Vitamin D_3 Dose Requirement that Raises 25-Hydroxyvitamin D to Desirable Level in Overweight and Obese Elderly

Dania S Bacha[1], Maya Rahme[1], Laila Al-Shaar[2], Rafic Baddoura[3], Georges Halaby[4], Ravinder J Singh[5], Ziyad R Mahfoud[6], Robert Habib[7], Asma Arabi[1], and Ghada El-Hajj Fuleihan[1]

1. Department of Internal Medicine, Division of Endocrinology, Calcium Metabolism and Osteoporosis Program, Beirut, Lebanon.

2. Scholars in Health Research Program, Department of Internal Medicine, American University of Beirut, Beirut, Lebanon.

3. Department of Rheumatology, Hotel Dieu de France, Beirut, Lebanon.

4. Department of Endocrinology, Hotel Dieu de France, Beirut, Lebanon.

5. Division of Clinical Biochemistry and Immunology, Mayo Clinic, Rochester, Minnesota.

6. Department of Population Health Sciences, Weill Cornell Medicine, NY, USA Weill Cornell Medicine, Doha, Qatar.

7. STS Research Center, The Society of Thoracic Surgeons, Chicago, Illinois.

Corresponding author's contact information:

Ghada El-Hajj Fuleihan, MD, MPH, FRCP

Professor of Medicine

Calcium Metabolism and Osteoporosis Program

WHO Collaborating Center for Metabolic Bone Disorders

American University of Beirut, Beirut-Lebanon

P.O. Box: 113-6044/C8

Email: gf01@aub.edu.lb

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ABSTRACT

Purpose: To investigate the impact of two vitamin D doses, bracketed between the IOM recommended dietary allowance (RDA) and the upper tolerable limit, on vitamin D nutritional status in elderly individuals.

Methods: This is a post-hoc analysis on data collected from a 12-month, double-blinded, randomized control trial. 221 ambulatory participants (≥ 65 years), with a mean BMI of 30.2 kg/m^2, and a mean baseline serum 25-hydroxyvitamin D [25(OH)D] level of 20.4 ± 7.4 ng/ml, were recruited from 3 out-patient centers in Lebanon. They all received 1,000 mg of elemental calcium from calcium citrate daily, and the daily equivalent of 600 IU or 3,750 IU, of vitamin D_3.

Results: Mean 25-hydroxyvitamin D [25(OH)D] level at 12 months was 26.0 ng/ml with low dose and 36.0 ng/ml with high dose, of vitamin D_3. The proportion of participants reaching a value ≥ 20 ng/ml was 86% in the low dose, and 99% in the high-dose arms, with no differences between genders. The increment of 25(OH)D per 100 IU/day was 1ng/ml with the low dose, and 0.41 ng/ml with the high dose. Serum 25 (OH)D levels at 1 year were highly variable in both treatment arms. Baseline 25(OH)D level and vitamin D dose, but not age, BMI, gender, nor season, were significant predictors of serum 25(OH)D level post-intervention.

Conclusion: The IOM RDA of 600 IU/day does not bring 97.5% of ambulatory elderly individuals above the desirable threshold of 20 ng/ml. Country-specific RDAs are best derived taking into account the observed variability and predictors of achieved 25(OH)D levels.

Key words: vitamin D, elderly, RDA, desirable level, IOM, guidelines

BACKROUND

Vitamin D is a steroid hormone known for its critical role in bone metabolism and musculoskeletal health (1, 2). Ample evidence also demonstrates its extra-skeletal physiologic actions (1). Vitamin D deficiency is a worldwide health problem that affects all populations (3, 4). It is particularly common in the Middle East, despite the region's abundant sunlight (5, 6). Vitamin D supplementation is a universally accepted therapeutic approach; but guidelines vary widely globally (7). The Institute of Medicine (IOM) recommends that a daily vitamin D_3 dose of 600 IU is adequate to bring 97.5% of the population to a pre-specified desirable 25-hydroxyvitamin D [25(OH)D] level of 20 ng/ml (8). The Endocrine Society 2011 proposes that the daily dietary intake should be 1,000-2,000 IU to reach a target level above 30 ng/ml (9). Some reasons behind this lack of consensus include scarcity of clinical trials-based evidence, different target populations, varied desirable 25(OH)D levels, and discordant approaches to the vitamin D dose-response relationship (10, 11).

Randomized controlled trials (RCTs) clearly demonstrate substantial variability in reported in levels achieved for comparable doses, within, and between ethnic groups (12-14). This raises questions regarding the applicability of the IOM recommended daily allowance (RDA) to non-US populations. Furthermore, there has been a rising trend for hypervitaminosis D due to over-supplementation in both developing and developed countries (15, 16).

In this study, we capitalize on data available from a completed vitamin D trial, to investigate the impact of two doses of vitamin D, bracketed between the IOM RDA and the upper tolerable limit (UTL), on vitamin D nutritional status, in elderly ambulatory individuals.

METHODS

Objective and Aims

We aim to:

1. Test the hypothesis whether the RDA recommended by the IOM brings 97.5% of our study population above the desirable cut-off value of 20 ng/ml.
2. Compare the proportion of subjects in each treatment dose who reach the desirable 25(OH)D cut-off levels, as recommended by the 2010 IOM report (20 ng/ml), and the 2011 ES guidelines (30 ng/ml).
3. Evaluate the inter-individual variability in serum 25(OH)D levels achieved, within and between treatment arms.
4. Investigate predictors of serum 25(OH)D levels achieved in response to vitamin D supplementation.

Study Design

This is a post-hoc analysis on data collected from a double-blinded RCT (NCT01315366) conducted at the American University of Beirut Medical Center (AUBMC), Hotel Dieu de France (HDF), and Rafic Hariri Governmental University Hospital (RHUH), to investigate the impact of vitamin D on two primary outcomes: indices of insulin resistance (17), and indices of bone and mineral metabolism (18). Recruitment, prescreening, and screening procedures were performed at all centers between January 2011 and July 2013, enrollment and protocol implementation were exclusively conducted at AUBMC and ended in July 2014.

Study Population

The study group consisted of 221 ambulatory elderly (≥ 65 years), who were overweight or obese (body mass index (BMI) >25 kg/m^2), and had a baseline serum 25(OH)D level ranging between 10 and 30 ng/ml. Study subjects were recruited from the out-patient clinics or through advertisements

posted at AUBMC, HDF, RHUH, and health dispensaries of the Ministry of Social Affairs, all from the greater Beirut Area.

Exclusion criteria included pre-diabetes on oral hypoglycemic medications, diabetes (fasting plasma glucose ≥ 126 mg/dl or HbA1C ≥ 6.5%), chronic heart failure (stage III or IV), liver failure or cirrhosis, chronic kidney disease (estimated glomerular filtration rate (eGFR) <30 ml/min), cancer, or autoimmune diseases. Participants were also excluded if they had conditions, or were on medications, known to affect bone metabolism, such as osteomalacia, history of kidney stones, fragility fractures, or a 10 years fracture risk (FRAX) exceeding 10% for major osteoporotic fractures using the Lebanon Fracture Risk Assessment Tool (FRAX) version 3.08 (https://www.shef.ac.uk/FRAX/tool.jsp).

Study Drug

All subjects received four tablets of calcium citrate daily (Citracal D: 250 mg elemental calcium and 125 IU vitamin D_3/tablet) amounting to a total of 1,000 mg of elemental calcium and 500 IU of vitamin D_3 daily. Additionally, each subject received 2 pills identical in shape, size, color, smell, and taste, taken once weekly, consisting of placebo in the low dose arm and vitamin D_3 (Euro D: 10,000 IU/pill) in the high dose arm. All the pills were provided by Europharm, Canada. Based on the certificate of analysis provided by Europharm to the Canadian regulatory agencies for all trial lots, the actual vitamin D_3 content was 150 IU and 11,000 IU in the Citracal D and Euro D tablets, respectively. Therefore, participants received a total daily vitamin D_3 equivalent of 600 IU in the low dose arm 3,750 IU in the high dose arm. Daily calcium intake was assessed through a detailed food-frequency questionnaire about the consumption of several dairy products.

All the study drugs were stored and dispensed in identical boxes at the central pharmacy in AUBMC, where the senior pharmacist implemented randomization and allocation concealment with stratification by gender and center (17). Allocation was concealed based on a simple randomization approach, and all the participants and study team members were blinded to the drug assignment until the completion of the trial and data entry.

The protocol was approved by the Institutional Review Board (IRB) at each center, and all participants provided written informed consent. An external Data Safety Monitoring Board, (DSMB, see acknowledgements), reviewed the final protocol and monitored the trial safety.

Study Visits and Measurements

Participants presented for follow-up every 3 months, where weight, height, and vital signs were measured, questionnaires were administered, study bottles were returned, and refill bottles were provided. At each visit (3, 6, and 12 months), study subjects were asked about adverse events, medication intake, and pill counts. Each participant was also contacted by phone every 2 weeks to encourage compliance, which was calculated as the percentage of full possible dose [(total number of study drug pills taken/total number of pills provided) x100].

Serum 25(OH)D levels were assayed at 0, 3, 6 and 12 months. Blood samples were allowed to clot for 30 minutes, centrifuged for 20 minutes, and immediately processed, or stored at −80°C, depending on the assay. Serum 25(OH)D was run using Liquid Chromatography Mass Spectroscopy (LC-MS) at the Mayo Clinic Clinical Laboratories, (Mayo Clinic, Rochester, Minnesota). That laboratory participates in the vitamin D quality assurance program, DEQAS. The LCMS methodology in that laboratory is directly traceable to NIST, Mayo Lab = 0.9599×NIST − 1.3716, R^2 = 0.9922. Vitamin D assays were run in batches at study completion, and samples drawn at serial time points for each hormone were included within the same assay for each study subject. Intra-assay CVs are 3.8%, 2.4%, and 4.7% at 25, 54, and 140 ng/ml, respectively. Inter-assay CVs are 6.4%, 6.8%, and 5.0% at 24, 52, and 140 ng/ml, respectively.

Sample Size Calculation

The trial's sample size was calculated based on a post-hoc analysis from a vitamin D and calcium trial previously conducted in overweight elderly Caucasian participants (19). Based on an anticipated between-arms mean ± standard deviation difference in insulin resistance index (HOMA-IR) of 0.9 ±

2, a power of 80%, and a significance level of 0.025 (taking into account the two primary outcomes), the total sample size needed was calculated to be 222. Allowing for a possible dropout rate of 30%, 257 subjects were enrolled in the study.

Statistical Analyses

The analyses were performed on all randomized subjects who completed the 1-year trial. For almost all variables, data was normally distributed, based on an inspection of histograms and stem leaf plots. Variables were appropriately summarizes as mean ± standard deviation (SD) or N (%). Serum 25(OH)D levels were compared between groups using the independent *t* test and within the same group using paired *t* test. Categorical variables (proportions) were compared using Pearson chi-squared test or Fisher's exact test, as applicable. Analysis of variance (ANOVA) was used for comparison between different seasons. Scatter plots tracing each individual's response to therapy were created by GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA). Dynamically-fit sigmoidal curves illustrating the percentage of patients reaching a spectrum of desirable 25(OH)D levels, in both arms, were generated using Sigmaplot version 15.0 (Systat Software Inc., San-Jose, CA). Bland-Altman plots showing the overlap in delta 25(OH)D between the low and high dose arms were also generated using SigmaPlot. Multivariate linear and logistic regression analyses were performed to explore possible predictors of mean serum 25(OH)D levels at 1 year, and the likelihood of reaching a level above the preset threshold values of 20, and 30 ng/ml. Predictors assessed were age, gender, baseline BMI, season, having a baseline serum 25(OH)D >20 ng/ml, and the vitamin D dose. We calculated the mean delta 25(OH)D per 100 IU at 1 year in each of low dose and high dose arms, unadjusted and after adjustment for significant predictors. Given that the relationship between vitamin D dose and serum 25(OH)D level is linear at doses between 400-1,600 IU/day (14, 21), we calculated the dose that would bring 97.5% of our cohort to a serum 25(OH)D level ≥ 20 ng/ml. A p-value of ≤0.05 was considered statistically significant, and was not adjusted for multiplicity of testing. Data analyses were performed using IBM SPSS version 26.0 (IBM Corporation, Armonk, NY).

RESULTS

Baseline characteristics

Originally, 129 and 128 elderly participants were randomly assigned to receive the high and low-dose of vitamin D_3, respectively. 35 subjects (14%) did not complete the study, and 1 participant refused to share samples with collaborators (for LC-MS assay). There was no statistical difference in the drop-out rate between the two treatment arms. Participants' baseline characteristics and comorbidities did not differ in the remaining 221 participants from the originally randomized group of 257 subjects (data not shown). The amount of blood samples from 1 subject in the low-dose arm and 5 subjects in the high-dose arm was not sufficient at follow-up; hence, no outcome data is available for these subjects.

The remaining overall study group consisted of 122 women and 99 men with a mean age of 71.1 ± 4.7 years, a BMI of 30.2 ± 4.5 kg/m^2, and a mean baseline serum 25(OH)D level of 20.4 ± 7.4 ng/ml. 111 participants received the low dose, and 110 received the high dose (Table 1). Age, gender, baseline BMI, dietary calcium intake, baseline mean serum 25(OH)D levels, and season of recruitment did not differ across treatment arms.

Response to vitamin D3 supplementation

The mean 25(OH)D levels reached after 12 months were 26.0 ± 6.9 ng/ml in the low dose arm and 36.0 ± 9.7 ng/ml in the high dose arm ($p<0.001$), both of which did not differ significantly between genders (Supplement 1) (20) . The wide-ranging spread of participants' serum 25(OH)D levels at baseline and after 1 year of supplementation, with each dose, is shown in Figure 1.

Proportion of subjects above desirable 25(OH)D cut-off levels at entry and one year

At randomization, overall, 50% of the participants had a 25(OH)D level ≥ 20 ng/ml, and only 10% had a level ≥ 30 ng/ml (Supplement 1) (20). The proportions of participants above the pre-specified cut-off levels did not differ significantly between treatment arms, with the exception of significantly higher proportion of women above 20 ng/ml in the low-dose arm (63%) compared to the high-dose

arm (44%) (p=0.043, Supplement 1) (20). A higher proportion of women (63%) were above 20 ng/ml, as compared to men (44%), in the low dose arm only (p=0.051, Supplement 1) (20).

At one year, there was a significant increase in the proportion of subjects above specific cut-offs, with both doses (p<0.001, Figure 2). The proportion of participants reaching the cut-off value of 20 ng/ml was 86% in the low-dose arm and 99% in the high-dose arm (p<0.001, Figure 2), with no differences across genders (Supplement 1) (20). The proportion of subjects reaching a level ≥30 ng/ml remained low at 26% in the low-dose arm, but increased to 73% in the high-dose arm (p<0.001, Figure 2), with no gender differences in both treatment arms (Supplement 1) (20). Figure 3 illustrates the proportion of subjects reaching a spectrum of desirable serum 25(OH)D levels at one year. There was a clear shift of the baseline sigmoidal curve to the right after supplementation, both with the low dose, and more substantially with the high dose.

Predictors of response to vitamin D_3 at 1 year

Unadjusted Analyses:

Baseline serum 25(OH)D level, vitamin D_3 dose received, and having a baseline 25(OH)D level ≥ 20 ng/ml, but not age, gender, season, or BMI, predicted both the serum 25(OH)D level at 1 year and the likelihood of achieving a serum 25(OH)D level of ≥ 20 ng/ml; however, having a baseline level of ≥ 20 ng/ml did not significantly predict the likelihood of achieving a level of ≥ 30 ng/ml (Supplement 3) (20). The most powerful predictor was vitamin D treatment dose with a model R^2 of 7-28% (Supplement 3) (20).

Multivariate Adjusted Analyses:

Baseline 25(OH)D levels (p<0.001) and treatment arm (p<0.001) remained the only significant predictors of serum 25(OH)D levels after 1 year of supplementation, after adjusting for other covariates, including age, gender, season, and baseline BMI, with an R^2 of 36.2% (Table 2). Similarly, treatment with high vitamin D dose and baseline serum 25(OH)D level were the only significant predictors for achieving a 25(OH)D level ≥ 20 ng/ml and ≥ 30 ng/ml at 1 year, after adjusting for the

same covariates (Table 2). Highest odds ratio estimates were noted in the high dose treatment arm, where subjects were 29 times more likely to achieve a level ≥ 20 ng/ml, and 9 times more likely to achieve a level ≥ 30 ng/ml, as compared to those receiving the lower dose (Table 2).

Inter-individual variability in serum 25(OH)D levels achieved at one year

Scrutiny of the individual serum 25(OH)D values at one year, and the difference in 25(OH)D levels between 12 months and baseline, reveal high variability in the magnitude and direction of response to both doses (Figure 1). To evaluate the impact of baseline serum 25(OH)D levels and dose on the response to therapy, we performed regression analyses of the change (delta) in serum 25(OH)D level with changing baseline 25(OH)D levels, for each dose. As the baseline 25(OH)D levels increased, the change in serum 25(OH)D levels at one year decreased, and with both doses (Figure 4A). However, for any baseline serum 25(OH)D level, there could be substantial variance and overlap in the delta vitamin D achieved, within, and between treatment groups (Figure 4A). The mean increment with the low dose was 6 ng/ml, with a wide range between -13 to 26 ng/ml (Figure 4B); and 15 ng/ml with the high dose, ranging between -13 and 41 ng/ml (Figure 4C).

The 25(OH)D delta/100 IU was 1.00 ± 1.26 ng/ml in the low dose, and less than half, with a mean of 0.41 ± 0.27 in the high dose, ($p<0.001$) in unadjusted analyses. This variable did not significantly differ by gender or BMI cut-off categories (above vs below 30 kg/m^2) (Supplement 2) (20). The 25(OH)D delta/100 IU was 0.99 ± 0.70 ng/ml in the low dose arm after adjusting for baseline 25(OH)D level.

Projected RDA in our cohort

Considering our observed increment of 1 ng/ml per 100 IU/day at low doses, we calculated that if participants in the low dose arm received 1,100 IU/day instead of 600 IU/day, their achieved 25(OH)D level would increase by 5ng/ml, allowing 97.5% of individuals to reach a desirable level of 20 ng/ml.

DISCUSSION

This one-year double-blinded controlled vitamin D trial reveals that in elderly subjects with an overall baseline serum 25(OH)D level of 20.4 ng/ml, the achieved 25(OH)D level after 1 year of supplementation was 26.0 ng/ml and 36.0 ng/ml with low and high doses, respectively. Importantly, it demonstrates that the recommended IOM daily dose of 600 IU of vitamin D_3 only brings 86% of elderly overweight or obese ambulatory Lebanese individuals to a serum 25(OH)D level at, or above, the desirable cut-off of 20 ng/ml. However, a higher dose of 3,750 IU/day, that is below the UTL of 4,000 IU/day, brings more than 99% of the study population to such target. Baseline 25(OH)D level and vitamin D dose were the only significant predictors of 25(OH)D level post-intervention. The increments per 100 IU vitamin D_3/day were 1.00 ng/ml/100 IU with the low dose, and 0.41 ng/ml/100 IU with the high dose.

Although hypovitaminosis is a global problem, there is still lack of consensus over the recommended intake that prevents patients from developing musculoskeletal health outcomes, such as bone loss or hip fractures (8). Several international agencies, such as the Institute of Medicine (IOM) (8), Nordic Council of Ministers (NORDEN) (22), the German Nutrition Society (23), and the European Food Safety Authority (EFSA) (24), established their recommendations based on a common target 25(OH)D level of 20 ng/ml. Yet, their recommendations for desirable dose still varied widely, reflecting the uncertainty of the evidence regarding dose response. The IOM's and NORDEN's RDAs, which should meet the needs of "nearly all" healthy individuals (i.e. 97.5%), aged 50-70, were 600 IU/day and 400 IU/day, respectively. The German Nutrition Society and the ESFA proposed "Adequate Intake (AI)" values of 800 IU/day and 600 IU/day, respectively.

Multiple randomized control trials tried to establish the optimal vitamin D dose that achieves a target desirable level. Our findings are consistent with those reported in a recent small 6-month trial by Shirvani et al. who investigated the effect of 3 vitamin D doses (600, 4,000, and 10,000 IU/day) in 30 healthy young adults with a mean BMI < 30 kg/m2 and mean baseline level of 17.1 ± 5.9 ng/ml. A vitamin D dose of 600 IU/day corrected 25(OH)D level to ≥20 ng/ml in 71% of the participants, but

brought only 14% to ≥ 30 ng/ml. However, higher doses of 4,000 and 10,000 IU/day allowed all participants to reach a serum 25(OH)D level ≥30 ng/ml (25). Another trial on 225 free-living Caucasian adults >64 years demonstrated that 600 IU/day was adequate for 99% of the participants to achieve a 25(OH)D level of ≥ 20 ng/ml (26). Heaney et al. investigated several vitamin D doses (0, 1,000, 5,000, and 10,000 IU/day) in 67 healthy men (mean age: 38.7 ± 11.2 years) in Nebraska in order to identify the steady state cholecalciferol input during winter that sustains the baseline autumn 25(OH)D level of 20.8 ng/ml. He concluded that in addition to the daily contribution from food and tissue stores, healthy men would need around 500 IU/day to sustain their starting 25(OH)D level (27). Gallagher et al. investigated a wide range of vitamin D doses (400-4,800 IU/day) in 163 healthy post-menopausal women in Nebraska, (mean age: 67 years), and found that 800 IU/day is needed to achieve a level ≥ 20 ng/ml. The dose-response relationship was curvilinear, plateauing at a level of around 44.9 ng/ml in subjects receiving doses greater than 3,200 IU/day (14). Smith et al. combined the analysis from the VitaDAS and ViDOS studies, and showed in a best fitting model, that in Caucasians, 800 IU/day and 2,400 IU/day are required to reach serum 25(OH)D levels of 20 ng/ml and 30 ng/ml, respectively (12). A trial of 126 Chinese adults >60 years showed that 2,000 IU/day is required to allow >86% of individuals to achieve the ES recommended level of 30 ng/ml (28). In another trial of 92 healthy post-menopausal Indian women (mean age: 54.8 years), only 16% and 66.67% of participants achieved a level ≥20 ng/ml with 500 IU and 1,000 IU per day, respectively (29). An RCT of 328 healthy African American women (43-59 years old) showed that 1,640 IU/day was required to achieve 20 ng/ml in 97.5% of the participants (30), possibly reflecting the need for a higher dose in subjects with lower baseline levels.

According to a meta-analysis conducted in the Middle East and North Africa (MENA) region, which included 2 trials in the elderly (age >65 years) and 17 in adults (18-65 years), only 89% receiving high dose (>2,000 IU/day) and 71% of participants receiving intermediate (800-2,000 IU/day) doses, reached a desirable threshold of 20 ng/ml (31). Another recent meta-regression analysis investigating region- and age-specific responses to vitamin D supplementation showed that adults in the MENA region require 1,229 IU/day to reach ≥ 30 ng/ml, whereas European adults (65-85 years old) can

achieve the same level with only 797 IU/day (32). Shab-Bidar et al. also conducted a meta-regression analysis including 7,150 individuals (mean age: 65.8) from several regions including US, Europe, Australia, and India, and reported that a dose of 800 IU/day is required to bring >97.5% of subjects to 20 ng/ml (33). To avoid the limitations inherent to meta-analysis of aggregate data, Cashman et al. conducted an IPD meta-regression using 7 winter-time North European RCTs to derive estimates of recommended vitamin D intakes. The ages in these trials varied widely, with only one elderly trial (mean age: 70 years), while others were in children, adolescents and younger adults. The IPD-derived estimate that brought >97.5% of the population to ≥ 20 ng/ml was ~26 µg/day (1040 IU/day), after adjusting for age and baseline level, whereas a standard meta-regression analysis of aggregate data from the same RCTs (assessed by several international agencies) had estimated the vitamin D requirement to be ~14 µg/day (560 IU/day) (21). Considering that the relationship between vitamin D dose and serum 25(OH)D level is linear at doses between 400 to 1,600 IU/day (14, 21) and the mean increment per 100 IU at low doses is 1 ng/ml, we project that the RDA in the overweight and obese Lebanese elderly would be 1,100 IU/day. Such projection is based on mean increments in serum 25(OH)D levels and does not take into account individual variations in response to vitamin D supplementation. Importantly, our projection is consistent with that reported by the IPD MA by Cashman et al.(21).

Baseline 25(OH)D level and vitamin D dose were the only significant predictors of 25(OH)D level post-intervention, accounting for 36.2% of the variance in response to therapy In line with our results, several previous studies consistently reported that the vitamin D dose (31-37) and baseline 25(OH)D level (31-35) are significant predictors of the achieved level post-intervention. Exceptionally, one meta-analysis conducted by Autier et al. on 6,207 Caucasians older than 50 receiving median vitamin D3 dose of 800 IU/day (range: 200-10,000 IU/day) revealed an inverse association between baseline 25(OH)D level and increments of serum 25(OH)D, but it did not reach statistical significance (38). Conversely, another global study of 7,564 postmenopausal women from 5 continents showed that the achieved level was higher for individuals with a baseline level <25 nmol/L (<10 ng/ml), compared to those >50 nmol/L (>20 ng/ml) (39). This heterogeneity in results could be owed to variations in

ethnicity and age across studies. Other factors such as age (32, 33), type of vitamin D (32, 38), trial duration (33), as well as BMI and season (35) were significant predictors of achieved serum 25(OH)D level in the literature, but they did not reach statistical significance in our trial. This may be explained by the relatively narrow range for age and BMI, in an exclusively older cohort, with entry criteria stipulating a BMI ≥25 kg/m^2. The impact of ethnicity on the achieved level post supplementation was explored by Mo et al., where he showed that vitamin D dose and baseline 25(OH)D level consistently and significantly predicted the achieved level in adults from different regions including Europe, North America, Asia, and the MENA region, whereas age was a significant predictor only in Europe (32). It was otherwise difficult to dissect the influence of ethnicity on achieved level, as many meta-analyses included trials from diverse regions/ethnicities, and did not account for this variable.

Our 25(OH)D increments per 100IU/day were 1 ng/ml for subjects randomized to 600 IU/ml and 0.41 ng/ml for subjects randomized to 3,750 IU/day. This trend is similar to what has been reported in the literature. Gallagher et al. noted increments of 1.6 ng/ml per 100 IU in postmenopausal older women randomized to 400 IU/day, and 0.6 ng/ml per 100 IU in women randomized to 4,800 IU/day (14). Mo et al. also demonstrated that among the elderly, there was an inverse trend between the dose administered and the achieved level, where the increment was 3.3 nmol/L per 100 IU/day (1.3 ng/ml per 100 IU/day) with the low weighted mean dose of 606 IU, whereas it was 1.7 nmol/L per 100 IU/day (0.68 ng/ml per 100 IU/day) with higher doses of 3,900 IU/day (32). He also showed that the increment per 100 IU/day is dependent on both the baseline 25(OH)D level (where it was highest in Asian adults who have a lower baseline than North Americans and Europeans), as well as age (where it was highest in the elderly (>64 years) compared to adults (18-64 years) receiving the same low dose) even though the weighted mean baseline 25(OH)D level in the elderly was higher than that of the adults (32). The increment per 100 IU/day also varies by body size, with smaller increments reported in individuals with higher BMI (40). Chakhtoura et al. also demonstrated that the increment per 100 IU/day was lowest (0.4-0.5 ng/ml) with high (3,750 IU) and intermediate (1,000 IU) doses, and highest (1 ng/ml) with low doses (600 IU) (31).

Our study has few potential limitations. It was limited geographically to the Greater Beirut area; however, study subjects were recruited from health centers that draw from 30-40% of the Lebanese population. The baseline 25(OH)D level (20.4 ng/ml) is representative of the general Lebanese population and is comparable to baseline levels in many other elderly cohorts worldwide (32, 33, 38). Our cohort consists of overweight and obese individuals (mean BMI: 30.2 kg/m^2); therefore, our findings are not generalizable to a population of leaner elderly individuals. However, our results apply to a large proportion of males and females older than 65 years worldwide. Indeed, the overweight and obesity rates average at 40% in males and 35% in females in developing countries, and at around 65% for males and females in developed countries (41, 42). The trial lacked a placebo arm because it would be unethical to have a control group without vitamin D supplementation given that more than half of our elderly cohort had a 25(OH)D level below 20 ng/ml. Importantly, the low dose corresponds to the currently recommended RDA by the IOM for adults and elderly until age 70 years, and thus serves as an appropriate surrogate control in our cohort. Our study duration was only 1 year, but vitamin D levels plateau within few weeks of administration (14, 43). We measured serum total 25(OH)D levels, and not the free or bioavailable metabolites. However, we have recently shown in this trial cohort that free and bioavailable 25(OH)D levels are not superior to total 25(OH)D in predicting bone health outcomes (44). We did not test the efficacy of an intermediate dose, for eg 2,200IU/day, due to lack of sufficient funding.

Our study has several important strengths, including its double-blinded design, its clinical relevance from recruiting high-risk elderly individuals, having included both genders, and having recruited across all seasons. We used vitamin D_3, the most commonly used form of vitamin D, measured its actual content in the tablets administered, and used LC-MS for serum 25(OH)D measurements. We also ensured high compliance of study subjects. Importantly, our choice of the doses of vitamin D also serves as a crucial strength, as the low dose (600 IU/day) is anchored at the IOM-recommended dose, and the high dose (3,750 IU/day) is below the IOM UTL.

In conclusion, in elderly overweight and obese Lebanese ambulatory subjects, with a mean 25(OH)D level of 20 ng/ml, the IOM recommended dose of 600 IU/day is not sufficient to bring 97.5% of the

cohort above the desirable threshold of 20 ng/ml. The trial fills a major knowledge gap, and provides evidence for care pathways and guidance for defining adequate vitamin D intake for ambulatory elderly subjects, from this region. We project that a dose of 1,100 IU/day would approximate the RDA for the elderly Lebanese. A dose response curve adjusted by age and baseline 25(OH)D level would best be suited to identify the lowest dose that surely allows individuals in the Middle East to achieve the desirable level.

Data Availability Statement:

Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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Figure Legends:

Figure 1 – Change in each participant's 25(OH)D level (ng/ml) from baseline to follow-up after 12 months of supplementation with low and high doses of vitamin D_3. Median 25(OH)D levels in the low-dose arm are 20 ng/ml and 25 ng/ml at baseline and after 12 months, respectively. Median 25(OH)D levels in the high-dose arm are 18.5 ng/ml and 35 ng/ml at baseline and after 12 months, respectively.

Figure 2 - Proportion of subjects with a serum 25(OH)D level ≥ 20 ng/ml and ≥ 30 ng/ml at baseline and after 1 year of supplementation with (A) low dose of 600 IU/day and (B) high dose of 3,750 IU/day. †Paired t-test p-value between baseline and after 1 year is <0.001. ‡Pearson chi-square p-value between high and low doses is <0.001.

Figure 3 - Dynamically-fit sigmoidal curves illustrating the % of subjects reaching a spectrum of desirable serum 25(OH)D levels at 1 year overall (A), in the low dose arm (B), and the high dose arm (C).

Figure 4 -**A.** Bland-Altman plot illustrating the overlap in delta 25(OH)D between the high dose and low dose arms. **B.** Corresponding Bland-Altman plot of the low dose arm showing regression analysis of the delta 25(OH)D ng/ml with changing baseline 25(OH)D levels (y=-0.60x + 18.0 (R2=30%)). **C.** Corresponding Bland-Altman plot of the high dose arm showing regression analysis of the delta 25(OH)D ng/ml with changing baseline 25(OH)D levels (y=-0.58x +27.3 (R2=19%).

Table 1 – Baseline characteristics of the study group overall, by dose, and gender.

	Overall	Low Dose (600 IU/day)	High Dose (3,750 IU/day)	P-value [a]
All Subjects	n=221	n=111	n=110	
Age (years)	71.1 ± 4.7	70.9 ± 4.6	71.2 ± 4.8	0.712
Gender (F/M)	122/99	59/52	63/47	0.538
BMI (kg/m^2)	30.2 ± 4.5	29.9 ± 4.6	30.6 ± 4.3	0.286
Calcium Dietary Intake (mg/day)	419.7 ± 282.2	442.2 ± 304.7	396.9 ± 257.0	0.234
Serum 25(OH)D (ng/ml)	20.4 ± 7.4	20.1 ± 6.9	20.6 ± 7.9	0.555
Season				0.904
Jan-March	72 (32.6)	34 (30.6)	38 (34.5)	
Oct-Dec	27 (12.2)	13 (11.7)	14 (12.7)	
Apr-Jun	66 (29.9)	35 (31.5)	31 (38.2)	

Jul-Sep	56 (25.3)	29 (26.1)	27 (24.5)	
Men:	n=99	n=52	n=47	
Age (years)	72.4 ± 5.5	72.1 ± 5.2	72.8 ± 5.8	0.535
BMI (kg/m^2)	28.7 ± 3.3	28.6 ± 3.2	28.9 ± 3.4	0.624
Calcium Dietary Intake (mg/day)	412.6 ± 266.9	410.7 ± 256.2	414.6 ± 281.2	0.943
Serum 25(OH)D (ng/ml)	19.5 ± 6.3	18.6 ± 6.0	20.4 ± 6.6	0.174
Women:	n=122	n=59	n=63	
Age (years)	69.9 ± 3.7	69.9 ± 3.8	70.0 ± 3.6	0.937
BMI (kg/m^2)	31.5 ± 4.9	31.1 ± 5.3	31.8 ± 4.6	0.436
Calcium Dietary Intake (mg/day)	425.4 ± 295	469.9 ± 341.6	383.7 ± 238.9	0.107
Serum 25(OH)D (ng/ml)	21.1 ± 8.2	21.3 ± 7.5	20.9 ± 8.8	0.762

a. P value: independent t-test comparing means between low dose and high dose of vitamin D supplementation.

Table 2 – Multivariate analysis of predictors for response to vitamin D_3 supplementation at 1 year.

	Predictors of serum 25(OH)D levels			**Predictors for 25(OH)D level ≥ 20 ng/mL**			**Predictors for 25(OH)D level ≥ 30 ng/mL**		
	Adjusted β (95% CI)	p-value	R^2 (%)	Adjusted OR (95 % CI)	p-value	R^2 (%)	Adjusted OR (95 % CI)	p-value	R^2 (%)
Age (years)	-0.02 (-0.26, 0.21)	NS	-	0.96 (0.83, 1.10)	NS	-	1.02 (0.95, 1.09)	NS	-
Female	0.37 (-1.97, 2.72)	NS	-	0.33 (0.09, 1.28)	NS	-	0.99 (0.49, 2.01)	NS	-
Baseline BMI (kg/m^2)	-0.07 (-0.33, 0.19)	NS	-	0.99 (0.87, 1.12)	NS	-	1.02 (0.94, 1.10)	NS	-
Season (vs. Jan-Mar)	-0.16 (-1.08, 0.75)	NS	-	-	NS	-	-	NS	-
Apr-Jun				1.61 (0.16, 16.8)	NS	-	0.85 (0.28, 2.64)	NS	-
Oct-Dec				0.31 (0.08, 1.20)	NS	-	0.58 (0.26, 1.29)	NS	-
Jul-Sep				1.89 (0.28, 12.56)	NS	-	0.69 (0.30, 1.61)	NS	-
Baseline Serum 25(OH)D level (ng/ml)	**0.41 (0.26, 0.56)**	**<0.001**	-	**1.17 (1.06, 1.29)**	**0.001**	-	**1.09 (1.04, 1.14)**	**0.001**	-
Treatment arm (high-dose)	**9.77 (7.63, 11.91)**	**<0.001**	-	**28.79 (3.24, 256.08)**	**0.003**	-	**8.74 (4.58, 16.68)**	**<0.001**	-
Overall Model		**<0.001**	36.2		**<0.001**	15.4 and 36.2		**<0.001**	26.2 and 34.9

Abbreviations: NS: not significant.

Figure 1

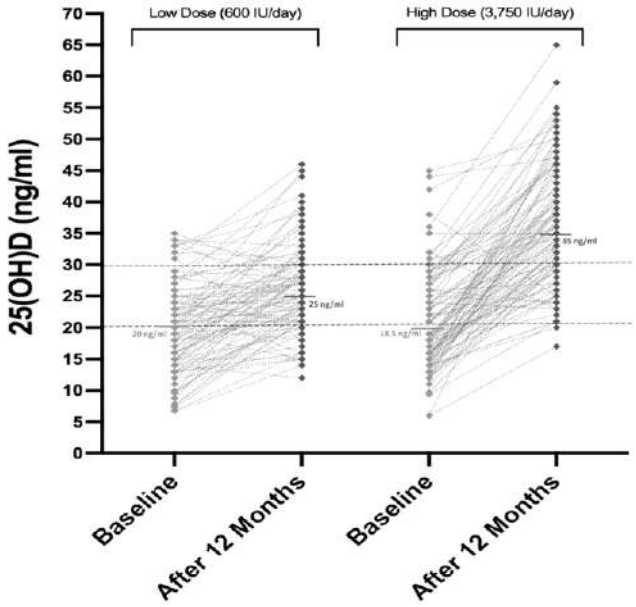

Figure 2

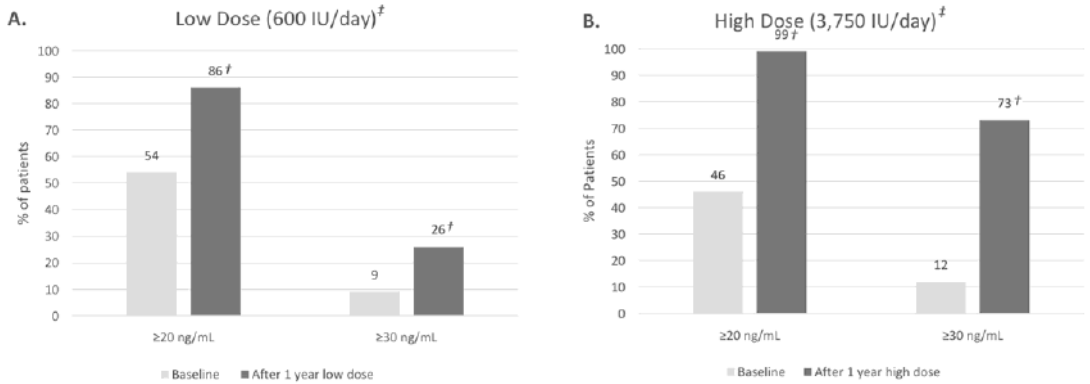

Figure 3

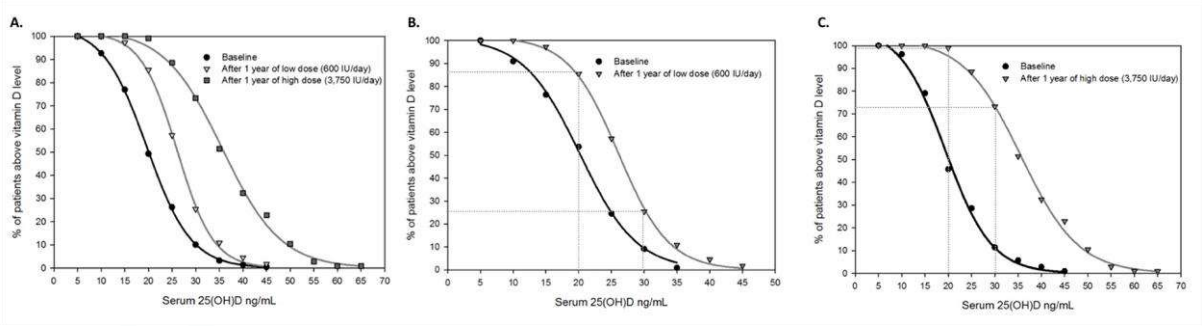

Figure 4