Systematic review and meta-analysis of calcium intake and bone mineral density

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Abstract:

Objective:

To determine whether increasing calcium intake from dietary sources has effects on bone mineral density (BMD), and if so, whether they are similar to the effects of calcium supplements on BMD.

Design

Random-effects meta-analysis of randomised controlled trials (RCTs).

Data sources

Ovid Medline, Embase, Pubmed and references from relevant systematic reviews. Initial searches undertaken in July 2013 and updated in September 2014.

Eligibility criteria for selecting studies

RCTs of dietary sources of calcium or calcium supplements (with or without vitamin D) in participants >50y with BMD at the lumbar spine, total hip, femoral neck, total body or forearm as an outcome.

Results

We identified 59 eligible RCTs: 15 studied dietary sources of calcium (n=1533) and 51 calcium supplements (n=12257). Increasing calcium intake from dietary sources increased BMD by 0.6-1.0% at the total hip and total body at 1y and by 0.7-1.8% at these sites and the lumbar spine and femoral neck at 2y. There was no effect on BMD at the forearm. Calcium supplements increased BMD by 0.7-1.8% at all 5 skeletal sites at 1y, 2y, and >2.5y, but the size of the BMD increase at later time-points was similar to the increase at 1y. Increases in BMD were similar in trials of dietary sources of calcium vs. calcium supplements (except at

the forearm), in trials of calcium monotherapy vs. co-administered calcium and vitamin D, in trials with calcium doses of ≥1000 vs. <1000 mg/d and ≤500 vs. >500 mg/d, and in trials where the baseline dietary calcium intake was <800 vs. ≥800 mg/d.

Conclusions

Increasing calcium intake from dietary sources or by taking calcium supplements produces small, non-progressive increases in BMD, which are unlikely to translate into fracture prevention. There appears little justification for recommending increased calcium intake for bone health.

Introduction

Maintaining a calcium intake of at least 1000-1200 mg/d has long been recommended for older individuals to treat and prevent osteoporosis.[1,2] Calcium supplements are commonly taken to achieve such intakes, which are considerably higher than the average dietary calcium intake in older people in Western countries of 700-900 mg/d. Recently, concerns have emerged about the risk-benefit profile of calcium supplements. The small reductions in total fractures[3] seem outweighed by the moderate risk of minor side-effects such as constipation, coupled with the small risk of significant side-effects such as cardiovascular events,[4-6] kidney stones,[7] and hospitalisation with acute gastrointestinal symptoms.[8] Consequently, some experts have recommended that older people increase their calcium intake through their diet, and only take supplements when that is not feasible.[9] In an accompanying systematic review of calcium intake and fractures, we concluded that there was no evidence currently that increasing dietary calcium intake reduces fracture risk.[10] We identified only 2 small randomised controlled trials (RCTs) of dietary calcium intake reporting fracture as an outcome. However, numerous cohort studies assessed the relationship between dietary calcium, milk or dairy intake and fracture risk, and the majority of these reported associations were neutral.[10]

The putative mechanism by which calcium intake affects bone health is by increasing bone mineral density (BMD). BMD is a surrogate endpoint for fracture risk that allows biological effects to be explored in RCTs of modest size. We investigated whether the results of RCTs with BMD as an endpoint support the recommendations to increase dietary calcium intake to prevent osteoporosis. We undertook a systematic review and meta-analysis of RCTs of dietary sources of calcium or calcium supplements in older adults (>50 years) to determine whether increasing calcium intake from dietary sources has effects on BMD, and if so, whether they are similar to the effects of calcium supplements on BMD.

Methods

Literature search

As part of a broader search for studies of calcium intake and health, we searched Ovid Medline and Embase in July 2013 and updated the search using Pubmed and Embase in September 2014 for RCTs of calcium, milk, or dairy intake, or calcium supplements with BMD as an endpoint. We also hand-searched recent systematic reviews, meta-analyses and any other articles included in our review for other relevant articles. The full text of the search is described in the accompanying paper[10] and Appendix Table 1.

Study selection

We included RCTs in participants aged >50 years at baseline with BMD measured by dual energy x-ray absorptiometry (DXA), or precursor technology such as photon absorptiometry. We included studies that reported bone mineral content (BMC) because BMD is obtained by dividing BMC by bone area and therefore the two are very highly correlated. We excluded studies where most participants at baseline had a major systemic pathology other than osteoporosis, such as renal failure or malignancy. We included studies of calcium supplements used in combination with other treatment provided that the other treatment was given to both arms (eg calcium plus vitamin K vs. placebo plus vitamin K), and included studies of co-administered calcium and vitamin D supplements (CaD). We treated RCTs of hydroxyapatite as a dietary source of calcium because it is made from bone and contains other minerals, hormones, protein, and amino-acids in addition to calcium. One author (WL or MB) screened titles and abstracts and two authors independently (WL, MB, or VT) screened the full-text of potentially relevant studies. The flow of articles is shown in Appendix Figure 1.

Data extraction and synthesis

We extracted information from each study on participant characteristics, study design, funding source and conflicts of interest, and BMD at the lumbar spine, femoral neck, total hip, forearm, and total body. Data were extracted by a single author (VT) and checked by a second author (MB). Risk of bias was assessed as recommended in the Cochrane Handbook.[11] Any discrepancies were resolved through discussion.

The primary endpoints were the percentage change in BMD from baseline at the five BMD sites. We categorised the studies into 3 groups by duration: '1 year'- duration <18 months; '2 years'- duration ≥18 months and ≤2.5 years; and '>2.5 years'. For studies that presented absolute data rather than percentage change from baseline, we calculated the mean percentage change from the raw data and the standard deviation of the percentage change using the approach described in the Cochrane Handbook.[11] Where data were presented only in figures, we used digital callipers to extract data. In four studies in which mean data but not measures of spread were reported,[12-15] we imputed the standard deviation for the percent change in BMD for each site from the average site- and duration- specific standard deviations of all other studies included in our review. We pre-specified subgroup analyses based on the following variables: dietary calcium intake vs. calcium supplements; risk of bias; calcium monotherapy vs. CaD; baseline age (<65 years); gender; community vs. institutionalised participants; baseline dietary calcium intake <800 mg/d; baseline 25-hydroxyvitamin D <50 nmol/L; calcium dose (≤500 vs. >500 mg/d and <1000 vs. ≥1000 mg/d); and vitamin D dose <800 IU/d.

Statistics

We pooled the data using random effects meta-analyses and assessed for between-study heterogeneity using the I^2 statistic (I^2 >50% was considered significant heterogeneity).

Funnel plots and Egger's regression model were used to assess for the likelihood of publication bias. We included RCTs of calcium with or without vitamin D in the primary analyses. RCTs in which supplemental vitamin D was provided to both treatment groups, so that the groups only differed in treatment by calcium, were included in calcium monotherapy subgroup analyses, while RCTs comparing co-administered CaD with placebo or controls were included in the CaD subgroup analyses. We included all available data from trials with factorial designs or multiple arms. Thus, for factorial RCTs, we included all study arms involving a comparison of calcium versus no calcium in the primary analyses and the calcium monotherapy subgroup analysis, but only arms comparing CaD with controls in the CaD subgroup analysis. For multi-arm RCTs, we pooled data from the separate treatment arms for the primary analyses, but each treatment arm was only used once. We undertook analyses of pre-specified subgroups using a random-effects model when there were 10 or more studies in the analysis and 3 or more studies in each subgroup and performed a test for interaction between subgroups. All tests were two-tailed and p-values <0.05 were considered statistically significant. All analyses were performed using Comprehensive Meta-Analysis (Version 2, Biostat, Englewood New Jersey, USA).

Results

Baseline characteristics

We identified 59 RCTs of calcium intake that reported BMD as an outcome.[7,12-70] Dietary sources of calcium were studied in 15 RCTs (n=810 calcium, n=723 controls),[16-30] and calcium supplements in 51 RCTs (n=6547 calcium, n=5710 controls).[7,12-15,17,19-22,26,28,31-70] The study design and selected baseline characteristics of the RCTs are shown in Table 1, and Appendix Tables 2-3. Of the 15 RCTs of dietary sources of calcium, 10 used milk or milk powder, 2 dairy products, and 3 hydroxyapatite preparations. Of the 51 RCTs of calcium supplements, 36 studied calcium monotherapy, 13 co-administered CaD, and 2 were multi-

arm studies of both. Other features of the RCTs are summarised in Table 2. The majority of RCTs studied calcium without vitamin D in community-dwelling women aged <70 years with baseline dietary calcium intake <800 mg/d and had a duration of ≤2 years. A calcium dose of >500 mg/d was used in the majority of RCTs, but a higher proportion of calcium supplement RCTs used a dose of ≥1000 mg/d. Appendix Table 4 shows our assessment of risk of bias. Of the 15 RCTs of dietary sources of calcium, we assessed 2 as low risk of bias, 6 as moderate risk, and 7 as high risk. Of the 51 RCTs of calcium supplements, we assessed 19 as low risk of bias, 12 as moderate risk, and 20 as high risk.

Primary analyses

Figures 1-5 and Table 3 show the results of the meta-analyses. Increasing calcium intake from dietary sources increased BMD by 0.6-1.0% at the total hip and total body at 1 year and by 0.7-1.8% at these sites and the lumbar spine and femoral neck at 2 years. There was no effect on BMD at the forearm. When we restricted the analyses to the 12 RCTs of milk or dairy products by excluding 3 RCTs of hydroxyapatite, there was little change in the results. Calcium supplements increased BMD by 0.7-1.4% at all five skeletal sites at 1 year, by 0.8-1.5% at 2 years and by 0.8-1.8%/y at >2.5 years (range of duration of trials 3-5 years). Using Egger's regression model and visual inspection of Funnel plots, data appeared skewed toward positive results with increased calcium intake from dietary sources or supplements in about half of analyses that included 5 or more studies, raising the possibility of publication bias. Seven multi-arm RCTs included a dietary source of calcium arm and a calcium supplement arm,[17,19-22,26,28] allowing a direct comparison of the interventions. There were no statistically significant between-group differences in BMD at any site in any individual trial, and there were also no statistically significant between-group differences in BMD at any site or any time-point in the pooled analyses (Appendix Table 5). We also tested for differences between the results of the RCTs of dietary sources of calcium and the RCTs of calcium supplements

by comparing the 2 groups in subgroup analyses (Table 3). There were no differences between the groups at any time-point at the lumbar spine, total hip, or total body. At the femoral neck, there were greater increases in BMD at 1 year in the calcium supplement RCTs, but greater increases in the RCTs of dietary sources of calcium at 2 years. At the forearm, there were increases in BMD in the calcium supplement RCTs but no effect in the RCTs of dietary sources of calcium.

Subgroup analyses:

We carried out additional subgroup analyses when there were 10 or more trials in an analysis and 3 or more trials in each subgroup. These criteria allowed analyses to be carried out in the trials of dietary sources of calcium only on the 1 year lumbar spine results. For the calcium supplement RCTs, we carried out analyses on the 1 year and 2 year lumbar spine, femoral neck, and forearm results, and the 1 year total body result. Appendix Table 6 shows that there were no consistent differences between subgroups based on calcium monotherapy vs. CaD, age, risk of bias, calcium dose of ≥1000 mg/d vs. <1000 mg/d, calcium dose of ≤500 mg/d vs. >500 mg/d, vitamin D dose, baseline dietary calcium intake or baseline 25-hydroxyvitamin D level. There were insufficient trials to carry out subgroup analyses based on gender and residence (community vs. institution).

Discussion

Increasing calcium intake from dietary sources slightly increased BMD (by 0.6-1.8%) over 1-2 years at all sites, except the forearm where there was no effect. Calcium supplements increased BMD to a similar degree at all sites and all time-points (by 0.7-1.8%). In the RCTs of calcium supplements, the increases in BMD were present by 1 year, but there were no further increases in BMD subsequently, thus the increases in BMD from baseline at both 2 and >2.5 years at each site were similar to the increases at 1 year. The increases in BMD with

dietary sources of calcium were similar to the increases in BMD with calcium supplements, except at the forearm, in both direct comparisons of the two interventions in multi-arm studies and in indirect comparisons of the two interventions through subgroup analyses. The increases in BMD were similar in trials of calcium monotherapy and CaD, consistent with a recent meta-analysis reporting that vitamin D monotherapy had no effect on BMD.[71] There were no differences in BMD changes in our subgroup analyses between trials with calcium doses of ≥1000 mg/d and <1000 mg/d or doses of ≤500 mg/d and >500 mg/d, and in populations with baseline dietary calcium intake of <800 mg/d and ≥800 mg/d. Overall, the results suggest increasing calcium intake, whether from dietary sources or by taking calcium supplements, provides a small, non-progressive increase in BMD, without any ongoing reduction in rates of BMD loss beyond 1 year. The similar effects of increased dietary intake and supplements suggests that the non-calcium components of the dietary sources of calcium do not directly impact on BMD.

The strength of this meta-analysis is its comprehensive nature, involving 59 RCTs, and assessing the effects of both dietary calcium sources and calcium supplements on BMD at 5 skeletal sites and at 3 time-points. The size of the review permitted a comparison of the effects on BMD of different sources of calcium intake, dietary sources or supplements, and also the effects in important subgroups such as those defined by dose of calcium, use of co-administered vitamin D, and baseline clinical characteristics. An important limitation is that BMD is only a surrogate for the clinical outcome of fracture. However, we undertook the review because many of the subgroup analyses in the dataset of trials with fracture as an endpoint have very limited power,[10] and a comparison between RCTs of dietary sources of calcium and calcium supplements with fracture as the endpoint is not possible because there are only 2 small RCTs of dietary sources of calcium that reported fracture data.[10]

The absence of any interaction with baseline dietary calcium intake or a dose-response relationship suggests that increasing calcium intake through dietary sources or by supplements does not correct a dietary deficiency (in which case greater effects would be seen in those with the lowest intakes or the highest doses). An alternative possibility is that increasing calcium intake has a weak anti-resorptive effect. Calcium supplements reduce markers of bone formation and resorption by about 20%,[62,65,72] and increasing milk intake also reduces bone turnover by a similar amount.[73] It is possible that suppression of bone turnover by this amount leads to the small increases in BMD observed.

Increases in BMD of about 1-2% over 1-5 years are unlikely to translate into clinically meaningful reductions in fractures. The average rate of BMD loss in older post-menopausal women is approximately 1%/year. So the effect of increasing calcium intake is to prevent, about 1-2 years of normal BMD loss, and if calcium intake is increased for more than 1 year, it will slow down but not stop BMD loss. These modest increases in BMD are smaller than observed with weak anti-resorptive agents such as etidronate[74] and raloxifene,[75] of which etidronate does not reduce vertebral or non-vertebral fractures, and raloxifene reduces vertebral but not non-vertebral fractures.[76] In contrast, potent anti-resorptive agents such as alendronate, zoledronate, and denosumab increase BMD by 6-9% at the spine, and 5-6% at the hip over 3 years,[77-80] and these changes are associated with reductions in vertebral fracture of 44-70%, hip fracture of 35-41%, and non-vertebral fractures of 15-25%.[76] The magnitude of fracture reduction predicted by the small increases in BMD we observed with increased calcium intake are consistent with the findings of our systematic review of calcium supplements and fracture.[10] We observed small (<15%), inconsistent reductions in total and vertebral fracture overall, but no reductions in fractures in the large RCTs at lowest risk of bias, and no reductions in forearm or hip fractures.

The results of this meta-analysis of calcium intake and BMD should be considered together with the systematic review of calcium intake and fracture risk.[10] The results of the 2 reviews suggest that: (1) increasing calcium intake by dietary sources or supplements has small effects on BMD that are unlikely to translate into clinically meaningful fracture reductions; (2) dietary calcium intake is not associated with risk of fracture and there is no evidence currently that increasing dietary calcium intake prevents fractures; and (3) calcium supplements have small, inconsistent benefits on fracture. These small benefits are outweighed by adverse effects of increased cardiovascular risk,[5,6] kidney stones,[7] and acute gastrointestinal symptoms[8] giving rise to an unfavourable risk-benefit profile for calcium supplements. Thus, there is little justification for clinicians, advocacy organisations and health policymakers to recommend increasing calcium intake for bone health or fracture prevention, either through dietary sources or by use of calcium supplements.

The large number of RCTs studying increased calcium intake and BMD and the consistency of the results across different populations, in studies using higher or lower doses of calcium, and in studies of dietary calcium sources or calcium supplements does not reveal any obvious evidence gap. Any future trials conducted should have a strong rationale as to why the results are likely to differ from the large body of existing trial evidence. It is usually recommended that anti-resorptive agents are co-prescribed with calcium and vitamin D, although RCTs of such agents have demonstrated reductions in fracture risk[81-83] and the expected increases in BMD[64,84-86] without the co-administration of calcium and vitamin D. RCTs clarifying the role of calcium and vitamin D in individuals using anti-resorptive agents might be valuable. For most individuals concerned about their bone health, dietary calcium intake does not require close scrutiny. For those at high risk of fracture, treatment should be focused on agents with proven anti-fracture efficacy.

What this paper adds Box

What is already known on this subject

- Older people are recommended to take at least 1000-1200 mg/d of calcium to treat and prevent osteoporosis.
- Many people take calcium supplements to meet these recommendations.
- Recent concerns about the safety of calcium supplements have led experts to recommend increasing calcium intake through food rather than by taking supplements, but the effect of increasing dietary calcium intake on bone health is not known.

What this study adds

- Increasing calcium intake either by dietary sources or supplements has small, non-progressive effects on bone density.
- These effects are unlikely to translate into clinically meaningful reductions in fractures
- When these results are considered together with those of the systematic review on calcium intake and fracture, there appears little justification for recommendations to increase calcium intake for bone health or fracture prevention, either through dietary sources or by use of calcium supplements.

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Conflicts of Interest:
All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: the study was funded by the Health Research Council (HRC) of New Zealand. MB is the recipient of a Sir Charles Hercus Health Research Fellowship; IR has received research grants and/or honoraria from Merck, Amgen, Lilly and Novartis; all other authors have no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Contributorship:
MB, WL, VT, AG and IR designed the research. WL and MB performed the literature searches. VT and MB extracted or checked data. MB performed the analyses. MB and VT drafted the paper. All authors critically reviewed and improved it. MB is the guarantor for the article. All authors had access to all the data and take responsibility for the integrity of the data and the accuracy of the data analysis**.**

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Transparency statement
MB affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained

Data sharing: all data are included in the paper or supplementary appendix- there are no additional data available

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Table 1: Study design and selected baseline characteristics of eligible trials

Trial	Design	Calcium dose (mg/d)	Vitamin D dose (IU/d)	Duration	Care setting	Total participants[a] (N)	Participants[b] Calcium/ Controls (N)	Gender (% Female)	Mean Age (y)
Dietary calcium trials									
	2 arm RCT								
Recker 1985[16]	Milk; control	NS		2 y	Community	30	16/14	100	59
	4 arm RCT								
Polley 1987[17]	dairy; Ca; dairy/salt restrict; control	≥1250		9 m	Community	269	58/52	100	57
	2*2 factorial RCT								
	Ex/milk; ex/control; sed/milk;								
Nelson 1991[18]	sed/control	831		1 y	Community	41	18/18	100	60
	3 arm RCT								
Chevalley 1994[19]	OMC/D; CaD; P/D	800	300,000 IM stat	18 m	Community	93	31/31	85	72
	4 arm RCT								
Prince 1995[20]	Milk; Ca; Ca/ex; P	1000		2 y	Community	168	42/42	100	63
	3 arm RCT								
Storm 1998[21]	Milk; Ca; P	NS		2 y	Community	40	20/20	100	71
	3 arm RCT								
Castelo-Branco 1999[22]	OHC; Ca; control	3320		2 y	Community	60	17/16	100	55
	2 arm RCT								
Cleghorn 2001[23]	Milk; control	700		1 y	Community	142	56/59	100	52
	2 arm RCT								
Lau 2001[24]	Milk; control	800		24 m	Community	200	95/90	100	57
	2 arm RCT								
Chee 2003[25]	Milk; control	1200		24 m	Community	200	91/82	100	59
	3 arm RCT								
Albertazzi 2004[26]	OHC; Ca: P	500		6 m	Community	153	52/50	100	68
	2 arm RCT								
Daly 2006[27]	Milk; control	1000	800	2 y	Community	167	85/82	0	62
	3 arm RCT								
Manios 2007[28]	Dairy; Ca: control	1200	300	12 m	Community	112	39/36	100	61

	2*2 factorial RCT								
Kukuljan 2009[29]	Milk; milk/ex; ex; control	1000	800	12 m	Community	180	90/90	0	61
	3 arm RCT								
Gui 2012[30]	Milk; soy milk; control	250		18 m	Community	141	100/41	100	56
Calcium supplement trials									
	3 arm RCT								
Recker 1977[31]	Ca; HRT; control	1040		2 y	Community	60	22/20	100	57
	2 arm RCT								
Lamke 1978[32]	Ca; P	1000		12 m	Community	40	19/17	100	60
	2*2 factorial RCT								
Smith 1981[33]	CaD; ex; ex/CaD; P	750	400	3 y	Institution	80	21/30	100	82
	4 arm RCT								
Hansson 1987[12]	30mg NaF/Ca; 10mg NaF/Ca; Ca; P	1000		3 y	NS	50	25/25	100	66
	4 arm RCT								
Polley 1987[17]	Ca; dairy; dairy/salt restrict; control	1000		9 m	Community	269	40/52	100	57
	3 arm RCT								
Riis 1987[34]	Ca; HRT; P	2000		2 y	Community	43	14/11	100	51
	2 arm RCT								
Smith 1989[35]	Ca; P	1500		4 y	Community	169	70/77	100	51
Dawson-Hughes	3 arm RCT								
1990[36]	Ca; Ca; P	500		2 y	Community	361	158/93	100	58
	2 arm RCT								
Fujita 1990[37]	Ca; control	900		2 y	Institution	32	12/20	100	80
	2 arm RCT								
Orwoll 1990[38]	CaD ; P	1000	1000	3 y	Community	86	41/36	0	58
	3 arm RCT	1000 or							
Elders 1991[39]	Ca; Ca; P	2000		2 y	Community	295	198/97	100	NS
	3 arm RCT								
Prince 1991[40]	Ca/ex; ex; HRT	1000		2 y	Community	80	39/41	100	57
	2 arm RCT								
Chapuy 1992[41]	CaD; P	1200	800	18 m	Institution	3270	27/29	100	84
	2*2 factorial RCT								
Lau 1992[42]	Ca; Ca/ex; ex/P; P	800		10 m	Institution	50	27/23	100	76

	2 arm RCT								
Reid 1993[43]	Ca vs P	1000		2 y	Community	135	61/61	100	58
	3 arm RCT								
Aloia 1994[44]	CaD; HRT/CaD; P/D	600	400	2.9 y	Community	118	34/36	100	52
	3 arm RCT								
Chevalley 1994[19]	CaD; OMC/D; P/D	800	300,000 IM stat	18 m	Community	93	31/31	89	72
	2*2 factorial RCT								
Strause 1994[45]	Ca; Ca/minerals; minerals; P	1000		2 y	Community	113	27/32	100	66
	4 arm RCT								
Prince 1995[20]	Ca; Ca/ex; milk; P	1000		2 y	Community	168	42/42	100	63
	3 arm RCT								
Fujita 1996[46]	Ca; Ca; P	900		2 y	Institution	58	38/20	100	81
	4 arm RCT								
Perez-Jaraiz 1996[47]	Ca; HRT; calcitonin; control	1000		1 y	Community	52	26/26	100	50
	2 arm RCT								
Recker 1996[48]	Ca; P	1200		4.3 y	Community	197	91/100	100	74
Dawson-Hughes	2 arm RCT								
1997[49]	CaD; P	500	700	3 y	Community	445	187/202	55	71
	3 arm RCT								
Baeksgaard 1998[50]	CaD; CaD/multivitamins; P	1000	560	2 y	Community	160	65/63	100	62
	2 arm RCT								
Ricci 1998[51]	Ca; P	1000		6 m	Community	43	15/16	100	58
	2 arm RCT								
Riggs 1998[52]	Ca; P	1600		4 y	Community	236	119/117	100	66
	3 arm RCT								
Storm 1998[21]	Ca; milk; P	1000		2 y	Community	40	20/20	100	72
	3 arm RCT								
Castelo-Branco 1999[22]	Ca; OHC; control	2500		2 y	Community	60	19/16	100	54
	2 arm RCT								
Ruml 1999[53]	Ca; P	800		2 y	Community	63	25/31	100	52
	4 arm RCT								
Fujita 2000[54]	Ca; Ca; Ca; P	900		4 m	NS	38	32/6	100	55
	3 arm RCT								
Peacock 2000[13]	Ca; 25OHD; P	750		4 y	Community	438	126/135	72	74

	3 arm RCT								
Son 2001[55]	Ca; alphacalcidiol; P	1000		10 m	Community	69	22/21	100	72
	3 arm RCT								
Chapuy 2002[56]	CaD; CaD; P	1200	800	2 y	Institution	610	393/190	100	85
	2 arm RCT								
Grados 2003[57]	CaD; P	500	400	12 m	Community	192	95/97	100	75
	3 arm RCT								
Albertazzi 2004[26]	Ca; OHC; P	500		6 m	Community	153	51/50	100	68
	2 arm RCT								
Doetsch 2004[58]	CaD; P	1000	800	12w	Community	30	16/14	NS	NS
	4 arm RCT		300,000 IM stat						
Harwood 2004[14]	CaD; CaD; D; control	1000	or 800	12 m	Community	150	75/75	100	81
	2 arm RCT								
Meier 2004[59]	CaD; control	500	500	6 m	Community	55	27/16	67	56
	3 arm RCT								
Riedt 2005[60]	CaD/w-loss; D/w-loss; w-maintain	1200	400	6 m	Community	55	23/24	100	61
	2 arm RCT								
Jackson 2006[7]	CaD; P	1000	400	7 y	Community	2431	1230/1201	100	62
	2 arm RCT								
Prince 2006[61]	Ca; P	1200		5 y	Community	1460	730/730	100	75
	2 arm RCT								
Reid 2006[62]	Ca; P	1000		5 y	Community	1471	732/739	100	74
	2*2 factorial RCT								
Bolton-Smith 2007[63]	CaD; CaD/vit K; vit K; P	1000	400	2 y	Community	244	99/110	100	68
	3 arm RCT								
Bonnick 2007[64]	CaD/alend; CaD; alend/D	1000	400	2 y	Community	563	282/281	100	66
	2 arm RCT								
Hitz 2007[15]	CaD; P	1200	1400	12 m	Community	122	34/45	83	68
	3 arm RCT;								
Manios 2007[28]	Ca; dairy; control	600		12 m	Community	112	26/36	100	62
	3 arm RCT	600 or							
Reid 2008[65]	Ca; Ca; P	1200		2 y	Community	323	216/107	0	56
	3 arm RCT								
Zhu 2008[66]	Ca; CaD; P	1200	1000	5 y	Community	120	79/41	100	75

Chailurkit 2010[67,68]	2 arm RCT Ca; P	500		2 y	Community	404	178/165	100	66
Karkkainen 2010[69]	2 arm RCT CaD; control	1000	800	3 y	Community	593	287/306	100	67
Nakamura 2012[70]	3 arm RCT Ca; Ca; P	250 or 500		2 y	Community	450	281/137	100	60

[a] Total number of randomised participants in all treatment arms

[b] Number of participants in relevant arms from trial in whom bone mineral density was reported

Abbreviations: RCT- randomised controlled trial; Ca- calcium; restrict- restriction; ex- exercise; sed- sedentary; OMC- ossein-mineral complex; D- vitamin D; CaD- co-administered calcium and vitamin D; P- placebo; IM –intramuscular; OHC- ossein-hydroxyapatite complex; HRT- hormone replacement therapy; NaF- sodium fluoride; 25OHD- 25-hydroxyvitamin D; w-loss – weight loss, w-maintain- weight maintenance; vit K- vitamin K; Alend- alendronate; NS –not stated

Table 2: Summary of selected characteristics of eligible trials

Characteristics of randomised controlled trials	Dietary sources of calcium (n=15)	Calcium supplements (n=51)
Agent studied		
Calcium monotherapy	11 (73)	36 (71)
Calcium with vitamin D	4 (27)	13 (25)
Multiarm study with calcium or calcium and vitamin D	0 (0)	2 (4)
Calcium dose ≥1000 mg/d	6 (40)	34 (67)
Calcium dose ≤ 500 mg/d	2 (13)	7 (14)
Duration ≤2 years	15 (100)	37 (73)
Duration ≥3 years	0 (0)	13 (25)
Community dwelling participants	15 (100)	45 (88)
Majority of participants female	13 (87)	48 (94)
Baseline mean age ≥70 years	2 (13)	18 (35)
Baseline mean dietary calcium intake < 800 mg/d	9/13 (69)	26/39 (67)

Data are number of trials (%).

Table 3: Pooled analyses of trials of dietary sources of calcium and calcium supplements

		Trials of dietary sources of calcium				Calcium supplement trials				
Site	Time-Point (y)	Studies (N)	Participants (N)	BMD difference[a] (95% CI)	P	Studies (N)	Participants (N)	BMD difference[a] (95% CI)	P	P[b] (interaction)
Lumbar spine	1	11	1260	0.6 (-0.1, 1.3)	0.08	27	3866	1.2 (0.8, 1.7)	<0.001	0.13
	2	8	816	0.7 (0.3, 1.2)	0.001	21	6115	1.1 (0.7, 1.6)	<0.001	0.19
	>2.5	0				8	3861	1.0 (0.3, 1.6)	0.003	
Femoral neck	1	8	1035	0.3 (-0.3, 0.9)	0.30	19	2651	1.2 (0.7, 1.8)	<0.001	0.02
	2	7	783	1.8 (1.1, 2.6)	<0.001	14	2415	1.0 (0.5, 1.4)	<0.001	0.05
	>2.5	0				5	2257	1.5 (0.2, 2.0)	0.025	
Total hip	1	6	900	0.6 (0.3, 1.0)	0.001	7	1159	1.4 (0.6, 2.3)	0.001	0.08
	2	5	689	1.5 (0.7, 2.4)	<0.001	7	4366	1.3 (0.8, 1.8)	<0.001	0.63
	>2.5	0				6	3835	1.2 (0.5, 1.9)	0.001	
Forearm	1	4	418	0.0 (-0.4, 0.5)	0.85	10	791	1.0 (0.2, 1.8)	0.014	0.04
	2	2	171	0.1 (-0.3, 0.4)	0.65	10	857	1.5 (0.5, 2.6)	0.005	0.01
	>2.5	0				5	437	1.8 (0.2, 3.4)	0.025	
Total Body	1	3	433	1.0 (0.3, 1.8)	0.009	10	1255	0.7 (0.4, 1.1)	<0.001	0.47
	2	2	358	0.9 (0.5, 1.3)	<0.001	6	3901	0.8 (0.5, 1.1)	<0.001	0.67
	>2.5	0				7	4164	0.8 (0.5, 1.1)	<0.001	

[a] Weighted mean difference between-groups in percentage change in bone mineral density (BMD) from baseline.

[b] Test for interaction between subgroup of trials of dietary sources of calcium and subgroup of calcium supplement trials

Abbreviation: CI- confidence interval

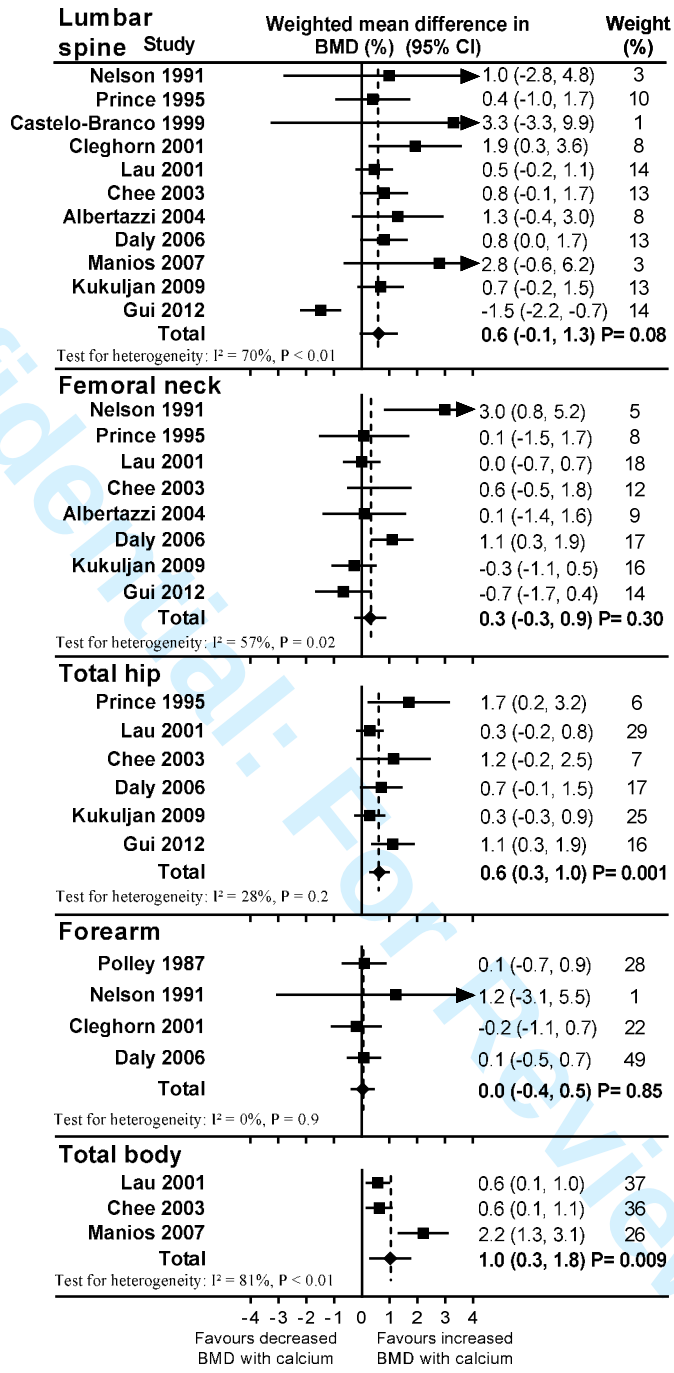


Figure 1: random effects meta-analyses of the effect of dietary sources of calcium on the percentage change in bone mineral density (BMD) from baseline at 1 year.

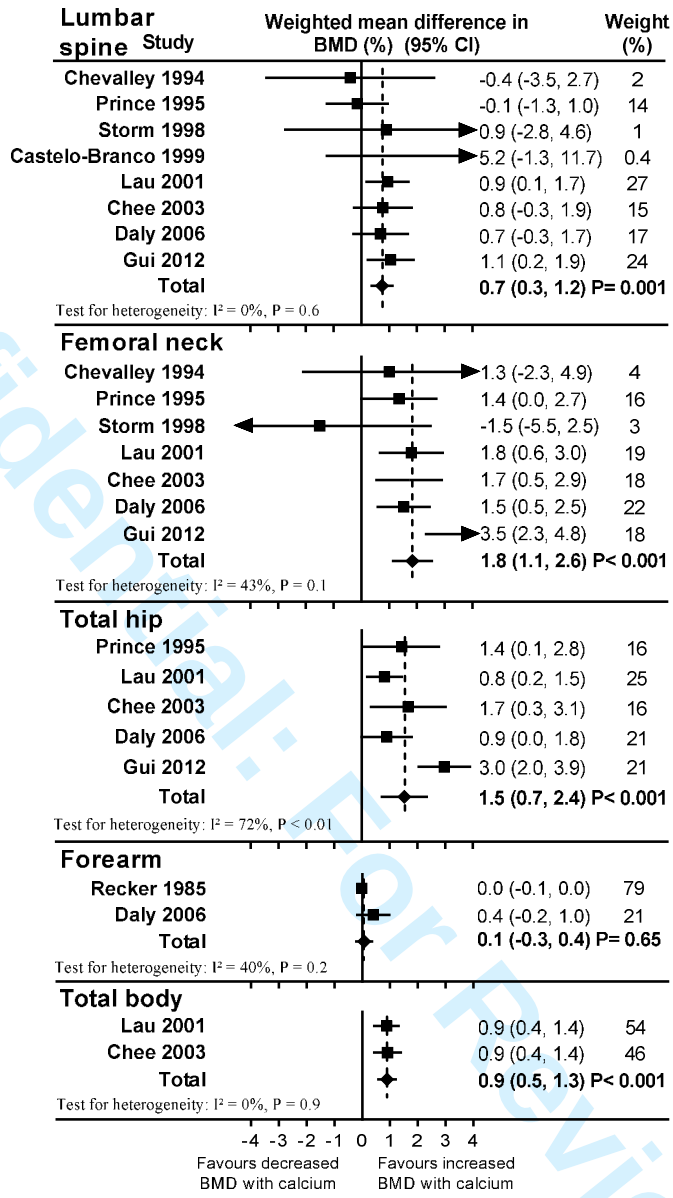


Figure 2: random effects meta-analyses of the effect of dietary sources of calcium on the percentage change in bone mineral density (BMD) from baseline at 2 years.

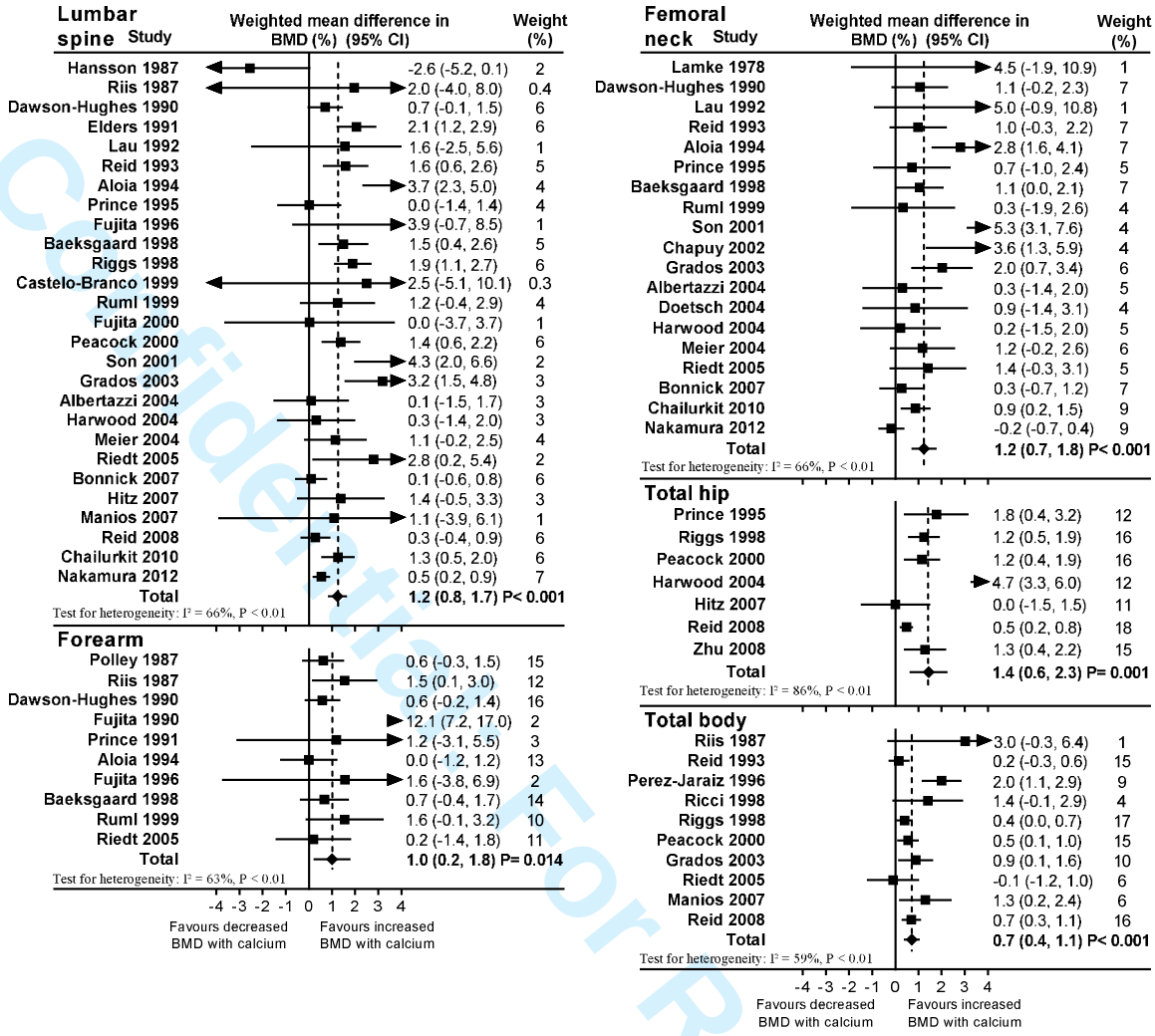


Figure 3: random effects meta-analyses of the effect of calcium supplements on the percentage change in bone mineral density (BMD) from baseline at 1 year.

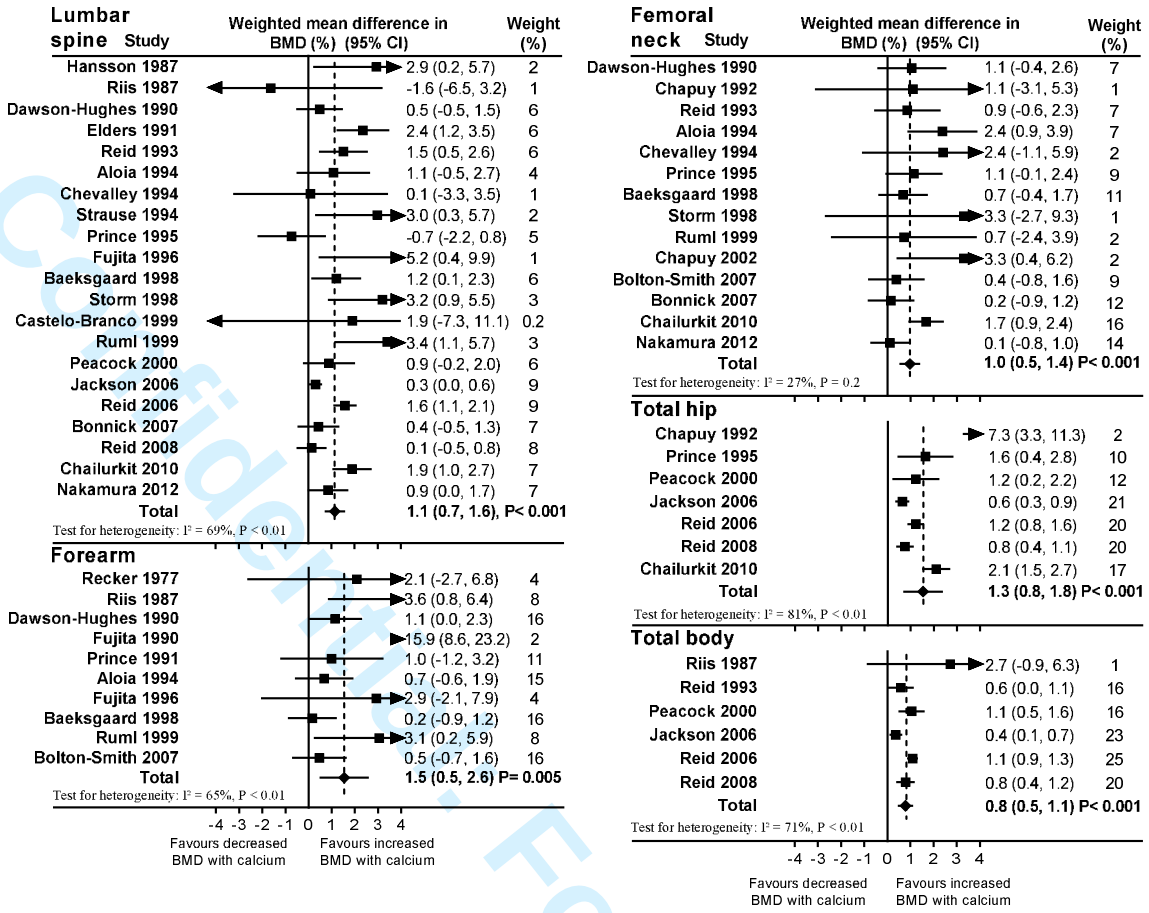


Figure 4: random effects meta-analyses of the effect of calcium supplements on the percentage change in bone mineral density (BMD) from baseline at 2 years.

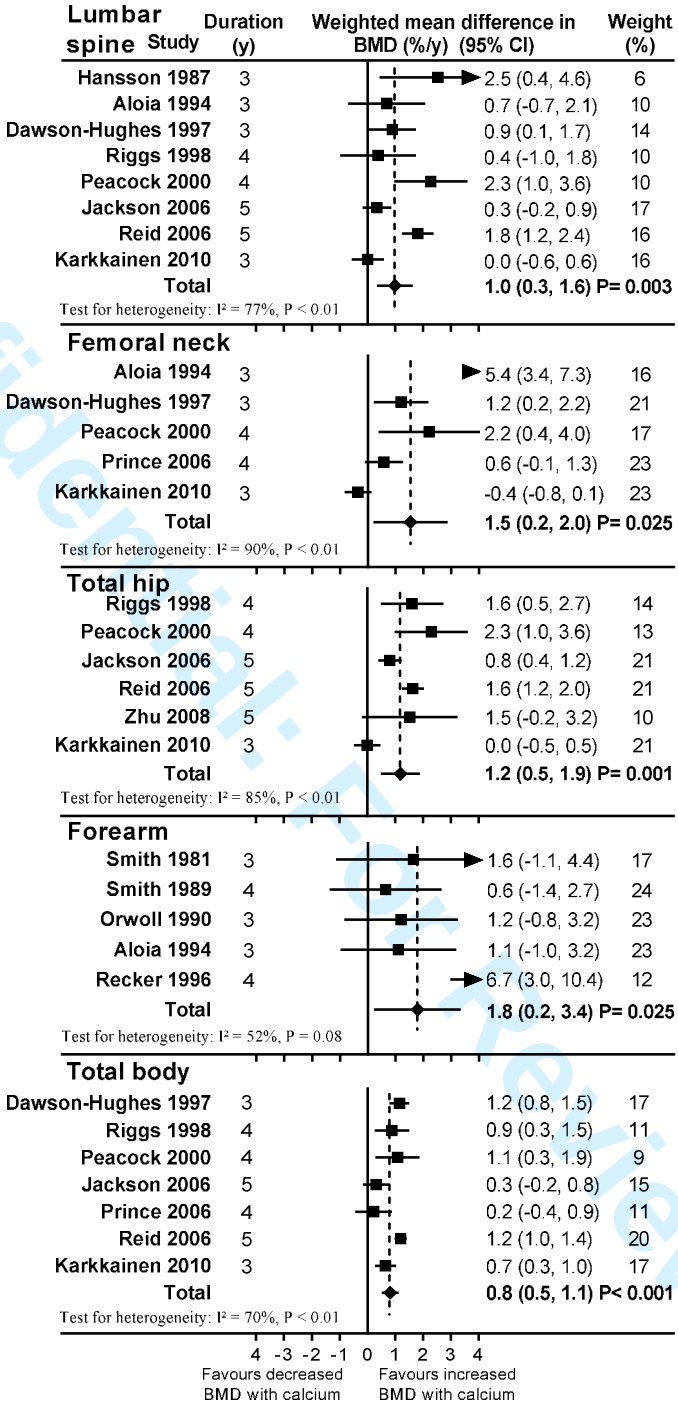


Figure 5: random effects meta-analyses of the effect of calcium supplements on the percentage change in bone mineral density (BMD) from baseline in studies >2.5 years

Appendix

Appendix Table 1: Literature searches

In July 2013, we searched Ovid Medline and Embase since inception for English language studies of calcium, milk, or dairy intake, or calcium supplements that reported on a broad range of skeletal and non-skeletal endpoints including BMD. The full text of the search was designed with assistance from a professional librarian and is shown below. We also identified 120 systematic reviews or meta-analyses on these topics from the search and hand-searched these articles and any other articles included in our review for other relevant articles. In September 2014, we updated the results with a focused search (no language restrictions) of Pubmed (below) and Embase for studies with fracture or BMD as an endpoint.

Ovid Medline search July 2013

1. Randomized Controlled Trials as Topic/
2. randomized controlled trial.pt. or randomi?ed controlled trial.mp. or Randomized Controlled Trial/
3. controlled clinical trial.pt. or Controlled Clinical Trial/
4. Random Allocation/
5. Double-Blind Method/
6. Single-Blind Method/
7. clinical trial.pt. or exp Clinical Trials/
8. multicenter study.pt. or multicenter study.tw.
9. or/1-8
10. (clinical adj trial$).tw.
11. ((singl$ or doubl$ or trebl$ or tripl$) adj (mask$ or blind$)).tw.
12. placebo$.tw. or Placebos/ or (control adj (arm or group)).tw.
13. (allocat$ adj2 random$).tw.
14. or/10-13
15. 9 or 14
16. case report.tw.
17. letter/
18. historical article/
19. or/16-18
20. 15 not 19
21. Epidemiologic studies/
22. (case control or case-control).tw. or exp case control studies/
23. cohort.tw. or exp Cohort Studies/
24. (Follow up or follow-up).tw. or Follow-Up Studies/
25. (observational adj (study or studies)).tw.
26. Longitudinal.tw. or Longitudinal Studies/
27. (prospective adj (study or studies)).tw. or Prospective Studies/
28. (retrospective adj (study or studies)).tw. or Retrospective Studies/
29. ((cross sectional or cross-sectional) adj (study or studies or analy$)).tw. or Cross-Sectional Studies/
30. ((cross-over or crossover) adj (study or studies)).tw. or Cross-Over Studies/
31. risk factor$.tw. or Risk factors/
32. exp Calcium, Dietary/
33. milk.tw. or Milk/
34. (dairy or cheese).tw. or dairy products/ or butter/ or cheese/ or ice cream/ or margarine/
35. yoghurt.tw. or cultured milk products/ or yoghurt/
36. Milk Substitutes/ or Soy Milk/
37. calcium.tw. or exp Calcium/
38. (calcium adj3 supplement$).mp.
39. Dietary Supplements/ and calcium.mp.
40. calcium carbonate$.mp. or exp Calcium Carbonate/
41. calcium citrate$.mp. or exp Calcium Citrate/
42. calcium phosphate$.mp. or exp Calcium Phosphates/
43. calcium gluconate$.mp. or exp Calcium Gluconate/
44. hydroxyapatite$.mp. or exp Hydroxyapatites/
45. fracture$.tw. or exp Fractures, Bone/
46. osteoporo$.tw. or Osteoporosis/ or Osteoporosis, Postmenopausal/

47. (bone density or BMD).tw. or Bone Density/
48. bone mineral density.tw.
49. (bone adj (mass or strength or loss or accret$ or remodel$ or resorp$)).tw.
50. (bone mineral content or BMC).tw.
51. (cardiovascular adj (disease$ or event$ or acute)).tw.
52. isch$ heart disease$.tw. or Myocardial Ischemia/
53. (acute coronary syndrome or ACS).tw. or Acute Coronary Syndrome/
54. unstable angina.tw. or Angina, Unstable/
55. coronary artery disease$.tw. or Coronary Artery Disease/
56. (myocardial infarct$ or MI or AMI).tw. or Myocardial Infarction/
57. (cerebrovascular adj (accident$ or disease$ or acute)).tw. or exp Cerebrovascular Accident/
58. stroke$.tw. or exp Stroke/
59. mortality.tw. or Mortality/ or Hospital Mortality/ or Mortality, Premature/
60. fatal outcome.tw. or Fatal Outcome/
61. death$.tw. or Death/ or Cause of Death/ or Death, Sudden, Cardiac/
62. cancer$.tw. or exp Neoplasms/dh, pc, et, di, ep
63. ((risk$ or occurrence$ or case$ or incidence) adj3 (cancer$ or neoplasm$ or malignan$ or adenocarcinom$ or carcinom$ or melanom$ or lymphom$ or leuk?emi$ or myelodysplas$ or myelom$ or sarcom$)).tw.
64. ((breast or prostate or colo$ or rect$) adj3 (neoplasm$ or cancer$ or tumo$ or malignan$ or carcinom$ or adenocarcinom$)).tw.
65. Breast Neoplasms/
66. Prostatic Neoplasms/
67. Colonic Neoplasms/ or Colorectal Neoplasms/
68. Adenomatous Polyps/ or Colonic Polyps/ or Intestinal Polyps/
69. ((colo$ or rect$) adj3 (adenoma$ or polyp$)).tw.
70. (weight adj2 (body or gain or increas$ or rise$ or rose or loss or reduc$ or fall or fell or change)).tw. or Weight Loss/
71. blood pressure.tw. or Blood Pressure/
72. Hypertension/ or hypertension.tw.
73. cholesterol.tw. or Cholesterol/ or Cholesterol, Dietary/
74. Cholesterol, VLDL/ or Cholesterol, LDL/ or Cholesterol,HDL/
75. ((serum or blood) adj calcium).tw.
76. ((kidney or renal) adj (calcul$ or stone$ or lithiasis)).tw. or Kidney Calculi/
77. ((gastrointestinal or GI) adj (discomfort or side-effect$ or side effect$)).tw.
78. ((abdominal or stomach) adj (pain or cramp$)).tw. or Abdominal Pain/
79. constipation.tw. or Constipation/
80. indigestion.tw. or Dyspepsia/
81. Diarrhea/ or diarrhea.tw.
82. Flatulence/ or bloating.tw.
83. or/20-31
84. or/32-44
85. or/45-82
86. and/83-85
87. limit 86 to english language
88. limit 87 to animal
89. limit 87 to human
90. 88 not 89
91. 87 not 90
92. *Dialysis/ or *hemodialysis/ or *peritoneal dialysis/
93. 91 not 92
94. limit 93 to (addresses or bibliography or biography or comment or congresses or consensus development conference or consensus development conference, nih or dictionary or directory or duplicate publication or editorial or in vitro or interview or lectures or letter or news or newspaper article or "review")
95. 93 not 94
96. limit 95 to ("all adult (19 plus years)" or "middle age (45 to 64 years)" or "middle aged (45 plus years)" or "all aged (65 and over)" or "aged (80 and over)")

Pubmed search September 2014
(dietary calcium OR calcium intake OR milk OR dairy OR calcium supplement* OR calcium supplement) AND (fracture OR bone density OR bone mineral)

Appendix Table 2: Baseline age, dietary calcium intake and vitamin D status in eligible studies.

Study	Age (y)		Dietary calcium (mg/d)		25-hydroxyvitamin D (nmol/l)	
	Calcium	Control	Calcium	Control	Calcium	Control
Dietary Calcium Trials						
Recker 1985[16]	59 (5)	59 (4)	679 (185)	821 (250)	NA	NA
Polley 1987[17]	58 (5)	57 (5)	669 (190)	709 (181)	NA	NA
Nelson 1991[18]	60 (7)	60 (7)	NA	NA	71(8)	70(9)
Chevalley 1994[19]	72 (7)	72 (6)	692 (328)	510 (273)	59(30)	64(28)
Prince 1995[20]	63 (4)	63 (4)	778 (335)	787 (312)	77(35)	85(43)
Storm 1998[21]	71 (5)	71 (5)	644 (224)	699 (286)	64(36)	60(30)
Castelo-Branco 1999[22]	54 (4)	56 (4)	800 (600)	800 (700)	NA	NA
Cleghorn 2001[23]	52 (3)	52 (3)	967 (187)	918 (170)	NA	NA
Lau 2001[24]	57 (2)	57 (2)	499 (261)	455 (195)	NA	NA
Chee 2003[25]	59 (4)	59 (3)	470 (214)	466 (220)	69(16)	68(16)
Albertazzi 2004[26]	68 (6)	68 (5)	563 (231)	679 (194)	NA	NA
Daly 2006[27]	62 (8)	62 (8)	997 (419)	883 (343)	77(23)	76(24)
Manios 2007[28]	61 (4)	61 (5)	665 (246)	717 (180)	70(22)	64(23)
Kukuljan 2009[29]	62 (8)	60 (7)	975 (410)	1031 (381)	87(31)	85(40)
Gui 2012[30]	56 (4)	57 (4)	NA	NA	NA	NA
Calcium Supplement Trials						
Recker 1977[31]	57 (4)	57 (4)	503 (192)	597 (250)	NA	NA
Lamke 1978[32]	60 (3)	60 (3)	NA	NA	NA	NA
Smith 1981[33]	82 (7)	82 (7)	NA	NA	NA	NA
Hansson 1987[12]	67	67	NA	NA	NA	NA
Polley 1987[17]	57 (5)	57 (5)	714 (171)	717 (180)	NA	NA
Riis 1987[34]	50 (2)	50 (2)	NA	NA	86 (32)	95 (15)
Smith 1989[35]	51 (7)	51 (7)	666 (207)	691 (267)	NA	NA
Dawson-Hughes 1990[36]	58 (5)	58 (5)	NA	NA	NA	NA
Fujita 1990[37]	81 (5)	81 (5)	NA	NA	NA	NA
Orwoll 1990[38]	55 (13)	55 (13)	1159 (576)	1159 (576)	60 (17)	52 (15)
Elders 1991[39]	NA	NA	1150 (197)	1151 (327)	45 (10)	45 (10)
Prince 1991[40]	57 (4)	57 (4)	850 (344)	780 (293)	64 (18)	81 (30)
Chapuy 1992[41]	84 (6)	84 (6)	511 (172)	514 (158)	40 (27)	33 (22)
Lau 1992[42]	77 (2)	77 (2)	260 (68)	256 (57)	52 (20)	66 (18)
Reid 1993[43]	58 (5)	58 (5)	760 (300)	730 (290)	NA	NA
Aloia 1994[44]	53 (3)	53 (3)	492 (62)	471 (165)	66 (22)	64 (26)
Chevalley 1994[19]	72 (6)	72 (6)	656 (317)	510 (273)	56 (28)	64 (28)
Strause 1994[45]	66 (8)	66 (8)	535 (285)	614 (287)	NA	NA
Prince 1995[20]	63 (4)	63 (4)	822 (286)	787 (312)	77 (38)	85 (43)
Fujita 1996[46]	79 (9)	79 (9)	NA	NA	28 (7)	25 (3)
Perez-Jaraiz 1996[47]	50 (5)	50 (5)	NA	NA	NA	NA
Recker 1996[48]	73 (7)	73 (7)	422 (197)	444 (187)	63 (15)	65 (22)
Dawson-Hughes 1997[49]	72 (5)	72 (5)	716 (338)	742 (359)	77 (36)	71 (28)
Baeksgaard 1998[50]	62	62	889 (404)	863 (389)	NA	NA
Ricci 1998[51]	58 (9)	58 (9)	706 (267)	602 (217)	NA	NA
Riggs 1998[52]	66 (3)	66 (3)	711 (276)	717 (295)	76 (26)	74 (25)

Storm 1998[21]	71 (5)	71 (5)	603 (250)	699 (286)	69 (32)	60 (30)
Castelo-Branco 1999[22]	56 (4)	56 (4)	700 (600)	800 (700)	NA	NA
Ruml 1999[53]	52 (4)	52 (4)	584 (164)	637 (208)	NA	NA
Fujita 2000[54]	50 (12)	50 (12)	NA	NA	NA	NA
Peacock 2000[13]	73 (8)	73 (8)	595 (303)	598 (279)	64 (24)	61 (30)
Son 2001[55]	72 (4)	72 (4)	405 (122)	458 (106)	46 (19)	44 (10)
Chapuy 2002[56]	86 (8)	86 (8)	558 (234)	556 (246)	22 (14)	23 (17)
Grados 2003[57]	75 (8)	75 (8)	697 (296)	671 (273)	17.5	17.5
Albertazzi 2004[26]	68 (5)	68 (5)	651 (225)	679 (194)	NA	NA
Doetsch 2004[58]	NA	NA	NA	NA	NA	NA
Harwood 2004[14]	81 (5)	81 (5)	NA	NA	30 (17)	29 (13)
Meier 2004[59]	58 (11)	58 (11)	NA	NA	75 (28)	77 (23)
Riedt 2005[60]	62 (6)	62 (6)	1033 (317)	1025 (307)	61 (19)	71 (28)
Jackson 2006[7]	62 (7)	62 (7)	1148 (654)	1154 (658)	NA	NA
Prince 2006[61]	75 (3)	75 (3)	910 (330)	920 (318)	NA	NA
Reid 2006[62]	74 (4)	74 (4)	861 (390)	853 (381)	52 (19)	52 (19)
Bolton-Smith 2007[63]	68 (6)	68 (6)	1052 (234)	1057 (263)	62 (16)	57 (16)
Bonnick 2007[64]	66 (9)	66 (9)	1257 (585)	1227 (574)	NA	NA
Hitz 2007[15]	68 (9)	68 (9)	793 (313)	785 (347)	53 (17)	57 (28)
Manios 2007[28]	61 (5)	61 (5)	531 (392)	710 (257)	63 (33)	64 (22)
Reid 2008[65]	57 (10)	57 (10)	900 (490)	800 (360)	91 (32)	95 (32)
Zhu 2008[66]	75 (3)	75 (3)	991 (351)	1046 340)	68 (25)	67 (34)
Chailurkit 2010[67,68]	66 (4)	66 (4)	342 (223)	306 (240)	70 (15)	69 (16)
Karkkainen 2010[69]	67 (2)	67 (2)	928 (487)	888 (490)	50 (18)	49 (17)
Nakamura 2012[70]	60 (6)	60 (6)	496 (126)	488 (138)	45 (15)	44 (14)

Data are mean (standard deviation). NA- not available

Appendix Table 3: Baseline bone mineral density in eligible studies

Study	BMD measurement method and DXA brand	Lumbar Spine BMD (g/cm^2)		Total Hip BMD (g/cm^2)		Femoral Neck BMD (g/cm^2)		Forearm BMD (g/cm^2)		Total Body BMD (g/cm^2)	
		Calcium	Control	Calcium	Control	Calcium	Control	Calcium	Control	Calcium	Control
Dietary Calcium Trials											
Recker 1985[16]	P	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Polley 1987[17]	P	NA	NA	NA	NA	NA	NA	1.04 (0.15)[a]	1.04 (0.19)[a]	NA	NA
Nelson 1991[18]	P	1.11 (0.16)	1.12 (0.18)	NA	NA	0.81 (0.13)	0.82 (0.13)	0.62 (0.07)	0.63 (0.12)	NA	NA
Chevalley 1994[19]	P	0.98 (0.21)	1.03 (0.19)	NA	NA	0.71 (0.14)	0.75 (0.12)	NA	NA	NA	NA
Prince 1995[20]	D-H	0.89 (0.13)	0.87 (0.14)	0.86 (0.12)	0.83 (0.11)	0.75 (0.09)	0.69 (0.09)	NA	NA	NA	NA
Storm 1998[21]	D-L	1.05 (0.18)	1.10 (0.22)	NA	NA	0.79 (0.13)	0.80 (0.09)	NA	NA	NA	NA
Castelo-Branco 1999[22]	D-L	1.01 (0.10)	1.01 (0.11)	NA	NA	NA	NA	NA	NA	NA	NA
Cleghorn 2001[23]	D-N	1.05 (0.17)	1.11 (0.14)	NA	NA	NA	NA	0.47 (0.06)	0.48 (0.05)	NA	NA
Lau 2001[24]	D-H	0.83 (0.11)	0.89 (0.10)	0.76 (0.09)	0.80 (0.10)	0.66 (0.08)	0.70 (0.09)	NA	NA	0.92 (0.07)	0.96 (0.07)
Chee 2003[25]	D-L	1.00 (0.13)	1.03 (0.15)	0.84 (0.10)	0.90 (0.12)	0.79 (0.09)	0.84 (0.10)	NA	NA	1.05 (0.07)	1.08 (0.08)
Albertazzi 2004[26]	D-L	1.06 (0.12)	1.05 (0.12)	NA	NA	0.83 (0.08)	0.83 (0.10)	NA	NA	NA	NA
Daly 2006[27]	D-L	1.22 (0.16)	1.21 (0.16)	1.02 (0.12)	1.04 (1.12)	0.95 (0.12)	0.95 (0.10)	0.79 (0.07)	0.78 (0.07)	NA	NA
Manios 2007[28]	D-L	1.09 (0.17)	1.05 (0.20)	NA	NA	NA	NA	NA	NA	1.13 (0.07)	1.12 (0.08)
Kukuljan 2009[29]	D-L	1.22 (0.15)	1.24 (0.15)	1.02 (0.08)	1.02 (0.10)	0.92 (0.07)	0.94 (0.08)	NA	NA	NA	NA
Gui 2012[30]	D-H	0.96 (0.09)	0.95 (0.07)	0.87 (0.09)	0.87 (0.08)	0.72 (0.09)	0.72 (0.08)	NA	NA	NA	NA
Calcium Supplement Trials											
Recker 1977[31]	P	NA	NA	NA	NA	NA	NA	0.93 (0.16)	0.80 (0.20)	NA	NA
Lamke 1978[32]	X-S	NA	NA	NA	NA	2.58 (0.46)[a]	2.58 (0.46)[a]	NA	NA	NA	NA
Smith 1981[33]	P	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hansson 1987[12]	P	2.69[a]	2.75[a]	NA	NA	NA	NA	NA	NA	NA	NA
Polley 1987[17]	P	NA	NA	NA	NA	NA	NA	1.06 (0.18)[a]	1.04 (0.19)[a]	NA	NA
Riis 1987[34]	P	0.86 (0.11)	0.87 (0.10)	NA	NA	NA	NA	37 (4.9)[b]	36 (4.3)[b]	3054 (344)[c]	2957 (358)[c]
Smith 1989[35]	P	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dawson-Hughes 1990[36]	P	1.08 (0.17)	1.08 (0.13)	NA	NA	0.79 (0.13)	0.79 (0.10)	0.61 (0.13)	0.61 (0.10)	NA	NA
Fujita 1990[37]	P	NA	NA	NA	NA	NA	NA	0.35 (0.10)	0.38 (0.08)	NA	NA

Orwoll 1990[38]	P	NA	NA	NA	NA	NA	NA	1.24 (0.18)[a]	1.29 (0.14)[a]	NA	NA
Elders 1991[39]	P	0.88 (0.13)[d]	0.89 (0.13)[d]	NA	NA	NA	NA	NA	NA	NA	NA
Prince 1991[40]	P	NA	NA	NA	NA	NA	NA	0.53 (0.06)	0.53 (0.05)	NA	NA
Chapuy 1992[41]	D-H	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lau 1992[42]	D-N	0.70 (0.11)	0.65 (0.15)	NA	NA	0.53 (0.11)	0.51 (0.09)	NA	NA	NA	NA
Reid 1993[43]	D-L	1.02 (0.14)	1.05 (0.14)	NA	NA	0.85 (0.09)	0.86 (0.10)	NA	NA	1.06 (0.09)	1.06 (0.08)
Aloia 1994[44]	P	1.21 (0.18)	1.20 (0.15)	NA	NA	0.84 (0.15)	0.87 (0.09)	0.64 (0.06)	0.66 (0.09)	NA	NA
Chevalley 1994[19]	P	1.00 (0.25)	1.03 (0.19)	NA	NA	0.76 (0.15)	0.75 (0.12)	NA	NA	NA	NA
Strause 1994[45]	D-H	0.91 (0.19)	0.93 (0.15)	NA	NA	NA	NA	NA	NA	NA	NA
Prince 1995[20]	D-H	0.88 (0.12)	0.87 (0.14)	0.84 (0.10)	0.83 (0.11)	0.70 (0.09)	0.69 (0.09)	NA	NA	NA	NA
Fujita 1996[46]	D-L	0.62 (0.13)	0.62 (0.18)	NA	NA	NA	NA	0.45 (0.09)	0.50 (0.13)	NA	NA
Perez-Jaraiz 1996[47]	D-N	NA	NA	NA	NA	NA	NA	NA	NA	38 (4)[e]	38 (5)[e]
Recker 1996[48]	P	NA	NA	NA	NA	NA	NA	0.71 (0.13)[c]	0.75 (0.14)[c]	NA	NA
Dawson-Hughes 1997[49]	D-L	1.16 (0.19)	1.15 (0.20)	NA	NA	0.89 (0.12)	0.87 (0.11)	NA	NA	1.11 (0.10)	1.10 (0.09)
Baeksgaard 1998[50]	D-N	0.90 (0.20)	0.93 (0.17)	NA	NA	0.73 (0.13)	0.73 (0.12)	0.37 (0.07)	0.37 (0.08)	NA	NA
Ricci 1998[51]	D-L	NA	NA	NA	NA	NA	NA	NA	NA	1.11 (0.09)	1.15 (0.08)
Riggs 1998[52]	D-H	0.90	0.92	0.81	0.81	NA	NA	NA	NA	1.02	1.03
Storm 1998[21]	D-L	1.10 (0.22)	1.10 (0.22)	NA	NA	0.77 (0.18)	0.80 (0.09)	NA	NA	NA	NA
Castelo-Branco 1999[22]	D-L	1.00 (0.10)	1.01 (0.11)	NA	NA	NA	NA	NA	NA	NA	NA
Ruml 1999[53]	D-H/D-L/P	0.90 (0.12)	0.90 (0.09)	NA	NA	0.73 (0.12)	0.68 (0.09)	0.63 (0.08)	0.66 (0.06)	NA	NA
Fujita 2000[54]	D-N	0.81 (0.16)	0.88 (0.13)	NA	NA	NA	NA	NA	NA	NA	NA
Peacock 2000[13]	D-L	1.07 (0.19)	1.08 (0.20)	0.85 (0.14)	0.86 (0.13)	0.80 (0.15)	0.79 (0.13)	NA	NA	1.07 (0.11)	1.07 (0.10)
Son 2001[55]	D-L	0.87 (0.16)	0.83 (0.15)	NA	NA	0.61 (0.11)	0.62 (0.10)	NA	NA	NA	NA
Chapuy 2002[56]	D-H/S-H	NA	NA	0.68 (0.15)	0.71 (0.13)	0.59 (0.13)	0.62 (0.18)	0.31 (0.07)	0.31 (0.07)	NA	NA
Grados 2003[57]	D-L/D-H/D-N	0.91 (0.19)	0.90 (0.19)	NA	NA	0.68 (0.11)	0.66 (0.11)	NA	NA	0.99 (0.11)	0.98 (0.12)
Albertazzi 2004[26]	D-L	1.09 (0.14)	1.05 (0.12)	NA	NA	0.83 (0.07)	0.83 (0.10)	NA	NA	NA	NA
Doetsch 2004[58]	D-N	NA	NA	NA	NA	0.59 (0.11)	0.59 (0.07)	NA	NA	NA	NA
Harwood 2004[14]	D-H	0.86 (0.12)	0.85 (0.13)	0.64 (0.10)	0.65 (0.08)	0.57 (0.09)	0.56 (0.06)	NA	NA	NA	NA
Meier 2004[59]	D-H	1.03 (0.15)	0.96 (0.25)	NA	NA	1.00 (0.22)	0.88 (0.17)	NA	NA	NA	NA
Riedt 2005[60]	D-L	0.97 (0.09)	1.00 (0.09)	NA	NA	0.82 (0.09)	0.86 (0.11)	0.62 (0.08)	0.64 (0.08)	1.10 (0.08)	1.12 (0.08)
Jackson 2006[7]	D-H	NA	NA	0.87 (0.14)	0.86 (0.14)	NA	NA	NA	NA	NA	NA
Prince 2006[61]	D-H	NA	NA	0.82 (0.12)[f]	0.82 (0.12)[f]	0.69 (0.11)[f]	0.69 (0.10)[f]	NA	NA	0.85 (0.10)[f]	0.86 (0.10)[f]
Reid 2006[62]	D-L	1.06 (0.18)	1.05 (0.18)	0.86 (0.14)	0.85 (0.13)	NA	NA	NA	NA	1.04 (0.09)	1.03 (0.09)
Bolton-Smith 2007[63]	D-H	NA	NA	NA	NA	0.83 (0.11)	0.83 (0.11)	0.60 (0.08)	0.60 (0.08)	NA	NA

Bonnick 2007[64]	D-H	0.78 (0.08)	0.77 (0.08)	NA	NA	0.65 (0.10)	0.65 (0.10)	NA	NA	NA	NA
Hitz 2007[15]	D-H	0.93 (0.18)	0.84 (0.20)	0.74 (0.11)	0.70 (0.14)	NA	NA	NA	NA	NA	NA
Manios 2007[28]	D-L	1.09 (0.27)	1.05 (0.20)	NA	NA	NA	NA	NA	NA	1.08 (0.11)	1.12 (0.08)
Reid 2008[65]	D-L	1.26 (0.17)	1.24 (0.16)	1.08 (0.14)	1.08 (0.13)	NA	NA	NA	NA	1.26 (0.09)	1.26 (0.10)
Zhu 2008[66]	D-H	NA	NA	0.81 (0.09)	0.83 (0.13)	NA	NA	NA	NA	NA	NA
Chailurkit 2010[67,68]	D-L	1.01 (0.12)	1.02 (0.14)	0.88 (0.10)	0.89 (0.11)	0.79 (0.10)	0.80 (0.10)	NA	NA	NA	NA
Karkkainen 2010[69]	D-L	1.04 (0.17)	1.05 (0.17)	0.95 (0.14)	0.95 (0.13)	0.87 (0.13)	0.87 (0.12)	NA	NA	1.07 (0.09)	1.08 (0.09)
Nakamura 2012[70]	D-H	0.90 (0.15)	0.93 (0.16)	NA	NA	0.69 (0.10)	0.70 (0.10)	NA	NA	NA	NA

Values are means (standard deviations). Abbreviations: BMD- bone mineral density. NA - not available. P- photon absorptiometry; D- dual energy x-ray absorptiometry (DXA); H- Hologic device; L- Lunar device; N- Norland device; X-S X-ray spectrophotometry; S- single x-ray absorptiometry

[a] bone mineral content (g/cm)

[b] bone mineral content (units)

[c] bone mineral content (g)

[d] units geq hydroxyapatite/cm^2

[e] bone mineral content/ body weight (g/kg)

[f] measured at 1 year

Appendix Table 4: Risk of bias assessment for eligible trials

Study	Random sequence generation described	Allocation concealment	Blinding of participants/ personnel	Blinding of BMD assessment	Differential loss to follow-up	Selective reporting	BMD as primary endpoint	Overall risk of bias[a]	Funding	Conflicts of interest
Dietary calcium trials										
Recker 1985[16]	NS	NS	N	N	N	N	Y	High	IF, Ind	NS
Polley 1987[17]	NS	NS	N	N	Y	N	Y	High	IF, Tab	NS
Nelson 1991[18]	NS	NS	Y	Y	Y	N	Y	High	IF, Ind	NS
Chevalley 1994[19]	NS	NS	Y	Y	N	N	Y	Low	IF, Tab	NS
Prince 1995[20]	NS	Y	N	N	NS	N	Y	Moderate	IF, Tab	NS
Storm 1998[21]	NS	NS	N	Y	N	N	Y	High	Ind	NS
Castelo-Branco 1999[22]	Y	NS	N	N	N	N	Y	High	NS	No
Cleghorn 2001[23]	NS	NS	N	N	N	N	Y	Moderate	Ind, Tab	NS
Lau 2001[24]	NS	NS	N	NS	N	N	Y	Moderate	Ind	NS
Chee 2003[25]	NS	NS	N	N	Y	N	Y	High	Ind	NS
Albertazzi 2004[26]	Y	Y	Y	Y	N	N	Y	Low	Ind, Tab	No
Daly 2006[27]	Y	NS	N	Y	N	N	Y	Moderate	IF, Ind, Tab	No
Manios 2007[28]	NS	NS	N	N	N	N	Y	Moderate	Ind	Yes
Kukuljan 2009[29]	NS	NS	N	NS	N	N	Y	Moderate	IF, Ind, Tab	No
Gui 2012[30]	Y	NS	N	Y	Y	N	Y	High	IF, Tab	No
Calcium supplement trials										
Recker 1977[31]	NS	NS	N	Y	NS	N	Y	High	IF	No
Lamke 1978[32]	NS	NS	NS	NS	N	N	Y	Moderate	NS	NS
Smith 1981[33]	NS	NS	Y	Y	N	N	Y	High	NS	NS
Hansson 1987[12]	NS	NS	NS	NS	N	N	Y	Moderate	IF	NS
Polley 1987[17]	NS	NS	N	N	Y	N	Y	High	IF, Tab	NS
Riis 1987[34]	Y	NS	Y	Y	N	N	Y	Moderate	NS, Tab	NS
Smith 1989[35]	NS	NS	Y	Y	N	N	Y	Low	IF, Ind	NS
Dawson-Hughes 1990[36]	NS	NS	Y	Y	NS	N	Y	High	IF, Ind, Tab	NS
Fujita 1990[37]	N	N	NS	NS	Y	N	Y	High	NS	NS

Orwoll 1990[38]	Y	NS	Y	Y	NS	N	Y	Low	IF, Ind	NS
Elders 1991[39]	NS	NS	NS	NS	Y	N	Y	High	IF	NS
Prince 1991[40]	Y	Y	Y	Y	NS	N	Y	Low	IF, Tab	NS
Chapuy 1992[41]	NS	NS	NS	NS	N	N	N	High	IF, Tab	NS
Lau 1992[42]	NS	NS	NS	NS	N	N	Y	Moderate	Ind, Tab	NS
Reid 1993[43]	NS	NS	Y	Y	NS	N	Y	Low	IF, Tab	NS
Aloia 1994[44]	Y	NS	Y	Y	NS	N	Y	Low	IF, Tab	NS
Chevalley 1994[19]	NS	NS	Y	Y	N	N	Y	Low	IF, Tab	NS
Strause 1994[45]	NS	NS	Y	Y	N	N	Y	High	IF, Ind	NS
Prince 1995[20]	NS	Y	Y	Y	NS	N	Y	Low	IF, Tab	NS
Fujita 1996[46]	N	N	Y	Y	N	N	Y	High	NS	NS
Perez-Jaraiz 1996[47]	NS	NS	N	N	N	N	Y	High	NS	NS
Recker 1996[48]	NS	NS	Y	Y	NS	N	Y	Low	IF, Ind, Tab	NS
Dawson-Hughes 1997[49]	NS	NS	Y	Y	NS	N	Y	Low	IF	NS
Baeksgaard 1998[50]	NS	NS	Y	Y	N	N	Y	Low	NS, Tab	NS
Ricci 1998[51]	NS	NS	Y	Y	N	N	Y	High	IF	NS
Riggs 1998[52]	NS	NS	Y	Y	N	N	Y	Low	IF, Tab	NS
Storm 1998[21]	NS	NS	Y	Y	N	N	Y	Moderate	Ind	NS
Castelo-Branco 1999[22]	Y	NS	N	N	N	N	Y	High	NS	No
Ruml 1999[53]	NS	NS	NS	NS	Y	N	Y	High	IF, Tab	No
Fujita 2000[54]	NS	Y	Y	Y	NS	N	Y	High	Tab	NS
Peacock 2000[13]	NS	NS	Y	Y	N	N	Y	Moderate	IF, Ind, Tab	NS
Son 2001[55]	NS	NS	NS	NS	NS	N	Y	Moderate	IF	NS
Chapuy 2002[56]	NS	NS	Y	Y	N	N	N	High	Ind	NS
Grados 2003[57]	NS	NS	Y	Y	N	N	Y	Moderate	Ind, Tab	Yes
Albertazzi 2004[26]	Y	Y	Y	Y	N	N	Y	Low	Ind, tab	No
Doetsch 2004[58]	NS	NS	Y	Y	NS	N	N	High	IF, Ind	NS
Harwood 2004[14]	Y	Y	N	N	N	N	Y	High	Ind	NS
Meier 2004[59]	NS	NS	N	N	Y	N	Y	High	NS	No
Riedt 2005[60]	NS	NS	Y	Y	NS	N	Y	Moderate	IF	No
Jackson 2006[7]	NS	NS	Y	Y	N	N	N	Low	IF	Yes
Prince 2006[61]	Y	Y	Y	Y	NS	Y	N	High	IF	No
Reid 2006[62]	Y	Y	Y	Y	N	N	N	Low	IF, Tab	NS
Bolton-Smith 2007[63]	Y	NS	Y	Y	N	N	Y	Low	IF, Tab	No

Bonnick 2007[64]	NS	NS	Y	Y	N	N	Y	Moderate	Ind	Yes
Hitz 2007[15]	NS	Y	Y	Y	NS	N	Y	Moderate	IF, Ind, Tab	No
Manios 2007[28]	NS	NS	N	N	N	N	Y	High	Ind	Yes
Reid 2008[65]	Y	Y	Y	Y	N	N	Y	Low	IF, Tab	Yes
Zhu 2008[66]	Y	Y	Y	Y	N	N	Y	Low	IF	No
Chailurkit 2010[67,68]	Y	Y	Y	Y	N	N	Y	Low	IF, Tab	No
Karkkainen 2010[69]	NS	Y	N	N	N	N	N	Moderate	IF, Tab	No
Nakamura 2012[70]	Y	Y	Y	Y	N	N	Y	Low	IF, Ind, Tab	No

[a] For each study, we assessed an overall risk of bias based on the features of the study design in the Table. We gave the greatest weighting in assessing a study as higher risk to lack of blinding, high rate of drop out or differential drop out, small size of study, non-random selection of subsets of participants for bone density measurement, and other specific biases as described in the Table. Funding source and conflict of interests were not included in our assessment.

BMD = bone mineral density; NS = not stated; IF- funding by grants from independent funders; Ind- funded by grants from industry and/or run by industry; Tab- study tablets provided by pharmaceutical company or milk provided by industry.

Appendix Table 5: Pooled analyses of multi-arm trials permitting a comparison of a calcium supplement arm with a dietary source of calcium arm

Site	Time-Point (y)	Studies (N)	Participants (N)	BMD difference[a] (95% CI)	P
Lumbar spine	1	4	288	-0.8 (-1.7,0.2)	0.12
	2	4	215	-0.3 (-1.3,0.6)	0.46
Femoral neck	1	2	187	0.4 (-0.7,1.4)	0.50
	2	3	179	0.7 (-1.3,2.8)	0.47
Total hip	1	1	84	0.1 (-1.2,1.3)	0.91
	2	1	84	0.2 (-1.0,1.4)	0.75
Forearm	1	1	98	0.5 (-0.2,1.3)	0.16
Total body	1	1	65	-0.9 (-2.0,0.2)	0.12

[a] Weighted mean difference between-groups in percentage change in bone mineral density (BMD) from baseline in multi-arm randomised controlled trials with a calcium supplement arm and a dietary source of calcium arm. Results are for the calcium supplement arm compared to the dietary calcium supplement arm.

Abbreviation: CI- confidence interval

Appendix Table 6: Subgroup analyses

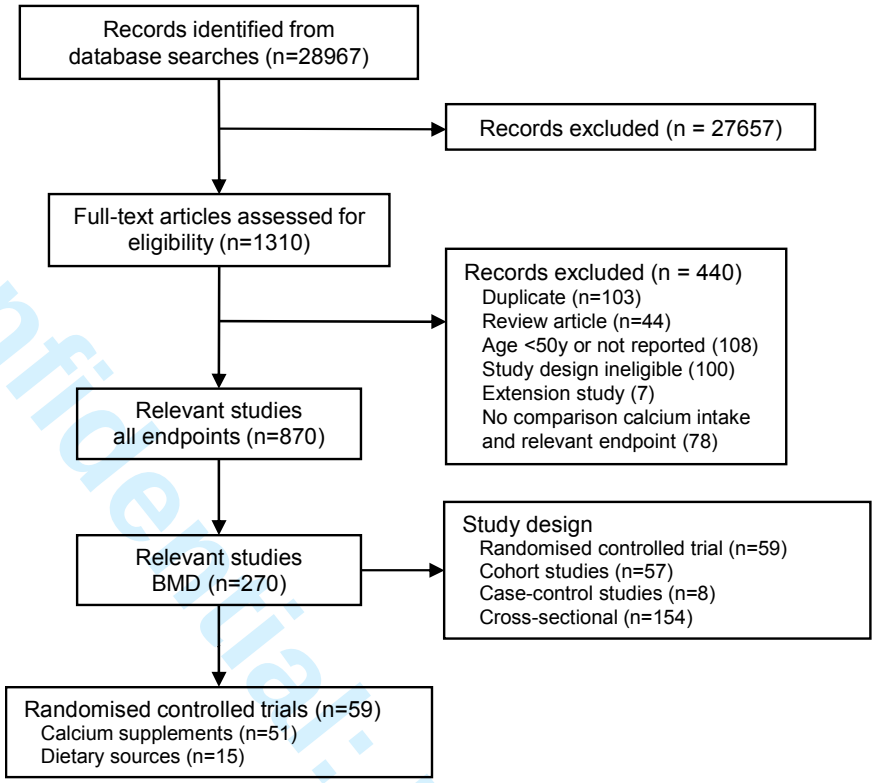
Site/ Time-point/ Intervention	Subgroup	Studies (N)	BMD difference[a] (95% CI)	P[b]
Calcium monotherapy vs Co-administered CaD				
LS/1/diet	Ca mono	8	0.5 (-0.4,1.5)	0.62
	CaD	3	0.8 (0.2,1.4)	
LS/1/supp	Ca mono	21	1.3 (0.8,1.7)	0.81
	CaD	7	1.1 (0.2,2.1)	
LS/2/supp	Ca mono	18	1.3 (0.8,1.8)	0.007
	CaD	3	0.4 (0.1,0.8)	
FN/1/supp	Ca mono	13	1.3 (0.5,2.0)	0.86
	CaD	7	1.2 (0.4,1.9)	
FN/2/supp	Ca mono	9	1.2 (0.6,1.7)	0.16
	CaD	5	0.5 (-0.1,1.2)	
TB/1/supp	Ca mono	7	0.6 (0.2,1.0)	0.21
	CaD	3	1.1 (0.5,1.7)	
Risk of bias				
LS/1/supp	Low	9	1.2 (0.6,1.8)	0.76
	Mod/High	18	1.3 (0.7,1.9)	
LS/2/supp	Low	10	0.9 (0.4,1.4)	0.10
	Mod/High	11	1.7 (0.9,2.6)	
FN/1/supp	Low	7	0.9 (0.2,1.6)	0.22
	Mod/High	12	1.6 (0.8,2.4)	
FN/2/supp	Low	8	1.0 (0.4,1.6)	0.60
	Mod/High	6	0.7 (-0.1,1.6)	
TB/1/supp	Low	3	0.4 (0.1,0.7)	0.06
	Mod/High	7	1.0 (0.5,1.6)	
F/1/supp	Low	3	0.4 (-0.4,1.2)	0.17
	Mod/High	7	1.4 (0.2,2.5)	
F/2/supp	Low	4	0.5 (-0.2,1.1)	0.02
	Mod/High	6	3.6 (1.1,6.1)	
Calcium dose (mg/d)				
LS/1/diet	≥1000	6	0.8 (0.3,1.2)	0.68
	<1000	5	0.5 (-0.9,1.8)	
LS/1/supp	≥1000	15	1.1 (0.4,1.7)	0.61
	<1000	14	1.3 (0.8,1.8)	
LS/2/supp	≥1000	13	1.0 (0.4,1.7)	0.83
	<1000	9	1.1 (0.6,1.7)	
FN/1/supp	≥1000	11	1.4 (0.5,2.2)	0.64
	<1000	9	1.1 (0.3,1.9)	
FN/2/supp	≥1000	8	0.7 (0.2,1.2)	0.28
	<1000	6	1.2 (0.4,2.1)	
TB/1/supp	≥1000	7	0.6 (0.1,1.0)	0.29
	<1000	4	0.9 (0.5,1.3)	
F/1/supp	≥1000	5	0.7 (0.2,1.3)	0.25
	<1000	5	1.9 (0.0,3.9)	
F/2/supp	≥1000	5	0.8 (-0.1,1.8)	0.13
	<1000	5	2.7 (0.5,4.9)	
Vitamin D dose (IU/d)				
Age (y)				
LS/1/supp	50-65	15	1.2 (0.7,1.7)	0.97
	65+	12	1.2 (0.5,2.0)	
LS/2/supp	50-65	12	0.8 (0.3,1.3)	0.08
	65+	9	1.5 (0.9,2.2)	
FN/1/supp	50-65	10	1.1 (0.4,1.7)	0.36
	65+	8	1.6 (0.6,2.7)	
FN/2/supp	50-65	7	0.8 (0.3,1.4)	0.58
	65+	7	1.1 (0.3,2.0)	
TB/1/supp	50-65	7	0.9 (0.3,1.5)	0.24
	65+	3	0.5 (0.2,0.8)	
F/2/supp	50-65	7	1.1 (0.3,1.8)	0.23
	65+	3	5.6 (-1.7,13.0)	
Baseline dietary calcium intake (mg/d)				
LS/1/diet	<800	5	0.7 (0.2,1.1)	0.54
	≥800	4	0.9 (0.3,1.5)	
LS/1/supp	<800	14	1.7 (1.1,2.3)	0.14
	≥800	6	0.9 (0.1,1.7)	
LS/2/supp	<800	10	1.5 (1.0,2.0)	0.08
	≥800	7	0.8 (0.2,1.4)	
FN/1/supp	<800	10	1.7 (0.7,2.6)	0.11
	≥800	4	0.7 (0.1,1.4)	
FN/2/supp	<800	9	1.4 (0.6,2.1)	0.10
	≥800	4	0.5 (0.0,1.1)	
F/1/supp	<800	3	0.6 (-0.1,1.3)	0.96
	≥800	3	0.6 (-0.3,1.4)	
F/2/supp	<800	3	1.3 (-0.1,2.8)	0.25
	≥800	3	0.4 (-0.4,1.1)	
Calcium dose (mg/d)				
LS/1/diet	>500	9	0.7 (0.4,1.1)	0.72
	≤500	3	1.5 (-2.8,5.9)	
LS/1/supp	>500	20	1.3 (0.7,1.9)	0.62
	≤500	7	1.1 (0.5,1.7)	
LS/2/supp	>500	18	1.2 (0.7,1.7)	0.89
	≤500	3	1.1 (0.3,1.9)	
FN/1/supp	>500	12	1.6 (0.7,2.5)	0.16
	≤500	7	0.8 (0.2,1.5)	
FN/2/supp	>500	11	0.9 (0.4,1.4)	0.97
	≤500	3	0.9 (-0.1,2.0)	
Baseline 25-hydroxyvitamin D (nmol/L)				
LS/1/supp	<50	6	2.0 (0.8,3.2)	0.41
	≥50	12	1.4 (0.8,2.0)	
LS/2/supp	<50	4	1.3 (0.1,2.4)	0.63
	≥50	9	1.0 (0.2,1.7)	
FN/1/supp	<50	5	2.0 (0.1,3.9)	0.58
	≥50	6	1.4 (0.6,2.2)	

FN/1/supp	<800	4	1.0 (0.3,1.7)	0.71	**FN/2/supp**	<50	3	1.2 (-0.9,3.3)	0.81
	≥800	3	1.5 (-0.7,3.7)			≥50	6	1.4 (0.9,2.0)	

[a] Weighted mean difference in percentage change in bone mineral density (BMD) from baseline.

[b] Test for interaction between subgroups

Abbreviation: CI- confidence interval; Ca mono- calcium monotherapy, CaD- calcium and vitamin D; LS- lumbar spine; FN- femoral neck; TB- total body; F- forearm; supp- calcium supplement trials; diet- dietary calcium intake trials.



Appendix Figure 1: flow of studies

Protocol of a systematic review of whether the effects of calcium supplements are different from the effects of dietary calcium intake for a series of endpoints

Background

Calcium supplements have been widely used to supplement dietary calcium intake in the prevention and treatment of osteoporosis. Calcium supplements typically are taken either once or twice daily at a dose of 500-600mg/tablet. It has generally been assumed that calcium taken from supplements in this manner is equivalent to calcium obtained from food as part of the diet.

In randomised controlled trials (RCTs), calcium supplements increase the risk of myocardial infarction and stroke by a small amount, findings confirmed in some but not all observational studies. By contrast, there is no evidence to date linking dietary calcium intake with increased cardiovascular risk. One potential explanation for this difference is that calcium supplements but not food sources of calcium increase serum calcium levels for about 4-6h, and serum calcium is associated with increased atherosclerosis, cardiovascular events, and cardiovascular mortality.

Therefore, we hypothesize that calcium supplements may not be equivalent to dietary calcium intake. We will undertake a systematic review to determine whether the effects of calcium supplements differ from the effects of dietary calcium intake for endpoints that are known or to be influenced by the use of calcium supplements.

Aims

1. The broad aim is to answer the question are the effects of calcium supplements different from the effects of dietary calcium intake on the incidence of fractures, cardiovascular events, cancer, on changes in bone mineral density, body weight, serum cholesterol, blood pressure and serum calcium, and on the incidence of kidney stones, and gastrointestinal side-effects.

2. Specifically for each endpoint we will address three questions:
    i. What is the effect of calcium supplements or modifying dietary calcium intake on the endpoint in RCTs?
    ii. What is the relationship between calcium supplements or dietary calcium intake and the endpoint in longitudinal studies?
    iii. What is the relationship between calcium supplements or dietary calcium intake and the endpoint in cross-sectional studies?

    Each of these questions will be addressed in a hierarchical order- ie. the greatest weight will be given to the results of RCTs, and evidence from longitudinal studies will only be given primacy if there are inadequate data from RCTs (and likewise, cross-sectional studies will only be given primacy where there are inadequate data from RCTs and longitudinal studies).

3. The endpoints will also be considered in a hierarchical fashion in the following order of importance.

    Primary therapeutic endpoints:
    i. Fractures
        a. Total fractures
        b. Hip fractures
        c. Vertebral fractures
        d. Forearm fractures

    ii. Bone mineral density
        a. Lumbar spine
        b. Total hip
        c. Femoral neck
        d. Total body

e. Forearm

Primary adverse endpoints:

i. Cardiovascular events
   a. Myocardial infarction
   b. Stroke
   c. Ischaemic heart disease (or synonym) to capture acute coronary events
   d. Cerebrovascular disease (or synonym) to capture acute cerebrovascular events.

ii. Mortality

iii. Kidney stones

iv. Gastrointestinal side-effects
   a. Constipation
   b. Acute gastrointestinal symptoms

Secondary therapeutic events:

i. Cancer
   a. Total cancers except non-melanoma skin cancers
   b. Colorectal adenoma
   c. Colorectal carcinoma
   d. Breast cancer
   e. Prostate cancer

ii. Blood pressure

iii. Cholesterol and its fractions

iv. Weight

v. Serum calcium

Methods

The review will be conducted and reported in compliance with the PRISMA guidelines for RCTs and the MOOSE guidelines for observational studies.

Literature search

The electronic databases MEDLINE (OvidSP) and EMBASE will be searched from inception to Jan 2013 for studies meeting the inclusion criteria. The reference lists of papers meeting the inclusion criteria, and recent related systematic reviews, will be screened for additional potentially relevant papers that may have been missed by our electronic searches.

Inclusion criteria:

- Languages: English only
- Study design: RCTs, prospective longitudinal observational studies, and cross-sectional studies.
- Report at least one endpoint of interest

RCTs:

- Minimum age of trial participants at baseline ≥50 years.
- Trial population is men, women or both.
- Trial has a randomized controlled design.

Prospective longitudinal observational studies:

- Minimum age of trial participants at baseline or the majority of follow-up occurs in participants ≥50 years
- Participant population is men, women or both.
- Calcium intake and/or supplement use assessed at baseline.

Cross-sectional studies:

- Minimum age of trial participants at baseline ≥50 years.
- Participant population is men, women or both.
- Calcium intake and/or supplement use assessed at baseline.

Exclusion criteria:

- Studies in which most subjects have a major systemic pathology other than osteoporosis (e.g., renal failure, malignancy) are not eligible.
- Only one publication from each study (with respect to each endpoint) will be considered, to avoid duplication of data in the meta-analysis. The largest study that otherwise conforms to the inclusion and exclusion criteria will be included for RCTs, and the longest duration study will be included for cohort studies
- Used calcium in combination with other treatment (eg,fluoride, hormones, or antiresorptive therapy), unless the combination treatments are given to both arms such that the only difference between the groups is calcium.

Screening

For the first level of screening, one reviewer will read the titles of all the citations retrieved from the electronic database searches and remove all citations that were clearly not related to the review questions asked or were duplicates. The second level of screening will involve abstract review by one reviewer, and all unrelated citations will be removed. Full-text articles will be obtained for the remaining abstracts that are potentially relevant. Unrelated articles will again be removed. The full text of all remaining potentially relevant articles will be independently assessed by two reviewers for eligibility, and any disagreements resolved by consensus.

Data extraction strategy

We will assess the method of every study using a four-item checklist: reporting of randomisation method; allocation concealment; blinding of outcome assessment; and completeness of follow-up. The criteria were drawn from the Cochrane Collaboration guidelines (Cochrane handbook for systematic reviews of interventions 5.1.0: The Cochrane Collaboration, 2011). Assessment of quality of a cohort study will use the Newcastle Ottawa Scale.

Data from eligible papers will be extracted by one reviewer into a form, and reviewed by a second reviewer. Any disagreements will be resolved by consensus. Events, total numbers, and p-values will be recorded for discrete data. Means (with standard errors), standard deviations, numbers, and p-values will be recorded for continuous data.

Statistical analyses

- Funnel plots will be used to assess publication bias
- Random effects model will be used to pool study data. Heterogeneity will be assessed using Cochran's Q statistic and the I^2 statistic> 50% will be used as a threshold indicating significant heterogeneity.
- All tests will be two tailed, with significance level set at 5%.
- Analyses will be undertaken using CMA (or Stata SE v.11).