

Randomized comparison of adequate vitamin D intake versus 100 mcg (4000 IU) per day: Biochemical responses and effects on well-being 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 kimbal2@uwindsor.ca

Amanda Hu amanda.hu@utoronto.ca

Paul Walfish walfish@mshri.on.ca

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

Abstract

Background

100 mcg (4000 IU)/d vitamin D is safe for healthy adults, and the official adequate intake (AI) for older adults is 15 mcg (600 IU)/d.

Methods

We compared effects of these doses on biochemical responses and sense of well-being in a blinded, randomized trial. In Study 1, 62 outpatients were started on vitamin D in December 2001. Biochemical responses were followed at subsequent visits that were part of clinical care, and 37 patients completed a well-being questionnaire in early December and late February. In Study 2, 66 patients were started and 37 completed a well-being questionnaire in both December 2002 and February.

Results

In Study 1, where 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 ± 30 nmol/L for the 15 mcg/d group, and 112 ± 41 nmol/L for the 100 mcg/d group.

Parathyroid hormone was significantly lower with both doses. Between December and February, well-being score improved for the 100-mcg/d group, based on 1-tail paired t-test. In Study 2, in which summer 25(OH)D averaged 39 ± 9 nmol/L, both doses significantly improved well-being scores between December and February (two-tail $p < 0.001$).

Conclusion

The highest AI for vitamin D was effective in bringing summertime 25(OH)D to >40 nmol/L in most patients; it lowered PTH, and improved sense of well-being in patients with low 25(OH)D. The 100 mcg/d dose produced more consistent effects. Since it was

ethically necessary to offer the patients at least a meaningful dose of vitamin D, we cannot rule out placebo effects on well-being. This work confirms the safety and efficacy of 100 mcg/d of vitamin D in patients who needed additional vitamin D.

Background

Vitamin D has the potential to affect many tissues. Therefore, it may affect many aspects of health and well-being. Recent opinions in the context of osteoporosis prevention are that 25(OH)D should exceed 72 nmol/L, and that adult consumption of vitamin D should be about 25 mcg (1000 IU)/d [1]. According to the criterion that a recommended dietary allowance (RDA) is an intake “adequate to meet the known nutritional needs of practically all healthy persons”[2], 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)/d of vitamin D3.

We have reported cross-sectional relationships between vitamin D intakes, 25(OH)D, 1,25(OH)₂D and PTH in endocrine outpatients [5]. 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 highest current AI for vitamin D, and 100 mcg (4000 IU)/d.

In addition monitoring biochemical effects, we enquired about participants' subjective aspects of well-being because brain possesses the enzyme that can produce 1,25(OH)₂D, the biologically active form of vitamin D [6,7] and it possesses the appropriate receptors to respond to this [8-10]. Electroencephalographic readings

change with season, especially in women [11]. One study has reported that vitamin D reduced depression in people with seasonal affective disorder [12]. One study of healthy students concluded that 10 or 20 mcg (400 or 800 IU)/day for only 5 days during winter improved mood [13]. In men with prostate cancer, 50 mcg (2000 IU)/day vitamin D improved functionality and quality of life [14]. A large placebo controlled, randomized study that showed fracture prevention with 20 mcg (800 IU)/d of vitamin D reported improved self-reported health for women, but not men [15]. In community-dwelling healthy older American men with relatively high 25(OH)D levels who were randomized to 25 mcg (1000 IU)/d vitamin D or placebo, there was no effect on health perception [16]. Likewise, in healthy American women supplemented with 10 mcg (400 IU)/d vitamin D or placebo were not different in terms of perceived mood changes with season [17]. In frail elderly, a 4-month randomized study of multivitamin supplementation (5 mcg (200 IU) /d vitamin D) failed to produce an effect on well-being [18]. Hence, the season, dose, duration of the study, as well as the age, sex, and general health of the population studied may all play a role in whether there is an improved sense of well-being with vitamin D supplementation.

Depression scores at northern latitudes are generally worst between December to February [19], coincident with the nadir in 25(OH)D levels [20,21]. Thus, we compared the effects during these months of two doses of supplementary vitamin D on biochemical responses and measures of well-being of patients prescreened to be at high risk of vitamin D insufficiency.

Methods, Materials & Patients

Materials

Vitamin D₃ doses were prepared in two concentrations: 700 mcg/mL or 95 mcg/mL. For this, we used crystalline cholecalciferol (vitamin D₃, USP Grade, Sigma, St Louis) as previously described [22]. 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 pathlength 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 at or below 60 nmol/L, because these patients would have been expected to develop winter 25(OH)D concentrations <40 nmol/liter. 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 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 [22,23].

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 well-being, 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 well-being score for Study 1 was the total number of “YES” responses to

these questions. A lower score (out of 6) reflected “better” well-being.

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 well-being score for Study 2 was the total number of “YES” responses to these questions, with a lower score (out of 16) reflecting “better” well-being. 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 well-being were done and presented using both the intent-to-treat approach with 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 well-being 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 well-being 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, we detected significant suppression of PTH 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)/d 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)/d resulted in average 25(OH)D concentrations of 112 (\pm 41) nmol/L with a minimum non-outlier value of 69

nmol/L (note, winter minima would be lower than the summer/fall values presented for data >6 mo beyond the baseline).

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 increase was 2.2 nmol/L/mcg/d, (25th and 75th percentile values were 0.6, 4.1 nmol/L/mcg/d respectively). For the higher dose group, the median increase was 0.6 nmol/L/mcg/d (25th and 75th percentile values were 0.4, 0.9 nmol/L/mcg/d respectively).

Study 1 Effects on well-being.

Table 1 summarizes the scores for well-being, based on six questions. For the patients enrolled Study 1, mean 25(OH)D concentrations prior to December 2001 were 49 ± 9 nmol/L for the higher dose group, and 46 ± 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 null hypothesis prior to the study was the one-tailed question of whether the higher dose of vitamin D has a better effect on well-being 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 resulted in a significant improvement in well-being, compared to the effect of 15 (600 IU)/day. This statistical conclusion was the same whether based on the intention-to-treat analysis or per protocol analysis, and upon either parametric or nonparametric statistical analysis.

Study 2 Effect on well-being.

Table 2 summarizes the results for scores for well-being, based on 16 questions.

For both dose groups of Study 2, 25(OH)D concentrations prior to December 2002 were 39 ± 9 nmol/L. Well-being improved from December to February for all new patients enrolled in the study ($p < 0.001$); well-being 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 well-being at the beginning of Study 2 ($p = 0.039$, compared to the corresponding Study-1 lower-dose group). We also calculated these data based on the subset of six questions used in Study 1. Analyses of Study 2 data using the questions giving the 6-point scale of well-being as in Study 1 resulted in 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 well-being in Study 1, there was no difference in effect between 15 mcg (600 IU)/day versus 100 mcg (4000 IU)/day.

Data summarized in Table 2 are presented as box-plots in **Figure 3** to highlight interactions between time on dose and well-being. After Month 0, the quartile values suggest that the response was greater (lower score) for participants with the higher dose than with the AI. For the pooled data of Figure 3, nonparametric correlation of well-being vs months on vitamin D indicated a significant decline (improvement in well-being) for participants consuming 100 mcg (4000 IU)/day but not for those consuming 15 mcg (600 IU)/day.

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 well-being to be less than that of the general population. 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 [29,30]. Since some patients were older than 70 y, we provided all patients with at least the AI for that age group, 15 mcg (600 IU)/d.

The greatest biochemical responses to the vitamin D occurred beyond six months of supplementation, and 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 [23,31], 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)/d. 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)/d that involved healthy volunteers who were mostly already sufficient in vitamin D [23,32].

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 [13]. 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)/d 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 well-being [26].

In Study 2, both dose groups exhibited highly statistically significant improvement in well-being 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 well-being 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 well-being. 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 well-being response of low-dose patients from Study 1 may reflect a cumulative effect of their vitamin D intake. Since there was no placebo, 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 well-being 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 well-being [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 as unlikely that a different questionnaire would have affected the sorts of changes we observed, or the conclusions about well-being 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/d. This work suffered from the ethical constraint that participants should not receive a placebo supplement. While this weakens the quality of evidence about well-being, 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 well-being. Patients having low 25(OH)D levels cannot be deprived of vitamin D, and the provision of the AI to these patients did appear to be effective. To demonstrate the largest *absolute* effects of vitamin D on well-being, 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 well-being 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 well-being of patients was not made worse by the consumption of the higher dose (instead, it improved). If well-being 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, well-being effects reported with short-term intervention and smaller doses of vitamin D [12-14], 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 well-being 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).

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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. Boxes indicate percentile values; median scores are indicated by heavy lines. The boxes with solid perimeters indicate data for new, Study-2 patients; the boxes with dashed-line perimeter indicate data for patients who had been consuming their vitamin D since December the previous year (from Study 1). Shaded boxes indicate the final data, for February, 2002. Statistical comparisons among these data are presented in Table 2.

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

46 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

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

FEBRUARY
2003

31 Continuers Complete
Both Questionnaires of
Winter 2002-3

48 New Participants
complete both
Questionnaires of Winter
2002-3

Figure 2

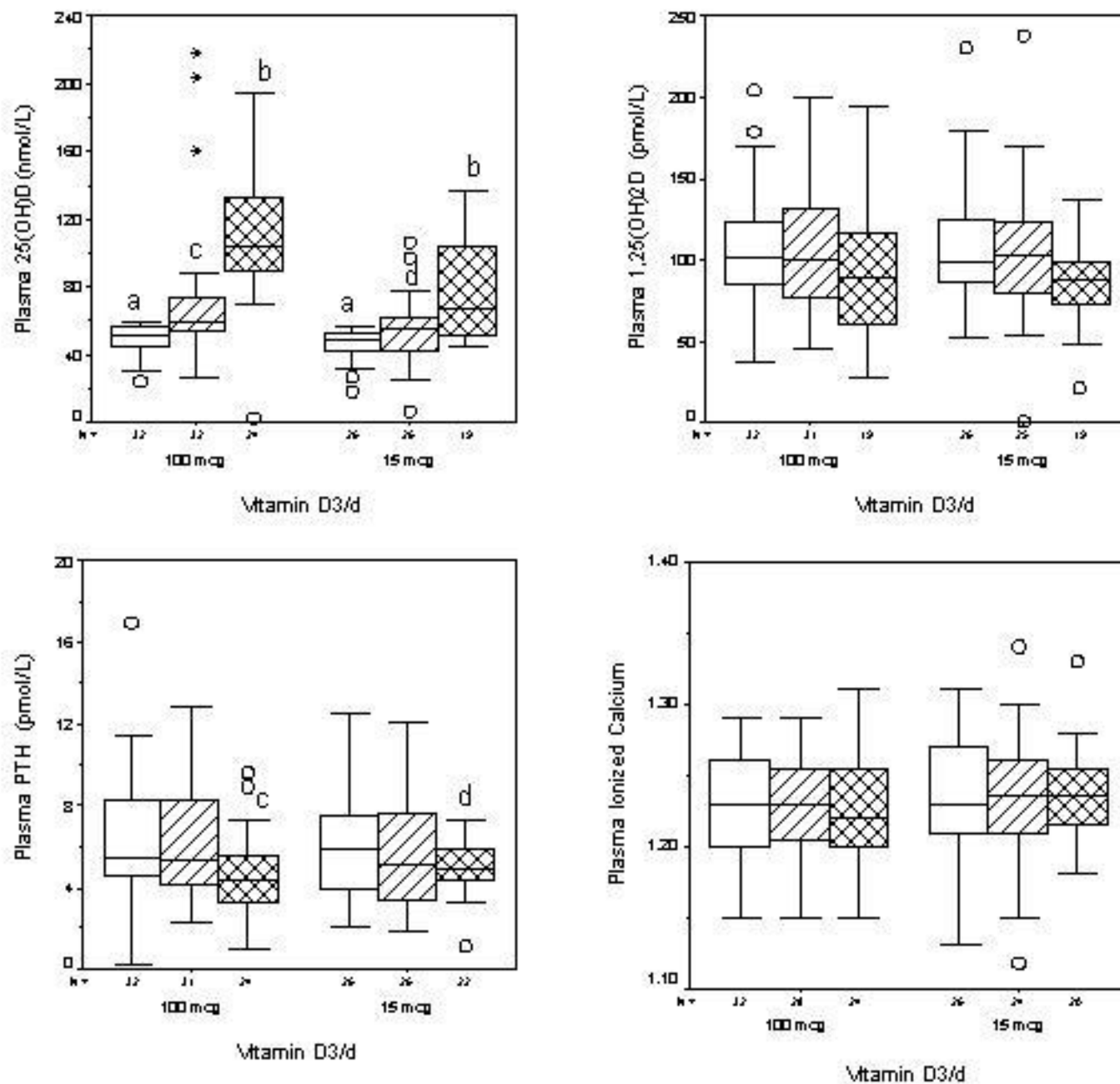
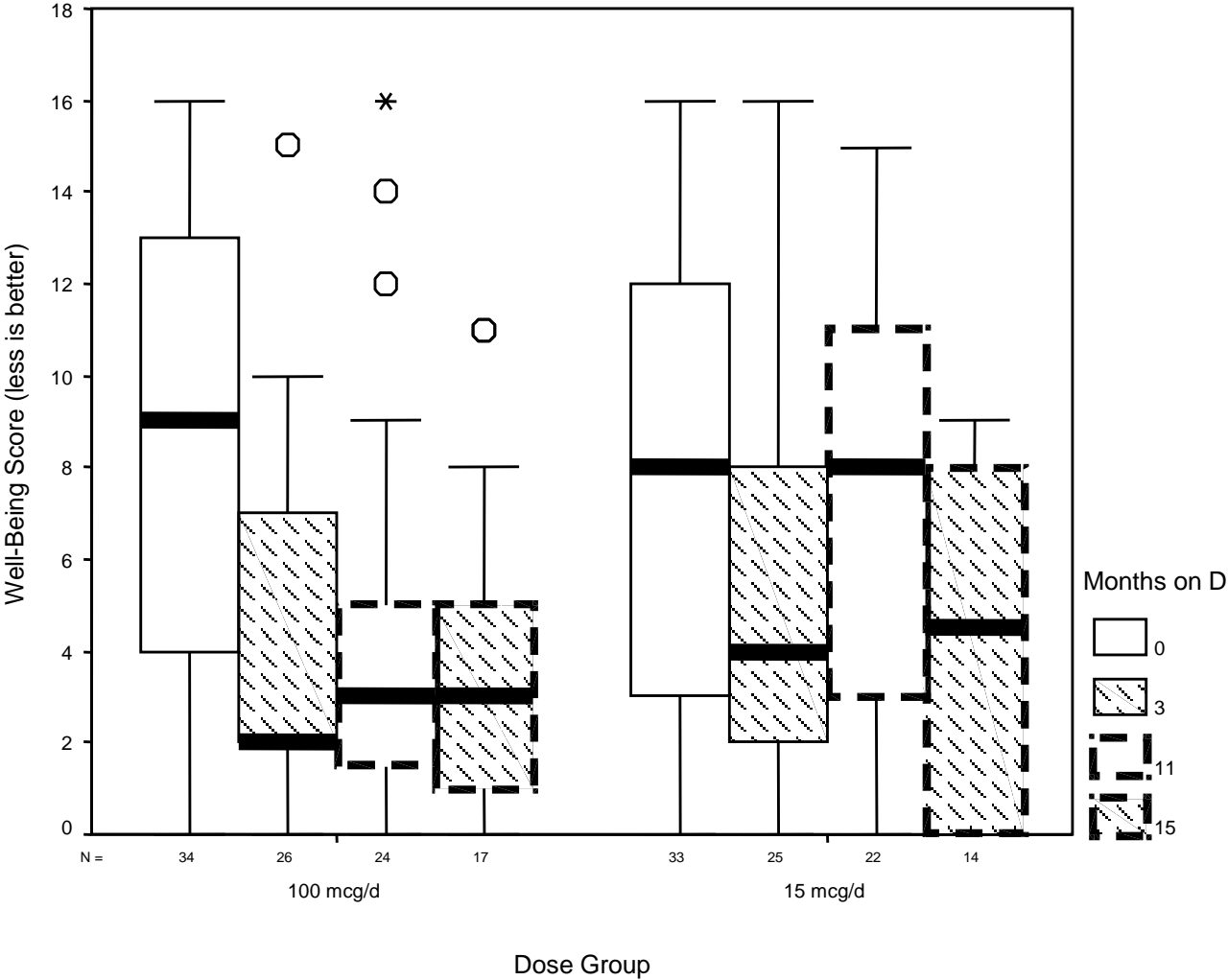


Figure 2

Figure 3



Additional files provided with this submission:

Additional file 1: Vieth Tables Wellbeing manuscript.doc : 67KB
<http://www.nutritionj.com/imedia/6006059533542013/sup1.doc>