

Randomized comparison of the vitamin D₃ adequate intake versus 100 mcg (4000 IU) per day: Biochemical responses and effects on wellbeing of patients.

Reinhold Vieth¹, Samantha Kimball, Amanda Hu, and Paul G. Walfish²

¹Departments of Laboratory Medicine and Pathology, ²Medicine, Pediatrics, and Otolaryngology, University of Toronto, and ¹Pathology and Laboratory Medicine, and ²Medicine and Endocrine Oncology Program, Mount Sinai Hospital, Toronto, Canada.

Email addresses:

Reinhold Vieth reinhold.vieth@utoronto.ca Corresponding author

Samantha Kimball skimball@uoguelph.ca

Amanda Hu amanda.hu@utoronto.ca

Paul Walfish walfish@mshri.on.ca

Phone 416-586-5920; Fax 416--586-8628

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)/d, 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 in summer 2001) with 25(OH)D <61 nmol/L were randomized and 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 randomized and 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 (\pm 9SD) nmol/L. Supplementation for more than 6 months produced mean 25(OH)D of 79 \pm 30 nmol/L for the 15 mcg/day group, and 112 \pm 41 nmol/L for the 100 mcg/day group. Both doses lowered plasma parathyroid hormone but had no effect on 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 \pm 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 offer the patients at least a meaningful dose of vitamin D, we cannot rule out a placebo wellbeing response, particularly with the lower dose. This work confirms the safety and efficacy of both 15 and 100 mcg/day vitamin D₃ 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 >70y. Resistance from manufacturers may also stem from the fact that no clinical study has yet specifically used 15 mcg (600 IU)/day of vitamin D₃.

We have published our findings of cross-sectional relationships between vitamin D intakes, 25(OH)D, 1,25(OH)₂D and PTH in endocrine outpatients [5]. We have also shown that 100 mcg (4000 IU)/day of vitamin D is a safe, physiologic dose for adults [6]. 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)/d.

In addition to monitoring their biochemical responses, we enquired about participants' subjective aspects of wellbeing. Aside from 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 [7,8]. The brain also possesses the appropriate receptors to respond to this [9-11]. Electroencephalographic readings change with season, especially in women [12]. One study has reported that vitamin D supplementation reduces depression in people with seasonal affective disorder better than does treatment with bright light [13]. One study of healthy students concluded that 10 or 20 mcg (400 or 800 IU)/day for only 5 days during winter improved mood [14]. In men with prostate cancer, 50 mcg (2000 IU)/day vitamin D improved functionality and quality of life [15]. In a large placebo-controlled, randomized study that showed that fractures are prevented with mcg (800 IU)/day of vitamin D the authors also reported that self-reported health improved significantly for women, but not men [16]. 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 [17]. 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 [18]. 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 [19]. 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.

Depression scores at northern latitudes are generally worst between December and February [20], coincident with the nadir in 25(OH)D levels [21,22]. Thus, we chose these months to compare the effects of two doses of supplementary vitamin D₃ 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 D₃ doses were prepared in two concentrations: 700 mcg/mL and 95 mcg/mL. For this, we used crystalline cholecalciferol (vitamin D₃, USP Grade, Sigma, St Louis) as previously described [23]. The crystalline vitamin D₃ was dissolved in US-Pharmacopoeia-grade ethanol and calibrated based on absorbance at 265nm 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 [5]. The following procedures followed were in accordance with the ethical standards of Mount Sinai Hospital on human experimentation and approval was obtained from its human research ethics committee. 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 these patients would have been expected to develop 25(OH)D concentrations <40 nmol/L by the next winter. 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 [6,23]. 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/d.

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 [24]. 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 [25].

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 D₃ 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 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 [26]:

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 [27]. 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 \pm 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.

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 (600IU)/day resulted in average 25(OH)D concentrations of 79 (\pm 30) nmol/L with a minimum non-outlier value of 44 nmol/L; 100 mcg (4000IU)/day resulted in average 25(OH)D concentrations of 112 (\pm 41) 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 ($p=0.011$, Mann-Whitney test; $p=0.003$, 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.

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 \pm 9 nmol/L for the higher dose group, and 46 \pm 9 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.

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 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. 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

Participants were selected because of low 25(OH)D concentrations prior to recruitment. 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 [28], there was reason to consider other non-bone-related effects of vitamin D in patients with such low 25(OH)D levels. It was necessary, from an ethical perspective, to offer at least a meaningful amount of vitamin D to the volunteers taking part in this research [29,30]. We provided all patients with at least the AI for the oldest age group, 15 mcg (600 IU)/day, since some patients were older than 70 years.

The greatest biochemical responses to the vitamin D occurred beyond 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 [6,31], 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

[1,5]. 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 [5]. 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 [32]. 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 who were mostly already sufficient in vitamin D [6,32].

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 [14]. 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 offered the benefit of 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), it was necessary to confirm the observations. The next winter, the protocol was refined (Study 2) to include a more stringent recruitment, requiring yet lower summer 25(OH)D

concentrations < 51 nmol/L, and additional questions relating to wellbeing [26].

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 can be attributed 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 [33]. Nonetheless, simple screening tools like ours do correlate with, and perform about as well as more complex, well-validated questionnaires [34]. Therefore, it is 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 having low 25(OH)D levels cannot be deprived of vitamin D, and the provision of the AI to these patients was moderately effective. 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 to 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 [13-15], we have found that the effects were sustainable for the longer term of one year – which would be very unlikely if this were simply a placebo effect. Sense of wellbeing or depressive symptoms should be important criteria for targeting an RDA for vitamin D, and these aspects of nutrition still require further study.

List of abbreviations

25-hydroxy-vitamin D, 25(OH)D ; 1,25-dihydroxy-vitamin D, 1,25(OH)₂D ; adequate intake, AI; recommended dietary allowance, RDA.

Competing interests none

Authors' contributions

Reinhold Vieth and Paul Walfish conceived this study. Paul Walfish was responsible for the clinical care of the patients. Amanda Hu and Samantha Kimball prepared vitamin D, prepared mailings, helped in designing the study, and maintained the data. Samantha Kimball and Reinhold Vieth performed statistical analyses and were responsible for writing the publication.

Acknowledgements

Financial Support. This work was supported by the Canadian Institutes for Health Research (RV), and by the Mount Sinai Hospital Foundation and Department of Medicine Research Fund (PGW) as well as support from the Temmy Latner Dynacare and Julius Kuhl Family Foundations (PGW).

References

1. Dawson-Hughes B, Heaney R, Lips P, Meunier P, Vieth R: **Vitamin D Round Table.** In *Nutritional Aspects of Osteoporosis*. Edited by Dawson-Hughes B, Heaney R, Burckhardt P. New York: Academic Press; 2004.
2. Yates AA: **Process and development of dietary reference intakes: basis, need, and application of recommended dietary allowances.** *Nutr Rev* 1998, **56**: S5-S9.
3. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes.: *Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D, and fluoride.* National Academy Press; 1997.
4. Vieth R, Fraser D: **Vitamin D insufficiency: no recommended dietary allowance exists for this nutrient.** *CMAJ* 2002, **166**: 1541-1542.
5. Vieth R, Ladak Y, Walfish PG: **Age-Related Changes in the 25-Hydroxyvitamin D Versus Parathyroid Hormone Relationship Suggest a Different Reason Why Older Adults Require More Vitamin D.** *J Clin Endocrinol Metab* 2003, **88**: 185-191.
6. Vieth R, Chan PC, MacFarlane GD: **Efficacy and safety of vitamin D(3) intake exceeding the lowest observed adverse effect level.** *Am J Clin Nutr* 2001, **73**: 288-294.
7. Wang JY, Wu JN, Cherng TL, Hoffer BJ, Chen HH, Borlongan CV *et al.*: **Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats.** *Brain Res* 2001, **904**: 67-75.
8. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM *et al.*: **Extrarenal Expression of 25-Hydroxyvitamin D(3)-1alpha-Hydroxylase.** *J Clin Endocrinol Metab* 2001, **86**: 888-894.
9. Langub MC, Herman JP, Malluche HH, Koszewski NJ: **Evidence of functional vitamin D receptors in rat hippocampus.** *Neuroscience* 2001, **104**: 49-56.
10. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F: **Vitamin D(3) and brain development.** *Neuroscience* 2003, **118**: 641-653.
11. Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D: **New clues about vitamin D functions in the nervous system.** *Trends Endocrinol Metab* 2002, **13**: 100-105.
12. Deldin PJ, Duncan CC, Miller GA: **Season, gender, and P300.** *Biol Psychol* 1994, **39**: 15-28.

13. Gloth FM, III, Alam W, Hollis B: **Vitamin D vs broad spectrum phototherapy in the treatment of seasonal affective disorder.** *J Nutr Health Aging* 1999, **3**: 5-7.
14. Lansdowne AT, Provost SC: **Vitamin D3 enhances mood in healthy subjects during winter.** *Psychopharmacology (Berl)* 1998, **135**: 319-323.
15. Van Veldhuizen PJ, Taylor SA, Williamson S, Drees BM: **Treatment of vitamin D deficiency in patients with metastatic prostate cancer may improve bone pain and muscle strength [In Process Citation].** *J Urol* 2000, **163**: 187-190.
16. Trivedi DP, Doll R, Khaw KT: **Effect of four-monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial.** *BMJ* 2003, **326**: 469-475.
17. Kenny AM, Biskup B, Robbins B, Marcella G, Bureson JA: **Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men.** *J Am Geriatr Soc* 2003, **51**: 1762-1767.
18. Harris S, Dawson-Hughes B: **Seasonal mood changes in 250 normal women.** *Psychiatry Res* 1993, **49**: 77-87.
19. Chin APM, de Jong N, Schouten EG, van Staveren WA, Kok FJ: **Physical exercise or micronutrient supplementation for the wellbeing of the frail elderly? A randomised controlled trial.** *Br J Sports Med* 2002, **36**: 126-131.
20. Mersch PP, Middendorp HM, Bouhuys AL, Beersma DG, van den Hoofdakker RH: **Seasonal affective disorder and latitude: a review of the literature.** *J Affect Disord* 1999, **53**: 35-48.
21. Vieth R, Cole DE, Hawker GA, Trang HM, Rubin LA: **Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it.** *Eur J Clin Nutr* 2001, **55**: 1091-1097.
22. Scharla SH: **Prevalence of subclinical vitamin D deficiency in different European countries.** *Osteoporos Int* 1998, **8 Suppl 2:S7-12**: S7-12.
23. Trang H, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R: **Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2.** *American J Clinical Nutrition* 1998, **68**: 854-848.
24. Vieth R, Carter G: **Difficulties with vitamin D nutrition research: objective targets of adequacy, and assays for 25-hydroxyvitamin D.** *Eur J Clin Nutr* 2001, **55**: 221-222.
25. Hollis BW, Kilbo T: **The assay of circulating 1,25(OH)2D using non-end-capped C18 silica: performance and validation.** In *Vitamin D: Molecular,*

Cellular and Clinical Endocrinology. Edited by Norman AW, Schaefer K, Grigoleit H-G, vHerrath D. Berlin: W deGruyter; 1988:710-719.

26. Thompson C, Cowan A: **The Seasonal Health Questionnaire: a preliminary validation of a new instrument to screen for seasonal affective disorder.** *J Affect Disord* 2001, **64**: 89-98.
27. Jones B, Jarvis P, Lewis JA, Ebbutt AF: **Trials to assess equivalence: the importance of rigorous methods.** *BMJ* 1996, **313**: 36-39.
28. Visser M, Deeg DJ, Lips P: **Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam.** *J Clin Endocrinol Metab* 2003, **88**: 5766-5772.
29. Brody BA, Dickey N, Ellenberg SS, Heaney RP, Levine RJ, O'Brien RL *et al.*: **Is the use of placebo controls ethically permissible in clinical trials of agents intended to reduce fractures in osteoporosis?** *J Bone Miner Res* 2003, **18**: 1105-1109.
30. Heaney RP: **Ethical issues in the design of osteoporosis clinical trials: the state of the question.** *J Bone Miner Res* 2003, **18**: 1117-1120.
31. Vieth R: **Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety.** *Am J Clin Nutr* 1999, **69**: 842-856.
32. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ: **Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol.** *Am J Clin Nutr* 2003, **77**: 204-210.
33. Garratt A, Schmidt L, Mackintosh A, Fitzpatrick R: **Quality of life measurement: bibliographic study of patient assessed health outcome measures.** *BMJ* 2002, **324**: 1417.
34. Schade CP, Jones ER, Jr., Wittlin BJ: **A ten-year review of the validity and clinical utility of depression screening.** *Psychiatr Serv* 1998, **49**: 55-61.

FIGURE LEGENDS

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

Figure 2. Effects of vitamin D₃ supplementation on biochemical responses of endocrine outpatients during one year. Open bars indicate pre-supplementation data; boxes with diagonal lines indicate data at 2-6 months; heaviest crosshatched 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, $p = 0.013$.

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.

Table 1. Statistical analysis of Study 1 scores of wellbeing.

<i>Dose of Vitamin D</i>	<i>Intent-to-treat analysis</i>				<i>Per-protocol analysis</i>		
	<i>N</i>	<i>Age</i>	<i>December Score out of 6; mean (SD)</i>	<i>February Score out of 6; mean (SD)</i>	<i>Number of female, male</i>	<i>December Score out of 6; mean (SD)</i>	<i>February Score out of 6; mean (SD)</i>
<i>mcg/day (IU/day)</i>	<i>Total in group, (% female)</i>				<i>Total in group, (% female)</i>		
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)

Table 2. Statistical analysis of Study 2 scores of wellbeing.

Dose of Vitamin D mcg/day (IU/day)	N Total in group, (% female)	Age yr (SD)	Intent-to-treat analysis 25(OH)D nmol/L (SD)			Per-protocol analysis		
			December Score (out of 16)	February Score (out of 16)		Total in group, (% female)	December Score (out of 16)	February Score (out of 16)
CONTINUERS FROM STUDY 1								
15 (600)	22 (77%)	54 (14)	69 (26)	7.2 (4.5)	4.4 (3.4)	15	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	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	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	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 Wilcoxon test, $p < 0.012$

c For New patients, low vs high dose group, unpaired t test $p = 0.188$; Mann-Whitney $p = 0.183$

Figure 1

SUMMER
2001
(Study 1)

333 Invitation Letters Sent in Summer
2001;
criterion 25(OH)D <61nM

93 Signed Consents
Received, patients
randomized, and 1st
questionnaire completed

37 Patients completed both
Questionnaires of Winter 2001-
2; i.e. per protocol

NOVEMBER
2002
(Study 2)

46 Continue with the study

324 Invitation Letter Sent in Summer
2002 to new patients;
critrriion 25(OH)D <51 nM

66 Signed Consents
Received, patients
randomized, and 1st
questionnaire completed

FEBRUARY
2003

31 Continuers Complete
Both Questionnaires of
Winter 2002-3

51 New Participants
complete both
Questionnaires of Winter
2002-3

Figure 2

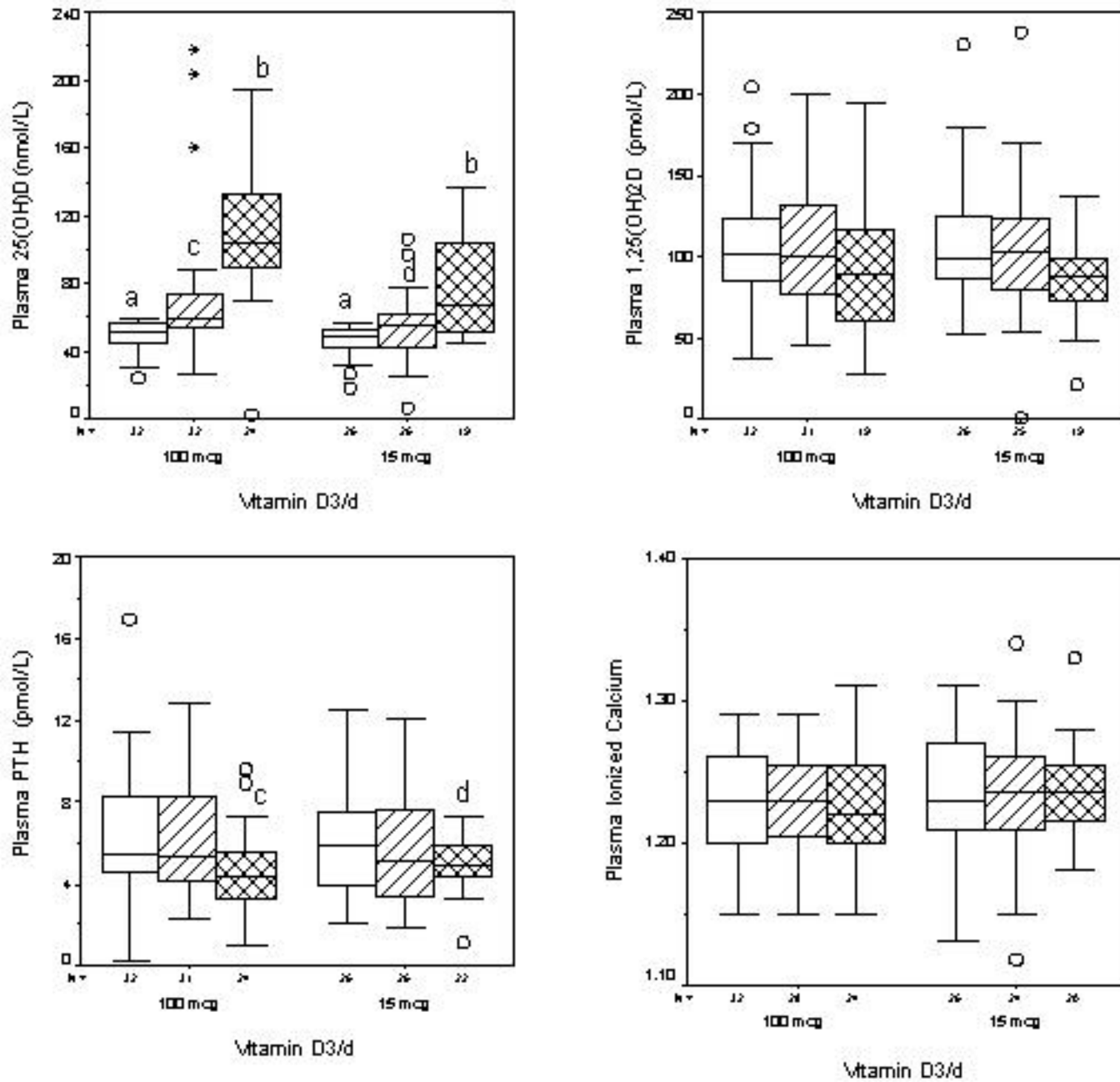


Figure 2

Figure 3

