

Original Research

Plasma Vitamin D and 25OHD Responses of Young and Old Men to Supplementation with Vitamin D₃

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Key words: vitamin D, cholecalciferol, intestinal absorption, 25-hydroxyvitamin D, aging, vitamin supplementation

Objectives: This study was conducted to determine whether there are age differences in the plasma parent vitamin D and 25-hydroxyvitamin D (25OHD) responses to eight weeks of supplementation with 20 $\mu\text{g}/\text{day}$ of vitamin D₃.

Methods: Twenty-five healthy young men (age 18–35) and 25 healthy older men (62–79) were randomly assigned to supplementation with 20 $\mu\text{g}/\text{day}$ of vitamin D₃ or to no intervention and followed for eight weeks. Plasma vitamin D₃ was measured by high performance liquid chromatography and 25OHD was measured by competitive protein binding.

Results: Both young and old men in the supplemented group had pronounced, rapid and similar increases in plasma vitamin D₃, whereas vitamin D₃ concentrations were stable in the control group. By the end of the eight-week adaptation period, plasma vitamin D₃ of young and old men had increased by 4.3 and 6.2 nmol/L respectively. In the supplemented group, mean 25OHD concentrations of both the young and old men increased during the study, and the magnitude of the change after eight weeks was nearly identical in the two age groups (22.5 and 22.1 nmol/L in the young and the old men, respectively). In the control group there was a modest decrease in 25OHD of both the young and old men.

Conclusions: There appears to be no age-related impairment among men in the absorption or metabolism of 20 $\mu\text{g}/\text{day}$ of vitamin D₃ taken orally for at least eight weeks.

INTRODUCTION

Aging reduces the capacity of skin to produce vitamin D when exposed to sunlight [1,2], but the degree to which aging affects the absorption and metabolism of orally consumed vitamin D is less clear. A single high oral dose of vitamin D₂ ($\geq 1250 \mu\text{g}$) produced similar vitamin D increases in young and old subjects [3,4] in two studies, and a smaller increase in 25-hydroxyvitamin D (25OHD) of old compared with young subjects in a third study [5]. Modest single doses of vitamin D₃ resulted in similar [6] or smaller [7] increases in vitamin D and 25OHD among older subjects. Age differences in the effects of prolonged supplementation with vitamin D have been much less studied. In a recent small study, we reported that, compared with young men (age 22–28 years), older men (age 65–73 years) had smaller increases in 25OHD after three weeks of

supplementation with 45 $\mu\text{g}/\text{day}$ of vitamin D₂ [8]. The purpose of the present study was to determine whether the age difference we observed previously would be present when a smaller vitamin D dose was given (20 $\mu\text{g}/\text{day}$) and when subjects were followed for a longer period (eight weeks). In addition, we have now studied vitamin D₃ instead of vitamin D₂ because the former appears to increase serum 25OHD more efficiently than vitamin D₂ [9].

MATERIALS AND METHODS

Subjects

Twenty-six young and 26 older men were randomized and enrolled in the study. Eligible subjects were 18 through 35 or

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62 through 79 years old, had no history of liver or kidney disease or other medical conditions or medications likely to affect vitamin D absorption or metabolism. One of two liver enzymes, alanine aminotransferase or aspartate aminotransferase, was slightly elevated in each of two young men in the supplemented group, and both were allowed to remain in the study. These enzymes were in the normal range for all other subjects. All subjects had a screening plasma 25OHD concentration between 37.4 and 87.4 nmol/L, consumed no more than 5 μg /day of vitamin D per day, had not used vitamin D or calcium supplements in the past six months, had body mass index between 18 and 38 kg/M^2 , usually consumed no more than three alcoholic drinks per day, had not traveled south of the Boston area (latitude 42°N) in the past 60 days and did not plan to travel south during the study, were not employed in outdoor occupations, and had not used a tanning lamp in the past 60 days. Despite the assertion of all subjects that they did not plan to travel south during the study, one young man went to Phoenix, AZ (latitude 33°N), and one older man went to the Dominican Republic (latitude 20°N) during the study. Both of these men were in the control group and have been excluded from all analyses.

Timing of Study Visits

Baseline study visits were conducted from December through mid-February, and the median baseline visit date was January 14th. Randomization was balanced over time: the number of subjects in each group who were measured before the median baseline visit date were six supplemented young, six supplemented old, five control young and eight control old. Follow-up visits took place four, six and eight weeks after baseline, and all study visits were completed by mid-April.

Biochemical Measurements

Fasting was not required prior to the blood draws. Blood was drawn between about 6 AM and 2 PM. For each subject, blood was drawn at the same time (within ten minutes) at each of the four study visits. Plasma vitamin D₃ was measured by high-performance liquid chromatography (HPLC) at Boston University under the direction of Dr. T.C. Chen as previously described [10]. Briefly, an ethyl acetate extract was sequentially exposed to a C-18 cartridge, normal phase HPLC (mobile phase hexane:isopropanol 99.2:0.8), and reverse phase HPLC (mobile phase 25% methanol). The quantity of vitamin D present was determined by its UV absorbance.

Plasma 25OHD was measured in the Nutrition Evaluation Laboratory at Tufts University (NEL) by the competitive protein binding (CPB) method of Chen *et al.* [11], without a preparatory chromatography step. This method results in higher 25OHD estimates than are achieved by HPLC because it does not remove compounds that result in nonspecific interference at the DBP binding site [12]. As we have reported previously, 25OHD values by HPLC are approximately 60% of 25OHD

values by our CPB method [8], whereas 25OHD by radioimmunoassay (Incstar) provides modestly higher estimates than our CPB method [13]. Plasma 1,25(OH)₂D was measured in the NEL with double-antibody radioimmunoassay kits from DiaSorin Inc (Stillwater, MN). Serum intact parathyroid hormone was measured in the NEL with Allegro intact radioimmunoassay kits from Nichols Institute (San Juan Capistrano, CA). Serum total calcium was measured with a Nova 7 calcium analyzer (Nova Biochemical, Waltham, MA).

Other Measurements

Vitamin D intake over the previous four weeks was estimated with a short food frequency questionnaire that has been used extensively in our laboratory. Body mass index was calculated from height measured with a wall-mounted stadiometer and weight was measured with a digital scale.

Statistical Analysis

The primary endpoints in this study were changes in plasma vitamin D and 25OHD after eight weeks of supplementation. Values at interim time points are shown graphically, but were considered of secondary importance because they are less likely than the final measurements to reflect long term responses to supplementation. Unadjusted mean values of subject characteristics and biochemistries are shown in the tables. The effects of supplementation group, age group and their interaction on characteristics and biochemistries were investigated with analysis of variance (ANOVA) in the total study sample ($n = 50$). Two ANOVA models were constructed for each dependent variable. In the first, supplementation group, age group and a supplementation by age group interaction term were included as independent variables. After determining that the interaction term was not significant (at the 0.05 level), a second model that included only supplementation group and age group as independent variables was run. Further analyses examined potential predictors of changes in vitamin D and 25OHD by adding potential predictors as independent variables to those already included in the second ANOVA model. Other analyses included paired *t* tests to determine whether within-group changes in selected variables differed from zero, and partial correlations of selected baseline biochemistries with their changes. *p* values less than 0.05 were considered to indicate statistical significance. Analyses were conducted with SPSS (SPSS Inc, Chicago, IL).

RESULTS

Mean body mass index was lower in the young men than in the older men (Table 1) and did not change significantly during the study in either group. Self-reported vitamin D intakes were substantially below recommended levels in all groups (Table 1). Baseline vitamin D intake and biochemical variables did not

Table 1. Baseline Characteristics of the 50 Subjects

	Mean \pm SD				p^a	
	Supplemented		Control		Age	Supplementation
	Young	Old	Young	Old		
N	13	14	12	11	50	50
Age (years)	28.7 \pm 4.6	72.8 \pm 4.5	26.9 \pm 3.7	70.3 \pm 5.1	<0.001	0.098
Body mass index (kg/m ²)	25.0 \pm 4.9	29.0 \pm 4.3	25.1 \pm 4.2	30.0 \pm 3.2	0.001	0.653
Vitamin D intake (μ g/day)	1.78 \pm 1.38	3.54 \pm 1.90	3.30 \pm 3.39	2.87 \pm 1.47	0.237	0.495
Plasma vitamin D ₃ (nmol/L)	3.14 \pm 1.88	2.00 \pm 2.11	1.65 \pm 1.24	1.98 \pm 2.05	0.395	0.160
Plasma 25(OH)D (nmol/L)	59.9 \pm 16.4	61.5 \pm 15.7	48.9 \pm 17.2	53.8 \pm 18.2	0.512	0.053
Plasma 1,25(OH) ₂ D (pmol/L)	94.2 \pm 16.2	97.2 \pm 23.7	111.0 \pm 15.6	96.7 \pm 32.3	0.447	0.212
Serum calcium (mmol/L)	2.45 \pm 0.06	2.41 \pm 0.11	2.43 \pm 0.06	2.40 \pm 0.11	0.136	0.469
Serum PTH (pmol/L)	3.35 \pm 1.01	4.61 \pm 2.23	4.03 \pm 1.71	4.47 \pm 2.83	0.130	0.634

^a From analysis of variance in which age and supplement group were included as independent variables. Preliminary analyses indicated no significant interactions of age group by supplementation group.

differ significantly by age group or by supplementation group (Table 1), and, among men in the supplemented group, mean baseline 25OHD concentrations were nearly identical.

Vitamin D₃

Both young and old men in the supplemented group had pronounced, rapid and similar increases in plasma vitamin D₃ (Fig. 1), whereas vitamin D₃ concentrations were stable in the control group. By the end of the eight-week adaptation period, plasma vitamin D₃ of young and old men had increased by 4.3 and 6.2 nmol/L respectively (Table 2, $p < 0.005$ for comparison with no change). These changes did not differ significantly by age group, and an age by supplement group interaction term was not statistically significant. Of age group, body mass index, vitamin D intake and baseline vitamin D₃, the only predictor of the change in vitamin D₃ was baseline vitamin D₃. There was no evidence of a supplement group by baseline vitamin D₃ interaction (p for interaction, 0.649), and in the group as a whole ($n = 50$) the correlation of the baseline value with the change, controlled for supplement group, was -0.36 , $p = 0.011$.

25OHD

Changes in 25OHD in the supplemented and control subjects over the eight study weeks are shown in Fig. 1, and mean eight-week changes are shown in Table 2. In the supplemented group, mean 25OHD concentrations of both the old and young men increased during the study ($p < 0.001$ for comparison with no change), and the magnitude of the change after eight weeks was nearly identical in the two age groups (Table 2). In the control group there was a modest decrease in 25OHD ($p < 0.05$ for comparison with no change) of both the young and old men. Among the supplemented men, mean 25OHD concentrations in the young and old subjects by the end of the study were 82.4 ± 11.8 and 83.6 ± 19.0 respectively, and all subjects had achieved 25OHD concentrations between 50 and 120 nmol/L.

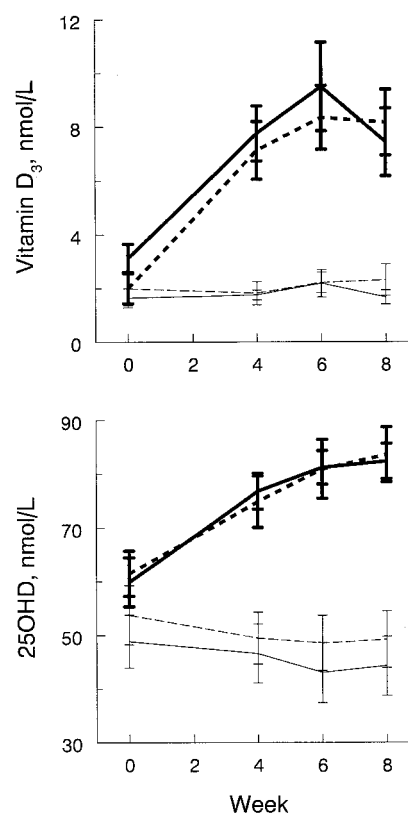


Fig. 1. Mean plasma vitamin D₃ and 25OHD concentrations in young supplemented men (bold solid line), old supplemented men (bold dashed line), young controls (light solid line) and old controls (light dashed line). Error bars indicate standard errors of the mean.

Of age group, BMI, vitamin D intake and baseline 25OHD, the only predictor of the change in 25OHD was baseline 25OHD, and it was predictive only in the supplemented group (p for treatment by baseline 25OHD interaction: 0.047). The linear associations of change in 25OHD with baseline 25OHD in subjects who did and did not receive supplementation are

Table 2. Eight-Week Changes in Laboratory Values

	Mean ± SD				<i>p</i> ^a	
	Supplemented		Control		Age	Supplementation
	Young	Old	Young	Old		
N	13	14	12	11		
Plasma vitamin D ₃ (nmol/L)	4.30 ± 4.39	6.16 ± 5.04	0.02 ± 1.47	0.33 ± 1.72	0.270	<0.001
Plasma 25OHD (nmol/L)	22.5 ± 14.7	22.1 ± 13.4	-4.6 ± 6.1	-4.5 ± 6.5	0.956	<0.001
Plasma 1,25(OH) ₂ D (pmol/L)	28.2 ± 42.6	6.2 ± 18.1	-3.6 ± 22.9	1.5 ± 11.8	0.224	0.023
Excluding 1 subject ^b	18.2 ± 23.4				0.492	0.027
Serum calcium (mmol/L)	-0.01 ± 0.10	-0.02 ± 0.10	-0.04 ± 0.08	-0.04 ± 0.07	0.875	0.315
Serum PTH (pmol/L)	0.24 ± 1.58	-1.07 ± 1.34	0.18 ± 1.72	0.13 ± 1.31	0.096	0.197

^a From analysis of variance in which age and supplement group were included as independent variables. Preliminary analyses indicated no significant interactions of age group by supplementation group.

^b One young man in the supplemented group had a 1,25(OH)₂D value of 228 pmol/L at the eight-week visit compared with 79, 115 and 94 at his previous visits and compared with the highest value of 146 pmol/L for all other subjects at all visits. The subject's other laboratory values were unremarkable.

illustrated in Fig. 2. Inclusion of quadratic terms did not appreciably improve the data fit.

1,25(OH)₂D and PTH

Vitamin D supplementation significantly increased mean 1,25(OH)₂D both before and after the exclusion of one outlying value (Table 2). Although the mean increase was somewhat greater among the younger subjects, the interaction of supplementation with age group did not reach statistical significance. Supplementation did not have any statistically significant effects on serum calcium or serum PTH (Table 2).

DISCUSSION

This study demonstrates that healthy young and old men have similar increases in plasma concentrations of parent vitamin D and 25OHD when they are supplemented with 20

µg/day of vitamin D₃. Although we did not measure vitamin D absorption directly, the similarity of parent vitamin D responses in the two age groups suggests that there is no age-related impairment in absorption of cholecalciferol at the dose given. Since the increases in parent vitamin D were similar, the corresponding similarity in 25OHD responses suggests that the transport and liver hydroxylation of parent vitamin D to 25OHD also remain intact during healthy aging. The minimal 25OHD changes in our control group indicate that use of a December through April measurement period was very effective in preventing any age-related difference in endogenous vitamin D synthesis.

This finding contrasts with those of our previous study in which young men had an almost 90% greater increase in 25OHD than older men after supplementation. The vitamin D dose given in the present study, 20 µg/day, was much lower than that given in the earlier study (120 µg/day), and it may be that a true age-related difference in vitamin D absorption or metabolism becomes apparent only at a moderately high dose. However, there is no obvious physiological reason that this should be the case. Another possible explanation for the different findings is that there is an age difference in the metabolism of vitamin D₂, a synthetic or plant-derived compound not normally present in human circulation, but not vitamin D₃, a physiologic compound in humans. There is evidence in both rats [14] and humans [9,15] that vitamin D₃ supplementation results in a greater increase in 25OHD than does a comparable dose of vitamin D₂, perhaps because of differing rates of enzymatic 25-hydroxylation in the mitochondrial fraction [16,17] or because some vitamin D₂ but no vitamin D₃ is metabolized to 24OHD instead of 25OHD [14]. Though speculative, it is possible that the relative importance of these mechanisms changes with age.

The results of this study are consistent with the preservation of intestinal fat absorption in aging. Vitamin D absorption is impaired in patients with intestinal fat malabsorption syndromes [18,19], but there is little evidence of a general decline

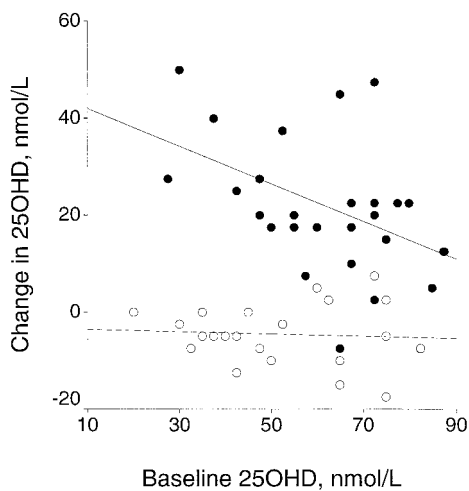


Fig. 2. Associations of the eight-week change in 25OHD with baseline 25OHD in supplemented subjects (*r* = -0.44, *p* = 0.021) and controls (*r* = -0.07, *p* = 0.764).

in fat absorption in healthy aging. On the contrary, Arora *et al.* in a study of over 100 healthy subjects given controlled diets on a metabolic ward demonstrated preservation of intestinal fat absorption over a wide age range [20].

The study was well-powered to detect meaningful supplement and age-related changes in vitamin D and 25OHD, but, consistent with previous studies, changes in 1,25(OH)₂D and PTH were much more variable, and our power to detect meaningful group differences in these measurements was limited. We observed no significant suppression of PTH with supplementation, but we did observe increases in 1,25(OH)₂D. Need *et al.* observed an inverse association between 25OHD and 1,25(OH)₂D in postmenopausal women with 25OHD concentrations up through 40 nmol/L but a positive association in women with higher 25OHD [21], such as the subjects we studied (about 60 nmol/L). They suggested that this may indicate that at low but not higher 25OHD concentrations, increased PTH-stimulated hydroxylation compensates for reduced vitamin D substrate. However, Barger-Lux *et al.* found no significant effect of vitamin D supplementation with 25 μg/day on 1,25(OH)₂D in young men [22] with baseline 25OHD and changes in 25OHD similar to those we observed.

CONCLUSION

There appears to be no age-related impairment among men in the absorption or metabolism of 20 μg/day of vitamin D₃ taken orally. Since estrogen status influences 25OHD concentrations, it is possible that the finding would have been different in women [23,24]. Although we found similar age responses to a 20 μg/day intake, this study cannot rule out the possibility that age differences in vitamin D absorption or metabolism do exist at lower vitamin D intake levels. The current adult dietary reference intakes for vitamin D are based on the assumption that “no vitamin D is available from sun-mediated cutaneous synthesis,” and range from 5 μg/day for ages 19–50 to 15 μg/day for ages 71 and older [25]. Studies that examine a variety of vitamin D doses are needed to determine whether age-group specific reference intakes are warranted for adults.

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