The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis

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Summary

Background Vitamin D insufficiency is associated with many disorders, leading to calls for widespread supplementation. Some investigators suggest that more clinical trials to test the effect of vitamin D on disorders are needed.

Methods We did a trial sequential meta-analysis of existing randomised controlled trials of vitamin D supplements, with or without calcium, to investigate the possible effect of future trials on current knowledge. We estimated the effects of vitamin D supplementation on myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, cancer, total fracture, hip fracture, and mortality in trial sequential analyses using a risk reduction threshold of 5% for mortality and 15% for other endpoints.

Findings The effect estimate for vitamin D supplementation with or without calcium for myocardial infarction or ischaemic heart disease (nine trials, 48 647 patients), stroke or cerebrovascular disease (eight trials 46 431 patients), cancer (seven trials, 48 167 patients), and total fracture (22 trials, 76 497 patients) lay within the futility boundary, indicating that vitamin D supplementation does not alter the relative risk of any of these endpoints by 15% or more. Vitamin D supplementation alone did not reduce hip fracture by 15% or more (12 trials, 27 834 patients). Vitamin D co-administered with calcium reduced hip fracture in institutionalised individuals (two trials, 3853 patients) but did not alter the relative risk of hip fracture by 15% or more in community-dwelling individuals (seven trials, 46 237 patients). There is uncertainty as to whether vitamin D with or without calcium reduces the risk of death (38 trials, 81 173).

Interpretation Our findings suggest that vitamin D supplementation with or without calcium does not reduce skeletal or non-skeletal outcomes in unselected community-dwelling individuals by more than 15%. Future trials with similar designs are unlikely to alter these conclusions.

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See Comment page 269

See Online for an audio interview with Mark Bolland

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Introduction

Findings from observational studies have shown vitamin D insufficiency to be associated with a wide variety of disorders such as fractures, ischaemic heart disease, cerebrovascular disease, and cancer.[1] Such findings have led to calls for widespread vitamin D supplementation.[1] However, some researchers have suggested that such recommendations should not be made without supportive trial data, and they have therefore called for randomised controlled trials of vitamin D supplementation with non-skeletal endpoints as primary outcomes.[2–5] Findings from several randomised controlled trials have already reported the effects of vitamin D supplementation on these outcomes, as secondary trial endpoints, and several meta-analyses of these randomised controlled trials have been done. Thus, the results of any future clinical trials will not be considered in isolation, but in the context of these existing data. Using trial sequential analysis, it is possible to model the changing precision in estimates of effects as trials are reported, and the likely effect of future trial results on the existing body of data.[6,7] This method allows identification of the point at which the body of evidence is sufficiently large and consistent to render further trials unnecessary, because of the low probability that they will affect the existing meta-analytic result. The futility analysis is analogous to the termination of a clinical trial when an interim analysis indicates that the collection of further data is highly unlikely to alter the interim result. We have used data from the most recent meta-analyses on myocardial infarction, stroke, cancer, fractures, and mortality to estimate the potential effect on current knowledge of the results of future randomised controlled trials of vitamin D supplementation.

Methods

Search strategy and selection criteria

We searched PubMed using the terms "vitamin D", "systematic review", and "meta-analysis" on Jan 31, 2013, for the most recent meta-analyses (those published since January, 2009) of the effects of vitamin D with or without calcium on cardiovascular events, cerebrovascular events, cancer, fracture, and mortality (appendix). We identified four trial-level meta-analyses for myocardial infarction and stroke,[8–11] three for cancer,[8,12,13] four for fracture,[8,12,14,15] and six for mortality.[8,10,11,14,16,17] We also reviewed recent reports on vitamin D by the International Agency for Research on Cancer, the Institute of Medicine, and the Endocrine Society, each of which included meta-analyses of randomised controlled trials of vitamin D.[18–20] We

See Online for appendix

identified all randomised controlled trials that were included in any of these meta-analyses or reports and that studied vitamin D (cholecalciferol or ergocalciferol) with outcome data for cardiovascular events, cerebrovascular events, cancer, fracture, or death. We excluded cluster randomised trials, trials of hydroxylated vitamin D or vitamin D analogues, trials that included other interventions only in the vitamin D group, trials of fortified dairy products, and trials in populations with chronic comorbidity other than osteoporosis or frailty (appendix).

Outcomes

We extracted outcome data from the original papers and then verified them against the reported data in the earliest meta-analysis in which the trial was included. We used results of intention-to-treat analyses throughout using numbers of participants with an incident event, resolving any differences by consensus between two investigators (MJB, AG). If previously unpublished data were used in a meta-analysis, we extracted those data from the meta-analysis. We extracted and included all data for the endpoints of interest that were reported in the original papers, irrespective of whether they were included in the identified meta-analyses. Following the approach of the Endocrine Society,[10] trials that reported data for myocardial infarction were analysed together with studies that reported data for ischaemic heart disease or cardiovascular events, as were trials reporting data for stroke and cerebrovascular disease.

We obtained data from 44 reports of 40 individual randomised controlled trials (table).[21–64] For one trial,[21] we did not include data for fractures and hospital admissions for cerebrovascular and coronary causes in our analysis because it was not clear whether the data were counts of total events or number of participants with events,[14] but we included causes of mortality. We obtained unpublished data for two trials[46,53] from meta-analyses of calcium supplements.[65,66] 32 (80%) of 40 trials reported baseline 25-hydroxyvitamin D (25OHD) concentrations, and in 23 (72%) of these 32 trials the average baseline 25OHD concentration was lower than 50 nmol/L. 34 (85%) of 40 trials reported 25OHD on treatment, with 31 (97%) of 32 trials reporting numerical increases in 25OHD from baseline, and 30 trials (94%) reporting 25OHD concentrations greater than 50 nmol/L in groups treated with vitamin D (table).

For all analyses, we assessed the effects of vitamin D, vitamin D plus calcium, and vitamin D with or without calcium separately. Randomised controlled trials in which calcium supplements were provided to both treatment groups, so that the groups only differed in treatment by vitamin D were included in the vitamin D analyses. Trials comparing co-administered calcium and vitamin D with placebo or controls were included in the vitamin D and calcium analyses. Several trials had factorial designs or more than two arms, permitting multiple comparisons.[21,29,39,40,46,53,59] For these trials, we included all available data from the study. For factorial studies, we included all study arms, which allowed a comparison of vitamin D versus no vitamin D in both the vitamin D analysis and the vitamin D with or without calcium analysis, but only included arms comparing co-administered vitamin D and calcium with placebo in the vitamin D and calcium analysis. For multi-arm studies, we pooled data from the separate treatment arms for the vitamin D with or without calcium analyses, but each treatment arm was only used once.

Statistical analysis

We did traditional meta-analyses in which data were pooled with random-effects models, and assessed whether there was heterogeneity between results of the subgroup of trials of vitamin D and the subgroup of trials of vitamin D plus calcium. Within each subgroup, we assessed statistical heterogeneity between summary data using the I^2 statistic (I^2>50%). We assessed publication bias using funnel plots and Egger's test (appendix). We then did cumulative meta-analyses, in which we added the results of each trial sequentially by date and calculated updated pooled effect estimates. We used Comprehensive Meta-analysis (version 2) for all statistical analyses. All tests were two-tailed and $p<0·05$ was regarded as significant. Finally, we did trial sequential analysis.[6,7] Cumulative meta-analyses are at risk of false-positive results because of repetitive statistical testing, a situation analogous to repeated interim assessments in a randomised controlled trials. Trial sequential analysis maintains the overall risk of type-1 error at 5%, and also reports the information size, an estimate of the optimum sample size for statistical inference from a meta-analysis, after taking into account heterogeneity of included studies. Trial sequential analysis provides estimates of treatment effects, and thresholds for statistical significance and futility (ie, an effect is not statistically significant despite an optimum sample size) taking into account multiple statistical tests. For our analyses, we chose to calculate thresholds using a 15% risk reduction for all events, except for mortality for which we used a 5% risk reduction. We think these thresholds are the smallest effects that are clinically relevant for an individual—smaller benefits are unlikely to be attractive because the absolute benefit of treatment is small, and there is a high likelihood of no benefit for an individual. For smaller thresholds, the optimum sample size increases substantially and is generally much larger than the number of participants in the current meta-analysis, which could preclude the calculation of futility boundaries. For meta-analyses of trials with low heterogeneity, we assumed between-trial heterogeneity of 15%, and for meta-analyses of trials with high heterogeneity, we used the value derived from the random-effects meta-analysis. We did these analyses using Trial Sequential Analysis (version 0.9 beta).

	Participants (vitamin D / no vitamin D)	Age (years)	Sex (percentage female)	Duration	Treatment groups	Dose (vitamin D or vitamin D + calcium [for CaD])	Primary endpoint	Secondary endpoints	Baseline 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*	Achieved 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*
Inkovaara et al, 1983[21]	181/146	80	83%	1 year	2×3 factorial†: vitamin D, calcium, methandienone placebo	1000 IU per day/3 g per day	Biochemistry	IHD, CBVD, death	NS	NS
Corless et al, 1985[22]	41/41	82	78%	40 weeks	vitamin D and placebo	9000 IU per day	ADL	Death	18/17 (all)	110/20 (all)
Chapuy et al, 1992[23,24]	1634/1636	84	100%	3 years	CaD and placebo	800 IU + 1·2 g per day	Fracture	Death	40/32·5 (73/69)	105/27·5 (73/69)
Ooms et al, 1995[25]	177/171	80	100%	2 years	Vitamin D and placebo	400 IU per day	BMD	Death	27/25 (all)	62/23 (all)
Lips et al, 1996[26]	1291/1287	80	74%	4 years	Vitamin D and placebo	400 IU per day	Fracture	Death	26/27 (270)	54/23 (96)
Dawson-Hughes et al, 1987[27]	187/202	71	55%	3 years	CaD and placebo	700 IU + 500 mg per day	BMD	Fracture, death	77/72 (313)	112/70 (313)
Baeksgaard et al, 1988[28]	80/80	62	100%	2 years	CaD and placebo	560 IU + 1 g per day	BMD	Death	NS	NS
Komulainen et al, 1998[29,30]	232/232	53	100%	5 years	2×2 factorial†: vitamin D, HRT, placebo	300 IU per day for 4 years then 100 IU per day	BMD	Fracture, MI, stroke, cancer, death	NS	NS
Krieg et al, 1999[31]	124/124	85	100%	2 years	CaD and control	880 IU + 1·1 g per day	BMD	Death	30/29 (34/38)	66/14 (34/38)
Pfeifer et al, 2000[32]	74/74	74	100%	1 year	CaD and calcium	800 IU + 1·2 g per day/1·2 g per day	Body sway	Fracture	26/25 (all)	66/43 (all)
Chapuy et al, 2002[33]	389/194	85	100%	2 years	CaD and placebo	800 IU + 1·2 g per day	Biochemistry	Fracture, death	22/23 (all)	75/15 (all)
Meyer et al, 2002[34]	569/575	85	76%	2 years	Vitamin D and placebo	400 IU per day	Fracture	Death	47/51 (31/34)	64/46 (31/34)
Bischoff et al, 2003[35]	62/60	85	100%	12 weeks	CaD and calcium	800 IU + 1·2 g per day/1·2 g per day	Falls	Fracture, death	31/29 (all)	66/29 (all)
Cooper et al, 2003[36]	93/94	56	100%	2 years	CaD and calcium	10 000 IU per week + 1 g per day/1 g per day	BMD	Death	82/83 (all)	81/70 (73/80)
Latham et al, 2003[37]	121/122	79	65%	6 months	Vitamin D plus exercise and placebo plus exercise	300 000 IU (one-off dose)	Health	Falls, death	38/48 (all)	60/48 (all)
Trivedi et al, 2003[38]	1345/1341	75	24%	5 years	Vitamin D and placebo	100 000 IU every 4 months	Fracture	IHD, CBVD, cancer, death	NS	74/53 (124/114)
Avenell et al, 2004[14,39]	70/64	77	83%	46 months	2×2 factorial†: vitamin D, calcium, control	800 IU per day + 1 g per day	Compliance	Fracture, death	NS	NS
Harwood et al, 2004[40]	113/37	81	100%	1 year	IM vitamin D, IM vitamin D plus calcium, CaD, control	300 000 IU/300 000 IU + 1g per day/800 IU + 1 g per day	BMD	Fracture, death	29/30 (all)	45/32 (71/32)
Meier et al, 2004[41]	30/25	56	58%	2 years	CaD and control	500 IU + 500 mg per day	BMD	Death	75/77 (27/16)	82/64 (27/16)
Aloia et al, 2005[42]	104/104	61	100%	3 years	CaD and calcium	800 IU per day for 2 years then 2000 IU per day + 1·2–1·5 g per day/1·2–1·5 g per day	BMD	Death	47/43 (all)	87/NS (all)
Brazier et al, 2005[43]	95/97	75	100%	1 years	CaD and placebo	800 IU + 1g per day	Adverse events	MI, stroke, death	18/18 (all)	72/27 (all)
Flicker et al, 2005[44]	313/312	83	95%	2 years	CaD and calcium plus placebo	10 000 IU per week then 1000 IU per day + 600 mg per day/600 mg per day	Falls	Fracture, death	NS	NS
Porthouse et al, 2005[45]	1321/1993	77	100%	25 months	CaD and control	800 IU per day + 1 g per day	Fracture	Death	NS	NS

(Table continues on next page)

	Participants (vitamin D / no vitamin D)	Age (years)	Sex (percentage female)	Duration	Treatment groups	Dose (vitamin D or vitamin D + calcium [for CaD])	Primary endpoint	Secondary endpoints	Baseline 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*	Achieved 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*
(Continued from previous page)										
Grant et al, 2005[46]	2649/2643	77	85%	45 months	2×2 factorial†: vitamin D, calcium, placebo	800 IU per day/1 g calcium per day	Fracture	MI, stroke, cancer, death	38 (60)	62/44 (60)
WHI trials, 2006–07[47–49]	18176/18106	62	100%	7 years	CaD and placebo	400 IU + 1 g per day	Fracture	MI, stroke, cancer, death	48 (357)	61/NS‡ (227/221)
Bolton-Smith et al, 2007[50]	62/61	69	100%	2 years	CaD and placebo	400 IU + 1 g per day	BMD	Fracture, death	57/63 (all)	75/49 (all)
Broe et al, 2007[51]	99/25	89	73%	5 months	Vitamin D and placebo	200, 400, 600, or 800 IU per day	Falls	Death	48/53 (All)	63/60 (all)
Burleigh et al, 2007[52]	100/103	83	59%	1 month	CaD and calcium	800 IU + 1·2 g per day/1·2 g per day	Falls	Fracture, death	25/22 (54)	27/22 (NS)
Lappe et al, 2007[53]	446/734	67	100%	4 years	CaD, calcium, placebo	1100 IU per day + 1·4–1·5 g per day/1·4–1·5 g per day	BMD	MI, stroke, cancer, death	72/72 (All)	96/71 (All)
Lyons et al, 2007[54]	1725/1715	84	76%	3 years	Vitamin D and placebo	100 000 IU every 4 months	Fracture	Death	NS	80/54 (102)
Smith et al, 2007[55]	4727/4713	79	54%	3 years	IM vitamin D and placebo	300 000 IU every year	Fracture	Death	56·5 (43)	+21%/NS (NS)
Björkman et al, 2008[56]	150/68	85	82%	6 months	Vitamin D and placebo	5600 or 16 800 IU per week	Biochemistry	Death	22/23 (all)	60/25 (all)
Chel et al, 2008[57]	166/172	84	78%	4 months	Vitamin D and placebo	600 IU per day, 4200 IU per week, or 18 000 IU per month	Biochemistry	Death	25/25 (all)	63/27 (all)
Prince et al, 2008[58]	151/151	77	100%	1 year	CaD and calcium plus placebo	1000 IU + 1 g per day/1 g per day	Falls	Fracture, IHD, stroke, cancer, death	45/44 (all)	60/45–55§ (all)
Zhu et al, 2008[59]	39/81	75	100%	5 years	CaD, calcium, placebo	1000 IU per day + 1·2 g per day/1·2 g per day	BMD	Death	70/67 (all)	106/63 (all)
Pfeifer et al, 2009[60]	121/121	77	75%	20 months	CaD and calcium	800 IU + 1 g per day/1 g per day	Falls	Fracture	55/54 (all)	84/57 (all)
Lips et al, 2010[61]	114/112	78	NS	16 weeks	vitamin D placebo	8400 IU per week	Body sway	Death	34/35 (all)	65/35 (all)
Salovaara et al, 2010[62]	1718/1714	67	100%	3 years	CaD and control	800 IU + 1 g per day	Fracture	Death	50/49 (279/295)	75/56 (279/295)
Sanders et al, 2010[63]	1131/1125	76	100%	3–5 years	Vitamin D and placebo	500 000 IU every year	Fracture	CVD, cancer, death	53/45 (74/57)	55–74/40–50¶ (16–57/20–49)
Glendenning et al, 2012[64]	353/333	77	100%	9 months	Vitamin D and placebo	150 000 IU every 3 months	Falls	Death	65/67 (20/20)	75/61 (20/20)

25OHD=25-hydroxyvitamin D. IHD=ischaemic heart disease. CBVD=cerebrovascular disease. CaD=co-administered calcium and vitamin D. ADL=activities of daily living. HRT=hormone replacement therapy. MI=myocardial infarction. NS=not stated. CVD=cardiovascular disease. IM=intramuscular. BMD=bone mineral density.*25OHD concentrations were measured in subgroups of participants for most studies, except those labelled "all", in which measurements were done in all participants; where a single number is reported, the number of participants with measurements was not reported by treatment group. †Factorial study with all possible combinations of the interventions (2×2= four treatment groups, 2×3= eight treatment groups). ‡25OHD concentrations were stated to be 28% higher in the CaD group than controls, or about 61 nmol/L (based on reported baseline values). §Achieved 25OHD concentrations were about 60 nmol/L in winter–spring and summer–autumn in the vitamin D group, and 45 nmol/L in winter–spring and 55 nmol/L in summer–autumn in the control group. ¶25OHD concentrations measured annually, ranging from 55–74 nmol/L in the vitamin D group, and about 40–50 nmol/L in the control group; the number of participants with measurements at each timepoint ranged from 16–57 and 20–49, respectively.

Table: **Study characteristics**

Role of the funding source

The sponsor of this study had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the paper; or decision to submit the manuscript for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Figure 1 shows the results of traditional meta-analyses of the effects of vitamin, vitamin D and calcium, and vitamin D with or without calcium on myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, and total cancer (non-skeletal endpoints). We found no significant heterogeneity between the results of trials of vitamin D and trials of

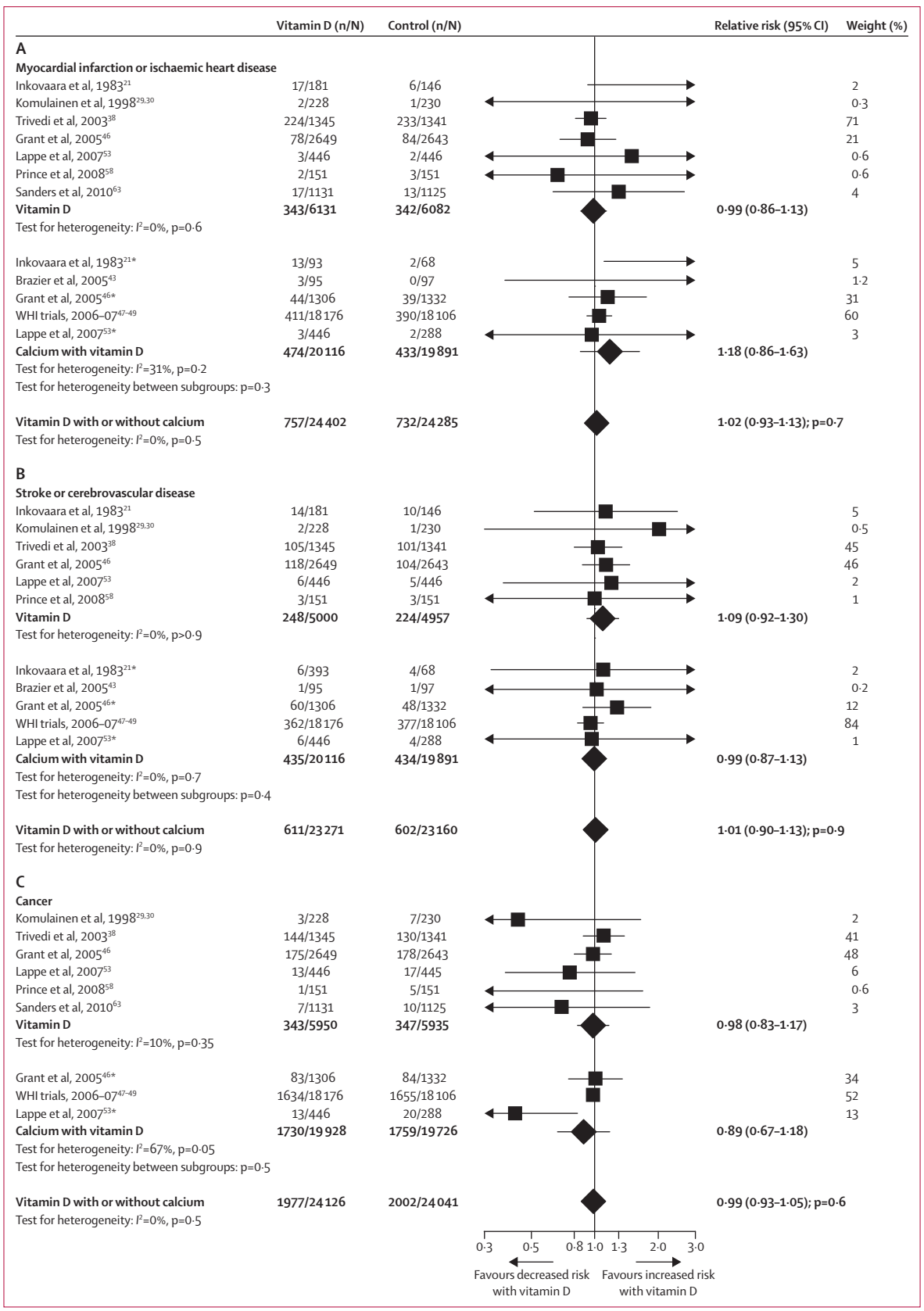

Figure 1: **Random effects meta-analyses of vitamin D, vitamin D with calcium, and vitamin D with or without calcium on non-skeletal endpoints**

*Multi-arm or factorial studies permitting a separate comparison of vitamin D with calcium and placebo.

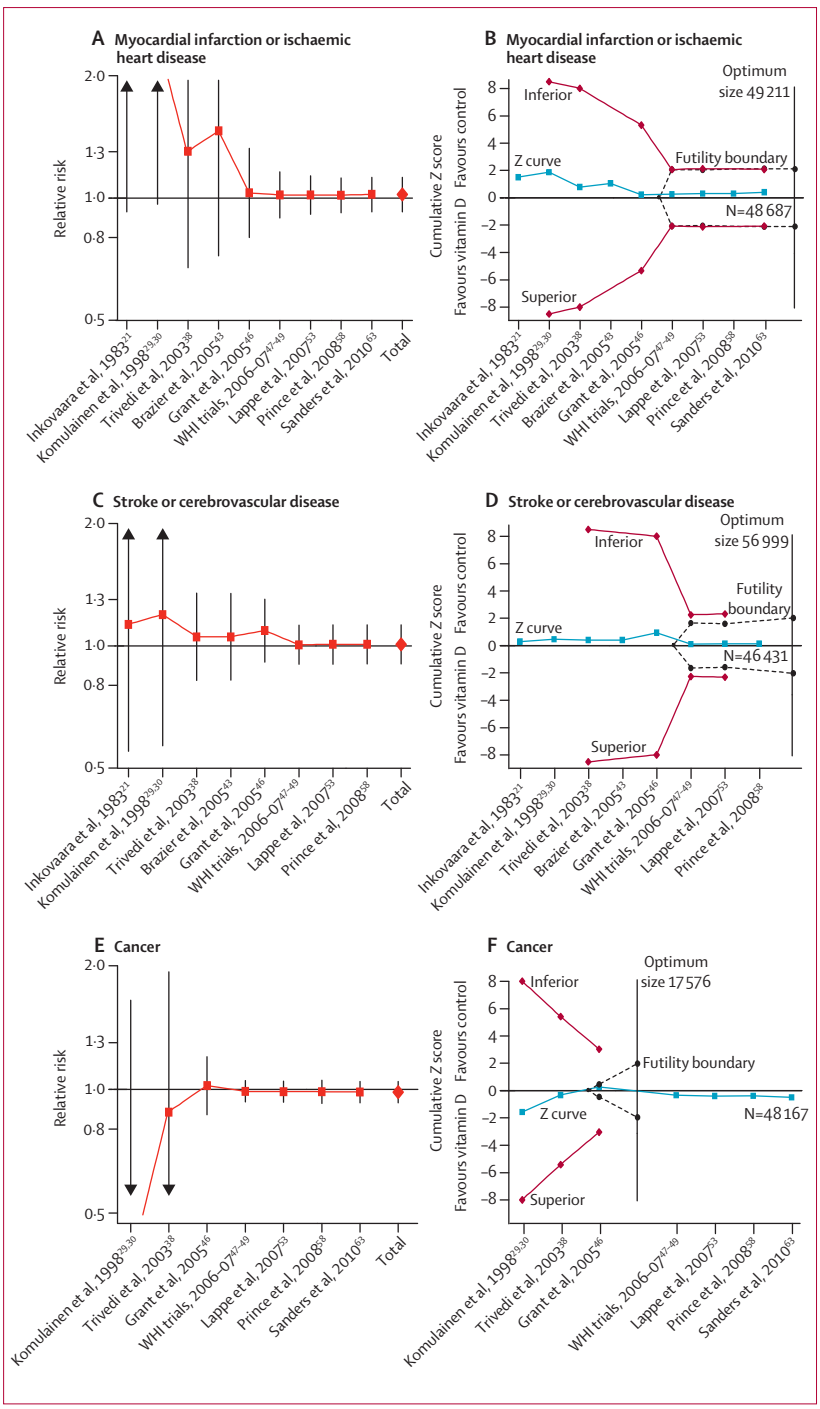

***Figure 2*: Cumulative random effects meta-analyses and trial sequential analyses of vitamin D with or without calcium on non-skeletal endpoints**

Cumulative random effects meta-analyses for myocardial infarction or ischaemic heart disease (A), stroke or cerebrovascular disease (C), and cancer (E). Trial sequential analyses for myocardial infarction or ischaemic heart disease (B), stroke or cerebrovascular disease (D), and cancer (F). For trial sequential analyses, the *Z* curve is a measure of treatment effect, and the boundaries are thresholds for statistical significance adjusted for heterogeneity of trial results and multiple statistical testing. A treatment effect outside the statistical significance boundary (red line) indicates that there is reliable evidence of a treatment effect, and a treatment effect within the futility boundary (dotted line) indicates that there is reliable evidence of no treatment effect. Optimum size indicates the calculated optimum sample size for statistical inference and N indicates the number of participants in the meta-analysis.

vitamin D and calcium for any these outcomes (figure 1). Vitamin D with or without calcium had no effect on myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, or cancer (figure 1). Results were similar to the pooled analyses when we considered separately outcomes for myocardial infarction (five trials, 43 116; relative risk 1·04, 95% CI 0·91–1·17; p=0·6) and ischaemic heart disease or cardiovascular disease (four trials, 5571; 1·12, 0·78–1·62; p=0·5), and outcomes for stroke (six trials, 43 418; 1·00, 0·88–1·13; p>0·9), and cerebrovascular disease (two trials, 3013; 1·05, 0·82–1·34; p=0·7). Subgroup analyses did not show statistically significant interactions between baseline 25OHD concentrations, achieved 25OHD concentrations, or treatment duration for any of the endpoints (appendix).

Figure 2 shows cumulative meta-analyses and trial sequential analyses for the effects of vitamin D with or without calcium on myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, and total cancer. The pooled sample size for the trial sequential meta-analysis exceeded the calculated optimum sample size for the cancer endpoint, closely approximated (99%) the optimum sample size for myocardial infarction or ischaemic heart disease, and was 81% of the optimum sample size for stroke or cerebrovascular disease. For each endpoint, the effect estimate lay within the futility boundary, providing evidence that vitamin D supplementation does not alter the relative risk of any of these endpoints by 15% or more.

We did additional sensitivity analyses. We repeated the trial sequential analyses using a threshold of 10% risk reduction. The optimum sample size increased by 2–3 times for each endpoint. For myocardial infarction or ischaemic heart disease, the effect estimate lay between the inferiority and futility boundary, whereas for both stroke or cerebrovascular disease and cancer, there was insufficient information to calculate futility boundaries. Data from two re-analyses of the Women's Health Initiative calcium and vitamin D trial[11,67] suggested that widespread use of personal calcium and vitamin D supplements in the trial might have obscured adverse effects of calcium and vitamin D on cardiovascular events and beneficial effects on cancer. We repeated the trial sequential analyses using results from those re-analyses restricted to women not using personal calcium supplements. For myocardial infarction or ischaemic heart disease and stroke or cerebrovascular disease, the effect estimate lay between the inferiority and futility boundary, whereas for cancer the effect estimate lay within the futility boundary.

Figure 3 shows the results of the traditional meta-analyses for total fracture and hip fracture (skeletal endpoints). We found statistically significant heterogeneity between the results of trials of vitamin D and trials of vitamin D and calcium for hip fracture (p=0·004), but not for total fracture (p=0·4). Vitamin D with or without calcium had no effect on total fracture

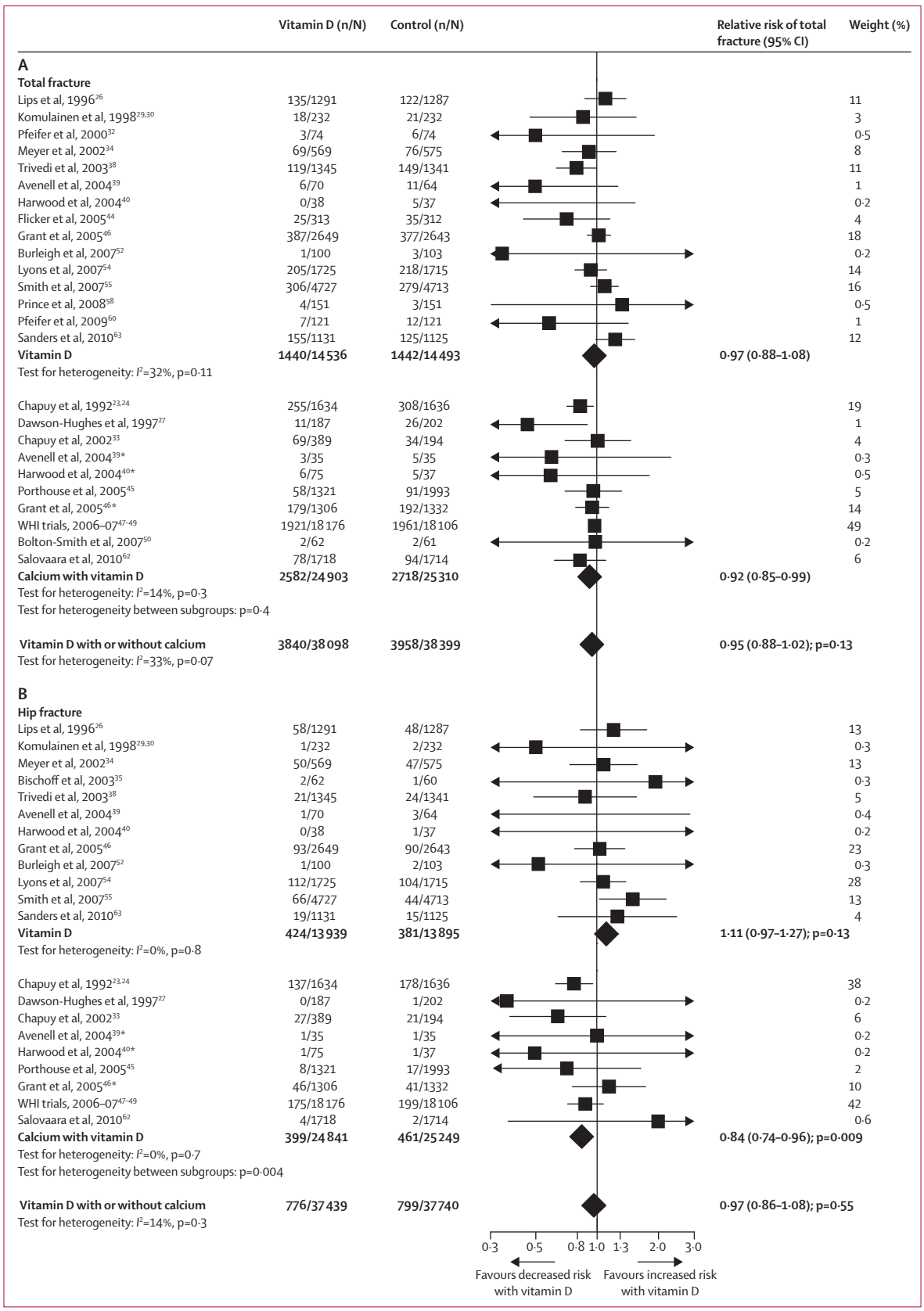

***Figure 3*: Random effects meta-analyses of vitamin D, vitamin D with calcium, and vitamin D with or without calcium on skeletal endpoints**

*Multi-arm or factorial studies permitting a separate comparison of vitamin D with calcium and placebo.

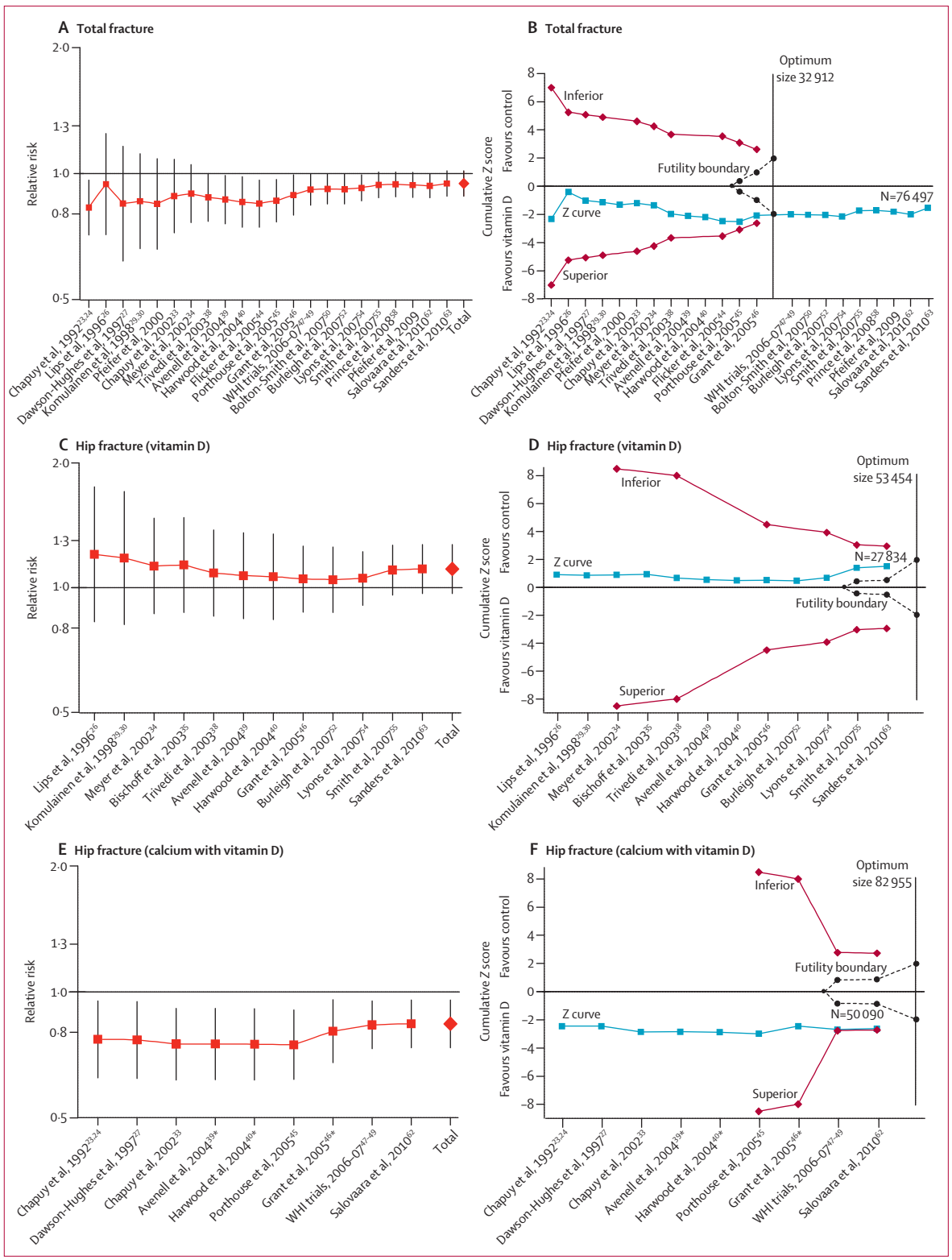

Figure 4: **Cumulative random effects meta-analyses and trial sequential analyses of vitamin D with or without calcium on skeletal endpoints**

Cumulative random effects meta-analyses for total fracture (A), hip fracture (vitamin D; C), and hip fracture (calcium and vitamin D; E). Trial sequential analyses for total fracture (B), hip fracture (vitamin D; D), and hip fracture (calcium and vitamin D; F). For trial sequential analyses, the *Z* curve is a measure of treatment effect, and the boundaries are thresholds for statistical significance adjusted for heterogeneity of trial results and multiple statistical testing. A treatment effect outside the statistical significance boundary (red line) indicates that there is reliable evidence of a treatment effect, and a treatment effect within the futility boundary (dotted line) indicates that there is reliable evidence of no treatment effect. Optimum size indicates the calculated optimum sample size for statistical inference and N indicates the number of participants in the meta-analysis.

(figure 3). Vitamin D had no effect on hip fracture, but co-administered vitamin D and calcium reduced hip fractures (figure 3).

Figure 4 shows cumulative meta-analyses and trial sequential analyses for the effects of vitamin D with or without calcium on total fracture. Because of the

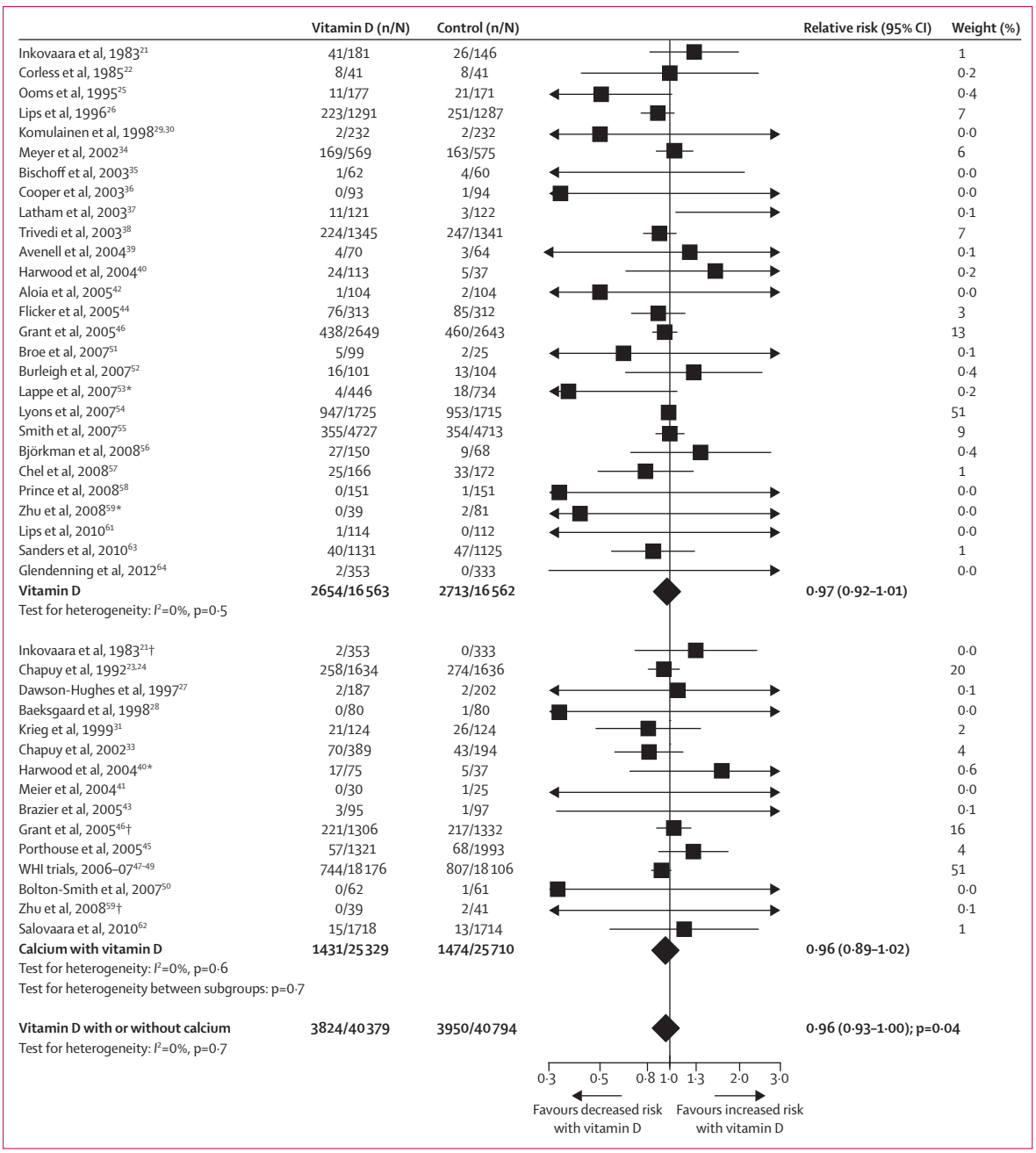

Figure 5: **Random effects meta-analyses of vitamin D, vitamin D with calcium, and vitamin D with or without calcium on mortality**
*Control group includes both placebo arm and calcium monotherapy arm. †Multi-arm or factorial studies permitting a separate comparison of vitamin D with calcium and placebo.

heterogeneity in results for hip fracture, these analyses are presented separately for vitamin D and vitamin D with calcium. For total fracture, the calculated optimum sample size was exceeded and the effect estimate lay within the futility boundary, indicating that vitamin D does not alter the relative risk of total fracture by 15% or more. For vitamin D and hip fracture, the pooled sample size was 52% of optimum and the effect estimate lay between the futility and inferiority boundary, indicating uncertainty as to whether vitamin D increases the relative risk of hip fracture. These findings indicate that vitamin D does not reduce hip fracture by 15% or more. For vitamin D with calcium and hip fracture, the pooled sample size was 60% of optimum and the effect estimate lay between the futility and superiority boundary, indicating uncertainty as to whether vitamin D with

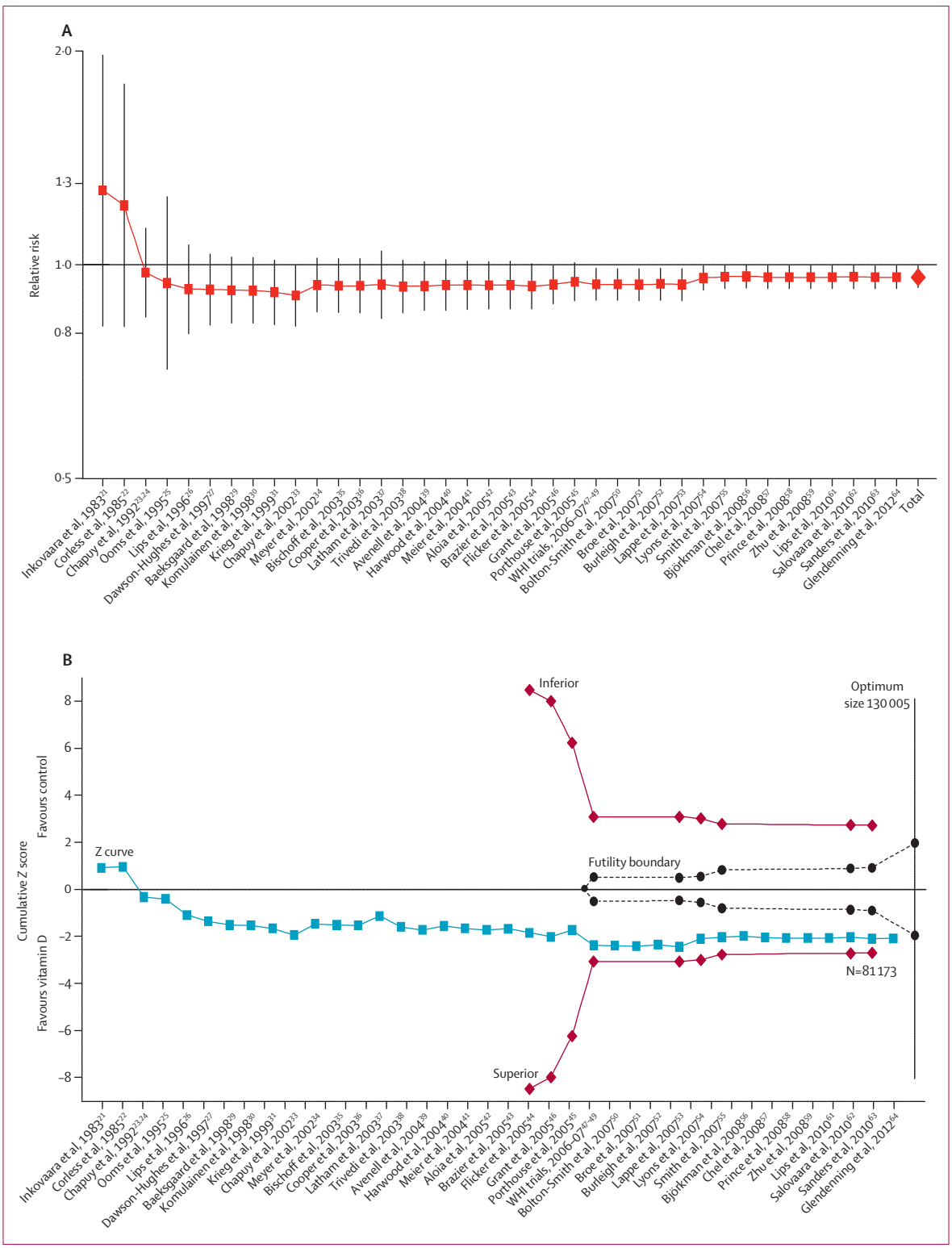

Figure 6: **Cumulative random effects meta-analysis (A) and trial sequential analyses (B) of vitamin D with or without calcium on mortality**
For trial sequential analyses, the Z curve is a measure of treatment effect, and the boundaries are thresholds for statistical significance adjusted for heterogeneity of trial results and multiple statistical testing. A treatment effect outside the statistical significance boundary (red line) indicates that there is reliable evidence of a treatment effect, and a treatment effect within the futility boundary (dotted line) indicates that there is reliable evidence of no treatment effect. Optimum size indicates the calculated optimum sample size for statistical inference and N indicates the number of participants in the meta-analysis.

calcium decreases the relative risk of hip fracture by 15% or more.

In additional sensitivity analyses, we repeated the trial sequential analyses using a threshold of 10% risk reduction. The optimum sample size roughly doubled for each endpoint. For total fracture, the effect estimate lay within the futility boundary; for vitamin D and hip fracture, there was insufficient information to calculate futility boundaries; and for vitamin D with calcium and hip fracture, the effect estimate lay between the futility and superiority boundary. Previous meta-analyses have suggested that vitamin D with calcium reduces fracture incidence in individuals living in institutions but not those living in the community.[14] To find out the effect of vitamin D in community-dwelling individuals we repeated our trial sequential analyses after excluding seven trials that included institutionalised individuals.[23,33–35,44,52,54] For total fracture, the effect estimate lay within the futility boundary; for vitamin D and hip fracture, the effect estimate lay between the futility and inferiority boundary; and for vitamin D with calcium and hip fracture, the effect estimate lay within the futility boundary. In trial sequence analyses of the trials that included institutionalised individuals, for vitamin D with calcium and hip fracture (two trials[23,24,33]), the effect estimate lay below the superiority boundary. Finally, in traditional random-effects models of the 11 trials with total fracture as a primary endpoint, the relative risk for vitamin D with or without calcium was 0·97 (0·91–1·04; p=0·43), and in the 11 trials with total fracture as a secondary endpoint only, the relative risk was 0·75 (0·60–0·93; p=0·01).

Figures 5 and 6 show the results of the traditional meta-analyses, cumulative meta-analysis, and trial sequential analysis for the effects of vitamin D with or without calcium on mortality. We found no statistically significant heterogeneity between the results of trials of vitamin D and trials of vitamin D with calcium for mortality. In traditional meta-analyses, vitamin D with or without calcium reduced the risk of death (figure 5). However, in the trial sequential analysis, the pooled sample size was 60% of optimum, and the effect estimate lay between the futility and superiority boundary, indicating uncertainty as to whether vitamin D with calcium decreases the relative risk of mortality by 5% or more (figure 6). We repeated the trial sequential analyses using a threshold of 10% risk reduction, and although the optimum sample size decreased by 75%, the results were similar. Use of lower thresholds (3% or 4%) increased the optimum sample size substantially.

Discussion

Our analyses suggest that there is reliable existing evidence that supplementation of vitamin D with or without calcium does not reduce the incidence of myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, cancer, total fractures, or hip fractures in community-dwelling individuals by more than 15%. Vitamin D with calcium reduced hip fracture incidence in two trials of institutionalised individuals. There is uncertainty as to whether vitamin D with or without calcium has small effects on mortality. Further trials that are similar in design to existing trials are unlikely to alter these results.

For skeletal endpoints, we saw contrasting results. Vitamin D with or without calcium had no effect on total fracture in traditional meta-analyses. Trial sequential analysis suggested that vitamin D with or without calcium does not decrease total fracture by 15% or more, and that results from similar future trials are unlikely to alter these findings. For hip fracture, there is insufficient evidence to confidently ascertain whether vitamin D increases hip fracture incidence or has no effect, but similar future trials are unlikely to alter the finding that it does not reduce hip fracture incidence. Vitamin D with calcium decreased hip fracture incidence by 16% in the traditional meta-analysis, but trial sequential analysis suggested that there is uncertainty in this finding, and sensitivity analyses suggested that any benefit is restricted to institutionalised individuals. The two trials that were most influential in these analyses were done in elderly French women with low baseline 25OHD concentrations and calcium intakes (resulting in secondary hyperparathyroidism), by the same group of investigators.[23,24,33] In community-dwelling individuals, trial sequential analyses suggested that vitamin D with or without calcium does not decrease total fracture or hip fracture by 15% or more.

For mortality, vitamin D with or without calcium reduced the risk of death by 4% in traditional meta-analyses, but trial sequential analysis suggested that uncertainty remains in this finding.

We included 40 randomised controlled trials of older men and women with a range of risks for all endpoints. Our dataset also had a broad range of doses of administered vitamin D, and most trials were done in populations with 25OHD concentrations lower than 50 nmol/L and achieved 25OHD concentrations of 50 nmol/L or greater with vitamin D supplements (appendix). The absence of effect of vitamin D could be because the populations studied have not had low enough vitamin D concentrations to benefit from supplementation. This seems unlikely because most trials had baseline 25OHD concentrations lower than 50 nmol/L, which is widely thought to indicate vitamin D insufficiency.[19] Trials of vitamin D supplementation in individuals with more pronounced vitamin D deficiency might produce different results. However, before such trials are undertaken, there should be strong evidential support underpinning the trial rationale, particularly in view of the absence of effects seen in studies done thus far.

Another possible explanation for the present null findings is that 25OHD concentrations did not increase sufficiently in groups treated with vitamin D for benefits to occur. This explanation also seems unlikely, because 25OHD concentrations increased after vitamin D

supplementation in most studies and were greater than 50 nmol/L in almost all trials (and much higher in several trials; appendix). Furthermore, most of the evidence linking vitamin D insufficiency and non-skeletal events comes from observational studies. These studies report that small increments in 25OHD concentrations within the pre-treatment and post-treatment ranges seen in the trials analysed here are associated with decreased rates of cardiovascular events and cancer. Therefore, some benefits should have been seen in the trials we analysed if the findings from the observational studies are generalisable to randomised controlled trials, although these trials might not have detected maximum benefits of vitamin D supplements. For these reasons, future trials with similar study designs to those in our dataset, or those that only differ by dose of vitamin D, are unlikely to produce differing results from the trials we analysed, or substantially alter the findings of our meta-analyses.

It is also possible that vitamin D supplementation affects the incidence of one or more of the endpoints in our analyses, but that our meta-analyses are underpowered to detect the effects. An important question is what effect a positive result (reduction in risk in the vitamin D intervention arm) from a future large trial would have on the existing meta-analyses. A small effect size could alter the overall estimate, but the sample size needed for such a trial to do so is impractically large (usually >50 000 participants). For example, investigators doing a large ongoing randomised clinical trial of vitamin D estimated that a 5-year, 20 000-person, placebo-controlled randomised clinical trial would have only 52% power to detect a 12·5% reduction in cancer incidence.[68] The issue of sample size is particularly relevant for analysis of mortality, in which the optimum sample size in our trial sequential analysis for a 5% risk reduction is greater than 130 000 participants, increasing to greater than 200 000 participants for a 4% risk reduction, and greater than 350 000 participants for a 3% risk reduction. If the effect size in a future trial is large (eg, >20% risk reduction), the inclusion of the trial would substantially increase the heterogeneity of the results in the meta-analysis. The use of a random-effects model means that such a trial would not receive sufficient weighting in the pooled analyses to alter the pooled result substantially. Thus, if such a positive result were reported, it would be so different from those from that of previous studies that it probably should not be pooled with results of existing studies.

The efficacy thresholds we chose for the primary trial sequential analyses (15% risk reduction) could be too high. At an individual level, small treatment effects are unlikely to be attractive to patients because the absolute benefit does not justify the effort of taking the treatment. At a population level, however, small effects could produce substantial benefits if the outcome is common, and the treatment is used widely and is safe. However, this justification leads to a somewhat circular argument. A strong evidence base is needed before widespread treatment can be introduced. Available evidence does not lend support to vitamin D supplementation and it is very unlikely that the results of a future single randomised clinical trial will materially alter the results from current meta-analyses. Thus, several large randomised controlled trials with results that differ substantially from trials included here would be needed to provide convincing evidence that any small treatment effect (<15% risk reduction) is a real finding. The consistency of results in trials done so far suggests that the likelihood that such results will be reported is low. Furthermore, the absence of positive findings in large number of trials completed thus far suggests that similar future trials will have a high chance of null or negative results and therefore might be viewed as a low priority by research funders.

A limitation of our analysis is that in many of the included studies the outcomes reported were not the primary endpoint of the study. Data for these secondary endpoints might not have been collected in the same manner or subjected to the same amount of scrutiny as data for the primary endpoint in the trials. This possibility is unlikely to introduce a differential bias between the groups. We assessed the effect of type of study endpoint on the effect of vitamin D with or without calcium on total fracture. Pooled analyses of trials with fracture as a secondary endpoint suggested beneficial effects for vitamin D, whereas we saw no effect in trials with fracture as the primary endpoint. This suggests the level of endpoint might affect results, but, for total fracture, inclusion of studies with fracture only as a secondary endpoint did not bias results toward null findings, and might indicate publication bias.

In view of our findings, there is little justification for prescribing vitamin D supplements to prevent myocardial infarction or ischaemic heart disease, stroke or cerebrovascular disease, cancer, or fractures, or to reduce the risk of death in unselected community-dwelling individuals. Investigators and funding bodies should consider the probable futility of undertaking similar trials of vitamin D to investigate any of these endpoints.

Contributors

MJB, AG, GDG, and IRR had the idea for and designed the study. MJB and AG acquired the data. MJB, AG, GDG, and IRR did the analysis and interpretation of data. MB wrote the first draft of the paper, with subsequent revisions from AG, GDG, and IRR. MJB and GDG did the statistical analysis.

Conflicts of interest

We declare that we have no conflicts of interest.

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