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

Mark J Bolland, Andrew Grey, Greg D Gamble, Ian R Reid

Summary

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

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 effect 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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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. Such findings have led to calls for widespread vitamin D supplementation. 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. 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 trials on the existing body of data. 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, three for cancer, four for fracture, and six for mortality. 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.
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,26 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).21–44 For one trial,26 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,26 but we included causes of mortality. We obtained unpublished data for two trials26,31 from meta-analyses of calcium supplements.32–34 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.21,23,39,40,46,51,59 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 I² statistic (I²>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.67 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-I 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).
<table>
<thead>
<tr>
<th>Participants (vitamin D / no vitamin D)</th>
<th>Age (years)</th>
<th>Sex (percentage female)</th>
<th>Duration</th>
<th>Treatment groups</th>
<th>Dose (vitamin D or vitamin D + calcium [for CaD])</th>
<th>Primary endpoint</th>
<th>Secondary endpoints</th>
<th>Baseline 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*</th>
<th>Achieved 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inkovaara et al, 1983&lt;sup&gt;21&lt;/sup&gt;</td>
<td>181/146</td>
<td>80</td>
<td>83%</td>
<td>1 year</td>
<td>2×3 factorial: vitamin D, calcium, methandienone placebo</td>
<td>1000 IU per day/3 g per day</td>
<td>Biochemistry</td>
<td>IHD, CBVD, death</td>
<td>NS</td>
</tr>
<tr>
<td>Coffless et al, 1985&lt;sup&gt;21&lt;/sup&gt;</td>
<td>41/41</td>
<td>82</td>
<td>78%</td>
<td>40 weeks</td>
<td>vitamin D and placebo</td>
<td>9000 IU per day</td>
<td></td>
<td>ADL</td>
<td>Death</td>
</tr>
<tr>
<td>Chapuy et al, 1992&lt;sup&gt;22,23&lt;/sup&gt;</td>
<td>163/1636</td>
<td>84</td>
<td>100%</td>
<td>3 years</td>
<td>CaD and placebo</td>
<td>800 IU + 1.2 g per day</td>
<td>Fracture</td>
<td>Death</td>
<td>40/32 (73/69)</td>
</tr>
<tr>
<td>Ooms et al, 1995&lt;sup&gt;22&lt;/sup&gt;</td>
<td>17/171</td>
<td>80</td>
<td>100%</td>
<td>2 years</td>
<td>Vitamin D and placebo</td>
<td>400 IU per day</td>
<td></td>
<td>BMD</td>
<td>Death</td>
</tr>
<tr>
<td>Lips et al, 1996&lt;sup&gt;22&lt;/sup&gt;</td>
<td>1291/1287</td>
<td>80</td>
<td>74%</td>
<td>4 years</td>
<td>Vitamin D and placebo</td>
<td>400 IU per day</td>
<td></td>
<td></td>
<td>Fracture</td>
</tr>
<tr>
<td>Dawson-Hughes et al, 1987&lt;sup&gt;27&lt;/sup&gt;</td>
<td>187/202</td>
<td>71</td>
<td>55%</td>
<td>3 years</td>
<td>CaD and placebo</td>
<td>700 IU + 500 mg per day</td>
<td></td>
<td></td>
<td>BMD</td>
</tr>
<tr>
<td>Baeksgaard et al, 1988&lt;sup&gt;28&lt;/sup&gt;</td>
<td>80/80</td>
<td>62</td>
<td>100%</td>
<td>2 years</td>
<td>CaD and placebo</td>
<td>560 IU + 1 g per day</td>
<td></td>
<td></td>
<td>BMD</td>
</tr>
<tr>
<td>Komulainen et al, 1998&lt;sup&gt;29,30&lt;/sup&gt;</td>
<td>232/232</td>
<td>53</td>
<td>100%</td>
<td>5 years</td>
<td>2×2 factorial: vitamin D, HRT, placebo</td>
<td>300 IU per day for 4 years then 100 IU per day</td>
<td>BMD</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Krieg et al, 1999&lt;sup&gt;31&lt;/sup&gt;</td>
<td>124/124</td>
<td>85</td>
<td>100%</td>
<td>2 years</td>
<td>CaD and control</td>
<td>880 IU + 1.1 g per day</td>
<td>BMD</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Pfeifer et al, 2000&lt;sup&gt;32&lt;/sup&gt;</td>
<td>74/74</td>
<td>74</td>
<td>100%</td>
<td>1 year</td>
<td>CaD and calcium</td>
<td>800 IU + 1.2 g per day</td>
<td></td>
<td>Body sway</td>
<td>Fracture</td>
</tr>
<tr>
<td>Meyer et al, 2002&lt;sup&gt;33&lt;/sup&gt;</td>
<td>389/194</td>
<td>85</td>
<td>100%</td>
<td>2 years</td>
<td>CaD and placebo</td>
<td>800 IU + 1.2 g per day</td>
<td>Biochemistry</td>
<td></td>
<td>Fracture, death</td>
</tr>
<tr>
<td>Bischoff et al, 2003&lt;sup&gt;34&lt;/sup&gt;</td>
<td>569/575</td>
<td>85</td>
<td>76%</td>
<td>2 years</td>
<td>CaD and placebo</td>
<td>800 IU + 1 g per day</td>
<td>Fracture</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Cooper et al, 2003&lt;sup&gt;35&lt;/sup&gt;</td>
<td>93/94</td>
<td>56</td>
<td>100%</td>
<td>2 years</td>
<td>CaD and calcium</td>
<td>10 000 IU per week + 1 g per day</td>
<td>BMD</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Latham et al, 2003&lt;sup&gt;36&lt;/sup&gt;</td>
<td>121/122</td>
<td>79</td>
<td>65%</td>
<td>6 months</td>
<td>Vitamin D plus exercise and placebo plus exercise</td>
<td>300 000 IU (one-off dose)</td>
<td>Health</td>
<td>Falls, death</td>
<td>38/48 (all)</td>
</tr>
<tr>
<td>Trivedi et al, 2003&lt;sup&gt;37&lt;/sup&gt;</td>
<td>1345/1341</td>
<td>75</td>
<td>24%</td>
<td>5 years</td>
<td>Vitamin D and placebo</td>
<td>100 000 IU every 4 months</td>
<td>Fracture</td>
<td></td>
<td>IHD, CBVD, cancer, death</td>
</tr>
<tr>
<td>Avenell et al, 2004&lt;sup&gt;38,39&lt;/sup&gt;</td>
<td>70/64</td>
<td>77</td>
<td>83%</td>
<td>46 months</td>
<td>2×2 factorial: vitamin D, calcium, placebo</td>
<td>800 IU per day + 1 g per day</td>
<td>Compliance</td>
<td>Fracture, death</td>
<td>NS</td>
</tr>
<tr>
<td>Harwood et al, 2004&lt;sup&gt;40&lt;/sup&gt;</td>
<td>113/37</td>
<td>81</td>
<td>100%</td>
<td>1 year</td>
<td>IM vitamin D, IM vitamin D plus calcium, CaD, control</td>
<td>300 000 IU/300 000 IU + 1 g per day/800 IU + 1 g per day</td>
<td>BMD</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Meier et al, 2004&lt;sup&gt;41&lt;/sup&gt;</td>
<td>30/25</td>
<td>56</td>
<td>58%</td>
<td>2 years</td>
<td>CaD and control</td>
<td>500 IU + 500 mg per day</td>
<td>BMD</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Alosa et al, 2005&lt;sup&gt;42&lt;/sup&gt;</td>
<td>104/104</td>
<td>61</td>
<td>100%</td>
<td>3 years</td>
<td>CaD and calcium</td>
<td>800 IU per day for 2 years then 2000 IU per day + 1 g per day/600 mg per day</td>
<td></td>
<td>Fracture</td>
<td>Death</td>
</tr>
<tr>
<td>Brazer et al, 2005&lt;sup&gt;43&lt;/sup&gt;</td>
<td>95/97</td>
<td>75</td>
<td>100%</td>
<td>1 years</td>
<td>CaD and placebo</td>
<td>800 IU + 1 g per day</td>
<td>Fracture</td>
<td></td>
<td>MI, stroke, death</td>
</tr>
<tr>
<td>Flicker et al, 2005&lt;sup&gt;44&lt;/sup&gt;</td>
<td>313/312</td>
<td>83</td>
<td>95%</td>
<td>2 years</td>
<td>CaD and calcium plus placebo</td>
<td>10 000 IU per week then 1000 IU per day + 600 mg per day</td>
<td>Fracture</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Porthouse et al, 2005&lt;sup&gt;45&lt;/sup&gt;</td>
<td>1321/1993</td>
<td>77</td>
<td>100%</td>
<td>25 months</td>
<td>CaD and control</td>
<td>800 IU per day + 1 g per day</td>
<td>Fracture</td>
<td></td>
<td>Death</td>
</tr>
</tbody>
</table>

(Table continues on next page)
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 vitamin D with or without calcium.

Table: Study characteristics

(Continued from previous page)

<table>
<thead>
<tr>
<th>Participants (vitamin D / no vitamin D)</th>
<th>Age (years)</th>
<th>Sex (percentage female)</th>
<th>Duration</th>
<th>Treatment groups</th>
<th>Dose (vitamin D or vitamin D + calcium [for CaD])</th>
<th>Primary endpoint</th>
<th>Secondary endpoints</th>
<th>Baseline 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*</th>
<th>Achieved 25OHD concentrations in nmol/L; vitamin D/no vitamin D (N)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant et al, 2005*</td>
<td>264/2641</td>
<td>77</td>
<td>85%</td>
<td>45 months</td>
<td>800 IU per day/1 g calcium per day</td>
<td>Fracture</td>
<td>MI, stroke, cancer, death</td>
<td>38 (60)</td>
<td>62/44 (60)</td>
</tr>
<tr>
<td>Whit trials, 2006–07‡</td>
<td>18175/18106</td>
<td>62</td>
<td>100%</td>
<td>7 years</td>
<td>CaD and placebo</td>
<td>Fracture</td>
<td>MI, stroke, cancer, death</td>
<td>48 (357)</td>
<td>61/NS1 (222/221)</td>
</tr>
<tr>
<td>Bolton-Smith et al, 2007*</td>
<td>62/61</td>
<td>69</td>
<td>100%</td>
<td>2 years</td>
<td>CaD and placebo</td>
<td>BMD</td>
<td>Fracture, death</td>
<td>57/63 (all)</td>
<td>75/49 (all)</td>
</tr>
<tr>
<td>Broe et al, 2007*</td>
<td>99/25</td>
<td>89</td>
<td>73%</td>
<td>5 months</td>
<td>Vitamin D and placebo</td>
<td>Falls</td>
<td>Death</td>
<td>48/53 (All)</td>
<td>63/60 (all)</td>
</tr>
<tr>
<td>Burleigh et al, 2007†</td>
<td>100/103</td>
<td>83</td>
<td>59%</td>
<td>1 month</td>
<td>CaD and calcium</td>
<td>Falls</td>
<td>Fracture, death</td>
<td>25/22 (54)</td>
<td>27/22 (NS)</td>
</tr>
<tr>
<td>Lappe et al, 2007†</td>
<td>446/734</td>
<td>67</td>
<td>100%</td>
<td>4 years</td>
<td>CaD, calcium, placebo</td>
<td>1100 IU per day/1·4–1·5 g per day/1·4–1·5 g per day</td>
<td>BMD</td>
<td>MI, stroke, cancer, death</td>
<td>72/72 (All)</td>
</tr>
<tr>
<td>Lyons et al, 2017†</td>
<td>175/175</td>
<td>84</td>
<td>76%</td>
<td>3 years</td>
<td>Vitamin D and placebo</td>
<td>100 000 IU every 4 months</td>
<td>Fracture</td>
<td>Death</td>
<td>NS</td>
</tr>
<tr>
<td>Smith et al, 2007†</td>
<td>4727/4713</td>
<td>79</td>
<td>54%</td>
<td>3 years</td>
<td>IM vitamin D and placebo</td>
<td>300 000 IU every year</td>
<td>Fracture</td>
<td>Death</td>
<td>56/5 (43)</td>
</tr>
<tr>
<td>Bjorkman et al, 2008‡</td>
<td>150/68</td>
<td>85</td>
<td>82%</td>
<td>6 months</td>
<td>Vitamin D and placebo</td>
<td>5600 or 16 800 IU per week</td>
<td>Biochemistry</td>
<td>Death</td>
<td>22/23 (all)</td>
</tr>
<tr>
<td>Chel et al, 2008‡</td>
<td>166/172</td>
<td>84</td>
<td>78%</td>
<td>4 months</td>
<td>Vitamin D and placebo</td>
<td>600 IU per day, 2400 IU per week, or 18 000 IU per month</td>
<td>Biochemistry</td>
<td>Death</td>
<td>25/25 (all)</td>
</tr>
<tr>
<td>Prince et al, 2008‡</td>
<td>151/151</td>
<td>77</td>
<td>100%</td>
<td>1 year</td>
<td>CaD and calcium plus placebo</td>
<td>1000 IU + 1 g per day/1·4–1·5 g per day</td>
<td>Fracture</td>
<td>IHD, stroke, cancer, death</td>
<td>45/44 (all)</td>
</tr>
<tr>
<td>Zhu et al, 2010‡</td>
<td>39/81</td>
<td>75</td>
<td>100%</td>
<td>5 years</td>
<td>CaD, calcium, placebo</td>
<td>1000 IU per day + 1·2 g per day/1·2 g per day</td>
<td>BMD</td>
<td>Death</td>
<td>70/67 (all)</td>
</tr>
<tr>
<td>Pfeifer et al, 2009‡</td>
<td>121/121</td>
<td>77</td>
<td>75%</td>
<td>20 months</td>
<td>CaD and calcium</td>
<td>800 IU + 1 g per day/1 g per day</td>
<td>Falls</td>
<td>Fracture</td>
<td>55/54 (all)</td>
</tr>
<tr>
<td>Lips et al, 2010‡</td>
<td>114/112</td>
<td>78</td>
<td>NS</td>
<td>16 weeks</td>
<td>vitamin D placebo</td>
<td>8400 IU per week</td>
<td>Body sway</td>
<td>Death</td>
<td>34/35 (all)</td>
</tr>
<tr>
<td>Salovaara et al, 2010†</td>
<td>1718/1714</td>
<td>67</td>
<td>100%</td>
<td>3 years</td>
<td>CaD and control</td>
<td>800 IU + 1 g per day</td>
<td>Fracture</td>
<td>Death</td>
<td>50/49 (279/295)</td>
</tr>
<tr>
<td>Sanders et al, 2010†</td>
<td>1131/1125</td>
<td>76</td>
<td>100%</td>
<td>3–5 years</td>
<td>Vitamin D and placebo</td>
<td>500 000 IU every year</td>
<td>Fracture</td>
<td>CVD, cancer, death</td>
<td>52/45 (74/57)</td>
</tr>
<tr>
<td>Glendenning et al, 2012‡</td>
<td>353/353</td>
<td>77</td>
<td>100%</td>
<td>9 months</td>
<td>Vitamin D and placebo</td>
<td>150 000 IU every 3 months</td>
<td>Falls</td>
<td>Death</td>
<td>65/67 (20/20)</td>
</tr>
</tbody>
</table>

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 65 nmol/L in winter-spring and 55 nmol/L in summer-autumn in the control group. ¶25OHD concentrations measured annually, ranging from 55–74/24 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.
**A**

**Myocardial infarction or ischaemic heart disease**

- **Inkovaara et al, 1983** [21]
  - 17/181 vs 6/146: 2
- **Komulainen et al, 1998** [29,30]
  - 10/146 vs 2/230: 0.5
- **Trivedi et al, 2003** [38]
  - 224/1345 vs 233/1341: 0.71
- **Grant et al, 2005** [46]
  - 78/2649 vs 84/2643: 2
- **Lappe et al, 2007** [53]
  - 3/446 vs 2/446: 0.6
- **Prince et al, 2008** [58]
  - 7/151 vs 3/151: 0.6
- **Sanders et al, 2010** [63]
  - 17/1131 vs 13/1125: 4

**Vitamin D**

- 343/6131 vs 342/6082: (0.99, 0.86–1.13)

Test for heterogeneity: $I^2=0\%$, $p=0.6$

**B**

**Stroke or cerebrovascular disease**

- **Inkovaara et al, 1983** [21]*
  - 13/93 vs 26/88: 5
- **Brazier et al, 2005** [43]
  - 3/95 vs 0/97: 2
- **Grant et al, 2005** [46]*
  - 44/1306 vs 39/1332: 31
- **WHI trials, 2006–07** [47–49]
  - 362/18176 vs 377/18106: 84
- **Lappe et al, 2007** [53]*
  - 3/446 vs 2/288: 3

**Calcium with vitamin D**

- 474/20116 vs 433/19891: 1.18 (0.86–1.63)

Test for heterogeneity: $I^2=31\%$, $p=0.2$

Test for heterogeneity between subgroups: $p=0.3$

**C**

**Cancer**

- **Komulainen et al, 1998** [29,30]
  - 3/228 vs 7/230: 2
- **Trivedi et al, 2003** [38]
  - 144/1345 vs 130/1341: 41
- **Grant et al, 2005** [46]
  - 175/26643 vs 178/2643: 48
- **Lappe et al, 2007** [53]
  - 13/446 vs 17/445: 6
- **Prince et al, 2008** [58]
  - 7/151 vs 5/151: 6
- **Sanders et al, 2010** [63]
  - 7/1131 vs 10/1125: 3

**Vitamin D**

- 343/5950 vs 347/5935: 0.98 (0.83–1.17)

Test for heterogeneity: $I^2=10\%$, $p=0.35$

**Calcium with vitamin D**

- 1730/19928 vs 1759/19726: 0.89 (0.67–1.18)

Test for heterogeneity: $I^2=67\%$, $p=0.05$

Test for heterogeneity between subgroups: $p=0.5$

**Vitamin D with or without calcium**

- 1977/24126 vs 2002/24041: 0.99 (0.93–1.05)

Test for heterogeneity: $I^2=0\%$, $p=0.6$

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

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**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.
**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.*
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 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

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

(figure 3).
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 calcium...
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. 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. 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 total fracture, the effect estimate lay within the futility boundary; for vitamin D 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. 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. 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), 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 73%, 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. 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. 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. 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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The puzzling world of vitamin D insufficiency

In the past decade, not only skeletal but also non-skeletal actions of vitamin D have been frequently reported—rickets, it seems, was merely the tip of the iceberg. Vitamin D deficiency might result in a higher incidence of osteoporotic fractures, cancer, cardiovascular diseases, diabetes, infections, neuropsychiatric disorders, and a higher risk of death. A mild form of vitamin D deficiency has also been introduced—referred to as vitamin D insufficiency.

At what concentrations of serum 25-hydroxyvitamin D, the accepted barometer of vitamin D status, should physicians recommend supplementation? Current recommendations vary widely, with some proponents suggesting an optimum serum concentration of 100–150 nmol/L, an amount that would classify nearly all individuals as insufficient. However, it is not evident to what extent serum 25-hydroxyvitamin D can serve as a valid biomarker of effect—ie, if the concentrations relate to health outcomes via a causal pathway and can serve as a valid predictor of such outcomes. Additionally, differences in assay performances, seasonal variation, fat mass, nutritional status (eg, calcium intake), and individual bioavailability of 25-hydroxyvitamin D by genetically determined concentrations of vitamin-D-binding protein render detection of uniform threshold effects troublesome. Low serum 25-hydroxyvitamin D concentrations have also been associated with frailty, which increases the likelihood of reverse causation bias in observational studies. Furthermore, forming guideline recommendations for vitamin D intake or serum 25-hydroxyvitamin D concentrations on the basis of changes in calcium absorption or serum parathyroid hormone concentrations seen in short-term interventional studies of vitamin D supplementation is difficult.

Despite these uncertainties regarding measurement and definition of thresholds for vitamin D insufficiency, the appealing idea that a high intake of vitamin D prevents illness has been lent strong support by several leading scholars in the field. Furthermore, these ideas have had a major effect on health practitioners as well as on the general population. A massive demand now exists for the measurement of blood concentrations of 25-hydroxyvitamin D. Supplemental use of vitamin D in the past decade has become frequent: for instance, in the USA during the period 2002–2011 the sales of vitamin D supplements increased by more than ten times, from US$42 million to $605 million. Can these supplement users expect a healthier and longer life than if they lived their life without vitamin D supplementation? Is more better?

In this issue of The Lancet Diabetes & Endocrinology, Mark Bolland and colleagues present findings from their meta-analysis of placebo-controlled randomised control trials using multiple non-skeletal and skeletal endpoints. The investigators used traditional meta-analyses to assess available evidence, and then did a trial sequential analysis to assess both treatment effects and the theoretical alteration on risk estimates by future trials. In conventional meta-analyses, the effect of vitamin D supplementation (with or without calcium) on the different outcomes was not significant for myocardial infarction, stroke, and cancer. In trial sequential analyses, the effect of vitamin D supplementation (with or without calcium) on all of these non-skeletal outcomes was below the futility boundary of 15%; for mortality, whether there was an effect at a boundary of 5% was unclear. Of particular interest is their finding that future studies are not likely to change these pooled estimates—ie, that the body of evidence is already sufficiently large. Of skeletal endpoints, only a clear reduction in the risk of hip fracture was seen for the combination of calcium and vitamin D in elderly nursing home residents, probably because these elderly people have lower serum concentrations of 25-hydroxyvitamin D combined with a low calcium intake. Vitamin D supplementation without the addition of calcium did not reduce hip fracture or total fracture risk. This information adds to the findings of a meta-analysis from the same research group showing no major effect of vitamin D supplementation on bone mineral density. No effect modification by higher dose, duration of treatment or low baseline 25-hydroxyvitamin D concentrations (<50 nmol/L as suggested by Institute of Medicine) was noted. However, chronic late-onset diseases often have long induction periods, and, theoretically, short randomised control trials (lasting about 5 years or less) will be able to assess only whether progress of the disease is modified by vitamin D supplementation. Several researchers have claimed that higher vitamin D doses are needed to achieve a clear positive effect on health but high annual doses of vitamin D actually increase the...
risk of fractures and falls. Some think that the use of these high intermittent doses are not physiological and therefore the results from these studies are claimed not to be compelling. The debate is likely to continue and trials with high daily doses are ongoing.

Nevertheless, existing evidence does not lend support to the commonly held belief that vitamin D supplementation in general prevents osteoporosis, fractures, and non-skeletal diseases. Consequently, the impression that vitamin D is a sunshine vitamin and that increasing doses lead to improved health is far from clear. Without stringent indications—ie supplementing those without true insufficiency—there is a legitimate fear that vitamin D supplementation might actually cause net harm. A report from the Institute of Medicine also emphasized that there might be risks from both low and high concentrations of vitamin D and that there might be a U-shaped curve of risk, as has been noted with other vitamin supplements. Until more information is available, it would be prudent to choose a cautious approach to vitamin D supplementation and to put more emphasis on the development of evidence-based cutoff points for vitamin D inadequacy.

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I declare that I have no conflicts of interest.

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