of this subgroup reduced the real serum 25OHD difference between surveys in men by 5.9 nmol/L, so that the predicted age-standardized mean accounting for assay differences for NHANES III was only 1.1 nmol/L higher than the predicted mean accounting for biological and behavior factors for NHANES 2003–2004. In women, adjusting for these population changes resulted in a predicted age-standardized mean for NHANES 2003–2004 that was 1.6 nmol/L higher than the predicted mean for NHANES III.

DISCUSSION

A comparison of the observed serum 25OHD concentrations between NHANES III and NHANES 2000–2004 suggests that a decline in measured vitamin D status may have occurred

in the population over the past 10–15 years, with age-standardized mean serum 25OHD values observed in NHANES 2000–2004 being roughly 5–20 nmol/L lower than those seen in NHANES III (1988–94) for persons ages 12 years and older. Accounting for confounding from assay differences reduced the difference in serum 25OHD between NHANES III and NHANES 2000–2004 by approximately 10 nmol/L. Thus, most of observed difference in serum 25OHD between NHANES III and NHANES 2000–2004 appears to be an artifact of assay changes rather than an actual decline in serum 25OHD concentrations. However, the remaining difference appears to represent a true decline in vitamin D status of the population since NHANES III.

Our analyses to identify potential biological and behavioral factors that contributed to the actual decline in serum 25OHD values between surveys suggest that changes in BMI, milk intake and sun protection may have played a role, at least in the subgroup of non-Hispanic white adults for whom the analyses were conducted. We focused on these factors because they are related to serum 25OHD and they appeared to have changed in a direction that is consistent with a decline in serum 25OH in this population group. These findings may be relevant for subsequent discussions regarding approaches to address the decline in serum 25OHD. In addition, finding that changes in relevant biological and behavioral factors in the population appear to explain some portion of the serum 25OHD difference that remained between surveys after accounting for confounding factors supports the likelihood that the remaining serum 25OHD difference is real rather than being due to other, uncontrolled confounding factors. More research is needed to examine the relative effects of confounding vs. behavioral and biological factors in other population subgroups. Such analyses were precluded in the present study due to lack of data for some of the relevant factors in other population groups and the need to account for differences between surveys in the race/ethnic composition by season.

The impact of using different assays to measure serum 25OHD has been described previously. Binkley et al (16) found mean serum 25OHD values varied as much as two-fold when different assay types were used to measure the same set of blood samples. Variations in results from the same method when used in different laboratories have also been described (17). Our results suggest that changes of about 12% in the same assay over time can also impact serum 25OHD concentrations even when performed in the same laboratory. Standard reference materials (SRMs) for serum 25OHD are currently being developed by the National Institute of Standards and Technology (18), which should improve agreement between assay methods. It is important to note that the assay adjustment used in the present study was based on the current version of the RIA assay because the NHANES III-era assay version could not be reconstructed. However, without a standard reference material for serum 25OHD, it is not clear which assay version is the best in terms of assessing nutritional status.

In addition to examining trends in serum 25OHD, we looked at serum 25OHD concentrations in the current NHANES by selected demographic characteristics. These data fill an important gap for some groups in whom data have previously been scanty, in particular children, adolescents and pregnant women (19,20). In the present study, mean serum 25OHD was highest in children ages 1–5 years, intermediate in children ages 6–11 years, and lowest in adolescents ages 12–19. Weng et al. (21) found a similar pattern by age in their sample of apparently healthy children and adolescents. Pregnant women had higher serum 25OHD than nonpregnant women of the same age in NHANES 2000–2004. Recent community-based studies of vitamin D status in pregnant women in the US have reported that low vitamin D status is common in this group, but they have not included comparisons with non-pregnant women (20,22).

It is important to note that statistical adjustments to serum 25OHD data for assay differences or biological and behavior factors that changed between NHANES III and NHANES 2000–2004 were made in the present study only. The publicly-released serum 25OHD data for

NHANES III and NHANES 2000–2004 available on the NHANES website (www.cdc.gov/nchs/nhanes.htm) are the observed, unadjusted values. We recommend that researchers who use the publicly-released data to make comparisons between surveys should consider the confounding effects of assay differences and changes in population demographics in their analyses.

This study has limitations. The analyses to assess the relative contribution of confounding factors versus changes in vitamin-D-related biological and behavioral risk factors in the population were limited to adults age 20–59 years due to lack of sun protection data in other age groups. These analyses were further limited to non-Hispanic whites to avoid confounding due to differences in the race/ethnic composition of the sample by season between surveys. A correction factor was needed to account for a shift in the 25OHD assay quality control pools that occurred while the assay comparison study was being conducted. The impact of the biological and behavior factors was also indirectly estimated using regression to predict mean values, so that results depend on the robustness of the underlying models. The models to assess changes in vitamin-D-related factors in the population were limited to factors for which nationally representative data were available, so some potentially important factors could not be considered. For example, dietary intake of vitamin D was only represented indirectly by the milk intake variable in the model because direct estimates of dietary vitamin D intake from food are not available from NHANES 2000–2004. The fact that some of the observed difference in serum 25OHD between NHANES III and NHANES 2003–2004 in men was not explained by our models suggests that additional variables may have played a role.

Other study limitations include the potential nonresponse bias in both NHANES datasets, since not all those who were selected to participate in the survey did so. Nonresponse bias in NHANES is reduced by a nonresponse adjustment factor included in the calculation of the sample weights. However, about 5–10% of those who came to the mobile exam centers did not have serum 25OHD data in the two surveys, and this nonresponse is not addressed by the sample weight adjustments. Finally, some important at-risk groups, such as institutionalized persons and people living in the northern US during the winter, were not included in the NHANES sampling frame by design.

In summary, age standardized mean serum 25OHD concentrations were significantly lower in 2000–2004 than in 1988–1994 in all groups examined when based on observed values. Accounting for assay changes noticeably reduced the difference between surveys. However, mean serum 25OHD remained significantly lower in males (except Mexican Americans) in NHANES 2000–2004 than in NHANES III even after adjusting for assay differences. This remaining difference likely represents a real decline in vitamin D status. Changes in BMI, milk intake and sun protection appeared to contribute to this decline in a subgroup of non-Hispanic white adults. The possibility that trends in overweight, sun protection, and milk intake may continue supports the need to continue monitoring the serum 25OHD status of the population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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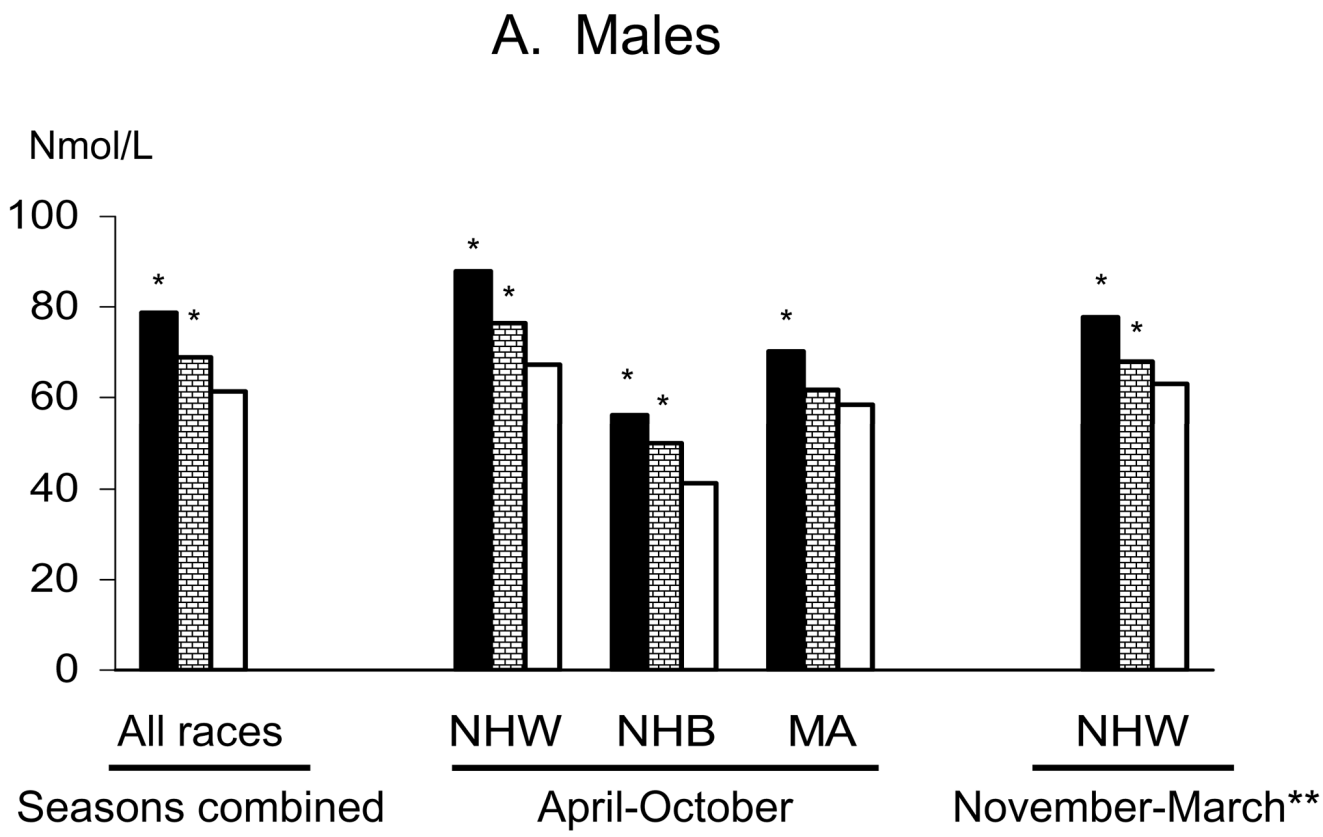


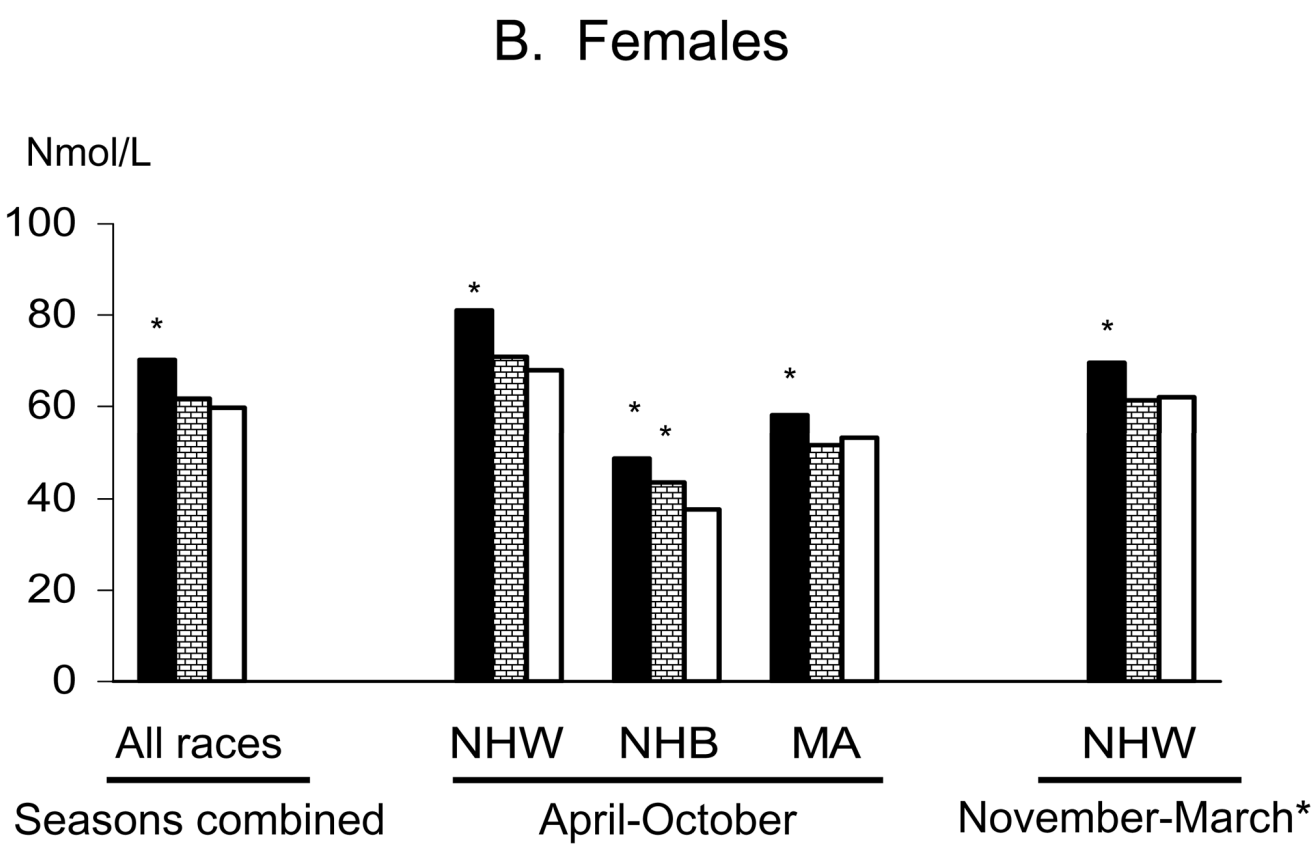


Figure 1.

Age-standardized mean serum 25OHD by sex and season of blood collection among persons age 12 years and older: NHANES III (as originally assayed and as predicted if current assay used) versus NHANES 2000–2004

NHW = Non-Hispanic white NHB = Non-Hispanic black MA = Mexican

* P < 0.05 comparing NHANES III to NHANES 2000-2004 based on t-tests

**Comparison for November-March limited to Non-Hispanic whites only due to significant different in proportion of nonwhites between NHANES III and NHANES 2000-2004 for the November-March sample.

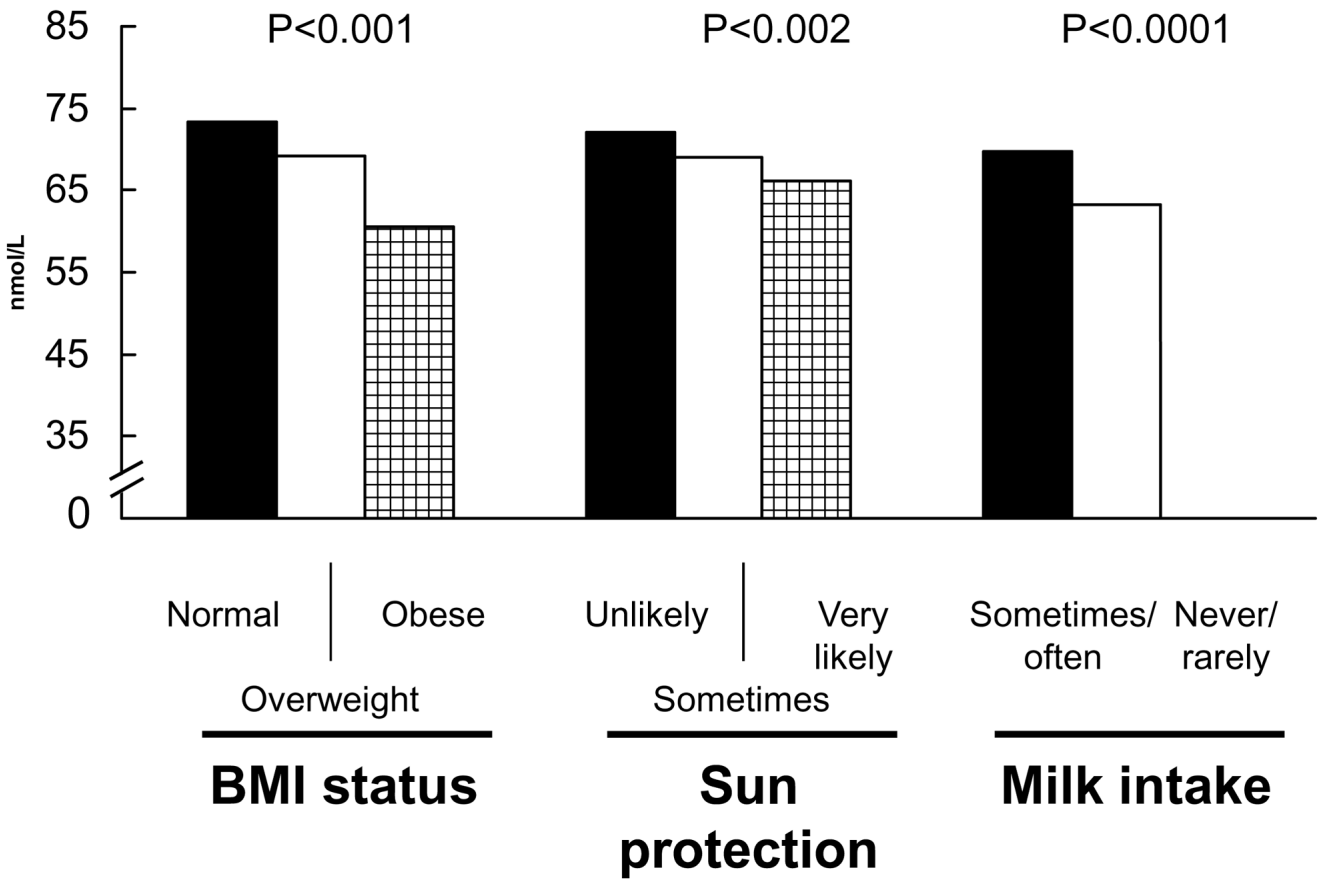
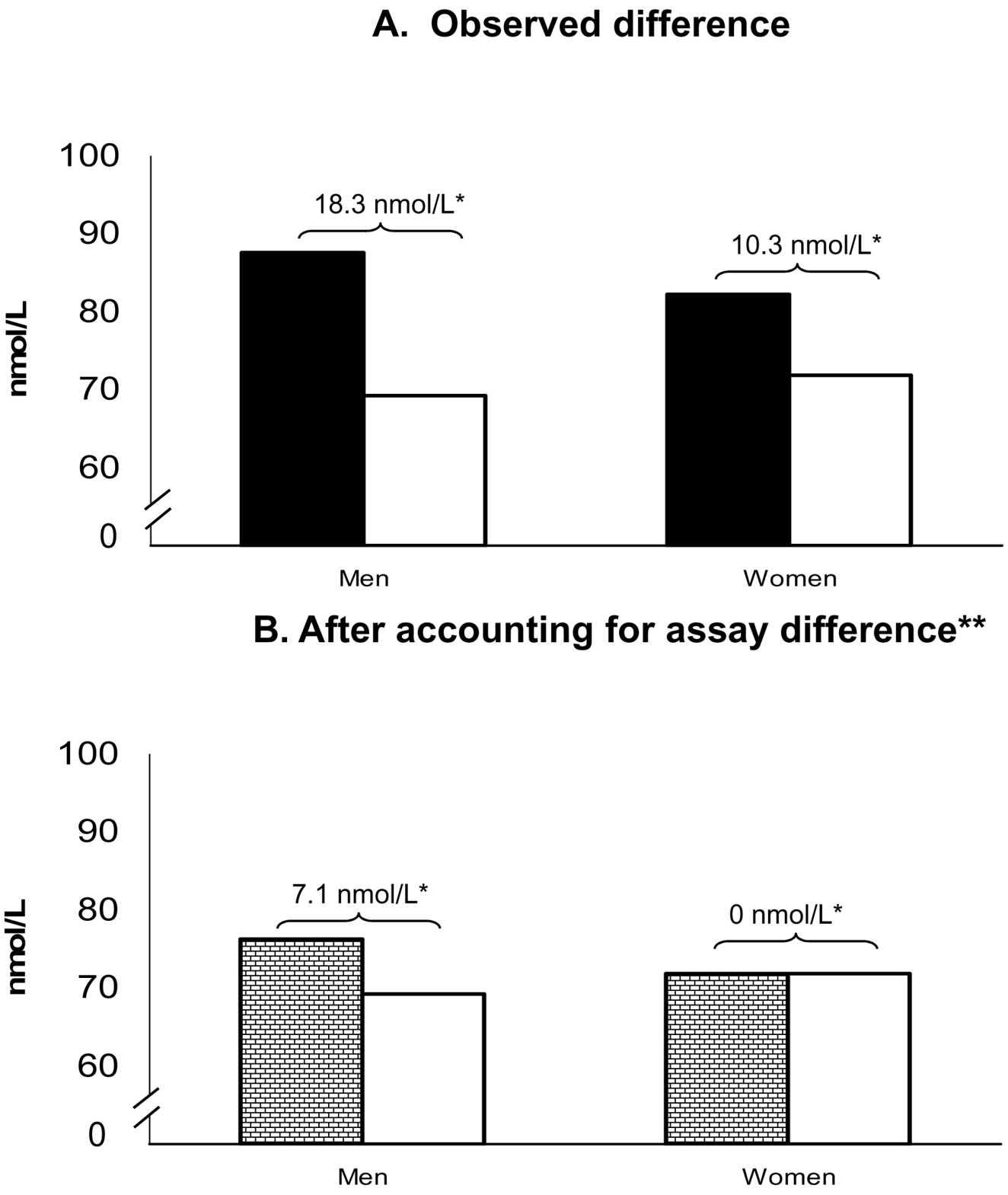


Figure 2.
Mean age- and sex-adjusted serum 25OHD by selected variables for non-Hispanic whites ages 20–59 years: NHANES 2003–2004
P values from overall F test for variable from linear regression.



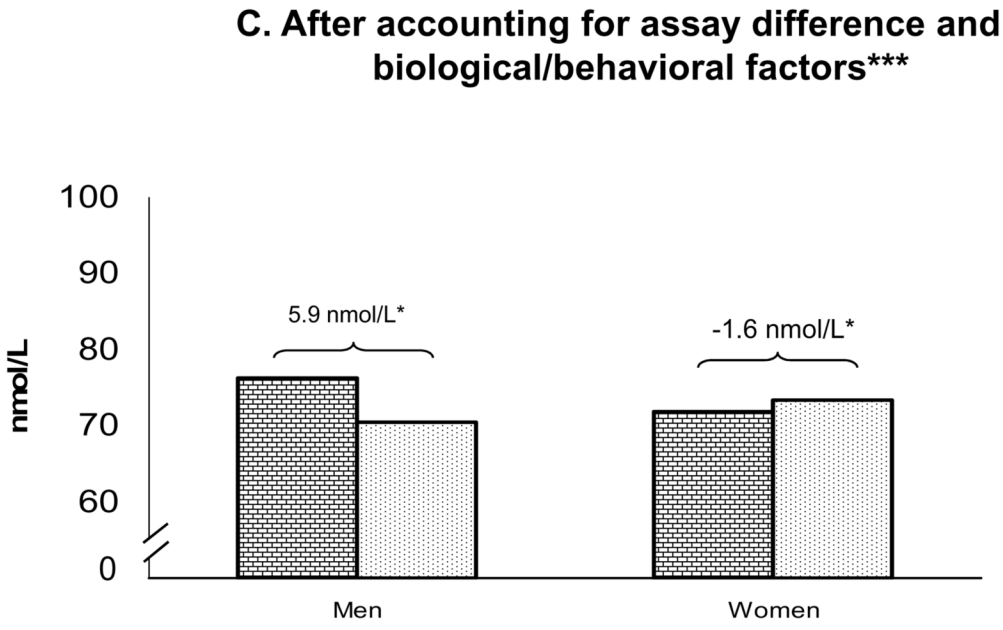


Figure 3.

Observed and predicted age-standardized mean serum 25OHD by sex among non-Hispanic whites age 20–59 years examined in April-October: NHANES III (1988–1994) versus NHANES 2003–2004

* Calculated as NHANES III mean minus NHANES 2003-04 mean

** Mean predicted for NHANES III 1988-1994 if current RIA was used.

*** Mean predicted for NHANES III 1988-1994 assuming current RIA was used and mean predicted for NHANES 2003-2004 if mean BMI and milk consumption rates from NHANES III and sun protective behavior rates from 1992 NHIS applied.

Table 1

Adjusted mean serum 25OHD[1] by selected characteristics for persons age 1 year and older: NHANES 2000–2004

	n	Mean (nmol/L)	SEM (nmol/L)	p-value[2]
Age (y)[†]				0.001
1–5	895	76.43	1.58	
6–11	2285	70.02	1.09	
12–19	5361	63.86	0.98	
20–49	5454	62.06	0.84	
50–69	3215	59.22	0.99	
70+	2340	57.45	0.76	
Sex				0.01
Male	9873	62.91	0.81	
Female	9677	61.54	0.85	
Race/ethnicity[‡]				0.001
NonHispanic white	8055	66.87	0.89	
NonHispanic black	5020	40.14	0.88	
Mexican American	5086	53.94	0.93	
Season				0.001
November-March	7824	58.86	0.86	
April-October	11726	63.76	1.01	
Pregnancy status (Women ages 13–56 y)				0.001
No	4886	61.50	1.53	
Yes	739	69.52	0.97	

[1] Means for each characteristic have been adjusted for all other characteristics shown in the table.

[2] p value for overall F test for this variable from linear regression.

[†] Means in each age category differs significantly from the mean of the preceding age category based on t-tests, $p<0.05$

[‡] Means in each race-ethnic group differs significantly from that of the other two race-ethnic groups based on t-tests, $p<0.05$

Table 2

Selected variables related to serum 25OHD by survey for non-Hispanic white persons age 20–59 years and older: NHANES III versus NHANES 2003–2004

	1988–1994			2003–2004		
Variable	**n**	**Mean or percent**	**SE[1]**	**n**	**Mean or percent**	**SE[1]**
Latitude (degrees North)	3433	37.86	0.50	1323	37.19	0.73
Body mass index (kg/m^2)	3433	26.24	0.16	1307	28.01†	0.23
Dietary calcium (mg/day)	3344	901.47	13.21	1263	973.26†	21.78
Sex (%)						
Male	1616	50.89	0.62	676	50.61	0.96
Female	1817	49.11	0.62	647	49.39	0.96
Season of blood collection (%)						
November-March	1121	30.81	3.93	364	28.23	5.95
April-October	2312	69.19	3.93	959	71.77	5.95
Milk consumption (%)						
Never/rarely	773	21.93	0.86	364	27.52 †	1.49
Sometimes/often	2645	78.07	0.86	955	72.48	1.49
Type of milk usually consumed (%)						
Whole milk	877	27.49	1.93	263	22.45	2.17
2% milk	1303	43.91	1.83	455	43.87	1.9
1% milk	255	9.46	1.22	113	11.56	1.57
Skim or nonfat milk	578	19.13	1.32	220	22.12	2.03
Vitamin-mineral supplement use in past month (%)						
Yes	1419	42.15	1.12	686	53.74†	2.73
No	2014	57.85	1.12	636	46.26	2.73
Activity level compared to peers (%)						
More	1044	31.86	1.05	410	32.12	1.82
Less	784	23.22	1.17	334	23.39	1.38
Same	1539	44.92	1.16	574	44.49	1.7

† P-value < 0.05 based on t-tests for comparison between 1988–1994 and 2003–2004

[1] Standard error of the mean orpercent

Table 3

Prevalence of sun protection by survey for adults ages 20–59 years of age: NHIS 1992 versus NHANES 2003–2004

	NHIS 1992			NHANES 2003–2004		
Practice sun protection	**n**	**Percent**	**SE[1]**	**n**	**Percent**	**SE[1]**
Men						
Very likely or always/most	1572	41.9	1.04	650	47.5	2.02
Sometimes	1113	29.6	0.91	472	37.0†	1.04
Unlikely or Rarely/never	1104	28.5	1.06	223	15.5†	1.70
Women						
Very likely or always/most	2818	57.4	0.92	846	57.4	2.39
Sometimes	1235	25.3	0.78	421	29.8†	1.77
Unlikely or Rarely/never	855	17.4	0.73	180	12.7	1.52

†P-value < 0.05 based on t-tests

[1]Standard error of the mean orpercent