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Calcium Intake and Colorectal Adenoma Risk: Dose-Response Meta-Analysis of Prospective Observational Studies

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Novelty and Impact Statements

This is the first dose-response meta-analysis on the relationship between calcium intake and colorectal adenomas. By showing a continued reduction in risk of adenomas, particularly high-risk adenomas (≥ 1 cm in diameter, (tubulo)villous histology, dysplasia, or multiplicity), beyond 1,000 mg/day of total calcium intake, our results suggest that calcium may have chemopreventive potential against colorectal neoplasia, irrespective of baseline total calcium intake over a wide range.

Abstract

Evidence from randomized controlled trials suggests that calcium may protect against recurrence of colorectal adenomas, which could lead to the subsequent prevention of cancer. Yet, because the trials used only a large single dose and were of small sizes, knowledge of the dose-response relationship and influence on high-risk adenomas is limited. To address these issues, we conducted linear and non-linear dose-response meta-analyses primarily based on prospective observational studies published up to July 2014 identified from PubMed and Embase. Summary relative risks (RRs) and 95% confidence intervals (CIs) were calculated for total and supplemental calcium intake, respectively, using a random-effects model. For total calcium intake, summary RR for each 300mg/day increase was 0.95(95% CI=0.92-0.98; I^2=45%; 8 studies with 11,005 cases; range of intake=333-2,229 mg/day). Evidence of non-linearity was indicated: approximately, compared to 550 mg/day of total calcium intake, the summary RR was 0.92(95% CI=0.89-0.94) at 1000 mg/day and 0.87(95% CI=0.84-0.90) at 1450 mg/day ($P_{non\text{-}linearity}$<0.01). Associations were stronger for high-risk adenomas (≥1 cm in diameter, (tubulo)villous histology, dysplasia, or multiplicity): approximately, compared to 550 mg/day of total calcium intake, the summary RR was 0.77(95% CI=0.74-0.81) at 1000 mg/day and reduced to 0.69(95% CI=0.66-0.73) at 1450 mg/da ($P_{non\text{-}linearity}$<0.01). For supplemental calcium intake, summary RR of total adenoma risk for each 300mg/day increase was 0.96(95% CI=0.93-0.99; I^2=0%; 3 studies with 4,548 cases; range of supplementation=0-1,366 mg/day). In conclusion, calcium intake may continue to decrease the risk of adenomas, particularly high-risk adenomas, over a wide range of calcium intake.

Introduction

Colorectal cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide, excluding non-melanoma skin cancer.[1] The majority of colorectal cancers are preceded by adenomas[2] and thus, targeting adenomas is an effective way to prevent colorectal cancer. While screening endoscopy that detects and removes asymptomatic adenomas has been suggested to reduce colorectal cancer incidence rates and mortality[3, 4], a significant proportion of people with initial adenomectomy develop recurrent adenomas within three years.[5] Thus, there is a pressing need to identify modifiable factors that could reduce the risk of adenoma occurrence (first time diagnosis of adenomas) and recurrence (development of adenomas after undergoing previous adenomectomy).

Available evidence suggests that calcium may have chemopreventive potential against adenomas. In a meta-analysis of three randomized controlled trials (RCTs), compared to people assigned to a placebo group, those assigned to take 1,200-2,000 mg of calcium supplements without co-administered vitamin D over 3-4 years had an approximately 20% reduced risk of adenoma recurrence.[6] Given that the beneficial effect manifested within a short duration of intervention and that the evidence came from RCTs, the current gold-standard study design for establishing a causal relationship, a role of calcium in the prevention of adenomas appears to be promising.

However, each of the RCTs included tested only a large dose of supplemental calcium and thus, critical information in developing guidelines for chemoprevention by calcium, such as the dose-response relationship, is missing. Given some concern on the suggested harm of high-dose calcium supplements on cardiovascular disease,[7, 8] identification of the dose-response relationship would also help answer an important clinical question if regular use of low-dose calcium supplements could lead to protection against adenomas and thus, ultimately, colorectal cancer. Moreover, the propensity of adenomas to progress, left unremoved, varies greatly by size, histology, grade and number.[2] While the Calcium Polyp Prevention Study suggested a greater benefit for high-risk adenomas,[9] the majority of endpoints in the trials of recurrent adenomas were likely to be solitary, small tubular adenomas, which have a low propensity to progress.[2] Thus, we conducted a dose-response meta-analysis of prospective observational studies, addressing potential heterogeneity in the relationship by adenoma subtypes.

Methods

For the design, analysis, and reporting of this study, the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist[10] was followed for meta-analysis of prospective observational studies. Two authors (DL and NK) participated in literature search, study selection, and data extraction independently. Inconsistency between researchers was resolved through discussion.

Literature Search

PubMed and Embase databases were searched for studies published up to July 2014. Detailed search terms are provided (Supplementary Table 1). The language was limited to English and no other restrictions were imposed. Abstracts and unpublished results were not included. The reference lists of selected reviews and meta-analyses, and all the articles included in our analysis were also reviewed for additional studies.

Study Selection

To be included, studies had to be an observational study (e.g. cross-sectional, case-control, or cohort study) investigating the relationship between calcium intake and colorectal adenomas. For dose-response meta-analysis, studies had to provide the following information: a quantitative measure of calcium intake for at least 3 categories with the estimates of RRs (odds ratio, rate ratio, or hazard ratio), 95% confidence interval (CI), category-specific or total number of cases, and category-specific or total number of either noncases or person-years. When there were several publications from the same cohort, the publication with the largest number of cases was selected. Authors of two cohort studies[11, 12] were contacted for additional data and one[11] of the two studies could be incorporated in dose-response meta-analysis. The procedure of study selection, including reasons for exclusion, is summarized in Figure 1.

Data Extraction

From each study, the following information was extracted: the most fully adjusted RR and corresponding 95% CI in each category of calcium intake, category-specific range of calcium intake, category-specific or total number of cases, category-specific or total number of person-years (for rate ratio or hazard ratio) or noncases (for odds ratio), types of calcium intake (total=dietary+supplemental, dietary, supplemental), mean or median calcium intake of source population at study entry, subtypes of adenomas by anatomic location and propensity to progress to colorectal cancer (high risk adenoma characterized by large size of ≥ 1 cm in diameters, (tubulo)villous histology, dysplasia, or multiplicity vs. small adenomas), first author's name, publication year, study design, study name, country of the study population, sex, age at enrollment, sample size, number of cases, study period, dietary assessment method (type, whether it had been validated), adjustment variables, temporality (i.e. prospective: diet was assessed prior to participants' knowledge of adenoma status; retrospective: diet was assessed prior to participants' knowledge of adenoma status).

Statistical Analysis

Across cross-sectional, case-control, or cohort studies, cohort studies are generally least prone to biases. However, the asymptomatic nature of adenomas reduced methodological distinctions across the study designs in the investigation of calcium intake and adenoma risk. For instances, as the true case and control status can be determined only at time of endoscopy,

most studies defined the source population as subjects who underwent an endoscopy. Furthermore, as the timing of endoscopy was not necessarily guided by symptoms, time from study baseline to adenoma detection is rather arbitrary. Thus, like the other study designs, most cohort studies used logistic regression only accounting for whether or not an event happens rather than Cox regression incorporating both whether or not and when an event happens. For these reasons, one of the most important methodological distinctions narrowed down to temporal relationship between the assessment of calcium intake and participants' knowledge about their adenoma status. As studies that prospectively assessed calcium intake are less prone to recall bias than those that retrospectively assessed, prospective studies constituted our primary analysis.

Furthermore, out of the three possible types of calcium intake (total, dietary, supplemental), total calcium intake is the exposure measure that is most relevant to exploring a dose-response relationship between calcium intake and adenoma risk. Thus, our primary meta-analysis included prospective studies that investigated total calcium intake. To enhance the comparability of our results with RCTs that tested the effect of supplemental calcium, a dose-response meta-analysis was also conducted based on prospective studies that investigated supplemental calcium.

Linear and non-linear dose-response meta-analyses were conducted. For linear dose-response meta-analysis assuming a linear relationship between calcium intake and adenoma risk, the method described by Greenland and Longnecker[13] was used to calculate study-specific RRs (linear slopes) and 95% CIs from the correlated RRs and 95% CIs extracted across categories of calcium intake. In estimating study-specific linear trends, several approximations were made: the midpoint of calcium intake in each category was assigned to the corresponding RR; the width of the open-ended extreme categories was assumed to be the same as that of the adjacent interval; when the distributions of person-years or non-cases were not provided but analyzed based on quantiles, they were equally divided across the quantiles; for studies[14, 15] that showed results separately for distal and rectal adenomas or for men and women, category-specific RRs and variances were combined using a fixed effects model based on inverse variance weight to obtain combined estimates for colorectal adenomas or for both sexes, before estimating the study-specific RR and 95% CI; for one study[16] that used the sixth lowest category of calcium intake as the reference, the method by Hamling et al[17] was used to estimate new RRs and 95% CIs setting the lowest category as the new reference. Then, the estimated study-specific RRs and variances were pooled using a random effects model to calculate the summary RR and 95% CI. Forest plots of the linear dose-response meta-analysis were presented for RRs for each 300mg/day increment of calcium intake (the unit equivalent to calcium content in one serving (250 mL) of milk).

To examine potential non-linear relationship between calcium intake and adenoma risk, non-linear dose-response meta-analysis was performed based on the restricted cubic spline approach.[18, 19] Of note, this approach requires that studies have more than three categories of calcium intake. For each study, cubic splines were modeled with three knots fixed at percentiles (10%, 50%, and 90%) of the whole distribution of calcium intake, accounting for correlation across category-specific RRs and 95% CIs within each study.[18] The reference was set to 550 mg/day, the lowest value of the reported calcium intakes that were concentrated in lower extremes. Then, the derived curves were combined using multivariate random-effects meta-analysis.[20] The p-value for nonlinearity was obtained from the test of the null hypothesis that the regression coefficient of the second spline transformation was equal to zero.

Heterogeneity in the relationship between calcium intake and adenomas across studies was assessed by I^2 and Q test.[21] Subgroup analyses and meta-regression were conducted by *a*

priori selected variables related to etiologic heterogeneity and potential effect modifiers to identify sources of heterogeneity; by variables concerning methodological characteristics to assess study quality. Potential for small study effects,[22, 23] such as publication bias, was assessed visually using funnel plots and statistically using Egger's test.[24] Upon the detection of statistically significant evidence of small study effects, contour-enhanced funnel plot was plotted that distinguishes areas of the statistical significance and non-significance of the funnel plot using contour.[25, 26] By presenting each study in the context of statistical significance, this plot helps determine if the cause of asymmetry is attributable to publication bias based on statistical significance.[25] To explore robustness of the results, diverse sensitivity analyses including the influence analysis and highest vs. lowest meta-analysis that pooled RRs for the extreme categories of calcium intake using a random effects model were performed. For statistical significance, two-sided α was set at P=0.05. All statistical analyses were conducted using STATA 12 (StataCorp, College Station, TX).

Results

Total calcium intake

A total of 14 studies[11, 14-16, 27-36] were eligible for the inclusion in dose-response meta-analysis of total calcium intake and adenoma risk (Supplementary Table 2). Across the studies, types of adenomas investigated varied. The study by Lieberman et al.[27] specifically investigated advanced adenomas and the other 13 studies primarily examined total adenomas with some studies[11, 16, 27, 29, 34, 35] further conducting subgroup analysis by adenoma subtypes. Two studies[35, 36] exclusively investigated recurrent adenomas and the other 12 studies examined occurrent adenomas. While accumulating evidence suggests that calcium may be more protective against advanced adenomas,[9, 11, 16, 29, 35] there is no sufficient a priori evidence to suspect that the effect of calcium may differ by recurrent vs. occurrent adenomas. Thus, dose-response meta-analysis of total calcium intake and adenoma risk was based on 13 studies[11, 14-16, 28-36] excluding the study by Lieberman et al.,[27] which was included only in the subgroup analysis for high-risk adenomas.

Out of the 13 studies,[11, 14-16, 28-36] eight studies[11, 14, 16, 29, 31, 34-36] qualified for prospective studies, including a total of 11,005 cases with category-specific midpoints of total calcium intake ranging from 333 to 2,229 mg/day; the remaining five studies were classified as retrospective studies (2,401 cases, range=285-1405 mg/day).

In the linear dose-response meta-analysis of eight prospective studies,[11, 14, 16, 29, 31, 34-36] each 300 mg/day increase in total calcium intake was associated with an approximately 5% decreased risk of adenomas (RR=0.95, 95% CI=0.92-0.98), with moderate heterogeneity (I^2=45%, $P_{heterogeneity}$=0.08) (Figure 2A). The linear association was stronger among retrospective studies (RR=0.91, 95% CI=0.82-1.01), but it was not statistically significant and had a greater degree of heterogeneity (I^2=58%, $P_{heterogeneity}$=0.05) (Figure 2B). There was no evidence of heterogeneity by prospective vs. retrospective assessment of calcium intake ($P_{heterogeneity}$=0.67). In sensitivity analyses such as excluding one study at a time and including one additional prospective studies[37] that reported dietary calcium intake instead of total calcium intake, the results did not change materially (data not shown).

In the non-linear dose-response meta-analysis, after excluding one study[31] that analyzed calcium intake in three categories only, seven prospective studies[11, 14, 16, 29, 34-36] were included (10,828 cases, range=333-2,229 mg/day). Moderate non-linearity was apparent, with adenoma risk decreasing more steeply in the lower range of total calcium intake than in the higher range ($P_{non\text{-}linearity}$<0.01) (Figure 3A). Approximately, compared to 550 mg/day of total calcium intake, the summary RR was 0.92 (95% CI=0.89-0.94) at 1000 mg/day and further reduced to 0.87 (95% CI=0.84-0.90) at 1450 mg/day.

Although the number of prospective studies was limited, small study effects, such as publication bias, were indicated by asymmetry in the funnel plot and Egger's test (P<0.01)(Figure 4). Several sensitivity analyses were performed. First, when an equivalent analysis was run using a fixed effects model, which gives less weight to small studies compared to a random effects model, the association was attenuated but still statistically significant (RR=0.96, 95% CI=0.95-0.98, I^2=45%, $P_{heterogeneity}$=0.08). Second, contour-enhanced funnel plot indicated that potential missing studies were likely to be located in both statistically significant and non-significant areas, reducing the possibility of publication bias based on statistical significance to explain the observed asymmetry (Figure 4). Third, when highest vs. lowest meta-analysis was performed including two additional prospective studies[12, 37] that were not eligible for dose-response analysis due to insufficient information, a significant inverse association

persisted (RR=0.84, 95% CI=0.78-0.92) with no evidence of heterogeneity (I^2=0%, $P_{heterogeneity}$=0.47) and no evidence of small study effects(P_{Egger}=0.35) (Supplementary Figure 1).

To explore if the inverse association with calcium was stronger against high-risk adenomas than against other subtypes, subgroup analysis was performed by differential propensity to progress to colorectal cancer. Consistent with the prior evidence,[9, 11, 16, 29, 35] the linear association was stronger with high-risk adenomas (RR=0.89, 95% CI=0.85-0.94, I^2=17%, $P_{heterogeneity}$=0.30, 6 prospective studies[11, 16, 27, 29, 34, 35] with 2,685 cases, range=333-1,822 mg/day) than with small adenomas (RR=0.97, 95% CI=0.94-1.01, I^2=0%, $P_{heterogeneity}$=0.91, 3 prospective studies[11, 16, 29] with 3,540 cases, range=333-1,822 mg/day) (Figure 2B). Between-subgroup heterogeneity by high-risk vs. small adenomas was statistically significant ($P_{heterogeneity}$=0.02). When the non-linear dose-response curve was plotted among high-risk adenomas ($P_{non-linearity}$<0.01) (Figure 3B), the overall slope was much steeper compared with that for total adenomas. Approximately, compared to 550 mg/day of total calcium intake, the summary RR was 0.77 (95% CI=0.74-0.81) at 1000 mg/day and further reduced to 0.69 (95% CI=0.66-0.73) at 1450 mg/day.

Supplemental calcium intake

Out of the eight prospective studies[11, 14, 16, 29, 31, 34-36] included in the meta-analysis on total calcium intake, three studies[14, 35, 36] reported sufficient information for dose-response meta-analysis of supplemental calcium intake and adenoma risk. All of the three studies were from the U.S. where supplement usage is relatively high. They included a total of 4,548 cases with category-specific midpoints of supplemental calcium intake ranging from 0 to 1,366 mg/day (Supplementary Table 2). In the linear dose-response meta-analysis, each 300 mg/day increase in supplemental calcium intake was associated with an approximately 4% decreased risk of adenomas (RR=0.96, 95% CI=0.93-0.99), with no evidence of heterogeneity (I^2=0%, $P_{heterogeneity}$=0.53) (Figure 2C). The small number of studies included precluded a meaningful influence analysis. Albeit low-powered due to the small number of studies included, Egger's test provided no evidence of small study effects, such as publication bias (P=0.44). The funnel plot also displayed a symmetrical shape. Non-linear dose-response meta-analysis was not conducted, as two out of the three studies had only three categories of calcium intake and thus were not eligible for the restricted cubic spline approach.

Subgroup analyses

For the linear dose-response meta-analysis of total calcium intake and adenomas based on the eight prospective studies,[11, 14, 16, 29, 31, 34-36] subgroup analyses were performed to explore sources of the observed moderate heterogeneity (Supplementary Table 3). A statistically significant inverse association persisted in most of the subgroups, suggesting robustness of the inverse association. There was evidence of between-subgroup heterogeneity by number of cases ($P_{heterogeneity}$=0.03), with a stronger inverse association observed in the subgroup of studies with ≤1000 cases of adenomas. This is consistent with the observed evidence of small study effects, but an inverse association was still statistically significant in the subgroup of studies with >1000 cases of adenomas. The magnitude of an inverse association was similar regardless of adjustment for confounding by vitamin D status (determined by intake or sun exposure).

Discussion

In our dose-response meta-analyses of prospective observational studies, each 300 mg/day increase in total calcium intake was associated with an approximately 5% decreased risk of adenoma occurrence/recurrence within the 333-2,229 mg/day range of total calcium intake. While a non-linear dose-response relationship was indicated with adenoma risk decreasing less steeply at higher levels of calcium intake, the degree of curvature was mild and thus, overall, a linear association reasonably approximated the shape of the relationship. The linearity was further supported by the subgroup analyses that showed equivalent results between two subgroups classified by mean/median baseline calcium intakes of study populations. Of note, a stronger association was observed for high-risk adenomas having a high propensity to progress to colorectal cancer, with an 11% reduction in adenoma risk for each 300 mg/day increase in calcium intake. This magnitude of risk reduction is consistent with the results of our previous linear dose-response meta-analysis that found a 8% decreased risk of colorectal cancer associated with each 300 mg/day increase in calcium intake.[38]

The strength of the linear association might have been overestimated due to small study effects, but several lines of evidence suggest robustness of the quantitative finding. First, in the dose-response meta-analysis of supplemental calcium intake in which no evidence of small study effects was found, results were virtually the same (RR=0.96, 95% CI=0.93-0.99). Second, in the meta-analysis[6] of RCTs that compared calcium supplementations of 1200-2000 mg/day (weighted average: 1400 mg/day) with placebo, its pooled estimate of 20% reduction in adenoma risk is consistent with that estimated from our linear dose-response meta-analysis of prospective observational studies. Given 4-5% reduction per 300 mg/day increase in calcium intake, 19-23% reduction in adenoma risk is predicted by 1400 mg/day difference in calcium intake. Considering that heterogeneity was low in each meta-analysis of prospective observational studies on supplemental calcium and of RCTs on calcium supplements, such consistency in estimates after accounting for dose of calcium intake serves as strong evidence for robustness of our quantification of the linear dose-response relationship.

The detailed investigations of the association over a wide range of calcium intake by adenoma subtypes, particularly among high-risk adenomas, distinguish our meta-analysis from the meta-analysis of RCTs.[6] As adenomas are an etiologically heterogeneous disease with differential potential to progress colorectal cancer[2, 9], a certain subtype could be more responsive to the chemopreventive effect of calcium. In the meta-analysis of RCTs that included a total of 407 adenomas, the power to perform subgroup analyses by adenoma subtypes was limited. For instance, in their analysis confined to large adenomas (> 0.9 cm), the confidence interval was wide (RR=0.78, 95% CI=0.50-1.22).[6] In contrast, our dose-response meta-analysis on high risk adenomas included 2,685 adenomas and showed a statistically significant association, which was more pronounced than the association with small tubular adenomas. Furthermore, as the RCTs exclusively investigated recurrent adenomas, only the role of calcium intake after the diagnosis of an adenoma was able to be examined. Although the pathophysiology of occurrent and recurrent adenomas is likely to overlap, there are some differences, for example, in bowel location propensity.[39] In addition occurrent adenomas may better capture the effect of earlier dietary intake. Our meta-analysis performed a subgroup analysis to examine occurrent adenomas separately and observed a potential benefit that earlier calcium intake may confer.

Findings from our meta-analysis may inform the role of calcium in the colorectal carcinogenesis. In light of the well-established natural history of colorectal carcinogenesis by which the progression from the normal epithelium, to hyperproliferative epithelium, to aberrant cryptic foci, to small adenomas, to large adenomas, and finally to adenocarcinomas occurs over a

long duration, up to 30 to 40 years[2], the presence of an inverse association between calcium and adenoma risk suggests that calcium may act on an early stage of the pathway. The protective role of calcium in the prevention of adenomas is also supported by several biological mechanisms. First, calcium in the colorectal lumen binds to and precipitates with secondary bile acids or ionized fatty acids, protecting the mucosa from their carcinogenic effects[40, 41]. Second, calcium has been suggested to reduce cell proliferation and promote cell differentiation and apoptosis, as a rise in extracellular calcium leads to an increase in cytosol calcium concentration of colonic epithelia cells, which in turn modulates signaling pathways related to such cell cycles[42-45]. Third, given that mutations in the APC/β-catenin pathway are a common early hallmark in the colorectal carcinogenesis and that calcium was shown to induce favorable changes in the APC/β-catenin pathway[46], calcium may prevent the initiation of the neoplastic pathogenesis.

Our meta-analysis has several limitations. First, the small number of prospective studies included precludes a definitive conclusion related to the shape of relationship and subgroup analyses. The non-linearity is strongly driven by data points in extreme values of the exposure, but we had sparse data in the high extreme of calcium intake, as shown by inner ticks on the x axis in Figure 3. Similarly, our subgroup analyses were low-powered to identify a statistically significant source of between-subgroup heterogeneity. Second, as studies on dietary intake are prone to substantial measurement error, our meta-analyses were also affected by measurement error within each study included. Additional measurement error was introduced due to assumptions inevitable in conducting dose-response meta-analyses, such as assigning midpoint of category-specific calcium intake to the corresponding RR and equating the width of open-ended extreme categories with that of the adjacent interval. While the direction of bias cannot be predicted, measurement error is generally anticipated to attenuate the true effect[47], particularly since the dietary information was collected before participants' knowledge of case status. Despite diverse sources of measurement error, bias due to small study effects discussed above and bias due to measurement error are anticipated to direct opposite, offsetting each other to some degrees. Thus, our quantification of 5% reduced risk in adenoma risk for each 300 mg/day increase in calcium intake maybe a reasonable approximation.

Another limitation relates to the inclusion of prevalent cases, particularly in the occurrence studies. As most participants did not systematically undergo baseline endoscopy, some of them might have been harboring asymptomatic adenomas. Thus, the cases detected were likely to be a mixture of incident and prevalent adenomas. Inclusion of prevalent cases elicits several concerns. The first is survival bias, that is, less fatal disease subtypes are over-represented because individuals with prevalent disease have to survive up to study baseline in order to be included. However, as adenomas are benign by themselves, existing adenomas were unlikely to have affected survival up to the study baseline, ameliorating the concern. Second, because the precise timing of the appearance of the adenoma is not known, the time relation between calcium intake and adenoma appearance is unclear. However, considering that people's diet tend to track over time, it might be acceptable to assume that calcium intake measured at study baseline were likely to reflect distant past diet prior to adenoma initiation. Furthermore, as the majority of adenomas are asymptomatic, existing adenomas would not have induced people to change their calcium intake, which diminishes the likelihood of reverse causation.

Our meta-analysis has several strengths as well. To the best of our knowledge, this is the first meta-analysis that identified and quantified the dose-response relationship between calcium intake and adenoma risk over the wide range of calcium intake. By showing consistency between meta-analysis of cohort studies and that of RCTs in terms of magnitude after adjusted

for dose, direction, and statistical significance of the association, our study provides strong evidence supporting a protective role of calcium in the prevention of adenomas. As our primary dose-response meta-analysis included only prospective studies, recall bias is unlikely to have biased the results. Indeed, the stronger linear association observed among retrospective studies might have been driven by recall bias. While case-control studies that assessed diet intake before endoscopy were parts of our primary dataset of the eight prospective studies, as both cases and controls were sampled from the well-defined primary source population of subjects who underwent an endoscopy, there is less potential for selection bias. Although confounding cannot be entirely ruled out in observational studies, the observation of the association for total and supplemental calcium intake after adjustment for multiple factors and in diverse populations (e.g. North America, Europe, Asia) argues against a confounding factor. Arguably, vitamin D might be the most likely confounder, but the results were similar across subgroups defined by adjustment for confounding by vitamin D status, for supplemental calcium intake, and in a European/Asian studies where milk is not systematically fortified with vitamin D.

In conclusion, within the range of 333-2,229 mg/day total calcium intake, the risk of adenomas continued to decrease with an increasing calcium intake. Given the dose-response relationship and evidence from RCTs, calcium may be an effective chemopreventive agent against adenomas, particularly high-risk adenomas. Despite the anticipated efficacy and affordability of calcium supplements, several questions remain to be answered before clinical recommendations are made widely. Given a broadly linear association of calcium intake with adenoma risk, especially advanced adenomas, and possible harm of high-dose calcium supplements on cardiovascular disease,[7, 8] RCTs testing the effect of long-term use of low-dose calcium supplements on advanced adenoma or colorectal cancer, with additional assessment of the risk-benefit balance in terms of colorectal cancer, cardiovascular disease, and osteoporosis are warranted.

Conflict of Interest

Dr. Greenwood reports grants from Danone and personal fees from American Institute for Cancer Research / World Cancer Research Fund, outside the submitted work. The other authors declare none.

Reference List

1. International Agency for Research on Cancer. Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/Pages/fact_sheets_population.aspx. Accessed July 15, 2014.

2. Giovannucci E, Wu K. Cancers of the colon and rectum *Cancer Epidemiology and Prevention*, 3 ed. New York: Oxford University Press, 2006: 809-29.

3. Schoen RE, Pinsky PF, Weissfeld JL, Yokochi LA, Church T, Laiyemo AO, Bresalier R, Andriole GL, Buys SS, Crawford ED, Fouad MN, Isaacs C, et al. Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy. *N Engl J Med* 2012;**366**: 2345-57.

4. Keum N, Giovannucci EL. Folic acid fortification and colorectal cancer risk. *Am J Prev Med* 2014;**46**: S65-72.

5. Neugut AI, Jacobson JS, Ahsan H, Santos J, Garbowski GC, Forde KA, Treat MR, Waye J. Incidence and recurrence rates of colorectal adenomas: a prospective study. *Gastroenterology* 1995;**108**: 402-8.

6. Shaukat A, Scouras N, Schunemann HJ. Role of supplemental calcium in the recurrence of colorectal adenomas: a metaanalysis of randomized controlled trials. *Am J Gastroenterol* 2005;**100**: 390-4.

7. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ* 2011;**342**: d2040.

8. Weaver CM. Calcium Supplementation: Is Protecting Against Osteoporosis Counter to Protecting against Cardiovascular Disease? *Curr Osteoporos Rep* 2014;**12**: 211-8.

9. Wallace K, Baron JA, Cole BF, Sandler RS, Karagas MR, Beach MA, Haile RW, Burke CA, Pearson LH, Mandel JS, Rothstein R, Snover DC. Effect of calcium supplementation on the risk of large bowel polyps. *J Natl Cancer Inst* 2004;**96**: 921-5.

10. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;**283**: 2008-12.

11. Oh K, Willett WC, Wu K, Fuchs CS, Giovannucci EL. Calcium and vitamin D intakes in relation to risk of distal colorectal adenoma in women. *Am J Epidemiol* 2007;**165**: 1178-86.

12. Kampman E, Giovannucci E, Van't Veer P, Rimm E, Stampfer MJ, Colditz GA, Kok FJ, Willett WC. Calcium, vitamin D, dairy foods, and the occurrence of colorectal adenomas among men and women in two prospective studies. *American Journal of Epidemiology* 1994;**139**: 16-29.

13. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 1992;**135**: 1301-9.

14. Peters U, Chatterjee N, McGlynn KA, Schoen RE, Church TR, Bresalier RS, Gaudet MM, Flood A, Schatzkin A, Hayes RB. Calcium intake and colorectal adenoma in a US colorectal cancer early detection program1-3. *American Journal of Clinical Nutrition* 2004;**80**: 1358-65.

15. Tseng M, Murray SC, Kupper LL, Sandler RS. Micronutrients and the risk of colorectal adenomas. *Am J Epidemiol* 1996;**144**: 1005-14.

16. Massa J, Cho E, Orav EJ, Willett WC, Wu K, Giovannucci EL. Total calcium intake and colorectal adenoma in young women. *Cancer Causes Control* 2014;**25**: 451-60.

17. Hamling J, Lee P, Weitkunat R, Ambuhl M. Facilitating meta-analyses by deriving relative effect and precision estimates for alternative comparisons from a set of estimates presented by exposure level or disease category. *Stat Med* 2008;**27**: 954-70.

18. Harrell FE, Jr., Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. *J Natl Cancer Inst* 1988;**80**: 1198-202.

19. Orsini N, Greenland S. A procedure to tabulate and plot results after flexible modeling of a quantitative covariate. *Stata Journal* 2011;**11**: 1-29.

20. White IR. Multivariate random-effects meta-analysis. *Stata Journal* 2009;**9**: 40-56.

21. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;**21**: 1539-58.

22. Sterne JA, Gavaghan D, Egger M. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. *J Clin Epidemiol* 2000;**53**: 1119-29.

23. Nuesch E, Trelle S, Reichenbach S, Rutjes AW, Tschannen B, Altman DG, Egger M, Juni P. Small study effects in meta-analyses of osteoarthritis trials: meta-epidemiological study. *BMJ* 2010;**341**: c3515.

24. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**: 629-34.

25. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J Clin Epidemiol* 2008;**61**: 991-6.

26. Palmer TM, Peters JL, Sutton AJ, Moreno SG. Contour-enhanced funnel plots for meta-analysis. *Stata Journal* 2008;**8**: 242-54.

27. Lieberman DA, Prindiville S, Weiss DG, Willett W. Risk factors for advanced colonic neoplasia and hyperplastic polyps in asymptomatic individuals. *JAMA* 2003;**290**: 2959-67.

28. Miller EA, Keku TO, Satia JA, Martin CF, Galanko JA, Sandler RS. Calcium, dietary, and lifestyle factors in the prevention of colorectal adenomas. *Cancer* 2007;**109**: 510-7.

29. Yamaji T, Iwasaki M, Sasazuki S, Sakamoto H, Yoshida T, Tsugane S. Association between plasma 25-hydroxyvitamin D and colorectal adenoma according to dietary calcium intake and vitamin D receptor polymorphism. *Am J Epidemiol* 2012;**175**: 236-44.

30. Fu Z, Shrubsole MJ, Smalley WE, Wu H, Chen Z, Shyr Y, Ness RM, Zheng W. Lifestyle factors and their combined impact on the risk of colorectal polyps. *Am J Epidemiol* 2012;**176**: 766-76.

31. Boyapati SM, Bostick RM, McGlynn KA, Fina MF, Roufail WM, Geisinger KR, Wargovich M, Coker A, Hebert JR. Calcium, vitamin D, and risk for colorectal adenoma: dependency on vitamin D receptor BsmI polymorphism and nonsteroidal anti-inflammatory drug use? *Cancer Epidemiol Biomarkers Prev* 2003;**12**: 631-7.

32. Levine AJ, Harper JM, Ervin CM, Chen YH, Harmon E, Xue S, Lee ER, Frankel HD, Haile RW. Serum 25-hydroxyvitamin D, dietary calcium intake, and distal colorectal adenoma risk. *Nutr Cancer* 2001;**39**: 35-41.

33. Martinez ME, McPherson RS, Annegers JF, Levin B. Association of diet and colorectal adenomatous polyps: dietary fiber, calcium, and total fat. *Epidemiology* 1996;**7**: 264-8.

34. Kesse E, Boutron-Ruault MC, Norat T, Riboli E, Clavel-Chapelon F. Dietary calcium, phosphorus, vitamin D, dairy products and the risk of colorectal adenoma and cancer among French women of the E3N-EPIC prospective study. *Int J Cancer* 2005;**117**: 137-44.

35. Hartman TJ, Albert PS, Snyder K, Slattery ML, Caan B, Paskett E, Iber F, Kikendall JW, Marshall J, Shike M, Weissfeld J, Brewer B, et al. The association of calcium and vitamin D with risk of colorectal adenomas. *J Nutr* 2005;**135**: 252-9.

36. Martinez ME, Marshall JR, Sampliner R, Wilkinson J, Alberts DS. Calcium, vitamin D, and risk of adenoma recurrence (United States). *Cancer Causes Control* 2002;**13**: 213-20.

37. Hyman J, Baron JA, Dain BJ, Sandler RS, Haile RW, Mandel JS, Mott LA, Greenberg ER. Dietary and supplemental calcium and the recurrence of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 1998;**7**: 291-5.

38. Keum N, Aune D, Greenwood DC, Ju W, Giovannucci EL. Calcium intake and colorectal cancer risk: Dose-response meta-analysis of prospective observational studies. *Int J Cancer* 2014.

39. Martinez ME, Sampliner R, Marshall JR, Bhattacharyya AK, Reid ME, Alberts DS. Adenoma characteristics as risk factors for recurrence of advanced adenomas. *Gastroenterology* 2001;**120**: 1077-83.

40. Wargovich MJ, Eng VW, Newmark HL. Calcium inhibits the damaging and compensatory proliferative effects of fatty acids on mouse colon epithelium. *Cancer Lett* 1984;**23**: 253-8.

41. Newmark HL, Wargovich MJ, Bruce WR. Colon cancer and dietary fat, phosphate, and calcium: a hypothesis. *J Natl Cancer Inst* 1984;**72**: 1323-5.

42. Lamprecht SA, Lipkin M. Cellular mechanisms of calcium and vitamin D in the inhibition of colorectal carcinogenesis. *Ann N Y Acad Sci* 2001;**952**: 73-87.

43. Buset M, Lipkin M, Winawer S, Swaroop S, Friedman E. Inhibition of human colonic epithelial cell proliferation in vivo and in vitro by calcium. *Cancer Res* 1986;**46**: 5426-30.

44. Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. *Nat Rev Cancer* 2003;**3**: 601-14.

45. Rey O, Young SH, Jacamo R, Moyer MP, Rozengurt E. Extracellular calcium sensing receptor stimulation in human colonic epithelial cells induces intracellular calcium oscillations and proliferation inhibition. *J Cell Physiol* 2010;**225**: 73-83.

46. Ahearn TU, Shaukat A, Flanders WD, Rutherford RE, Bostick RM. A randomized clinical trial of the effects of supplemental calcium and vitamin D3 on the APC/beta-catenin pathway in the normal mucosa of colorectal adenoma patients. *Cancer Prev Res (Phila)* 2012;**5**: 1247-56.

47. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing with dietary measurement error in nutritional cohort studies. *J Natl Cancer Inst* 2011;**103**: 1086-92.

Figure legends

Figure 1. Flowchart for study selection

Figure 2. Linear dose-response meta-analyses of calcium intakes and adenoma risk. RR=relative risk; CI=confidence interval. (A) Total calcium intake and total adenomas; (B) Total calcium intake and adenomas by subtypes; (C) Supplemental calcium intake and total adenomas

Figure 3. Non-linear dose-response meta-analysis of total calcium intake and adenoma risk (reference=550mg/day) (A) Total adenomas ($P_{non\text{-}linearity}<0.01$); (B) High-risk adenomas ($P_{non\text{-}linearity}<0.01$). RR=relative risk
Legend:
Inner ticks on the x axis represent data points contributed by the studies included in the meta-analysis

Figure 4. Contour-enhanced funnel plot.

Figure 1

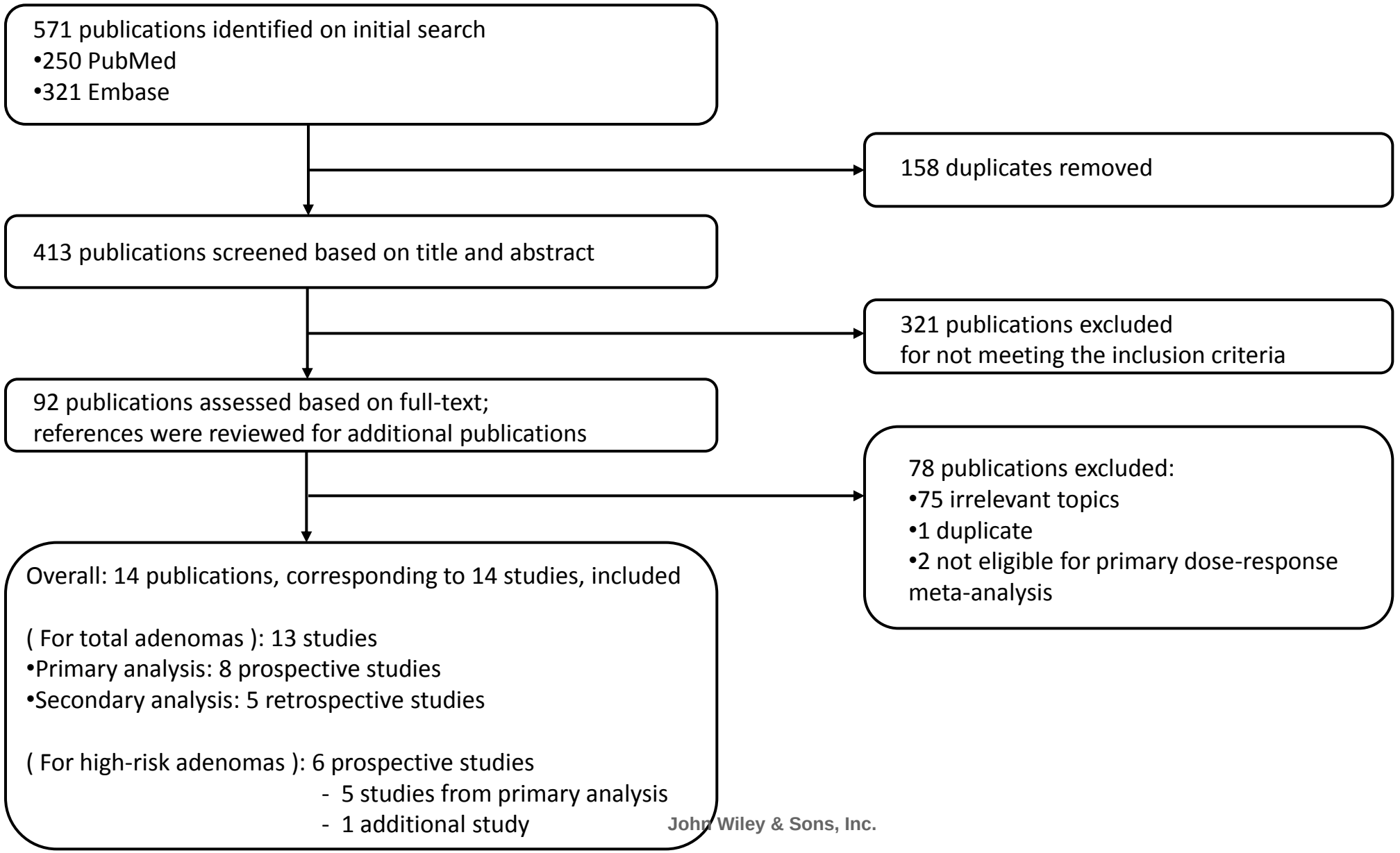


Figure 2A

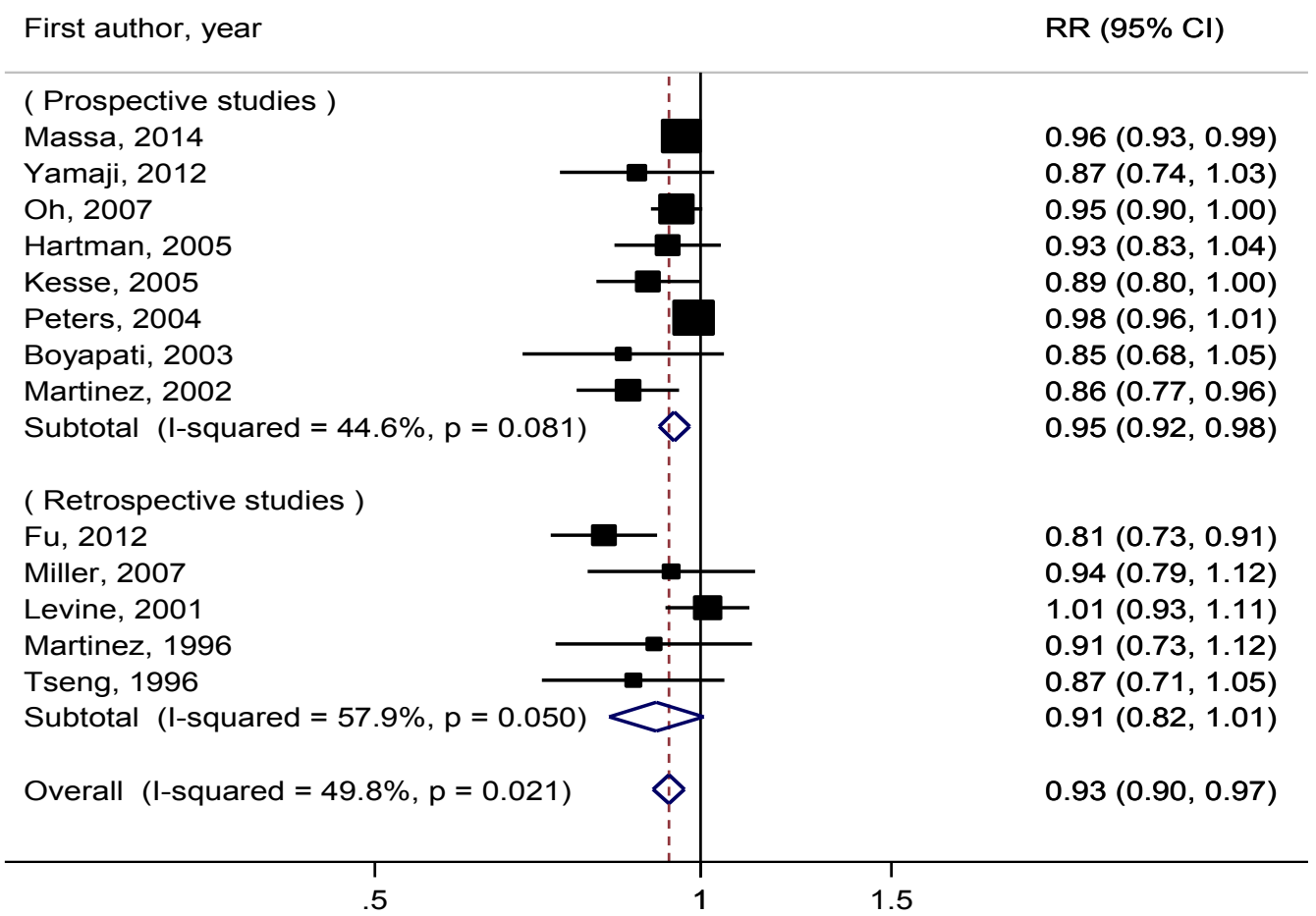


Figure 2B

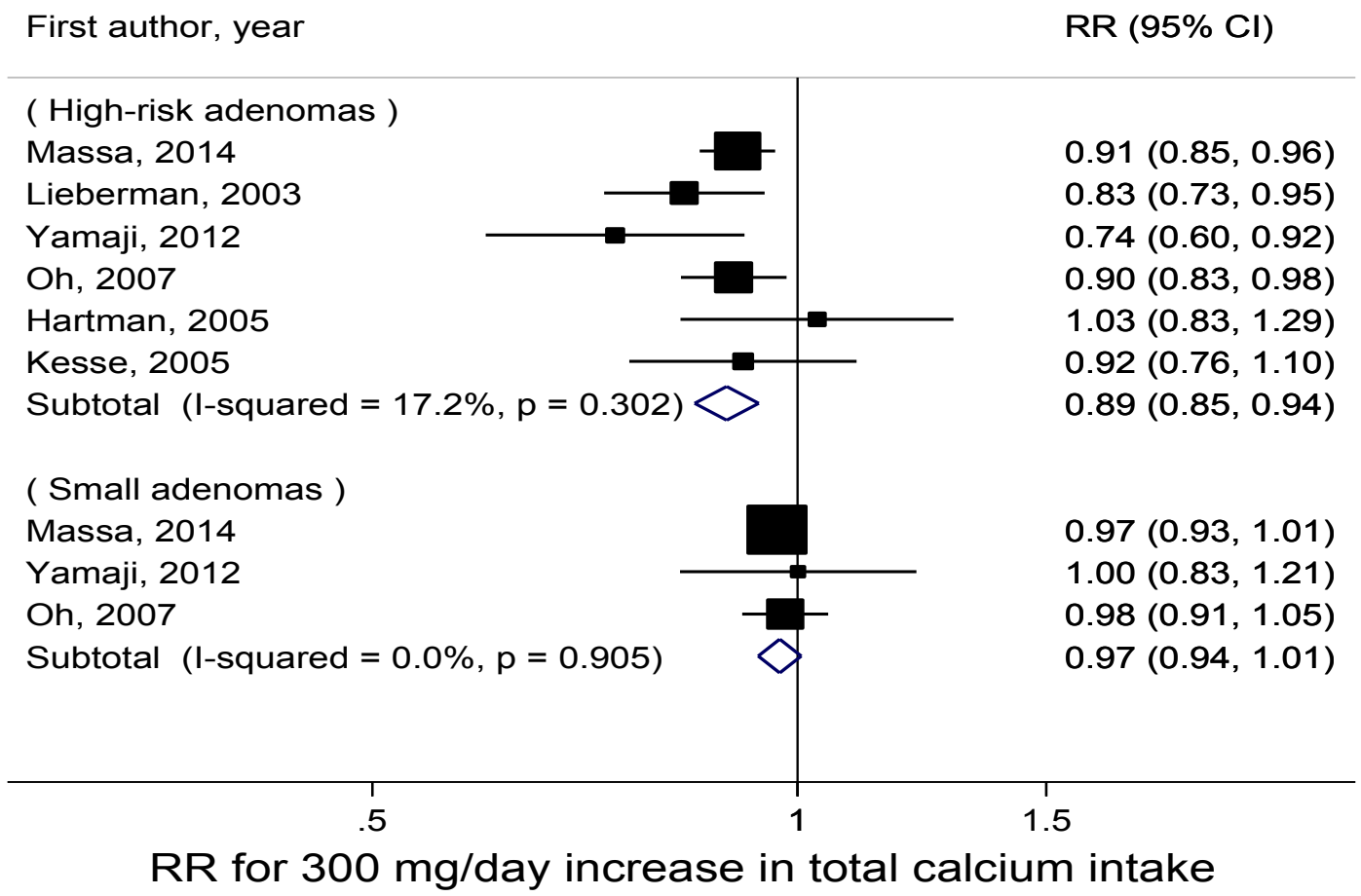

First author, year
RR (95% CI)
(High-risk adenomas)
Massa, 2014
0.91 (0.85, 0.96)
Lieberman, 2003
0.83 (0.73, 0.95)
Yamaji, 2012
0.74 (0.60, 0.92)
Oh, 2007
0.90 (0.83, 0.98)
Hartman, 2005
1.03 (0.83, 1.29)
Kesse, 2005
0.92 (0.76, 1.10)
Subtotal (I-squared = 17.2%, p = 0.302)
0.89 (0.85, 0.94)
(Small adenomas)
Massa, 2014
0.97 (0.93, 1.01)
Yamaji, 2012
1.00 (0.83, 1.21)
Oh, 2007
0.98 (0.91, 1.05)
Subtotal (I-squared = 0.0%, p = 0.905)
0.97 (0.94, 1.01)
.5
1
1.5
RR for 300 mg/day increase in total calcium intake

Figure 2C

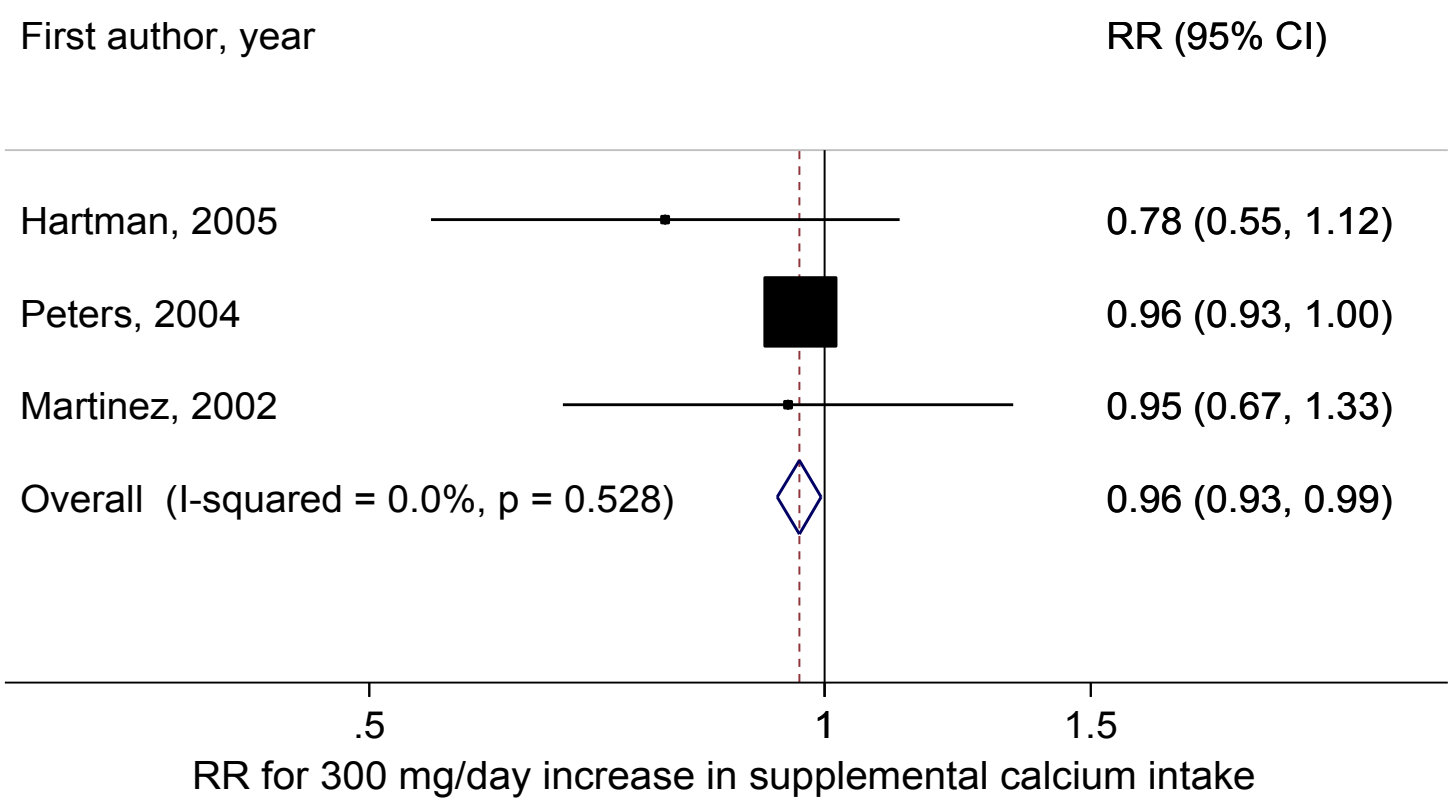

First author, year
RR (95% CI)
Hartman, 2005
0.78 (0.55, 1.12)
Peters, 2004
0.96 (0.93, 1.00)
Martinez, 2002
0.95 (0.67, 1.33)
Overall (I-squared = 0.0%, p = 0.528)
0.96 (0.93, 0.99)
.5
1
1.5
RR for 300 mg/day increase in supplemental calcium intake

Figure 3A

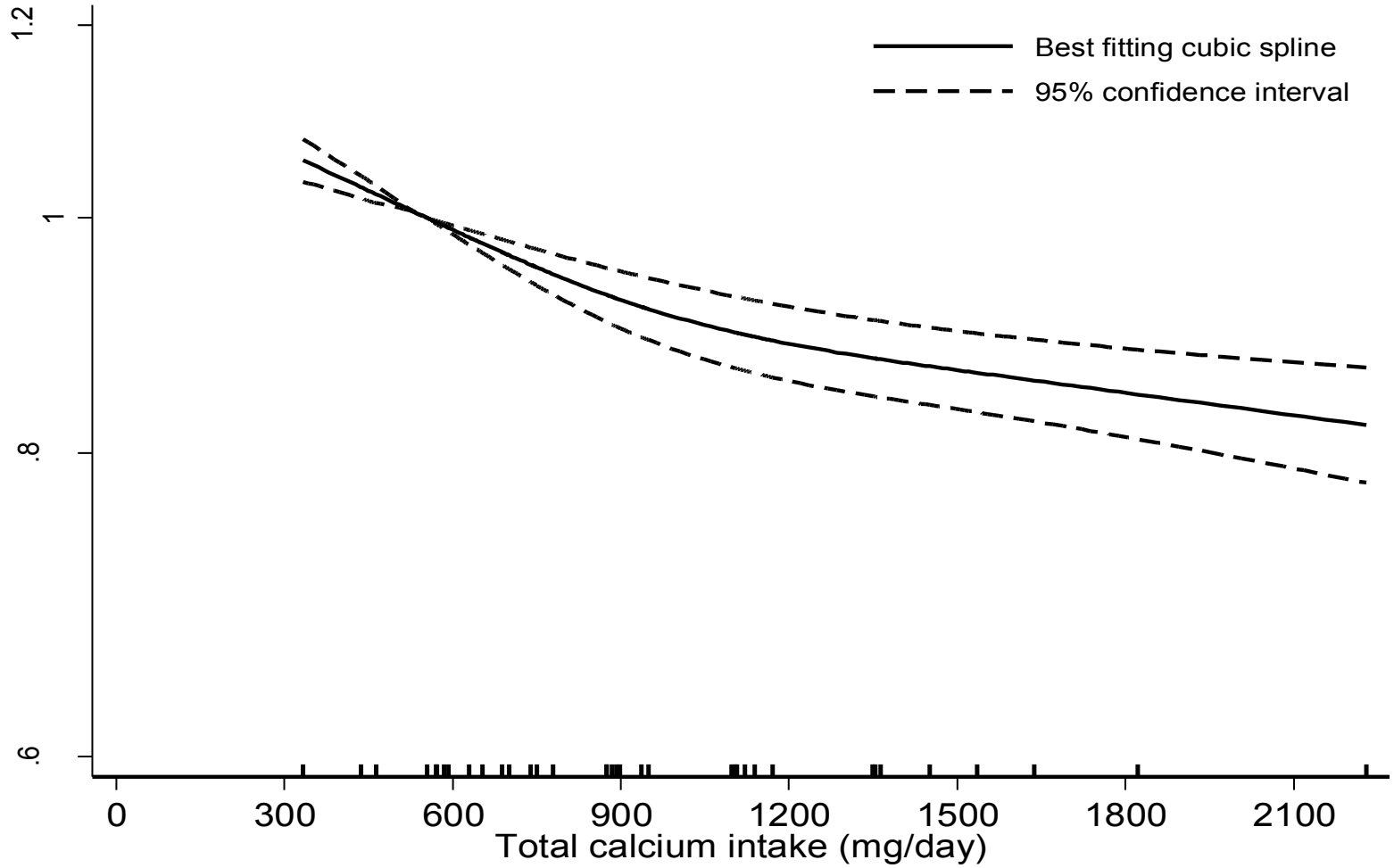

Best fitting cubic spline
95% confidence interval
1.2
1
.8
.6
0
300
600
900
1200
1500
1800
2100
Total calcium intake (mg/day)

Figure 3B

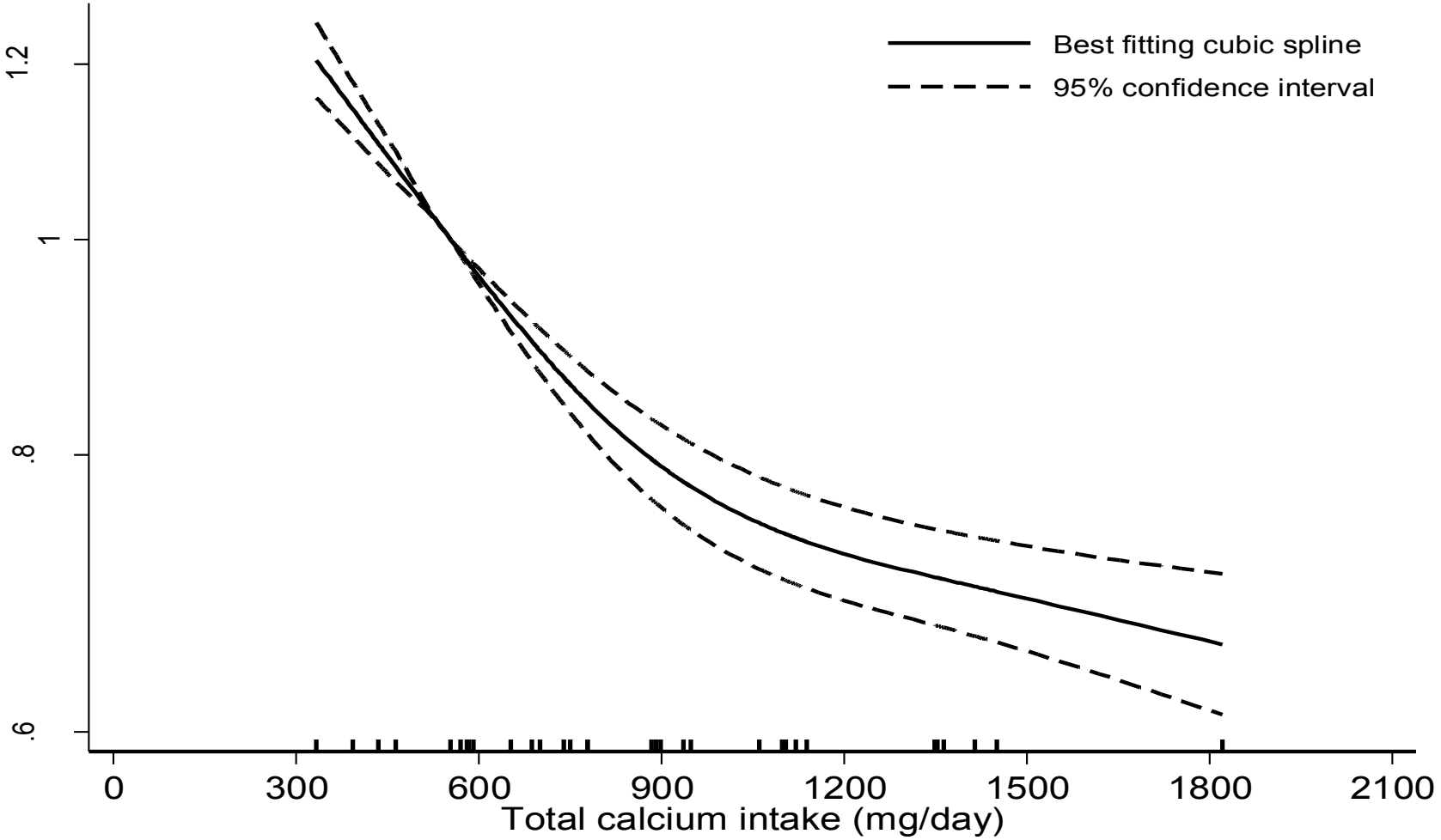
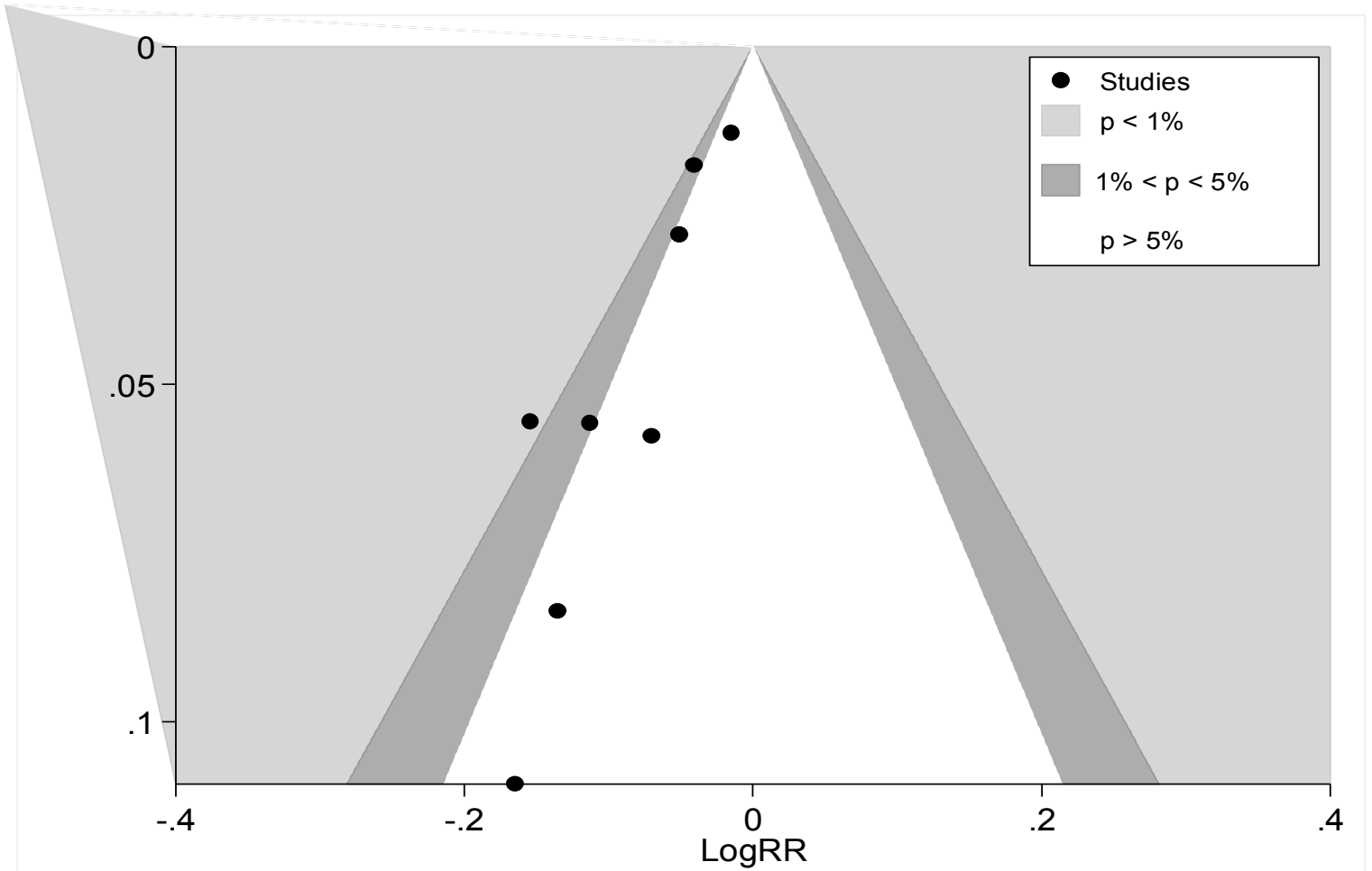

Best fitting cubic spline
95% confidence interval
1.2
1
.8
.6
0
300
600
900
1200
1500
1800
2100
Total calcium intake (mg/day)

Figure 4



Supplementary Table 1. Database Search Strategy

PubMed	(calcium[tw] AND (intake[tw] OR dietary[tw] OR diet[tw] OR food[tw] OR foods[tw] OR supplementation[tw] OR supplement[tw] OR supplements[tw])) AND (("Adenomatous Polyps"[Mesh] OR "Adenoma"[Mesh] OR adenoma[tw] OR adenomas[tw] OR adenomatous[tw] OR "Polyps"[Mesh:NoExp] OR "Intestinal Polyps"[Mesh] OR polyp[tw] OR polyps[tw]) AND ("Intestine, Large"[Mesh] OR "Colorectal Neoplasms"[Mesh:NoExp] OR "Colonic Neoplasms"[Mesh:NoExp] OR "Rectal Neoplasms"[Mesh:NoExp] OR colon[tw] OR rectum[tw] OR colonic[tw] OR rectal[tw] OR colorectal[tw] OR colo rectal[tw]))
Embase	('calcium intake'/de OR (calcium:ab,ti AND (intake:ab,ti OR diet*:ab,ti OR food*:ab,ti OR supplement*:ab,ti))) AND ('colorectal adenoma'/exp OR 'intestine polyp'/exp OR ('adenoma'/exp OR adenoma:ab,ti OR adeonmas:ab,ti OR adenomatous:ab,ti OR polyp*:ab,ti) AND ('large intestine tumor'/exp OR 'colon'/exp OR 'rectum'/exp OR colon:ab,ti OR rectum:ab,ti OR colonic:ab,ti OR rectal:ab,ti OR colorectal:ab,ti OR 'colo rectal':ab,ti))

Supplementary Table 2. Main characteristics of observational studies included in the dose-response meta-analyses

First author, Year, Country, Reference	Source population	Study period	No. cases, No. controls Sex, Age	Total mean intake at study entry (mg/d)	Adenoma outcome	Calcium type, Highest vs. lowest calcium intake (mg/d)	OR/HR (95% CI)	Variables adjusted for
Prospective studies								
Massa, 2014, USA [16]	Colonoscopy/sigmoidoscopy-based	1991-2007	2,273 39,130 female 26-60yrs	1,133	occurrence	Total 1,822 vs. 436	OR: 0.82 (0.61, 1.10)	age, family history of colorectal cancer, reason for endoscopy, height, BMI, physical activity, smoking, aspirin use, UV-B flux, folate, vitamin B6, alcohol, unprocessed red meat, processed meats, total energy intake
Yamaji, 2012, Japan [29]	Colonoscopy-based	2004-2005	737 703 combined M:50-79yrs F:40-79yrs	570	occurrence	Total 937 vs. 333	OR: 0.67 (0.47, 0.95)	age, screening period, sex, season of blood collection, smoking, alcohol, BMI, family history of colorectal cancer, NSAID use, total energy intake, height
Oh, 2007, USA [11]	Sigmoidoscopy-based	1980-2002	2,747 45,368 female 34-59yrs	732	occurrence	Total 1,451 vs. 584	OR: 0.88 (0.74, 1.04)	age, BMI, smoking , alcohol, family history of colon cancer, history of previous endoscopic screening, aspirin use, physical activity, menopausal status, hormone use, total energy intake, fiber, red meat, folate, phosphorus, vitamin D
Kesse, 2005, France [34]	E3N-EPIC	(1993-1995) to 1997	516 5,320 female 52.8yrs	1,035	occurrence	Total 1,348 vs. 688	HR: 0.80 (0.62, 1.03)	age, sex, educational level, smoking, family history of colon cancer, BMI, physical activity, total energy intake, alcohol
Hartman, 2005,	With prior adenomec	1 or 4yrs since	754 1,151	981	recurrence	Total 1,354 vs. 592	OR: 0.86 (0.62, 1.18)	age, NSAID use, sex, total energy intake, intervention assignment, sex,

USA [35]	tomy	(1991-1994)	combined 61.1yrs 754 1,151			Supplemental 199 vs. 0	OR: 0.83 (0.65, 1.05)	intervention group
Peters, 2004, USA [14]	Sigmoidoscopy-based	(1993-2001) to 2003	3,162 34,817 combined 55-74yrs 3,155 34,811	1,171	occurrence	Total 2,229 vs. 572 Supplemental 1,366 vs. 0	OR: 0.90 (0.77, 1.04) OR: 0.74 (0.57, 0.95)	age, screening center, sex, total energy intake, ethnic origin, educational level, smoking, alcohol, aspirin and ibuprofen use, physical activity, BMI, red meat, folate, fiber
Boyapati, 2003, USA [31]	Colonoscopy-based	1995-1997	177 288 combined 56yrs	826	occurrence	Total 1,247 vs. 417	OR: 0.64 (0.35, 1.15)	age, sex, total energy intake
Lieberman, 2003, USA [27]	Colonoscopy-based	1994-1997	312 1,359 combined 50-75yrs	780	occurrence	Total 1,415 vs. 393	OR: 0.51 (0.31, 0.83)	age, total energy intake, family history, BMI, smoking, alcohol, physical activity, NSAID use
Martinez, 2002, USA [36]	With prior adenomectomy	3.1yrs	639 665 NR 66yrs 639 665	1,062	recurrence	Total 629 vs. 1,638 Supplemental 300 vs. 0	OR: 0.62 (0.42, 0.90) OR: 0.94 (0.67, 1.33)	age, sex, number of colonoscopies, history of polyps prior to baseline, aspirin use, fiber, vitamin D, location and number of polyps at baseline, total energy intake
Retrospective studies								
Fu, 2012, USA [30]	Colonoscopy-based	2003-2010	1,271 3,269 combined	971	occurrence	Total 1,268 vs. 758	OR: 0.74 (0.60, 0.90)	age, sex, study sites, race, educational level, indication for colonoscopy, recruitment before or after colonoscopy, year of

			56.8yrs					recruitment, smoking, NSAID use, total energy intake
Miller, 2007, USA [28]	Colonoscopy-based	1998-2000	218 473 combined 56.2yrs	916	occurrence	Total 1,100 vs. 300	OR: 0.85 (0.53, 1.37)	age, race, sex, total energy intake
Levine, 2001, USA [32]	Sigmoidoscopy-based	1991-1993	518 553 combined 61.8yrs	900	occurrence	Total 1,404 vs. 285	OR: 1.05 (0.74, 1.49)	age, sex, race, clinic, sigmoidoscopy date, total energy intake, BMI, fiber, saturated fat, multivitamin use
Tseng, 1996, USA [15]	Colonoscopy-based	1988-1991	237 783 combined 60.2yrs	780	occurrence	Total 1,323 vs. 329	OR: 0.71 (0.36, 1.38)	age, BMI, total energy intake, smoking, alcohol, supplement use, family history of colon cancer, total fat, fiber
Martinez, 1996, USA [33]	Colonoscopy/sigmoidoscopy-based	1991-1993	157 480 combined 54.7yrs	775	occurrence	Total 1,386 vs. 357	OR: 0.7 (0.30, 1.30)	age, sex, race, BMI, smoking, family history, NSAID and aspirin use, fiber, total fat

Abbreviations: BMI, body mass index; F, females; M, males; No., number; NR, not reported; NSAID, non-steroidal anti-inflammatory drug; yrs, year

Supplementary Table 3. Subgroup analyses for linear dose-response meta-analysis of prospective studies

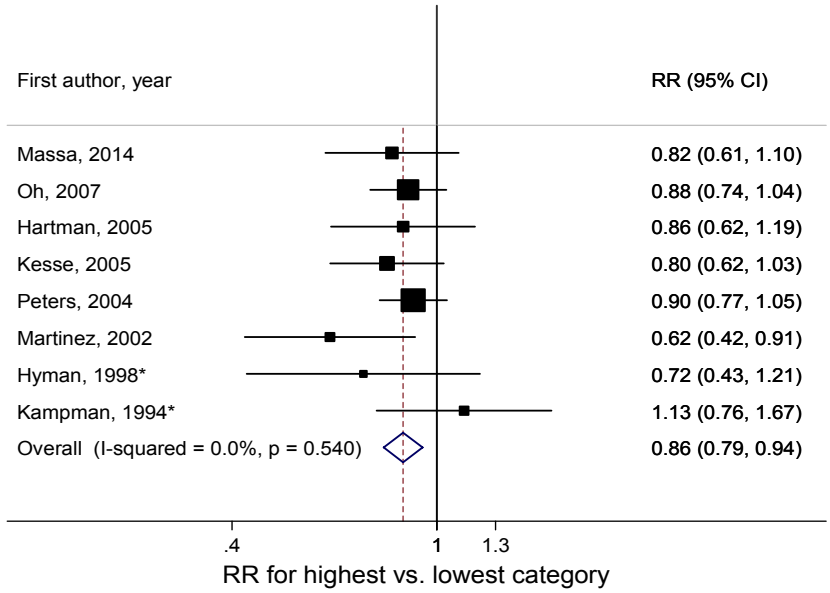
Subgroups	No. of studies	RR*(95% CI)	I^2(%)	$P_{heterogeneity}$ Within subgroup	$P_{heterogeneity}$ Between subgroups
All studies	8	0.95 (0.92, 0.98)	45	0.08	NA
1) By etiologic heterogeneity					
Sites of adenomas					
Distal colorectum	2	0.98 (0.95, 1.01)	28	0.24	0.17
Entire colorectum	6	0.92 (0.88, 0.96)	26	0.13	
Endpoints of adenomas					
Occurrence	6	0.96 (0.93, 0.99)	34	0.18	0.16
Recurrence	2	0.89 (0.82, 0.97)	9	0.29	
2) By potential effect modifiers					
Baseline age					
> 60yrs	4	0.93 (0.86, 1.00)	64	0.04	0.98
≤ 60yrs	4	0.95 (0.93, 0.98)	0	0.45	
Sex					
Women	3	0.95 (0.93, 0.98)	0	0.47	0.74
Combined	5	0.92 (0.85, 0.99)	60	0.04	
Geographical location					
USA	6	0.95 (0.92, 0.99)	46	0.10	0.21
Europe	1	0.89 (0.80, 1.00)	NA	NA	
Asia	1	0.87 (0.74, 1.03)	NA	NA	
Fortification of dairy products with vitamin D					
Yes	6	0.95 (0.92, 0.99)	46	0.10	0.20
No	2	0.89 (0.81, 0.97)	0	0.82	
Baseline calcium intake					
> 1000 mg/day	4	0.95 (0.91, 0.99)	67	0.03	0.61
≤ 1000 mg/day	4	0.94 (0.89, 0.98)	13	0.62	
3) By methodological characteristics					
Measures of association					
Hazard ratio	1	0.89 (0.80, 1.00)	NA	NA	0.40
Odds ratio	7	0.95 (0.92, 0.98)	44	0.10	
No. of cases					
> 1000	3	0.97 (0.95, 0.99)	11	0.33	0.03
≤ 1000	5	0.89 (0.84, 0.94)	0	0.85	

Use of validated dietary questionnaire					
Yes	7	0.94 (0.91, 0.97)	12	0.34	0.14
No	1	0.99 (0.96, 1.01)	NA	NA	
Adjustment for confounders					
BMI, PA					
Yes	5	0.96 (0.94, 0.99)	34	0.20	0.08
No	3	0.89 (0.83, 0.95)	0	0.56	
Smoking, Alcohol intake					
Yes	5	0.96 (0.94, 0.99)	34	0.19	0.09
No	3	0.89 (0.82, 0.96)	0	0.52	
Any vitamin D					
Yes	4	0.94 (0.90, 0.98)	37	0.19	0.69
No	4	0.94 (0.89, 1.01)	44	0.15	
Intakes of red meat, fiber and folate					
Yes	2	0.98 (0.95, 1.01)	28	0.24	0.17
No	6	0.92 (0.88, 0.96)	26	0.24	
NSAID use					
Yes	7	0.95 (0.92, 0.98)	44	0.10	0.40
No	1	0.89 (0.80, 1.00)	NA	NA	
Family history of colorectal cancer					
Yes	5	0.95 (0.92, 0.98)	0	0.46	0.75
No	3	0.93 (0.86, 1.02)	70	0.04	
History of endoscopy prior to study entry					
Yes	2	0.91 (0.83, 1.01)	64	0.10	0.49
No	6	0.96 (0.93, 0.99)	34	0.18	

Abbreviations: BMI, body mass index; NA, not applicable; PA, physical activity; No, number; NSAID, non-steroidal anti-inflammatory drug; yr, year

*RR for a 300mg/day increase in total calcium intake

Supplementary Figure 1.



* represents the two studies that were not eligible for dose-response meta-analysis due to insufficient information

MOOSE Checklist

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	While evidence from randomized controlled trials suggests that calcium may protect against the recurrence of colorectal adenomas, because the trials used only a large dose of calcium supplements and were of small sizes, knowledge of the dose-response relationship and influence on high-risk adenomas is limited.
√	Hypothesis statement	Calcium may continue to decrease the risk adenomas, particularly high-risk adenomas, over a wide range of calcium intake.
√	Description of study outcomes	Colorectal adenomas, which precedes the majority of colorectal cancers, the third most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide, excluding non-melanoma skin cancer.
√	Type of exposure or intervention used	Calcium intake (total and supplemental)
√	Type of study designs used	We included prospective observational studies for the primary analysis.
√	Study population	We placed no restriction.
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the two investigators DL and NK were indicated in the author list.
√	Search strategy, including time period included in the synthesis and keywords	Search was done to include studies published up April 2014. Detailed search strategy was provided in the supplementary material.
√	Databases and registries searched	PubMed and Embase
√	Search software used, name and version, including special features	We did not employ a search software. EndNote was used to merge retrieved articles and eliminate duplicates.
√	Use of hand searching	We hand-searched the reference lists of studies included in this analysis and those of selected reviews and meta-analyses.
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in Figure1.
√	Method of addressing articles published in languages other than English	We restricted the language to English.
√	Method of handling abstracts and unpublished studies	We excluded abstracts and unpublished results.
√	Description of any contact with authors	Authors of two publications were contacted for necessary information and one author provided the requested data.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the method section.
√	Rationale for the selection and coding of data	Studies had to provide all the data required for dose-response meta-analysis.
√	Assessment of confounding	We extracted the most fully adjusted RRs; conducted subgroup

		analyses and meta-regression by adjustment for important confounders.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Study quality was assessed by conducting subgroup analyses and meta-regression by variables concerning methodological characteristics.
√	Assessment of heterogeneity	Heterogeneity in the relationship between calcium intake and the risk of adenomas across studies was assessed by Q test and quantified by I^2.
√	Description of statistical methods in sufficient detail to be replicated	Description of dose-response meta-analysis was detailed in the method section.
√	Provision of appropriate tables and graphics	We included two tables (summary characteristics of the studies included, summary results from subgroup analyses) and four figures (flow chart, linear dose-response meta-analysis, non-linear dose-response meta-analyses, funnel plot).
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figure 2
√	Table giving descriptive information for each study included	Table 1
√	Results of sensitivity testing	Table 2
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented for all summary estimates.
Reporting of discussion should include		
√	Quantitative assessment of bias	Measurement errors are likely to underestimate the true effect, but small study effects may have led to overestimation. Thus, some cancellation of biases is expected.
√	Justification for exclusion	We excluded retrospective studies to minimize recall and selection bias.
√	Assessment of quality of included studies	We reported that none of the methodological aspects was a statistically significant source of heterogeneity.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Alternative explanations were thoroughly discussed in the limitations.
√	Generalization of the conclusions	Calcium intake was associated with reduced risk of adenomas, particularly high-risk adenomas, over a wide range of calcium intake. By preventing adenoma formation, calcium may have chemopreventive potential against colorectal neoplasias.
√	Guidelines for future research	Future studies had to examine if regular use of low-dose calcium supplements leads to protection against colorectal cancer, with additional assessment of the risk-benefit balance in terms of colorectal cancer, cardiovascular disease, and osteoporosis.
√	Disclosure of funding source	We declared no external funding for this work in the acknowledgement section.