

Calcium Absorptive Effects of Vitamin D and Its Major Metabolites*

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ABSTRACT

The absorptive response to graded doses of vitamin D₃, 25(OH)D, and 1,25(OH)₂D was measured in healthy adult men after treatment periods of eight, four, and two weeks, respectively. While no relationship was found between baseline absorption and serum vitamin D metabolite levels, all three vitamin D compounds significantly elevated ⁴⁵Ca absorption from a 300 mg calcium load given as part of a standard test meal. 1,25(OH)₂D was active even at the lowest dose (0.5 μg/day), and the slope was such that doubling of absorption would occur at an oral dose of approximately 3 μg/day. 25(OH)D was also active in elevating absorption and did so without raising total 1,25(OH)₂D levels. On the basis of the dose response curves for

1,25(OH)₂D and 25(OH)D, the two compounds exhibited a molar ratio for physiological potency of approximately 100:1. The absorptive effect of vitamin D₃ was seen only at the highest dose level (1250 μg, or 50,000 IU/day) and was apparently mediated by conversion to 25(OH)D. Analysis of the pooled 25(OH)D data from both the 25(OH)D- and vitamin D₃-treated groups suggests that approximately one eighth of circulating vitamin D-like absorptive activity under untreated conditions in winter may reside in 25(OH)D. This is a substantially larger share than has been predicted from studies of *in vitro* receptor binding. (*J Clin Endocrinol Metab* 82: 4111–4116, 1997)

SINCE THE discovery and elucidation of the activity of 1,25-dihydroxycholecalciferol, [1,25(OH)₂D], the major precursor vitamin D compounds, cholecalciferol (vitamin D₃) and 25-hydroxycholecalciferol [25(OH)D] have generally been considered to be physiologically unimportant except insofar as they constitute the precursor pool for the hormonally active form of the vitamin. In various receptor-binding or tissue culture assays they exhibit molar potencies that are, respectively, 3–4 and 6–7 orders of magnitude lower than 1,25(OH)₂D (1, 2). Nevertheless, *in vitro* studies have shown that 25(OH)D promotes calcium absorption in its own right (3). Moreover, when calcium absorption has been measured in humans *in vivo*, using sensitive calcium tracer techniques, circulating 25(OH)D levels have generally correlated better with observed absorption efficiency than have levels of 1,25(OH)₂D (4–7).

Those findings suggested that 25(OH)D may itself promote calcium absorption at physiological concentrations. That conclusion was directly supported by a pair of studies, in which Brickman *et al.* (8) and Colodro *et al.* (9) measured ⁴⁷Ca absorption in response to graded doses of 25(OH)D and of 1,25(OH)₂D. They found, in normal subjects, a molar potency for 25(OH)D only two orders of magnitude lower than for of 1,25(OH)₂D. In view of the fact that physiological concentrations of 25(OH)D are typ-

ically three orders of magnitude greater than those of 1,25(OH)₂D, this relative potency suggested that 25(OH)D might account for a larger fraction of the vitamin D-like activity in the circulating plasma than was generally considered likely and could therefore explain the results of clinical studies.

For these reasons, we judged that the relative roles of vitamin D and its metabolites in the normal calcium economy needed to be re-evaluated. Accordingly, the purposes of the present study were 1) to determine the dose-response relationships of vitamin D and its principal metabolites, using calcium absorption efficiency in healthy young adults as the response variable; 2) to compare the findings with the earlier, provocative results of Colodro *et al.* (9); and 3) to estimate the relative contributions of 25(OH)D and 1,25(OH)₂D to calcium absorption under physiological conditions.

Subjects and Methods

Subjects

The subjects were men who were free of health problems and who took no medications known to affect calcium metabolism or skeletal physiology, as determined by interview. To limit the effects of nonstudy sources of vitamin D, we performed the tests in midwinter and excluded candidates who either reported usual milk consumption of more than 0.47 L/day (16 fl oz/day) or who would be exposed to sunlight (via travel to a sunny climate) during the study or in the preceding month. We accepted several candidates who regularly used daily multivitamins (typically containing 10 μg of vitamin D, equivalent to 400 IU), but who agreed to abstain from them during their participation in the study and for at least one week before entering it. The Creighton University Institutional Review Board approved the protocol, and each subject gave informed consent. After entering, two subjects withdrew for personal reasons. The 116 who finished the study included two Hispanics, three blacks, five subjects of Asian origin, and 106 whites. Table 1 presents data

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characterizing them. There were no significant differences in anthropometric variables or serum chemistries in the men assigned to the three agents. However, baseline absorption fraction for those assigned to 25(OH)D was slightly, but significantly, lower than for those receiving 1,25(OH)₂D (0.250 vs. 0.289).

Study protocol

The study was planned to measure within-individual changes in calcium absorption efficiency in response to three doses each of vitamin D₃, 25(OH)D, and 1,25(OH)₂D. Dosage levels and duration of treatment for the nine groups were as follows:

- Vitamin D₃ at 25, 250, and 1250 µg/d¹ for eight weeks
- 25(OH)D at 10, 20, and 50 µg/d² for four weeks
- 1,25(OH)₂D at 0.5, 1.0, and 2.0 µg/d³ for two weeks

Subjects were assigned to one of the three vitamin D compounds on the basis of personal scheduling considerations, but were allocated to dosage level within compound by use of a random number scheme. Vitamin D₃ capsules in each dosage were custom-manufactured by Tishcon Corp. (Westbury, NY) and their potency confirmed by analysis of their vitamin D₃ content. 25(OH)D was given in the form of Calderol (provided without charge by Organon, Inc., West Orange, NJ), and 1,25(OH)₂D was given as Rocaltrol (provided without charge by Roche Laboratories, Nutley, NJ). Materials were repackaged by qualified pharmacy personnel in fully-labeled, child-resistant vials. The subjects were instructed to take their capsules by mouth at bedtime.

Subjects returned their medication vials at the time of the second absorption test, and the remaining pills were tallied. Compliance was very high (mean: 98.9%; median: 100%). Actual daily dosage, used in certain of the analyses, was calculated from the number of pills consumed, divided by the number of days on treatment.

Each subject was tested twice—immediately before and after his prescribed treatment period. At both visits, the following measurements were made: total calcium, vitamin D₃, 25(OH)D, and 1,25(OH)₂D in fasting serum; height by stadiometer; weight, in light clothing without shoes, by use of an electronic digital strain gauge scale (Healthometer, Bridgeview, IL); and calcium absorption efficiency. Thus, the serum samples obtained for vitamin D metabolite determinations were collected just a few minutes before performing each calcium absorption test. All visits took place between January 3 and April 3, to coincide with the annual nadir of circulating 25(OH)D levels related to sunlight intensity in the temperate northern hemisphere, and thus to minimize the effect of variations in solar vitamin D.

Calcium absorption testing

Calcium absorption efficiency was measured as absorption fraction by use of a 5-hr single isotope method described previously (10–11). This method measures total absorption, both active and passive, and has been shown to yield the same results as produced by classical balance methods adjusted for endogenous fecal calcium losses (12). The standard deviation of the difference between replicate measurements in the same individual with this method is 0.042. As each dosage group contained 12–14 individuals, that level of precision meant that we had a power of 0.90 to find a difference of 0.043 or greater, within each treatment group.

The calcium load was 7.5 mmol, given as milk and labeled with approximately 185 kBq (equivalent to 5.0 µCi) ⁴⁵Ca contained in a submicrogram quantity of high specific activity ⁴⁵CaCl₂ (Amersham, Arlington Heights, IL). The tracer was added to the milk 16 hr before dosing, and individual servings were sealed and refrigerated until use. The test load was given in the morning as a part of a standard breakfast, after an overnight fast, and with abstinence from alcohol for at least 3 days. Three subjects who identified themselves as milk-intolerant took two caplets of Lactaid Extra Strength dietary supplement (McNeil Consumer Products Co., Ft. Washington, PA) with the test load (at both tests). The breakfast consisted of three slices of unenriched white bread toast, buttered, with a serving of water, coffee, or tea (no sugar was allowed). The meal was consumed under supervision. Halfway through

TABLE 1. Subject descriptive data at baseline^a

	Median	Inter-quartile range	
		From	To
Age (yr)	27.2	25.5	30.4
Height (m)	1.78	1.74	1.83
Weight (kg)	81.4	73.8	88.7
Body mass index (kg/m ²)	25.3	23.8	27.3
Fasting serum Ca (mmol/L)	2.41	2.37	2.46
Fasting serum P (mmol/L)	1.26	1.16	1.35
Serum vitamin D (nmol/L)	8	5	10
Serum 25(OH)D (nmol/L)	69	53	84
Serum 1,25(OH) ₂ D ₃ (pmol/L)	91	79	103
Ca absorption fraction	0.295	0.251	0.352

^a *n* = 116 for all variables except serum vitamin D, for which *n* = 69.

the breakfast, the labeled milk was consumed. To assure full ingestion of the tracer, the milk container was rinsed repeatedly with distilled water and the rinsings consumed. Fluid intakes were standardized to provide a similar degree of gastric distention for all participants. At the second visit (post-treatment), correction was made for residual serum calcium radioactivity from the earlier test.

The absorption method has been calibrated in female subjects against the double-isotope reference method (13), by adjusting for body size (and, thus, volume of distribution) using empirical power functions of weight and height (10, 11). To estimate absorption fraction in the male subjects in the present study, we adjusted the results from the use of this algorithm upwards by a factor of 1.15, to reflect the relative difference in body water for males and females of the same body mass index (14). However, for the principal purposes of the present study, we collected data on within-subject change. Therefore, none of the statistical analyses are dependent upon the method of calculation of absorption, nor upon adjustment for male-female body compositional differences. We use adjusted fractional absorption in what follows solely to facilitate discussion of the results in terms of a familiar physiological phenomenon.

Analytical methods

Serum Ca was measured by atomic absorption spectrophotometry (model 2380, Perkin-Elmer Corp., Norwalk, CT). Serum samples for vitamin D metabolites were protected from light during processing, frozen promptly at -20 C, and shipped on dry ice to the Vitamin D Skin and Bone Research Laboratory of the Boston University Medical Center for analysis. The Vitamin D₃ assay involved lipid extraction of the serum specimens, fractionation by use of rapid reverse-phase cartridge chromatography and high-performance liquid chromatography, and quantitation by use of ultraviolet absorptiometry, with a detection limit of 2.6 nmol/L (15). Vitamin D levels were measured in all subjects assigned to receive vitamin D itself but in only a 40% random sample of the others. The assay for 25(OH)D [25(OH)D₂ and 25(OH)D₃] involved ethanol extraction of the serum, followed by a protein-binding assay, with a detection limit of 12.5 nmol/L (16). The assay for 1,25(OH)₂D [1,25(OH)₂D₂ and 1,25(OH)₂D₃] involved solvent extraction of the serum, followed by a radio-receptor assay, with a detection limit of 12 pmol/L (17). Pre- and post-treatment samples for a given subject were tested in the same run.

Statistical methods

The CRUNCH software package, version 4.04 (CRUNCH Software Corp., Oakland, CA) was used for all statistical analyses. Measured changes for each treatment group were tested against a null hypothesis of no difference both by use of the Student's *t* test for matched pairs, and by standard regression models (in which both dose and log of dose were used as the X-variable). Baseline values for the various variables in the three treatment groups prior to dose randomization were tested by ANOVA. Dose-response relationships were tested by standard bivariate and multivariate linear regression.

¹ equivalent to 1 000, 10 000, and 50 000 IU/day, and 0.065, 0.65, and 3.25 nmol/day, respectively.

² equivalent to 0.025, 0.05, and 0.125 nmol/day.

³ equivalent to 1.2, 2.4, and 4.8 nmol/day.

Results

Baseline relationships

Relationships between vitamin D metabolite levels and absorption fraction at baseline were tested by both simple univariate regression and by multiple regression models, combining serum 1,25(OH)₂D and 25(OH)D levels as independent variables. Even with the pooled sample ($n = 116$), no statistically significant relationships with baseline absorptive performance were found for serum levels of either metabolite.

Changes in calcium absorption efficiency

Table 2 provides summary data for within-subject changes in calcium absorption fraction, both in absolute values and as a percent of baseline, for each of the nine treatment groups. Significant increases in calcium absorption efficiency occurred in seven of the nine groups. The highest dose of 1,25(OH)₂D elevated absorption efficiency by 73% and the highest dose of vitamin D by 44 percent. Out of the nine treatment groups, the only ones failing to show a significant absorptive increase were those who received the two lower doses of vitamin D₃. While 1,25(OH)₂D treatment elevated serum 1,25(OH)₂D levels as expected, neither 25(OH)D nor vitamin D₃ produced changes in serum 1,25(OH)₂D levels (Table 3). Thus the increases in absorption efficiency for 25(OH)D and for vitamin D₃ were not due to conversion to circulating total 1,25(OH)₂D.

Dose-response relationships

Figure 1 compares the relationships of doses of 1,25(OH)₂D and 25(OH)D and percent change in calcium absorption fraction, and Table 4 presents the parameters of the relationships using absolute absorptive change (rather

than percent change). (In Table 4, "adjusted dose" refers to the assigned dose, adjusted both for compliance and for body weight.)

For all regressions, the r -value was slightly improved when adjusted dose was used instead of assigned dose. As is visually evident, the slope for 1,25(OH)₂D was substantially greater than that for 25(OH)D, so the two relationships are displaced from one another by differing amounts at differing doses. Thus, to estimate the relative potency of 25(OH)D and 1,25(OH)₂D, we calculated the quantity of each required to produce a 25% increase in absorption efficiency. (While arbitrary, the 25% figure nevertheless reflects a physiologically interesting change.) As shown by the *dashed line* in Fig. 1, a mean increase of 25% in absorption efficiency was produced by both 50 $\mu\text{g/day}$ of 25(OH)D and 0.5 $\mu\text{g/day}$ 1,25(OH)₂D, for a relative molar potency of the two compounds *in vivo* of 1:100. The zero effect doses for the two lines (*i.e.* the X-axis intercepts) differ by a ratio of only 1:16.

25(OH)D and calcium absorption efficiency

In multiple regression models of the absorptive change in the vitamin D₃-treated subjects, testing serum levels of all three compounds as independent variables, only post-treatment serum 25(OH)D proved to be significantly associated with absorption fraction. Accordingly, to explore further the relationship between 25(OH)D and calcium absorption, the data from the vitamin D₃ and 25(OH)D-treated subjects were pooled. Figure 2 shows the relationship of change in circulating 25(OH)D produced by treatment and change in calcium absorption efficiency. (The *open circles* represent the subjects who took 25(OH)D, and the *closed circles*, those who took vitamin D₃.) As can be seen, the largest increases in circulating 25(OH)D were produced by vitamin D₃ (high dose). As the figure also suggests, the two groups can be

TABLE 2. Calcium absorption fraction; pretreatment values^a and changes with treatment^b

Agent: Vitamin D ₃					
25 $\mu\text{g/d}$ ($n = 13$)		250 $\mu\text{g/d}$ ($n = 11$)		1250 $\mu\text{g/d}$ ($n = 14$)	
Pre-treatment	Change	Pre-treatment	Change	Pre-treatment	Change
0.330 \pm 0.12	+0.014 \pm 0.01 +7.89 \pm 4.3	0.306 \pm 0.07	+0.001 \pm 0.02 +3.10 \pm 5.8	0.280 \pm 0.08	+0.113 \pm 0.01 ^c +44.02 \pm 6.8
Agent: 25(OH)D					
10 $\mu\text{g/d}$ ($n = 13$)		20 $\mu\text{g/d}$ ($n = 14$)		50 $\mu\text{g/d}$ ($n = 14$)	
Pre-treatment	Change	Pre-treatment	Change	Pre-treatment	Change
0.292 \pm 0.06	+0.031 \pm 0.01 ^d +9.79 \pm 3.7	0.293 \pm 0.06	+0.046 \pm 0.01 ^e +17.37 \pm 5.1	0.278 \pm 0.04	+0.065 \pm 0.01 ^e +24.99 \pm 4.1
Agent: 1,25(OH) ₂ D					
0.5 $\mu\text{g/d}$ ($n = 12$)		1.0 $\mu\text{g/d}$ ($n = 13$)		2.0 $\mu\text{g/d}$ ($n = 12$)	
Pre-treatment	Change	Pre-treatment	Change	Pre-treatment	Change
0.338 \pm 0.07	+0.076 \pm 0.01 ^f +23.77 \pm 4.0	0.338 \pm 0.06	+0.216 \pm 0.02 ^c +65.09 \pm 5.5	0.320 \pm 0.06	+0.233 \pm 0.03 ^c +72.55 \pm 10.5

Note: all P -values are with respect to zero change; d, day.

^a mean \pm SD.

^b mean \pm SEM; absorption fraction adjusted for male body composition (see text); the second row in each cell is the mean (\pm SEM) of the individual percent changes from baseline.

^c $P < 0.0001$.

^d $P < 0.05$.

^e $P < 0.01$.

^f $P < 0.001$.

TABLE 3. Serum 1,25(OH)₂D₃ (pmol/L): pre-treatment values^a and changes with treatment^b

Vitamin D ₃					
25 µg/d (n = 13)		250 µg/d (n = 11)		1250 µg/d (n = 14)	
Pre-treatment	Change	Pre-treatment	Change	Pre-treatment	Change
94.7 ± 24	-6 ± 6	84.4 ± 17	2 ± 8	95.0 ± 21	-11 ± 7
25(OH)D ₃					
10 µg/d (n = 13)		20 µg/d (n = 14)		50 µg/d (n = 14)	
Pre-treatment	Change	Pre-treatment	Change	Pre-treatment	Change
90.6 ± 24	+11 ± 6	86.7 ± 16	+3 ± 5	93.1 ± 18	+7 ± 4
1,25(OH) ₂ D ₃					
0.5 µg/d (n = 13)		1.0 µg/d (n = 13)		2.0 µg/d (n = 12)	
Pre-treatment	Change	Pre-treatment	Change	Pre-treatment	Change
94.6 ± 14	10 ± 4 ^c	90.6 ± 18	46 ± 7 ^d	96.8 ± 27	60 ± 6 ^e

Note: all *P*-values are with respect to zero change.

^a mean ± SD.

^b mean ± SEM.

^c *P* < 0.05.

^d *P* < 0.001.

^e *P* < 0.0001.

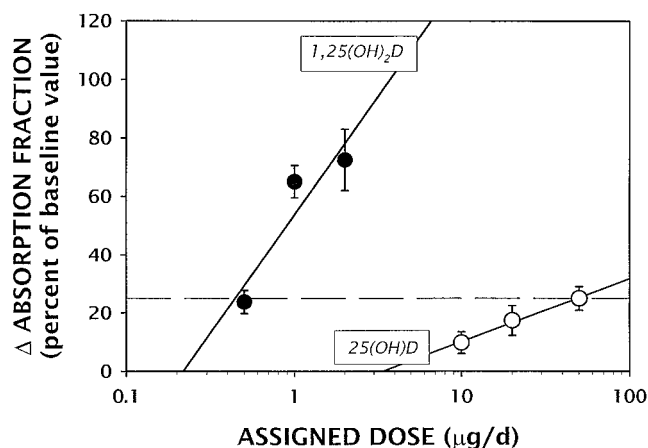


FIG. 1. Log-dose response relationships for 1,25(OH)₂D and 25(OH)D. The response is expressed as the percent change from baseline (untreated) values. The horizontal, dashed line represents a 25% increase in absorption fraction. (Copyright Robert P. Heaney, 1997. Reproduced with permission.)

represented by the same regression line. (This approach was validated by performing the regression calculations separately for the two treatment groups. The slopes and intercepts were numerically very close and did not differ significantly from one another.) The effect of change in 25(OH)D was apparent even when the analysis was restricted to the lower end of the distribution (e.g. an increase in 25(OH)D of <200 nmol/L). For this subgroup, the mean change in absorption efficiency was $+0.030 \pm 0.006$, SEM; *P* < 0.01). The slope of the composite regression, shown in Fig. 2, means that absorption fraction changed by $+0.000146$ for every nmol/L increase in serum 25(OH)D.

The relationship between serum 25(OH)D and absorption in the groups treated with 25(OH)D and vitamin D₃ was also evaluated using absolute post-treatment values for both variables (rather than changes in each evoked by treatment). Figure 3 presents the results. The slope of absorption on 25(OH)D level was $+0.0000849$, substantially the same as

TABLE 4. Regression parameters describing the relationships of change in Ca absorption fraction (as the *y* variable) and the log of vitamin D metabolite dosage (as the *x* variable)

	Slope	Intercept	<i>r</i>
25(OH)D ₃ (n = 41)			
Assigned dosage	0.0215	-0.0187	0.3036 ^b
Adjusted dosage	0.0217	0.0763	0.3163 ^c
1,25(OH) ₂ D ₃ (n = 38)			
Assigned dosage	0.1335	0.1848	0.7689 ^d
Adjusted dosage	0.1331	0.7695	0.8087 ^d

Values are expressed as µg/kg/d and are derived both from assigned dosages and from dosage adjusted for body weight and subject compliance.

^a *P* < 0.001.

^b *P* = 0.054.

^c *P* < 0.05.

^d *P* < 0.0001.

found via analysis of treatment-induced changes. When multiple regression models were constructed, serum 1,25(OH)₂D level did not contribute significantly; nevertheless, when values were adjusted in the model for 1,25(OH)₂D, the slope was $+0.0000934$.

Discussion

We show here that both Vitamin D₃ and 25(OH)D produce dose-dependent increases in calcium absorption efficiency in healthy adult men, and that they do so without evoking a detectable rise in circulating total 1,25(OH)₂D. The effect of vitamin D₃ appears to be explainable largely, if not entirely, by conversion to 25(OH)D, there being no correlation between circulating vitamin D₃ levels and absorption after adjusting for serum 25(OH)D. Thus, these findings confirm previous reports (4–7, 9) that exogenous 25(OH)D exerts absorptive activity in humans in its own right. As shown in Figure 4, our dose-response data for 25(OH)D are remarkably similar to those reported earlier by Colodro *et al* (9). Further, as reported above and shown in Fig. 1, the dose-response data indicate that the absorption-promoting activ-

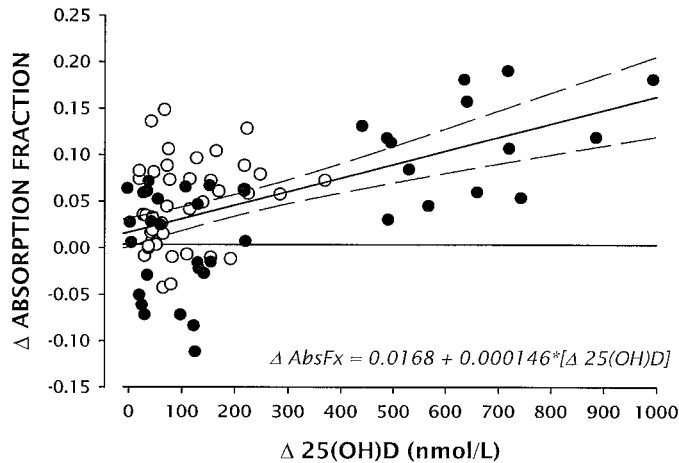


FIG. 2. Absolute change in absorption fraction expressed as a function of induced change in serum 25(OH)D. The solid symbols represent values in subjects receiving vitamin D₃ and the open symbols, those receiving 25(OH)D. The least squares regression line and its 95% confidence interval are shown, as well as the zero, or no-change, line. (Copyright Robert P. Heaney, 1997. Reproduced with permission.)

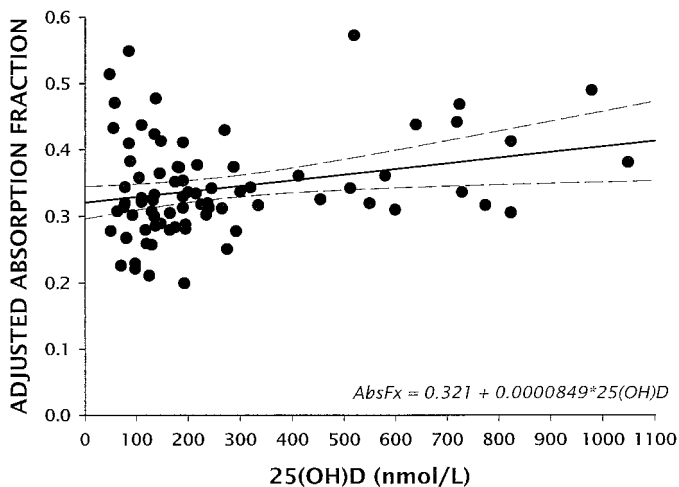


FIG. 3. Absolute value for absorption fraction at the second test, expressed as a function of absolute serum 25(OH)D at that same visit. The least squares regression line and its 95% confidence limits are indicated. (Copyright Robert P. Heaney, 1997. Reproduced with permission.)

ity of an increment in circulating 25(OH)D is 1/100th as great as that of 1,25(OH)₂D. This is very close to the value of 1/125 reported by Colodro *et al.* (9).

In brief, it is clear that calcium absorption efficiency in healthy adults can be increased by elevating circulating levels of 25(OH)D, and that when given orally, 25(OH)D exerts an effect on absorption, relative to that of 1,25(OH)₂D, which is substantially greater than would be predicted from the receptor binding affinities of the two substances. What is less clear is precisely how 25(OH)D may be acting and how much absorptive activity under physiological conditions can be attributed to 25(OH)D, and how much to 1,25(OH)₂D. There is a related question regarding how much of basal absorptive activity is vitamin D-mediated and how much is passive.

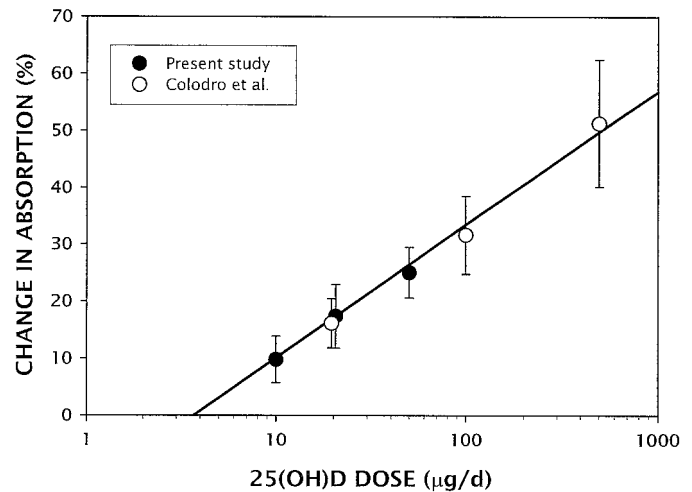


FIG. 4. Comparison of present study data for absorptive response to 25(OH)D with previously published data by Colodro *et al.* (8). As can be seen, the relationship is effectively identical in the two studies. (Copyright Robert P. Heaney, 1997. Reproduced with permission.)

The absence of a detectable relationship between D-metabolite level and absorption fraction under baseline (untreated) conditions complicates any attempt to address these issues. A partial explanation lies in the fact that the absorption method used measures total absorption, passive as well as active. At calcium loads of the size used in this study, about half of total absorption is probably passive (18, 19), *i.e.* not vitamin D-mediated; variations around that partition, coupled with analytical variability, might well obscure an underlying relationship, given values for both variables only within the normal range. However, the clear effects of induced increments in D-metabolite levels (which serve to expand both ranges) may permit an approach to partition of absorptive activity between the D metabolites at baseline.

The slope of absorption fraction on serum 25(OH)D, adjusted for 1,25(OH)₂D, ranged from +0.0000849 in the univariate model to +0.0000934 in the multivariate model. The corresponding slope from our earlier study, in which we had also augmented 25(OH)D levels (6), was +0.00070. Because the confidence limits of neither slope excluded the other, we pooled the two estimates, weighting for differences in sample size, to yield a composite value of +0.000299 (*i.e.* absorption fraction rises by an increment of 0.000299 for every rise in serum 25(OH)D of 1 nmol/L). Because the mean 25(OH)D level at baseline was 68 nmol/L, this approach suggests that, of the total absorption value (0.298), about 0.020 was related to circulating 25(OH)D.

A similar analysis of the absorption activity related to 1,25(OH)₂D yielded an estimate of 0.128. The total for 1,25(OH)₂D and 25(OH)D is thus 0.128 + 0.020, or 0.148, *i.e.* just about half of actual total absorption. This total is consistent with previous estimates of passive absorption in the range of 10–20% of intake (18–20). Thus, taking this approach, about one eighth of total circulating vitamin D absorptive activity in these subjects can be attributed to 25(OH)D, and seven eighths to 1,25(OH)₂D.

The relatively small slopes of absorption fraction on

serum 25(OH)D levels discussed in the foregoing would seem to be somewhat at variance with the dose-response effect seen in Fig. 1. There, a 50 μg dose of 25(OH)D evoked a 25% increase in absorption efficiency. Yet the rise in serum 25(OH)D required to produce a 25% increase in absorption (*i.e.* +0.0745), from the equation in Fig. 2 (*i.e.* a change of +395 nmol/L), is substantially higher than the mean change actually produced in serum 25(OH)D at the 50 μg dose level. In other words, the absorptive response appears greater than would be expected for the evoked rise in serum 25(OH)D. This discrepancy opens up the possibility that some of the absorptive effect of these agents was mediated by a first-pass effect on the intestinal mucosa during the compound's own absorption. Mortensen and Charles (21) have reported what may be a similar first pass effect of vitamin D itself. (The concentration of the D-metabolite within the mucosa could, of course, be many orders of magnitude higher at the time of its absorption than its subsequent serum level.)

Alternatively, there may have been some conversion of vitamin D₃ to 5,6-trans-vitamin D₃, as has been reported in both rats and humans (22–24). This metabolite is a pseudo 1 α (OH) analog of 1,25(OH)₂D₃ and has been reported to increase intestinal calcium transport in anephric rats (22). Yet another explanation, suggested by Veith (25) as the basis for the enhanced calcium absorption of vitamin D toxicity, is based on the fact that 25(OH)D binds more tightly than 1,25(OH)₂D to circulating D-binding protein (DBP). Elevated 25(OH)D₃ levels, therefore, could displace 1,25(OH)₂D from DBP and thereby increase its free concentration. Thus 1,25(OH)₂D would be more active, and absorption would rise, without a change in the total measured 1,25(OH)₂D concentration.

As noted earlier, we performed these studies in late winter to minimize variations in vitamin D status in our subjects. Nevertheless, there is no reason to suspect actual vitamin D insufficiency in any of them. All subjects were healthy and well-nourished. Moreover, at no dose did vitamin D produce an increase in total serum 1,25(OH)₂D level. This is in contrast with the study of Francis *et al.* (4) who found that giving less than 1000 IU of extra vitamin D to older women in the north of England produced changes in both 1,25(OH)₂D and absorption fraction. We found no such changes at the 1000 IU treatment level, and we interpret this difference to mean, therefore, that the changes in absorption produced by both 25(OH)D and vitamin D in this study do not represent simple repair of a nutritional deficit. Rather than enhancing 1- α hydroxylation by expanding the precursor pool for 1,25(OH)₂D, these compounds, at the dosages used, produce an absorptive effect in their own right. Clinicians and investigators using these compounds at high doses will want to be aware of this effect.

In conclusion, both 25(OH)D and vitamin D₃ itself, in large doses, can raise calcium absorption efficiency. Neither compound produces an increase in circulating total 1,25(OH)₂D. The absorptive effect of vitamin D₃ seems mainly to be mediated by its conversion to 25(OH)D. Whether this effect occurs directly by binding to the vitamin D receptor, by a topical (first pass) effect in the intestine, by formation of the 5,6-trans metabolite, or by increasing free 1,25(OH)₂D is still to be resolved. Calculations from the data produced in this study suggest that

up to one eighth of total vitamin D absorptive activity under conditions of health may be the result of circulating 25(OH)D. This activity of 25(OH)D, plus its relatively long half time in plasma, may be the reason 25(OH)D activity is often reported to be better correlated with absorption than the more labile serum 1,25(OH)₂D (4–7).

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