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CEBP Focus: Biomarkers and Biospecimens

Long-term Variation in Serum 25-Hydroxyvitamin D Concentration among Participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Abstract

Molecular epidemiologic studies of vitamin D and risk of cancer and other health outcomes usually involve a single measurement of the biomarker 25-hydroxyvitamin D [25(OH)D] in serum or plasma. However, the extent to which 25(OH)D concentration at a single time point is representative of an individual's long-term vitamin D status is unclear. To address this question, we evaluated within-person variability in 25(OH)D concentrations across serum samples collected at three time points over a 5-year period among 29 participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Blood collection took place year-round, although samples for a given participant were collected in the same month each year. The within-person coefficient of variation and intraclass correlation coefficient were calculated using variance components estimated from random effects models. Spearman rank correlation coefficients were calculated to evaluate agreement between measurements at different collection times (baseline, +1 year, +5 years). The within-subject coefficient of variation was 14.9% [95% confidence interval (CI), 12.4-18.1%] and the intraclass correlation coefficient was 0.71 (95% CI, 0.63-0.88). Spearman rank correlation coefficients comparing baseline to +1 year, +1 year to +5 years, and baseline to +5 years were 0.65 (95% CI, 0.37-0.82), 0.61 (0.29-0.81), and 0.53 (0.17-0.77), respectively. Slightly stronger correlations were observed after restricting to non-Hispanic Caucasian subjects. These findings suggest that serum 25(OH)D concentration at a single time point may be a useful biomarker of long-term vitamin D status in population-based studies of various diseases. *Cancer Epidemiol Biomarkers Prev; 19(4); 927–31. ©2010 AACR.*

Introduction

Evidence from *in vitro* and animal studies suggests that the steroid prohormone vitamin D may play an important protective role against cancer development and metastasis (1). These experimental findings have led to several epidemiologic studies investigating associations with cancer risk for prediagnostic blood levels of 25-hydroxyvitamin D [25(OH)D], the primary circulating form of vitamin D and a clinical biomarker for vitamin D status. Some prospective studies have reported associations between low circulating 25(OH)D and increased risk of various cancers (2-4). Most, but not all, studies investigating prediagnostic serum or plasma 25(OH)D concentration and colorectal cancer risk are suggestive of a protective effect (5-10); however, the totality of the evidence for other malignancies is inconclusive (8-12). A potential limitation of these studies is their reliance on 25(OH)D measurements in a single sample of serum or plasma from each individual. Although levels of 25(OH)D are known to vary seasonally (highest in the summer and fall, lowest in winter and spring) in accordance with dermal vitamin D production following sun exposure, relatively little is known about the degree to which an individual's season-specific level of 25(OH)D varies over time, and whether vitamin D concentration at a given time point is representative of an individual's long-term vitamin D status (13).

To better understand the within-person variability of 25(OH)D over time, we compared 25(OH)D measurements from serum samples collected at study baseline, 1 year, and 5 years later among 29 participants in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Blood samples within PLCO were collected year-round, although serial samples from each participant were collected in the same month each year during the 5-year follow-up period. Because of this collection protocol, this study provides a unique opportunity to assess the variability over time of 25(OH)D measurements within the same season.

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Materials and Methods

The design, methods, and goals of the PLCO have been described previously (14). This study used stored serum from a sample of 29 cancer-free subjects previously selected to support evaluations of long-term stability for various analytes. Subjects provided nonfasting baseline blood samples that were processed and frozen within 2 h of collection and stored at −70°C. Measurements of 25(OH)D were done on 100-μL aliquots of serum collected from these subjects at study baseline, the first annual follow-up health examination (+1 y), and the fifth annual follow-up examination (+5 y). Of the 29 subjects, 28 had serum from baseline, 29 had serum from +1 y, and 26 had serum from +5 y. All subjects had serum from at least two time points, and 25 subjects had serum from all three time points. Measurements of 25(OH)D (in nmol/L) were done at Heartland Assays, Inc. (Ames, IA) by competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25-OH Vitamin D TOTAL Assay (DiaSorin S.p.A., Saluggia, Italy). All 83 samples were measured in a single batch.

Using variance components computed from a random effects model of log-transformed 25(OH)D concentrations, we calculated the coefficient of variation (CV) on the original scale to assess the extent of within-person variability across the three time periods (15). The intraclass correlation coefficient (ICC) for log-transformed 25(OH)D concentration was calculated to evaluate the degree of between-person variability relative to total variability, including within-person variability over time [i.e., $\sigma^2_{between}/(\sigma^2_{between} + \sigma^2_{within})$]. Unadjusted Spearman rank correlation coefficients were calculated to perform pairwise evaluations of agreement between measurements at different time points (baseline versus +1 y, +1 y versus +5 y, baseline versus +5 y, and average of baseline/+1 y versus +5 y). The aforementioned analyses were also conducted including only non-Hispanic Caucasian subjects ($n = 23$).

Table 1. Descriptive statistics and estimates of within-subject variability and agreement between serum concentrations of 25(OH)D measured at three time points (baseline, +1 y, and +5 y) over a 5-y period

Analysis	*n*	Values
Season of blood draw*		
January-March	7	
April-June	6	
July-September	11	
October-December	5	
Summary of 25(OH)D measures at:		
Baseline		
Mean (SD)	28	60.0 (14.6) nmol/L
Median	28	60.2 nmol/L
Range	28	33.7-99.6 nmol/L
+1 y		
Mean (SD)	29	59.8 (15.9) nmol/L
Median	29	59.5 nmol/L
Range	29	28.6-94.2 nmol/L
+5 y		
Mean (SD)	26	56.6 (16.2) nmol/L
Median	26	55.1 nmol/L
Range	26	24.1-98.2 nmol/L
Measures of agreement		
CV (95% CI)	29	14.9% (12.4-18.1%)
ICC (95% CI)	29	0.71 (0.63-0.88)
Spearman rank correlation coefficients (95% CI)		
Baseline vs. +1 y	28	0.65 (0.37-0.82)
+1 y vs +5 y	26	0.61 (0.29-0.81)
Baseline vs. +5 y	25	0.53 (0.17-0.77)
Baseline/+1 y average vs. +5 y	25	0.65 (0.34-0.83)

*All participants had the serial blood draws during the same calendar month in each year. On average, samples were collected within a 13-d window (range, 2-26 d).

Results

The average age at baseline among subjects included in this analysis was 61 years (range, 55-70 years), and over half of the participants were male ($n = 18$). Most participants ($n = 23$) were non-Hispanic Caucasian; the remaining six participants were Asian. Participants from each of the 10 PLCO screening centers throughout the United States were represented in the study sample.

Statistics summarizing within-person variability and agreement of 25(OH)D are summarized in Table 1. The within-person CV across the three time points was 14.9% [95% confidence interval (CI), 12.4-18.1%] over the 5-year period, and the ICC was 0.71 (95% CI, 0.63-0.88). Spearman rank correlation coefficients comparing baseline to +1 year, +1 year to +5 years, and baseline to +5 years were 0.65 (95% CI, 0.37-0.82), 0.61 (0.29-0.81), and 0.53 (0.17-0.77), respectively. We also calculated the Spearman rank correlation coefficient comparing the average of the baseline and +1-year measurements against the +5-year measurements, and found that the correlation was slightly higher than when either baseline or +1 year was considered independently in relation to +5-year measurements (Spearman rank correlation coefficient = 0.65; 95% CI, 0.34-0.83). Plots of 25(OH)D measurements for each pairwise comparison are shown in Fig. 1.

After restricting to non-Hispanic Caucasian subjects, the within-person CV was 15.5% (95% CI, 12.5-19.2%) and the ICC was 0.75 (95% CI, 0.64-0.90). We observed slightly stronger correlations when we restricted to non-Hispanic Caucasian subjects (Spearman rank correlation coefficients = 0.72, 0.69, 0.58, and 0.70 for baseline versus +1 year, +1 year versus +5 years, baseline versus

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+5 years, and average of baseline/+1 year versus +5 years, respectively). Correlations were generally consistent after stratification by sex (data not shown).

Discussion

Although serum 25(OH)D concentrations are often used to characterize vitamin D levels in population-based studies, the extent to which a single measurement is representative of long-term vitamin D status is unclear. The findings of this study suggest that measurements of 25(OH)D from serum collected at the same time of the year are reasonably stable in healthy subjects over a 5-year period. We observed relatively low within-subject variability and fairly high correlations in 25(OH)D measured from samples collected at study baseline, after 1 year, and after 5 years (Fig. 2).

To evaluate the effect of within-person variability on relative risk estimates in studies of vitamin D status and cancer risk, we used the following formula derived from Rosner et al. (16) to estimate the degree of relative risk attenuation:

$$RR_{observed} = \exp\,[ICC \times \ln(RR_{true})]$$

Given the ICC of 0.71 that was observed in this study, we estimated that for true relative risks of 1.5, 2.0, and 2.5,

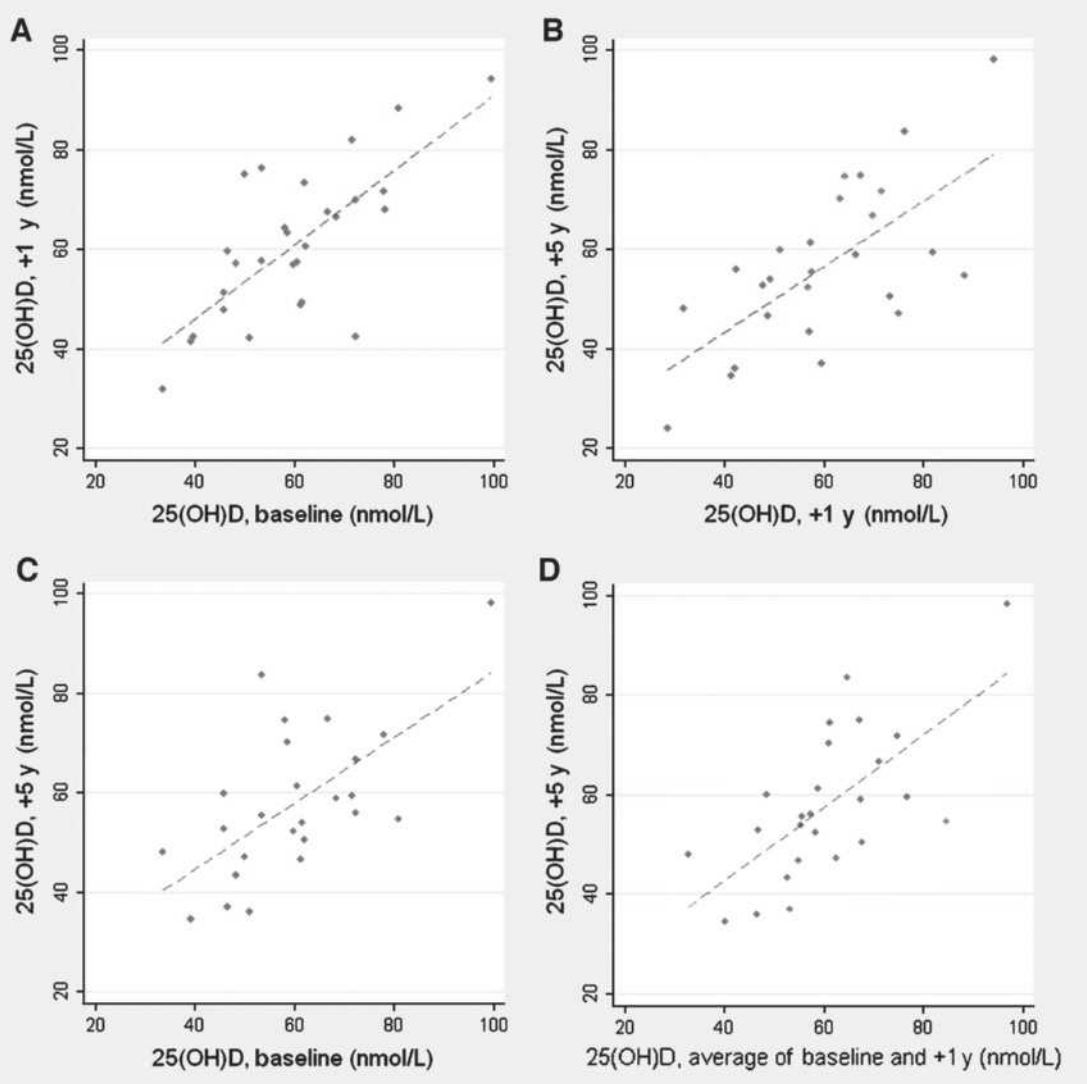

Figure 1. Scatterplots and fitted lines for serum 25(OH)D concentration at baseline versus +1 y (A), +1 y versus +5 y (B), baseline versus +5 y (C), and average of baseline/+1 y versus +5 y (D).

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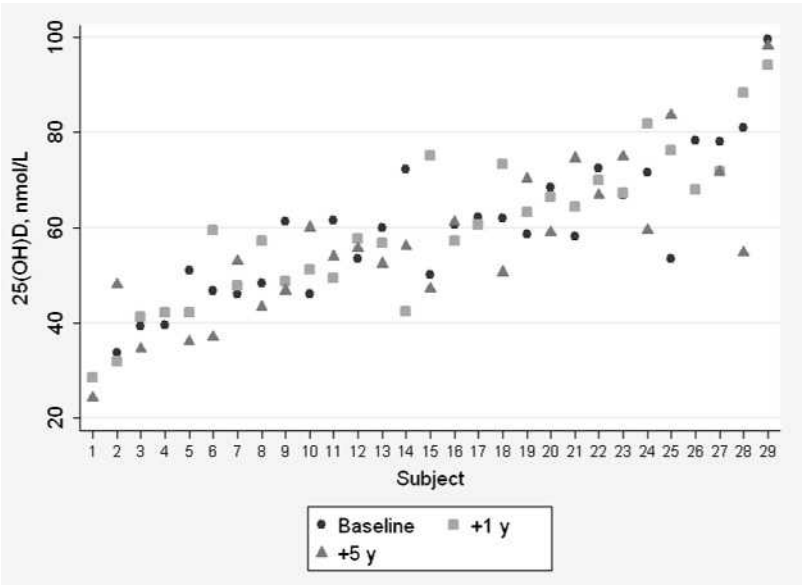

Figure 2. Serial measurements of serum 25(OH)D concentration at baseline, +1 y, and +5 y for each subject.

we would expect observed relative risks of 1.3, 1.6, and 1.9, respectively. Investigators should consider this attenuation when designing studies of various diseases in relation to long-term vitamin D status to ensure that they have adequate statistical power.

If possible, investigators might also consider taking multiple 25(OH)D measurements from each subject at different times to reduce misclassification of vitamin D status due to within-person variability over time (17), although this may not be feasible in many studies. In this study, we observed a higher correlation when the average of the baseline and +1 year measurements was compared with 25(OH)D concentration at +5 years. The average of two measurements taken 1 year apart may thus provide a better assessment of long-term vitamin D status than a single measurement, although the increase in agreement observed in this study was relatively small.

Seasonal variation in 25(OH)D concentration has been well-described (18, 19). However, to our knowledge, relatively few studies have evaluated temporal variability beyond seasonal effects. Platz et al. (11) measured 25(OH)D concentrations in two blood samples obtained an average of 3.0 years apart from 144 cancer-free men in the Health Professionals Follow-up Study. They reported a Pearson correlation coefficient of 0.70, which is consistent with the level of agreement observed in this study. Rejnmark et al. (20) evaluated variability between serial measurements using the same assay at three time points over a 4-year period among 187 postmenopausal women in the Danish Osteoporosis Prevention Study; median within-person CVs ranged from 13% to 19%.

An important strength of the present study is the use of serum from PLCO blood specimens collected up to 5 years apart, at the same time each year, which provided a unique opportunity to control for seasonal variation in 25(OH)D while assessing variability over a long period. However, the 5-year range in serial specimens limits the generalizability of our findings, in that the level of agreement of a single measure with 25(OH)D levels over longer time periods remains unclear. It is worth noting that a 5-year time window may be biologically relevant to the relationship between vitamin D and cancer, in that vitamin D has been postulated to exert effects which influence late-stage carcinogenesis, metastasis, and survival for some cancers (1). This study is also limited by its small size, although the 95% CIs surrounding the CV and ICC estimates still support the inference of low within-person variability and high agreement in 25(OH)D across the time points.

In conclusion, the findings from this study suggest that serum 25(OH)D concentration at a single point in time may be a useful biomarker of season-specific vitamin D status over a 5-year period. Further studies are needed to evaluate the extent of within-person variability of 25(OH)D over a longer time.

Disclosure of Potential Conflicts of Interest

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