Longitudinal Changes in Serum 25-Hydroxyvitamin D in Older People

Horace M. Perry III, M. Horowitz, J.E. Morley, Ping Patrick, Bruno Vellas, Richard Baumgartner, and Phillip J. Garry

Cross-sectional studies have suggested that serum 25-hydroxyvitamin D (25OHD) levels decline with aging. We have examined this putative decline in a longitudinal study using participants in the New Mexico Aging Process Study. 25OHD levels were measured in participants in whom serum samples were available between 1980 to 1982 and 1989 to 1994 (37 men and 99 women). The available data for these visits included age, gender, and the date the sample was obtained. Questionnaires assessing physical activity and vitamin D intake were administered at the visits. A seasonal variation ($r = .25$, $P < .05$) in 25OHD was demonstrated in the whole group of subjects. In 25 subjects who were not receiving vitamin D supplementation at either time and had samples obtained in the same season, both serum 25OHD ($P < .05$) and physical activity ($P < .05$) decreased over a mean period of 11.4 years. In 23 subjects who had samples obtained in the same season but used vitamin D supplements at both times, there was no change in serum 25OHD. Mean summer 25OHD levels did not change with the duration of study. On the other hand, the mean serum 25OHD declined with the duration of study when measured from winter to winter or spring to spring. Multiple regression analysis demonstrated that the month, activity level, vitamin D supplementation, and gender ($P < .001$) were independent determinants of serum 25OHD levels. This study confirms that aging is associated with a reduction in serum 25OHD, and suggests that this decrease is a reflection of reduced sun exposure rather than aging per se. The reduction in serum 25OHD was the result of decreasing winter and spring 25OHD serum concentrations. It is clear that vitamin D supplementation can prevent the age-related decline in 25OHD levels.

A SERIES of cross-sectional studies have suggested that serum 25-hydroxyvitamin D (25OHD) concentrations decrease slowly across a broad age spectrum.[1-4] The etiology of this decrease appears to be multifactorial. The ability of the skin to photosynthesize vitamin D diminishes with age,[5] as well as the intake of dietary foods fortified with vitamin D (particularly milk),[6-8] vitamin D absorption,[9] and physical activity[10,11] with resultant sun exposure. It is also well established that there are seasonal differences in serum 25OHD levels in most populations.[12-16] Vitamin D insufficiency has been demonstrated to have a role in bone loss in older women.[17] Similarly, vitamin D and calcium supplementation increases bone mineral density.[17-21] Such vitamin D supplementation has been shown to decrease fracture rates in older women in some,[20-23] but not all[24] studies.

There are numerous cross-sectional investigations, but we are not aware of any longitudinal studies examining the effect of age on serum 25OHD; we have therefore examined the effect of aging on serum 25OHD in a longitudinal manner. In addition, we attempted to determine whether it is aging per se or some other factor associated with aging that is primarily responsible for any decline in serum 25OHD, and whether these effects are modified by vitamin D supplementation.

From the Geriatric Research, Education and Clinical Center, St. Louis Veterans Affairs Medical Center, St. Louis; Division of Geriatric Medicine, St. Louis University Medical School, St. Louis, MO; Clinical Nutrition Program, School of Medicine, University of New Mexico, Albuquerque, NM; Department of Geriatric Medicine, University of Toulouse, Toulouse, France; and Department of Medicine, University of Adelaide, Adelaide, Australia.
Submitted October 5, 1998; accepted February 4, 1999.
Address reprint requests to Horace M. Perry III, MD, St. Louis University, Department of Internal Medicine, Division of Geriatric Medicine, 1402 S Grand Blvd, Room M238, St. Louis, MO.

0026-0495/99/4808-0017$0.00/0

SUBJECTS AND METHODS

Subjects

One hundred thirty-six subjects (37 men and 99 women) participating in the New Mexico Aging Process Study who had serum samples taken within 3 years of entry into the study (1980 to 1982) and in 1989 and/or 1994 were investigated. A total of 555 sera were obtained. Of 136 participants, one woman who took between 10,000 and 14,000 IU vitamin D each day was excluded. Among the other participants, 45% were taking vitamin D supplements at the initial visit, 52.3% at the final visit, and 26.5% during both evaluations.

The New Mexico Aging Process Study is a longitudinal study of nutrition and aging that began in 1979.[25] The participants are caucasians with above-average income and education who reside predominantly in the area of Albuquerque, NM. Ninety-six percent of the participants are non-Hispanic whites, and 4% are of Hispanic origin; this cohort is not a population-based sample, since the area is approximately one-third Hispanic. Entrance criteria for the study excluded individuals with serious disease(s), including cancer (except for skin) within the past 5 years, recent myocardial infarction, and chronic obstructive pulmonary disease, and treatment with chemotherapeutic, cardiac, respiratory, or antipsychotic medication. Although continued participation was not contingent upon the maintenance of good health, the subjects in the cohort would be considered to have better than average health. All participants provided informed consent, and the study was approved by the Human Subjects Research Review Committee of the University of New Mexico School of Medicine.

To evaluate the effects of aging on serum 25OHD, data from two subgroups were examined. Group A included subjects who did not take vitamin D supplements at any time and in whom both blood samples were obtained during the same season (n = 25, eight men and 17 women). Group B included subjects who were taking oral vitamin D supplements throughout the study and in whom both blood samples were obtained during the same season (n = 23, seven men and 16 women).

Measurement of Serum 25OHD

Measurements were made on single serum samples in the College of American Pathologists–certified Geriatric Medicine Laboratory at St. Louis University. Samples were stored at −70°C, and all specimens from a single individual were analyzed in the same assay. Serum 25OHD concentrations were measured in duplicate using a commer-

Metabolism, Vol 48, No 8 (August), 1999: pp 1028-1032

Table 1. Demographic Variables and Serum 25OHD Levels in Subjects With or Without Vitamin D Supplementation Throughout the Study

Variable	Group A (n = 25)		Group B (n = 23)	
	Baseline	Final	Baseline	Final
Age (yr)	70.6 ± 0.7	82.2 ± 0.8	71.0 ± 0.4	82.8 ± 0.4
Weight (kg)	60.9 ± 1.7	60.0 ± 3.9	65.4 ± 1.0	63.0 ± 1.1
Physical activity (score)	18.7 ± 0.9	14.5 ± 1.3*	18.9 ± 0.6	13.8 ± 0.6*
Serum 25OHD (ng/mL)	26.2 ± 2.2	21.5 ± 1.9*	27 4 ± 1.0	27.7 ± 1.1†

NOTE. Group A were subjects who did not use vitamin D supplements; group B used vitamin D supplements.

*$P < .05$ v baseline.

†$P < .05$, group A v group B.

cially available, competitive protein-binding assay (Nichols Institute, San Juan Capistrano, CA). The intraassay coefficient of variation is 7.7% and the interassay coefficient of variation 11%, as previously reported.[4] The lower limit of detection for serum 25OHD was 4 ng/mL.

Demographic Variables

Physical activity and dietary intake of vitamin D and vitamin D supplements (in international units) were estimated and body weight was measured at the same time blood samples were obtained. Supplemental vitamin D intake was derived not only from multivitamin intake but also from a variety of other over-the-counter vitamin or herbal preparations. Activity levels, both work-related and recreational, were determined by questionnaire as previously described.[13,26,27] Activity was scored from 1 to 35, such that increasing activity yielded a greater score.

Statistical Analysis

Statistical analyses were performed using a commercially available statistics package (Statistica; Statsoft, Tulsa, OK). The month in which each specimen was obtained was coded as follows: January = 1, February = 2, . . . December = 12. The season was coded as follows: winter from January 1 to March 31 (coded as 1), spring from April 1 to June 30 (coded as 2), and summer from July 1 to October 31 (coded as 3). Very few samples were obtained between October 31 and December 31, and these accordingly were not coded. Male gender was coded as 1 and female as 2. A paired Student t test, Pearson's correlation, and multiple regression analysis were used to examine relationships between the measured variables To evaluate potential seasonal variation of serum 25OHD, the best-fit cosinor function for all serum 25OHD concentrations and the month/season they were obtained was derived, as described previously.[28] In the multiple regression analysis, the month

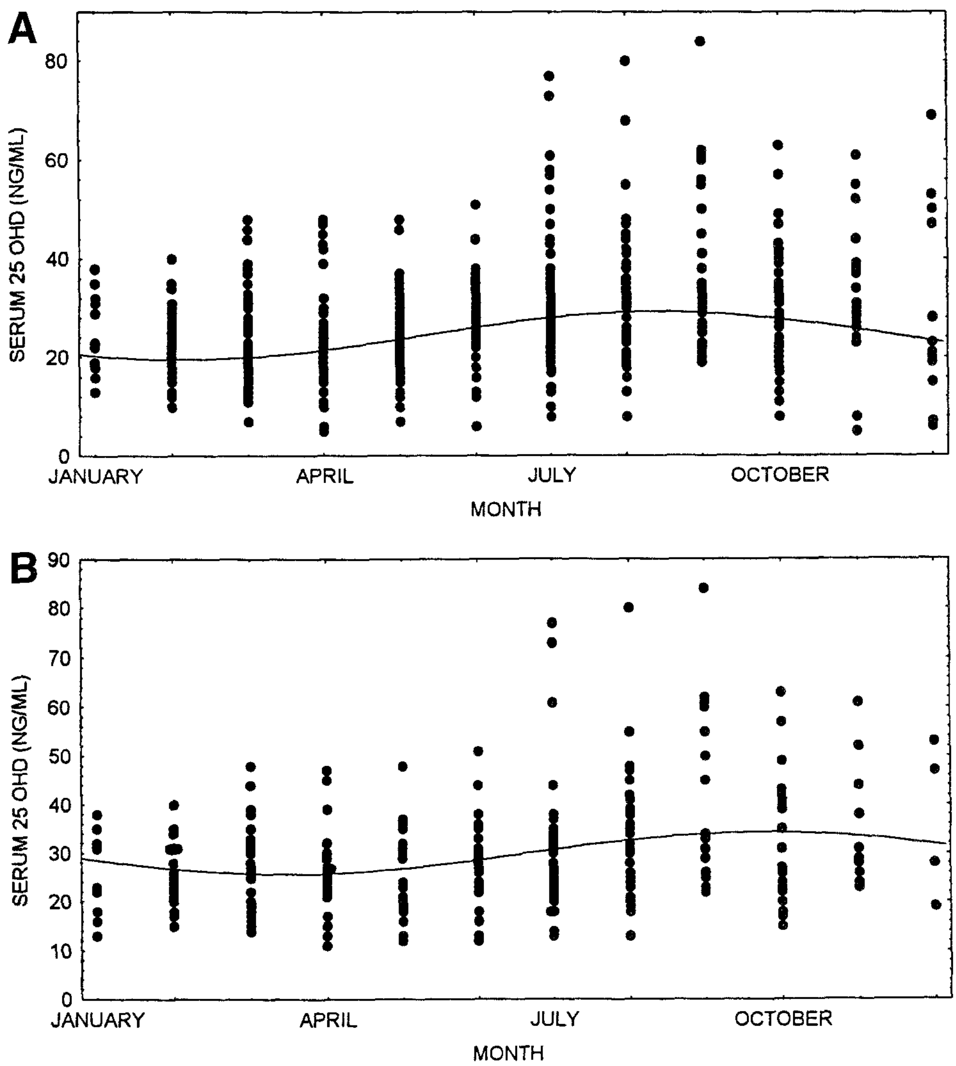

Fig 1. Seasonal variation in serum 25OHD levels during the entire study in (A) subjects who were not taking vitamin D supplements ($r = .324$, $P < .001$) and (B) subjects who were taking vitamin D supplements ($r = .245$, $P < .001$).

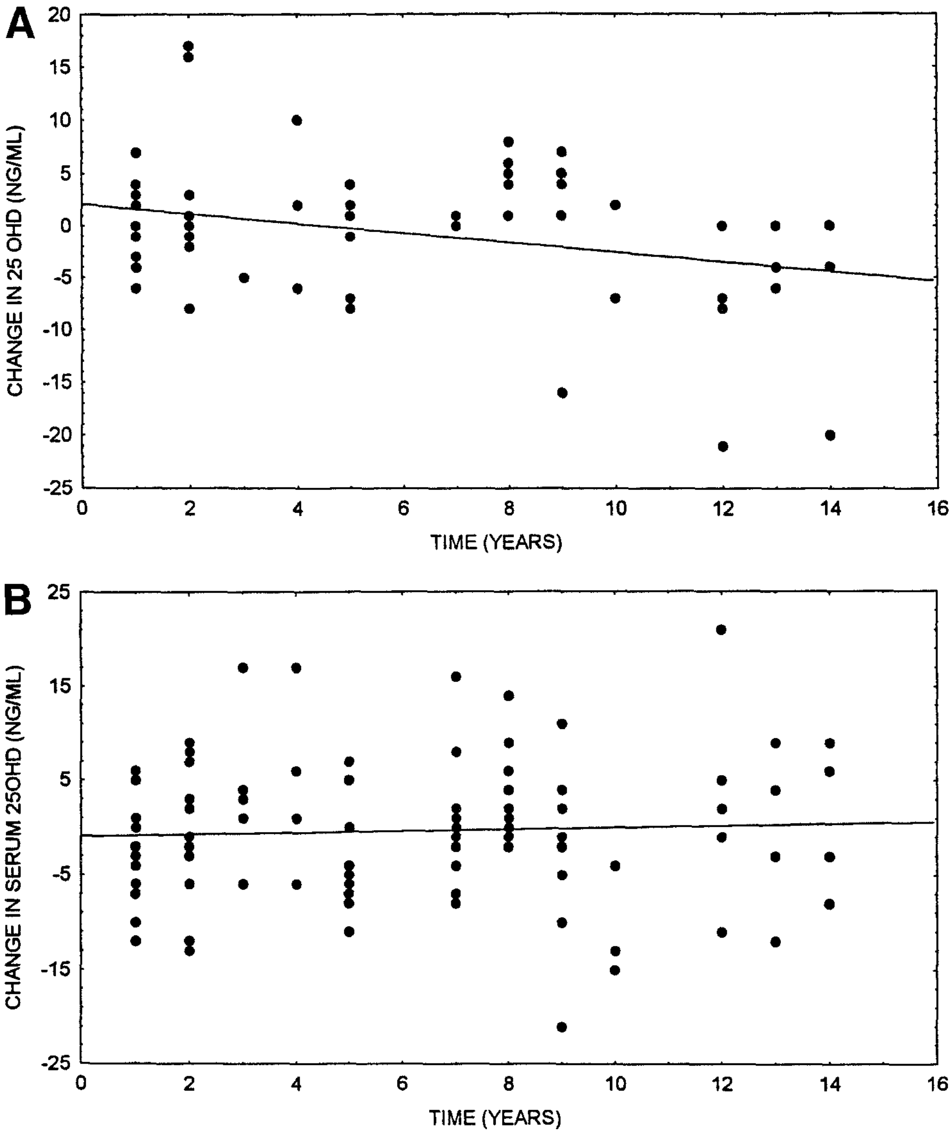

Fig 2. Duration of time in study versus change in serum 25OHD levels obtained throughout the study in subjects tested in the same season. (A) Winter and spring differences ($r = -.307$, $P = .012$). (B) Summer differences ($r = .052$, NS).

in which the sample was obtained was corrected using this cosinor function (corrected month). A P value less than .05 was considered significant. Data are presented as the mean ± SEM.

RESULTS

In subjects who did not take vitamin D supplements (group A), there was a significant decrease in both serum 25OHD and physical activity between the beginning and end of the study ($P < .05$; Table 1). At baseline, serum 25OHD was 12 ng/mL or less (below the lower limit of normal in our laboratory) in two of the 25 subjects, and at follow-up study, it was 12 ng/mL or less in four subjects. Mean dietary vitamin D was 124 IU/d at the beginning of the study and 112 IU/d at the end (nonsignificant [NS]). The baseline serum 25OHD concentration ($r = .42$, $P < .04$) was related to the follow-up serum 25OHD ($r = .69$, $P < .001$), but not to age ($r = -.07$, NS), physical activity ($r = .33$, $P = .16$), dietary vitamin D intake ($r = .26$, NS), or body weight ($r = -.06$, NS). The final serum 25OHD was also related to season ($r = .47$, $P = .02$) and dietary vitamin D intake ($r = .61$, $p = .013$), but not to age ($r = -.07$, NS), physical activity ($r = -.03$, NS), or body weight ($r = -.19$, NS). Physical activity was inversely but nonsignificantly related to age ($r = -.28$, NS) in this subgroup.

In subjects who took vitamin D supplements (group B) at the beginning and end of the study, there was no significant change in serum 25OHD, but a reduction ($P < .05$) in physical activity was observed (Table 1). Baseline serum 25OHD was not significantly higher in group B compared with group A, although the final serum 25OHD was ($P < .05$). In group B, baseline serum 25OHD was significantly related to both the supplemental dose of vitamin D ($r = .54$, $P = .01$) and total vitamin D intake (supplemental plus dietary, $r = .73$, $P = .001$) and the follow-up serum 25OHD level ($r = .46$, $P = .03$), but not to age ($r = .23$, NS), physical activity ($r = .05$, NS), body weight ($r = -.14$, NS), or season ($r = .10$, NS). The final serum 25OHD level also was not related to season ($r = -.19$, NS) or any of the other demographic variables.

Regardless of whether participants took supplemental vitamin D, a seasonal variation in serum 25OHD was evident, with peak levels for both the baseline and the final visit in July to October (Fig 1). In the baseline sample, serum 25OHD correlated with the (cosinor-corrected) month ($r = .42$, $P < .001$), activity level ($r = .22$, $P = .021$), follow-up 25OHD level ($r = .53$, $P < .001$), and dose of vitamin D supplement ($r = .21$, $P = .016$), but not age ($r = -.08$, $P = .35$). The final serum 25OHD was also related to the (cosinor-corrected) month ($r = .21$, $P = .017$), activity level ($r = .27$, $P = .002$), and mean dose of vitamin D ($r = .23$, $P = .027$). There was no significant difference in the mean dose of vitamin D supplementation between the study entry and conclusion (215 ± 28 *v* 193 ± 19 IU, respectively).

In an effort to investigate more closely the effect of age on serum 25OHD, we examined all samples obtained in the same season from a given individual who was not on vitamin D supplementation at the time the samples were obtained. Samples obtained either winter to winter or spring to spring (Fig 2A) demonstrated that the duration of time in the study was associated with decreasing serum 25OHD ($r = -.307$, $P = .012$). Figure 2B demonstrates no significant change in serum 25OHD from summer to summer with the duration of the study ($r = .052$, NS). Age did not predict a decline in 25OHD in samples from winter, spring, or summer (data not shown).

Multiple regression analysis was performed to determine the independent predictors for any serum 25OHD samples for which all parameters were available for analysis. This analysis demonstrated that the physical activity level, dose of vitamin D supplementation, corrected month (using the cosinor function), and gender were independent predictors of serum vitamin D levels (Table 2).

Table 2. Multiple Regression Analysis of Determinants of Serum 25OHD Levels in 135 Subjects (n = 363 samples)

Factor	β	*P*
Cosinor-corrected month	.210	<.001
Physical activity level	.124	.015
Dose of vitamin D	.93	<.001
Gender	−.117	.017

NOTE. For the entire analysis, $r = .423$, $P < .0001$.

DISCUSSION

A decrease in the serum 25OHD concentration with increasing age has been reported in cross-sectional studies.[1-4] In this longitudinal study, we have confirmed that serum 25OHD decreases significantly with age, at a rate of 4.3 ng/dL decade, in subjects who are not taking vitamin D supplements. Vitamin D supplementation prevented this decrease in serum 25OHD levels.

It is of interest that (corrected) month, vitamin D supplementation, and physical activity were independent predictors of serum 25OHD concentrations, but not age per se, despite a considerable age range. These results suggest that despite the series of detrimental changes in 25OHD metabolism reported with age, the major predictors of the 25OHD concentration, apart from vitamin D intake, are related to sunlight exposure, ie, month, physical activity level, and gender. Therefore, the age-related changes in the ability of the skin to photosynthesize vitamin D and the decreased intestinal absorption of vitamin D with aging[5,9] may play only a minor role in the age-related decline in serum 25OHD. These findings are partially in concert with those of Dawson-Hughes et al,[15] who reported that gender, time spent outdoors, and vitamin D intake were the major predictors of serum 25OHD levels. Our results differ in that their group also reported an effect of weight and age. Their subjects were from a significantly more northern latitude, which might account for these differences, since we observed significant changes only in winter and spring (Fig 2). Other groups have also demonstrated a significant relationship between gender and serum 25OHD.[12-14]

Our study also confirms a seasonal variation in 25OHD concentrations that is well described in older persons in cross-sectional and longitudinal studies,[12-17] ie, season was a significant factor in predicting serum 25OHD levels. Vitamin D supplementation increased the baseline but did not abolish the effect of season.

In conclusion, we have demonstrated a decrease in serum 25OHD over time. Despite this demonstration, the independent predictors of serum 25OHD were not related to age, but rather to vitamin D supplementation, gender, activity, and month of sample obtainment. We reconcile these differences by suggesting that the changes observed with age were related to decreased activity, which would result in less sunlight exposure.

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