What factors modify the effect of monthly bolus dose vitamin D supplementation on 25-hydroxyvitamin D concentrations?

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What factors modify the effect of monthly bolus dose vitamin D supplementation on 25-hydroxyvitamin D concentrations?

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Highlights

- The 25(OH)D response to vitamin D supplementation differs between studies.
- There is little research on factors that modify the 25(OH)D response to vitamin D.
- We investigated effect modifiers in participants in a vitamin D supplement trial.
- Age, sex, 25(OH)D, BMI and sun exposure modify the 25(OH)D response to vitamin D.

Abstract

The increasing use of vitamin D supplements has stimulated interest in identifying factors that may modify the effect of supplementation on circulating 25-hydroxyvitamin D (25(OH)D) concentrations. Such information is of potential interest to researchers, clinicians and patients when deciding on bolus dose of vitamin D supplementation. We carried out a large randomized controlled trial of 5,110 adults aged 50-84 years, of European/Other (84%), Polynesian (11%) and Asian (5%) ethnicity, to whom we gave a standard dose of vitamin D_3 supplements (200,000 IU initially, then 100,000 IU monthly) which was taken with high adherence. All participants provided a baseline blood sample, and follow-up blood samples were collected at 6 months and annually for 3 years in a random sample of 441 participants, and also at 2 years in 413 participants enrolled in a bone density sub-study. Serum 25(OH)D was measured by LC/MSMS. Mixed model analyses were carried out on all 854 participants providing follow-up blood samples in multivariable models that included age, sex, ethnicity, body mass index (kg/m^2), tobacco smoking, alcohol intake, physical activity, sun exposure, season, medical prescription of high-dose vitamin D_3 (Cal.D.Forte tablets), asthma/COPD and the study treatment (vitamin D or placebo). The adjusted mean difference in 25(OH)D in the follow-up points between vitamin D supplementation and placebo groups was inversely related (all p for interaction <0.05) to baseline 25(OH)D, BMI, and hours of sun exposure, and higher in females, elders, and those with high frequency of alcohol, medical prescription of vitamin D, and asthma/COPD. The mean difference was not significantly related to ethnicity (p=0.12), tobacco (p=0.34), and vigorous activity (p=0.33). In summary, these data show that vitamin D status, BMI, sun exposure hours, sex and asthma/COPD

modify the 25(OH)D response to vitamin D supplementation. By contrast, ethnicity, tobacco smoking, and vigorous activity do not.

Keywords: randomized controlled trial, 25-hydroxyvitamin D; vitamin D supplementation; effect modification.

1. Introduction

Vitamin D is a fat-soluble secosteroid and is an inactive prohormone. The active form of vitamin D is 1α, 25-dihydroxyvitamin D_3, which acts as a classic hormone when it is released into the circulation [1]. By binding to the vitamin D receptor, it can regulate many functions, including cell proliferation, inflammation, antioxidant defences and calcium homeostasis [1]. Vitamin D status is mainly ascertained through measurement of the total blood 25-dihydroxyvitamin D (25(OH)D) levels, which is the major circulating form of vitamin D and with a half-life of 2-3 weeks [2, 3]. The main source of vitamin D is the synthesis of vitamin D_3 in the skin by solar UV irradiation [4]. In adults, dietary sources only contribute about 5%-10% of the requirement of vitamin D [4].

Although the optimal level of serum 25(OH)D is a topic of ongoing research, many researchers and organizations categorise a serum 25(OH)D level of less than 50 nmol/L as vitamin D deficiency [5-7]. Based on this definition, vitamin D deficiency is widespread across different locations around the world [5, 8, 9]. People with vitamin D deficiency can be brought above this specified level cheaply and easily with supplementation or food fortification [7, 10, 11]. Given it is more cost-effective to provide supplementation or fortify food with vitamin D than test 25(OH)D levels [12], it is useful to know whether there are differences in how people respond to vitamin D supplementation. This will help to ascertain if people with certain characteristics need more vitamin D supplements than others.

However, the determinants of the 25(OH)D response to vitamin D supplementation have been reported to differ across studies [13, 14]. A recent meta-analysis of 136 randomized controlled trials (RCTs) suggested that the higher dose of vitamin D supplements, higher baseline 25(OH)D levels and older age were significant predictors of higher achieved 25(OH)D levels after vitamin D supplementation [15]. A secondary analysis of a large RCT with 2187 older adults also revealed lower baseline 25(OH)D levels associated with larger increases in serum 25(OH)D levels in response to vitamin D supplementation, along with other associated factors include being female, optimal supplement adherence, winter season [16], but not age. In addition, a recent RCT with 133 menopausal women

demonstrated that larger changes in serum 25(OH)D levels from vitamin D supplementation were associated with higher dose of vitamin D supplements, lower baseline 25(OH)D level, early spring season [17], but did not exam other possible predictors. As summarised by Mazahery [13], 3 out of 20 studies failed to show any significant association between baseline 25(OH)D levels and increases in serum 25(OH)D levels in response to vitamin D supplementation; 6 out of 15 studies failed to show any relationships between BMI and response to vitamin D supplements. These inconsistent results could be attributed to variations in participants, sample size, dose of vitamin D supplement, different types of vitamin D, adherence of vitamin D supplements, and methodological considerations (e.g. regression to the mean, uncontrolled confounders). In addition, other factors may modify the 25(OH)D response.

Given the mixed evidence and the possibility of other influencing factors, we investigated which modify the effect of a monthly large dose of vitamin D supplementation on serum 25(OH)D levels using a subsample of the large Vitamin D Assessment (ViDA) study in New Zealand. The large sample provided by the ViDA study enables variables, including lifestyle, to be investigated which have previously received little attention. It also provides an opportunity to resolve debate about some variables which may or may not affect changes in people's circulating 25(OH)D concentrations from bolus dose vitamin D supplementation which is used clinically in some countries such as New Zealand.

2. Methods

2.1. Study design

We performed a longitudinal analysis of two subsamples of the ViDA study. The ViDA study recruited 5110 participants into a randomized, double-blinded, placebo-controlled trial to evaluate the effect of monthly large dose of vitamin D_3 supplementation on a range of health outcomes, including cardiovascular disease, acute respiratory infection, falls and fractures. The study methods have been described in detail elsewhere [18]. In brief, adults aged 50 to 84 years were recruited mainly (97%) from the patient registers of family physicians in Auckland during 2011-2012 and followed up to July 2015. Inclusion criteria were: (a) age 50-84 years, (b) ability to give informed consent, (c) resident in Auckland at recruitment, and (d) anticipated residence in New Zealand for the 4-year study period (2011-2015). Exclusion criteria were: (a) current use of vitamin D supplements (age 50-70 years >600 IU/d; 71-84 years >800 IU/d); (b) having a psychiatric disorder that prevented participation in the study; (c) history of hypercalcemia, nephrolithiasis, sarcoidosis, parathyroid disease, or gastric bypass surgery; (d) enrolled in another study which could affect participation in the vitamin D study; and (e) baseline serum calcium >2.50 mmol/L. The study was approved by the Multi-region Ethics Committees, Wellington (MEC/09/08/082), and all the participants provided written informed consent.

In the current analysis, participants comprised two subsamples of the ViDA study. A randomly selected sample who agreed to return at 6, 12, 24 and 36 months after randomization to provide a blood sample to measure serum calcium corrected for albumin (ADVIA analyzer, Siemens Healthcare Diagnostics, Tarrytown, NY) [19], on fresh blood) and 25(OH)D levels (in stored blood samples, measured in the same batch for each participant),and physical measurements (called the annual sample, n=441), and a randomly selected bone density sample agreed to have total body bone density at baseline and one more bone density measurement and a blood sample for 25(OH)D levels at two years after baseline (called the bone density sample, n=413).

2.2. Baseline assessment

All baseline assessments occurred within one month of each participant's eligibility being confirmed, and were carried out between April 2011 and November 2012. The baseline assessment lasted one to two hours, and the following information was collected: demographic status (age, sex, ethnicity), lifestyle (alcohol drinking frequency in the last 12 months, tobacco smoking, vigorous activity in a typical week during the past 3 months, sun exposure hours in a typical week during the last three months), and past medical history told by a doctor. Participants with asthma were those who said at their baseline assessment they had been informed by a doctor as having asthma, and who also were prescribed inhaled asthma medication (corticosteroids and/or beta-adrenergics) in the period 12 months prior baseline to 36 months after. Participants with chronic obstructive pulmonary disease (COPD) at baseline were defined as those having a ratio of forced expiratory volume in one second (FEV_1) divided by forced vital capacity (FVC) of <0.70 from spirometry, and who had smoked > 100 cigarettes in their whole life. The Ministry of Health Pharmaceutical Information Database [20] was accessed to capture prescription drugs, including high dose of vitamin D prescriptions (50,000 IU Cal.D.Forte tablets). Height (nearest 0.1 cm) was measured with a stadiometer, and weight (nearest 0.1 kg) was measured with digital scales, with both measurements performed without shoes and in light clothing. Body mass index (BMI) was calculated by the formula of weight in kilograms divided by height in meters squared. Participants were classified into normal weight (BMI <25.0 kg/m^2), overweight (BMI=25.0-29.9 kg/m^2) and obese (BMI≥30.0 kg/m^2) in this study. A non-fasting blood sample was collected from all the participants at baseline to screen for hypercalcemia, and stored at -80°C for later measurement of 25(OH)D concentrations.

2.3. Randomization and Intervention

After the baseline assessment, eligible participants were randomized to receive soft-gel oral capsules of either 100,000 IU vitamin D_3 or identical placebo (by Tishcon Corporation, Westbury, New York, USA). The randomization list was generated by a statistician not involved in outcome measures, by

strata of ethnicity and 5-year age categories, within randomly assigned blocks of 8, 10, or 12. Two capsules, with a 200,000-IU loading dose of vitamin D_3, or placebo, were given in the first mail-out after randomization, and thereafter, participants received one capsule monthly with 100,000-IU vitamin D_3 or placebo until the end of follow-up (July 2015). Investigators and participants were blinded until the end of follow-up.

2.4. Follow-up

A one-page duplex printed monthly questionnaire (with a reply-paid envelope) and one capsule were mailed to participants monthly until July 2013, and a four-monthly questionnaire and four capsules were mailed four-monthly thereafter. The monthly and four-monthly questionnaires collected information on the adherence of capsule use and non-hospital outcomes for cardiovascular conditions, infections, fall and fractures. A capsule was only recorded as being taken if the participant confirmed they had done so in the returned questionnaire or if otherwise confirmed by phone call or email. Additional blood samples to enable testing of 25(OH)D concentrations were collected at 6, 12, 24 and 36 months after baseline interview in the randomly selected annual sample and at 24 months in the randomly selected bone density sample. The blood samples were stored with those collected at baseline.

2.5. Serum 25(OH)D concentration

Serum 25(OH)D concentration (combining $25(OH)D_2$ and $25(OH)D_3$) at baseline and follow-up were both measured by liquid chromatography-tandem mass spectrometry 180 (LC-MS/MS, AB Sciex API 4000, Framingham, MA) in a local laboratory participating in the Vitamin D External Quality Assessment Scheme program (www.deqas.org). The assay did not measure 3-epimer but showed good agreement with NIST standard reference material 972. The interassay coefficient of variation for the assay is 12.7% at levels of 25-50 nmol/L and 8% at 100 nmol/L. Measurement of standards were, on average, within 4.1% (SD 2.9%) of target values. Baseline and follow-up blood samples from each person were tested in the same batch. Considering that the majority of vitamin D studies have selected

50 or 75 nmol/L as cut-offs for vitamin D deficiency, and the potential non-linear relationship between baseline 25(OH)D levels and their response to vitamin D supplementation, baseline 25(OH)D levels were categorized to <50.0 nmol/L, 50.0-74.9 nmol/L and ≥75.0 nmol/L in this analysis.

2.6. Statistical analysis

Multivariable mixed model analyses with a random intercept (which allows the intercepts to differ between the subjects) were carried out on all participants providing follow-up blood samples. In the analysis, all 25(OH)D levels were used as the continuous dependent variable (including the baseline 25(OH)D measurement), and age, sex, ethnicity, BMI, alcohol drinking frequency, tobacco smoking, vigorous activity, sun exposure, baseline 25(OH)D levels, enrolled season, medical prescription of high-dose vitamin D_3 (50,000 IU Cal.D.Forte tablets), asthma/COPD, follow-up time (baseline, 6-month, 1-year, 2-year, 3-year), study treatment (vitamin D or placebo), interaction between study treatment and follow-up time (study treatment * follow-up time), and further 3-way interactions between those baseline factors (age, sex, ethnicity, BMI, alcohol drinking frequency, tobacco smoking, vigorous activity, sun exposure, baseline 25(OH)D levels, enrolled season, medical prescription of high-dose vitamin D3), study treatment and follow-up time were included as independent variables [20]. The modification effects of the duration of monthly vitamin D supplementation (follow-up time) on serum 25(OH)D levels were reflected by an interaction test between study treatment and follow-up time. The modification effects of the above baseline factors on the effect of monthly vitamin D supplementation on 25(OH)D levels were reflected by 3-way interactions between those baseline factors, study treatment and follow-up time. The adjusted mean difference [MD] between vitamin D supplementation and placebo groups and 95% confidence interval [CI] of 25(OH)D levels at each follow-up time point were used to reflect the response to vitamin D supplement [21]. Considering the sample size of this subsample, we chose multivariable model analyses for the whole group, and did not do separate subgroup analyses by level of for each baseline variable. All analyses were performed using SAS version 9.4 (Cary, NC). A two-sided $p \leq 0.05$ was considered statistically significant.4

3. Results

3.1 Study participants

From all 5110 participants of the ViDA study, a randomly selected annual sample of 441 participants (out of 518 invited) and a randomly selected bone density sample of 413 participants (out of 616 invited) were included in the current analysis. In total, 854 eligible participants with baseline plus one or more follow-up blood samples were randomized into vitamin D supplementation (n=432) or placebo (n=422) groups (Figure 1). During the follow-up periods, not all the selected participants returned to provide a blood sample at each interview, with 83% returning at 6 months (367 out of 441), 90% at 1-year (n=396 out of 441), 93% at 2-year (795 out of 854) and 76% at 3-year (334 out of 441). The proportions of participants who returned at each follow-up time point were similar between the vitamin D and placebo groups (see appendix Table).

3.2 Baseline characteristics

The baseline characteristics of all the 854 participants are shown in **Table 1**. Most were male (61%) or of European/Other ethnicity (84%), and the mean (SD) age was 67 (8) years. A total of 257 participants (30%) were obese, and 402 (47%) were overweight. In the last 12 months, around one in four drank alcohol daily (23%). Most participants were never smokers (52%), followed by ex-smokers (41%) and current smokers (7%). As shown in Table 1, the baseline characteristics of participants were comparable between the vitamin D supplementation and placebo groups.

3.3 Serum 25(OH)D levels

In all 854 participants, mean (SD) baseline 25(OH)D level was 59 (24) nmol/L and 40% had 25(OH)D levels <50.0 nmol/L. As shown in Table 2, there was no difference in mean observed 25(OH)D levels between vitamin D and placebo groups at baseline (59 vs 59 nmol/L, p=0.87). However, in all the

follow-up time points, the vitamin D supplementation group had significantly higher 25(OH)D levels than the placebo group (mean differences ranged from 53 nmol/L to 69 nmol/L between two groups) (Table 2).

3.4 Factors modifying 25(OH)D response

As shown in Table 3, in the follow-up period, the adjusted mean difference in 25(OH)D levels between the two treatment groups ranged from 50.9 nmol/L at 6 months and 63.1 nmol/L at 3-year follow-up ($p_{interaction}=0.004$). After adjusting for the co-variables, the factors that significantly modified the effect of monthly vitamin D supplementation on mean difference in 25(OH)D levels compared to placebo were sex ($p_{interaction}=0.002$), age ($p_{interaction}=0.03$), baseline 25(OH)D levels ($p_{interaction}<0.001$), BMI ($p_{interaction}=0.002$), alcohol drinking frequency ($p_{interaction}=0.03$), sun exposure hours ($p_{interaction}<0.001$), season ($p_{interaction}<0.001$), medical prescription of 50,000 IU vitamin D ($p_{interaction}<0.001$) or asthma/COPD ($p_{interaction}<0.001$). However, the mean difference in 25(OH)D levels between vitamin D supplementation and placebo groups was not related to ethnicity ($p_{interaction}=0.12$), tobacco smoking ($p_{interaction}=0.34$), or vigorous activity ($p_{interaction}=0.33$). Neither were there significant interactions between the annual sample and the bone density sample (sample * treatment, $p_{interaction}=0.94$; sample * time, $p_{interaction}=0.23$).

As shown in Figure 2, male participants had a lower mean difference in 25(OH)D levels between treatment groups at each follow-up time point than female participants. Baseline 25(OH)D levels were inversely related to mean difference in 25(OH)D levels between treatment groups, with the highest response in participants with vitamin D deficiency (<50 nmol/L), followed by participants with 50.0-74.9 nmol/L and ≥ 75.0 nmol/L at 1-year and 2-year follow-up. Similar inverse associations at follow-up were observed between BMI and mean difference in 25(OH)D levels between treatment groups (highest response in participants with normal BMI, followed by overweight and obese). Sun exposure hours also were associated inversely with mean difference in 25(OH)D levels (lower response to vitamin D supplementation in participants with higher sun exposure hours). Although there was a

significant interaction between response in 25(OH)D levels and season, no clear pattern was observed. In addition, participants who were older, drank alcohol more frequency, were not medically prescribed 50,000 IU vitamin D, or had asthma/COPD had significantly higher 25(OH)D responses to vitamin D supplementation.

4. Discussion

Given ongoing interest in identifying factors that may modify the effect of bolus dose vitamin D supplementation on circulating 25(OH)D levels, we investigated the longitudinal relationship between baseline factors and the difference in 25(OH)D levels between vitamin D supplementation and placebo groups in two subsamples of ViDA study participants with follow-up blood measures. In this community-based study of 854 eligible participants, the mean difference in 25(OH)D levels between vitamin D supplementation and placebo groups was inversely related to baseline 25(OH)D levels, BMI, hours of sun exposure; varied with season; were higher with female sex, older age, daily alcohol drinking, medical prescription of 50,000 IU vitamin D and among those with asthma/COPD. By contrast, the mean difference was not significantly related to ethnicity, tobacco smoking, and vigorous activity.

Response factors for mean difference in serum 25(OH)D between vitamin D supplementation and placebo groups have been studied previously in adults, but with conflicting results. A recent systematic review with 20,884 participants from 136 randomized controlled trials found, after adjusting for vitamin D supplementation dose, that baseline 25(OH)D levels were positively related to post-intervention serum 25(OH)D levels in adults participants (68 studies), pregnant women (12 studies) and children (19 studies) [15]. In that review, the mean change in the post-intervention of 25(OH)D levels ranged from 0.6 (95%CI=0.5-0.7) to 0.9 (95%CI=0.7-0.1) nmol/L for 1 nmol/L increase in the baseline 25(OH)D levels [15]. A systematic review of changes in serum 25(OH)D levels in mainly Caucasian participants aged 50 years or over (which included 74 studies) found a non-significant inverse association between increases in 25(OH)D levels and baseline 25(OH)D levels (mean change = -0.12, 95%CI=-0.33, 0.10, p=0.28), after controlling for dose, type of vitamin D and calcium supplement [22]. The inconsistent findings between our study and the above systematic reviews could be attributed to the heterogeneity across studies in these reviews (e.g. ethnicity difference) and limitations of the meta-regression method (e.g. limited number of trials and co-variables controlled) [23]. By contrast, as reviewed by Mazahery et al [13], 17 out of 20 interventional studies reported a significant inverse relationship between post-

intervention 25(OH)D levels and baseline 25(OH)D levels. All of the three non-significant trials had small sample sizes (ranging from 53 to 61) and a narrow range of baseline 25(OH)D levels. The inverse relationship was seen between baseline 25(OH)D levels and difference in 25(OH)D levels between two groups may be related to the conversion of vitamin D being saturable. Heaney et al [24] suggest that at lower 25(OH)D concentrations, levels rise rapidly as the conversion to 25(OH)D is a first order reaction. Once the hepatic hydroxylases become saturated the reaction switches to zero order and the conversion of vitamin D to 25(OH)D slows [24]. In addition, there appears to be have a counter-regulatory mechanism which is acting to prevent very high 25(OH)D levels [25].

BMI has been reported to modify the 25-hydroxyvitamin D response to vitamin D supplementation. A comprehensive literature review concluded that 10 out of 18 intervention studies reported that higher BMI or body fat was associated with a smaller difference in 25(OH)D levels in response to vitamin D supplementation [13]. The failure of some studies to show any interaction with BMI have been largely attributed to their small sample size, small dose of vitamin D supplements, and not have enough participants in different BMI categories. One explanation for the interaction with BMI is that vitamin D is fat soluble, and that the more body fat a person has, the more vitamin D is stored (and sequestered) in fat tissues. The tightly bound vitamin D is unable to be released into the body as needed, and resulting in lower circulating levels [26, 27]. In addition, as suggested, obese adults (BMI >30 kg/m^2) required two to three times more vitamin D supplementation to reach the same increase in 25(OH)D level as normal weighted adults [5, 11, 28].

Having asthma/COPD significantly modified the mean difference of 25(OH)D levels in response to vitamin D supplement ($p_{interaction} < 0.001$). Participants with asthma/COPD had a higher 25(OH)D difference between two groups at each follow-up point, except 3-year follow-up. Recently, individual participant data meta-analyses have found that vitamin D supplementation significantly reduces the rate of asthma exacerbation in patients with baseline 25(OH)D levels <25 nmol/L [29, 30]. This relationship and possible mechanisms between asthma/COPD and a possible high response to vitamin D supplementation merits further investigation.

Season is another factor that can modify the mean difference in 25(OH)D levels in response to vitamin D supplementation. A seasonal effect on mean difference of 25(OH)D levels has been reported in the past, with the highest response to vitamin D supplementation in winter or spring [13, 16, 17]. However, we did not observe a clear pattern in our study, except for the highest response to monthly vitamin D supplementation in summer (December to February). This might due to chance given that only 26 participants enrolled in summer season in our analysis.

In addition to the above factors, we found that hours of sun exposure, prescription of high-dose vitamin D supplementation, alcohol drinking frequency, age and sex significantly modified the response to vitamin D supplementation. The inverse association between higher sun exposure hours and lower response to vitamin D supplementation can be partly explained by the relationship between sun exposure hours and 25(OH)D level. As reported previously [31-33], length of sun exposure hours was highly related to serum 25(OH)D levels. Similar to sun exposure hours, prescription of high-dose vitamin D supplementation is also highly related to serum 25(OH)D levels. This might indicate that those factors are independent determinants of response to vitamin D supplement, or they might reflect the residual effect of baseline 25(OH)D levels. The decreased 25(OH)D response in men, independent of BMI, could be due to their increased muscle mass and greater sequestration of vitamin D into fat-soluble skeletal muscle cells, compared to women [34]. This mechanism may also explain the inverse association between 25(OH)D response and age as muscle mass decreases with increasing age [35]. The explanation for the interaction between alcohol drinking frequency and response to vitamin D supplementation is unclear and we recognize that this could be a chance finding ($p=0.03$). Nevertheless, we did not find ethnicity, smoking and vigorous activity were independent effect modifiers of the increase in 25(OH)D levels in response to vitamin D supplementation. The reason for the lower increase in 25(OH)D response at 2-years compared to 1-year and 3-years is unclear (Table 3 and Figure 2). It is not due to inclusion of participants from the bone survey at 2-years for this time point as mean 25(OH)D concentrations at 2-years were similar for both the annual and bone density samples (Table 2).

Strengths of the current study include recruitment of participants from the community which increases the external validity of the findings, the wide range of potential factors explored in statistical models, and the measurement of serum 25(OH)D using the current gold-standard LC-MS/MS method. The ViDA study had both high retention (87% still participating in the final follow-up) and high adherence (84% of capsules reported taken in questionnaires). The significantly higher 25(OH)D levels in the follow-up period for the vitamin D group also confirmed this good adherence. Further, the annual sample and bone-density sample combined resulted in a large sample size comprising 854 people, larger than most previous RCTs which investigated change in 25(OH)D levels in response to vitamin D supplementation. In addition, regression to the mean was adjusted by including baseline 25(OH)D levels as a co-variable in the mixed model [21]. There are also some limitations in this study. First, there are missing data in the follow-up period, but any bias from these was minimised by the use of the mixed model method under the missing at random mechanism [36]. Second, although a range of co-variables at baseline has been controlled in our analysis, the changes of those co-variables were not incorporated in the analysis. Participants in both treatment and placebo group could take low-dose vitamin D and calcium; but this might bias the increases in 25(OH)D levels in response to vitamin D treatment towards the null by increasing 25(OH)D levels of the placebo group. Third, the 25(OH)D assay used in this study has not been formally standardised against a reference method, which decreases the comparability of the 25(OH)D data in our study with that from other studies which have been standardized. However, the same assay was used to measure 25(OH)D in both study arms which ensures the validity of our results from the comparison between the vitamin D and placebo groups at each time point.

5. Conclusion

In summary, in a subsample of the ViDA study with 854 older adults in New Zealand, we examined 13 baseline factors that could potentially modify the difference of 25(OH)D levels in response to bolus dose vitamin D supplementation. We confirmed that several demographic and physiological variables – such as age, sex, baseline 25(OH)D status, BMI, sun exposure hours and asthma/COPD – modify the

effect of 25(OH)D levels in response to bolus dose vitamin D supplementation. By contrast, ethnicity, tobacco smoking, and vigorous activity do not. These significant modifying factors should be taken into account in individuals with vitamin D deficiency (e.g. 25OHD levels < 50 nmol/L) to estimate the adequate vitamin D supplementation dose and frequency for achieving sufficient 25(OH)D levels.

Authors statement

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Conflicts of interest

All authors declare no conflict of interest.

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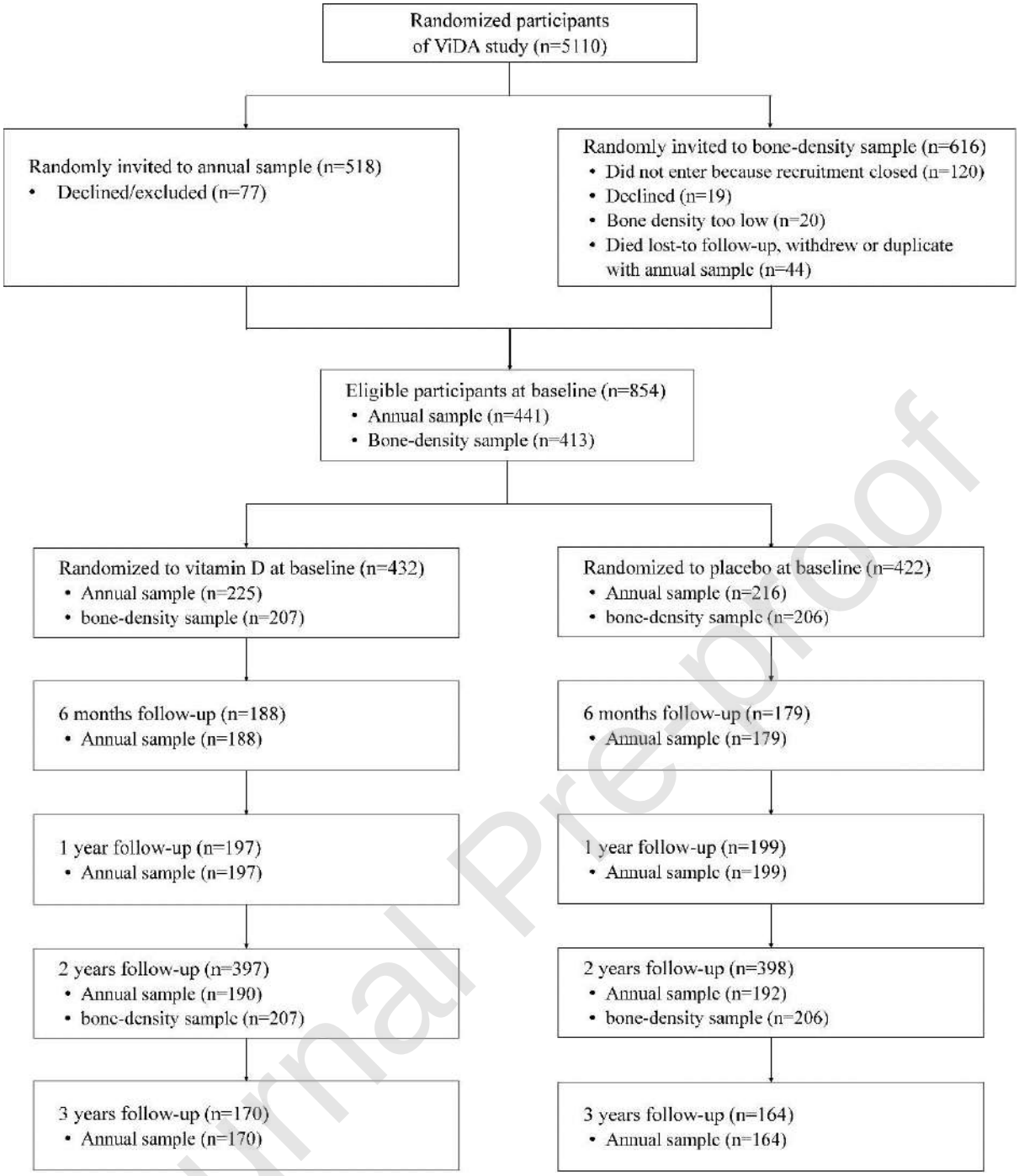
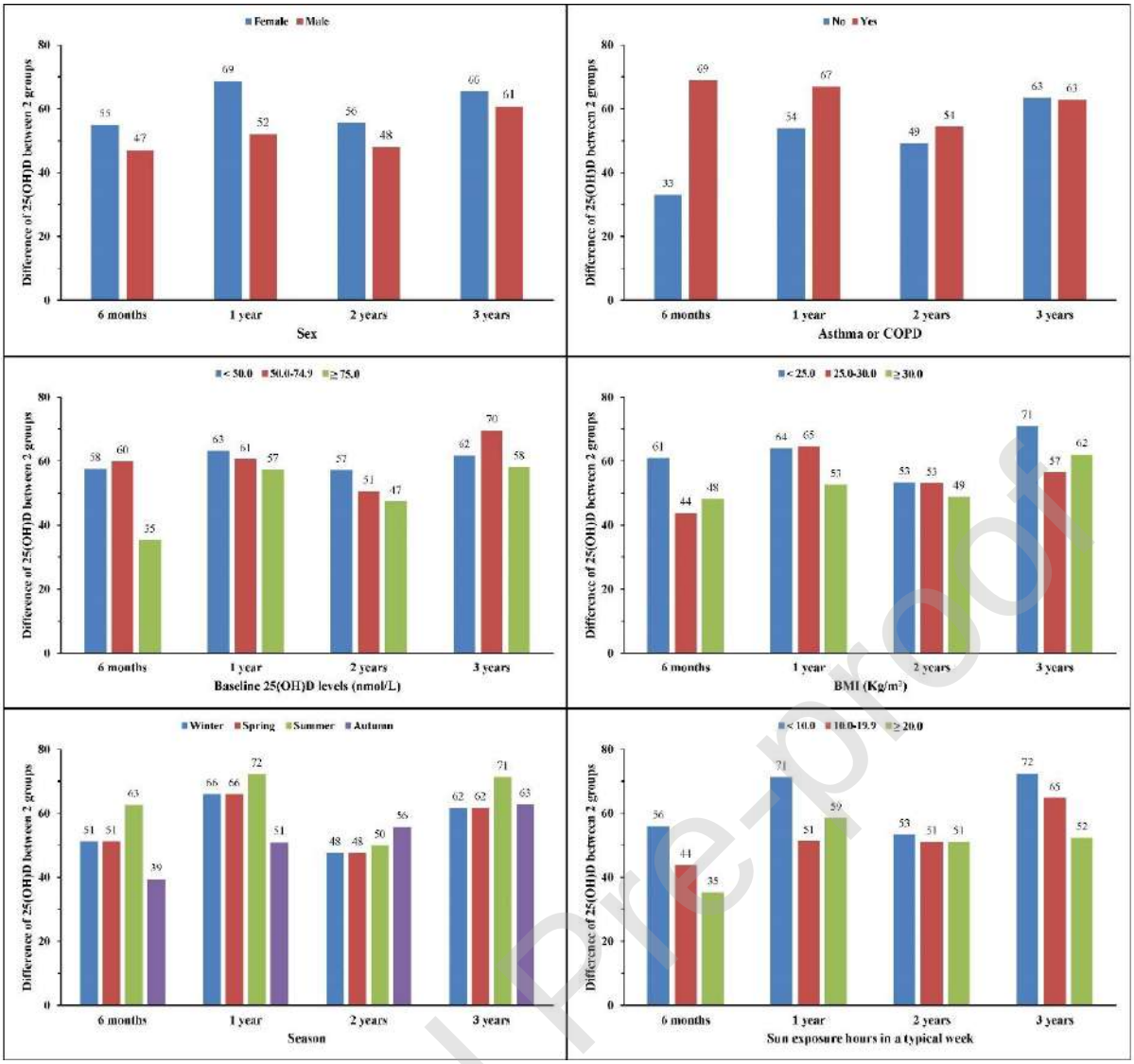


Figure 1 Eligible participants in this study



Notes, 25(OH)D, 25-hydroxyvitamin D (nmol/L); BMI, body mass index; Winter, June to August; Spring, September to November; Summer, December to February; Autumn, March to May; Mean difference of 25(OH)D levels between two groups were adjusted for age, sex, ethnicity, baseline 25(OH)D levels, BMI, vigorous activity in a typical week, sun exposure hours in a typical week, enrolled season, prescription of vitamin D and asthma/COPD.

Figure 2 Adjusted mean difference in 25-hydroxyvitamin D levels between the two treatment groups (placebo as reference)

Table 1 Baseline characteristics of participants

	All participants (n=854)	Placebo group (n=422)	Vitamin D group (n=432)	p value
Sex, n (%)				0.63
Female	331 (38.8)	167 (39.6)	164 (38.0)	
Male	523 (61.2)	255 (60.4)	268 (62.0)	
Age (years), n (%)				0.52
50-54	88 (10.3)	43 (10.2)	45 (10.4)	
55-64	220 (25.8)	100 (23.7)	120 (27.8)	
65-74	406 (47.5)	205 (48.6)	201 (46.5)	
75-84	140 (16.4)	74 (17.5)	66 (15.3)	
Ethnicity, n (%)				0.86
European/Other	719 (84.2)	359 (85.1)	360 (83.3)	
Mäori	45 (5.3)	20 (4.7)	25 (5.8)	
Pacific	50 (5.9)	23 (5.5)	27 (6.3)	
South Asian	40 (4.7)	20 (4.7)	20 (4.6)	
BMI (kg/m^2), n (%)				0.08
Normal weight (<25.0)	195 (22.8)	90 (21.3)	105 (24.3)	
Overweight (25.0-29.9)	402 (47.1)	190 (45.0)	212 (49.1)	
Obese (≥30.0)	257 (30.1)	142 (33.6)	115 (26.6)	
Alcohol drinking frequency in the last 12 months, n (%)				0.39
None	113 (13.2)	52 (12.3)	61 (14.1)	
< 4 times monthly	249 (29.2)	134 (31.8)	115 (26.6)	
< 7 times weekly	300 (35.1)	146 (34.6)	154 (35.6)	
Daily	192 (22.5)	90 (21.3)	102 (23.6)	
Tobacco smoking, n (%)				0.22
Never smoker	448 (52.5)	209 (49.5)	239 (55.3)	
Ex-smoker	348 (40.7)	184 (43.6)	164 (38.0)	
Current smoker	58 (6.8)	29 (6.9)	29 (6.7)	
Vigorous activity in a typical week during the past 3 months (hour), n (%)				0.36
None	329 (38.5)	157 (37.2)	172 (39.8)	
1-2	236 (27.6)	126 (29.9)	110 (25.5)	
≥2	289 (33.8)	139 (32.9)	150 (34.7)	
Sun exposure in a typical week during the past 3 months (hours), n (%)				0.47
<10	383 (44.8)	198 (46.9)	185 (42.8)	
10-<20	279 (32.7)	134 (31.8)	145 (33.6)	
≥20	192 (22.5)	90 (21.3)	102 (23.6)	
25-hydroxyvitamin D level (nmol/L), n (%)				0.56
<50.0	337 (39.5)	166 (39.3)	171 (39.6)	
50.0-74.9	309 (36.2)	147 (34.8)	162 (37.5)	
≥75.0	208 (24.4)	109 (25.8)	99 (22.9)	

Season, n (%)				0.55
Winter (June – August)	379 (44.4)	187 (44.3)	192 (44.4)	
Spring (September-November)	349 (40.9)	171 (40.5)	178 (41.2)	
Summer (December-February)	26 (3.0)	10 (2.4)	16 (3.7)	
Autumn (March-May)	100 (11.7)	54 (12.8)	46 (10.6)	
Prescription of vitamin D in the past 6 months before baseline *, n (%)				0.37
No	849 (99.4)	421 (99.8)	428 (99.1)	
Yes	5 (0.6)	1 (0.2)	4 (0.9)	
Asthma/COPD				0.06
No	709 (83.0)	340 (80.6)	369 (85.4)	
Yes	145 (17.0)	82 (19.4)	63 (14.6)	

Notes, n, number of participants; Current smoker, someone has smoked greater than 100 cigarettes in their lifetime; Ex-smoker, someone has smoked greater than 100 cigarettes in their lifetime; Never smoker, someone who has not smoked greater than 100 cigarettes in their lifetime and does not currently smoke; *, 50,000 IU vitamin D (Cal.D.Forte Tablets); COPD, Chronic obstructive pulmonary disease.

Table 2 Observed 25-hydroxyvitamin D levels at baseline and follow-up points

Time point	No. of participants	Vitamin D group		Placebo group		p value
		n	mean (SD)	n	mean (SD)	
All participants						
Baseline	854	432	58.8 (24.0)	422	59.0 (23.2)	0.87
6-month follow-up	367	188	128.7 (42.1)	179	75.3 (31.4)	<0.001
1-year follow-up	396	197	119.3 (44.9)	199	60.3 (28.0)	<0.001
2-year follow-up	795	397	130.2 (33.8)	398	62.5 (25.3)	<0.001
3-year follow-up	334	170	135.5 (39.8)	164	66.1 (28.9)	<0.001
Annual sample						
Baseline	441	225	61.5 (24.4)	216	61.4 (23.7)	0.87
6-month follow-up	367	188	128.7 (42.1)	179	75.3 (31.4)	<0.001
1-year follow-up	396	197	119.3 (44.9)	199	60.3 (28.0)	<0.001
2-year follow-up	382	190	131.5 (39.1)	192	65.5 (27.2)	<0.001
3-year follow-up	334	170	135.5 (39.8)	164	66.1 (28.9)	<0.001
Bone density sample						
Baseline	413	207	55.7 (23.2)	206	56.6 (22.4)	0.87
2-year follow-up	413	207	128.9 (28.1)	206	59.8 (23.1)	<0.001

Notes, n, number of participants; SD, standard deviation; 25-hydroxyvitamin D levels in nmol/L.

Table 3 Adjusted mean difference at each follow-up time point in 25-hydroxyvitamin D levels between the two treatment groups (placebo as reference)

	Adjusted difference in 25(OH)D levels (nmol/L) between two groups (vitamin D-placebo) MD (95%CI), p					p value for interaction
	Baseline	6-month	1-year	2-year	3-year	
All participants	0.6 (-27.3, 28.6), 0.96	50.9 (21.0, 80.9), <0.001	60.3 (41.8, 78.9), <0.001	51.7 (38.4, 65.1), <0.001	63.1 (45.6, 80.6), <0.001	0.004
Sex						0.002
Female	1.1 (-26.9, 29.2), 0.93	55.0 (24.9, 85.2), <0.001	68.7 (49.4, 88.1), <0.001	55.6 (41.6, 69.5), <0.001	65.5 (47.2, 83.8), <0.001	
Male	0.1 (-28.2, 28.4), 0.99	46.9 (16.0, 77.7), 0.003	52.0 (32.6, 71.3), <0.001	47.9 (34.2, 61.7), <0.001	60.7 (42.2, 79.2), <0.001	
Age (years)						0.03
50-54	-1.6 (-30.8, 27.6), 0.91	39.4 (6.9, 71.8), 0.01	64.0 (43.0, 84.9), <0.001	48.2 (32.1, 64.4), <0.001	58.9 (37.9, 79.9), <0.001	
55-64	1.1 (-27.4, 29.6), 0.94	56.8 (26.0, 87.7), <0.001	55.5 (35.8, 75.1), <0.001	45.8 (31.4, 60.2), <0.001	69.1 (50.2, 88.0), <0.001	
65-74	0.1 (-28.2, 28.4), 1.00	42.2 (12.0, 72.5), 0.006	55.8 (36.3, 75.2), <0.001	50.9 (36.6, 65.3), <0.001	57.0 (37.9, 76.0), <0.001	
75-84	3.0 (-26.5, 32.4), 0.84	65.4 (32.1, 98.6), <0.001	66.2 (42.3, 90.1), <0.001	62.0 (45.5, 78.6), <0.001	67.5 (44.3, 90.7), <0.001	
Baseline 25(OH)D levels (nmol/L)						<0.001
< 50.0	0.9 (-27.2, 29.1), 0.95	57.6 (26.3, 88.9), <0.001	63.2 (43.7, 82.6), <0.001	57.2 (43.4, 71.1), <0.001	61.7 (42.7, 80.8), <0.001	
50.0-74.9	-1.6 (-30.2, 27.0), 0.91	59.9 (29.5, 90.3), <0.001	60.6 (40.5, 80.7), <0.001	50.6 (36.3, 65.0), <0.001	69.5 (49.9, 89.2), <0.001	

≥ 75.0	2.5 (-26.0, 31.0), 0.86	35.3 (3.9, 66.8), 0.03	57.3 (36.4, 78.2), <0.001	47.4 (32.5, 62.2), <0.001	58.1 (38.5, 77.6), <0.001	
BMI (kg/m^2)						<0.001
< 25.0	0.5 (-28.4, 29.4), 0.97	60.9 (29.1, 92.7), <0.001	63.9 (42.9, 84.9), <0.001	53.3 (38.4, 68.2), <0.001	70.9 (50.8, 90.9), <0.001	
25.0-29.9	0.8 (-27.6, 29.1), 0.96	43.7 (13.2, 74.2), 0.005	64.6 (45.2, 84.0), <0.001	53.1 (39.1, 67.2), <0.001	56.5 (37.6, 75.4), <0.001	
≥ 30.0	0.6 (-27.4, 28.7), 0.96	48.2 (17.3, 79.2), 0.002	52.5 (32.6, 72.5), <0.001	48.8 (34.5, 63.0), <0.001	62.0 (42.7, 81.2), <0.001	
Alcohol drinking frequency in the last 12 months						0.03
None	-0.3 (-29.1, 28.5), 0.98	49.4 (16.2, 82.5), 0.004	45.1 (24.0, 66.2), <0.001	47.7 (32.4, 63.0), <0.001	58.1 (36.7, 79.6), <0.001	
< 4 times monthly	2.4 (-25.9, 30.7), 0.87	50.0 (19.0, 81.0), 0.002	56.0 (35.8, 76.2), <0.001	53.6 (39.1, 68.0), <0.001	65.0 (45.0, 85.1), <0.001	
< 7 times weekly	0.7 (-28.1, 29.4), 0.96	60.2 (28.4, 92.1), <0.001	71.0 (49.5, 92.5), <0.001	48.4 (33.4, 63.4), <0.001	58.7 (38.3, 79.1), <0.001	
Daily	-0.3 (-29.2, 28.7), 0.98	44.2 (13.2, 75.2), 0.005	69.3 (47.9, 90.7), <0.001	57.3 (42.1, 72.6), <0.001	70.6 (49.5, 91.7), <0.001	
Sun exposure in a typical week during the past 3 months (hours)						<0.001
<10	0.2 (-28.2, 28.7), 0.99	55.9 (25.1, 86.6), <0.001	71.3 (51.2, 91.4), <0.001	53.3 (39.1, 67.4), <0.001	72.4 (53.4, 91.4), <0.001	
10-<20	1.6 (-26.6, 29.8), 0.91	43.8 (13.1, 74.5), 0.005	51.3 (31.9, 70.6), <0.001	51.0 (36.9, 65.1), <0.001	64.7 (45.8, 83.6), <0.001	
≥20	0.0 (-28.5, 28.6), 1.00	53.2 (21.7, 84.6), <0.001	58.5 (37.9, 79.1), <0.001	51.0 (36.2, 65.7), <0.001	52.3 (32.0, 72.5), <0.001	
Season						<0.001
Winter	-0.3 (-28.3, 27.8), 0.99	50.8 (20.3, 81.2), 0.001	52.3 (33.2, 71.4), <0.001	54.0 (40.7, 67.4), <0.001	56.8 (38.8, 74.8), <0.001	
Spring	0.0 (-27.9, 28.0), 1.00	51.2 (20.1, 82.3), 0.001	66.0 (46.0, 86.0), <0.001	47.5 (34.0, 61.0), <0.001	61.7 (43.0, 80.3), <0.001	
Summer	2.8 (-31.0, 36.6), 0.87	62.6 (21.7, 103.4), 0.003	72.2 (43.8, 100.6), <0.001	49.9 (23.9, 75.9), <0.001	71.3 (40.1, 102.4), <0.001	
Autumn	-0.1 (-29.1, 29.0), 1.00	39.3 (8.5, 70.1), 0.01	50.9 (30.4, 71.3), <0.001	55.6 (39.9, 71.3), <0.001	62.7 (43.5, 82.0), <0.001	
Prescription of vitamin D						<0.001
No	0.7 (-8.3, 9.7), 0.89	69.2 (56.7, 81.6), <0.001	78.1 (66.0, 90.1), <0.001	72.8 (62.5, 83.1), <0.001	87.6 (73.5, 101.7), <0.001	
Yes	0.6 (-53.2, 54.4), 0.98	32.7 (-24, 89.9), 0.26	42.6 (10.4, 74.8), 0.01	30.7 (9.5, 51.9), 0.005	38.6 (11.7, 65.6), 0.005	
Asthma/COPD						<0.001
No	0.6 (-27.3, 28.6), 0.97	33.0 (3.0, 63.0), 0.03	53.8 (34.8, 72.7), <0.001	49.2 (35.8, 62.7), <0.001	63.4 (45.8, 81.0), <0.001	
Yes	0.6 (-28.1, 29.3), 0.97	68.9 (37.2, 101.6), <0.001	66.9 (46.1, 87.7), <0.001	54.3 (39.3, 69.2), <0.001	62.8 (42.3, 83.4), <0.001	

Notes, 25(OH)D, 25-hydroxyvitamin D; MD, mean difference CI, confidence interval; BMI, body mass index; Winter, June to August; Spring, September to November; Summer, December to February; Autumn, March to May; COPD, Chronic obstructive pulmonary disease; Mean difference of 25(OH)D levels between two groups were adjusted for age, sex, ethnicity, baseline 25(OH)D levels, BMI, vigorous activity in a typical week, sun exposure hours in a typical week, enrolled season, prescription of vitamin D and asthma/COPD.

Appendix Table: The number (percent) of participants who returned at each follow-up time point.

Time	Annual sample			Bone density sample		
	Total	**Vitamin D**	**Placebo**	**Total**	**Vitamin D**	**Placebo**
Baseline	441 (100)	225 (100)	216 (100)	413 (100)	207 (100)	206 (100)
6 months	367 (83.2)	188 (83.6)	179 (82.9)			
1 year	396 (89.8)	197 (87.6)	199 (92.1)			
2 years	382 (86.6)	190 (84.4)	192 (88.9)	413 (100)	207 (100)	206 (100)
3 years	334 (75.7)	170 (75.6)	164 (75.9)			