This is the authors' final version, post peer-review, of an article published in *Am J Clin Nutr* 2014;100(3):746-55. The definitive version is available from acjn.nutrition.org

Cardiovascular disease and vitamin D supplementation; trial analysis, systematic review and meta-analysis

John A. Ford MBChB, Graeme S MacLennan MSc, Alison Avenell MD, Mark Bolland PhD, Andrew Grey MD, Miles Witham PhD, for the RECORD trial group

Health Services Research Unit, University of Aberdeen (JAF, GSM, AA)

Department of Medicine, University of Auckland (MB, AG)

Section of Aging and Health, University of Dundee (MW)

Corresponding author and reprint requests:

John Ford
Norwich Medical School
Faculty of Medicine and Health Sciences
University of East Anglia
Chancellors Drive
Norwich, NR4 7TJ
UK
Email: john.ford@uea.ac.uk
Tel: +441603591269
Fax: +441603593752

Short title

Vitamin D and cardiovascular disease

Names for PubMed indexing: Ford, MacLennan, Avenell, Bolland, Grey and Witham

Funding

The MRC funded the central organisation of RECORD, and Shire Pharmaceuticals funded the drugs, which were co-funded and manufactured by Nycomed. Shire Pharmaceuticals and Nycomed were given the opportunity to comment on the penultimate version of the RECORD main trial report in the Lancet only. However, the trial was conducted, analysed and reported independently of all the funders. The Health Services Research Unit, University of

Aberdeen, is funded by the Chief Scientist Office of the Scottish Government Health and Social Care Directorates.

Abbreviations

BNP = brain natriuretic peptide

CI = confidence interval

CrI = credible interval (Bayesian statistics)

25(OH)D = 25-hydroxyvitamin D

ED71 = 1α,25-dihydroxy-2β-(3-hydroxypropyloxy)vitamin D_3

HR = hazard ratio

IQR = interquartile range

IU = international units

2MD = 2-Methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3

MI = myocardial infarction

PTH = Parathyroid hormone

RAAS = renin-angiotensin-aldosterone system

RCT = randomized controlled trial

RR = risk ratio

SD = standard deviation

Abstract

Background

Low 25(OH) vitamin D status is associated with increased cardiovascular events in epidemiological studies.

Objective

The aim was to assess if vitamin D supplementation reduces cardiac failure, myocardial infarction (MI) and stroke, through analysis of the RECORD randomized controlled trial (RCT), and systematic review and meta-analysis.

Design

Two analyses were undertaken. Firstly, a trial analysis. RECORD was a factorial RCT comparing vitamin D_3 (800IU daily), calcium (1,000mg daily), vitamin D plus calcium and placebo. Cardiovascular events were collected throughout the trial and three years' post-trial follow-up. Data were analysed using Cox regression.

Secondly, a systematic review. MEDLINE, EMBASE, CENTRAL, conference abstracts and on-going trials were searched for RCTs evaluating vitamin D from 1980 to 2013. RCTs with at least one year of follow-up and participants' mean/median age of 60 years or older were included. Meta-analyses were based on a Bayesian fixed-effects model using a complementary log-log link function to account for varying length of follow-up.

Results

Trial analysis showed that for the 5292 participants in the RECORD trial the hazard ratios for vitamin D compared to no vitamin D for cardiac failure, myocardial infarction and stroke were

HR 0.75 (95%CI 0.58, 0.97), HR 0.97 (95%CI 0.75,1.26), and HR 1.06 (95%CI 0.8, 1.32) respectively.

Twenty-one studies met the inclusion criteria for the systematic review (n=13,033). Estimated hazard ratios (credible intervals) for vitamin D compared to placebo or control for on-study events for cardiac failure, myocardial infarction and stroke were HR 0.82 (Crl 0.58, 1.15), HR 0.96 (Crl 0.83, 1.10) and HR 1.07 (Crl 0.91, 1.29) respectively.

Conclusion

Vitamin D supplementation might protect against cardiac failure in older people, but does not appear to protect against MI or stroke.

Key words: vitamin D, cardiovascular diseases, heart failure, meta-analysis

Introduction

Low 25-hydroxyvitamin D [25(OH)D] status has been associated with cardiovascular disease in epidemiological studies (1). Lower 25(OH)D levels are seen in patients with higher blood pressure, metabolic syndrome, heart failure and stroke than in patients without these disorders (1). Suggested pathophysiological mechanisms by which vitamin D deficiency could lead to cardiovascular disease, including heart failure, are overactivity of the renin-angiotensin-aldosterone system (RAAS); endothelial dysfunction; direct effects on calcium flux leading to decreased myocyte contractility; hyperparathyroidism, which is associated with left ventricular hypertrophy; promotion of chronic inflammation; and increased risk of metabolic syndrome and type 2 diabetes (1). The causality of these relationships is debated however, and the quality of the available studies criticised (2). Known risk factors for cardiovascular disease, including smoking, obesity, inactivity (thus reduced sun exposure) and advanced age, are associated with lower 25(OH)D, making dissection of the causal role of low 25(OH)D status in cardiovascular disease difficult. Finally, recent evidence suggests that 25(OH)D may be a negative acute-phase reactant, thus chronic disease may lead to low 25(OH)D, even in the presymptomatic phases of cardiovascular disease (3).

There have been several previous systematic reviews evaluating vitamin D supplementation and cardiovascular outcomes, but these reviews have not focussed on cardiac failure (4-8), an area of growing interest (9-10). The most recent reviews found no effect on cardiovascular mortality (RR 0.98, 95%CI 0.90 to 1.07) (4) or on the incidence of myocardial infarction (MI) (RR 1.02 95%CI 0.93 to 1.13) or stroke (RR 1.05 95%CI 0.88 to 1.25) (6). Wang and colleagues found a statistically nonsignificant reduction in cardiovascular disease with moderate to high doses of vitamin D (RR 0.90, 95%CI 0.77 to 1.05) (8). None of these systematic reviews

examined the effect of supplementation on chronic heart failure; an area of growing interest (9,10).

Previous systematic reviews used a narrow search strategy by including cardiovascular terms, and included vitamin D plus calcium versus placebo or control RCTs, wrongly assuming no effect of calcium on cardiovascular disease (11,12), nor sought unpublished data. We undertook an extensive search for new published and unpublished trial data using a broad search strategy, and included unpublished data from the RECORD trial for the secondary prevention of fractures (13).

Methods

RECORD Trial

Study design and participants

Full details of the RECORD study (ISRCTN 51647438) have been published (13). This was a factorial trial that randomized 5,292 participants with previous fracture to oral vitamin D_3 (800 IU daily) plus calcium (1,000 mg daily as calcium carbonate), vitamin D_3 alone, calcium alone or placebo. Participants were recruited between Feb 1, 1999 and Mar 31, 2002. The primary outcome was low-trauma fracture. Major inclusion criteria were age 70 years or over and fracture in the past ten years. Exclusion criteria included cognitive impairment, daily supplement intake of vitamin D or calcium (maximum 200 IU and 500 mg respectively) and bone altering medications. Ethics approval was obtained from the Multicentre Research Ethics Committee for Scotland and from the local research ethics committee of each hospital, and participants gave written informed consent.

Randomization and masking

Participants were randomized by a central computerised system that minimised by age (below 80 years or 80 years and above), sex, time since initial fracture (three months and less or more than three months) and type of fracture (proximal femur, distal forearm, clinical vertebral or other). Participants were allocated to daily doses of vitamin D_3 800 IU, calcium 1000 mg, combined vitamin D_3 plus calcium or placebo. Allocation remained concealed until final analysis. Tablets were posted to participants every four months. Participants and researchers were blinded to the intervention.

Procedures

Deaths attributed to cardiovascular or cerebrovascular disease were pre-specified as outcomes in the main trial protocol (http://www.thelancet.com/protocol-reviews/02PRT-35),. In addition, cardiovascular outcome data were collected from questionnaires, hospital and family doctors' reports, nominated friends or family, as well as death certificates. After trial closeout, data were only collected from the main cause of death from death registrations, provided by the General Register Office for Scotland for all UK participants; these data were collected independently of the trial as part of routine national statistics. On-study data collected during the RECORD trial were adjudicated by researchers independent from the trial, with advice from cardiologists.

All participants alive at trial closure were included in a three year 'off-study' post-intervention follow-up period.

Three pre-specified outcomes comparing vitamin D (with or without calcium) with no vitamin D (with or without calcium) supplementation as per factorial trial design from both on-study (24 to 62 months follow up) and off-study periods (three years after trial closure) were assessed: time to first cardiac failure, time to first MI, time to first stroke, and time to first composite outcome of cardiac failure, MI or stroke. Inclusion of the off-study period was justified because it allowed the potential lag effect of vitamin D to be examined, where remodelling could occur several years before clinically overt heart failure.

The following definitions were used;

- cardiac failure – "heart failure", "pulmonary oedema" or synonymous terms, or any of the ICD-9 codes: 125.5, 111.0, 142.0, 142.7, 142.8, 142.9, 150.0, 150.1, 150.9

- myocardial infarction – “myocardial infarction” or “heart attack”, or ICD-9 code 410
- stroke - “stroke”, “cerebral infarction”, “intracerebral hemorrhage”, “subarachnoid hemorrhage”, or “cerebrovascular accident”, or any of the ICD-9 codes 430, 431, 433, 434
- composite – cardiac failure, myocardial infarction or stroke, as defined above

Statistical analysis

RECORD trial outcomes were analysed in a time-to–event framework using Cox proportional hazards regression models. The potential for any effect modification due to an interaction with calcium was explored in subgroup analysis and summarised graphically using a forest plot presenting the treatment effect in the calcium subgroup, the no-calcium subgroup and the interaction effect (which tests the difference between these subgroups). Sensitivity analysis explored the effects of compliance with treatment allocation. A *post hoc* analysis of fatal events was undertaken by replicating the primary analysis. All estimates of treatment effects are presented as hazard ratios (HR) and 95% confidence intervals. Further details of the regression model and compliance sensitivity analysis can be found in supplementary material.

Systematic review and meta-analyses

Data sources and searches

A systematic search for randomized trials of vitamin D supplementation was undertaken. Published studies were identified from MEDLINE (January 2005 to February 2013), EMBASE (January 2006 to February 2013) and CENTRAL (January 1980 to February 2013). MEDLINE search terms shown in supplementary material were adapted as appropriate for other

databases. The references of included studies and published systematic reviews were screened. Grey literature was identified from hand searching conference abstracts of the American Society for Bone and Mineral Research 2007 to 2012. The International Clinical Trials Registry Platform was searched for unpublished and on-going trials.

Study selection

Only RCTs including participants with a mean/median age of equal to or more than 60 years (older age reflecting higher risk of vitamin D deficiency) and at least one-year follow up were included. Any vitamin D or vitamin D analogue intervention was eligible, as we were looking for a class effect. Co-administration with other medications, such as calcium, was allowed provided that the comparator group received the same medication. There were no language restrictions. Studies assessing vitamin D supplementation in participants selected solely on the basis of renal impairment (estimated glomerular filtration rate, eGFR, 60ml/min/1.73m^2), steroid-induced osteoporosis or psoriasis were excluded.

To locate unpublished data authors were contacted for studies that met the inclusion criteria but did not report cardiovascular outcomes, or were completed but unpublished. Authors were also contacted to resolve any uncertainties in the published data.

Data extraction and quality assessment

Data were extracted by one author, double-checked by a second reviewer and discrepancies resolved through discussion. Data were extracted per patient rather than per event. Risk of bias within studies was assessed using the Cochrane risk of bias tool (14).

Data synthesis and analysis

The RCTs included in the study reported outcomes at varying lengths of follow-up. Standard meta-analysis ignores variation in follow-up that may be sub-optimal when longer follow-up results in more events, as is the case here. Therefore a Bayesian fixed-effects model using a complementary log-log link function to account for varying length of follow-up was used. Further details can be found in supplementary information. Results are presented as hazard ratios and 95% credible intervals, based on fixed effects models. Random effects models were also run and compared to the fixed models using the residual deviance. Traditional random effects meta-analysis models were run in Stata using only the proportions of participants experiencing events. These are presented as risk ratios and 95% confidence intervals for comparison. Forest plots are presented for illustrative purposes. All analyses, both for the trial analysis and meta-analysis, were undertaken in Stata 12.(15)

Results

RECORD Trial

Full details of the recruitment and participant flow for the RECORD trial are published elsewhere (13). There were 2,649 participants randomized to vitamin D and 2,643 to no vitamin D. The groups were similar at baseline (**Table 1**). The mean age was 77.5 years (SD 5.6). Most participants were white and female. Only a small number of participants had diabetes or were smokers. In the vitamin D group 438 participants died during the on-study period compared with 460 in the no vitamin D group. Median time from randomization to final post-trial follow up was 6.2 years in the vitamin D group (IQR 5.1 to 7.0) and 6.2 years in the no vitamin D group (IQR 4.9 to 7.0).

Descriptive information on outcomes for the entire follow-up period and estimated treatment effects are presented in **Table 2** and **Supplementary Table 1**. The risk of first cardiac failure was lower in the vitamin D group compared to no vitamin D, adjusted HR 0.75 (95%CI 0.58, 0.97; p = 0.027) (Table 2 and **Supplementary Figure 1**). There was no evidence of a difference in risk for MI (HR 0.97 95%CI 0.75, 1.26; p= 0.84), stroke (HR 1.06, 95% CI 0.85, 1.32; p = 0.61) or the composite outcome (HR 0.92, 95%CI 0.80, 1.08; p = 0.32).

Risk of fatal cardiac failure was lower in the vitamin D group compared to no vitamin D (adjusted HR 0.70, 95%CI 0.53, 0.91; p = 0.009) but risk of fatal events was not lower for other outcomes (Table 2).

According to the pre-specified definition of adherence, 2,268 (42.9%) participants were adherent. Adherence was similar between the vitamin D group (43.8%) and the no vitamin D

group (42.0%). For the composite outcome the hazard ratio adjusted for adherence was 0.99 (95%CI 0.59, 2.31). This was similar to the analysis that was not adjusted for adherence (HR 0.92, 95%CI 0.80, 1.08).

Interaction between vitamin D and calcium was found to be small, but there was considerable uncertainty (**Supplementary Figure 2**).

Systematic review and meta-analysis

Literature searching identified 8,907 records (**Supplementary Figure 3**). The full texts of 197 articles were assessed and 132 articles were excluded. The commonest reason for study exclusion was comparison of calcium plus vitamin D versus placebo. Fifty-six studies met the inclusion criteria. Eight studies reported suitable cardiovascular outcomes in the published report (16-23). Authors of eleven studies provided supplementary data on cardiovascular events (24-33). Unpublished data from Avenell 2004 (34) were available locally from the Health Services Research Unit, University of Aberdeen. For consistency with the other trials, on-study data from the analysis of the RECORD study was included in the primary meta-analysis. In total, 21 studies met the inclusion criteria for meta-analysis. Three studies did not report any event in either arm and therefore did not contribute to the meta-analysis (23, 24, 32).

Characteristics of included studies are shown in **Table 3**. Thirteen studies included only female participants, one study only male and the remaining both. Eight studies included only participants at higher risk of fracture, i.e. previous fracture, osteoporosis or osteopenia. Cholecalciferol was used in ten studies. Doses of cholecalciferol were given daily, monthly or

yearly; expressed as dose per day, doses ranged from 800 IU to 4000 IU. Calcitriol was used in four studies and doses ranged from 0.25 µg to 0.50 µg per day and two studies included a dose escalation protocol. Ergocalciferol was used in two studies. In five studies a vitamin D analogue was used (doxercalciferol, alfacalcidol, 2MD, or ED71). In 13 studies the follow-up period was 12 months. The follow-up of the remaining studies ranged from a median of 17.6 months to 6.2 years.

Studies were generally of low or unclear risk of bias (**Supplementary Table 2**). Xia 2009 and Avenell 2004 were open label studies (27, 34).

In total 13,033 participants were included. Mean ages ranged from 61 years to 77 years (**Supplementary Table 3)** Baseline 25(OH)D was recorded in eleven studies, ranging from 24 to 80 nmol/l.

Vitamin D did not significantly reduce the risk of cardiac failure compared with no vitamin D (68 vs 80 events, HR 0.82, credible interval, CrI 0.58, 1.15, **Table 4**). There was no statistically significant difference in MI or stroke events between vitamin D and no vitamin D (320 vs 334 events, HR 0.96, CrI 0.83, 1.10; 251 vs 226 events, HR 1.07, CrI 0.91, 1.29, respectively). There was low statistical heterogeneity throughout. Figures 1, 2 and 3 shows forest plots produced by the traditional random effects meta-analysis for illustrative purposes.

In a *post hoc* sensitivity analysis, only trials evaluating cholecalciferol or ergocalciferol were examined in meta-analysis. The results were virtually identical (myocardial infarction HR 0.95,

CrI 0.82, 1.10; stroke HR 1.08, CrI 0.91, 1.29). There were no trials of vitamin D analogues which provided data on cardiac failure.

Funnel plot inspections did not suggest publication bias.

In the sensitivity analysis, including off-study events from the RECORD trial, risk of cardiac failure event was statistically significantly lower in vitamin D compared to the no vitamin D group (overall HR 0.79, CrI 0.59, 0.99). No statistically significant differences were found for MI (HR 0.99, CrI 0.87, 1.11) or stroke (HR 1.07, CrI 0.91, 1.24).

Discussion

Analysis of the whole follow-up period of the RECORD trial showed a statistically significant, clinically important reduced risk of cardiac failure events with vitamin D, but that vitamin D had no significant effect on MI, stroke or the composite outcome. Meta-analysis found that vitamin D did not reduce the risk of cardiac failure during the on-study periods in the trials, but inclusion of the RECORD off-study events generated a statistically significant effect. No statistically significant difference was found in the meta-analysis for MI or stroke. There was no indication of adverse effects of vitamin D on cardiovascular disease.

What do these results mean?

The results suggest that there is insufficient evidence to support vitamin D supplementation for the reduction of cardiovascular events, but raise the possibility that it might impact on heart failure. This might occur by preventing the development of heart failure, or mitigating its progression. The key drivers for heart failure in older patients are ischemic heart disease and hypertension (35), but the lack of effect of vitamin D on MI does not support this mechanism. This may suggest that vitamin D affects the chronic pathogenesis of heart failure. A systematic review of trials of vitamin D supplementation found that there may be a beneficial effect on blood pressure (36); this may be of particular significance in this study as hypertension is a common cause of heart failure in older women (37).

If vitamin D does not prevent the onset of heart failure, it could mitigate the severity of the syndrome once established. Existing trial data are contradictory. Vitamin D supplements improved echocardiographic markers of heart failure and pro-inflammatory cytokines in a RCT in Egyptian infants with 25(OH)D of 35 nmol/L (38). In a RCT of adults with cardiac failure (baseline 25(OH)D 36 nmol/L(39), cholecalciferol 2000 IU/d improved pro-inflammatory

cytokines, but had no significant effect on echocardiographic parameters or N-terminal propeptide of brain natriuretic peptide (BNP). In a small, short-term trial Witham and colleagues found that vitamin D supplementation (100,000 IU ergocalciferol every 10 weeks) improved BNP compared to placebo, but had no effect on symptoms, exercise capacity or quality of life in older patients with heart failure despite low baseline 25(OH)D (mean 21 nmol/L)(40).

Vitamin D supplementation reduces parathyroid hormone (PTH), which is known to be vasculotoxic and associated with left ventricular hypertrophy (1). In a cohort study (n=864), Hagstrōm and colleagues found that high PTH levels were associated with increased cardiac failure hospitalisations (HR for 1-SD increase of PTH, 1.41, 95% CI 1.12, 1.77) (41).

Vitamin D could reduce cardiac failure through the RAAS system.(42) In a very large cohort of individuals without heart failure, low vitamin D status was associated with increased RAAS activation (43). However, in randomised trials Witham and colleagues (40) and Boxer and colleagues (44) found no significant effect on RAAS in patients with heart failure, perhaps in part due to the high prevalence of RAAS system blocker use in heart failure patients. The mechanism is not clear, postulated mechanisms include upregulation of vascular endothelial growth factor, and mediation through calcium myocyte handling with improved cardiac muscle strength (1). It was not possible to explore J or U shaped associations between outcomes and 25(OH)D levels because of the limited 25(OH)D data in RECORD.

Context of these results

Previous systematic reviews (4, 6, 8) of RCTs of vitamin D on cardiovascular endpoints failed to report significant benefits, but may have been subject to potential bias through limited

searching and failure to obtain unpublished data. Some reviews included trials which compared calcium and vitamin D versus placebo or control, as well as trials of vitamin D alone, which is problematic because calcium has been found to increase the risk of cardiovascular events (11,12). In the Women's Health Initiative (WHI) trial (45), co-administered calcium and vitamin D had no effect on CHF in the entire cohort, but might reduce the risk of CHF in women at low risk of cardiovascular disease, but not in those at high risk (46).

A Cochrane review of RCTs evaluating vitamin D and overall mortality, undertook a subgroup analysis of cardiovascular mortality and found no difference, although overall mortality was slightly reduced (RR 0.97, 95% CI 0.94 to 0.99). (4). Given the findings of this review we decided to test the robustness of the RECORD Trial results to a post-hoc sensitivity analysis to explore the potential influence of death from all other causes within a competing risks framework. These results (not presented) were practically identical to the results in Table 2 and are robust to death from other causes. Similarly, Elamin and colleagues in a meta-analysis including randomised controlled trials, found no difference for MI or stroke (6). Wang and colleagues, undertook a meta-analysis of two trials and found a non-statistically significant reduction in cardiovascular outcomes (8). In a trial sequential meta-analysis, Bolland and colleagues found that vitamin D did not reduce skeletal or non-skeletal outcomes by more than 15%.(47)

Strengths and weaknesses

RECORD was a trial of secondary prevention of fractures. Osteoporosis has been associated with an increased risk of cardiovascular disease (48), so the participants in RECORD may have been at higher risk of cardiovascular events than the general population, although the

participants were mainly women and only 8% were diabetic. Although cardiovascular outcomes were prespecified, RECORD was not designed as a cardiovascular trial and event were no verified against participants' medical records. Compliance with tablets was poor reducing the examination of efficacy, but this reflects likely compliance in clinical practice. Data after trial close out were only collected from death certificates, so non-fatal events were missed. However, the longer follow-up did allow the potential lag effect of vitamin D to be examined. We used a robust search strategy for the systematic review. Studies were found which reported cardiovascular events in the full text, but not abstract or title in databases. Unpublished data from 12 trials were included. However, the method of collecting cardiovascular data varied between studies. Meta-analysis was driven by two studies (13,21). Therefore results are sensitive to the population and vitamin D dose of these studies.

Future research

Further mechanistic studies are really needed to explore the mechanisms by which vitamin D could influence the development or progression of heart failure in high risk groups. Sufficiently powered, high quality RCTs are needed to investigate the relationship between cardiovascular disease and vitamin D. The VITAL trial randomizes 20,000 healthy participants to cholecalciferol 2,000 IU/d or placebo for five years with primary outcomes including MI, stroke, and death from cardiovascular disease (49). . However, participants are allowed to take non-protocol supplements of up to 800 IU/d of vitamin D and 1g/d of calcium. The ViDA trial (http://www.anzctr.org.au/) is assessing effects of 100,000IU/month cholecalciferol on cardiovascular disease in 5100 men and women aged 50-84 years. The FIND trial (http://clinicaltrials.gov/show/NCT01463813), is examining effects of 1,600IU/d, 3,200IU/d or placebo on cardiovascular disease in 18,000 men and women 60y and over. An on-going trial (http://clinicaltrials.gov/ct2/show/NCT01326650) will randomize 1,000 participants with

cardiac failure to vitamin D or control and measure symptom improvement and mortality at three years. However, no studies are currently aiming to prevent heart failure in patients at high risk (e.g. MI with reduced ejection fraction). These trials are using high doses of vitamin D, though none have set vitamin D deficiency as an entry criterion.(50)

In conclusion, long-term RECORD trial results demonstrate that vitamin D, compared to no vitamin D, resulted in a statistically significant reduction in cardiac failure events. In meta-analysis there was evidence to suggest that vitamin D supplementation may reduce cardiac failure events in older people when these RECORD data are included, but not for on study trial data alone.

Acknowledgements

We thank the patients who took part in the RECORD study, without whose help this study would not have been possible. We would also like to thank the following study authors for supplying additional data and clarifications: Cyrus Cooper, Itsuo Goari, Hazel Inskip, Wim Janssens, Glenn Liu, Per Magnusson, JoAnn Manson, Toshio Matsumoto, Susan Ott, Kerrie Sanders, Klaus Witte, Weino Xia and Kun Zhu. We thank Daryll Archibald for checking data extraction in trials involving AA.

Authors contributions

AA, GSM and MW conceived the idea. All authors contributed to the research design. JF, AA and GSM undertook the hands on research including data collection and statistical analysis. JF wrote the first draft of the paper. All authors made major significant contributions to re-drafting. AA had primary responsibility for the final content.

***RECORD TRIAL GROUP:**

RECORD Trial Management Group

Health Services Research Unit, University of Aberdeen, Aberdeen, UK (AM Grant, A Avenell, MK Campbell, AM McDonald, GS MacLennan, GC McPherson); University of Southampton, UK (FH Anderson); MRC Epidemiology Resource Centre, University of Southampton, UK (C Cooper); Newcastle University, UK (RM Francis); Glasgow Caledonian University, UK (C Donaldson); The Hull York Medical School, Hull, UK (WJ Gillespie); Royal Infirmary of Edinburgh, UK (CM Robinson); Department of Health Sciences, York, UK (DJ Torgerson); Queens Medical Centre, Nottingham, UK (WA Wallace).

Further members of the RECORD Trial Group are listed in the RECORD trial.

388

389 **Conflicts of interest**

390 AA and GSM took part in two of the trials in the systematic review. MW took part in one of
391 the trials. JAF, GSM, MB, AG, MW, AA have no other conflicts of interest to declare. Details of
392 conflicts of interest for other members of the RECORD Trial Group are provided in reference
393 No 13.

394

395 **Ethics**

396 Ethical approval for the RECORD study was granted from the Multicentre Research Ethics
397 Committee for Scotland.

398

399 No published protocol exists

References

1. Beveridge LA, Witham MD. Vitamin D and the cardiovascular system. Osteoporosis Int 2013;24:2167-80.

2. Sokol SI, Tsang P, Aggarwal V, Melamed ML, Srinivas VS. Vitamin D status and risk of cardiovascular events: Lessons learned via systematic review and meta-analysis. Cardiol Rev 2011;19:192-201.

3. Reid D, Toole BJ, Knox S, Talwar D, Harten J, O'Reilly DS, Blackwell S, Kinsella J, McMillan DC, Wallace AM. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. Am J Clin Nutr 2011;93:1006-11.

4. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, Bjelakovic M, Gluud C. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database of Systematic Reviews 2014, Issue 1. Art. No.: CD007470. DOI: 10.1002/14651858.CD007470.pub3.

5. Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, Lichtenstein A, Patel K, Raman G, Tatsioni A et al. Vitamin D and calcium: A systematic review of health outcomes. Evid Rep Technol Assess (Full Rep) 2009:183:1-420.

6. Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A et al. Vitamin D and cardiovascular outcomes: A systematic review and meta-analysis. J Clin Endocrinol Metab 2011;96:1931-42.

7. Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Systematic review: Vitamin D and cardiometabolic outcomes. Ann Int Med 2010;152:307-14.

8. Wang L, Manson JE, Song Y, Sesso HD. Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. Ann Int Med 2010;152:315-23.

9. Witham MD. Vitamin D in chronic heart failure. Curr Heart Fail Rep 2011;8(2):123-30.

10. Meems LM, van der Harst P, van Gilst WH, de Boer RA. Vitamin D biology in heart failure. Curr Drug Targets 2011;12:29-41.

11. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events. BMJ 2010;341:c3691.

12. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events. BMJ 2011;342:2040.

13. Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, Anderson FH, Cooper C, Francis RM, Donaldson C et al. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people. Lancet. 2005 May 7-13;365:1621-8.

14. Higgins JPT, Altman DG, Sterne JAC. Assessing risk of bias in included studies. In: Higgins JPT, Green S, editors. Cochrane Handbook. 5.1.0 ed. John Wiley & Sons, Ltd; 2011.

15. Stata. Stata statistical software: Release 12, 2011.

16. Gallagher JC, Fowler SE, Detter JR, Sherman SS. Combination treatment with estrogen and calcitriol in the prevention of age-related bone loss. J Clin Endocrinol Metab 2001;86:3618-28.

17. Gallagher JC, Sai A, Templin T, Smith L. Dose response to vitamin D supplementation in postmenopausal women a randomized trial. Ann Int Med 2012;156:425-37.

18. Aloia JF, Vaswani A, Yeh JK, Ellis K, Yasumura S, Cohn SH. Calcitriol in the treatment of postmenopausal osteoporosis. Am J Med 1988;84:401-408.

19. Ott SM, Chesnut III CH. Calcitriol treatment is not effective in postmenopausal osteoporosis. Ann Int Med 1989;110:267-74.

20. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson CG. Annual high-dose oral vitamin D and falls and fractures in older women. JAMA 2010;303:1815-22.

21. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community. BMJ 2003;326:469.

22. Majima T, Komatsu Y, Shimatsu A, Satoh N, Fukao A, Ninomiya K, Matsumura T, Nakao K. Efficacy of combined treatment with raloxifene and alfacalcidol on bone density and biochemical markers of bone turnover in postmenopausal osteoporosis. Endocr J 2008;55:127-34.

23. Attia S, Eickhoff J, Wilding G, McNeel D, Blank J, Ahuja H, Jumonville A, Eastman M, Shevrin D, Glode M et al. Randomized, double-blinded phase II evaluation of docetaxel with or without doxercalciferol in patients with metastatic, androgen-independent prostate cancer. Clin Cancer Res 2008;14:2437-43.

24. DeLuca HF, Bedale W, Binkley N, Gallagher JC, Bolognese M, Peacock M, Aloia J, Clagett-Dame M, Plum L. The vitamin D analogue 2MD increases bone turnover but not BMD in postmenopausal women with osteopenia. J Bone Miner Res 2011;26:538-45.

25. Gorai I, Tanaka Y, Hattori S, Iwaoki Y. Assessment of adherence to treatment of postmenopausal osteoporosis with raloxifene and/or alfacalcidol in postmenopausal japanese women. J Bone Miner Metab 2010;28:176-84.

26. Matsumoto T, Miki T, Hagino H, Sugimoto T, Okamoto S, Hirota T, Tanigawara Y, Hayashi Y, Fukunaga M, Shiraki M, Nakamura T. A new active vitamin D, ED-71, increases bone mass in osteoporotic patients under vitamin D supplementation. J Clin Endocrinol Metab 2005;90:5031-6.

27. Xia WB, Zhang ZL, Wang HF, Meng XW, Zhang Y, Zhu GY, Xing XP, Liu JL, Wang LH, Jiang Y et al. The efficacy and safety of calcitriol and/or caltrate D in elderly chinese women with low bone mass. Acta Pharmacol Sin 2009;30:372-8.

28. Zhu K, Devine A, Dick IM, Wilson SG, Prince RL. Effects of calcium and vitamin D supplementation on hip bone mineral density and calcium-related analytes in elderly ambulatory australian women. J Clin Endocrinol Metab 2008;93:743-9.

29. Lehouck A, Mathieu C, Carremans C, Baeke F, Verhaegen J, van Eldere J, Decallonne B, Bouillon R, Decramer M, Janssens W. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease. Ann Int Med 2012;156:105-20.

30. Toss G, Magnusson P. Is a daily supplementation with 40 microgram vitamin D(3) sufficient? Eur J Nutr 2012;51:939-45.

31. Macdonald HM, Wood AD, Aucott LS, Black AJ, Fraser WD, Mavroeidi A, Reid DM, Secombes KR, Simpson WG, Thies F. Hip bone loss is attenuated with 1000 IU but not 400 IU daily vitamin D3. J Bone Miner Res 2013;doi:10.1002/jbmr.1959.

32. Prince RL, Austin N, Devine A, Dick IM, Bruce D, Zhu K. Effects of ergocalciferol added to calcium on the risk of falls in elderly high-risk women. Arch Int Med 2008;168:103-8.

33. Witham MD, Price RJ, Struthers AD, Donnan PT, Messow CM, Ford I, McMurdo ME. Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension. JAMA Int Med 2013;doi:10.1001/jamainternmed.2013.9043.

34. Avenell A, Grant AM, McGee M, McPherson GC, Campbell MK, Group RTM. The effects of an open design on trial participant recruitment, compliance and retention--a randomized controlled trial comparison with a blinded, placebo-controlled design. Clin Trials 2004;1:490-8.

35. McDonagh TA, Morrison CE, Lawrence A, Ford I, Tunstall-Pedoe H, McMurray JJ, Dargie HJ. Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population. Lancet 1997;350:829-33.

36. Witham MD, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure. J Hypertens 2009;27:1948-54.

37. Nieminen MS, Harjola VP, Hochadel M, Drexler H, Komajda M, Brutsaert D, Dickstein K, Ponikowski P, Tavazzi L, Follath F et al. Gender related differences in patients presenting with acute heart failure. Eur J Heart Fail 2008;10:140-8.

38. Shedeed SA. Vitamin D supplementation in infants with chronic congestive heart failure. Pediatr Cardiol 2012;33:713-9.

39. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure. Am J Clin Nutr 2006;83:754-9.

40. Witham MD, Crighton LJ, Gillespie ND, Struthers AD, McMurdo ME. The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure. Circ Heart Fail 2010;3:195-201.

41. Hagstrom E, Hellman P, Larsson TE, Ingelsson E, Berglund L, Sundstrom J, Melhus H, Held C, Lind L, Michaelsson K et al. Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. Circulation 2009;119:2765-71.

42. Schroten NF, Ruifrok WP, Kleijn L, Dokter MM, Sillje HH, Lambers Heerspink HJ, Bakker SJ, Kema IP, van Gilst WH, van Veldhuisen DJ et al. Short-term vitamin D3 supplementation lowers plasma renin activity in patients with stable chronic heart failure: an open-label, blinded end point, randomized prospective trial (VitD-CHF trial). Am Heart J 2013;166:357-364.

43. Tomaschitz A, Pilz S, Ritz E, Grammer T, Drechsler C, Boehm BO, Marz W. Independent association between 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin-angiotensin system. Clin Chim Acta. 2010;411:1354-60

44. Boxer RS, Kenny AM, Schmotzer MS, Vest M, Fiutem MS, Pina IL. A randomized controlled trial of high-Dose Vitamin D3 in patients with heart failure. Journal of the Am Coll Cardiol Heart Fail 2013;1:84-90.

45. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, Bassford T, Beresford SAA, Black HR, Blanchette P et al. Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 2006;354:669-83.

46. Donneyong MM, Taylor KC, Baumgartner RN, Myers J, Song WL, Martin LW. Calcium plus vitamin D supplementation and the risk of heart failure: The calcium and vitamin D trial of the women's health initiative study. Am J Epidemiol 2013;177:S128.

47. Bolland MJ, Grey A, Gamble GD, Reid IR. The eff ect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis. Lancet Diabetes Endocrinol 2014;2:307-20.

48. den Uyl D, Nurmohamed MT, van Tuyl LDH, Raterman HG, Lems WF. (Sub)clinical cardiovascular disease is associated with increased bone loss and fracture risk. Arthritis Res Ther 2011;13:doi:10.1186/ar3224.

49. Manson JE, Bassuk SS, Lee IM, Cook NR, Albert MA, Gordon D, Zaharris E, Macfadyen JG, Danielson E, Lin J et al. The VITamin D and OmegA-3 TriaL (VITAL). Contemp Clin Trials. 2012;33:159-71.

50. Pilz S, Rutters F, Dekker JM. Disease prevention: vitamin D trials. Science 2012;338:883.

Tables and figures

Table 1: RECORD baseline characteristics

Variable		Vitamin D N=2,649	Placebo N=2,643
Age in years - mean (SD)		77.5 (5.6)	77.4 (5.6)
Calcium		1,306(49.3)	1,311(49.6)
Female		2,240 (84.6)	2,241 (84.8)
White		2,629 (99.2)	2,623 (99.2)
Type of enrolling fracture	Proximal femur	459 (17.3)	445 (16.8)
	Distal forearm	924 (34.9)	922 (34.9)
	Clinical vertebral	4 (0.2)	4 (0.2)
	Other	1,262 (47.6)	1,272 (48.1)
Time since enrolling fracture	≥ 3 months	469 (17.7)	475 (18.0)
Diabetes mellitus		208 (7.9)	212 (8.0)
Oral hypoglycaemics		119 (4.5)	108 (4.1)
Insulin		40 (1.5)	50 (1.9)
Current smoker		298 (11.3)	320 (12.1)
Ambulant in community[1]		2,492 (94.1)	2,487 (94.1)
Oral steroids ≥ 7.5 mg prednisolone per day		49 (1.9)	44 (1.7)

Cell contents are n (%) unless otherwise stated; [1]able to walk outdoors unaccompanied

SD = standard deviation.

Table 2: Estimated effects of vitamin D on outcomes for on-trial plus off-trial

	Outcome	Vitamin D N=2,649	Placebo N=2,643	HR[1] (95%CI)	P value
Fatal and non-fatal events	Cardiac failure	102	136	0.75 (0.58, 0.97)	0.027
	MI	114	117	0.97 (0.75, 1.26)	0.84
	Stroke	160	149	1.06 (0.85, 1.32)	0.61
	Composite outcome	339	363	0.92 (0.80, 1.08)	0.32
Fatal events only	Cardiac Failure	89	127	0.70 (0.53, 0.91)	0.009
	MI	87	88	0.99 (0.73, 1.33)	0.92
	Stroke	102	101	0.99 (0.75, 1.30)	0.94
	Composite outcome	256	291	0.87 (0.73, 1.03)	0.11

HR = hazard ratio, CI = confidence interval, MI = myocardial infarction, [1] Cox regression adjusted for: age (younger than 80 years or 80 years and older), sex, time since fracture (previous three months or longer), type of fracture (proximal femur, distal forearm, clinical vertebral or other), diabetic status and smoking status

Table 3: Study characteristics

Study Country	Participants	Interventions given to all participants	Intervention	Comparator	Primary outcome	Follow-up
Aloia 1988 USA (18)	Postmenopausal women with osteoporosis aged 50 to 80 years	Vit D 400 IU daily (unspecified) and calcium 1,000 mg dietary intake	Calcitriol 0.50 µg daily with dose escalation if necessary	Placebo	Bone biopsy, mineral & urinary measurements and radiographs	2 years
Attia 2008 USA (23)	Men with metastatic prostate cancer without starting chemotherapy	Docetaxel plus dexamethasone on cycle days 1, 8 & 15	Doxercalciferol 10 ug daily	Placebo	PSA, median progression free survival	Median 17.6 mths
Avenell 2004 UK (34)	Men and women aged ≥ 70 years with a previous low trauma osteoporotic fracture	None	Oral calcium 1g daily, oral cholecalciferol 800 IU daily or both	Placebo	Eligible participants recruited	1 year
Deluca 2011 USA (24)	Postmenopausal women with osteopenia aged 55-80 years	Cholecalciferol 600 IU daily	2MD 220 ug or 440 ug daily	Placebo	Percent change in lumbar BMD	1 year
Gallagher 2001 USA (16)	Women aged 65-77 years with no evidence of osteopenia	None	Calcitriol 0.25 ug twice a day HRT alone, or HRT plus calcitriol	Placebo	Femoral and spine BMD	3 years
Gallagher 2012 USA (17)	White postmenopausal women aged 57 to 90 years with vitamin D insufficiency	Daily calcium to maintain intake of 1200 to 1400 mg	Cholecalciferol 400, 800, 1600, 2400, 3200, 4000, or 4800 IU once daily	Placebo	25-(OH)D and PTH	1 year

Gorai 2010 Japan (25)	Postmenopausal women living in Japan	None	Alfacalcidol 1.0 ug daily or raloxifene 60 mg plus vitamin D daily	Raloxifene 60 mg daily	Adherence to treatment	1 year
Lehouck 2012 Belgium (29)	Current or former smokers >50 years with COPD	None	Cholecalciferol 100 000 IU monthly	Placebo	Time to first exacerbation	1 year
Majima 2008 Japan (22)	Postmenopausal women living in Japan	None	Alfacalcidol 1.0 ug daily or raloxifene 60 mg plus alfacalcidol daily	Raloxifene 60 mg daily	BMD	1 year
Matsumoto 2005 Japan (26)	Postmenopausal women with osteoporosis over 60 years old	Cholecalciferol 400 IU/d if 25(OH)D < 50 nmol/l or with 200 IU/d if ≥ 50 nmol/l	ED-71 0.5, 0.75, or 1.0 ug daily	Placebo	Change in lumbar BMD	1 year
Ott 89 USA (19)	Postmenopausal women with at least two compression fractures	Calcium to maintain intake of 24.9 mmol/day	Calcitriol 0.25 ug twice daily with dose escalation if needed	Placebo	Change in BMD	2 years
Prince 2008 Australia (32)	Women with vitamin D deficiency aged 70 to 90 years	Calcium 1000 mg daily	Ergocalciferol 1000 IU daily	Placebo	Incidence of falls	1 year
RECORD 2005 UK(13)	Men and women over 70 years with previous low trauma fracture	None	Cholecalciferol 800 IU daily, calcium 1 g daily or both	Placebo	Low-energy fractures	Median 6.18 years
Sanders 2010 Australia (20)	Women over 70 years with high risk of fracture	None	Cholecalciferol 500 000 IU once yearly	Placebo	Numbers of falls and fractures	3-5 years

Toss 2011 Sweden (30)	Community-dwelling men and women aged 55-85 years	None	Cholecalciferol 1,600 IU plus 1,000 mg calcium per day	Calcium 1,000mg per day	Serum 25(OH)D	1 year
Trivedi 2003 UK (21)	Men and women aged 65-85 from British doctors' register & general practice register	None	Cholecalciferol 100 000 IU every four months	Placebo	Fracture incidence and total mortality	5 years
Witham 2013 UK(33)	Men and women aged over 70 years with BP more than 140 mmHg systolic and 25(OH)D less than 75	None	Cholecalciferol 100,000 IU every three months	Placebo	Change in blood pressure	1 year
Witte (unpublished) UK	Men and women with stable cardiac failure	None	Cholecalciferol 4000 IU daily	Placebo	Left ventricular function	1 year
Macdonald 2012 UK (31)	Postmenopausal women living in Scotland	None	Cholecalciferol 400 IU per day or 1000 IU per day	Placebo	Serum lipid profile, estimate of insulin resistance, inflammatory biomarkers, and blood pressure	1 year
Xia 2009 China (27)	Postmenopausal women over 65 years living in China	None	Calcitrol 0.25 µg plus Caltrate D (calcium 600 mg and 125 IU cholecalciferol) daily	Caltrate D alone (calcium 600 mg and 125 IU cholecalciferol) daily	Percent change lumbar and hip BMD	1 year

Zhu 2008 Australia (28)	Women aged over 70 years selected from electoral register	None	Calcium 1200mg with ergocalciferol 1000 IU daily	Calcium 1200mg with placebo vitamin D daily	Hip BMD	5 years

AE = adverse event, NIH = National Institute of Health, BMD = bone mineral density, IU = international units, NR = not reported, PTH = parathyroid hormone, BP = blood pressure, FEV1 = forced expiratory volume in 1 second, COPD = chronic obstructive pulmonary disease, QoL = quality of life, HRT = hormone replacement therapy, ALP = alkaline phosphatase , ALAT= alanine aminotransferase, GGT = gamma-glutamyl transpeptidase, TSH = thyroid stimulating hormone, BALP = bone alkaline phosphatase, TRACP5b = tartrate-resistant acid phosphatase 5b, 25(OH)D = 25-hydroxyvitamin D, PSA = prostate specific antigen

Table 4: Meta-analysis results including on-trial only results from RECORD

Outcome	**RR[3] (95% CI)**	**HR[2] (95% CrI[1])**
Cardiac failure	0.83 (0.60, 1.13)	0.82 (0.58, 1.15)
MI	0.96 (0.83, 1.10)	0.96 (0.83, 1.10)
Stroke	1.09 (0.92, 1.30)	1.07 (0.91, 1.29)

[1] CrI is credible interval [2] Calculated using traditional random effects meta-analysis methods

[3] Calculated using Bayesian fixed-effects model to combine both HRs and RRs

Figure legend page

Figure 1: Cardiac failure forest plots for illustrative purposes including on-trial only results from RECORD

Figure 2: Stoke forest plots for illustrative purposes including on-trial only results from RECORD

Figure 3: Myocardial infarction forest plots for illustrative purposes including on-trial only results from RECORD