Calcium with or without vitamin D for prevention of cardiovascular disease: An updated systematic review and meta-analysis for the National Osteoporosis Foundation

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Abstract (Annals of Internal Medicine)

Background: Effects of high calcium intake on cardiovascular disease (CVD) outcomes diverge widely.

Purpose: A focused update and reanalysis of two broader systematic reviews to inform a joint position statement from two organizations.

Data Sources: MEDLINE®, the Cochrane Central, and Scopus (including EMBASE) through May 2015 for English-language, peer reviewed publications.

Study Selection: We included randomized controlled trials (RCTs), prospective cohort, and nested case–control studies reporting any CVD event or mortality endpoints. Analyses of combinations of calcium and micronutrients other than vitamin D were excluded.

Data Extraction: We extracted data on study and participant characteristics, calcium exposures (including supplements), and statistical analyses, including adjustments for confounding. Risk of bias was assessed using standardized checklists. Differences were resolved by consensus.

Data Synthesis: We included a total of four RCTs (in 9 publications) and 21 cohort studies. Overall, RCTs did not find significant differences in risks of CVD events or mortality between calcium (with or without vitamin D supplementation) and placebo groups. Dose-response meta-regression analyses of cohort studies did not find significant linear or non-linear relationships between levels of dietary or total calcium intake and the risks of CVD mortality or stroke. The random-effects meta-analysis also showed no significant associations between adequacy of calcium intake (≥1000 vs. < 1000 mg/d) and the risks of CVD mortality (pooled adjusted hazard ratio = 1.01; 95% CI 0.94 to 1.07; I^2 = 49%), stroke mortality (pooled adjusted hazard ratio = 1.04; 95% CI 0.96 to 1.14; I^2 = 0%), or total stroke (pooled adjusted hazard ratio = 1.02; 95% CI 0.94 to 1.10; I^2 = 6.8%).

Limitations: CVD outcomes were secondary endpoints in all trials; and meta-analysis of cohort studies was limited by potential residual confounding, ecological bias, and misclassifications of calcium exposures.

Conclusion: Calcium intake levels (dietary and supplemental sources) are not associated with CVD risks among generally healthy adults within the tolerable upper intake levels (ULs, 2000 to 3000 mg/d).

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Background

Calcium is an essential nutrient that is required for maintaining bone health. A small proportion of total body calcium (less than 1%) is also required for vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion. Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany (1). Calcium is found in some foods (e.g., dairy and some dark green vegetables) but vitamin D is naturally present in few foods (e.g., fatty fish and egg yolks). Both calcium and vitamin D are available in dietary supplements. In 2011, the Food and Nutrition Board of the Institute of Medicine (IOM FNB) updated the dietary reference intake values (DRIs) for calcium and vitamin D for healthy populations, based on a rigorous and comprehensive review of the scientific data, and a risk assessment framework that considered a wide range of chronic disease and other indicators to assess nutrient adequacy (1, 2). The IOM Committee concluded that the evidence that calcium or vitamin D reduced risks of nonskeletal chronic disease outcomes (including cardiovascular disease outcomes) was inconsistent, inconclusive, and did not meet criteria for establishing cause-and-effect relationships. Thus, the 2011 calcium DRIs are largely based on calcium balance studies of populations aged 1 to 50 years old and observational and clinical trial evidence on bone health outcomes after age 50. For calcium, Recommended Dietary Allowances (RDAs; covering requirements of ≥97.5% of the population) and the tolerable upper intake levels (ULs; the highest daily intake of the nutrient that is likely to pose no risk) range from 1000 to 1300 mg/d (RDAs) and from 2000 to 3000 mg/d (ULs), respectively, for life-stage groups 18 years or older. For vitamin D, RDAs range

from 600 to 800 IU/d and UL is 4000 IU/d for life-stage groups 18 years or older. It should be noted that UL is intended to reflect long-term chronic intake and to be used for public health purposes. Unlike RDAs, UL is not intended as a target intake; rather, the risk for harm begins to increase once intakes surpass this level.

Adequate intakes of calcium and vitamin D are critical for maintaining bone health, particularly for older adults. Data from previous systematic reviews showed that combined calcium and vitamin D supplementation can reduce risk of fractures in older adults (3, 4) but more recent systematic reviews found inconsistent effects across randomized controlled trials (5, 6). Furthermore there are emerging concerns on the effects of high calcium (with or without vitamin D) intake from foods and supplements on cardiovascular disease (CVD) outcomes (7-9). Specifically, a meta-analysis of both study-level and patient-level data from randomized controlled trials showed that calcium with or without vitamin D supplementation increased the risk of myocardial infarction (relative risk = 1.24; 95% CI,1.07 to 1.45) and stroke (relative risk = 1.15; 95% CI, 1.00, 1.32) (10, 11). However many researchers have questioned the strength of the body of evidence linking supplemental calcium intake with CVD risk, noting that the CVD outcomes have not been the primary endpoint of any calcium or calcium and vitamin D supplementation trials to date (12, 13).

To inform a joint position statement from the National Osteoporosis Foundation (NOF) and the American Society for Preventive Cardiology (ASPC), NOF commissioned a focused update and reanalysis of two broader systematic reviews (evidence reports) of the effects of calcium and vitamin D on a wide range of clinical and intermediate outcomes (6, 14). This update addresses the effects of calcium intake (dietary or supplement sources), alone or in combination with vitamin D, on cardiovascular disease (CVD) among generally healthy adults.

Methods

This systematic review implemented the same methodology as the 2009 evidence report examining the effects of calcium and vitamin D (alone or in combination with each other) on 17 health outcomes across all life stages that was produced to inform the IOM committee charged to update the DRI for calcium and vitamin D (14). In 2014, the Agency for Healthcare Quality and Research (AHRQ) commissioned an update of the 2009 evidence report focusing on studies of vitamin D alone or in combination with calcium (6). Thus, the effects of calcium intake (from foods or supplements) alone on CVD were not updated in the 2014 evidence report.

The methodological details have been described previously in the two evidence reports (6, 14). Methods related to the present update are summarized as follows. Two reviewers performed abstract and full-text screening and risk of bias assessment independently. Disagreements between the reviewers were discussed until both parties were in agreement.

Data Sources and Searches

MEDLINE®, the Cochrane Central, and Scopus (including EMBASE) databases were searched from 2009 to May 2015 for both 1) prospective cohort or nested case-control (or case-cohort) studies reporting an association between calcium intake (dietary or supplement sources) and risk of incident CVD (cardiac, cerebrovascular, or peripheral vascular events, and new hypertension); and 2) randomized controlled trials (RCTs) on the effect of increasing calcium intake (by food courses or supplements) on the same outcomes. Analyses of combinations of calcium and micronutrients other than vitamin D were excluded. Studies or analyses that did not quantify the amount of calcium in the interventions or exposures were also

excluded. The literature search strategy was adapted from the 2009 evidence report (14) but focused on calcium exposures and CVD outcomes.

Study Selection

Study eligibility was restricted to peer-reviewed, English-language studies of generally healthy adults (with the exception of hypertension, no more than 20% of study participants could have known CVD). Exceptions were also made for elderly populations (>60 years of age) since the prevalence of CVD is high in this population. Studies restricted to pregnant women, people with diabetes, or those on dialysis were excluded. Reference lists of relevant systematic reviews were cross-checked with our list of included studies to ensure no relevant studies were missed. All CVD event or mortality outcomes (defined by the original authors) were included.

Data Extraction and Risk of Bias (Quality) Assessment

All data in the 2009 and 2014 evidence reports (6, 14) are publicly accessible on Pubmed® and Pubmed Health. Relevant data from the studies included in the two evidence reports were extracted from their evidence tables (Appendix C of the evidence reports) and included in this update. Data from studies published after the evidence reports were extracted by one reviewer and confirmed by at least one other using the same data extraction form. The risk of bias of RCTs and prospective cohort studies were assessed separately, with the same assessment tools used in the 2009 and 2014 evidence reports. However, to be consistent with the current methodology recommended in the Cochrane Handbook, an overall quality grade was not assigned for each study in this update(15).

Data Synthesis

We synthesized RCTs and cohort studies separately. However our conclusions were based on the totality of the body of evidence. For RCTs, we did not perform a meta-analysis because trials reported outcomes with heterogeneous definitions. For cohort studies, we performed meta-analyses when four or more studies reported analyses of similar exposure-and-outcome relationships. If more than one analysis model was reported in a study, we focused on the model that adjusted for the most potential confounders. Many cohort studies had multiple analyses reporting different calcium exposures or CVD outcomes of interest. We carefully planned our dose-response meta-regression and meta-analysis to ensure that study populations did not overlap within each analysis.

We performed linear and non-linear dose-response meta-regressions to examine the associations between calcium intake levels and the risks of CVD using a two-stage hierarchical regression model implemented in '*dosresmeta' R* package (16, 17). The method was first formalized by Greenland and Longnecker (18); the authors described how to approximate the covariances of reported log relative risks and how to use them to efficiently estimate an exposure-disease relation. The study specific estimates are combined through multivariate random-effects meta-analytical models, to obtain a pooled dose-response association. In addition, we performed random-effects meta-analyses (19) to examine the risk of CVD in adults with calcium intake levels greater or equal to the RDA (≥1000 mg/d) compared to those with intake less than the RDA. We tested for heterogeneity using Cochran's Q statistic (considered significant when $P < 0.10$) and quantified the extent of heterogeneity with the I^2 index.

To facilitate the dose-response meta-regressions and random-effects meta-analyses, for each study, we back-calculated the "effective counts" of events in each category of calcium intake

level based on the pertinent adjusted log relative risk (versus a reference exposure category), their variance, and the total number of participants per exposure category and by solving a set of non-linear equations (20). The effective counts of events are such that when used in a logistic regression with the exposure categories as the sole predictors, they result in the same log relative risk (coefficients) variances and covariances as those from the original adjusted model. The mean or median value per exposure category of calcium intake levels is also needed for dose-response meta-regressions. When it was not reported, we selected the midpoint between exposure category thresholds, and for the open categories we imputed a mean intake that was 20% lower for the lowest quintile threshold or 20% higher for the highest quintile threshold, respectively. For random-effects meta-analysis, we regrouped the exposure categories based on the mean or median value (≥1000 vs. <1000 mg/d) and calculated adjusted relative risk and its standard error using a 2-by-2 table of the effective counts of events and people at risk in each study.

All extracted and calculated data of the included cohort studies are available in the **Supplemental Data File**. Analyses were conducted using Stata SE 13 software (Stata Corp., College Station, TX) and *R* version 3.2.5 (The R Foundation for Statistical Computing). All P values were two-tailed and $p< 0.05$ was considered significant.

Role of the Funding Source

Support for this research was provided through an unrestricted educational grant from the National Osteoporosis Foundation (NOF) through the support of Pfizer Consumer Healthcare. The authors were blinded of the corporate funder until the final manuscript was submitted to the NOF. The funder reviewed the draft evidence synthesis for drafting the position statement, but had no role in study selection, quality assessment, data analysis or writing of the manuscript.

Results

Search results

We included a total of four RCTs (in 9 publications (11, 21-28)) and 21 cohort studies (29-49). **Appendix Figure 1** shows the summary of evidence searches and study selection flow for this update.

Randomized Controlled Trials

Two RCTs (reported in 7 publications) examined the effects of calcium plus vitamin D supplementation (11, 21-26), and three trials examined the effects of calcium supplementation alone (21, 27, 28). Of these 5 trials, one (the RECORD trial) used a 2-by-2 factorial design of calcium and vitamin D, and therefore contributed to both comparisons (calcium vs. placebo; calcium plus vitamin D vs. placebo) (21). CVD outcomes were secondary endpoints in all RCTs. (**Table 1**) The overall risk of bias of the included RCT publications was low to moderate. Common limitations were a high rate of loss to follow-up (>20%), unclear reporting of allocation concealment, and discrepancies in result reporting. (**Appendix Figure 2)**

Effects of Calcium plus Vitamin D Supplementation on CVD

Overall, two RCTs (the Women's Health Initiative [WHI] and RECORD trials) did not find significant differences in risks of CVD events or mortality between calcium plus vitamin D supplementation and placebo groups. Individual trial results are shown in **Appendix Table 1**.

Multiple publications analyzed data from the WHI trial (11, 22-26). The WHI trial randomized 36,282 postmenopausal U.S. women (50-79 years of age) to either 1,000 mg calcium plus 400 IU vitamin D_3 daily or placebo. Five of the publications examined CVD outcomes at the end of 7 years of supplementation (11, 23-26), and the sixth paper (22) included CVD outcomes

5 years post-intervention. Outcomes examined across these papers included myocardial infarction (MI), coronary heart disease (CHD), total heart disease, total CVD, CVD death, CHD death, cerebrovascular death, coronary artery bypass grafting or percutaneous coronary intervention, confirmed angina, hospitalized heart failure, stroke (ischemic, hemorrhagic, or other), transient ischemic attack, and heart failure. Across multiple publications, post hoc subgroup analyses were performed to compare effects among women with any use of personal calcium supplements during the trial with effects among women with no use of personal calcium supplements, among different age groups, or between low and high baseline CVD risk groups. Except for two analyses, most subgroup analyses did not find significant differences between groups. One subgroup analysis showed that use of personal calcium supplements altered the effect of calcium and vitamin D on CVD (11). In post-menopausal women not taking personal calcium supplements, the hazard ratios with calcium and vitamin D were 1.13 to 1.22 for CVD endpoints. By contrast, in women taking personal calcium supplements, the hazard ratios for these endpoints with calcium and vitamin D were 0.83 to 1.08. Another subgroup analysis found a lower risk of heart failure with calcium and vitamin D supplementation in the subgroup of postmenopausal women without pre-existing heart failure precursors at baseline (hazard ratio = 0.63; 95% CI, 0.46 to 0.87), but no significant effect of supplementation was observed in those with these conditions (hazard ratio = 1.06; 95% CI, 0.90 to 1.24) (23). (**Appendix Table 1)** The RECORD trial examined the effects of 3 years of daily supplementation with 1000 mg calcium, 800 IU vitamin D_3, or both on CVD deaths and cerebrovascular disease deaths among 5,292 patients (85% female, >70 years of age) recruited from fracture clinics or orthopedic wards in England and Scotland (21). Calcium plus vitamin D supplementation had no significant effect on all vascular disease deaths compared with placebo (risk ratio = 0.99; 95% CI, 0.82 to 1.20).

Effects of Calcium Supplementation on CVD

Three RCTs examined the effects of supplementation with calcium alone on different CVD outcomes (21, 27, 28), and thus the results cannot be pooled. The Calcium Intake Fracture Outcome Study (CAIFOS) from Western Australia examined the effects of 1200 mg of calcium carbonate daily for 5 years on risks of atherosclerotic vascular disease among 1460 elderly women (>70 years of age) recruited from the general population (27). The Auckland calcium study randomized 1,471 postmenopausal women (>55 years of age) to 5 years of daily supplementation with 1000 mg of calcium citrate or placebo and examined the outcomes of MI and stroke five years post-intervention (28). The RECORD trial (described above) reported the effects of calcium supplementation alone on CVD and cerebrovascular deaths (21). Overall, none of the studies found a significant effect of calcium supplementation on CVD outcomes. (**Appendix Table 1**)

Prospective Cohort Studies

Twenty-one cohort studies examined the relationships between calcium intake (from foods or supplements) levels and the risks for CVD outcomes among adults living in the U.S. (29, 30, 32, 34, 40, 45, 46), Europe (35-38, 41, 44), Asia (33, 39, 42, 43, 47-49), and Australia (31). None evaluated the interaction or combination of calcium and vitamin D intake on CVD outcomes. The baseline ages ranged from 17 to 99 years old; of which, two cohorts exclusively enrolled elderly (>60 years of age) (33, 38). Cohort sample sizes ranged from 755 to 388,229, and follow-up durations ranged from 8 to 28 years. (**Table 2**) Calcium intake were assessed by food frequency quesitonnaires in most cohorts except two (38, 45). Most of the cohort studies reported CVD mortality outcomes assessed by death certificates, ICD codes, medical records, or self-report. The overall risk of bias of these studies was moderate. Common limitations were lack

of justification of final statistical models, unclear reporting of blinding of outcome assessors, not designating which outcomes were primary, and not reporting time from exposure assessment to CVD outcome assessment. (**Appendix Figure 3)**

All, except four (38, 40, 48, 49), cohort studies reported at least one CVD outcome that was pooled in a meta-analysis. The results from these four studies are summarized narratively, followed by corresponding meta-analysis results.

Relationships between Calcium Intake Levels and Risks of CVD Mortality

Twelve cohort studies were included (29, 31-33, 35, 37, 39, 42, 44-47). Individual study results are shown in **Appendix Figure 4**: Panels a and b display analyses examining the associations of dietary (foods only, panel a) and total (foods and supplements, panel b) calcium intake levels with the risks of CVD or ischemic heart disease (IHD) mortality. Dose-response meta-regression analyses did not find significant linear or non-linear relationships between levels of dietary (250 to 2000 mg/d) or total calcium (400 to 2400 mg/d) intake and the risks of CVD or IHD mortality. (**Table 3; Appendix Figure 5**)

Of the 12 cohorts, nine reported data that allowed meta-analysis to examine the risk of CVD or IHD mortality among adults who had calcium intakes greater or equal to the RDA (≥1000 mg/d) compared to those with intakes less than the RDA. The three cohort studies not included in the meta-analysis were conducted in Asian countries (33, 42, 47), and the highest intake levels in these cohorts were less than 1000 mg/d. The random-effects meta-analysis of nine cohort studies showed no significant associations between adequacy of calcium intake and the risks of CVD or IHD mortality (pooled adjusted hazard ratio = 1.01; 95% CI 0.94 to 1.07; I^2 = 49% for dietary calcium, and pooled adjusted hazard ratio = 1.01; 95% CI 0.95 to 1.08; I^2 = 0% for total calcium from food and supplements). (**Appendix Figure 6; Appendix Figure 7**) Among these

cohorts included in the meta-analysis, only one (46) showed that dietary calcium intake levels greater than 1000 mg/d (reported mean calcium intake level in quintile 5 was 1247 mg/d for men and 1101 mg/d for women) were associated with a higher risk of CVD mortality (adjusted hazard ratio = 1.06; 95% CI, 1.00, 1.14 for women, and adjusted hazard ratio = 1.10; 95% CI, 1.04 to 1.16). This study (AARP Diet and Health cohort) also found that supplemental calcium intake (≥1000 mg/d) was associated with an elevated risk of CVD mortality compared to no supplemental intake (adjusted relative risk = 1.20; 95% CI, 1.05 to 1.36), and total calcium intake had a U-shaped association with total CVD mortality in men but not in women, with increased CVD mortality observed at calcium intakes of 1500 mg/d and higher (46). In contrast, a cohort (Nurses' Health Study) that could not be pooled in the meta-analysis found lower risks of CVD events or mortality among women who took more than 1000 mg/d of calcium supplements compared to those who did not take calcium supplements (adjusted relative risk = 0.82; 95% CI, 0.74 to 0.92) (40).

Relationships between Calcium Intake Levels and Risks of Stroke

Eleven cohort studies assessed the association of calcium with stroke risk (30, 34, 36, 37, 39, 41-43, 45-47). Individual study results are shown in **Appendix Figure 8**, which display analyses examining the associations of dietary or total calcium intake levels with the risks of stroke mortality (panel a) and total stroke (panel b). Dose-response meta-regression analyses did not find significant linear or non-linear relationships between levels of dietary or total calcium intake (200 to 2200 mg/d) and the risks of stroke mortality or total stroke. (**Table 3; Appendix Figure 9**)

Of the 11 cohorts, eight contributed data to a meta-analysis to examine the risk of stroke mortality (3 studies) or total stroke (5 studies) among adults whose calcium intake level greater

or equal to the RDA (≥1000 mg/d) compared with those with lower calcium intake. The three cohort studies (42, 43, 47) that could not be included in the meta-analysis were conducted in Asia countries, and the highest intake levels in these cohorts were less than 1000 mg/d. The random-effects meta-analysis showed no significant associations between adequacy of calcium intake and the risks of stroke mortality (pooled adjusted hazard ratio = 1.04; 95% CI 0.96 to 1.14; I^2 = 0%) (**Appendix Figure 10)** or total stroke (pooled adjusted hazard ratio = 1.02; 95% CI 0.94 to 1.10; I^2 = 6.8%) **(Appendix Figure 11**).

Among three additional cohort studies that did not report sufficient data for dose-response meta-regression or meta-analysis, none found significant associations between calcium intake levels and the risks of stroke events or mortality (38, 48, 49).

Conclusions

On the basis of the aggregate internal validity, precision of risk estimates, and consistency of results from both RCTs and cohort studies, we conclude that calcium intake levels (dietary and supplement sources) are not associated with CVD risks among generally healthy adults within the tolerable upper intake levels (ULs, 2000 to 3000 mg/d). Limited evidence from post hoc subgroup analyses and one large population-based cohort study in older adults suggests that calcium supplements may increase the CVD risks in older adults compared to the risks of supplement non-users but may be beneficial among people with low baseline CVD risks. It is important to note that the total calcium intake levels from foods and supplements are not well estimated, and post hoc subgroup analyses and secondary outcomes can be hard to interpret when the primary outcome of the trial is insignificant (50). The available RCT data are not sufficient to draw a firm conclusion regarding the balance between benefits and harms of

calcium with or without vitamin D supplements in the prevention of osteoporosis and cardiovascular disease.

Our findings are inconsistent with previous meta-analyses of RCTs (10, 11) and cohort studies (51-53). Many differences in the data synthesis methods may account for the apparent discordant results and conclusions. We believe our findings are more valid than previous meta-analyses for several reasons. Previous meta-analyses of RCTs included some unpublished data, did not appraise the risk of bias, and combined trials of calcium supplements alone with those of calcium plus vitamin D supplements. None of the included RCTs to date was designed to examine the effects of calcium (with or without vitamin D) supplementation on CVD outcomes as primary endpoints. The ascertainment of the CVD outcomes in some RCTs included in the previous meta-analyses was patient self-reported "adverse events" without verification (10, 11). All three previous meta-analyses of cohort studies (51-53) reported a non-linear dose-response between calcium intake levels and stroke risks. However the dose-response meta-regression methods were unclear in two meta-analyses (52, 53), and their results are likely to be wrong due to the limitations of the statistical package (*glst* command) for dose-response meta-analysis implemented in Stata (54). As pointed out by Liu et al (17), *glst* does not provide solutions to pool studies with different reference exposure doses, which is the case in all the dose-response meta-analyses of calcium intake and CVD risks. Moreover, the two meta-analyses also performed naïve "high versus low" or extreme quantile meta-analyses, which produces uninterpretable pooled results because the ranges of highest and lowest quantile categories of calcium intake varied substantially across studies. An empirical evaluation of meta-analytic approaches for nutrient and health outcome dose-response data discouraged using meta-analytic

approaches that use only data from extreme exposure categories because the results typically are biased away from null (55).

Despite using novel approaches to synthesize data from cohort studies, our dose-response meta-regressions and meta-analyses had several limitations. In addition to the concerns on the overall moderate risk of bias of the included cohort studies, potential residual confounding, ecological bias, and misclassifications of calcium exposures further limited our confidence in the meta-analysis results. Since different cohort studies adjusted for different sets of confounders, using the most adjusted risk estimates in the meta-analyses assumed that the different adjustments across studies would not affect the meta-analytic results – an assumption that we cannot verify without conducting simulation studies. With these caveats in mind, we still believe our dose-response meta-analysis of cohort studies strengthens the causal reasoning and widens the generalizability. Our novel meta-analysis using effective counts reduced the heterogeneity by standardizing the comparisons of the calcium intake levels across studies, and enabled us to evaluate policy relevant questions by conducting comparisons above or below the RDA.

Systematic review and meta-analysis play an important role in evidence-based medicine. Apparently conflicting conclusions across multiple meta-analyses of the same topic can cause uncertainty within the healthcare community and confusion among the general public. To increase the transparency, reduce research waste, minimize potential biases, and facilitate updating and translation of evidence-based information to practice or policy, we recommend future systematic reviews and meta-analyses make all data publicly available.

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