The Calgary Vitamin D Study

Safety of High-Dose Vitamin D Supplementation: Secondary Analysis of a Randomized Controlled Trial

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Abstract

Context: Over 3% of adults report vitamin D intakes ≥4000 IU/day, but the safety of this practice is unknown.

Objective: To establish whether vitamin D doses up to 10000 IU/day are safe and well-tolerated.

Design: The Calgary Vitamin D Study was a three-year double-blind RCT.

Setting: Single-centre study at the University of Calgary, Canada.

Participants: Healthy adults (n=373) aged 55-70 with serum 25-hydroxyvitamin D 30-125 nmol/L.

Interventions: Participants were randomized 1:1:1 to vitamin D3 400, 40000 or 10000 IU/day. Calcium supplementation was initiated if dietary calcium intake was <1200mg/day.

Main Outcome Measures: In these pre-specified secondary analyses, changes in serum 25-hydroxyvitamin D, calcium, creatinine, 24-hour urine calcium excretion, and incidence of adverse events were assessed. Between-group differences in adverse events were examined using incident rate differences and logistic regression.

Results: Of 373 participants (400:124, 4000:125, 10000:124), 49% were male, mean (SD) age was 64 (4) years, and 25-hydroxyvitamin D 78.0 (19.5) nmol/L. Serum calcium, creatinine, and 24-hour urine calcium excretion did not differ between treatments. Mild hypercalcemia (2.56-2.64 mmol/L) occurred in 15 (4%) participants (400:0%, 4000:3%, 10000:9%, p=0.002); all cases resolved on repeat testing. Hypercalciuria occurred in 87 (23%) participants (400:17%, 4000:22%, 10000:31%, p=0.011). Clinical adverse events were experienced by 365 (97.9%) participants and were balanced across treatment arms.

Conclusions: The safety profile of vitamin D supplementation is similar for doses of 400, 4000 and 10000 IU/day. Hypercalciuria was common and occurred more frequently with higher doses. Hypercalcemia occurred more frequently with higher doses but was rare, mild, and transient.

Précis

In this pre-specified analysis from the Calgary Vitamin D Study, safety profiles of vitamin D 400, 4000, and 10000 IU/day were similar, although episodes of mild hypercalcemia and hypercalciuria were more frequent with higher doses.

Introduction

Despite a lack of convincing benefit to individuals who are not vitamin D deficient (1-3), enthusiasm for vitamin D supplementation is widespread. Estimates indicate that over half of US adults take a vitamin D supplement (4), and more than 3% (>7 million individuals) consume ≥4000 IU/day (5). The safety of this practice is unclear.

When taken at very high doses (i.e. >25000 IU/day), vitamin D supplementation may precipitate hypercalcemia, hypercalciuria, nephrolithiasis, renal dysfunction, and soft tissue calcification (6,7). The Institute of Medicine (IOM) report on dietary reference intakes for calcium and vitamin D set the tolerable upper intake limit at 4000 IU/day (6), a dose likely to pose no risk of adverse health effects to almost all individuals in the general population. It has been suggested that the tolerable limit could be increased to 10000 IU/day, as hypercalcemia is rarely encountered at lower doses (6,8-10), and most reports of other manifestations of vitamin D toxicity (i.e. lethargy, confusion, vomiting, arrhythmia, nephrocalcinosis) are limited to doses exceeding 40000 IU/day (9). Previously available data regarding the safety of doses >4000 IU/day have been primarily limited to small, heterogeneous, short-term studies (11-13). However, a recent one-year trial comparing supplementation with vitamin D 10000 or 600 IU/day in 132 postmenopausal women also receiving calcium carbonate 1200 mg/day, found that the 10000 IU/day dose was associated with a 3.6-fold increased risk of developing hypercalciuria (14).

Our group conducted a three-year, double-blind, randomized controlled trial to examine the skeletal effects and safety of vitamin D3 supplementation with 400, 4000 or 10000 IU/day. Compared to 400 IU/day, no bone strength benefits and a small dose-dependent decrease in bone density was observed with doses of 4000 or 10000 IU/day; episodes of mild hypercalcemia and hypercalciuria were also more frequent with these higher doses (15). Here, we report the complete pre-specified safety outcomes

from this study (16). We hypothesized that supplementation with up to 10000 IU/day would be safe and well tolerated.

Methods

Trial Design

The study design (16) and primary outcomes (volumetric bone density and strength)(15) have been described in detail elsewhere. Briefly, in this double-blind study, 373 healthy adults were recruited into either a pilot (n=62) or main (n=311) cohort and randomized 1:1:1 to receive 400, 4000, or 10000 IU oral vitamin D3 (cholecalciferol) daily for three years. Primary outcomes and select safety outcomes have been published for the main cohort (15); as previously reported, the pilot cohort received the same study intervention as the main cohort but did not undergo primary outcome measurements. Therefore, the present study describes all pre-specified and exploratory safety outcomes for both the pilot and main cohorts (n=373). All decisions about which participants to include in these analyses were made prior to unblinding and data analysis; all 373 individuals who received the study intervention were included in safety analyses to reduce the likelihood of failing to detect uncommon but clinically important adverse events.

Study visits and data collection took place at a single centre (McCaig Institute for Bone & Joint Health, Cumming School of Medicine, University of Calgary, Canada). Prior to initiation, the trial was registered at clinicaltrials.gov (NCT01900860), with approval granted by the Conjoint Health Research Ethics Board at the University of Calgary (ID: 24882) and a Letter of No Objection obtained from Health Canada. An independent Data and Safety Monitoring Board had access to unblinded data and completed interim safety analyses at regular intervals.

Participants

Healthy men and postmenopausal women between age 55-70 years residing in or near Calgary, Canada were recruited via letters and advertisements. Eligible participants had lumbar spine and total hip bone mineral density T-scores >-2.5, assessed using dual x-ray absorptiometry. Exclusion criteria consisted of: serum 25-hydroxyvitamin D (25OHD) <30 or >125 nmol/L, serum calcium >2.5 or <2.10 mmol/L, consumption of vitamin D supplements >2000 IU/day within the previous 6 months, use of bone-active medication within the past 2 years, disorders known to affect vitamin D metabolism. All participants provided written informed consent.

Intervention and Procedures

Participants were randomized to receive vitamin D3 400, 4000, or 10000 IU/day for three years (16). These intervention doses were selected because the IOM has set the recommended daily intake at 600 IU/day for adults aged 51-70 years -- which for most individuals can be achieved with a 400 IU supplement given that dietary vitamin D intake averages 200 IU/day -- and the tolerable upper intake limit at 4000 IU/day (6), although it has been argued that up to 10000 IU/day is safe (8-10). Vitamin D3 was provided by Ddrops® Canada (Woodbridge, Ontario, Canada) as a bottled liquid, with each bottle containing a metered dropper. Quality control procedures and administration technique have been previously described (16). All participants self-administered 5 drops of vitamin D3 liquid orally each day, regardless of treatment allocation. Concentration of vitamin D3 liquid varied according to treatment arm (400: 80 IU/drop, 4000: 800 IU/drop, 10000: 2000 IU/drop). Participants and outcome assessors were blinded as to treatment allocation, and serum 25OHD measurements throughout the study.

Participants were permitted to take up to 200 IU/day of vitamin D in addition to the study intervention (e.g. a multivitamin). Dietary calcium and vitamin D intake were evaluated at screening using a food frequency questionnaire (17). If dietary calcium intake was less than the recommended 1200 mg/day

(18), a daily supplement containing either 300mg or 600mg elemental calcium was provided to approximate a total daily intake of 1200 mg.

Data Collection

Study visits took place at baseline and at 3, 6, 12, 18, 24, 30 and 36 months. At each visit, medication use, multivitamin and additional vitamin D supplement intake (up to 200 IU/day) were monitored, and a fasting morning blood sample was collected and analyzed for the following parameters: serum 25OHD, calcium, albumin, parathyroid hormone (PTH), creatinine, AST, ALT. All clinical chemistry measurements were performed at a centralized laboratory (Calgary Laboratory Services [CLS]). Assay platforms and procedures have been described previously (16). Fasting serum 25OHD was measured by DiaSorin Liaison XL system (DiaSorin, Stillwater, MN); CLS participates in the Vitamin D External Quality Assessment Scheme (DEQAS). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation (19). Participants underwent 24-hour urine collections for calcium and creatinine at baseline and months 12, 24 and 36. A second void urine sample was collected and calcium:creatinine ratio determined at months 6, 18, and 30, and at other time points in cases where the participant could not provide a 24-hour urine sample or required follow-up testing after hypercalciuria was detected.

Hypercalcemia was defined as total serum calcium level exceeding normal range (>2.55 mmol/L), liver enzyme elevation as AST or ALT >1.5 times the upper limit of normal, and renal dysfunction as serum creatinine >133 µmol/L (20).A 24-hour urine calcium excretion exceeding 7.5 mmol/day was used to as the cutoff for hypercalciuria for participants under 75kg body weight;a weight-based cutoff >0.1 mmol/kg/day was used for those over 75kg (21). The presence of any of these biochemical abnormalities prompted review of testing protocols (i.e. fasting for blood draw, appropriate 24-hour urine collection procedure) with the participant, followed by repeat testing. In the case of detected hypercalcemia or hypercalciuria, if there was no evidence of violation of the testing protocol, the

participant was advised to reduce their supplemental calcium intake (or dietary calcium intake if not taking a supplement) prior to repeat testing. When hypercalcemia occurred, an ECG was performed if the participant endorsed symptoms of hypercalcemia. Participants were asked to discontinue the study intervention if repeat testing demonstrated persistent hypercalcemia, liver enzyme elevations, or renal dysfunction. The second void urine calcium:creatinine ratio served as a safety flag for the identification of significant hypercalciuria. A ratio of ≥1.0 mmol/mmol at month 6, 18 or 30 prompted review of the participant's next 24-hour urine calcium excretion by a study physician. A ratio of ≥1.0 mmol/mmol done in follow-up of an elevated 24-hour urine calcium excretion resulted in discontinuation of the study treatment.

At the baseline visit, fall and fracture status were ascertained via questionnaire. Participants were asked whether they had sustained any falls within the past year, and whether they had sustained any fractures since age 50. At each subsequent study visit, participants were asked to report any new or ongoing medical issues and hospitalizations. All reported adverse events (AEs) were reviewed and classified by organ system and by seriousness and severity; classification was performed by a study physician (EOB or DAH). The following clinical AEs of special interest were defined *a priori*: serious AEs, skin and non-skin cancer diagnoses, low-trauma fractures , falls, nephrolithiasis. Serious AEs were defined as events that were fatal or life-threatening, or which resulted in hospitalization or prolongation of existing hospital stay, persistent or significant incapacity or disability (22).In individuals who reported a fracture, a description of the mechanism of fracture was obtained. Low-trauma fractures were defined as clinical fractures of any bone (excluding fingers and toes) that occurred as the result of a fall from standing height or less. Morphometric vertebral fractures were not assessed. At each study visit, participants were asked whether they had sustained any falls since the last visit. Falls resulting from sports participation were not included in analyses. Serious AEs, cancer diagnoses, low-trauma fractures, and

nephrolithiasis were adjudicated by a study physician (DAH or EOB) via review of the participant's medical chart and relevant imaging. Decisions to discontinue the study intervention on the basis of clinical AEs were made by the study physicians, in conjunction with the participant and their clinical care team.

Outcomes

Pre-specified safety outcomes were: changes in biochemical parameters (serum 25OHD, serum calcium, serum creatinine, 24-hour urine calcium excretion), occurrence of biochemical and clinical AEs (hypercalcemia, hypercalciuria, liver enzyme elevation, renal dysfunction, decline in eGFR of >10 mL/min/1.73 m^2, deaths, serious AEs, AEs leading to study withdrawal, nephrolithiasis, falls, low-trauma fractures, skin and non-skin cancer diagnoses). The incidence of infections and, specifically, upper respiratory tract infections were exploratory outcomes.

Statistical Methods

Biochemical safety parameters were evaluated using descriptive statistics for each treatment outcome at each time point. The distributions of the continuous variables were described using boxplots. For each biochemical and clinical AE, we tabulated the total number of occurrences in each treatment arm and the proportion of participants in each treatment group who experienced the AE. For all pre-specified AEs with an overall prevalence ≤4% and ≤96%, we examined formally for between-group differences for trend in proportions using logistic regression (23). Incidence rate differences with 95% confidence intervals (CIs) were calculated, using the 400 IU/day group as the referent. Student's *t*-tests were used to evaluate differences in mean 25OHD and PTH levels during states of hypercalcemia and normocalcemia, and during states of hypercalciuria and normocalciuria. P-values <0.05 were considered

statistically significant and were not adjusted. Analyses were undertaken with R version 3.4 (R Project for Statistical Computing).

Results

We enrolled 373 participants (49.1% male) with mean (SD) serum 25OHD of 78.0 (19.5) nmol/L between August 2013 and November 2014. Data collection was completed as planned in December 2017. Flow of participants through the study is outlined in Figure 1. The three treatment groups were comparable with respect to baseline characteristics (Table 1). All randomized participants received at least one dose of the study treatment and were included in safety analyses. In total, 36 (9.7%) participants discontinued the study intervention prematurely (400: 8.9%, 4000: 8.0%, 10000: 12.1%). Premature treatment discontinuation resulted from an AE in 20 (5.4%) participants (400: 4.0%, 4000: 4.8%, 10000: 7.3%). Supplementary Table 1 summarizes the AEs resulting in treatment discontinuation. Fewer than 1% of vitamin D doses were missed by active study participants, with adherence rates of 99.6%, 99.7% and 99.1% for the 400, 4000 and 10000 IU/day groups, respectively. A total of 263 (70.5%) participants (400: 75.0%, 4000: 63.2%, 10000: 73.4%) were started on calcium supplementation at the time of randomization in order to achieve a total daily calcium intake of approximately 1200 mg. During the study, 86 (32.7%) of calcium supplement takers (400: 24.7%, 4000: 34.2%, 10000: 39.6%) discontinued their supplemental calcium. Of the 110 participants who did not start a calcium supplement at baseline, 20 (18.2%) initiated calcium supplementation during the study.

Changes in Biochemical Parameters

Figure 2 depicts serum 25OHD, calcium, PTH, and creatinine concentrations and 24-hour urinary calcium excretion throughout the study.

At month 3, mean (SD) 25OHD levels were 76 (17), 114 (22), and 187 (38) nmol/L in the 400, 4000 and 10000 groups respectively. The highest individual 25OHD level (343 nmol/L) occurred at month 18 in a participant taking 10000 IU/day, and no participants discontinued the study treatment based on elevated 25OHD. In the 10000 group, peak mean (SD) 25OHD concentration achieved in the study was 198 (42) nmol/L, occurring at month 18. As previously reported (15), following data collection and unblinding, it was determined that two lots of the investigational product provided to participants in the 10000 IU/day group, beginning between months 18 and 24, and continued up until month 36, had suffered from varying degrees of premature degradation; as a result, the 10000 group actually received doses estimated between 2000 and 10000 IU/day throughout this timeframe. Despite this problem, the 10000 group mean 25OHD remained at or above the levels achieved by the 4000 IU group (Figure 2).

The three treatment groups did not differ throughout the study in terms of serum calcium or creatinine concentrations, or 24-hour urinary calcium excretion (Figure 2). As previously reported for a subset of the study population (15) and shown in Figure 2, PTH levels decreased between baseline and month 18, being lowest in the 10000 IU group.

Biochemical Adverse Events

Table 2 demonstrates the proportion of participants in each group with biochemical AEs. Three biochemical AEs had appropriate overall prevalence for formal between-group statistical testing; of these, two (hypercalcemia [p=0.002] and hypercalciuria [p=0.011]) were significantly different between groups and exhibited a dose-response effect, while one (decline in eGFR by more than 10 mL/min/1.73 m^2 throughout the study [p=0.316]) did not differ between treatment arms. Pre-specified biochemical AEs that were not formally tested owing to very low overall prevalence were: creatinine >133 µmol/L (0.5%), and ALT or AST >1.5x ULN (1.9%).

Sixteen episodes of mild hypercalcemia (serum calcium 2.56-2.64 mmol/L) occurred in 15 participants; hypercalcemia was most frequent in the 10000 group (p=0.002 for trend), as shown in Table 2. Comparing concurrent 25OHD and PTH concentrations during episodes of hypercalcemia (n=16) and normocalcemia (n=2467), 25OHD concentrations were higher in states of hypercalcemia (mean [SD]: 154 [23] versus 124 [53] nmol/L, p=0.02), while PTH concentrations were lower but not significantly different (17.3 [6.2] vs 20.2 [7.1] ng/L, p=0.10) in states of hypercalcemia and normocalcemia. Twelve episodes of hypercalcemia occurred within the first 12 months, and the remaining 4 episodes occurred at month 30 (Supplementary Appendix (24)). One participant in the 10000 group experienced two episodes of transient hypercalcemia, at month 6 and month 30. Ten (67%) of participants with hypercalcemia were taking a calcium supplement at the time of calcium measurement. Hypercalcemia resolved on follow-up testing in all cases; calcium intake was reduced prior to follow-up testing in 10 of these cases (this involved discontinuation of a calcium supplement in 8 cases and a decrease in dietary calcium intake in 2 cases). Two participants in the 10000 group withdrew from the study based on hypercalcemia (Supplementary Appendix (24)); a diagnosis of primary hyperparathyroidism was suspected in one.

Hypercalciuria was observed in 4.3% of participants at baseline, none of whom had a calcium:creatinine ratio of ≥1.0 mmol/mmol. Figure 2 demonstrates 24-hour urine calcium excretion throughout the study. Following randomization and administration of the study intervention, 123 episodes of hypercalciuria occurred in 87 (23.3%) participants. At least one episode of hypercalciuria occurred in 21 (16.9%), 28 (22.4%) and 38 (30.6%) participants in the 400, 4000 and 10000 groups respectively (Table 2, Figure 3). Comparing concurrent 25OHD and PTH concentrations during episodes of hypercalciuria (n=123) and normocalciuria (n=936), 25OHD concentrations were higher (mean [SD]: 137 [55] versus 121 [50]

nmol/L, p<0.0001) and PTH concentrations lower (17.1 [5.8] vs 20.2 [7.0] ng/L, p<0.0001) in states of hypercalciuria. All of these participants had spot urine calcium:creatinine ratio <1.0 mmol/mmol on follow-up testing. However, recurrent episodes of elevated 24-hour urine calcium excretion were common, occurring in 5 (4.0%), 8 (6.4%) and 14 (11.3%) participants in the 400, 4000 and 10000 groups, respectively. Timing of hypercalciuria is shown in the Supplementary Appendix (24). No participants discontinued the study treatment due to hypercalciuria.

Incident rate differences for pre-specified biochemical safety parameters, with the 400 group as the referent, are shown in Figure 3. Informal interpretation of the CIs of the incidence rate differences agree with the formal statistical testing of the trend in proportions.

Clinical Adverse Events

Table 2 summarizes the clinical AEs experienced by study participants. A total of 2580 AEs were reported by 365 (97.9%) participants over 1063 person-years of follow-up. Five AEs had appropriate overall prevalence for formal statistical testing; no statistically significant between-group differences were identified (all serious AEs [p=0.695], falls [p=0.599], fractures [p=0.425], skin cancer [p=0.943] and infections [p=0.190]). Clinical AEs with very low overall prevalence (nephrolithiasis [2.9%], cancer [3.2%]) or extremely high overall prevalence (all AEs [97.9%]) were not formally examined. Figure 3 demonstrates incidence rate differences for the eight pre-specified AEs of clinical interest, with the 400 group as the referent.

Serious Adverse Events

Overall, 59 serious AEs occurred in 49 (13.1%) participants. These included 22 events (18 participants) in the 400 group, 16 events (12 participants) in the 4000 group, and 21 events (19 participants) in the

10000 group. One death (presumed myocardial infarction), occurred at month 22: this was a participant in the 400 group who had discontinued the study intervention at month 15 but continued to attend follow-up visits until the time of death. The Supplementary Appendix (24) provides a summary of serious AEs.

Adverse Events of Special Interest

Falls, low-trauma fractures, nephrolithiasis, and cancer diagnoses were considered clinical AEs of special interest. Number of falls and fractures was similar across treatment groups, as was the proportion of participants experiencing either event (Table 2). Two participants (400: 0, 4000: 1, 10000: 1) had renal colic diagnosed. The participant from the 4000 group presented with clinical features of renal colic at month 17, at which time plain film x-rays demonstrated a possible left-sided calculus. This participant continued the study intervention and went on to experience a second episode of renal colic at month 36, at which time computed tomography confirmed an 11mm left-sided renal calculus. The participant from the 10000 group presented with clinical features of renal colic at month 20, at which time computed tomography demonstrated a 3mm left-sided renal calculus. This participant subsequently discontinued the study intervention and was moved to the intent-to-treat group. Neither of these participants had hypercalcemia or hypercalciuria at any time during the three years of the study, and neither passed a kidney stone. Although both had radiographic evidence of nephrolithiasis, we cannot exclude the possibility that the stones were present before study entry.

New cancer diagnoses were comparable between treatment arms (Table 2): 19 cases of nonmelanomatous skin cancer (Supplementary Appendix (24)), and 12 cases of non-skin cancer

(including melanoma) were diagnosed (3 gastrointestinal, 3 prostate, 2 lymphoma, 1 melanoma, 1 breast cancer, 1 bladder cancer, 1 papillary thyroid cancer).

Other Adverse Events of Clinical Relevance

Incidence of reported infections, and specifically upper respiratory tract infections, did not vary between treatment groups (Table 2). Of interest, one participant from the 10000 group with psoriasis reported complete resolution of his skin lesions within 12 months of starting the study intervention.

Discussion

At very high doses, vitamin D has been associated with the development of toxicity in the form of hypercalcemia, hypercalciuria, renal dysfunction, and nephrolithiasis (6,9). In healthy adults randomized to take vitamin D3 400, 4000 or 10000 IU/day for three years, the safety profile was similar across the range of doses assessed. We observed a dose-dependent increase in incidence of hypercalcemia, although episodes were rare -- affecting fewer than 5% of participants -- and always transient. Hypercalciuria was also dose-dependent and affected almost a quarter of participants.

Prior studies have assessed the risk of developing hypercalcemia with daily vitamin D supplementation, concluding that doses of up to 4000 IU/day are not associated with increased risk. For example, in a trial of healthy postmenopausal women (n=2303) randomized to daily supplementation with vitamin D (2000 IU/day) plus calcium (1500mg/day) or placebo for four years, hypercalcemia was infrequent, occurring in 0.5% of the treatment group and 0.2% of the placebo group (25). In another study of 61 adults who were supplemented with vitamin D 1000 or 4000 IU/day for two to five months, no hypercalcemia or differences in serum calcium levels were observed (26). Conversely, in a 2016 meta-analysis of 48 trials, there was an increased risk of hypercalcemia with vitamin D supplementation compared to placebo (relative risk:1.94, 95% CI:1.09-2.18) (27). The studies included in this meta-analysis were widely variable in terms of vitamin D formulation, dose, and administration interval, although the majority of trials evaluated vitamin D3, and the average dose studied was 2354 IU/day (27). The authors did not observe a dose-response relationship between vitamin D supplementation and risk of hypercalcemia, although the average daily vitamin D dose in the analyzed trials was well below 4000 IU (27). Most previous studies evaluating vitamin D supplementation with more than 4000 IU/day also suggest a low incidence of hypercalcemia. For example, Heaney *et al* supplemented 67 healthy men with either 1000, 5500 or 11000 IU/day vitamin D for 20 weeks and reported no episodes of hypercalcemia (11). In a study of 12

patients with multiple sclerosis treated with progressively increasing vitamin D doses up to 280000 IU/week (average 40000 IU/day) for 28 weeks, no cases of hypercalcemia were observed (13). Most recently, Aloia and colleagues randomized 132 postmenopausal women to daily vitamin D 600 IU or 10000 IU (all participants also received 1200 mg/day calcium as carbonate) for one year and found no difference in the incidence of hypercalcemia between groups (14). In the present study, median serum calcium concentrations were similar for each of the three vitamin D doses throughout three years of follow-up. Hypercalcemia was most common in participants taking vitamin D 10000 IU/day, although episodes were infrequent, mild, and transient. Two thirds of these episodes occurred in participants taking a calcium supplement, discontinuation of which invariably resulted in normalization of serum calcium.

The development of hypercalcemia in persons taking vitamin D 4000 or 10000 IU/day might be more dependent on calcium intake than on vitamin D. In their 2016 meta-analysis, Malihi *et al* noted that hypercalcemia was more common in studies where calcium and vitamin D supplements were co-administered, compared with studies of vitamin D alone (27). In their 12 month comparison of vitamin D 600 and 10000 IU/day, Aloia *et al* (14) observed hypercalcemia in 23% of women taking vitamin D 10000 IU/day, versus the 7% prevalence that was observed in the 10000 IU/day group over the first 12 months of the present study. Aloia *et al* reported a median daily calcium intake of just under 2000 mg (1200 mg supplement plus diet), whereas our study aimed for an estimated total intake of 1200 mg daily. The resolution of hypercalcemia with reduction in calcium intake that we observed in the present study suggests that calcium intake may be a stronger mediator of the development of hypercalcemia than vitamin D intake. Our findings suggest that individuals who take high-dose vitamin D (i.e. ≥4000 IU/day), in addition to supplemental calcium or high dietary calcium intake, should be monitored within the first year of starting this regimen, with reduction in calcium intake if hypercalcemia is observed.

High vitamin D intake has also been associated with excess urinary calcium excretion, with or without hypercalcemia (14,27,28). Malihi *et al* found an increased risk of hypercalciuria with vitamin D supplementation (relative risk:1.64, 95% CI:1.06-2.53),(27) and Aloia and colleagues reported higher risk of hypercalciuria in individuals randomized to 10000 IU/day compared to 600 IU/day (odds ratio:3.6, 95% CI:1.39-9.3) (14). In the present study, hypercalciuria was identified in 4.3% of participants at baseline, comparable with previous reports suggesting a prevalence of 5-10% (29). Throughout the three-year study, prevalence of hypercalciuria was dose-dependent, affecting almost one third of the 10000 IU/day group. Follow-up spot urine testing demonstrated that these participants invariably had urine calcium:creatinine ratios less than 1.0 mmol/mmol, indicating against the presence of severe hypercalciuria. Similarly, Kimball and colleagues did not observe elevations in urinary calcium:creatinine ratios (i.e. >1.0 mmol/mmol) in 12 adults treated with vitamin D doses of up to 280000 IU/week for 28 weeks (13). Importantly, our results highlight a discrepancy between the 24-hour urinary calcium excretion and fasting second void calcium:creatinine ratio cut-offs chosen for the diagnosis of hypercalciuria (30); the clinical relevance of this discrepancy is uncertain, particularly in terms of long-term renal outcomes. However, as 24-hour urinary calcium excretion is more reflective of oral calcium intake (via diet and supplement) than fasting spot urine assessment -- corroborated by our observation that 24-hour urinary calcium excretion normalized in most cases following reduction in calcium intake -- clinicians should use 24-hour urinary calcium assessments to monitor calciuria in individuals receiving high-dose vitamin D supplements, with a view to reducing calcium intake if hypercalciuria is detected.

We did not find higher risk of clinically relevant renal sequelae (renal dysfunction and nephrolithiasis) with increasing vitamin D dose. Although our study was not powered to detect differences in these events, which occurred infrequently, our results are in keeping with a large observational study that did

not find a relationship between vitamin D intake and incident nephrolithiasis (31), as well as a four-year interventional study of vitamin D plus calcium versus placebo in 2303 postmenopausal women which reported similar incidence of renal calculi in the treatment group (1.4%) and the placebo group (0.9%) (25), and a recently published meta-analysis (27). In contrast, the Women's Health Initiative demonstrated a 17% increase in the risk of nephrolithiasis with combined vitamin D (400 IU/day) and calcium carbonate (1000 mg/day) compared to placebo, with 2.5% of women in the treatment group and 2.1% of women in the placebo group reporting kidney stones over an average of 7 years of follow-up (32). Given the relatively low vitamin D dose and relatively high calcium supplement dose evaluated in the Women's Health Initiative study, it is possible that calcium supplementation has a larger influence on kidney stone formation than vitamin D supplementation.

Intermittent administration of large doses of vitamin D (equating to average daily doses of 800 to 2000 IU) has been associated with an increased risk of falls and/or fractures in some studies (33-35) but not others (36). We did not observe a dose-dependent difference in the incidence of falls or low-trauma fractures, although both outcomes were infrequent in our population of healthy adults without osteoporosis.

A myriad of non-skeletal effects of vitamin D have been postulated (37). Specifically, vitamin D has been shown to have several immunomodulatory actions, and the possibility has been raised that vitamin D supplementation might impact upon the pathogenesis of immune-related conditions including infections, autoimmune disorders such as psoriasis and multiple sclerosis, and some cancers (37). For example, findings from a recent meta-analysis (38) and a systematic review of meta-analyses (37) indicate that vitamin D supplementation may protect against the development of upper respiratory tract infections, and topical vitamin D analogues have been successfully used in the treatment of psoriasis

(39). However, current evidence does not support vitamin D supplementation for the prevention or treatment of other non-skeletal conditions, including cancer (37,40,41). In our study, we did not observe a dose-dependent effect of vitamin D supplementation on the incidence of upper respiratory tract infections, dermatologic conditions, or cancers. Cancer was diagnosed in 3.2% of our study cohort over three years of follow-up, comparable to the 4.7% incidence reported by Lappe *et al* in their four-year study of vitamin D (2000 IU/day) plus calcium (1500 mg/d) versus placebo in healthy postmenopausal women (25). In the present study, prostate cancer was diagnosed in three participants taking vitamin D 10000 IU/day, although given the long latency period of prostate cancer (15-20 years), it is unlikely that these cancers were related to the study intervention.

Cardiovascular events were balanced among intervention groups in this healthy population. Although the present study was not powered to detect differences in cardiovascular event rates, two recent trials have demonstrated no effect of vitamin D supplementation on the incidence of cardiovascular events (41,42).

Our findings should be interpreted in the context of some limitations. First, safety was a pre-specified secondary outcome of this study. As such, the trial was not designed to detect rare AEs. Second, clinical AEs were elicited from participants in a passive manner, and only select clinical AEs (serious AEs, fractures, nephrolithiasis and cancer) were adjudicated. Third, while the three-year duration of this study was long in relation to many previous trials, vitamin D supplementation is generally commenced with a plan to be continued indefinitely, and it is possible that the safety profile may differ with longer-term supplementation. Fourth, two lots of the vitamin D preparation administered to the 10000 IU/day group between months 18 and 36 suffered from degradation, limiting our ability to draw conclusions about the safety of supplementation with vitamin D 10000 IU/day beyond 18 months, although the

mean 25OHD level remained above the 4000 IU group at month 36. An additional limitation inherent to all studies of vitamin D supplementation is that we could not control for dietary intake (including fortified food sources) or skin synthesis via ultraviolet light exposure. However, our randomized controlled approach serves to mitigate bias introduced by these factors. It is also important to note that our study population consisted predominantly of individuals who were vitamin D-sufficient (25OHD >50 nmol/L) at baseline, and vitamin D supplementation may have a more favourable risk-to-benefit ratio in the setting of vitamin D insufficiency (40).

Our study has several strengths. To our knowledge, it is the largest randomized-controlled trial to evaluate the effects of vitamin D up to 10000 IU/day in healthy adults for more than 12 months. More than 90% of participants completed study follow-up, with >99% adherence. While not designed to detect rare AEs, the trial's sample size permitted evaluation of the principal manifestations of vitamin D toxicity (hypercalcemia, hypercalciuria, renal dysfunction) over a relatively long timeframe, and our findings thus represent a significant and necessary contribution to the literature regarding the safety of vitamin D supplementation.

Conclusions

In healthy adults who are not vitamin D deficient, daily vitamin D supplementation with doses of 400, 4000, and 10000 IU for up to three years is generally safe and well tolerated, although further study is required to determine whether use of high-dose vitamin D results in any long-term sequelae. This is particularly relevant for the dose of 10000 IU/day, as participants randomized to receive this dose in the present study actually received lower doses between 18 and 36 months. Over three years, supplementation with up to 10000 IU/day is associated with dose-dependent increases in hypercalciuria and with rare cases of transient hypercalcemia; these events typically resolve with reduction in calcium

intake. It may be prudent for clinicians to monitor for the development of hypercalcemia and hypercalciuria in individuals taking vitamin D ≥4000 IU/day and who use calcium supplements or have high dietary calcium intake, with a view to reducing calcium intake if hypercalcemia or hypercalciuria are observed. This study does not provide any evidence of dose-dependent effects of vitamin D on fractures, falls, infections, dermatologic conditions, or cancer.

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Authors' Contributions

EOB reviewed and adjudicated adverse events (with DAH), completed the preliminary data analysis and interpretation, and drafted the manuscript. LAB participated in data collection and cleaning, provided input regarding data analysis and interpretation, and revised the manuscript for important intellectual content. MSR completed the final statistical analysis, provided insights regarding data interpretation and created Figures 2 and 3. EMD contributed to the literature review and preliminary data analysis. SG and MK were responsible for trial coordination and data collection, and both participated in creation of the manuscript. SKB and DAH conceived of the study idea, designed the study, oversaw data collection, provided insights regarding data analysis and interpretation, and made major intellectual contributions to the manuscript.

Data sharing

The authors commit to making relevant anonymized patient-level data available to researchers who provide a methodologically sound proposal. Data will be available from 6 months following publication until 5 years following publication. To gain access, data requestors will need to sign a data access agreement.

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Figure Legends

Figure 1. Flow of participants through study

Figure 2. Box plots of three-year changes in serum 25-hydroxyvitamin D, serum calcium, serum parathyroid hormone (PTH), serum creatinine, and 24-hour urine calcium in healthy adults taking vitamin D 10000 IU (blue), 4000 IU (red) or 400IU/day (green) y. Boxes show medians and interquartile ranges. The whiskers show the adjacent values, which indicate where approximately 99% of the values of the data lie. Horizontal dashed lines represent the upper limit of the normal range for serum calcium (second panel), 133 µmol/L for serum creatinine (third panel), and 24-hour urine calcium excretion of 7.5 mmol/day (fourth panel).

Figure 3. Incidence rate differences in number of healthy individuals experiencing relevant biochemical (left panel) and clinical (right panel) adverse events (AEs) while taking vitamin D 400 (green), 4000 (red), or 10000 (blue) IU/day for three years, using 400 IU/day as the referent. Incidence rates reflect the number of participants experiencing the event per person-year of follow-up. Error bars represent 95% confidence intervals. Unit of measure is µmol/L for creatinine and mL/min for eGFR. Non-skin cancer includes melanoma.
eGFR = estimated glomerular filtration rate, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ULN = upper limit of normal, URTIs = upper respiratory tract infections

Table 1. Baseline characteristics of study population

Variable	10000 IU	4000 IU	400 IU
N	124	125	124
Male Sex (%)	61 (49.2 %)	58 (46.4 %)	64 (51.6 %)
Age (yrs)	62.0 (4.1)	62.7 (4.3)	62.0 (4.2)
Time since menopause (yrs)	12.5 (5.6)	11.4 (6.7)	11.9 (5.8)
Caucasian ethnicity (%)	117 (94.4 %)	118 (94.4 %)	115 (92.7 %)
Dietary calcium intake (mg/day)	639 (344)	624 (279)	600 (303)
Dietary vitamin D intake (IU/day)	188 (120)	178 (92)	166 (88)
BMI (kg/m^2)	27.2 (4.4)	27.8 (5.0)	27.7 (4.4)
Body fat (%)	33.0 (8.4)	34.3 (8.9)	34.1 (8.9)
Systolic blood pressure (mmHg)	126 (16)	130 (17)	128 (15)
Diastolic blood pressure (mmHg)	80 (10)	80 (9)	81 (8)
History of skin cancer (%)	16 (12.9 %)	9 (7.2 %)	13 (10.5 %)
History of non-skin cancer (%)[a]	12 (9.7 %)	9 (7.2 %)	7 (5.6 %)
History of cardiovascular condition (%)	16 (12.9 %)	14 (11.2 %)	24 (19.4 %)
Type 2 diabetes (%)	5 (4.0 %)	4 (3.2 %)	3 (2.4 %)
Rheumatoid arthritis (%)	1 (0.8 %)	2 (1.6 %)	2 (1.6 %)
Asthma (%)	11 (8.9 %)	10 (8.0 %)	6 (4.8 %)
Current smoker (%)	5 (4.0 %)	2 (1.6 %)	3 (2.4 %)
Fall(s) within past year (%)	19 (15.3 %)	22 (17.6 %)	27 (21.8 %)
Fracture(s) since age 50 (%)	23 (18.5 %)	16 (12.8 %)	23 (18.5 %)
Lumbar spine T-score	-0.1 (1.4)	0.1 (1.4)	0.0 (1.4)
Total hip T-score	0.0 (1.1)	0.1 (1.2)	0.0 (1.1)
Serum 25OHD (nmol/L)	78 (18)	80 (20)	76 (21)
Serum PTH (ng/L)	22.6 (7.4)	21.7 (6.4)	22.1 (7.4)
Serum calcium (nmol/L)	2.4 (0.1)	2.4 (0.1)	2.4 (0.1)
Serum phosphate (nmol/L)	1.0 (0.1)	1.0 (0.2)	1.0 (0.2)
Serum creatinine (µmol/L)	80 (14)	79 (14)	80 (14)
Estimated GFR (mL/min)	80 (11)	80 (12)	81 (11)
24-hour urine calcium (mmol/day)	4.2 (2.0)	4.6 (2.0)	4.2 (2.0)
Serum alkaline phosphatase (U/L)	70 (17)	67 (15)	70 (20)
Plasma CTX (ng/L)	345 (123)	343 (135)	334 (126)
Fasting blood glucose(mmol/L)	5.7 (0.7)	5.7 (0.3)	5.7 (0.5)
Hemoglobin A1C (%)	5.2 (1.5)	5.0 (0.8)	5.2 (1.2)

Values are presented as mean (SD) or n (n/N %)

[a]Includes melanoma

BMI = body mass index, 25OHD = 25-hydroxyvitamin D, PTH = parathyroid hormone, GFR = glomerular filtration rate, CTX = C-telopeptide of type 1 collagen

Table 2. Summary of relevant biochemical and clinical adverse events in healthy adults taking vitamin D 10000, 4000 or 400 IU daily for three years

	10000 IU (N=124)		**4000 IU** (N=125)		**400 IU** (N=124)	
	Total Events, n	Participants with Events, n (%)	Total Events, n	Participants with Events, n (%)	Total Events, n	Participants with Events, n (%)
Biochemical AEs						
Hypercalcemia	12	11 (9%)	4	4 (3%)	0	0 (0%)
Hypercalciuria	56	38 (31%)	40	28 (22%)	27	21 (17%)
Creatinine >133 µmol/L	4	2 (2%)	3	2 (2%)	3	3 (2%)
eGFR decline of >10 mL/min	-	53 (43%)	-	40 (32%)	-	47 (38%)
AST or ALT >1.5x ULN[a]	3	3 (2%)	3	3 (2%)	8	5 (4%)
Clinical AEs[b]						
All Clinical AEs	824	122 (98%)	920	122 (98%)	836	121 (98%)
Neurologic	24	19 (15%)	35	26 (21%)	33	21 (17%)
Ophthalmologic	22	17 (14%)	33	21 (17%)	34	22 (18%)
Otalaryngologic	206	87 (70%)	255	98 (78%)	220	93 (75%)
Cardiovascular	44	31 (25%)	39	29 (23%)	46	35 (28%)
Pulmonary	43	29 (23%)	54	34 (27%)	55	39 (31%)
Gastrointestinal	65	43 (35%)	82	51 (41%)	58	43 (35%)
Genitourinary	55	30 (24%)	37	24 (19%)	37	25 (20%)
Endocrine	12	11 (9%)	8	7 (6%)	6	6 (5%)
Hematologic	12	12 (10%)	4	4 (3%)	1	1 (1%)
Dermatologic	53	36 (29%)	65	49 (39%)	59	37 (30%)
Musculoskeletal	215	96 (77%)	257	96 (77%)	216	86 (69%)
Psychiatric	21	21 (17%)	12	11 (9%)	24	23 (19%)
Other[c]	52	35 (28%)	39	30 (24%)	47	37 (30%)
Serious AEs	21	19 (15%)	16	12 (10%)	22	18 (15%)
AEs of Special Interest						
Falls	10	8 (6%)	13	12 (10%)	5	5 (4%)
Low-trauma fractures	8	7 (6%)	3	3 (2%)	5	5 (4%)
Nephrolithiasis	2	1 (1%)	1	1 (1%)	0	0 (0%)
Non-skin cancer[d]	6	6 (5%)	4	4 (3%)	2	2 (2%)
Skin cancer	7	7 (6%)	3	3 (2%)	9	8 (7%)
Infections	287	101 (81%)	297	100 (80%)	277	106 (86%)
URTIs	166	80 (65%)	204	89 (71%)	194	89 (72%)

eGFR = estimated glomerular filtration rate, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ULN = upper limit of normal, AE = adverse event, URTIs = upper respiratory tract infections

[a]AST ULN = 32 IU/L for females and 40 IU/L for males, ALT ULN = 40 IU/L for females and 60 IU/L for males

[b]AEs and Serious AEs defined using the standard International Conference on Harmonization Good Clinical Practice definition

[c]AEs that do not localize to a single organ system (e.g. diffuse infectious symptoms, generalized allergic reactions, electrolyte abnormalities, fatigue, insomnia, weight changes)

[d]includes melanoma