A randomized double-blind study investigating dose-dependent longitudinal effects of vitamin D supplementation on bone health

Trial Protocol

Principal Investigators:

David Hanley (Endocrinologist), McCaig Institute for Bone and Joint Health

Steven Boyd (Biomedical Engineer), McCaig Institute for Bone and Joint Health

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Introduction

Vitamin D is necessary for normal bone health and calcium/phosphate metabolism. Although that has remained the main focus of the recent Institute of Medicine (IOM) report (1,2), there remains major controversy regarding the optimum level of intake or measured blood levels of vitamin D to provide optimum bone health (3,4). In addition, it is becoming apparent that vitamin D is important for a wide variety of cell functions in many tissues and organ systems. Vitamin D receptors are present in many tissues and so is the 1-alpha hydroxylase enzyme which is necessary to synthesize the most active vitamin D metabolite, 1, 25(OH)2 vitamin D. Although not as well studied as its effects on bone and mineral metabolism, it is clear that there is an association between vitamin D deficiency and a variety of medical disorders, including autoimmune disease (e.g. rheumatoid arthritis, type 1 diabetes mellitus, multiple sclerosis), cardiovascular disease and associated risk factors (e.g. hypertension, insulin resistance and type 2 diabetes), and mental health disorders (e.g. depression). There is a potential role for optimizing vitamin D physiology/status in the prevention of a variety of these common conditions. As pointed out repeatedly in the Endocrine Society Guidelines, it is not certain what dose (if any) of vitamin D is needed for the potential prevention benefits of these non-skeletal disorders (4).

For prevention of the major bone disorders of vitamin D deficiency (osteomalacia and rickets for severe deficiency; and milder deficiency contributing to the fractures of osteoporosis), there remains disagreement among experts as to the optimal dose of vitamin D to recommend. The IOM report suggested vitamin D deficiency was not as prevalent as many experts believe, and suggested that the bone benefits of vitamin D (prevention of rickets, osteomalacia, and fractures) would be achieved for 97.5% of the population by an intake (all sources) of 600 international units (IU) daily for adults under age 70 and 800 IU daily for adults over the age of 70. The IOM report indicates that the average Canadian receives between 200 and 300 IU daily from diet, so if sunlight exposure contributes little vitamin D, a supplement intake of 400 IU would be more than adequate for the general population under age 70 years. The Osteoporosis Canada Guidelines and Endocrine Society Guidelines set that dose recommendation moderately higher (400 to 2000 IU daily as a general recommendation). The IOM and Endocrine Society disagree on the interpretation of one of the few studies relating vitamin D levels in blood to bone histology and pathology. This study of 675 autopsies found the presence of microscopic signs of severe vitamin D deficiency (osteomalacia) in a large proportion of people dying of disorders unrelated to bone disease (mostly trauma) in Germany (5). The authors indicated they saw signs of osteomalacia (severe vitamin D deficiency) only in individuals whose serum 25-OH vitamin D (25OHD) was less than 75 nmol/L. The IOM re-interpreted the authors' conclusions, and suggested that 97.5% of the population would not have signs of severe vitamin D deficiency if the 25OHD levels were above 50 nmol/L, and used this finding to provide further support for their contention that a low dose supplement of vitamin D would be all that is needed to provide the bone benefits of vitamin D. The authors of the study and other experts do not agree with the IOM (4). A recent pooled analysis of vitamin D fracture prevention studies indicates that fracture prevention is only consistently seen when vitamin D intake is 800 IU

daily, or higher (6).

A major problem in the area of vitamin D and bone and mineral metabolism is that although clinicians and nutritionists have been trying to assess the vitamin D clinical trials the same way a pharmaceutical agent would be tested, no proper dose finding studies have been done, and dose-response studies have basically looked only at blood levels of 25-OHD and serum/urine calcium (1,3,4).

There have been no proper dose-finding studies for vitamin D in the examination of measureable effects upon bone, particularly with the kind of technology we propose to use. The upper range of vitamin D dose effects on bone has not been explored adequately. Attempting to define a dose for prevention of the most severe expression of bone complications of vitamin D deficiency (fractures, osteomalacia and rickets) is appropriate in formulating a public heath recommendation when the evidence supporting higher doses is not readily obtainable, as the IOM concluded (1). However, this is not the ideal way to define the optimal level of vitamin D intake for bone health or other potential benefits of vitamin D. Some have suggested that higher doses than the current recommendations may provide more benefit. Although the IOM raised the Tolerable (safe) Upper Intake Level (TUL) to 4,000 IU/day (TUL is a dose which would be free of adverse effects and would not require medical monitoring) (7), others have suggested that a more appropriate TUL would be 10,000 IU/day, and noted that apart from patients with disease that alter vitamin D metabolism (e.g. sarcoidosis), no cases of vitamin D toxicity have been clearly documented with doses less than around 40,000 IU/day (8). Doses above 4,000 IU/day are commonly used in patients with multiple sclerosis, in the belief that this may reduce the severity or frequency of relapses, and many people are consuming doses in the 5-10,000 IU/day range on the assumption of unproven health benefits. There have been very few well-designed studies examining the potential benefits of higher doses of vitamin D, but small studies are being performed in patients with multiple sclerosis and have suggested there may be beneficial effect on immune response in multiple sclerosis patients (9,10). Further, in one of these studies (11) an increasing dose of up to 40,000 IU/day was used before giving 10,000 IU daily for up to 9 months. The dose averaged to approximately 14,000 IU/day for one year, and was shown to have no appreciable effect on serum calcium or urine calcium excretion (11). In this study, the mean value of serum 25OHD was 179 nmol/L and almost all subjects achieved a level above 100 nmol/L (11).

The three doses chosen in our study are aimed at getting almost all subjects above target levels of serum 25OHD of 50 nmol/L (the IOM recommendation), 75 nmol/L (4,000 IU/day should do this), and 100 nmol/L (the 10,000 IU/day dose).

There is a strong association between vitamin D deficiency and a number of cancers. One small randomized controlled clinical trial found vitamin D supplementation at 1100 IU daily was associated with a significant reduction in all cancers (12), but the study has been criticized for being too small (IOM report). The IOM report also called attention to several studies showing an association between higher levels of 25OHD and pancreatic and prostate cancers. Although the present study is too small for cancer incidence to be a primary outcome variable, we will

capture cancers as adverse events and be able to identify any incidence trends in the three groups.

Because we have access to a state-of-the-art method of assessing cortical and trabecular bone without subjecting the patient to a bone biopsy (HR-pQCT), we feel it is appropriate to use it to assess the effects of increasing doses of vitamin D on bone, parameters of mineral metabolism and quality of life (QOL) and depression parameters. We propose to use the vitamin D dose recommended by IOM for adults under age 70 years (400 IU of supplement, assuming at least 200 IU from diet), 4,000 IU/day (the TUL identified as safe by the IOM and now adopted by Health Canada), and 10,000 IU/day (the proposed TUL by Hathcock et al. (8)). Although there is certainly evidence that 10,000 IU/day is safe, we will continue to monitor our subjects for changes in urine and serum calcium.

Hypotheses

1. It is hypothesized that vitamin D, in a dose-dependent manner, will suppress parathyroid hormone action, resulting in less bone turnover, and decreased cortical porosity, leading to improved bone strength as assessed by finite element analysis.
2. It is hypothesized that vitamin D, in a dose-dependent manner, will increase bone density in the central skeleton (spine, hip), as measured by the current standard method of dual X-ray absorptiometry (DXA).
3. It is hypothesized that vitamin D, in a dose-dependent manner, will have an impact on quality of life, including indices of depression, as measured by the SF-36 questionnaire and an appropriate index of depression.

Outcomes

Primary outcome will be a non-invasive assessment of bone density and strength, as measured by HR-pQCT.
Secondary outcomes will be trabecular and cortical bone, as measured by HR-pQCT, areal bone mineral density as measured by DXA, parameters of calcium metabolism, including biochemical markers of bone turnover, balance, and quality of life.

Study Design

A clinical trial open to healthy men and women 55 to 70 years of age, with serum 25[OH]D under 125 nmol/L and above 30 nmol/L.

The study will be a randomized, double-blind clinical trial. A total of up to 375 men and women will be randomly assigned to one of three treatment groups, each receiving one of the following daily doses: 400 IU vitamin D, 4,000 IU vitamin D, and 10,000 IU vitamin D. The study will be registered with the NIH Clinical Trials Registry.

We will include a pilot group which will consist of up to 75 men and women. This is necessary because, due to manufacturer error in the Canadian certification application, the second generation HR-pQCT (XtrememeCT2), which was to be used in the original study design has not yet been certified for use in Canada. We will therefore randomize up to 75 people in this pilot cohort. Pilot study subjects will be scanned using the HR-pQCT first generation (XtremeCT1).

Once the XtremeCT2 is certified we will scan the pilot group on both scanners at 6 and 12 months. (See further explanation of radiation involved using the two XtremeCT machines in the HR pQCT section). The objective of this pilot cohort will be to compare results from XtremeCT1 to XtremeCT2. This will allow us to (a) make a comparison on a cross-sectional basis and (b) determine whether there is any additional sensitivity to longitudinal changes using XtremeCT2 compared to XtremeCT1. Additionally, we will have a clean dataset of XtremeCT1 image data at 6 and 12 months which can be used to determine if there is an early treatment effect of groups A,B and C.

Inclusion and Exclusion Criteria

Women and men will be generally healthy and between 55 and 70 years of age; women will be at least 5 years post-menopause. Both men and women will have a baseline lumbar spine and total hip bone mineral density (BMD) T-score above –2.5 SD assessed using dual x-ray absorptiometry (DXA), and a serum 25-[OH] vitamin D (25OHD) of >30 nmol/L (12 ng/mL). The recent Institute of Medicine (IOM) report claimed that a level of 40 nmol/L (16 ng/mL) is indicative of adequate bone protective vitamin D nutrition for at least 50% of the population, 50 nmol/L (20 ng/mL) for 97.5% of the population; and that individuals under age 70 years would achieve an average serum 25OHD of 50 nmol/L by receiving 600 IU/day from all sources (diet plus supplements).

People will be excluded from the study if they are found to be at high risk (>20%) for fracture, as defined by the Canadian FRAX 10-year fracture risk calculator, or have taken bone active osteoporosis prescription drugs in the past 2 years (bisphosphonates) or 1 year (other osteoporosis prescription therapies).

The following is a list of inclusion/exclusion criteria:

Inclusion.

1. Healthy women and men between 55 and 70 years of age; women will be at least 5 years post-menopause. Presence of a chronic illness does not exclude participation if the condition is stable and managed by a physician.
2. Subjects will have a baseline lumbar spine and total hip bone mineral density (BMD) assessed using dual x-ray absorptiometry (DXA), and will be eligible if their T-score is above –2.5 SD.

Exclusion.

1. A serum 25-[OH] vitamin D (25OHD) of <30 nmol/L (<12 ng/mL) or >125 nmol/L (50 ng/mL).
2. Hypercalcemia (serum calcium >2.55 mmol/L), hypocalcemia (serum calcium <2.10 mmol/L) or eGFR <30 mL/min.
3. Surgical cure of Primary Hyperparathyroidism within the last year.
4. Known hypersensitivity or allergy to Vitamin D
5. Serum creatinine, AST, ALT, PTH, calcium, or alkaline phosphatase greater than 1.5 times the upper limit of normal at the screening visit
6. BMD exclusions:

(a) High (≥ 20%) 10-year risk for osteoporotic fracture, as defined by the World Health Organization's Canadian FRAX calculator.

(b) DXA T-score below or equal to –2.5 at lumbar spine, Total Hip or Femoral Neck

These individuals would be more likely to be prescribed osteoporosis drug by their primary care physician.

7. Have taken bone active osteoporosis prescription drugs in the past 2 years (bisphosphonates) or 1 year (other osteoporosis prescription therapies). Post-menopausal estrogen/progesterone therapy is not an exclusioin if the subject's intention is to carry on with this therapy for the proposed duration of the study, but if this therapy is stopped during the study the subject would be withdrawn from the study.
8. Any medical condition that would prevent participation in a clinical trial for a full three years.
9. Medications such as prednisone >2.5 mg daily (or equivalent); other bone active medications such as tamoxifen or aromatase inhibitors for breast cancer, or androgen deprivation therapy of prostate cancer.
10. Disorders known to affect vitamin D metabolism such as sarcoidosis or renal failure or malabsorption disorders (e.g. pancreatic insufficiency or celiac disease).
11. Regular (monthly or more frequent) use of tanning salons.
12. Consumption of vitamin D supplements at a dose ≥ 2000 IU/day for the past 6 months.
13. Active kidney stone disease (documented kidney stone within the last 2 years)

Calcium

All subjects will have adequate calcium intake as defined by the Institute of Medicine (total of 1200 mg/day). A brief dietary history will be taken and subjects will be instructed to take an appropriate dose of supplemental calcium if their daily intake is less than 1200 mg/day (the IOM's Recommended Daily Allowance for this study population).

Intervention Drug, Vitamin D3

For adults under age 70 years, the recent IOM report (1) recommends a total intake of 600 IU vitamin D/day will provide all the vitamin D needed for bone health, and since the typical Canadian diet contains between 200 and 300 units of vitamin D, the subjects in the lowest dose arm of our study will receive 400 IU/day. The other two groups will receive 10,000 IU and 4,000 IU, respectively. The 10,000 IU dose is the tolerable upper intake level (TUL) recommended by Hathcock et al (8) and 4,000 IU is the IOM's recommended TUL. Health Canada approval for the use of a daily 10,000 IU dose will be sought, and is expected to be provided based on past experience of the investigators.

Measurements

Screening

It is anticipated that an upper limit of 700 people from the Calgary region will need to be screened. The subjects will be recruited from the general population by means of posters, internet resources, and other means of public information (i.e., television advertisement). The screening process will ensure that subjects meet the inclusion criteria described above. Each participant into the study will be randomized into one of the three study arms, and there will be an equal number of men and women in each of those study arms. At screening, standard lab tests will be done including:

- Serum: AST, ALT, BUN, Creatinine, 25OHD, albumin, calcium, phosphate, Alkaline Phosphatase, glomerular filtration rate (GFR, calculated value from serum creatinine)
- Urine: calcium, creatinine, urinalysis for hematuria
- Parathyroid hormone (PTH) levels

Furthermore, an ECG will be performed at screening visit.

Annual Assessments

The screening study will provide a total of N=300 volunteers who will receive annual assessments. The pilot cohort up to 75 men and women will receive the same assessments as the original 300 subjects.

The exceptions to the annual assessment will be in the first year when a 3- and 6-month assessment will also be performed. Additionally, study visits at 18 and 30 months will be performed for additional safety monitoring of pertinent serum and urine biochemistryparameters (see testing as outlined below). The assessments will include a questionnaire (SF-36; described more later) and an assessment of biomarkers (described more later).

Sample size

The three study arms will each include up to 125 subjects (a total of 375 subjects). This number of subjects provides sufficient statistical power to assess a dose-dependent effect of vitamin D. The size of the study is also tempered by the capacity to perform annual measurements at our facilities for 375 people.

Blood and Urine Sampling

A total of 50 mL of whole blood will be drawn at any time point when blood sampling is scheduled. After the blood is drawn it will be centrifuged and then serum will be aliquotted into tubes. Some tests will be done as soon as possible after the blood draw for safety monitoring (e.g. serum calcium, creatinine, phosphate) or if the test item deteriorates with long term storage (e.g. serum PTH). Samples not needed for monitoring of safety (e.g. markers of bone turnover) will be frozen until completion of data collection at -80°C. Blood sampling will be performed in the screening cohort, and at time points of 3- and 6-month for the purposes of safety monitoring. Subsequently, blood sampling will be performed on an annual basis for three years. A fasting second voided urine specimen will be taken in the morning on each of the visits in the first year, for measurement of calcium to creatinine ratio to rule out hypercalciuria. Blood for DNA will be collected at baseline

Anthropometry

Height, weight and limb lengths (radius and tibia) of all subjects will be measured. For the height measurement subjects will be asked to remove their socks and shoes and stand with their heels together and arms at side in front of the stadiometer. Heel, buttocks, upper part of the back, but not necessarily the back of the head, are in contact with the wall. The subject will be instructed to look straight ahead, take a breath, and to stretch up a far as possible keeping their heels on the ground. It will be assured that the subject's heels are not elevated and the headboard will be brought down, crushing the hair. The height will be recorded twice to the nearest 0.1 cm. If the two readings are not within 0.4 cm, a third reading will be taken. For the weight measurements the subjects will be asked to remove their shoes and empty their pockets before stepping onto the scale. The weight will be recorded two times to the nearest 0.1 kg. The tibial length of both legs will be measured from the tibial plateau to the distal edge of the medial malleolus and the radius length will be measured as the distance from the ulnar styloid process to the olecranon process. These measurements will be obtained using a standard anthropometric tape measure and will be recorded to the nearest 0.1 cm. If the two readings are not within 0.4 cm, a third measurement will be taken.

DXA

Dual energy X-ray absorptiometry (DXA) will be used to scan the distal radius, lumbar spine and the proximal femur. From the scan of the lumbar spine and left proximal femur (~ 1 min), aBMD (g/cm2) will be used to determine a subject-specific T-score (aBMD compared to reference mean for a young healthy adult) and this value will be compared to standard World Health Organization criteria to classify each subject as normal (T-score>-1), osteopenic (-1 < T > -2.5) or osteporotic (T score < -2.5). The combined radiation dose associated is less than 25 µSv. Scans will be completed at baseline, months 12, 24 and 36. A whole-body scan will be performed to determine lean mass and body composition at baseline and at the end of the study (month 36).

HR-pQCT

High resolution peripheral quantitative computed tomography (HR-pQCT, XtremeCT, Scanco Medical, Switzerland) will be used to obtain bone quality parameters such as volumetric bone density, trabecular thickness (Tb.Th), trabecular number (Tb.N), cortical thickness (CtTh), cortical porosity (CtPo) and estimated bone strength from the finite element analysis at tibia and radius from all subjects. All scans will be performed at baseline, and at months 6, 12, 24, and 36. The pilot cohort will have duplicate scans on both the Xtreme CT1 and Xtreme CT2 machines as well as Xtreme CT2 scans at months 6, 12, 24 & 36.

The HR-pQCT measurements will provide high-resolution 3D images of the trabecular and cortical bone structure in 9 mm measurement regions of the tibia and radius (left and right). The nominal resolution of the system is 80 micrometers isotropic resolution (in-plane and between plane resolutions are the same). Participants will sit in a chair while placing their lower leg or their forearm in the XtremeCT system gantry while data is acquired. The local delivered dose from the HR-pQCT (XtremeCT1) system is 6.1 mGy per 9.020 mm image stack (Dr. Leszek Hahn, University of Calgary). At the tibia and the radius there are three different tissues, bone, skin and other tissues (fat, muscle) with corresponding weighting factors for effective dose of 0.01, 0.01 and 0.05 (according to ICRP91). The estimated fraction of total body skin, bone and other tissues exposed to the radiation are 1/1000, 1/500 and 1/1000. Therefore to calculate the effective dose the following equation is used:

> Effective dose = measured radiation * (weighting factor of tissue 1*percentage of tissue scanned + weighting factor of tissue 2*percentage of tissue scanned + weighting factor of tissue 3* percentage of tissue scanned)

To convert from mGy to µGy the effective dose must be divided by 1000.

> Effective dose = 6.1mG * (0.01*1/1000 + 0.01*1/500 + 0.05* 1/1000) = 6.1mGy *0.08 * 1/1000 = 0.5 µGy = 0.5µSv

This results in an effective dose of 0.5 µSv which is 1000 times lower then the measured dose. However, it is difficult to measure the effective dose from stray radiation so a value of 3 µSv for each examined (radius and tibia) is used for measurments on XtremeCT1. There is a slightly highter effective dose for XTremeCT2 because of the increased area scanned. We will use a maximum of 5µSv estimate for each examined area. The recommended effective dose limit for

occupational exposure to the foot (no data is available for foreleg) is 500mSv. Assuming that the recommended dose for the general public is 10 times lower, this results in a recommended dose of 50 mSv (ICRP). At the radiation type and the energy used with the XtremeCT the units Gy can be converted with a factor of 1 into Sv. If the measured dose and not effective dose is again used for saftey, this results in 50 mSv/6.1mSv = 8 measurements per subject per site per year. The maximum any subject in our study will be scanned is 4 times per site per year. This is half of the recommended dose.

Although only one scan is planned per year, a 2nd scan per scanning appointment may be used in special cases where subject movement artifact may occur.

As a point of reference, the normal background radiation that a person receives per year in Calgary is 2-3 mSv. A transatlantic flight will result in about 50 µSv effective dose and a chest X-ray results in about 20 µSv.

The outcome measures of the HR-pQCT scans are morphological parameters such as trabecular thickness (Tb.Th), trabecular number (Tb.N), bone volume ratio (bone volume / trabecular volume (BV/TV) and estimated bone strength from the finite element analysis. To determine morphological parameters from the scans, the trabecular portion has to be isolated from the cortical shell of the bone in order to analyze the components separately. The segmentation will be done by applying a contouring method using an auto-segmentation algorithm applied to the 3D HR-pQCT images. This analysis requires the transformation to binary images in which the pixels are either black (representing marrow) or white (representing bone tissue). This will be done by applying a Gaussian filter and threshold to the grayscale images. The binary images can then be analyzed and morphological parameters (cortical porosity, cortical thickness, bone volume to total volume ratio, trabecular thickness, trabecular spacing and trabecular number) can be determined. These standard HR-pQCT morphological outcomes were validated against gold-standard µCT imaging.

All data analysis is done post-measurement and therefore does not require subject participation. These measurements are performed on computers using software supplied by the HR-pQCT manufacturer and additional software developed by the principal investigator.

In summary, the maximum effective radiation dose to subjects being examined on XtremeCt2 is 10 (radius and tibia) (for each examination). The additional radiation dose to subjects in the pilot cohort at the 6 and 12 month time points: tibia and radius scan by XtremeCT1 will be 2 x 3µSv = 6 µSv. Please note that all radiation doses are approximate, as are any radiation doses for medical devices and are based on our testing done internally as well as manufacturer's provided data. As noted above these estimates are probably much greater than actual exposure.

Finite Element Analysis

In order to estimate bone strength, all HR-pQCT images will be put into a custom finite element program, in which a linear model can be created from the HR-pQCT scan using the voxel conversion approach. It incorporates the 3D microarchitecture and local density of the scanned bone region. Finite element analysis estimates the ultimate bone strength according to bone microarchitecture, defined boundary conditions and material properties of the model.

The models will be solved using custom large-scale finite element software (FAIM, v6) on a desktop workstation. Using our custom software, the tibia and radius models will require approximately 20 minutes to solve. Stiffness (N/mm) will be calculated as the reaction force (RFz) determined by the finite element model at 1% strain divided by the bone cross sectional area from the morphologic analysis. The stiffness value will be used to calculate apparent bone strength (ultimate stress, MPa) based on an established linear relationship.

Biomarkers

Blood serum will be assessed to investigate effects of vitamin D effect on calcium metabolism and bone: Serum PTH, 25OHD, calcium creatinine, phosphate, albumin.
Biomarkers of bone turnover will be assessed. N-terminal propeptide of type 1 collagen (PINP) is a bone formation marker and will be analyzed from serum using a commercial assay. C-telopeptide of type 1 collagen (CTx) is a bone resorption marker and will be analyzed from serum using a commercial assay. PINP and CTx measurements are considered the best currently available markers of the two components of bone turnover (10). Due to budget reasons we were unable to attain P1NP data.

Balance and Muscle Strength

The influence of vitamin D on the physical balance of the subjects will be semi-quantified by performing four balance tests at baseline and annually. These tests include standing with (a) two feet on the force platform with eyes open, (b) two feet on the force platform with eyes closed, (c) two feet on a foam pad with eyes open, and (d) two feet on a foam pad with eyes closed. All tests will be performed 3 times, and a blindfold will be used for all eyes-closed assessments. The total testing time is approximately 15 minutes.

Tests of strength will be performed at baseline and annually (grip strength and Timed Up and Go).

Questionnaires

All questionnaires will be administered by an interviewer. Semi-quantitative measures of quality of life (QoL), depression, and food frequency will be assessed on entry, and then annually thereafter.

a) Quality of Life (QoL) questionnaire: The QoL questionnaire will be the SF-36.
b) Depression questionnaire: Beck Depression Inventory (BDI-II) or PHQ-9.
c) Food Frequency questionnaire (FFQ): The FFQ records the food intake especially of nutrients rich in calcium in order to estimate calcium intake.

The depression questionnaire will be performed at 3 and 6 months to monitor whether there is an early change in depression score.

Statistical Analysis

Statistical analysis will be used for all data. All acquired data (e.g. bone quality parameters, bone density, bone markers) of the three groups will be compared. Refer to the statistical analysis plan for specific details. Results will be considered statistically significant at $p < 0.05$.

Safety

The main concern in safety of vitamin D consumption is the avoidance of toxicity in the form of hypercalcemia or renal damage, related to hypercalcemia and hypercalciuria. Two of the doses in this study are generally accepted as safe and not requiring any medical safety monitoring. The 10,000 IU daily dose exceeds the IOM recommended upper limit of safe, unsupervised vitamin D intake (hence, this trial includes clinical supervision as detailed below). Although the IOM report selected 4,000 IU/day as the TUL, there is reasonable evidence that 10,000 IU/day is not associated with toxicity in clinical trials (7,8,9), and some have proposed this dose as a more appropriate tolerable upper intake level (8). No cases of vitamin D toxicity have been clearly documented with doses less than around 40,000 IU/day (8). Further, in a small study examining the effect of vitamin D supplements on serum 25OHD, a dose of 10,000 IU/day caused an apparent plateau in 25OHD levels at around 250 nmol/L after 120 days of treatment (Heaney et al, “Additional Reference” # 4). This leads us to believe that if signs of vitamin D toxicity or excessive rise in 25OHD were to occur on this dose, we would expect them to occur within the first three or six months of the study and identified by the serum and urine testing. However, we will continue to monitor for safety at 12, 18, 24, 30 and 36 months.

We will monitor safety at each visit with a fasting morning sample collection for:

- Serum: AST, ALT, BUN, Creatinine, 25OHD, albumin, calcium, Phosphate, Alk Phos, glomerular filtration rate (GFR, calculated value from serum creatinine).
- Hemoglobin A1C and fasting glucose obtained at baseline, 3, 6, 12, 24 and 36 months.
- 24 hour urine calcium, and creatinine; urinalysis for hematuria. If the subject declines a 24 hour urine calcium measurement, a morning second voided fasting urine calcium to creatinine ratio will be measured.
- Parathyroid hormone (PTH) levels

Additionally, the routine interview will assess for any change in medical condition (e.g. the occurrence of kidney stones). An ECG will be performed for any patient who develops hypercalcemia during the treatment period.

Criteria for assessment of need for withdrawal/discontinuation of a subject from the trial:

1. Hypercalciuria is the first indication of vitamin D excess and occurs before the development of hypercalcemia. Hypercalciuria is defined by a 24 hour urine calcium exceeding 7.5 mmol/day (for individuals over 75 kg body weight, 24 hour urine exceeding 0.1 mmol/kg body weight/24 hours) or, for individuals not able to do a 24 hour calcium collection, a calcium to creatinine ratio ≥ 1.0 . These individuals will be questioned regarding excess calcium intake, and a repeat collection performed. If hypercalciuria persists on the second collection, the subject will be withdrawn from the study.

If calcium to creatinine ratio is used, a value of ≥1.0 will be the criterion for a similar sequence of investigations and if the repeated measurement is ≥1.0, the subject will be withdrawn from the study (see "Additional References" numbers 1 and 2 below).

2. Hypercalcemia, defined as a serum albumin-corrected calcium above 2.55 mmol/L (the upper limit of the normal range), will be treated the same way as hypercalciuria (repeated serum calcium after checking for excess calcium intake) and if hypercalcemia persists, the subject will be withdrawn from the study.

3. Serum 25OHD >450 nmol/L in the absence of any abnormality of calcium metabolism will be repeated, and if persistent, the subject will be withdrawn from the study. According to Hathcock et al (8) and Vieth (reference #3 in "Additional References"), no well-documented cases of vitamin D toxicity have been reported with levels under 500 nmol/L In the Phase I/II clinical trial of high dose vitamin D in multiple sclerosis ("Additional Reference" # 1), a mean peak level of 413 nmol/L was reached in the subjects receiving 40,000 IU daily, with no adverse effect on calcium metabolism or any other symptomatology]

4. A repeated rise in serum creatinine AST, ALT, or phosphate to above 1.5 times the upper limit of normal will be a criterion for withdrawal from the study.

A Data Safety and Monitoring Board (DSMB) will be set up, and Dr. Doreen Rabi has agreed to chair this.

Timeline and Reporting

The following outlines the timeline for the 3 year study, including the processes for acquiring ethics approval and screening. Thus, the total time to conduct the study is estimated to be 4 years. This timeline is approximate because the time for receiving approval from the ethics board is not predictable. All times will be adjusted according to when final protocol approval is in place.

Time (months)	Study (months)	Notes	Quest (min)	Blood (min)	DXA (min)	XCT (min)	Balance (min)	Total Visit (min)	Reports / Publications
0		Submit ethics protocol							
4		Ethics protocol approved							
6		Equipment and staff in place							
6		Commence screening	40	15	20			75	
12		Complete screening							
12	0	Start of main study protocol	40	15	30	30	15	130	
15	3	Safety check, 10000 IU		15				15	
18	6	Safety check, 10000 IU		15				15	
24	12	1 year follow-up	40	15	30	30	15	130	
36	24	2 year follow-up	40	15	30	30	15	130	
48	36	3 year follow-up	40	15	30	30	15	130	
51	39	Study completed, unblinding							Final report and publications

NOTE:
Timing of final approval of ethics protocol is beyond our control. All subsequent time points will be adjusted based on date of final approval.
Unblinding is required to provide scientific analysis of the results. This can only be done at end of study.
Each visit of 300 subjects will take approximately 5 months of intense data collection.

Annual reports will be provided to the study sponsors upon request including the following information:

1. A summary of current participants enrolled including descriptives of age and sex.
2. A report of the cumulative data points collected for outcome measures from HR-pQCT, DXA, biomarkers and balance. Since these data will be collected in a blind fashion, there will not be any quantitative results provided (i.e., we will not be able to separate the results into the three study arms for analysis of a dose-dependent response).

Outcome Measures

The following is a summary of all outcome measures that will be collected for the study.

HR-pQCT	BMD	Bone mineral density
	Ct.BMD	Cortical bone mineral density
	Tb.BMD	Trabecular bone mineral density
	BV/TV	Bone volume fraction
	Tb.Th	Trabecular thickness
	Tb.N	Trabecular number
	Tb.Sp	Trabecular separation
	Ct.Th	Cortical thickness
	Ct.Po	Cortical porosity
FEM	RFz	Total reaction force to 1% strain
	RFz.cort	Cortical bone reaction force
	RFz.trab	Trabecular bone reaction force
	Failure load	Maximum load until yield
	Stiffness	Apparent bone stiffness
DXA	Femoral_BMD	Areal bone mineral density
	L1-4_BMD	Areal bone mineral density
	Radius_BMD	Areal bone mineral density

Questionnaires	QoL	Quality of life, from SF-36
	Depression	Semi-quantitative depression
	FFQ	Food frequency questionnaire
Biomarker	serum calcium	biomarkers
	creatinine	biomarkers
	phosphate	biomarkers
	serum PTH	biomarkers
	25-[OH]D	biomarkers
	albumin	biomarkers
	P1NP	bone formation
	CTx	bone resorption
Anthropometry	height	cm
	weight	kg
	limb lengths	cm
Balance	CTSIB	Clin test sensory integ of balance
	CTSIB vs norm	Normative data comparison

Publications

The authors reserve the right to publish the results of this trial in a timely fashion at a top-rated peer-reviewed journal. The publication will be prepared whether or not there is a clear dose-dependent effect of vitamin D on measured health parameters. The study sponsors will not be authors on publications derived from this work.

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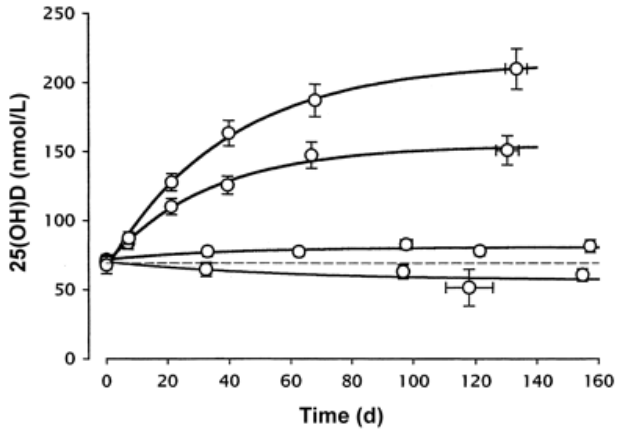

FIGURE 1.

Time course of serum 25-hydroxycholecalficerol [25(OH)D] concentration for the 4 dosage groups. The points represent the mean values, and error bars are 1 SEM. The curves are the plot of Equation *1*, fitted to the mean 25(OH)D_3 values for each dosage group. The curves, from the lowest upward, are for 0, 25, 125, and 250 μg cholecalciferol (labeled dose)/d. The horizontal dashed line reflects zero change from baseline.

Summary of Protocol Changes

Amendment 1 Approved August 29, 2013

	Protocol Modification
1	Removal of the Canadian Association of Radiology/Osteoporosis Canada 10-year fracture risk calculator (CAROC – available on the Osteoporosis Canada Website). Only use the World Health Organization's Canadian FRAX calculator.
2	Addition to exclusion criteria: 12. Consumption of vitamin D supplements at a dose ≥ 2000 IU/day for the past 6 months. 13. Active kidney stone disease (documented kidney stone within the last 2 years).
3	Addition to osteoporosis prescription drugs as part of the exclusion criteria point 7. Post-menopausal estrogen/progesterone therapy is not an exclusion if the subject's intention is to carry on with this therapy for the proposed duration of the study, but if this therapy is stopped during the study the subject would be withdrawn from the study.
4	Addition to screening labs Phosphate
5	Addition to blood sample Blood for DNA will be collected at baseline
6	Addition to HR-pQCT scan Added a scan at 6 months
7	Addition of muscle strength/function Tests of strength will be performed at baseline and annually (grip strength and Timed Up and Go).
8	Addition of more tests to the safety monitoring variables Phosphate, hemoglobin A1C and fasting glucose obtained at baseline, 3, 6, 12, 24 and 36 months.

Amendment 2 Approved January 17, 2014

	Protocol Modification
1	Addition of the pilot group and increase in the total number of participant's randomized. The pilot group were unable to be scanned with the second-generation HR-pQCT scanner at baseline. Participants were scanned with the first-generation HR-pQCT scanner at baseline and 6 months. At their 6-month appointment and beyond participants were scanned on the second-generation HR-pQCT scanner (6, 12, 24, 36). The only difference between the pilot and main cohort is the HR-pQCT scan at baseline. 100 additional participants may need to be screened. A total of 375 participants will be recruited, of which 300 completed the full protocol.
2	Change in serum 25(OH)D range for inclusion criteria. Serum 25(OH)D was changed to under 125 nmol/L and above 30 nmol/L.
3	Change to the total radiation dose for study duration. Due to the addition of one HR-pQCT scan the maximum does will be 5µSv.
4	Change to protocol to remove analysis of P1NP Due to budget reasons, we were unable to attain P1NP data.
5	Additional data analysis of HR-pQCT scans. From additional post processing of our HR-pQCT scans at baseline, 6, 12, 24, 36 months we will be able to observe vascular calcification throughout the trial.