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Author: Robert Clarke Connie Newman Joseph Tomson
Harold Hin Rijo Kurien Jolyon Cox Michael Lay Jenny Sayer
Michael Hill Jonathan Emberson Jane Armitage



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Highlights

- The BEST-D (Biochemical Efficacy and Safety Trial of vitamin D) trial will compare the biochemical and other effects of daily dietary supplementation with 100µg or 50µg vitamin D3 or placebo, when administered for 12 months, in 305 ambulant community-dwelling older people living in Oxfordshire, England.
- The primary analyses will compare 12-month mean plasma concentrations of 25(OH)D as well as the proportion of participants with a 12-month concentration >90 nmol/L between participants allocated 100µg and participants allocated 50µg daily. Secondary analyses will compare the two active doses (both separately and when combined) with placebo.
- Additional end-points include biochemical assessments of safety, blood pressure, arterial stiffness, falls, fractures, heel and wrist bone density, grip strength and physical performance and echocardiographic assessments of cardiac function in a random sample of participants.
- The results of this trial will help determine the optimum dose of vitamin D to test in a larger trial investigating whether vitamin D supplementation can reduce the risk of fractures, cardiovascular disease or cancer.

Estimation of the optimum dose of vitamin D for disease prevention in older people: rationale, design and baseline characteristics of the BEST-D trial

Robert Clarke^{1,*}, Connie Newman^{2,*}, Joseph Tomson^{1,*}, Harold Hin^{3,*}, Rijo Kurien¹, Jolyon Cox¹, Michael Lay¹, Jenny Sayer¹, Michael Hill¹, Jonathan Emberson¹, Jane Armitage^{1*} for the BEST-D Collaborative Group

1. Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, UK;[†]
2. Division of Endocrinology & Metabolism, New York University School of Medicine, New York, USA.
3. Hightown Surgery, Banbury, Oxfordshire, UK.

(*Contributed equally).

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[†]Investigators and staff in the BEST-D Collaborative Group are listed in the Appendix.

Address for correspondence: Dr Robert Clarke, Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Richard Doll Building, Roosevelt Drive, Oxford, OX3 7LF, England.

Tel: 01865-743743; Fax 01865-743822; Email: Robert.clarke@ctsu.ox.ac.uk

Conflict of interests: Robert Clarke, Connie Newman, Joseph Tomson, Harold Hin, Rijo Kurien, Jolyon Cox, Michael Lay, Jenny Sayer, Michael Hill, Jonathan Emberson, Jane Armitage declare that they have no conflict of interest.

Running title: Dose-finding trial of vitamin D

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Abstract

Background: Previous large trials of vitamin D for prevention of fractures and other disease outcomes have reported conflicting results, possibly because the doses tested were insufficient to maintain optimum blood levels of vitamin D (25[OH]D) predicted by the observational studies. This report describes the design and baseline characteristics of the BEST-D (Biochemical Efficacy and Safety Trial of vitamin D) trial which aims to establish the best dose of vitamin D to assess in a future large outcome trial.

Methods: The BEST-D trial will compare the biochemical and other effects of daily dietary supplementation with 100µg or 50µg vitamin D₃ or placebo, when administered for 12 months, in 305 ambulant community-dwelling older people living in Oxfordshire, England. The primary analyses will compare 12-month mean plasma concentrations of 25(OH)D as well as the proportion of participants with a 12-month concentration >90 nmol/L between participants allocated 100µg and participants allocated 50µg daily. Secondary analyses will compare the two active doses (both separately and when combined) with placebo. Additional end-points include biochemical assessments of safety, blood pressure, arterial stiffness, falls, fractures, heel and wrist bone density, grip strength and physical performance and echocardiographic assessments of cardiac function in a random sample of participants.

Results: About one-third of eligible participants agreed to participate in the trial. The mean age was 72 (SD 6) years with equal numbers of men and women. About one third reported a prior history of fracture or hypertension, one-fifth reported a prior

cardiovascular event, and one tenth reported diabetes or a fall in the previous 6 months.

Conclusions: The results of this trial will help determine the optimum dose of vitamin D to test in a larger trial investigating whether vitamin D supplementation can reduce the risk of fractures, cardiovascular disease or cancer.

Keywords: Vitamin D supplementation trial, 25-hydroxyvitamin D, parathyroid hormone.

Introduction

Osteoporosis causes substantial morbidity and mortality in older people, but whether chronic insufficiency of vitamin D is a reversible determinant of osteoporosis and related risk of fractures is controversial. Observational studies indicate that low plasma levels of 25-hydroxyvitamin D [25(OH)D] are associated with higher risks of fractures and with vascular and non-vascular mortality (1-4), but it is unclear if these associations are causal. Randomized trials assessing the effects on fracture and other health outcomes have generally failed to demonstrate beneficial effects of vitamin D supplementation (5-14). However, few such trials have used sufficient doses of vitamin D₃ to achieve and maintain what might be considered optimum plasma levels of 25(OH)D.

Although controversial, the available evidence suggests that the optimum plasma levels of 25(OH)D may be around 75 to 90 nmol/L. Firstly, parathyroid hormone (PTH) levels are linearly and inversely associated with plasma 25(OH)D levels until such levels reach about 75 nmol/L (15, 16). Secondly, prospective observational studies indicate that the risks of vascular and non-vascular mortality are lowest at plasma 25(OH)D levels of around 90 nmol/L (4). Thirdly, mean peak plasma 25(OH)D levels at the end of summer in young British adults are also about 90 nmol/L(17). At plasma 25(OH)D levels below a cut-off point of about 75 nmol/L, the average increase in 25(OH)D per 10µg of additional vitamin D₃ has been estimated to be about 7-10 nmol/L(16, 18). Hence, from a typical level of 25(OH)D of 55 (SD 26) nmol/L found in older UK adults (19), a dose of vitamin D₃ of at least 50µg (2000

IU), and possibly as much as 100µg (4000 IU), may be required to achieve and maintain blood levels >90 nmol/L throughout the year in most people in the UK (20).

The aims of the BEST-D(Biochemical Efficacy and Safety Trial of vitamin D) trial are to determine the optimum dose of vitamin D that is safe and effective to test in a large trial of vitamin D for prevention of fractures, vascular disease and cancer in older people living in the community. The primary objectives are to compare the effects on blood concentrations of 25(OH)D and the proportion of participants with 25(OH)D concentrations >90 nmol/L after one year of daily supplementation with 100 µg (4000 IU) or 50 µg (2000 IU) vitamin D3 versus placebo. The aim of the present report is to describe the rationale and design of the BEST-D trial and the baseline characteristics of the trial participants.

Methods

Inclusion and exclusion criteria

Ambulant community-dwelling men and women aged 65 years and older were identified from a single general practice in Oxfordshire and mailed an invitation letter to participate in the trial. The invitation letter included a screening questionnaire to check eligibility and a study information leaflet. Those who returned the screening questionnaire and were potentially eligible were provided with an appointment for a randomization study visit. **Figure 1** outlines the number of participants enrolled in each stage in the study.

People were ineligible if they were: nursing home residents; regular users of vitamin D supplements more than 10 µg (400 IU) vitamin D daily; prescribed calcium supplements, bisphosphonates, parathyroid hormone (PTH), or calcitonin; had medically diagnosed dementia or history of hypercalcaemia, hyperparathyroidism lymphoma, sarcoidosis, active tuberculosis or renal calculus; were judged by their doctor as likely to be poorly compliant with clinic visits or medication; or had a history of alcohol or substance misuse or a history that might limit their ability to take the study treatment (e.g. terminal illness). All participants provided written informed consent.

Outcomes

The co-primary outcomes are mean plasma 25(OH)D concentrations at 12 months and the percentage of participants with a 12-month 25(OH)D concentration >90 nmol/L. Secondary outcomes are: (i) mean plasma 25(OH)D concentrations at 1 and 6 months; (ii) percentage of participants with plasma 25(OH)D concentrations >90 nmol/L at 1 and 6 months; (iii) PTH within the reference interval (1.1-6.8 pmol/L) at 1, 6 and 12 months; (iv) albumin-corrected calcium concentrations above the reference interval (2.15-2.55 mmol/L) at 1, 6 and 12 months; and (v) mean plasma concentrations of albumin, phosphate, creatinine and alkaline phosphatase at 6 and 12 months; (vi) mean concentrations of lipids (total cholesterol, LDL-C, HDL-C, triglycerides, apo-B and apo-A1), high-sensitivity C-reactive protein (hsCRP), and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) at 6 and 12 months; (vii) mean levels of systolic and diastolic blood pressure, heart rate and arterial stiffness at 6 and 12 months; and (viii) echocardiographic measures of systolic

function, diastolic function and global cardiac function in a sub-set of 150 participants at 12 months. Tertiary outcomes are listed in the Web-Appendix, and include 12-month assessments of: (i) reported fractures at all sites and at specific sites; (ii) reported falls; (iii) muscle and joint pain; (iv) grip strength; (v) Short Physical Performance battery; (vi) Geriatric Depression Scores; (vii) respiratory infections; (ix) Height, weight and BMI; (x) bone density of wrist and heel; and (xi) estimated GFR and urinary albumin/creatinine ratio.

Sample size and statistical analyses

Based on the reported mean seasonally adjusted 25(OH)D levels of 55 (SD 20) nmol/L among post-menopausal women between the ages of 65 and 74 years (21), a dose of 50 µg of vitamin D3 may be required to increase the mean concentration of 25(OH)D by 25-35 nmol/L. However, there is uncertainty about whether the relationship between vitamin D dose and the increase in 25(OH)D is linear. The study was designed to have good power to detect even a small difference in mean 25(OH) concentrations at 12 months between the two active doses. Specifically, with 100 participants in each group and an assumed SD of 20 nmol/L, this study has 90% power (at $2p=0.01$) to detect a true difference in mean 25(OH)D between the two active doses at 12 months of just 11 nmol/L. (The minimum detectable difference will be even smaller than this due to the use of analysis of covariance to assess comparisons: see Web-Appendix.)

The primary efficacy assessment will be an “intention-to-treat” analysis among all randomized participants comparing daily dietary supplementation with vitamin D3 100µg vs vitamin D3 50µg on: (1) the mean 25(OH)D concentrations at 12 months

and (2) the proportion of individuals with levels of 25(OH)D above 90 nmol/L at 12 months. Pre-specified subgroup analyses of the co-primary outcomes will include subdivisions by: sex; age (<70 vs \geq 70 years); higher or lower body mass index (BMI); baseline plasma concentrations of 25(OH)D; estimated glomerular filtration rate; and estimated dietary calcium intake; or presence or absence of prior cardiovascular disease or cancer. No allowance will be made for multiple comparisons in the assessment of the co-primary endpoints with the statistical significance of each being based on a $2p < 0.05$ criterion.

Secondary and tertiary analyses of particular outcomes will compare the two active doses (separately and/or when combined) with placebo. (A detailed Statistical Analysis Plan is provided in the Web-Appendix). Allowance will be made in their interpretation for multiple hypothesis testing (taking into account, if relevant, the type of measure and evidence from other studies) (22, 23). But, the more extreme the p-value (or, analogously, the further the confidence interval is from zero) after any allowance has been made for the nature of the particular comparison, the more reliable the comparison and, hence, the more definite any finding will be considered.

Randomization and blinding

Table 1 shows a summary of the study procedures. Study visits were conducted by trained study nurses at the participant's home (but the final visit also included an additional visit to the General Practice: see below). At the first visit (month 0), eligibility was assessed and relevant medical history and medication, calcium intake, and baseline measures recorded. If willing and eligible, participants were randomized in a ratio of 1:1:1 to vitamin D3 100 μ g (4000 IU), vitamin D3 50 μ g

(2000 IU), or placebo daily. Randomization was performed by a central randomization service at the Clinical Trial Service Unit (CTSU), University of Oxford using a minimisation algorithm balanced for age group [65-69, 70-74, 75+ years], gender, body mass index (BMI), smoking history, ethnicity and history of fracture to assign a double-blind treatment allocation and a treatment allocation number. Vitamin D3 50 µg in soft gel capsules and matching placebo capsules were provided by Tischcon Corporation (Westbury, New York, USA), and packaged in labelled child-proof bottles by Sharp Clinical Services (Crickhowell, Powys, UK). At the randomization and 6 month visits, participants were provided with two bottles of medication, each containing 210 capsules (each containing 50 µg vitamin D3 or matching placebo) sufficient to last 7 months, and instructed to take one capsule from each bottle daily. Participants were asked to return all study treatment bottles at the 6 and 12 month visits.

Post-randomization follow-up

About 100 participants were randomly selected for a blood sample at 1 month (Table 1). All participants were to have follow-up visits from the study nurse at 6 and 12 months (Table 1). Compliance and adverse events were evaluated at each visit along with selected tests. Depression scores were assessed at 6 and 12 months using a 4-point Geriatric Depression Score (24). Spot urine samples were collected at the final home visit. Participants were invited to attend a special clinic at their local General Practice for a bone scan to assess heel and wrist bone density and a short physical performance battery (25).

Safety

At each visit non-serious adverse events (NSAEs) thought related to the study treatment or that resulted in the participant stopping treatment, and all serious adverse events (SAEs) were recorded in the electronic case report forms. Participants were provided with a 24-hour Freefone number to contact a clinician at CTSU should they wish to discuss trial-related medical problems.

Cardiovascular assessments

At each visit blood pressure and arterial stiffness were measured after 10 minutes of rest in the seated position (26). A finger probe (Pulsetrace PCA 2) placed on the right forefinger recorded the 'digital volume pulse' using photoplethysmography over 30-60 seconds. This was followed by blood pressure and brachial artery arterial stiffness measurements made over 2 minutes using a TensioClinic™ arteriograph. Left ventricular function was assessed by echocardiography in a randomly selected subset of 151 participants at the final visit (who provided separate consent for this examination). Any significant abnormalities were to be reported to the participant's GP.

Musculoskeletal parameters

A history of falls in the last 6 months and fractures at any time was recorded at randomization, 6 and 12 months. Participants were asked to rate from 1 to 10 their

level of physical activity, and the presence and severity of any muscle and, separately, joint pain, if present. Hand grip strength was assessed using a Jamar™ J00105 hydraulic dynamometer, as previously described (27). A modified short physical performance battery at the final visit evaluated the time taken for five consecutive chair rises, the time to walk 3 metres, and a balance score calculated from the time that balance was held in the tandem, semi-tandem, and side-by-side stances (25). Heel and wrist bone mineral density were measured using an OsteoSys EXA-3000 scanner (OsteoSYS, Seoul, Korea).

Laboratory analyses

Blood samples were collected at each visit into 2 standard 10 ml tubes (one containing EDTA and one lithium heparin) and at baseline and 12 months also into a PAXgene blood RNA tube (Pre-Analytix, Qiagen, Hombrechtiken, Germany). Samples were taken to the Horton Hospital Clinical Chemistry laboratory, Banbury, Oxfordshire, processed, fractionated into aliquots within 4 hours of blood collection and stored in a -80°C freezer. Lithium heparin plasma was used to measure plasma levels of calcium and albumin at the Horton Hospital Clinical Chemistry laboratory prior to starting study treatment. Plasma samples were stored at the Wolfson Laboratory in CTSU, Oxford for future determination of plasma levels of PTH, 25(OH)D, lipids, hsCRP and inflammatory cytokines at CTSU. Buffy coats were also stored from the randomization and 12 month visits for future extraction of DNA to measure markers related to vitamin D. Urine samples were collected at the final visit and frozen (-80 °C) for future measurement of urinary albumin to creatinine ratio.

Data collection and management

All questionnaire and clinical data were collected electronically using portable computers and encrypted to ensure confidentiality, data accuracy and protection of patient safety. BEST-D was conducted in accordance with the principles of the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH-GCP), and relevant regulations.

Ethics committee approval and funding

BEST-D received approval from the National Research Ethics Service (NRES) Committee South Central – Oxford B, the Thames Valley Primary Care Research Partnership, a Clinical Trial Authorisation from MHRA and is included on the National Institute for Health Research (NIHR) Trial portfolio. The University of Oxford acted as sponsor and funding was provided by the Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford and the British Heart Foundation.

Results

Characteristics of study participants

From the registered patients at the practice in this age group, 932 were invited to participate, 313 (33%) agreed to have a randomization visit from the study nurse and 305 participants were successfully randomized between 24 September 2012 and 14 March 2013 (**Figure 1**). Eight participants visited were not randomized (6 declined and 2 were ineligible) and 619 declined or ignored the invitation to participate. The final study visit for the last participant was held on 10 March 2014. Among surviving

participants, 296 completed a 6-month visit, 290 completed a 12-month visit, 272 had their bone density measured and 151 had cardiac echocardiography.

Baseline characteristics

Among the 305 participants, the overall mean age was 72 (SD 6) years, 51% were men, 14% had a prior history of IHD, 6% had stroke/TIA, 9% had diabetes and 39% had hypertension. Overall, 30% had any prior history of fracture and 13% had a history of a fall in the last 6 months. Table 2 also shows the use of medication for prevention of cardiovascular disease, mean levels of body mass index, blood pressure and grip strength; and estimated calcium intake. While use of anti-hypertensive drugs, anti-platelet agents and statins were appropriate for the proportions with prior vascular disease and diabetes in this general population, routine use of non-prescribed (“over-the-counter”) preparations of calcium and vitamin D supplements was uncommon (albeit this partly reflected the study design).

Discussion

Although severe vitamin D deficiency is associated with rickets in children and osteomalacia in adults (28), there is no consensus about routine supplementation with vitamin D for bone health. The UK dietary Reference Nutrient Intake (RNI) for vitamin D is 10 µg/day (400 IU) for adults aged 65 years and older and pregnant or lactating women. The RNI has not been established for otherwise healthy adults < 65 years old because of the assumption that sufficient vitamin D can be made after exposure to sunlight.

In 1992, Chapuy et al (5) reported that supplementation with 20 µg (800 IU) vitamin D plus calcium 1200 mg daily for 18 months significantly reduced non-vertebral fractures (160 vs 215; $p < 0.001$) including hip fractures (80 vs 110; $p = 0.004$) in 3,270 elderly women living in nursing homes or apartments for elderly people in France. Over the last 20 years, several large, long-term trials, and several study-level and individual participant-level meta-analyses have assessed the effect of vitamin D on fractures, but the results have been conflicting (6-14), possibly because the doses of vitamin D used were insufficient to maintain optimum levels of 25(OH)D. However, there have been few large trials of vitamin D that used adequate doses of vitamin D to achieve the blood levels of 25(OH)D that were predicted by the observational studies to be associated with lowest risk. While there is no consensus about the optimum levels of 25(OH)D for bone health, the available evidence suggests that it may be close to 90 nmol/L, the level reached by healthy UK adults at the end of the summer (17), and below which PTH levels may be elevated (15, 16) and the level in observational studies associated with the lowest risk of vascular and non-vascular mortality (4).

BEST-D is the first randomized placebo-controlled trial to assess the effects of two higher daily doses of vitamin D, 100 µg and 50 µg (equivalent to 4000 IU and 2000 IU), in older adults living in the UK, on plasma levels of 25(OH)D, PTH, calcium, phosphate and albumin. An additional advantage of choosing 4000 IU daily is to maximize mean levels and the proportion greater than 90 nmol/L of 25(OH)D levels after taking account of anticipated non-compliance of about 10-15% with allocated study treatment. The detailed clinical and biochemical monitoring at 0, 6 and 12 months should provide reliable data on the safety of higher doses of vitamin D.

The present study was designed to have sufficient power to detect differences in biochemical end-points. While the clinical end-points, including muscle pain, joint pain, self-rated physical activity, grip strength, balance, gait speed and bone density at the wrist and heel will be assessed, a much larger number of participants would be required to obtain significant differences by allocated treatment. The results of the BEST-D trial should establish the optimum daily dose of vitamin D to be used in a future randomized trial in the UK to evaluate whether vitamin D supplementation confers the musculoskeletal and other health benefits suggested by observational studies (29-31).

Appendix: BEST-D Collaborative Group

Writing Committee: Robert Clarke^{1,*}, Connie Newman^{2,*}, Joseph Tomson^{1,*}, Harold Hin^{3,*}, Rijo Kurien¹, Jolyon Cox¹, Michael Lay¹, Jenny Sayer¹, Michael Hill¹, Jonathan Emberson¹, Jane Armitage^{1,*} (*contributed equally).

¹ Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, UK;

². Division of Endocrinology & Metabolism, New York University School of Medicine, New York, USA.

³. Hightown Surgery, Banbury, Oxfordshire, UK.

Steering Committee: Jane Armitage (Chief Investigator and Chair), Harold Hin and Robert Clarke (Lead Investigators), Michael Hill (Laboratory Director), Joseph Tomson, Connie Newman, and Richard Haynes (Clinician Members), Anne Dawson (Lay Member), Jonathan Emberson (Statistician), Michael Lay (Computing) and Jenny Sayer (Administrative Coordinator).

Medication: Kevin Murphy.

Computing: Michael Lay, Jolyon Cox, Rijo Kurien.

Clinical Biochemistry, Horton Hospital, Banbury: Tim James and Peter Tandy and
Wolfson Laboratory, Clinical Trial Service Unit, Oxford: Michael Hill

Nurses: Lynn Peach, Enid Frost, Caroline Boulton, Barbara White.

Research Assistant: Jessica Hin.

Echocardiography: Joseph Tomson, Linda Arnold.

Administrators: Kate Gillingham, Jenny Sayer.

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Table 1: Schedule of study procedures

Study Procedure	Month			
	0	1	6	12
History				
• Medical history and consent	x			
• Assessment of dietary calcium	x			
• Assessment of fractures and falls	x		x	x
• Geriatric depression score (4 parts)			x	x
Physical measurements				
• Height and weight	x			x
• Blood pressure, arterial stiffness	x		x	x
• Grip strength, muscle pain	x		x	x
• Short physical performance				x
• Heel and wrist DEXA				x
• Echocardiography				x
Blood and urine collection				
• Plasma biochemistry*	x	x	x	x
• RNA markers	x			x
• Buffy coats for DNA markers	x		x	x
• Urinary albumin/creatinine				x
Study treatment				
• Issue study treatment	x		x	
• Assess compliance			x	x
• Monitor adverse events			x	x

* Total cholesterol, LDL-C, HDL-C, triglycerides, apolipoprotein A, apolipoprotein B, 25-hydroxy-vitaminD (25[OH]D); parathyroid hormone; (PTH), albumin, calcium, phosphate, alkaline

phosphatase, creatinine, C-reactive protein (CRP); n-terminal brain natriuretic peptide (nBNP). Plasma biochemistry measurements at 1 month will be analyzed in a sub-set of 100 participants.

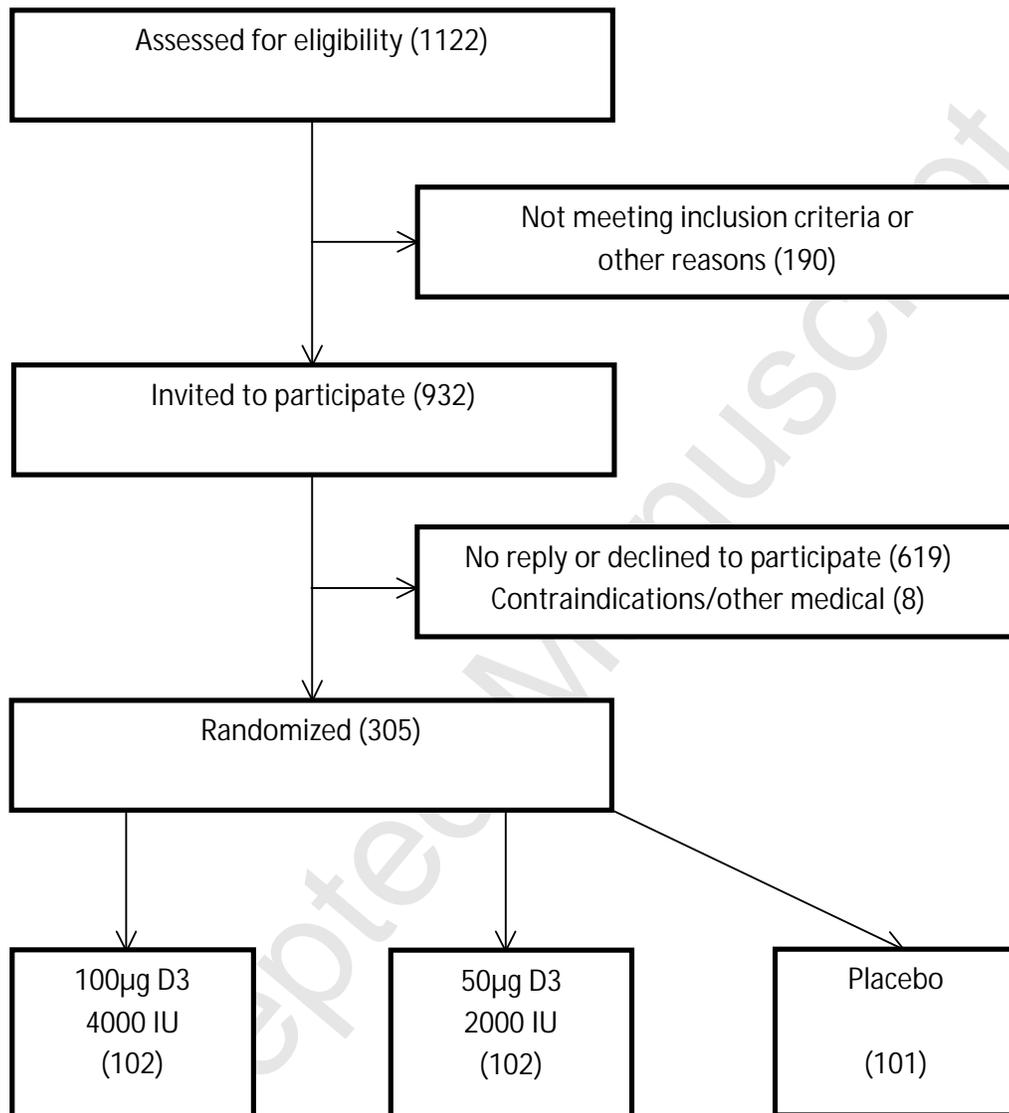
Table 2: Baseline characteristics of study participants, by allocated treatment

Mean (SD) or %	4000 IU/day (n=102)	2000 IU/day (n=102)	Placebo (n=101)
Age, years	71 (6)	72 (6)	72 (6)
Male	51%	50%	51%
Current smoker	7%	7%	7%
Dietary calcium (mg/day)	724 (287)	695 (292)	713 (302)
Prior disease			
Heart disease*	20%	11%	11%
Stroke/TIA	5%	8%	6%
Hypertension	39%	43%	35%
Diabetes	9%	9%	9%
Fracture	30%	29%	30%
Any fall in the past 6 months	13%	15%	12%
Medication			
Any antihypertensive drugs	43%	49%	40%
Statins	29%	27%	21%
Any anti-platelet drugs	18%	19%	15%
Vitamin D (≤ 400 IU/day)	6%	8%	11%
Calcium	2%	1%	3%
Physical measurements			
Height, cms	168 (10)	168 (10)	167 (10)
Weight, kgs	77 (17)	78 (15)	79 (15)
Body mass index, kg/m^2	27 (5)	27 (4)	28 (5)
Grip strength, kg	25 (11)	25 (11)	25 (11)

Mean (SD) or %	4000 IU/day (n=102)	2000 IU/day (n=102)	Placebo (n=101)
Systolic blood pressure, mm Hg	132 (22)	132 (17)	129 (18)
Diastolic blood pressure, mm Hg	77 (11)	77 (10)	76 (12)

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Figure 1: Selection of included participants in the BEST-D trial



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Lead Investigators:	Robert Clarke, Harold Hin
Endocrinology:	Connie Newman
Information Technology (IT) team:	Rijo Kurien, Michael Lay
Laboratory:	Michael Hill
Administrator:	Jenny Sayer
Statistics	Jonathan Emberson
Principal Investigator:	Jane Armitage