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Randomized comparison of the effects of the vitamin D_3 adequate intake versus 100 mcg (4000 IU) per day on biochemical responses and the wellbeing of patients

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Abstract

Background: For adults, vitamin D intake of 100 mcg (4000 IU)/day is physiologic and safe. The adequate intake (AI) for older adults is 15 mcg (600 IU)/day, but there has been no report focusing on use of this dose.

Methods: We compared effects of these doses on biochemical responses and sense of wellbeing in a blinded, randomized trial. In Study 1, 64 outpatients (recruited if summer 2001 25(OH)D <61 nmol/L) were given 15 or 100 mcg/day vitamin D in December 2001. Biochemical responses were followed at subsequent visits that were part of clinical care; 37 patients completed a wellbeing questionnaire in December 2001 and February 2002. Subjects for Study 2 were recruited if their 25(OH)D was <51 nmol/L in summer 2001. 66 outpatients were given vitamin D; 51 completed a wellbeing questionnaire in both December 2002 and February 2003.

Results: In Study 1, basal summer 25-hydroxyvitamin D [25(OH)D] averaged 48 ± 9 (SD) nmol/L. Supplementation for more than 6 months produced mean 25(OH)D levels of 79 ± 30 nmol/L for the 15 mcg/day group, and 112 ± 41 nmol/L for the 100 mcg/day group. Both doses lowered plasma parathyroid hormone with no effect on plasma calcium. Between December and February, wellbeing score improved more for the 100-mcg/day group than for the lower-dosed group (1-tail Mann-Whitney $p = 0.036$). In Study 2, 25(OH)D averaged 39 ± 9 nmol/L, and winter wellbeing scores improved with both doses of vitamin D (two-tail $p < 0.001$).

Conclusion: The highest AI for vitamin D brought summertime 25(OH)D to >40 nmol/L, lowered PTH, and its use was associated with improved wellbeing. The 100 mcg/day dose produced greater responses. Since it was ethically necessary to provide a meaningful dose of vitamin D to these insufficient patients, we cannot rule out a placebo wellbeing response, particularly for those on the lower dose. This work confirms the safety and efficacy of both 15 and 100 mcg/day vitamin D_3 in patients who needed additional vitamin D.

Background

Vitamin D nutrition can affect many aspects of health because its metabolites function at many tissues. For osteoporosis prevention, the recent consensus is that 25(OH)D should exceed 72 nmol/L, and that adult consumption of vitamin D should be about 25 mcg (1000 IU)/day [1]. A recommended dietary allowance (RDA) is an intake "adequate to meet the known nutritional needs of practically all healthy persons"[2]. According to this criterion, there is still no scientific basis for an RDA for vitamin D [3,4]. Controversies and ongoing concerns about exceeding the safe upper limit (UL) for vitamin D are probably why every major brand of multivitamins marketed for older adults still contains *less* than the adequate intake (AI) for adults >70 y. Resistance from manufacturers may also stem from the fact that no clinical study has yet specifically used 15 mcg (600 IU)/day of vitamin D_3.

Vitamin D consumption in the amount of 100 mcg (4000 IU)/day is safe and physiologic for adults [5-7]. We have characterized cross-sectional relationships between vitamin D intakes, 25(OH)D, $1,25(OH)_2D$ and PTH in endocrine outpatients [8]. Because circulating 25(OH)D was insufficient in 25% of those patients, i.e. it was less than 40-nmol/L (<16 ng/mL), we wanted to offer them vitamin D supplements and to determine whether there are demonstrable differences between the use of the highest current AI for vitamin D, and 100 mcg (4000 IU)/day.

In addition to monitoring their biochemical responses, we enquired about participants' subjective aspects of wellbeing. Among its many potential biological effects, vitamin D nutrition may influence the brain, because brain tissue possesses the enzyme that can produce $1,25(OH)_2D$, the biologically active form of vitamin D [9,10]. The brain also possesses the appropriate receptors to respond to this [11-13]. Electroencephalographic readings change with season, especially in women [14]. One study has reported that vitamin D supplementation reduces depression in people with seasonal affective disorder better than does treatment with bright light [15]. One study of healthy students concluded that 10 or 20 mcg (400 or 800 IU)/day for only 5 days during winter improved mood [16]. In men with prostate cancer, 50 mcg (2000 IU)/day vitamin D improved functionality and quality of life [17]. In a large placebo-controlled, randomized study that showed that fractures are prevented with 20 mcg (800 IU)/day of vitamin D the authors also reported that self-reported health improved significantly for women, but not men [18]. In community-dwelling healthy older American men with relatively high 25(OH)D levels who were randomized to 25 mcg (1000 IU)/day vitamin D or placebo, there was no effect on health perception [19]. Likewise, in healthy American women supplemented with 10 mcg (400 IU)/day vitamin D or placebo, there was not difference in terms of perceived mood changes with season [20]. In frail elderly, a 4-month randomized study of multivitamin supplementation (5 mcg (200 IU) /day vitamin D) failed to produce an effect on wellbeing [21]. Hence, the season, dose, duration of the study, as well as the age, sex, general health of the population studied and the 25(OH)D levels before starting vitamin D can all play a role in whether an improved sense of wellbeing is seen with vitamin D supplementation.

Most patients in the northern USA presenting with diffuse musculoskeletal symptoms exhibit 25(OH)D levels less than 50 nmol/L (20 ng/mL) [22]. Women in Saudi Arabia who present with low back pain commonly have 25(OH)D levels below 50 nmol/L, and in a Phase-2 study design with no control group, they responded remarkably well to treatment with oral 25(OH)D (5000 – 10,000 IU/day) [23].

Depression scores at northern latitudes are generally worst between December and February [24], coincident with the nadir in 25(OH)D levels [25,26]. Thus, we chose these months to compare the effects of two doses of supplementary vitamin D_3 on biochemical responses and measures of wellbeing of patients prescreened to be at high risk of vitamin D insufficiency during winter.

Methods, Materials & Patients

Materials

Vitamin D_3 doses were prepared in two concentrations: 700 mcg/mL and 95 mcg/mL. For this, we used crystalline cholecalciferol (vitamin D_3, USP Grade, Sigma, St Louis) as previously described [27]. The crystalline vitamin D3 was dissolved in US-Pharmacopoeia-grade ethanol and calibrated based on absorbance at 265 nm using a diode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA), and based on the vitamin D molar extinction coefficient of 18,300 AU/mol/L. Thus, the UV absorptivity at 264 nm was 33.4 and 5.0 AU/cm path-length respectively for the high and low dose.

Subjects (STUDY 1)

We previously reported on the biochemical characteristics of thyroid clinic outpatients [8]. The following procedures were followed in accordance with the ethical standards of Mount Sinai Hospital on human experimentation, approval was obtained from its human research ethics committee, and each participant signed an informed consent. Since current opinion is that desirable 25(OH)D concentrations should exceed 70 nmol/L [1], we offered to provide vitamin D to patients who, in spring and summer of 2001, had serum 25(OH)D <61 nmol/L, because we expected these patients to develop 25(OH)D concentrations <40 nmol/L by the next winter, based on what we

Table 1: Statistical analysis of Study 1 scores of wellbeing (Winter 2001–2002).

	Intent-to-treat analysis				*Per-protocol analysis*		
Dose of Vitamin D mcg/day (IU/day)	*N* Total in group, (% female)	*Age*	December 2001 Score out of 6; mean (SD)	February 2002 Score out of 6; mean (SD)	*N* Total in group, (% female)	December 2001 Score out of 6; mean (SD)	February 2002 Score out of 6; mean (SD)
15 (600)	32 (80%)	53 (14)	2.2 (2.0)	2.3 (2.3)	16 (80%)	2.4 (2.2)	2.3 (2.4)
100 (4000)	32 (83%)	55 (9)	2.0 (2.3)	1.1 (1.8) **a**	21 (83%)	1.5 (2.2)	1.0 (1.5) **bc**

a February scores for 100 mcg (4000 IU)/day were lower (better) than in the 15 mcg (600 IU)/day group by two-tail t-score p = 0.072; Mann-Whitney p = 0.072; these 2-tail values are equivalent to 1-tail significance.
b Paired t-test, December score vs February Score p = 0.097; or non-parametric Sign test, p = 0.109.
c Difference between dose groups by t-test p = 0.047; by Mann-Whitney test p = 0.072 (this 2-tail value is equivalent to 1-tail significance)

knew of seasonal 25(OH)D changes in Toronto [25,28]. In late summer 2001, we sent letters to 333 of these patients. Of those who signed the consent, approved by the ethics-review committee of Mount Sinai Hospital, 46 completed at least 3 months of vitamin D supplementation (Table 1). Participants were unpaid volunteers. They were not asked about intake of dietary supplements or vitamins, because the eligibility criterion was a low 25(OH)D that demonstrated a need for supplementation. Participants and their physician were blinded as to dose, which was either 95 mcg/week (4200 IU/week; 600 IU/day) or 700 mcg/week (28,000 IU/week; 4000 IU/day). Doses were in 1 ml ethanol solution, added with a syringe to a drink and consumed once per week as we have done in previous studies [5,27]. Because vitamin doses are usually described in their daily amounts, we express the weekly doses given here in their average daily amounts of 15 mcg/day or 100 mcg/day.

Biochemical Methods

We measured intact PTH on the DPC Immulite 2000 analyzer (DPC, Los Angeles, CA). Serum 25(OH)D was measured with the DiaSorin radioimmunoassay (Stillwater, MN) with which our laboratory consistently reported close to the mean of the DEQAS international proficiency survey for this analyte [29]. Serum $1,25(OH)_2D$ was measured with the classic, calf-thymus receptor assay, involving purification of analyte on Bond Elut C18OH cartridges (Varian, Harbor City, CA) and an internal standard to correct for losses during purification [30].

Questionnaire

To address the issue of whether the vitamin D supplementation affected sense of wellbeing, and in particular, whether consumption of 100 mcg/day offers benefits beyond those of consuming 15 mcg/day, the shipment of vitamin D was accompanied by a brief questionnaire, based on conventional depression-screening tools, and incorporating questions relating to energy and mood:

1. Has your general ENERGY LEVEL been less than average lately?

2. Has your MOOD been less than average lately?

3. Have you had problems sleeping, either too much or too little?

4. Have you lost interest or pleasure in things you normally enjoy doing?

5. Have you had a decrease in your ability to concentrate?

6. Have you lost/gained weight?

The wellbeing score for Study 1 was the total number of "YES" responses to these questions. A lower score (out of 6) reflected "better" wellbeing.

For those patients willing to continue taking the vitamin D, the dose originally assigned was continued through the winter 2002–2003, thereby overlapping their vitamin D supplementation with the patients in Study 2, and completing the same questionnaires as the patients in Study 2. Of the original 93 subjects who initially consented, 46 patients continued taking vitamin D_3 through to November 2002.

STUDY 2

At the end of summer, 2002, more patients of the outpatient endocrinology clinic were selected, this time based on 25(OH)D levels that had been measured as <51 nmol/L, and who had not participated previously. At the beginning of November 2002, invitation letters were mailed to 324 patients along with a consent form, and a new questionnaire. Of these, 14 were returned as changed mailing addresses, 243 did not respond. We received 67 returned, signed consents with completed questionnaires within

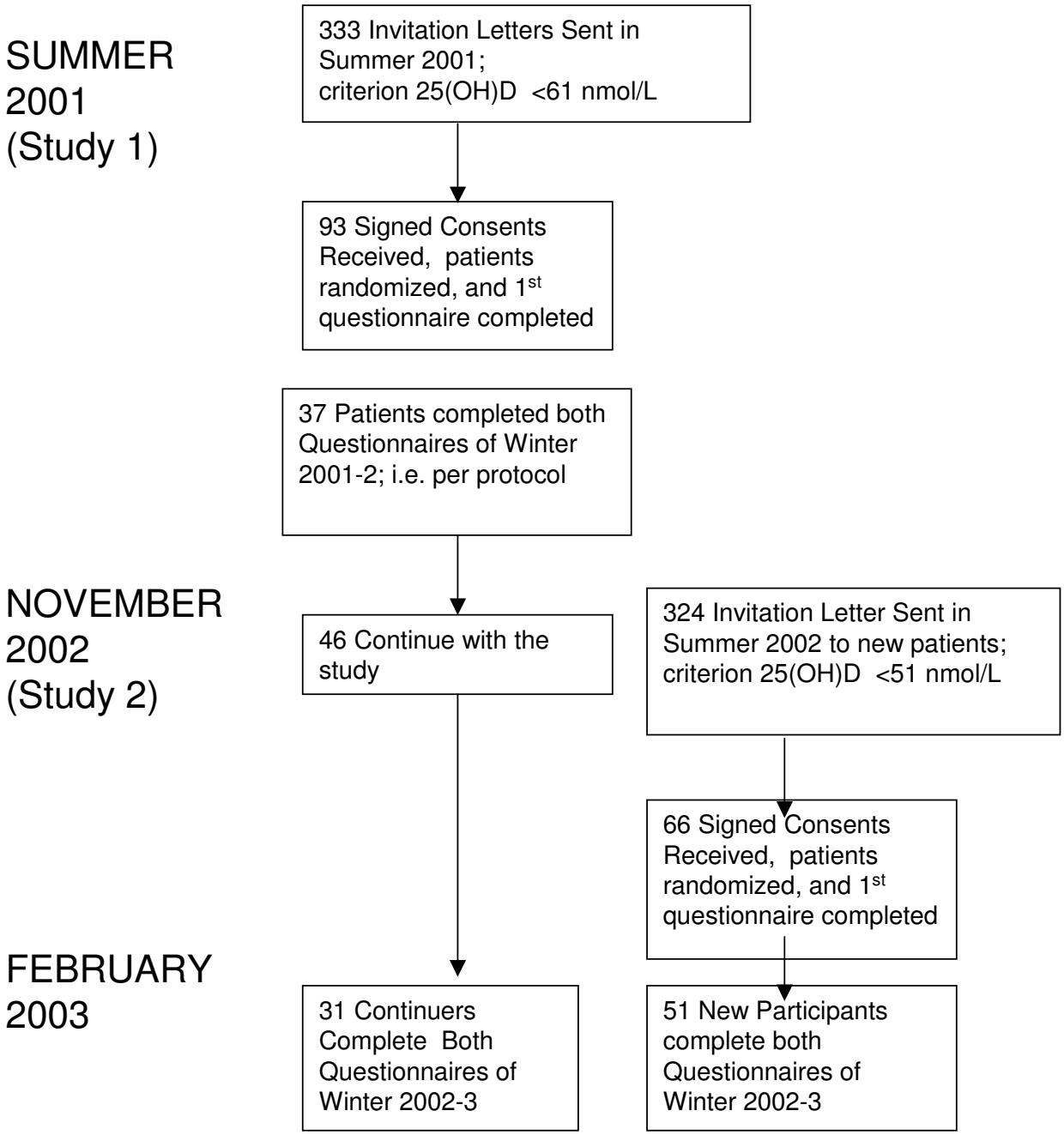

Figure 1
Flowchart showing numbers of patients during the duration of these studies.

the allotted time period (approximately 2 wks from mailing) (Figure 1).

Upon receipt of the completed consent, each patient was randomized as before. Ten questions were added to the

questionnaire, based upon the seasonal health questionnaire of Thompson and Cowan [31]:

7. Has your GENERAL HEALTH been less than average lately?

8. Have you felt less rested upon waking from sleep lately?

9. Have you experienced a down feeling or inappropriate guilt?

10. Have you felt less socially active lately?

11. Have you been indecisive lately?

12. Have you felt less productive or less creative lately?

13. Has your appetite increased or decreased?

14. Have you experienced any cravings for carbohydrates (bread, pasta, rice, sugary foods), more than normal?

15. Has it been more difficult to deal with daily stress?

16. Have you felt irritable or anxious lately?

The wellbeing score for Study 2 was the total number of "YES" responses to these questions, with a lower score (out of 16) reflecting "better" wellbeing. This was mailed at recruitment and in February 2003.

Statistical analysis

Statistical analysis and graphical presentation were carried out using SPSS version 11 (SPSS, Inc., Chicago, IL). As recommended by Jones et al, analyses pertaining to wellbeing were done and presented using both the intent-to-treat approach (all available data), as well as per-protocol, using only data for patients completing both December and February questionnaires [32]. For each of these, statistical analyses were done using both parametric, t-test comparisons, and equivalent non-parametric approaches, as specified in the following results section. For the wellbeing score of Table 2, the null hypothesis had been one tailed, i.e. that the higher dose would improve scores compared to the lower dose. Thus, although all p-values are presented here as 2-tailed, a one-tail null hypothesis was disproved if the 2-tail $p < 0.1$ for differences in the direction expected a-priori. Statistical analyses of longitudinal biochemical data are presented here as parametric assessments, using ANOVA. If ANOVA indicated that significant differences existed for the biochemistries, we performed 2-tail paired-t-tests because these were comparisons defined a priori, and not post-hoc comparisons. i.e. Since 25(OH)D levels had been expected to be higher after months of supplementing with vitamin D, the unexpected observation would have been to see no difference (i.e. beta error), the risk of which would have been increased with Bonferroni or Dunnett comparisons. Mean values are given with ±SD values. Correlation of wellbeing vs months on dose was done with Spearman's rank-order correlation coefficient, which measures association at the ordinal level.

Table 2: Statistical analysis of Study 2 scores of wellbeing (Winter 2002–2003).

	Intent-to-treat analysis					*Per-protocol analysis*		
Dose of Vitamin D mcg/day (IU/day)	*N* Total in group, (% female)	*Age* yr (SD)	25(OH)D nmol/L (SD)	December 2002 Score (out of 16)	February 2003 Score (out of 16)	*N* Total in group, (% female)	December 2002 Score (out of 16)	February 2003 Score (out of 16)
CONTINUERS FROM STUDY 1 (on Vit D since previous year)								
15 (600)	22 (77%)	54 (14)	69 (26)	7.2 (4.5)	4.4 (3.4)	15 (73%)	6.9 (4.8)	4.4 (3.4) **b**
100 (4000)	24 (84%)	56 (9)	126 (45) **a**	4.4 (4.4) **a**	4.0 (3.7)	16 (88%)	4.6 (4.6)	4.0 (3.7)
NEW PATIENTS FOR STUDY 2								
15 (600)	33 (68%)	48 (13)	39 (9)	8.0 (5.2)	5.4 (4.3)	25 (64%)	8.7 (5.5)	5.4 (4.3) **b**
100 (4000)	33 (85%)	50 (14)	39 (9)	8.4 (5.5)	3.9 (3.6) **c**	26 (89%)	8.1 (5.6)	3.9 (3.6) **bc**

a Different from 15 mcg (600 IU)/day group (the value above the mean marked by this footnote) by t-test $p < 0.04$; lower (better) than in the 600 IU/day group by Mann-Whitney $p = 0.039$.

b Paired t-test, December score vs February Score (the value to the left of the mean marked by this footnote) $p < 0.012$; also significant by the non-parametric equivalent to paired t-test, the Wilcoxan test, $p < 0.012$.

c For New patients, low vs high dose group, 2-tail unpaired t test $p = 0.188$; Mann-Whitney $p = 0.183$; i.e. not significantly different.

Results

Study 1. Biochemical responses

Results of biochemical tests are presented in Figure 2. For those patients in whom biochemistry data were tested within 2–6 months after starting vitamin D, both doses increased 25(OH)D significantly, with higher levels in the higher vitamin D dose group than in the lower dose group. In both groups, statistically significant suppression of PTH was detected only after 6 months of supplementation. While mean PTH was slightly lower for the 100 mcg/day group, PTH was not significantly different between dose groups. There were no significant differences in serum total or plasma ionized calcium concentrations, either over time, or between groups. There were no significant differences or changes in $1,25(OH)_2D$ concentrations between groups, or over time. Information relevant to determining nutrient intake requirements for adults is indicated by the bottom whiskers for 25(OH)D concentration measured beyond 6 months: 15 mcg (600 IU)/day resulted in average 25(OH)D concentrations of 79 (±30 SD) nmol/L with a minimum non-outlier value of 44 nmol/L; 100 mcg (4000 IU)/day resulted in average 25(OH)D concentrations of 112 (±41 SD) nmol/L with a minimum non-outlier value of 69 nmol/L (note that during winter, 25(OH)D levels should be lower than the summer/fall values presented for data >6 mo beyond the start of treatment).

Compared to the high-dose group, the median increase in 25(OH)D *per mcg vitamin D* intake was significantly larger in the lower dose group ($p = 0.011$, using the Mann-Whitney test; $p = 0.003$, using the t-test). For the lower dose group, the median 25(OH)D increase per mcg of vitamin D dose was 2.2 nmol/L/mcg/d, (25th and 75th percentile values were 0.6, 4.1 nmol/L/mcg/day respectively). For the higher dose group, the median 25(OH)D increase was 0.6 nmol/L/mcg/day (25th and 75th percentile values were 0.4, 0.9 nmol/L/mcg/day respectively).

Study 1 Effects on wellbeing (winter 2001–2002)

Table 1 summarizes the scores for wellbeing, based on six questions. For the patients enrolled in Study 1, mean 25(OH)D concentrations prior to December 2001 were 49 (±9 SD) nmol/L for the higher dose group, and 46 (±9 SD) nmol/L for the lower dose group (Figure 2). Based on the conventional two-tail analysis, none of the comparisons between doses or between December and February was statistically significant. However, the hypothesis at the outset of this research was the one-tailed question of whether the higher dose of vitamin D has a better effect on wellbeing than the lower dose. Therefore, we conclude from Study 1, with 95% confidence (based on 2-tail $p < 0.1$), that 100 mcg (4000 IU)/day of vitamin D did result in a significantly greater improvement in wellbeing, compared to the effect of 15 (600 IU)/day. This statistical conclusion was the same whether the analysis was based on the intention-to-treat analysis (analyses on the left side of Table 1) or per protocol analysis (analyses on the right side of Table 1), and the statistical conclusion was the same with either parametric or nonparametric statistical analysis.

Study 2 Effect on wellbeing (winter 2002–2003)

Table 2 summarizes the results for wellbeing, based on 16 questions. For each dose group of Study 2, 25(OH)D mean concentration prior to December 2002 was 39 (±9 SD) nmol/L. Wellbeing improved from December to February for all new patients enrolled in the study ($p < 0.001$); wellbeing also improved during this time for the lower-dose patients remaining on the protocol from the previous year ($p = 0.012$). There was no statistically significant change for the group that had been consuming 100 mcg (4000 IU)/day since the previous year. However, those consuming the higher dose for one year were already statistically at a lower (better) score for wellbeing at the beginning of December 2002 compared to the corresponding Study-1 lower-dose group (2-tail t-test, $p = 0.039$). We also compared the groups based on the subset of six questions used in Study 1; this produced the same statistical differences shown in Table 2 for all 16 questions. That is, in Study 2, and using the 6 questions that were the basis of wellbeing in Study 1, both doses lowered the total score, but this time, there was no difference in effect between 15 mcg (600 IU)/day versus 100 mcg (4000 IU)/day.

As a form of meta-analysis, to combine the wellbeing data of both Study cohorts in these experiments, we have summarized the data from Table 2 as box-plots in Figure 3. This figure highlights interactions between the duration of vitamin D supplementation, and wellbeing. After Month 0, the quartile values show that the response was greater (lower score) with the higher dose than with the AI dose of vitamin D. For the pooled data in the figure, the nonparametric correlation of wellbeing vs months on vitamin D indicated a significant decline (improvement in wellbeing) for participants consuming 100 mcg (4000 IU)/day ($p = 0.002$). However, for those consuming 15 mcg (600 IU)/day the correlation with time on the dose was not statistically significant.

Discussion

Since these were endocrine outpatients, we had expected their general perception of wellbeing to be less than that of the general population. Since older persons with 25(OH)D <50 nmol/L risk losing muscle strength [33] and development of musculoskeletal pain [22,23], there was reason to consider non-osteoporosis-related responses to vitamin D in patients with such low 25(OH)D levels. From an ethical perspective, patients

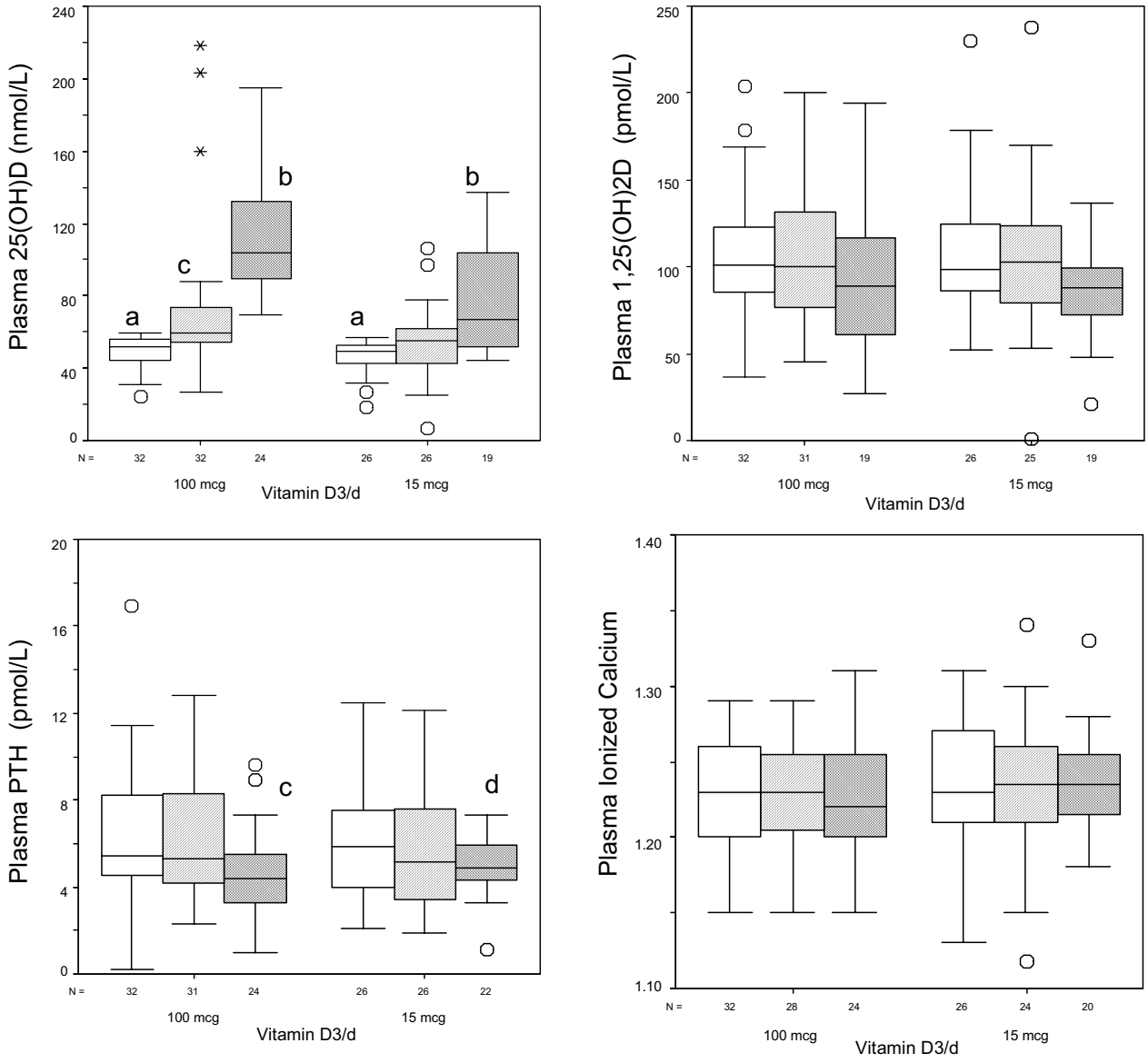

Figure 2
Biochemical responses to vitamin D_3 supplementation of endocrine outpatients during one year. Open bars indicate pre-supplementation data; boxes with intermediate shading indicate data at 2–6 months; darkest boxes, indicate data after >6 months of vitamin D. By the second visit after starting vitamin D, plasma 25(OH)D was higher in those taking 100 mcg/day than in those taking 15 mcg/day (values marked **b** differ significantly from the group's baseline values marked **a**, $p < 0.001$, by paired t-test). 25(OH)D values marked **b** differ significantly from each other, conventional Students t-test, $p = 0.006$). PTH values marked **c** differ significantly from the group's baseline value, $p = 0.003$; PTH values marked **d** differ significantly from the group's baseline value, $p = 0.013$.

who are selected because of low 25(OH)D levels should receive at least a meaningful amount of vitamin D [34,35]. We provided at least the vitamin D AI for the oldest age group, 15 mcg (600 IU)/day, because some of our

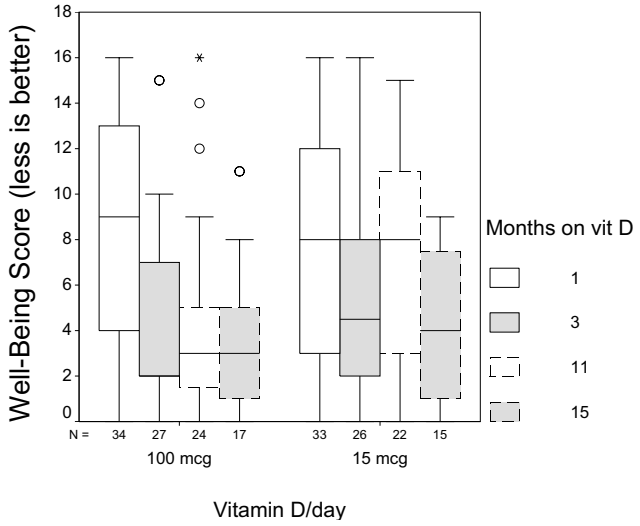

Figure 3
Cross-sectional presentation of the effect of duration of vitamin D supplementation on quartiles of well-being scores obtained during winter 2002–2003. The boxes with solid perimeters indicate new, Study-2 patients; the boxes with dashed-line perimeter indicate patients who had been consuming their vitamin D since December the previous year (from Study 1). Shaded boxes indicate the data for February, 2002. Spearman's non-parametric correlation of well-being vs months was significant and negative with the higher dose ($p = 0.002$), but the correlation was not significant for the lower dose ($p = 0.108$). Statistical comparisons among these data are presented in Table 2.

patients were older than 70 years, and because age does not affect the 25(OH)D response to a dose of vitamin D [8,36].

The greatest biochemical responses to the vitamin D occurred after six months of supplementation. During follow-up, there was no clear plateau in 25(OH)D (Figure 2). Lack of a plateau may reflect season, because the final samples for 25(OH)D in the figure were taken through the summer and autumn, when 25(OH)D levels should be higher than in winter. Differences between the first and the third box of each cluster in Figure 2 reflect the effects of the intervention, not the season, because these samples had been collected about one year apart. Future studies of vitamin D supplementation should take into account that it may take a year to reach stable 25(OH)D levels. Although previous work (including our own) has implied that plateau levels of 25(OH)D can occur within five months [5,37], the impression of a plateau reflects the time pattern of sampling; i.e. samples taken at short time intervals can give a false impression of a plateau.

Higher levels of 25(OH)D generally correlate with lower concentrations of PTH [1,8]. The present data confirm that both doses produced a significant suppression of PTH. The box-plots in Figure 2 suggest a somewhat greater PTH suppression with the higher dose of vitamin D, and we attribute the lack of a statistical difference in PTH between the dose groups to the relatively small sample sizes in this study. In our cross-sectional study of 1741 such patients we observed steady decreases in PTH as 25(OH)D increased [8]. There was no evidence of a change in plasma ionized calcium as a result of this relatively long-term use of vitamin D at a relatively high dose of 100 mcg (4000 IU)/day. We should point out that this dose is not high in the physiologic context, because it approximates what healthy men acquire daily, if they work outdoors [7]. The present data extend the time-frame for follow-up beyond what has been reported previously, and our focus was on patients who did require additional vitamin D; this contrasts with earlier studies of 100 mcg (4000 IU)/day that involved healthy volunteers, where most were already sufficient in vitamin D [5,7].

Lansdowne and Provost reported that 10 or 20 mcg (400 or 800 IU)/day of vitamin D, given for 5 days improved the mood of healthy Australian students during winter [16]. Their protocol provided a total of 100 mcg (4000 IU) vitamin D or less, which could not have produced a detectable change in 25(OH)D concentrations. The results we obtained in Study 1 indicated that the 100 mcg (4000 IU)/day dose of vitamin D resulted in fewer affirmative responses to questions that were mainly related to depression. However, since statistical significance was one-tailed – which we did regard as valid because the effect was in the direction hypothesized beforehand – we wanted to confirm the greater efficacy of the higher dose. The next winter, the protocol was refined (Study 2) to include a more stringent recruitment, requiring lower summer 25(OH)D concentrations (<51 nmol/L) and additional questions relating to wellbeing [31].

In Study 2, both dose groups exhibited highly statistically significant improvement in wellbeing between December 2002 and February 2003. The only patients who did not improve during the second winter were those who had been maintained on the higher dose of vitamin D for the 12 months leading up to December 2002, and whose wellbeing score had already improved during Study 1. Overall, both studies presented here were consistent with the expectation that higher vitamin D nutrition improves sense of wellbeing. The relatively greater improvement during Study 2 compared to Study 1 could be explained to the lower initial 25(OH)D concentrations of Study 2. The

eventual wellbeing response of low-dose patients from Study 1 may reflect a cumulative effect of their vitamin D intake. Since there was no placebo group used in this study, we cannot rule out other reasons for improvement. Questionnaire portions of this research were carried out entirely through the mail, with randomized blinded doses, and minimal direct contact between personnel and the participants; thus, it is not likely that investigator bias played a role. The winter was more severe during Study 2, so we doubt that weather would have explained the improved wellbeing reported during Study 2.

In retrospect, the SF-36 questionnaire, which is acceptable to the FDA as a measure of health outcome, would have been better to assess wellbeing [38]. Nonetheless, simple screening tools like ours do correlate with, and perform about as well as more complex, well-validated questionnaires [39]. Therefore, it as unlikely that a different questionnaire would have affected the sorts of changes we observed, or the conclusions about wellbeing in relation to vitamin D.

Conclusions

The present studies are the first to demonstrate, specifically, the efficacy of the highest current AI for vitamin D. They also demonstrate, in adults older than studied previously, the safety of longer-term vitamin D supplementation with 100 mcg/day. This work suffered from the ethical constraint that participants should not receive a placebo supplement. While this weakens the quality of evidence about wellbeing, we considered it important to report the findings, because they provide keys to the better design of subsequent research into effects of vitamin D on wellbeing. Patients known to have low 25(OH)D levels should not be deprived of vitamin D, and by providing these patients with the AI dose of vitamin D there seems to have been moderate improvement in wellbeing, albeit less of a response than with 100 mcg/day. To demonstrate the largest *absolute* effects of vitamin D on wellbeing, investigators would be advised to focus on a population with low initial 25(OH)D concentration <50 nmol/L. However, the *relative* question of whether a higher dose of vitamin D has a greater effect on wellbeing compared to the AI requires firstly, a larger sample size than was available for either of the present studies, and secondly, a focus on adults prescreened *not* to have the low initial 25(OH)D concentrations that we had specified in Study 2.

This work provides a new perspective about the safety of vitamin D. In the conventional sense, neither dose of vitamin D affected serum calcium levels. However, safety is also supported by the fact that reported wellbeing of patients was not made worse by the consumption of the higher dose (instead, it improved). If wellbeing had deteriorated in any way, this would have been accepted readily as a reason to keep vitamin D intake recommendations low. Although our work confirms the anti-depressant, wellbeing effects reported with short-term intervention and smaller doses of vitamin D [15-17], we found that with the higher dose, these effects were sustained for the longer term of one year – which seems unlikely to happen if this were simply a placebo effect. Effects on wellbeing or depressive symptoms should be important criteria for targeting an RDA for vitamin D, and these still require further study.

List of abbreviations

25-hydroxy-vitamin D or calcidiol, 25(OH)D; 1,25-dihydroxy-vitamin D or calcitriol, 1,25(OH)2D; adequate intake, AI; micrograms, mcg (the Greek letter mu, μ, is not used in this document because some software replaces it with "m", causing a 1000-fold error in the dose); recommended dietary allowance, RDA.

Competing interests

None declared.

Authors' contributions

RV and PW conceived this study. PW was responsible for the clinical care of the patients. AH and SK prepared vitamin D, prepared mailings, helped in designing the study, and maintained the data. SK and RV performed statistical analyses and were responsible for writing the publication.

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