THE UNIVERSITY OF AUCKLAND
FACULTY OF MEDICAL AND HEALTH SCIENCES
School of Population Health

hrc Health Research Council of New Zealand

ViDA
Vitamin D Assessment

STUDY OF VITAMIN D SUPPLEMENTATION

(THE ViDA STUDY)

STUDY PROTOCOL

Version 5.0: 4 November 2013
Approved by the Multi-Region Ethics Committee (MREC)
28 November 2013

STUDY PROTOCOL

Effect of vitamin D on cardiovascular and respiratory disease event rates
(Vitamin D Assessment [ViDA] Study)

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- Health Research Council of New Zealand (primary funder)
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1 PROTOCOL AMENDMENT HISTORY

VERSION (V)	VERSION DATE	ETHICS COMMITTEE APPROVAL DATE	CHANGES
V1 (Not stated on document)	Sep 2009 (Not stated on document)	30 Sep 2009	Title: Protocol for an Intervention Study to Investigate the Effect of Vitamin D Supplementation on Cardiovascular Disease and All -Cause Mortality
V2 (Not stated on document)	Oct 2010 (Not stated on document)	21 Oct 2010	CHANGES • Title change: Effect of vitamin D on cardiovascular and respiratory disease event rates • Reduced sample size from 7000 to 4000 • Broader age range 65-84yrs to 50-84yrs • Larger safety sample 5% to 10% of participants • Location: Recruitment will only be based in Auckland • Change of principal investigators – Professor Kay-Tee Khaw is now a full investigator; Associate Professor Ngaire Kerse and Professor David Schoenfield have withdrawn • Information sheet and consent version 3 • Information sheet and consent form for Safety sub study Version 3: 8
V3 (Not stated on document)	25 Mar 2011	22 Aug 2011	Title change: Effect of vitamin D on cardiovascular and respiratory disease event rates (Vitamin D Assessment (ViDA) Study) Correction of typographical errors, reformatting. Insertion: • Version(s) details • Table of contents • ANZTR number • More detail and/ or further clarification (e.g., insertion of tables) • Appendix: Protocol signature page
V4	07 May 2012		CHANGES 4.3 Participants Insertion • Source of potential participants broadened.

			Invitation extended to eligible people from the community who express interest in participating. • Recruitment period extended from December 2011 to September 2012 2.00 and 4.6 Intervention Deletion • TWO capsules in June. (Participants receive ONE capsule only for duration of study) • *Participant Information Form* revised to reflect this change. (Approved by MREC 8 May 2012) • 5.3 Data on fatal events and hospitalizations. Further clarification Details on how these unverified data will be made available to DMC in a timely fashion.
V5	October 2013	28 November 2013	CHANGES 2. Synopsis Inserted (underlined) <u>Additional data collected</u> • <u>Annual Sample Follow Up group</u> • <u>Psoriasis Sub Study</u> • <u>Bone Density Sub Study</u> • <u>Cholesterol Sub Study</u> 4.4 Baseline interview Name change (underlined item 7) • <u>BP +</u> (device) replaces Pulsecor 4.5 Deaths Deleted word (underlined): • <u>Annually</u> (each participant's NHI number will be used to search the Mortality data collected by the Ministry of Health) 4.5 Item 3 Non-hospital medical events (last paragraph) Insertion (number underlined): • mailed <u>four</u> monthly during follow up 4.6 Intervention Inserted sentence (underlined) • <u>From July 2013, study capsules were mailed to participants four monthly in batches of four capsules. (Previously study capsules were mailed each month along with monthly questionnaires.)</u>

			Inserted sentence (underlined) • From March 2014, one questionnaire will be mailed four monthly along with a batch of three capsules. This four monthly questionnaire will replace the one monthly questionnaire which will cease in November 2013. (Content of questionnaire remains the same – minor formatting changes only.) 4.6.2 Word change (underlined) • Their serum calcium results will only be seen by Mr Alistair Stewart and the Data Monitoring Committee to ensure research staff remain blind to treatment status. 4.7 Study period Amended month (underlined): • Recruitment will occur March 2011-December 2012 (Table 2 updated) • Four monthly mailouts.....continue until July 2015 plus an additional final mailout of the questionnaire in October 2015. 5. Safety • This substudy is referred to as the Annual Follow Up Group 5.2 Word inserted (underlined) • information 7. Data Management Insertion number (underlined) • The four monthly **11.2 Additional information collected** 11.2.1 Annual safety/compliance sample (section 5) now referred to as the Annual Follow-Up Group. MREC notified: 11 February 2011 MREC approved: 18 April 2011 Includes approval of a separate Annual Follow-Up Group Information Sheet V2.0 and Consent Form V4.0). 11.2.2 Additional information on the effect of vitamin D supplementation on psoriasis severity. Referred to as the Psoriasis Sub Study.

			MREC notified: 11 February 2011 MREC approved: 18 April 2011 Includes approval of a separate Information Sheet V2.0 and Consent Form V1. 11.2.3 Additional information on the effect of vitamin D supplementation on bone density. Referred to as the Bone Density Sub Study. MREC notified: 18 June 2012 MREC approved: 5 July 2013 Includes approval of document describing rationale and procedures and separate Information Sheet and Consent Form V1.18) 11.2.4 Additional information on the possible effect of serum cholesterol as a cardiovascular disease (CVD) risk factor as originally planned; to be linked with BP+ (formally Pulsecor) device measures of arterial stiffness. Referred to as the Cholesterol Sub Study.

2 SYNOPSIS

Study Title	Effect of vitamin D on cardiovascular and respiratory disease event rates
Abbreviated Name	**Vi**tamin **D A**ssessment Study(**ViDA**)
HRC ref. No.	10-400
ANZCTR No.	336777: U1111-1120-6224
Trial Design	Randomised, double blind, placebo-controlled trial
Trial Participants	Healthy adults aged 50-84 years
Planned Sample Size	5,100
Follow-up duration	4 years
Planned Trial Period	December 2010 to November 2015
Primary Objective	To determine if vitamin D supplementation (100,000 IU per month) reduces the incidence of cardiovascular disease compared with placebo.
Secondary Objectives	To determine if vitamin D supplementation, compared with placebo, reduces the incidence of: Respiratory infectious disease Non-vertebral fractures
Primary Endpoint	Incidence of cardiovascular disease (ICD-10 codes: I10-I82)
Secondary Endpoints	Incidence of respiratory infectious disease (ICD-10 codes: J00-J86) Incidence of non-vertebral fractures (ICD-10 codes: S22, S32, S42, S52, S62, S72, S82, S92, T12)
Additional Data Collected	Annual Follow-Up Group Psoriasis Sub Study Bone density Study Cholesterol Study
Investigational Medicinal Products	Vitamin D_3
Form	Softgel capsule
Dose	100,000 IU monthly for 4 years, starting one month after an initial oral loading dose of 200,000 IU.
Route	Oral

3 BACKGROUND

3.1 Health Significance

Cardiovascular disease (CVD) is the leading cause of mortality among New Zealanders. Each year, CV disease causes about 11,000 deaths[1] and 65,000 hospital discharges.[2] Infectious respiratory diseases (e.g., pneumonia, acute exacerbations of asthma/COPD) also are a huge burden, with about 24,000 hospital discharges annually in people ≥50 years.[2] There are ethnic disparities in both diseases with 2000-02 data showing 3-4 fold higher mortality among Maori for CV disease and COPD compared to non-Maori [www.maorihealth.govt.nz/moh.nsf].

Evidence from observational studies is rapidly increasing which indicates that low vitamin D status predicts increased risk of CVD;[3 4] while low vitamin D is associated with increased risk of respiratory infections.[5 6] There is an urgent need for clinical trials since attributable risk calculations using the burden of disease method suggest that up to 25-30% of CVD could be prevented by doubling the vitamin D levels of New Zealanders.[7]

Study findings could lead to new preventive strategies for people with low body vitamin D levels, such as Pacific, Maori, and older adult Pakeha, who all have an increased risk of CVD and respiratory infections. These might include recommending increased exposure to sunshine (that is compatible with the current public health policy to avoid sunburn because of concern about future skin cancers), increased vitamin D fortification of foods, and increased availability of higher-dose vitamin D supplementation (which is inexpensive and safe).

3.2 Overview of Vitamin D

3.2.1 Vitamin D Metabolism

Vitamin D enters our bodies in two forms: either as ergocalciferol (vitamin D_2) from plant foods, or cholecalciferol (vitamin D_3) from sun exposure or consumption of animal foods.[8]

Vitamin D_3 is synthesized in the skin by ultraviolet (UV) B radiation from the sun activating its precursor 7-dehydrocholesterol.[8] It then circulates in the blood to the liver where it is converted to its main form, 25-hydroxyvitamin D [25(OH)D], which has blood levels about 1000 times higher than the active metabolite, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D). The synthesis of 1,25-$(OH)_2$D is dependent on circulating levels of 25(OH)D.

3.2.2 5(OH)D Levels Required for Optimum Health

Vitamin D status is determined by measurement of serum 25(OH)D level. Until very recently, the cut-point for vitamin D insufficiency was considered to be a serum 25(OH)D level of 50 nmol/L.[9] However, there is increasing evidence that serum 25(OH)D levels should be 80-100 nmol/L for optimum health. This estimate comes from epidemiological studies which have observed that the risk of a range of diseases or markers – including bone mineral density, hip fracture, muscle strength, periodontal disease, diabetes, hypertension, coronary heart disease, lung function, colon and breast cancer, and all-cause mortality – is lowest at 25(OH)D levels >80 nmol/L.[10-19]

3.2.3 Dietary Intake Required to Achieve Optimum 25(OH)D Levels

The original aim of current recommendations for the daily intake of vitamin D was to prevent rickets, and these recommendations have been widely adopted around the world including New Zealand.[20] Recommended doses are, for example, 600 IU per day for people aged >70 years,[21] and 200-400 IU for younger adults.[22] However, recent research has shown that much higher doses are required to achieve the vitamin D levels now considered to be optimum. For example, to raise serum 25(OH)D from 50 to 80 nmol/L requires an additional.[23] 1700 IU of vitamin D_3 per day, while a dose of 4000 IU vitamin D_3 per day is required to raise serum 25(OH)D levels to 100 nmol/L.[24]

3.2.4 Vitamin D and New Zealand

Vitamin D levels in the New Zealand general population are, on average, lower than at similar latitudes elsewhere in the world. For example, mean serum 25(OH)D levels for participants aged ≥65 years in the New Zealand Adult National Nutrition Survey (women 43 nmol/L, men 55 nmol/L) were about 20 nmol/L lower than similarly aged participants the Third US National Health and Nutrition Examination Survey (NHANES III).[25 26] Moreover, 50% of women aged 25-64 years had serum 25(OH)D levels of ≤49 nmol/L and 50% of men had levels of ≤53 nmol/L.[26] The mean 25(OH)D levels were lower in Māori (42 nmol/L) and Pacific People (37 nmol/L) than in other ethnic groups.[26] A recent Christchurch study demonstrated that most, if not all, of 201 apparently healthy adults randomly selected from the electoral roll did not have adequate circulating 25(OH)D at some time in the year.[27] Even in February, only 12% had optimal (>80 nmol/L) plasma levels. The most likely explanation for the lower than expected vitamin D status in New Zealand is the Cancer Society's longstanding and widely-accepted advice to avoid sun exposure to prevent skin cancer. With one of the highest malignant melanoma rates in the world,[28] New Zealanders are very aware of sunlight exposure. The food supply in New Zealand is also not fortified with vitamin D, as in other countries such as the USA.[29] Vitamin D supplements in New Zealand are mainly available in small doses (mostly 100 IU multivitamin tablets which only increase serum 25(OH)D levels by 1-2 nmol/L[30]).Thus, current over-the-counter oral sources of vitamin D available to the general population in New Zealand are only able to increase serum 25(OH)D by very small amounts.

3.3 Vitamin D and Cardiovascular Disease

3.3.1 Historical Review

Within a couple of decades of the discovery of vitamin D in the 1920s by Mellanby in England and McCollum's group in Baltimore, [31] hypercalcaemia from high oral doses of were being reported. Cases of infantile hypercalcaemia and congenital supravalvular aortic stenosis in the 1950s and 1960s were attributed to a susceptibility to excess maternal vitamin D intake during pregnancy;[32 33] although a 1967 report by the American Academy of Paediatrics concluded that the hypothesis that vitamin D caused infantile hypercalcaemia was unproven.[34] The development of animal models of arteriosclerosis caused by hypervitaminosis D, studies in which mega-doses of 5000-10,000 IU/kg/day were given[35 36] – equivalent to daily doses of 350,000 to 700,000 IU for a 70kg adult human – supported medical opinion in the 1960s and 1970s that high intake of vitamin D might be a risk factor for vascular damage and CVD.[37-39] The prevailing opinion in the early 1970s, that vitamin D might be a cause of CVD, influenced early epidemiological studies. An ecological study of regions within England and Wales showed positive correlations between vitamin D intake measured in national surveys and standardized mortality ratios for ischemic heart disease (r=0.58) and cerebrovascular disease (r=0.49).[40] A case-control study of heart disease in Tromso (Norway) reported a significantly higher mean intake of vitamin D by cases compared to age- and sex-matched controls.[41]

However, the development of competitive protein-binding assays for 25(OH)D in the 1970s[42] revealed that diet contributed only a small proportion of vitamin D, with more than 80% synthesized from sun exposure.[43 44] This new method of measuring vitamin D status demonstrated in studies

from Germany and Denmark that cases of coronary heart disease actually did not have elevated, levels of serum 25(OH)D compared with controls.[45 46]

Importantly, the Tromso Heart Study which had previously reported higher dietary intake of vitamin D in cases,[41] found in a nested case-control comparison that myocardial infarction cases had a slightly lower serum 25(OH)D levels compared with controls matched for age and time of year, after correcting for vitamin D binding protein (p=0.02).[47]

3.3.2 Hypothesis that Sunlight and Vitamin D Protect against CVD

The hypothesis that sunlight and vitamin D may protect against CVD was published in 1981 by Dr Robert Scragg,[48] who is a Co-Investigator on the proposed RCT. Dr Scragg's paper, which was subsequently expanded,[49] drew together epidemiological data to propose that several CVD associations (increased rates in winter, at higher latitudes, at lower altitudes, among older people, among individuals with increased skin pigmentation such as African-Americans) were consistent with an inverse association between vitamin D status and CVD.

3.3.3 Epidemiological Studies of Vitamin D and Cardiovascular Disease

Since the publication of the vitamin D-CVD hypothesis,[48] a range of epidemiological study designs have been used to determine if vitamin D is associated with reduced risk of CVD. A population-based case-control study from NZ, restricted to incident cases providing blood samples within 12 hours of symptom onset, observed an inverse association between plasma 25(OH)D and risk of myocardial infarction, with the odds ratio for those in the highest 25(OH)D quartile being 0.30 (95%CI: 0.15, 0.61) compared with the lowest quartile.[50] In contrast, a hospital-based case-control study from India, which recruited prevalent cases of coronary artery disease whose vitamin D status may not have reflected that at the time of disease onset, reported a significantly (p<0.001) higher proportion of cases (59%) than controls (22%) had serum 25(OH)D levels above 222.5 nmol/L.[51] A case-control study from Cambridge (UK) found that mean Z score of 25(OH)D for incident stroke cases measured within 30 days of disease onset was significantly below that expected for a sample a healthy controls (-1.4, 95%CI: -1.7, -1.1; p<0.0001).[52] Cross-sectional analyses of the 1988-94 and 2001-04 NHANES surveys have shown that vitamin D deficiency is very common in people with prevalent CVD.[53 54]

Over the past 2 years, several cohort studies have reported inverse associations between vitamin D and risk of CVD. Participants in the Framingham Study Offspring cohort with baseline serum 25(OH)D levels <10 ng/mL (25 nmol/L) had an adjusted hazard ratio of 1.80 (95% CI: 1.05, 3.08) for CVD during the 5-year follow-up period, compared to those >15 ng/mL (37.5 nmol/L).[55] The Health Professionals Follow-up Study found that men with baseline plasma 25(OH)D levels ≤15 ng/mL (37.4 nmol/L) had an adjusted RR of 2.09 (95%CI: 1.24, 3.54) for myocardial infarction (fatal plus non-fatal) over 10 years follow-up compared to those with 25(OH)D < 30 ng/mL (74.9 nmol/L).[56] Participants in the lowest quartile of baseline serum 25(OH)D (<17.8 ng/mL [<44.4 nmol/L]) of the NHANES III Follow- up cohort had a 26% (95%CI: 8, 46) increased risk of all-cause mortality during a median 8.7 years follow-up, compared with those in the highest 25(OH)D quartile.[57] In further analyses of participants aged ≥65 years, we found that the increased all-cause

mortality from low vitamin D is mainly due to increased CVD mortality.[58] The Mini-Finland Health Survey found that participants in the highest vitamin D quintile at baseline (serum 25(OH)D >62 nmol/L for men and >56 nmol/L for women) had a 24% reduction in CVD mortality over the nearly 30 year follow-up period compared to those in the lowest quintile (<28 nmol/L for men and <23 nmol/L for women), adjusting for covariates.[59] The Hoorn study in the Netherlands found that participants in the lowest vitamin D quartile at baseline had an adjusted 5-fold increase in CVD mortality, and nearly 2-fold increase in all-cause mortality, during the follow-up period when compared to the other three vitamin D quartiles.[60]

3.3.4 Trials of Vitamin D Supplementation and Cardiovascular Disease

To date, the only clinical trial of vitamin D in the general population, the Women's Health Initiative (WHI), failed to detect any effect of vitamin D and calcium supplementation on CVD mortality and morbidity.[61] However, this study is not considered a proper test of the hypothesis that vitamin D protects against CVD, for the following reasons. Contamination was high as all participants including controls were allowed to take up to 1000 mg of calcium/day, and 600 IU of vitamin D/day (later raised to 1000 IU/day in 1999).[62] The daily intervention dose was 1000 mg of calcium and 400 IU of vitamin D. After a mean follow-up of 7 years, only 76% of women were still taking the study medication, and only 59% were taking 80% or more.[62] This poor compliance means that the 400 IU dose of vitamin D was effectively much less than the original study target – estimated to have been as low as 35% of the study target dose, or about 140 IU of vitamin D/day.[63] Although serum 25(OH)D levels increased by 28% in a small sample of intervention women (n=227) compared with control women (n=221),[64] no actual data have ever been reported on the change in 25(OH)D produced by the 400 IU daily dose. Controlled studies have shown that 25(OH)D increases by about 2.5 nmol/L for each 100 IU per day,[65] which suggests that mean serum 25(OH)D may have been up to 10 nmol/L higher in the vitamin D than control arm, although probably lower than this because of poor compliance. Thus, because of contamination and poor compliance, the difference in vitamin D status between intervention and control arms is likely to have been small, minimizing the possibility of seeing any effect from vitamin D.

Recent research has shown that much higher doses of vitamin D, than used in the WHI RCT, are required to achieve optimum vitamin D levels. For example, to raise serum 25(OH)D from 50 to 80 nmol/L requires an additional 1700 IU of vitamin D per day,[66] while a dose of 4000 IU [67] (100 µg) vitamin D_3 per day is required to raise serum 25(OH)D levels to 100nmol/L.

A recent meta-analysis of RCTs on bone health, which found that vitamin D supplementation reduces total mortality by 7%,[68] provides indirect evidence that vitamin D protects against CVD since this is the main cause of death in developed countries.

3.3.5 Possible Mechanisms by which Vitamin D may protect against Cardiovascular Disease

Low vitamin D status has been linked with a wide range of CVD including congestive heart failure,[69] peripheral vascular disease[70] and incident hypertension,[71] suggesting that its effects may occur amongst small blood vessels. Research on the possible mechanisms through which vitamin D may protect against CVD has recently been reviewed.[3 4 72] These include beneficial

effects of vitamin D on inflammatory processes and cytokines to prevent plaque and thrombosis formation; tissue remodelling involving reduction in matrix metalloproteases to prevent both cardiac hypertrophy and also proliferation of smooth muscle tissue to decrease arterial stiffness; down-regulation of the renin-angiotensin system to prevent hypertension; and decreased insulin resistance. More research is required to get a clearer picture of vitamin D's CV effects since the above mechanisms are overlapping.

3.4 Vitamin D and Respiratory Infection

3.4.1 Vitamin D Activates Cathelicidin Production to Protect Against Infection

Recently, vitamin D has been shown to have an important role in the innate immune system, which prevents infection without the need for immunological memory from previous exposure to the pathogen.[73] Innate immunity includes the production of antimicrobial peptides that are capable of killing viruses, bacteria and other organisms.[74 75] These peptides are produced on epithelial surfaces and within circulating white blood cells. Examples include human β- defensins 2 and 3 and cathelicidin (also known as hCAP-18 and LL-37).[75] The peptides are produced through the effect of Toll-like receptors, on the surface of macrophages and monocytes, causing cell activation when they recognize molecules derived from pathogens.[75 76] This activation results in expression of the genes that code for the vitamin D receptor, and for the 1α-hydroxylase enzyme that converts the pre-hormone, 25(OH)D, to the biologically active 1,25 dihydroxyvitamin D; which in turn activates the gene that produces cathelicidin.[76]

An increase in the concentration of cathelicidin in phagocytic vacuoles enhances the cells ability to kill microorganisms.[75] LL-37, the only human cathelicidin, has been identified in the following tissues: white cells, breast milk, skin, lung, saliva, and colon; and is active against a wide range of microbes, including bacteria (both gram positive and gram negative), fungi and viruses.[77]

3.4.2 Vitamin D and Tuberculosis

This new evidence linking vitamin D and cathelicidin[76] provides a possible explanation for the link between sun exposure, vitamin D and tuberculosis (TB).[78] There is increasing evidence that low body vitamin D levels may increase the risk of developing TB. For example, a hospital-based case-control study in London found that vitamin D deficiency was associated with an odds ratio of 2.9 (95%CI 1.3-6.5) for having active TB.[79] A case series of London TB patients showed that 56% had undetectable plasma 25(OH)D levels (<7 nmol/L).[80] A case-control study from West Africa observed lower mean serum 25(OH)D levels in cases (78 nmol/L) compared with controls (85 nmol/L; p<0.001).[81] Susceptibility to TB has been linked to vitamin D-receptor polymorphisms, with the presence of the *Fok*I F allele protecting against TB infection, and the *Taq*I t allele protecting against active disease but not infection.[82]

3.4.3 Epidemiological Studies of Rickets, Vitamin D and Respiratory Infection

The association between rickets and infection has been known since the 1960s.[83] Since then, numerous studies have reported that children with rickets commonly present to hospital with respiratory infections.[84-92] One of these studies observed lower serum 25(OH)D levels in cases of

acute severe lower respiratory infection requiring admission to hospital compared with controls: 23 *vs.* 38 nmol/L (p<0.0001).[92]

Exposure to sunshine and ultraviolet radiation (UV) B, which is the primary source of vitamin D in humans,[93] is also associated with respiratory infection markers. Sub-erythemal courses of UV radiation, administered twice a year for three years to Russian teenage athletes, resulted in fewer respiratory viral infections, fewer days of absences and shorter duration of illness, compared with non-irradiated athletes.[94] The irradiated subjects also had significant increases in salivary IgA, IgG and IgM compared with controls. A Dutch study found that children with low sun exposure were more likely to have a cough and a runny nose, compared to children with most sun-exposure.[95] The temporal association between seasonal changes in vitamin D levels and winter respiratory virus activity has led to the proposal that low vitamin D levels may have a causal role in the onset of influenza epidemics.[96 97]

Several observational studies have reported on vitamin D status and respiratory infection. A Turkish case-control study found that serum 25(OH)D levels were lower in neonatal cases of acute lower respiratory infection (23 nmol/L) than age-matched controls (41 nmol/L).[98] A Finnish cohort study found that young male soldiers with serum 25(OH)D levels <40 nmol/L at baseline had a 63% increased risk of absence from duty over the following 6 months due to respiratory infection compared with soldiers with levels >40 nmol/L (p=0.004).[99] A Canadian case-control study of children aged 1-25 months found no difference in mean serum 25(OH)D levels between cases of acute lower respiratory tract infection (77 nmol/L) and hospital controls (77 nmol/L).[100] However this finding is probably due to virtually all of the infants having high vitamin D diets through fortified formula or supplementation. A secondary analysis of the US NHANES III survey showed that, after adjusting for demographic and clinical characteristics, lower 25(OH)D levels were independently associated with self-reported URI in the past few days (compared to ≥75 nmol/L group: OR 1.36 [95%CI, 1.01-1.84] for <25 nmol/L and OR 1.24 [95%CI, 1.07-1.43] for 25-74 nmol/L groups).[101]

Vitamin D status is inversely associated with lung function,[102] and risk of respiratory infection is greatly increased in people with obstructive airway disease with low vitamin D. For example, we have found in NHANES that the association between serum 25(OH)D levels <25 nmol/L and upper respiratory infection was stronger among individuals with asthma (OR, 5.67) compared with those without asthma (OR, 1.24; P for interaction = 0.007).[6] Since most acute exacerbations of asthma/COPD are caused by respiratory infections,[103] asthma/COPD hospitalisations provide a useful outcome for testing the effect of vitamin D on respiratory infections.

3.4.4 Trials of Vitamin D Supplementation and Respiratory Infection

Observational studies of vitamin D and respiratory infection are likely to be affected by biases in measurement of both the relevant vitamin D level and the outcome, as well as potential confounding by exposure to sunlight (e.g. 'sickly' children might be less likely to spend time uncovered outside.) Good evidence of whether there is an effect of vitamin D on the risk of infection can be generated only by randomised controlled trials.

Two randomised controlled trials (RCTs) of vitamin D supplementation have indirectly examined its impact on respiratory infection. A study to prevent bone-loss in post-menopausal African-American women found that 8% of women on 800-2000 IU per day reported having cold or influenza symptoms over the 3 years follow-up compared with 25% of women on placebo ($p<0.002$).[104] The prevalence of URI symptoms in this study is undoubtedly underestimated due to the insensitive and imprecise manner in which these data were collected, although the double-blinded, randomised study design should have minimised reporting bias. In a sub-study of an RCT to prevent fractures with vitamin D supplementation, 3444 participants (mean age 77 years) were asked in winter if they had suffered an infection or received antibiotics during the previous week.[105] For intention-to-treat comparisons, there was a non-significant 10% reduction in the odds of reporting infection ($p=0.23$) and 16% reduction in the odds of reporting antibiotic use ($p=0.18$). Slightly stronger effects were observed for on-treatment per-protocol comparisons: 20% reduction in reporting infection ($p=0.06$) and 26% reduction in reporting antibiotics ($p=0.10$). Limitations of this study include the short outcome period of only one week, which reduced power, and its low dose of vitamin D (800 IU/day) which increased 25(OH)D levels from 38 nmol/L to only 62 nmol/L, well below the value of 80-100 nmol/L now considered to be associated with optimum health outcomes.[10 11 17 18] Another more recent RCT showed no benefit of vitamin D supplementation in decreasing the incidence or severity of URIs during winter.[106] However, this study was underpowered and used a relatively low dose of vitamin D without a loading dose.

3.5 Vitamin D and Fractures

There is currently uncertainty about whether vitamin D supplementation reduces the risk of non-vertebral fractures. An early meta-analysis of clinical trials concluded that a vitamin D dose >700 IU per day reduced fracture risk.[107] However, subsequent meta-analyses have come to a different conclusion – that it is calcium in combination that prevents fractures, not vitamin D by itself.[108 109] The whole area of this research is bedevilled by the use of combined calcium and vitamin D interventions, plus use of vitamin D doses (mostly ≤ 800 IU/day) which are now considered too low. Recently, a further meta-analysis, which evaluated the effective vitamin D dose taken by participants (after allowing for non-compliance) concluded that vitamin D reduces risk of non-vertebral fracture by 20%.[110] There is clearly a need for RCTs of large doses of vitamin D to determine with certainty whether it protects against fractures. Research is also needed to determine if any reduction in fractures is due to the effect of vitamin D on increasing bone density or on muscle function (e.g. strength, balance) by preventing falls.[111]

4 RESEARCH DESIGN AND METHODS

4.1 Hypothesis

The null hypotheses of this study are that vitamin D supplementation has no effect on the incidence of: CVD (primary outcome); respiratory infectious disease (secondary outcome); and non-vertebral fractures (secondary outcome).

4.2 Study Overview

The study is a double-blind, placebo-controlled RCT to evaluate the efficacy of vitamin D supplementation in reducing CVD morbidity and mortality (primary outcome), and incidence of

respiratory infections and non-vertebral fractures in 5,100 adults, age 50-84 years, followed for 4 years.

4.3 Participants

Participants will be recruited in Auckland from patient lists of family physicians. Participants will come from general practices belonging to the main PHOs in Auckland (East Tāmaki Health Care, Te Hononga, ProCare, and Harbour Health).

Eligible participants will be identified electronically from the registers at the above PHOs. Identification of names and addresses of eligible people from both sources will start 3 months before the budgeted start of the study to ensure that 5,100 people are recruited within the initial 18 month period. A personalized letter inviting people to participate, co-signed by their family doctor and Dr Scragg, along with the Ethics Committee approved participant information sheet, will be mailed to their homes, along with a 1-page sheet to enter their phone contact details and post back to the study team in a reply-paid envelope indicating their interest in participating. Participants will be phoned at home to check their eligibility and to make a time for their baseline interview at the School of Population Health, Tāmaki Innovation Campus. The recruitment strategy is based on previous experience in NZ for the BRIGHT trial by Prof N Kerse (46% response rate) and in Cambridge, UK (39% response rate).[112] We have budgeted for a 30% response rate – i.e., ≈ 14,700 people will be invited.

In addition, people from the community who contact the ViDA clinic expressing interest in partipating and who meet the age criteria, are invited to complete a ViDA registration form either electronically or by mail. These potential participants will be contacted by study personnel and their eligibily reviewed.

Inclusion criteria are men and women aged 50-84 years and resident in Auckland at recruitment. Exclusion criteria include: diagnosis of a terminal illness and/or in hospice care; intending to leave NZ during the follow-up period; taking vitamin D supplements (including cod liver oil) of >600 IU per day; history of renal stones, hypercalcaemia, or medical conditions that can cause hypercalcaemia; baseline serum calcium >2.50 mmol/L; or medications that affect vitamin D metabolism (e.g. anti-epileptics, tuberculosis medication).

Inclusion criteria:

1. Age 50-84 years;
2. Ability to give informed consent;
3. Resident in Auckland at recruitment;
4. Anticipated residence in New Zealand for the 4-year study period.

Exclusion criteria:

1. Current use of vitamin D supplements (>600 IU per day if aged 50-70 years; >800 IU per day if aged 71-84 years);
2. Diagnosis of psychiatric disorders that would limit ability to comply with study protocol – i.e., history of regular exacerbation of major psychosis (schizophrenia, bipolar disorder) in last 2 years;

3. History of hypercalcaemia, nephrolithiasis, sarcoidosis, parathyroid disease or gastric bypass surgery;
4. Enrolled in another study which could affect participation in the vitamin D study;
5. Serum calcium from baseline blood sample >2.50 mmol/L.

4.4 Baseline Interview

Eligible patients will be interviewed at the School of Population Health, Tāmaki Innovation Campus, within one month of each person being recruited. Baseline interviews (sufficient to recruit a sample of 5000) will be carried out over a 10-month period during Year 1 (months 3-12.) They will last about 60-90 minutes and are an important opportunity to establish rapport with participants to help ensure that a high proportion of participants remain in the study during the follow-up period. The following will be collected from each person:

1. **Obtain written informed consent:** the purpose of the study, what it involves, as well as its risks and benefits, will be re-iterated to participants prior to gaining written consent.
2. **Confirm eligibility:** the inclusion and exclusion criteria will be checked.
3. **Contact details collected:** full contact details, including name, address, date of birth, and of near family and neighbours to assist with follow-up.
4. **Current medication**: participants will be asked to bring all current medications to the and as a measure of CVD risk at baseline.
5. **Past medical history:** information will be collected for the presence of the following medical conditions (including age at diagnosis) – coronary heart disease, cardiac failure, cardiac arrhythmia, hypertension, hyperlipidemia, stroke, diabetes, chronic respiratory disease (asthma, chronic bronchitis, emphysema), cancer, renal disease (stones, microalbuminuria), sarcoidosis, and fracture (e.g. vertebra, forearm, hip).
6. **Lifestyle:** information will be collected on the following variables which affect the risk of the study outcomes – tobacco smoking, usual leisure-time physical activity (frequency non-drinkers from ex-drinkers),[114] and mood using Geriatric Depression Score.
7. **Blood pressure:** will be measured after 15 minutes rest with an Omron T9P oscillometric device on 3 occasions with approximately 30 seconds between readings, while the participant is seated;[115] followed by measurement of arterial waveform using the BP + device.
8. **Lung function:** (FEV1, FVC) will be measured (up to three times if necessary) with a spirometer.
9. **Anthropometry:** the following measurements will be made without shoes and in light clothing – height to the nearest 0.1cm; and weight to the nearest 0.1 kg.
10. **Muscle function:** walking speed and dynamic balance will be measured using a plate; muscle volume of the calf will be measured using ultrasound on a subsample.
11. **Blood sample:** 25 ml blood sample will be collected to measure serum calcium to screen for hypercalcaemia; remaining serum will be aliquoted and stored at -80^{0}C.

4.5 Endpoints and Endpoint Assessment

Information on study endpoints will be collected by the following strategies:

1. **Deaths:** each participant's NHI number will be used to search the Mortality data collected by the Ministry of Health since the beginning of the study to detect ALL deaths, including those

from the CV and respiratory events (listed below).

2. **Hospital admissions (discharges):** each participant's NHI number will be used to search annually the Hospital Discharge data collected by the NZ Ministry of Health since the beginning of the study to detect any discharges for the CV and respiratory events (listed below) that are primary and secondary outcomes, respectively, for this trial.
3. Non-hospital medical events: **a short questionnaire (1-page double-sided) will be mailed** every month during the follow-up period to each participant's home address – along with the monthly dose of vitamin D (or placebo) and a reply-paid envelope. Participants will indicate in the questionnaire whether they have been treated by a physician during the previous four months for any of the following medical events not resulting in admission to hospital or death: heart disease (including angina and arrhythmias), hypertension, hyperlipidemia, stroke, diabetes, acute respiratory infection, chronic respiratory disease, cancer, or renal stones. Participants who do not return a four monthly questionnaire will be contacted by phone to collect the questionnaire information. Family physicians may be contacted to verify any events not resulting in hospitalization or death that are related to the study outcomes (primary and secondary).

   The NHI number will be used to search Pharmac data for CV and antibiotic medications related to study outcomes; and to search Cancer Registry data for new cancer cases, so that the current study can be combined with other proposed international RCTs to determine if vitamin D protects against common cancers (colon, breast, prostate).

   Events with the following ICD-10 codes for CV disease will be collected: hypertensive diseases (I10-I15), heart diseases (I20-I25), pulmonary heart disease (I26-I28), other forms of heart disease (I30-I52), cerebrovascular diseases (I60-I69), diseases of arteries, arterioles and capillaries (I70-I79), and other venous embolism and thrombosis (I82). Relevant infectious respiratory disease ICD-10 codes are: acute upper respiratory infections (J00-J06), influenza and pneumonia (J10-J18), other acute lower respiratory infections (J20-J22), other diseases of upper respiratory tract (J30-J39), chronic lower respiratory diseases (J40-J47), and suppurative and necrotic conditions of lower respiratory tract (J85-J86). Codes for non-vertebral fractures are: S22, S32, S42, S52, S62, S72, S82, S92, and T12. Relevant disease events reported by participants in four monthly questionnaires, not resulting in hospitalization, will be validated against electronic family physician records for practices where this information can be extracted.

   All processing of data for endpoints – mailing of four monthly questionnaires, crossing NHI numbers with Ministry of Health mortality and hospital morbidity files – will be carried out by staff at the School of Population Health. Identified endpoints will be checked by a blinded Endpoint Committee (RS, CC, CL) using specified endpoint criteria.

4.6 Intervention

Following the baseline assessment, all participants will receive a blind placebo capsule in the mail with a 2-page questionnaire during a brief "run-in" period. Those who return the questionnaire will be randomly allocated to receive either vitamin D_3 (100,000 IU) or placebo. Participants will be given TWO capsules (of vitamin D or placebo) in the first mail-out after randomization (within 4

weeks of baseline assessment) to boost vitamin D levels in the treated group at the start of the intervention. Thereafter, monthly oral 2.5 mg (100,000 IU) doses of vitamin D_3 (cholecalciferol), or placebo, will be taken by participants throughout the 4-year follow-up period. Intakes equivalent to 3000 IU per day, or above, are required to raise serum 25-hydroxyvitamin D levels to 80-100 nmol/L, which are considered optimal for health. [10 102 116 117] Vitamin D and placebo capsules will be manufactured by Tishcon Corporation (Westbury, New York), which has supplied this medication in previous US research.[119]

From July 2013, study capsules have been mailed to participants four monthly in batches of four capsules. (Previously study capsules were mailed each month along with a monthly questionnaire.)

- From March 2014, one questionnaire will be mailed four monthly along with a batch of four capsules. This four monthly questionnaire will replace the one monthly questionnaire which will cease in November 2013. (Content of questionnaire remains the same – minor formatting changes only.)

4.6.1 Pilot Study of Monthly Vitamin D Dose

We have completed a pilot study to test the effectiveness of monthly 100,000 IU doses of vitamin D_3 in maintaining serum 25(OH)D levels at 80-100 nmol/L in all seasons of the year, and to monitor the safety of this dose to ensure it does not result in hypercalcaemia. Fifty-one people in the age range of 65-84 years (25 men, 26 women, mean age 73.2 years) were recruited from a retirement village in West Auckland in late January 2008. Starting then, they received twelve monthly doses of 100,000 IU of vitamin D3 (cholecalciferol) up to January 2009. Blood samples were collected every two months (including a baseline sample in January 2008), and results are shown in Table 1 below.

Table 1. Serum 25(OH)D and levels during monthly 100,000 IU vitamin D3 dosing

Time period	Dates	25-Hydroxyvitamin D3 (nmol/L)			Adjusted Calcium (mmol/L)		
(months)		N	Mean (SD)	Range	N	Mean (SD)	Range
0 (baseline)	28 Jan 08 – 29 Jan 08	51	85.5 (17.7)	51 – 124	51	2.25 (0.08)	2.12 – 2.48
2	27 Mar – 04 Apr	48	85.3 (13.4)	51 – 108	48	2.23 (0.06)	2.11 – 2.40
4	16 May – 06 Jun	49	80.8 (16.9)	44 – 120	49	2.22 (0.06)	2.13 – 2.42
6	24 Jul – 06 Aug	48	82.1 (14.2)	57 – 120	50	2.29 (0.08)	2.15 – 2.51
8	25 Sep – 30 Sep	48	84.0 (13.1)	60 – 114	46	2.19 (0.08)	2.04 – 2.42
10	27 Nov (only)	48	80.9 (12.8)	60 – 133	48	2.22 (0.08)	2.01 – 2.40
12	29 Jan 09 – 04 Feb 09	48	88.7 (16.9)	60 – 134	45	2.35 (0.08)	2.10 – 2.40

Mean serum 25-hydroxyvitamin D_3 levels stayed constant between 80-89 nmol/L. Unpublished data from a Christchurch laboratory which measures both vitamin D_3 and vitamin D_2 using liquid chromatography-tandem mass spectrometry indicates that 25-hydroxyvitamin D_2 levels in New

Zealand are low, being in the range of 3-5 nmol/L. Thus, the mean for total 25-hydroxyvitamin D (D_3 plus D_2) at the time of blood collection in our participants is probably in the range of 85-95 nmol/L. These values are likely to have been lower than the mean throughout the previous month because, firstly, blood samples were taken a month after ingestion of the most recent vitamin D dose and, secondly, serum 25(OH)D levels peak 7 days after a single 100,000 IU dose of vitamin D_3 and fall slowly thereafter. Importantly, there were no occurrences of elevated serum calcium levels (>2.60 nmol/L), with the highest calcium level being 2.51 nmol/L. Thus, the current dose was effective in avoiding the winter fall in serum 25(OH)D, and safe by not causing any hypercalcaemia.

In conclusion, monthly vitamin D doses of 100,000 IU are safe and effective in maintaining serum 25-hydroxyvitamin D levels above 80 nmol/L throughout the whole year.

4.6.2 Compliance

Compliance will be monitored by the four monthly questionnaire which will have a tick-box for each participant to indicate they have ingested the monthly capsule, and if they did not, a space to write down the reason for not ingesting the capsule. In addition, a 10% random sample (255 vitamin D_3, 255 placebo) will be selected to attend an annual interview where a blood sample will be collected to measure serum 25(OH)D (to monitor compliance) and serum calcium (to monitor safety). Their serum calcium results will only be seen by Mr Stewart and the Data Monitoring Committee to ensure that research staff remain blind to treatment status.

4.6.3 Discontinuation/ Withdrawal of Participants from Study

Each participant has the right to withdraw consent from participating from the study at any time. The reason for the withdrawal of consent will be recorded. In addition, the investigator may discontinue a participant from the study treatment at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study treatment or results in inability to continue to comply with study procedures
- Corrected plasma calcium levels <2.1 or >2.6 mmol/L (see safety below)
- Disease progression which requires discontinuation of the study treatment or results in inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up

Table 2. Study timeline

Activity	Year 1				Year 2				Year 3				Year 4				Year 5			
	March 2011 – Dec 2011				Jan 2012 – Dec 2012				Jan 2013 – Dec 2013				Jan 2014 – Dec 2014				Jan 2015 – Dec 2015			
Recruitment																				
Baseline assessment																				
Allocation to vitamin D/placebo																				
Intervention																				
Collect outcome data																				
Data management, quality control																				
Analyses: Data Safety and Monitoring																				
Analysis of outcome and dissemination																				

4.7 Study Period

Recruitment will occur during March 2011-December 2012 (see Table 2). Four monthly mail outs of the questionnaire and vitamin D/placebo capsules will continue until July 2015 plus an additional final mailout of the questionnaire in October 2015.
Outcome data from the Ministry of Health will be collected for the period ending September 2015, and processed over the next 3 months; with analyses carried out in December 2015, extending in to early 2016 because of extra time required to confirm causes of death.

5 SAFETY

Vitamin D intoxication is very rare, but can be caused by ingestion of very high doses. Although current data support the viewpoint that serum 25(OH)D levels must rise above 750 nmol/L to produce vitamin D toxicity, an upper limit of 250 nmol/L is used to ensure a wide safety margin.[121] Continuing oral intake in excess of 10,000 IU per day is required to produce a serum 25(OH)D of ≥250 nmol/L,[122] an intake much greater than in the proposed dosing regimen for this study. The safety of the chosen vitamin D dosing regimen is supported by the absence of toxicity in a variety of trials. These include trials that used vitamin D in doses ≥4,000 IU per day (often for >12 months),[118] in 100,000 IU monthly doses (see Auckland pilot data above), and following a 500,000 IU loading dose.[123]

Potential side effects from vitamin D will be monitored by the:

- inclusion of an open-ended question in our four monthly questionnaire which asks specifically about any side effects from vitamin D – any mention of symptoms known to be caused by vitamin D toxicity (such as anorexia, fever, thirst, vomiting, and weight loss) will be coded into the data set for that person;
- collection of blood samples 6-monthly in the first 12 months follow-up, then annually, from a 10% random sample of participants (255 vitamin D3, 255 placebo) to measure serum calcium

levels. This sub study is referred to as the Annual Follow Up Group. These data will be forwarded to the DMC to monitor for hypercalcaemia, along with any other potential adverse outcomes. Participants with corrected plasma calcium levels >2.6 mmol/L will be contacted by research staff and requested to provide a further blood sample for a second plasma calcium test. The results of both tests will then be forwarded to the Auckland Hospital Endocrinology Department for independent medical assessment by a designated specialist endocrinologist who will decide on a case by case basis the appropriate course of action, which may include the withdrawal of the participant from the study treatment and/or any ongoing medical assessment and treatment of the participant.

5.1 Adverse Event Reporting

Safety of exposure to vitamin D will be evaluated by the incidence of adverse events (AEs) and serious adverse events, which will be reported in tabular form giving absolute numbers and percentages. All observed or volunteered adverse events regardless of treatment group or suspected causal relationship to the investigational product will be reported as described in the following sections. Both open and closed reports will be sent every 6 months to the HRC Data Monitoring Committee.

5.2 Definition of Adverse Events

Adverse event data will be collated from the four monthly questionnaires and the blood sample from 10% of participants interviewed annually.

Four monthly questionnaire information (reported by participants):

- **Side-effects:** participants are able to record any symptoms they attribute to the vitamin D/placebo capsule they are taking.
- **CVD events:** heart attack, stroke, transient ischemic attack, irregular heartbeat, other.
- **Infections:** upper respiratory tract infection, chest infection, renal tract infection, skin infection, other.
- **Kidney stones**
- **Falls**
- **Fractures**

Annual blood sample (collected from 10%): will be measured for serum calcium and the proportion with calcium >2.6 mmol/L will be reported to the DMC.

5.3 Definition of a Serious Adverse Event

A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- **Results in death**
- **Is life-threatening (immediate risk of death)**
- **Requires inpatient hospitalisation**
- **Results in persistent or significant disability/incapacity**

Information on deaths and hospitalisations will be collected annually from mortality and hospital discharge files provided by the Ministry of Health, with linkage using participant NHI numbers. This information will not be available until at least 18 months into the study because of the timing

of the National databases in processing information.

In addition, unverified reports on fatal events and hospitalisations will be provided to the DMC 6 monthly to enable the Committee to review these data without having to wait for definitive reports from Ministry of Health linked data. As soon as it is available from the Ministry, this information will be collated and provided to the DMC in both open and closed reports.

This information will allow the DMC to monitor the safety of the study medication (and also its effectiveness) by comparing incidence rates of these between the intervention and placebo groups.

Because of the large sample size, with many deaths and hospitalisations expected among study participants, it is not feasible for the study investigators to review each adverse event and determine the relationship of the adverse event to the study medication (i.e., according to the standard classification as: not related, unlikely, possibly, probably or definitely related.)

6 STATISTICS

6.1 Sample Size

Sample size calculations are derived from the baseline age-distribution of a cohort aged 50-84 years at recruitment using data from the 2006 NZ Census (www.stats.govt.nz), and 2002 and 2003 mortality and hospital discharge data from the NZ Health Information Service of the Ministry of Health (www.moh.govt.nz); supplemented by non-hospital physician consultation rates for respiratory infections.[124 125] Our sample size calculations are based on a sample with 20% Maori, 20% Pacific and 60% European, which will have an annual CV disease event rate (for hospital discharges and mortality) of 6% for ages 50-84 years (similar proportion of sexes). Assuming that the event rate in healthy volunteers is 80% of this, a sample of 5000 people followed for 4 years has 80% power to detect a 20% reduction in CVD events, and 10% reduction in infectious respiratory disease events. This does not account for repeat disease events or GP consultations for CV and respiratory disease events (which will increase power). The study has an 80% chance of detecting a hazard ratio of 0.76 for non-vertebral fractures. Recruiting an extra 10% at baseline will allow for withdrawals which are expected to be low (1.2% per year) based on the study lead by Prof Khaw.[112]

Additional funding from the ACC has allowed us to recruit an extra 700 participants, increasing the baseline sample size to 5,100. Allowing for possible withdrawals this allows a reduction of 22% in non-vertebral fractures to be detected with 80% at the 5% level of significance.

6.2 Statistical Analysis

Analyses of the primary outcome (CVD) and two secondary outcomes (respiratory infection, non-vertebral fractures) will be conducted on an intention-to-treat basis. The key output from the modelling, both for the primary and secondary hypotheses, is the RR associated with the treatment relative to the placebo and this will be reported with its 95%CI.

Primary Hypothesis: CVD events: Assuming all CVD events – hospital discharge and death - have equal value, analysis will be by a repeated events survival analysis. The analysis will be a conditional

analysis – subsequent events being conditional on previous events – and the risk set being the individuals having had the same number of events at the time of exposure. This model, sometimes called the PWP model, is suitable for modelling the full time course of the recurrent event process.[126] The Cox regression proportional hazards model, using robust sandwich variance estimates and separate stratum for each subsequent event, to assess the difference in survival times in the two treatment groups. We will treat those who have died from a non-CVD cause as censored observations.

Secondary Hypotheses: Respiratory infections and non-vertebral fractures: the same methods will be used for respiratory infections and non-vertebral fractures as for CVD above. The analysis will be by a repeated events survival analysis, with subsequent events being conditional on previous events – and the risk set being the individuals having had the same number of events at the time of exposure.

Compliance will be assessed by testing the difference between the treated group and the placebo. An unpaired t-test will be used for both serum 25(OH)D (measured at the end of the study) and serum calcium. The level of serum 25(OH)D and serum calcium will also be associated with the information of tablet ingestion reported on the four monthly questionnaire.

Participants who stop returning their four monthly questionnaire will continue to be followed through their NHI number for mortality, hospital discharges, and prescriptions, but will be considered to have withdrawn from treatment from the trial. Sensitivity analyses of the effect of the loss of these participants will include; comparison of baseline characteristics with those not lost to follow up and testing the secondary hypothesis (2) on the assumption that these lost participants survive to the study end (likely to be a reasonable assumption except for those that emigrate). Also collection of hospital discharge may be possible for those lost to follow up and we can do a sensitivity analysis for the primary hypothesis using this information. Consent to do this will be collected at the baseline interview.

6.2.1 Censoring for Missing Information

For the CVD analysis, the time of non-cardiac death will be assumed to be a censoring time. In addition some participants will stop returning their four monthly questionnaires and be "lost to follow up" for the purpose of determining hospital and non-hospital CVD events. These participants will have partial data in that we will know whether they have had a fatal CVD event but not whether they have had a non-fatal CVD event. To avoid this problem the GP and hospital records of participants will still be examined after they withdraw from the trial. Consent to do this will be collected at the baseline interview. In the event that there is some selective censoring, an analysis is possible that makes use of all the data. In this analysis we separate fatal and non-fatal events as different event types and then patients without follow up for non-fatal events will not be in the risk set for non-fatal events after withdrawal but will be at risk for hospital events. For the respiratory analysis where there will be a great many more non-hospital respiratory events than hospital events, we will use the analysis where patients are censored at the time when we are unable to obtain information from all of their data sources. For total mortality all patients will have complete follow up using the NZ death registry and there will be complete follow up.

7 DATA MANAGEMENT

Interview data will be collected by trained research staff using computer assisted personal interview (CAPI) software, which immediately enters the data into an electronic database. The four monthly questionnaires will be checked for completeness and then scanned, so that data is extracted from them electronically. Macros and interim tabulations will be used to check data ranges and consistency. The database will be maintained by a dedicated database manager, who will merge the Ministry of Health databases with other interview and questionnaire data using participant NHI numbers.

8 ETHICS

8.1 Ethics Committee Approval

The protocol, informed consent form, participant information sheet and proposed advertising material have been approved by the Multi-region Ethics Committees, Wellington (MEC/09/08/082).

8.2 Participant Consent

Potential participants asked to participate in the study are entitled to choose whether or not to take part. Their decision is voluntary and they should be competent to understand what is involved. Consent forms are designed to assure the protection of participant rights. Written informed consent will be obtained from each participant before enrolment in the study.

Participants will receive adequate verbal and written information, and asked to bring a family member or friend to the interview to translate from English into their own language, if they require it. The verbal explanation will cover all the elements specified in the written information provided for patients. The investigator or co-worker will inform the patient of the aims, methods, anticipated benefits and potential hazards of the study including any discomfort it may entail. Participants will be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. Participants are entitled to withdraw their consent to participate at any time without penalty or loss of benefits to which they are otherwise entitled.

8.3 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified by initials, date of birth, NHI and a participants ID number. All documents will be stored securely and only accessible by trial staff and authorised personnel. Any data or samples that relate to participants and that leave the study site will be anonymised.

9 PUBLICATION POLICY

The principal investigator will co-ordinate dissemination of data from this study. All publications based on this study will be reviewed by each investigator prior to submission.

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11 APPENDIX

11.1 STUDY PROTOCOL SIGNATURE PAGE

I agree to adhere to the Study Protocol and to all the documents referenced.

I agree that the Study Protocol contains the necessary details for conducting the study.

Robert Scragg

_____

Principal Investigator
Professor Robert Scragg

_____

Signature

Date: 4 November 2013

11.2 ADDITIONAL INFORMATION COLLECTED

11.2.1 Annual Follow Up Group (Further details held in a separate document)

The initial annual safety/compliance sub study of a randomly selected group of 10% re tested at 6 months to monitor safety (section 5) is now referred to as the Annual Follow-Up Group.

10% of randomised participants are randomly selected to be invited to attend the study clinic 6 months post randomisation, at one year and then annually over 4 years. The clinic visit involves repeat: answering of the baseline questionnaire about current medications, lifestyle, mood, intake of food (and supplements) with high levels of vitamin D over past 3 months; repeat measurements of height, weight, lung function, muscle strength, walking speed and balance, blood pressure and heart rate variability; repeat collection of blood sample for vitamin D and calcium.

MREC notified: 11 February 2011
MREC approved: 18 April 2011
(Includes approval of a separate Annual Follow- Up group Information sheet V2.0 and Consent Form V4.0)

11.2.2 Psoriasis Sub Study (Further details held in a separate document)

50-100 randomised participants who have been told by a doctor that they have had psoriasis, have been invited to participate in a sub study to determine if vitamin D supplementation reduces the severity of psoriasis.

MREC notified: 11 February 2011
MREC approved: 18 April 2011
Includes approval of a separate Information Sheet V2.0 and Consent Form V1.

11.2.3 Bone Density Sub Study (Further details held in a separate document)

Approximately 400 randomised participants have been invited to participate in a sub study to determine if vitamin D supplementation affects bone loss.

MREC notified: 18 June 2012
MREC approved: 5 July 2013
Includes approval of document describing rationale and procedures; a separate Information Sheet and Consent Form V1.18)

11.2.4 Cholesterol Sub Study

Additional information on the possible effect of serum cholesterol as a risk factor for CHD is being obtained as planned. These data will be linked with BP + (formally Pulsecor) measures to determine if arterial stiffness is associated with major risk factors of CVD, and whether arterial stiffness is associated with the predicted 5-year absolute risk of CVD. Measurement of total cholesterol on all baseline blood samples is being undertaken.

Effect of vitamin D on cardiovascular and respiratory disease event rates (Vitamin D Assessment (ViDA) Study)

Statistical Analysis Plan

Prepared by Alistair Stewart

1. Introduction

The purpose of this Statistical Analysis Plan is to provide a detailed plan of the statistical analyses for the ViDA trial. The analyses here relate to the main analyses of treatment effect for the trial. Other analyses will be documented elsewhere.

2. Study Objectives
    a. Efficacy
        i. Primary

            To assess whether receiving once monthly vitamin D supplementation reduces the number of CVD events in a group of 50 to 84 year old males and females with varying skin colour recruited from General Practices in the Auckland area.
        ii. Secondary

            To assess whether receiving once monthly vitamin D supplementation reduces the number of deaths in a group of 50 to 84 year old males and females with varying skin colour recruited from General Practices in the Auckland area.

            In this same group the effect of treatment on infection events, falls and fractures will also be assessed.
    b. Safety

        To compare the safety of the treatment relative to placebo
3. Study Design

The study is a randomised placebo controlled trial of vitamin D supplementation in volunteer members of the population who attend invited General Practices in Auckland, New Zealand. Treatment will last for approximately 4 years and the outcomes during that time will be noted. Vitamin D supplementation consists of a 200,000 IU bolus as the initial dose and then 100,000 IU doses monthly. A placebo will be given to half the sample monthly. Treatment time is described as approximately because it will vary depending on when the participant is randomised – the average duration planned to be 4 years.

4. Study Endpoints
    a. Efficacy
        i. Primary

            Fatal and nonfatal CVD events as recorded in Government records.

            Infection events as recorded in Government and participants self-report.

            All falls and fractures recorded in Government and General Practice records.
        ii. Secondary

            All deaths recorded in Government records.

Government Records

- National Health Index – Date of death
- National Minimum Dataset – Hospital events
- Pharmaceutical Collection – Subsidised dispensing
- Mortality Collection – Details of death
- NZ Cancer registry – Primary malignant tumours diagnosed
- ACC - Injuries

b. Safety

Serious adverse events

Death

1. CVD death
2. All other deaths

Hospitalisations

1. CVD hospitalisations
2. Fractures hospitalisations
3. All other hospitalisations

Serum Ca levels in '10% Annual Sample'

5. Statistical Analysis

a. Introduction

i. General principles

All analyses will be based on the principle of 'intention to treat'. No adjustment will be made for multiple testing of the primary endpoint. No adjustments for multiplicity are planned for the secondary endpoints, adverse events or other endpoints. All statistical tests will be two tailed and if a fixed statistical cut point is need the 5% level of significance will be used. All analyses will be done using SAS version 9.2 or later.

Summaries of continuous variables which are approximately normally distributed will be presented as means and standard deviations and for skewed data as medians and 25th and 75th percentiles. Categorical variables will be presented as numbers and percentages.

1. Blinding

All participants are blind to their study treatment. All study personnel with participant contact are blind to the study treatment of all participants. All the steering committee members except the statistician are blind to the study treatments of all participants. The small team recruited to prepare the distribution letters will not be 'completely' blind to the study participant's treatment. As the capsules are stored in 24 boxes (12 for each treatment) there are subsets of participants in whom it is clear they are in the same arm of the study. The contents of the boxes are unknown to the letter packing team. This team, apart from the preparation of the letters, has no other involvement in the study.

2. Definition of end of follow up

The primary endpoint is to be assessed at the end of follow up. As the participants will not be followed for the same duration, because of the staggered entry to the study, end of follow up is to 31 July 2015 (this should give an average of 3.5 years follow up).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2011						0	0	0	0	0	0	0
2012	0	0	0	0	0	1	1	1	1	1	1	1

2013	1	1	1	1	1	2	2	2	2	2	2	2
2014	2	2	2	2	2	3	3	3	3	3	3	3
2015	3	3	3	3	3	4	4	4				

3. Missing data

The endpoint data from the Government records is the recording of an event. The lack of recording of an event will be taken as no event and not as missing data. Participants who leave the country, permanently, and so have little or no event data recorded will (if identifiable) be eliminated from the analyses.

Consideration will be given to using multiple imputation for missing predictor variables that will be used in multivariable models. Multiple imputation is the preferred method of missing data replacement.

ii. CONSORT statement

The CONSORT statement is acknowledged and the running of the reporting of the study will follow these guidelines.

iii. Participant disposition and baseline characteristics

The numbers of participants who were invited, fulfilled eligibility criterion, had a baseline assessment, were sent a pre randomisation letter and the numbers randomised will be summarised as shown in appendix 2. Reasons for exclusion will be given.

Baseline demographic variables such as age, ethnicity, gender, domicile code and relevant clinical variables (smoking status, baseline blood pressure, etc) will be summarised for each treatment group

b. Primary Endpoint analyses

i. Descriptive Analyses

Within treatments groups and strata the number of fatal and non fatal CVD events and the exposure time will be reported as numbers and as a relative risk with 95% confidence interval.

Within treatments groups and strata the number of infectious respiratory events (fatal and non-fatal) with the exposure time will be reported as numbers and as a relative risk with 95% confidence interval.

Within treatments groups and strata the number of falls and fractures with the exposure time will be reported as numbers and as a relative risk with 95% confidence interval.

Within treatments groups and strata the number of deaths and the exposure time will be reported as numbers and as a relative risk with 95% confidence interval.

ii. Primary Analysis (see individual documents)

1. Cardiovascular events
2. Infections
3. Falls and fractures

iii. Secondary Analysis (see individual documents)

1. Cardiovascular events
2. Infections
3. Falls and fractures

iv. Adjusted Analyses

The primary analysis will be repeated with further potential confounding variables included. These variables will include bmi, activity, ... etc

Sub-group analyses based on the participants baseline vitamin D levels will use a time adjusted vitamin D level. All baseline vitamin D levels will be adjusted to an August value. This will be done using a sinusoidal model with parameters derived from all baseline values.

Y (baseline vitamin D) = const + $a*\sin\theta + b*\cos\theta$ where θ is time of year

$Y = \text{const} + a'*\sin\theta'$

hence $a*\sin\theta + b*\cos\theta = a'*\sin\theta'$ where $\theta' = \theta + \phi$

maximum of Y is at $dY/d\theta = 0$

that is at $a*\cos\theta_m = b*\sin\theta_m$ so $\theta_m = \tan^{-1}(a/b)$

maximum Y is also at $\sin\theta_m' = 1$ so $\theta_m' = \pi/2$

as $\theta' = \theta + \phi$ then $\pi/2 = \tan^{-1}(a/b) + \phi$

$\phi = \pi/2 - \tan^{-1}(a/b)$

hence $6*\theta_m / \pi$ is the time of the year of the maximum and

$a*\sin\theta_m + b*\cos\theta_m$ the magnitude of the seasonal effect.

The variables for each participant for the regression analysis are:

$\sin(2\pi*(\text{month}-0.5)/12)$ and $\cos(2\pi*(\text{month}-0.5)/12)$

From the model the expected vitamin D level for the participant is calculated at the time of observation. The difference between the observed value and the expected value is the adjustment needed for that participant. Hence, using this adjustment and the expected value, available for any time of the year, the expected distribution for each participant can be calculated. Those with the mean of their distribution below 50 nmol/L (that is those with the distribution below 50 nmol/L for 6 months of the year) will be classified as vitamin D deficient and will be used in the sub-group analyses.

v. Further modelling

A further model building approach based on the adjusted analyses will be used to gain a more complete understanding of the data. How this will be done will be decided at the time. This hypothesis generating modelling will be done after the primary results paper has been submitted for publication.

In any of these analyses any convergence problems will be resolved by

means detailed in Lumley et al (2006).

c. Other outcomes
   i. There are very many other outcomes on which we have information. Methods of analysis of these data will be chosen at the time of analysis.

d. Safety Analyses
   i. The safety of the vitamin D supplementation will be assessed based on a number of outcomes
      1. Deaths
      2. All cause hospitalisations
      3. Serum calcium from the 10% 'annual visit' sample

   ii. The means of reporting of serious adverse events (SAE) is given in the ViDA standard operating procedure (SOP 6).

   iii. As the participants are an elderly group it is expected that there will be a substantial number of deaths and hospitalisations over the 4 year follow up period. Although total mortality is one of the secondary outcomes in the study, the difference in the incidence rate of deaths will be monitored during the course of the study. For the purposes of the safety assessment the incidence of death will be the simple, unadjusted incidence. As the trial will run for 4 years the probabilities associated with the O'Brien-Fleming with 4 assessment times will be used to assess this outcome. As this interim assessment is for the purpose of the DMC only these probabilities will not be used in reporting this outcome from the study.

   iv. For the final report, the maximum adjusted Ca value for each participant (in the 10% 'annual visit' sample) will be noted and used in the calculation of means and standard deviations by strata. Tables of the adjusted serum calcium levels will be constructed as necessary for the DMC. All participants with adjusted Ca>2.6 mmol/l at any time will be individually reported with their Ca history.

Independent Data Monitoring Committee (DMC)

i. The study will use an independent DMC to review the outcome and safety data in the context of the overall study. The DMC will determine the timing of these reviews. The study statistician will discuss with the DMC which data and analyses they wish to see and how often this is done.

Reference

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