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# Short-term variability of vitamin D-related biomarkers

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## Abstract

**Background—**Quantifying the variability of biomarkers is important, as high within-person variability can lead to misclassification of individuals. Short-term variability of important markers of vitamin D metabolism is relatively unknown.

**Methods—**A repeatability study was conducted in 160 Atherosclerosis Risk in Communities study participants (60% female, 28% black, mean 76 years). Fasting serum was drawn at two time points, a median of 6 (range 3-13) weeks apart. Vitamin D binding protein (VDBP) and 25-hydroxyvitamin D [25(OH)D] were measured by liquid chromatography mass spectrometry, fibroblast growth factor (FGF23) and parathyroid hormone (PTH) by enzyme-linked immunoassay, and calcium and phosphorus by Roche Cobas 6000. Free and bioavailable 25(OH)D were calculated. We calculated the within-person coefficient of variation ($CV_W$), intraclass correlation coefficient (ICC), Spearman rank correlation coefficient ($r$), and percent reclassified.

**Results—**The $CV_W$ was lowest for calcium (2.0%), albumin (3.6%), 25(OH)D (6.9%), VDBP (7.0%) and phosphorus (7.6%); intermediate for free 25(OH)D (9.0%) and bioavailable 25(OH)D (9.9%); and highest for PTH (16.7%) and FGF23 (17.8%). Reclassification was highest for PTH, VDBP and phosphorus (all 7.5%). The ICC and $r$ were highest (≥0.80) for 25(OH)D, free 25(OH)D, bioavailable 25(OH)D and PTH, but somewhat lower (~0.60-0.75) for the other biomarkers.

**Conclusions—**Six-week short-term variability, as assessed by CVw, was quite low for VDBP, calcium and phosphorus, but fairly high for FGF23 and PTH. As such, multiple measurements of

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FGF23 and PTH may be needed to minimize misclassification. These results provide insight into the extent of potential misclassification of vitamin D markers in research and clinical settings.

**Keywords**

vitamin D; short-term variability; vitamin D binding protein; fibroblast growth factor 23; parathyroid hormone

## Introduction

Compelling observational evidence(1-6) that low 25-hydroxyvitamin D [25(OH)D] is associated with diabetes, cardiovascular disease and cancer has prompted the U.S. National Institutes of Health to make strategic investments in randomized clinical trials to test whether vitamin $D_3$ supplementation reduces the risk of developing type 2 diabetes (the D2D Trial),(7) and heart disease, stroke and cancer (the VITAL trial).(8) Existing research has focused on total 25(OH)D; however, as highlighted in a 2015 U.S. Preventive Services Task Force Recommendation Statement,(9) it is possible that forms of vitamin D other than 25(OH)D may be relevant to human health. Some of the other biomarkers on the vitamin D pathway of potential clinical importance include fibroblast growth factor 23 (FGF23), vitamin D binding protein (VDBP), free 25(OH)D, bioavailable 25(OH)D, parathyroid hormone (PTH), calcium and phosphorus.

Biological measures often naturally vary within an individual on a day-to-day basis. This variation can result in misclassification in clinical settings, and in research may bias estimates of association. While the within person coefficient of variation (CVw) of some biomarkers of vitamin D status such as 25(OH)D, calcium and phosphorus are long-established, other biomarkers like VDBP, FGF23, free 25(OH)D and bioavailable 25(OH)D have only recently garnered interest,(10, 11) and their short-term variability has not yet been studied. For the more established biomarkers, older evaluations of their variability also require re-examination due to changes in laboratory methodology. For example, historically most studies measured 25(OH)D using immunoassay methods, whereas liquid chromatography tandem mass spectrometry (LC-MS/MS) is now considered to be far more accurate.(12) The newer LC-MS/MS methods typically also have less laboratory analytical imprecision, which would result in lower total variability.

As we await results of randomized clinical trials that will provide insight into whether vitamin D is causally associated with health benefits,(13) it is important to understand the biological variability of vitamin D biomarkers. This is especially relevant for 25(OH)D, which has a high prevalence of inadequacy (<20 ng/mL; 81% in blacks and 18% in whites according to the National Health and Nutrition Examination Survey (NHANES))(14) and is frequently measured in clinical settings.(15) Thus, the objective of this analysis was to quantify the short-term (6-week) within-person variability of fasting serum 25(OH)D, FGF23, VDBP, free 25(OH)D, bioavailable 25(OH)D, PTH, calcium and phosphorus in a community-based population. We also calculated the percent of the cohort for which the second measurement resulted in reclassification according to clinical cutpoints, laboratory reference values or concentrations outside the central $95^{th}$ percentile of the distribution.

## Materials and Methods

### Study Design and Study Population

The ARIC study is a community-based prospective cohort which began in 1987-1989 when a total of 15,792 predominantly black and white individuals, aged 45-64 years, were recruited from four U.S. field centers: suburban Minneapolis, Minnesota; Forsyth County, North Carolina; Washington County, Maryland; Jackson, Mississippi. Participants have been followed continuously since study initiation, and many have taken part in additional clinic examinations. The 5$^{th}$ clinic visit took place in 2011-2013, and was attended by 6,538 participants (66% of those still living). Institutional review boards at each site approved all procedures, and all study participants provided written informed consent.

During visit 5, a total of 200 participants (50 from each field center) were asked to return for a repeat 'quality control' visit approximately 4-8 weeks following the initial visit. These 200 individuals, who were representative of the age and gender distribution of the larger cohort of persons who attended ARIC visit 5, form the basis for the present analysis. We additionally excluded participants having a race with low sample numbers (Asians; N=2), missing or suspect information on SNPs needed for the calculation of bioavailable and free 25(OH)D (i.e. rs7041, rs4588; N=8), missing any biomarker data (N=6), and those with data points that were severe outliers (N=24) as identified by the iterative outlier removal approach.(16) Our final analytic sample includes 160 participants.

### Biomarker Measurement

Participants were asked to fast for 8 hours prior to the blood draw for both ARIC visit 5 and the repeat visit. Serum was stored at -70° C prior to measurement. Previously unthawed samples were used.

- VDBP was quantified using trypsin digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the University of Washington. This approach directly measures VDBP peptides.(11) The inter-assay analytical CV (calculated using laboratory controls) is 7.3-9.0%. VDBP was also measured using a monoclonal enzyme-linked immunosorbent assay (ELISA), produced by R&D Systems.
- 25(OH)$D_2$ and 25(OH)$D_3$ concentrations were measured using LC-MS/MS at the University of Minnesota.(17) The inter-assay analytical CVs were 5.3% for 25(OH)$D_2$ and 3.4% for 25(OH)$D_3$. Given the minimal (~6-week) time-span between measurements, 25(OH)$D_3$ concentrations were not corrected for known seasonality effects in the primary analyses. Total 25(OH)D was calculated as the sum of 25(OH)$D_2$ and 25(OH)$D_3$.
- Calcium and phosphorus were measured on the Roche Modular P800. Inter-assay analytical CVs were 2.3% and 2.2%.
- FGF23 was measured using a two site ELISA, manufactured by Kainos Incorporated. The inter-assay analytical CVs were 21.0% at a

concentration of 23 pg/mL, 9.7% at a concentration of 42 pg/mL and 7.8% at a concentration of 82 pg/mL.

- Albumin was measured as part of the glycated albumin assay by Asahi Kasei Pharma adapted to the Roche Modular P800 Chemistry Analyzer (Roche Diagnostics Corporation). Inter-assay analytical CVs were 1.9% at a concentration of 4.48 g/dL and 4.0% at a concentration of 2.5 g/dL.
- PTH was measured on a Roche Elecsys 2010. The inter-assay analytical CVs were 5.1% at a concentration of 33.10 pg/mL and 2.9% at a concentration of 204 pg/mL.
- Bioavailable and free 25(OH)D were calculated using a general formula(18) that has been tailored for 25(OH)D.(19) It incorporates serum VDBP, 25(OH)D and albumin concentrations, along with the binding affinities for 25(OH)D to albumin and VDBP. Binding affinities/constants are genotype-specific for VDBP.

## Statistical Analysis

For each biomarker we calculated the mean concentration at ARIC visit 5, the mean at the 6-week follow-up visit, the mean difference (repeat minus original) and the standardized mean difference (repeat/SD – original/SD). We also calculated the following measures of within-person total variability: within-person coefficient of variation ($CV_W$), intraclass correlation coefficient (ICC), index of individuality, and Spearman rank correlation coefficient ($r$).

As we have done previously,(20) to partition the total variance of the repeated measurements into the between-subject variance ($\sigma_{BS}^2$) and within-subject variance ($\sigma_{WS}^2$), we used linear mixed effects models with each biomarker as the dependent variable and the participant as a random effect. The between-person coefficient of variation ($CV_G$) was calculated as follows: $\left[\left(\sqrt{\sigma_{\mathrm{BS}}{}^2}\right)/\mu\right]*100$, where μ is the mean of all values (both original and repeat measurements). Similarly, we calculated the within-person coefficient of variation ($CV_W$): $\left[\left(\sqrt{\sigma_{\mathrm{WS}}{}^2}\right)/\mu\right]*100$. The $CV_W$ is a function of the within-person biological coefficient of variation and the analytical coefficient of variation (or the methods CV reported from the lab). We then calculated the index of individuality as $CV_W/CV_G$. (21, 22) We also calculated the ICC as: $\sigma_{BS}^2/(\sigma_{BS}^2 + \sigma_{WS}^2)$. We used bootstraping to estimate the 95% confidence intervals for estimates of $CV_W$, ICC, Spearman's rank correlation and the index of individuality using 200 replications. We also report the percent reclassified between the two visits according to clinical cutpoints or laboratory reference values, when they existed. For biomarkers that do not presently have clinical cutpoints, values falling within the central 95th percentiles (i.e. between 2.5% and 97.5%) were defined as normal. The distributions were defined based on the original exam values.

In sensitivity analyses, we additionally stratified by time between the initial and repeat visit (e.g. <6 weeks versus ≥6 weeks) and participant race (black versus white). Further sensitivity analyses were conducted where we accounted for seasonal variation in 25(OH)D

using the residuals approach.(23) All statistical analyses were conducted using Stata, version 13.0 (Stata Corp).

## Results

The 160 individuals in our final analytic sample were 60% female, 27.5% black., and had a mean (± SD) age of 76.3 ±4.9 years. The median age was 76 years with a range of 68 to 88 years. Participants who attended the repeat study visit were similar to the overall cohort who attended visit 5 in terms of age [75.8 ± 5.3 years], and percent female (58.8%) and black (23.6%). Relative to whites, black participants in the repeat study tended to have lower concentrations of 25(OH)D and higher concentrations of PTH, but had similar concentrations of other biomarkers (Table 1). Distributions of biomarkers at the original and repeat visits are presented in Supplemental Table 1.

Between the ARIC visit 5 blood draw and the repeat visit there were, on average, 6.4 weeks ± 2.1 [range: 3.3 to 13.3 weeks; $25^{th}$ and $75^{th}$ percentiles: 4.9 and 7.1 weeks]. Standardized differences between the visits were all near 0, indicating that there were no systematic blood processing or laboratory measurement issues between the visits (Table 2). The 6-week within-person variability ($CV_W$) was lowest for serum calcium (2.0%), albumin (3.6%), 25(OH)D (6.9%), VDBP (7.0%) and phosphorus (7.6%); intermediate for free 25(OH)D (9.0%) and bioavailable 25(OH)D (9.9%); and highest for PTH (16.7%) and FGF23 (17.8%). The ICC and *r* were highest for 25(OH)D, bioavailable 25(OH)D, free 25(OH)D, and PTH (all ≥0.80), but somewhat lower for VDBP, FGF23, calcium and phosphorus (~0.60 to 0.75). For the index of individuality, low values (defined by <0.6) were observed for 25(OH)D, free 25(OH)D, bioavailable 25(OH)D, PTH, calcium and albumin, while intermediate values (0.6 to <1.4) were observed for FGF23, phosphorus, and VDBP.

Overall results were similar in analyses stratified by race (Supplemental Table 2). The ICC and Spearman correlation (*r*) – both indices that are influenced by sample size(24) – were in some instances slightly higher in whites than blacks, which is consistent with our sample including more whites (n = 116) than blacks (n = 44). No noteworthy differences were observed when we stratified by length of time between measurements (Supplemental Table 3). Results for 25(OH)D were also virtually identical when we corrected for seasonal variation: CVw = 6.9, ICC = 0.95, Spearman Rank = 0.95, index of individuality = 0.22. Lastly, in Supplemental Table 4 we provide short-term variability results for VDBP as measured by the monoclonal immunoassay, which shows a higher ICC (0.95) and lower index of individuality (0.22) compared with LC-MS/MS.

To gauge the impact that individual-level variation may have on diagnostic misclassification, we examined the percent reclassified at the second measurement (Table 3). PTH, VDBP and phosphorus all had 7.5% reclassified. For FGF23 and 25(OH)D about 4% were classified, and for the other biomarkers approximately 3% or less were reclassified. Given our sample size relatively few individuals were reclassified, hence confidence intervals for the percent reclassified are wide. Reclassification for VDBP as measured by the monoclonal immunoassay is provided in Supplemental Table 5.

## Discussion

This evaluation of nine vitamin D pathway biomarkers revealed that six-week variability, as expressed by CVw, was quite low for serum VDBP, 25(OH)D, calcium, phosphorus, and albumin, intermediate for free and bioavailable 25(OH)D, but fairly high for FGF23 and PTH. Reclassification was highest for PTH, VDBP and phosphorus, although these results should be interpreted cautiously as precision was poor for these analyses. As such, multiple measurements of some biomarkers, such as FGF23 and PTH, may be needed to minimize misclassification. Variability was similar between blacks and whites, suggesting that although there may be racial differences in the absolute concentrations and the interrelations of these biomarkers,(25, 26) the short-term within-person stability of these biomarkers does not vary by race. These results provide insight into the extent of potential misclassification of vitamin D markers in both research and clinical settings.

CVw was identified *a priori* as the primary index of variability owing to its validity across a wide range of biomarker values. However, to allow for a more comprehensive understanding of the variability of these biomarkers, several other indicators of variability are reported herein, each with a different interpretation.(24) Standardized mean differences were all small, suggesting that there were no systematic differences between the 2 sets of readings (e.g. related to variation in blood processing and/or laboratory drift) thereby facilitating a systematic evaluation of short-term variability. Spearman rank correlations assess monotonicity of the two biomarker measurements, whereas ICC is an estimate of the fraction of the total measurement variability caused by variation among individuals. Overall, in the present analysis Spearman rank correlations and ICCs resulted in similar inferences. Notably, these indices are both highly influenced by the range of a biomarker among individuals.(24) Higher values are typically observed for biomarkers with a wider range, and correspondingly suggest that the within-person variance is low relative to the between-person variance. The index of individuality, which is the ratio of total within-person CV to between-person CV, can be useful for determining the value of repeating tests of a biomarker. In the present analysis, low indices (<0.6) were observed for several biomarkers (i.e. free 25(OH)D, bioavailable 25(OH)D, PTH, calcium and albumin), which suggests that repeating tests of these biomarkers will result in concentrations close to the first, and therefore provide little new information.(27) All of the other biomarkers explored had index of individuality values in the intermediate range, between 0.6-0.9, which suggests repeat measures may be more useful to minimize misclassification. The monoclonal immunoassay showed excellent within-person variability, however as we have shown previously this assay is biased by genotype;(10, 11) thus results for this assay should be interpreted with caution. The excellent within-person variability is most likely a result of the wide distribution of VDBP for this method, which results from the strong influence of genotype on the measurements (e.g., the poor reactivity of the Gc1f isoform in the assay).

This study provides the first evidence of the short-term variability of VDBP, and calculated free and bioavailable 25(OH)D. Recent studies have evaluated daily variability of FGF23, (28-31) and most have found no diurnal variation; however, no studies as of yet have assessed its short-term variability. Approximately 2-week biologic variability of 25(OH)D, calcium, phosphorus and albumin has been previously reported using data from the

NHANES III survey.(32) For 25(OH)D, the 2-week CVw and index of individuality reported in NHANES (i.e. 11.3, 0.30, respectively) (32) were roughly similar to those we observed in this ARIC sample (6.9, 0.22). The subsample of ARIC participants in the present study was on average 76 years old, while the NHANES population was, on average, 39 years old. The somewhat lower within-person variability among the older ARIC participants is not entirely surprising, since older populations may have less variability in their exposure to the sun and are known to have reduced dermal conversion of 7-dehydrocholesterol to vitamin $D_3$.(25) NHANES III also used a radioimmunoassay to measure 25(OH)D, while in the present ARIC sample we used LC-MS/MS. Prior work has demonstrated that 25(OH)D tracks reasonably well across longer time-periods.(33-35) Using data from more than 1,700 ARIC participants who had 25(OH)D measured in serum collected in 1990-1992 and in 1993-1995, we recently reported the 3-year correlation of 25(OH)D concentrations to be $r = 0.73$ in whites and $r = 0.66$ in blacks.(36) Our finding of high biological variability for PTH is consistent with that of prior work in individuals without chronic kidney disease, which has shown PTH to have a within-person variation of approximately 25%.(37, 38) For serum calcium, phosphorus and albumin, short-term variability was comparable between the ARIC and NHANES (32) samples (CVw/index of individuality): calcium ARIC = 2.0/0.56, NHANES = 2.3/0.55; phosphorus ARIC = 7.6/0.77, NHANES = 9.5/0.58; albumin ARIC = 3.6/0.58, NHANES = 3.5/0.46.

Quantifying the amount of within-person short-term variability inherent in these vitamin D markers provides insight into the extent of potential misclassification of vitamin D markers in both research and clinical settings. In these data, reclassification was highest for PTH, VDBP and phosphorus, although imprecision was high given the relatively few individuals reclassified. High within-person variability, or random fluctuations around a set point, can lead to false positive results at the individual level, and substantial overestimates of disease prevalence on a population level, especially if the biomarker is only measured once.(39, 40) Overestimates occur with greater short-term variability since the variance is larger, essentially flattening and widening the distribution and resulting in more individuals having concentrations beyond established cut-points. It is also possible that measurements from this study and resulting estimates of short-term variability could be incorporated into regression models (i.e. regression calibration which allows for the simultaneous correction of variation or measurement error in exposure(s) as well as accounts for the relationship of that variation with other risk factors) and could have a substantial effect on estimates of association.(41) Of course, understanding within-person variation is only one aspect of variability. Inter-laboratory bias remains a major concern in both clinical practice and research settings. As such, it is crucial that efforts by initiatives such as the Vitamin D Standardization Program – which seeks to standardize laboratory measurements such that results are accurate and comparable over time, location, and laboratory procedure – are implemented by laboratories. (42, 43)

These data have notable strengths, as well as limitations. The time-span between initial and repeat study visits varied between participants. Additionally, we may have been underpowered for the stratified analyses. Nevertheless, this study provides a comprehensive evaluation of the short-term variability of numerous markers of vitamin D metabolism. Also, our study sample was quite advanced in age (mean age 76.3 years). However, we believe our

results are generalizable to younger populations since we would not expect that intra-individual variability of these biomarkers would be different among the elderly versus younger adults, and our results were similar for the biomarkers previously reported in the younger NHANES sample.(32) Strengths of the study include the sample, which was community-based (as opposed to from a clinical setting) and was relatively large for a repeatability study. Presence of both blacks and whites in the sample is another important characteristic of the study, given the literature suggesting racial/ethnic differences in vitamin D metabolism.[21,22] For each biomarker, state-of-the-art assays for the samples from the original and repeat visits were conducted in the same lab and with the same instruments, which helped us isolate within-person variability from other sources of variability that could arise from storage time, freeze-thaw, machine calibration, and lot-to-lot variability of reagents.

In conclusion, over a 6-week time-frame, variability was quite low for serum VDBP, 25(OH)D, calcium, phosphorus, and albumin, intermediate for free and bioavailable 25(OH)D, but fairly high for FGF23 and PTH. As such, multiple measurements of some biomarkers, such as FGF23 and PTH, may be needed to minimize misclassification. These results provide insight into the extent of potential misclassification of vitamin D pathway biomarkers in research and clinical settings.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **25(OH)D** | 25-hyrdoxyvitamin D |
| **ARIC** | Atherosclerosis Risk in Communities Study |
| **Bioavailable 25(OH)D** | Bioavailable 25-hyrdoxyvitamin D |
| **SNP** | Single nucleotide polymorphism |
| **CV** | coefficient of variation |
| **FGF23** | fibroblast growth factor 23 |
| **Free 25(OH)D** | Free 25-hydroxyvitamin D |
| **NHANES** | National Health and Nutrition Examination Survey |
| **PTH** | parathyroid hormone |
| **VDBP** | vitamin D binding protein |

## Table 1

**Characteristics of repeatability study participants, overall and by race: The Atherosclerosis Risk in Communities Study, Visit 5 (2011-2013)**

| | Overall | Whites | Blacks |
|---|---|---|---|
| *N* | 160 | 116 | 44 |
| Age, years | 76.3 (4.9) | 76.2 (4.8) | 76.5 (5.2) |
| Female, % | 60.0 | 57.8 | 65.9 |
| VDBP, μg/mL | 264.5 (31.6) | 265.7 (33.5) | 261.3 (26.2) |
| 25(OH)D, ng/mL | 34.0 (11.1) | 35.7 (10.4) | 29.5 (11.6) |
| Calcium, mg/dL | 9.3 (0.4) | 9.4 (0.4) | 9.2 (0.4) |
| Phosphorus, mg/dL | 3.6 (0.4) | 3.5 (0.4) | 3.6 (0.4) |
| FGF23, pg/mL | 61.1 (20.9) | 62.2 (22.5) | 58.2 (15.8) |
| Albumin, g/dL | 4.1 (0.3) | 4.2 (0.3) | 3.9 (0.3) |
| PTH, pg/mL | 45.7 (18.5) | 43.4 (17.0) | 51.6 (21.0) |
| Free 25(OH)D, pg/mL | 10.4 (5.0) | 11.8 (4.7) | 6.9 (3.9) |
| Bioavailable 25(OH)D, ng/mL | 3.9 (1.9) | 4.4 (1.7) | 2.5 (1.6) |

Values are mean (standard deviation) or percentage.

VDBP = vitamin D binding protein, 25(OH)D=25-hyrdoxyvitamin D, FGF23 = fibroblast growth factor 23, PTH = parathyroid hormone

To convert mg/dL to mmol/L, multiply by 0.2495 for calcium and by 0.3229 for phosphorus.

**Table 2**

**Short-term variability in vitamin D-related biomarkers in 160 older adults: The Atherosclerosis Risk in Communities Study, Visit 5 (2011-2013)**

| | Original Exam Mean ± SD | Repeat Exam Mean ± SD | Difference (Repeat – Original) Mean ± SD | Standardized Difference* Mean ± SD | $CV_W$ (95% CI)¥ | ICC (95% CI)¥ | Spearman's rank correlation coefficient (r) (95% CI)¥ | Index of Individuality (95% CI)¥ |
|---|---|---|---|---|---|---|---|---|
| VDBP,µg/mL | 265 ± 32 | 257 ± 31 | -8 ± 25 | -0.1 ± 0.8 | 7.0 (6.3, 7.7) | 0.67 (0.62, 0.71) | 0.63 (0.53, 0.72) | 0.71 (0.63, 0.79) |
| 25(OH)D, ng/mL | 34.0 ± 11.1 | 33.5 ± 11.0 | -0.5 ± 3.3 | 0.0 ± 0.3 | 6.9 (6.2, 7.7) | 0.95 (0.95, 0.96) | 0.95 (0.93, 0.96) | 0.22 (0.19, 0.24) |
| Calcium, mg/dL | 9.3 ± 0.4 | 9.3 ± 0.4 | 0.0 ± 0.3 | 0.4 ± 0.7 | 2.0 (1.7, 2.2) | 0.76 (0.72, 0.80) | 0.71 (0.63, 0.78) | 0.56 (0.49, 0.63) |
| Phosphorus, mg/dL | 3.6 ± 0.4 | 3.5 ± 0.5 | 0.0 ± 0.4 | -0.4 ± 0.9 | 7.6 (6.7, 8.5) | 0.63 (0.56, 0.69) | 0.61 (0.51, 0.70) | 0.77 (0.67, 0.87) |
| FGF23, pg/mL | 61.1 ± 20.9 | 59.7 ± 19.4 | -1.4 ± 15.2 | 0.2 ± 0.8 | 17.8 (16.1, 19.6) | 0.71 (0.67, 0.76) | 0.66 (0.56, 0.74) | 0.63 (0.56, 0.70) |
| Albumin, g/dL | 4.1 ± 0.3 | 4.0 ± 0.3 | -0.1 ± 0.2 | 0.1 ± 0.7 | 3.6 (3.1, 4.0) | 0.75 (0.70, 0.80) | 0.75 (0.67, 0.81) | 0.58 (0.49, 0.66) |
| PTH, pg/mL | 45.7 ± 18.5 | 45.1 ± 19.2 | -0.6 ± 10.7 | -0.1 ± 0.6 | 16.7 (14.6, 18.8) | 0.84 (0.81, 0.87) | 0.80 (0.74, 0.85) | 0.44 (0.38, 0.50) |
| Free 25(OH)D, pg/mL | 10.4 ± 5.0 | 10.6 ± 5.2 | 0.1 ± 1.3 | 0.0 ± 0.3 | 9.0 (7.9, 10.1) | 0.97 (0.96, 0.97) | 0.96 (0.94, 0.97) | 0.19 (0.17, 0.21) |
| Bioavailable 25(OH)D, ng/mL | 3.9 ± 1.9 | 3.9 ± 2.0 | 0.0 ± 0.5 | -0.1 ± 0.3 | 9.9 (8.9, 11.0) | 0.96 (0.95, 0.97) | 0.95 (0.93, 0.96) | 0.21 (0.18, 0.23) |

*[Repeat value/SD] – [Original value/SD]

VDBP = vitamin D binding protein, 25(OH)D=25-hyrdoxyvitamin D, FGF23 = fibroblast growth factor 23, PTH = parathyroid hormone

¥The 95% CIs were bootstrapped with 200 replications

To convert mg/dL to mmol/L, multiply by 0.2495 for calcium and by 0.3229 for phosphorus.

**Table 3**

**Percent of participants reclassified with repeat biomarker measurements: The Atherosclerosis Risk in Communities Study, Visit 5 (2011-2013)**

| Biomarker | Cutpoint | | n (%) Abnormal | | | Reclassified* | |
|---|---|---|---|---|---|---|---|
| | Determination | Value | Original Exam | Repeat Exam | Both Exams | N | Percent (95% CI) |
| VDBP, μg/mL | Central 95%tile | 207.3 to 345.4 | 8 (5%) | 10 (6.3%) | 3 (1.9%) | 12 | 7.5 (4.3, 12.8) |
| 25(OH)D, ng/mL | Clinical | <20 | 17 (10.6%) | 19 (11.9%) | 15 (9.4%) | 6 | 3.8 (1.7, 8.2) |
| Calcium, mg/dL | Lab Reference | 8.4 to 10.2 | 3 (1.9%) | 3 (1.9%) | 2 (1.3%) | 2 | 1.3 (0.3, 4.9) |
| Phosphorus, mg/dL | Lab reference | 2.7 to 4.5 | 3 (1.9%) | 5 (3.1%) | 0 | 8 | 5 (2.5, 9.7) |
| FGF23, pg/mL | Central 95%tile | 28.6 to 116.6 | 8 (5%) | 3 (1.9%) | 2 (1.3%) | 7 | 4.4 (2.1, 9.0) |
| Albumin, g/dL | Lab reference | 3.5 to 5.0 | 1 (0.6%) | 2 (1.3%) | 0 | 3 | 1.9 (0.6, 5.7) |
| PTH, pg/mL | Clinical | ≥ 65 | 22 (13.8%) | 16 (10%) | 13 (8.1%) | 12 | 7.5 (4.3, 12.8) |
| Free 25(OH)D, pg/mL | Central 95%tile | 2.6 to 22.1 | 8 (5%) | 9 (5.6%) | 6 (3.8%) | 5 | 3.1 (1.3, 7.4) |
| Bioavailable 25(OH)D, ng/mL | Central 95% tile | 0.9 to 8.5 | 8 (5%) | 8 (5%) | 6 (3.8%) | 4 | 2.5 (0.9, 6.5) |

VDBP = vitamin D binding protein, 25(OH)D=25-hyrdoxyvitamin D, FGF23 = fibroblast growth factor 23, PTH = parathyroid hormone

* In a different category (abnormal versus normal) at the original exam and repeat visits.

To convert mg/dL to mmol/L, multiply by 0.2495 for calcium and by 0.3229 for phosphorus.