Calcium intake and the risk of stroke: an up-dated meta-analysis of prospective studies

Asia Pac J Clin Nutr 2015;24(2):xxx-xxx
doi: 10.6133/apjcn.2015.24.2.22

Running Title: Meta-Analysis of calcium intake and stroke risk

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ABSTRACT

Background and Purpose: Calcium intake has been associated with stroke risk in a prior meta-analysis, however, newly published results are inconsistent. Dairy food benefits on stroke incidence may involve a calcium-related mechanism. We have therefore updated this meta-analysis with particular references to any possibility of a calcium-mediated dairy food risk reduction of stroke risk. Methods: We searched multiple databases and bibliographies for prospective cohort studies. Reports with multivariate-adjusted relative risk (RR) and corresponding 95% confidence intervals (CI) for the association of calcium intake with stroke incidence were considered. Results: Ten studies with 371,495 participants and 10,408 stroke events were analyzed. The pooled analysis showed no statistically significant association of the risk of total stroke (RR=0.96; 95% CI: 0.89-1.04) and stroke subtypes with the highest and lowest calcium intake quantiles. Nevertheless, high dairy calcium intake was significantly associated with an approximately 24% reduction of stroke risk. (RR=0.76; 95% CI: 0.66-0.86). Furthermore, a long-term follow-up (≥14 years) was helpful to reduce the risk of stroke (RR=0.67; 95% CI: 0.51-0.88). Additionally, a non-linear dose-response relationship was predicted between calcium intake and stroke risk. Conclusions: Dairy calcium intake is inversely associated with stroke incidence. There is a non-linear dose-response relationship between calcium intake and stroke risk. However, when the follow-up time is long enough, the inverse relationship is independent of dose. Additional large cohort studies are required to illustrate this relationship in detail.

Key Words: stroke, calcium intake, meta-analysis, prospective cohort studies, dose-response
INTRODUCTION

Stroke is one of the leading causes of mortality and morbidity globally. Despite the advances achieved in recent years, stroke prevention remains a public health priority. Dietary intakes of potassium, calcium, and magnesium intake have been proved to be associated with stroke risk in previous studies. The mechanisms mainly involve regulation of blood cholesterol concentrations, blood pressure, insulin secretion and sensitivity, vasodilation, inflammation, thrombosis, and obesity. Several randomized controlled studies have showed that calcium supplementation reduces blood pressure. However, the association of calcium intake with risk of stroke remains controversial. Some studies suggested an inverse relation between the two, while the others reported no association.

Meta-analysis is a method to reduce the likelihood of false-positive or false-negative results by increasing the sample size, it is thought to be a valuable tool for studying unintended treatment effects. Although, previous meta-analyses of randomized controlled trials suggested that there was no association between calcium intake and stroke risk, the follow-up period in these studies was considerably short. In contrast, a meta-analysis of observational prospective studies demonstrated a U-shaped association between calcium intake and stroke risk. However, one recently published study did not find the same association. We conducted a detailed meta-analysis of prospective studies to investigate the relationship between levels of dietary calcium intake and stroke incidence.

METHODS

Data sources and searches

The search was conducted according to the recommendations of MOOSE (Meta-Analysis of Observational Studies in Epidemiology) guidelines. A systematical search of the electronic databases included Pubmed, Embase, Web of Science and the Cochrane Library using no language restrictions was performed for articles published between 1966 and 2014 July. The search terms used were: (“stroke” OR “cerebrovascular disease” OR “cerebrovascular accident” OR “intracranial hemorrhage” OR “subarachnoid hemorrhage” OR “CVD” OR “cerebral infarction”) AND (“calcium intake” OR “calcium consumption” OR “calcium supplement” OR “dietary calcium”).

The titles and abstracts of studies identified in the search were scanned to exclude any that were clearly irrelevant. The full texts of the remaining articles were read to determine whether they met the inclusion criteria. The reference lists of articles with information on the topic
were also reviewed to identify additional pertinent studies.

The study design was approved by the ethical committee of the 1st affiliated hospital of Zhengzhou University.

**Study selection and data abstraction**

Retrieved citations were reviewed independently by two authors at the title and abstract level. The inclusion criteria were set as follows: (1) the study design was prospective cohort-based; (2) the general population was studied; (3) the exposure of interest was calcium intake; (4) the outcome of interest was stroke incidence; (5) relative risk (RR), hazard ratio (HR) and the corresponding 95% confidence interval (CI) (or the data required to calculate them) were reported. We also extracted study characteristics for each trial.

**Statistical Methods**

We conducted meta-analyses combining risk ratios and hazard ratios for stroke. Relative risk (RR) was pooled. The $I^2$ statistic, a quantitative measure of inconsistency across studies, was also calculated. If $p < 0.10$, the between-study heterogeneity was considered statistically significant, and we chose the random-effects model to calculate the pooled RR. Conversely, if $p > 0.10$, the between-study heterogeneity was not considered significant, the fixed-effects model was applied. Mantel–Haenszel’s fixed effects method and Der-Simonian and Laird’s random effects method were used in STATA 12 software.

The range of calcium intake and the cutoffs for the categories varied between studies. Therefore, a dose-response relationship between calcium intake and risk of stroke was examined, using a method proposed by Greenland and Orsini.\(^{20,21}\) For all studies, the median or mean level of calcium for each category was assigned to each corresponding RR estimate. In studies that did not provide the number of cases and person-years in each exposure category, the variance-weighted least square regression model was used to estimate the slopes.

As characteristics of populations, ascertainment of stroke, and adjustments for confounding factors were not consistent between studies, we performed a sensitivity analysis to further explore the robustness of our results. To identify any study that may have exerted a disproportionate influence on the overall result, we investigated the influence of a single study on the overall risk estimate by omitting one study in each turn. Publication bias was quantified by Begg’s and the Egger’s tests.
RESULTS

Literature search and study characteristics
A total of 798 unique citations (non-duplicates) were retrieved from the PubMed, Embase, Web of Science and Cochrane Library Database. Most of these were excluded after the first screening based on abstracts or titles. After a full-text review, ten prospective cohort studies were identified that met the predefined inclusion criteria.

These ten studies included 371,495 participants and were published between 1996 and 2014. Four had been conducted in the United States, one in Taiwan, one in Finland, one in Japan, one in Sweden, one in Germany and one in The Netherlands. Dietary calcium intake was assessed by validated food frequency questionnaire (FFQ) in most studies and a 24-hour recall method in one study. The characteristics of included studies are listed in Table 1.

Five of the ten studies directly evaluated the relationship of calcium intake and risk of total stroke and different stroke subtypes, while three studies only focused only on specific stroke subtypes. Of these three studies, one study evaluated calcium intake and risk of ischemic stroke, intraparenchymal hemorrhage and subarachnoid hemorrhage, but not total stroke and two studies focused only on calcium intake and risk of ischemic stroke. The remaining studies evaluated only the risk of total stroke. Five of the ten studies also reported dairy calcium intake and risk of stroke.

Meta-analysis of stroke risk with dietary calcium intake
The heterogeneity was significant among the results of included studies (p = 0.003, I²=66.3%), Hence, a random-effects model was used for the analysis. The results showed that high calcium intake was not associated with the risk of total stroke (RR = 0.96; 95% CI: 0.89-1.04) (Figure 1).

Further, a subgroup analysis for different stroke subtypes indicated that highest or lowest calcium intake quartile has no protective effect in ischemic stroke (RR = 0.83; 95% CI: 0.66-1.03), intraparenchymal hemorrhage (RR = 1.04; 95% CI: 0.63-1.71) or subarachnoid hemorrhage (RR = 1.13; 95% CI: 0.77-1.64). We further explored the influence of gender and years of follow-up. No significant association was observed in either gender: male (RR = 1.05; 95% CI: 0.94-1.18) or female (RR = 0.98; 95% CI: 0.85-1.13). Interestingly, long-term calcium intake was observed to be significantly associated with stroke incidence. (14 years and above: RR = 0.67; 95% CI: 0.51-0.88; less than 14 years: RR = 0.99; 95% CI: 0.91-1.08) (Figure 2).
Meta-analysis of stroke risk with dairy calcium intake

As dairy calcium intake was also reported by the included studies, we further performed the analysis of dairy calcium intake and risk of stroke. High dairy calcium intake was significantly associated with an approximately 24% reduction in the risk of total stroke (RR=0.76; 95% CI: 0.66-0.86), while non-dairy calcium intake showed no effect (RR = 1.01; 95% CI: 0.82, 1.24) (Figure 3).

Meta-analysis of stroke risk with supplemental calcium intake

Four studies investigated the relationship between stroke risk and supplemental calcium, including one newly published article, however, no correlation was found (RR = 0.97; 95% CI: 0.86-1.10).

Dose-response analysis

Two studies used “lowest vs. highest” quartile to evaluate the calcium intake and risk of stroke, so these results could not be combined with others. Therefore, a dose-response analysis was performed in the other seven included studies. The results showed a non-linear relationship between calcium intake and stroke risk.

Sensitivity analysis

Sensitivity analysis was conducted to explore potential sources of heterogeneity among included studies. Upon exclusion of a study that separately evaluated calcium intake and different subtypes, the pooled RR become significant but still presented heterogeneity. Exclusion of any other single study did not alter the pooled RR significantly. The p values for the Begg’s and the Egger’s tests were both 0.175, suggesting a low probability of publication bias.

DISCUSSION

This meta-analysis of 10 prospective cohort studies involving 371,495 participants and 10,408 stroke events demonstrated no statistically significant difference in the risk of total stroke or stroke subtypes with calcium intake when the highest and lowest calcium intake quintiles were compared. Dose-response analysis also showed no inverse association between the two. Interestingly, a significant relationship was suggested when the follow-up period was longer than 14 years, however, more studies are required to confirm this conclusion. Furthermore, high dairy calcium intake was observed to be associated with a reduction of approximately
24% in the risk of total stroke.

Some potential explanations should be considered to illustrate the results of our meta-analysis. Firstly, it is possible that the inverse association between calcium intake and risk of stroke is stronger in specific subgroups of the population, such as patients with hypertension. The hypotensive effect of calcium has been well demonstrated by observational studies and large meta-analyses of randomized clinical trials. Previous studies reported that calcium intake was inversely associated with not only the risk of hypertension development but also systolic blood pressure levels. In one of our included studies, dairy calcium intake was also found to be inversely associated with a history of hypertension. As hypertension is one of the risk factors for stroke, the potential anti-hypertensive effect of calcium may reduce stroke risk in individuals with hypertension. However, according to the subgroup analysis of an included study, calcium intake was not associated with risk of stroke among women with a history of hypertension. Since the history of hypertension or baseline blood pressure level had been adjusted in the other included studies, the relationship between calcium intake and risk of stroke among hypertensive individuals could not be further investigated. Additional evidence is needed to further support this hypothesis.

Secondly, the effect of dietary calcium intake may vary among different populations. In our analysis, calcium intake in the Japanese population was much lower than that of American or European populations, and interestingly, an inverse association with stroke risk was observed only in Japanese populations. Currently, it remains unclear whether the reduction of stroke risk is related to a low dose or is population specific. Further studies of high-dose calcium and stroke risk in an Asian population can further illustrate this relationship. However, the possible protective effect of dietary calcium intake on stroke should not be neglected, especially in specific populations.

Thirdly, all but one of the included studies used FFQ as the evaluating instrument. Hence, the calcium intake can be considered the “daily calcium intake” which represents habitual calcium intake. It may require a long time to exhibit effects on cardiovascular system. This indicates that long-term follow-up is needed to observe the association between calcium intake and stroke risk. In our analysis, when follow-up time is longer than 14 years, an inverse association was predicted between the two, even when the dose of calcium intake was high.

An inverse association of stroke risk with calcium intake from dairy foods but not non-dairy sources was observed in our analysis. An earlier study conducted on a Chinese population cohort showed that dairy food intake reduced the mortality and stroke risk. Furthermore, calcium and vitamin-D intake contributed to the dairy-mortality association.
is suggested that calcium plays an important role in combination with potassium and magnesium in achieving an appropriate metabolic balance that reduces blood pressure and stroke incidence.\textsuperscript{26} It remains unclear how dairy calcium influences this process. One explanation is that the casein in milk and dairy foods enhances calcium absorption,\textsuperscript{27,28} which may result in higher efficiency of calcium usage in the body, and thus contribute to a protective effect against stroke. However, other factors in dairy foods may also account for the observed association. In addition to calcium and other minerals, milk contains fats and bioactive peptides that inhibit the angiotensin-converting enzyme and thus reduce the risk of stroke.\textsuperscript{25} Milk proteins have also been shown to delay stroke onset in stroke-prone spontaneously hypertensive rats model.\textsuperscript{29} However, additional evidence is needed to further clarify this hypothesis.

A meta-analysis exploring calcium intake and cardiovascular disease-related death predicts an U-shaped association.\textsuperscript{30} However, the author did not distinguish stroke incidence from mortality,\textsuperscript{16} which might have influenced the results. In the current study, we set up stricter criteria to distinguish the incidence of stroke from mortality. In addition, our up-dated meta-analysis included two recent studies.\textsuperscript{13,14} One of these studies predicted an inverse association between high calcium intake and stroke risk when only age and gender were adjusted. These findings are inconsistent with the result of the previous meta-analysis. The previous meta-analyses also suggested a weak positive association between high calcium intakes and stroke risk, but these were based on shorter follow-up periods. In the current study, additional aspects, such as the stroke subtype and years of follow-up were considered. Interestingly, when the follow-up time was sufficiently long, the individuals with high calcium intake were suggested to have reduced risk of stroke. Longitudinal studies with longer follow-up periods are required to verify our results.

There are certain limitations of this study. First, the analysis exhibited a significant heterogeneity among the studies in terms of sample size, duration of observation, number of events, and difference in dietary calcium intake between the groups being compared. As calcium intake is correlated with other healthy lifestyle and dietary factors, it is difficult to completely isolate the effect of calcium intake on stroke risk from other factors. This heterogeneity may lead to a reduced statistical power in detecting a possible association between dietary calcium and stroke. Second, misclassification bias may reduce the strength of the association.\textsuperscript{31} Misclassification of dietary calcium intake is inevitable, because the assessment of these factors is based on self-administered questionnaires in most studies. Publication bias is also a concern because our review was based solely on published studies.
that reported stroke outcomes.\textsuperscript{32} However, several reviewed studies reported null findings, suggesting that substantial selective reporting and publication of positive results was unlikely. Nevertheless, the Begg’s and Egger’s test indicates no evidence of substantial publication bias for the meta-analysis results.

CONCLUSION
In summary, the results of our meta-analysis indicated that while calcium intake had no apparent effect on stroke risk, dairy calcium intake was inversely associated with stroke risk. Additional large cohort studies particularly performed in special population such as people with hypertension are needed to further elucidate the potential role of calcium intake in the primary prevention of stroke.

DISCLOSURES
None of the authors have any conflicts of interest associated with this study. This study was supported by grants from 81471158 and U1404311 from the National Natural Science Foundation of China (to Drs. Yu-ming Xu and Drs Chang-he Shi), and the youth innovation fund of the first affiliated Hospital of Zhengzhou University (to Drs. Chang-he Shi)

REFERENCES
7. Umesawa M, Iso H, Ishihara J, Saito I, Kokubo Y, Inoue M, Tsugane S. Dietary calcium intake and


Figure 1. Meta-analysis of stroke risk with dietary calcium intake. * As data for total stroke was not available in the two studies, data for ischemic stroke was used.

Figure 2. Subgroup analysis of stroke risk with dietary calcium intake (L: follow-up year ≥14, S: follow-up year <14)
Figure 3. Meta-analysis of stroke risk with dairy calcium intake. (DC: dairy calcium intake, NDC: nondairy calcium intake.) * As data for total stroke was not available in the two studies, data for ischemic stroke was used.
### Table 1. Characteristics of included studies in our meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Location</th>
<th>Total Participants</th>
<th>Sex</th>
<th>Stroke Cases</th>
<th>Follow up (years)</th>
<th>Exposure Assessment</th>
<th>Dairy calcium reported</th>
<th>Comparison Range/median (mg/day)</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RD, et al. 1996 [5]</td>
<td>USA (Japanese ancestry)</td>
<td>3,150</td>
<td>Men</td>
<td>229#</td>
<td>22</td>
<td>24-hour recall Methods</td>
<td>Yes</td>
<td>Quintile (IV vs. I) (606-3109) vs (0-275)</td>
<td>Age, dietary potassium and sodium, alcohol, smoking, physical activity index, systolic blood pressure, total cholesterol, serum glucose, serum uric acid, hematocrit</td>
</tr>
<tr>
<td>Ascherio A, et al. 1998 [6]</td>
<td>USA</td>
<td>43,738</td>
<td>Men</td>
<td>328</td>
<td>8.0</td>
<td>FFQ</td>
<td>Yes</td>
<td>Quintile (V vs. I) 1400 vs 500</td>
<td>Age, total energy intake, smoking, alcohol consumption, history of hypertension, history of hypercholesterolemia, parental history of myocardial infarction before age 65 years, profession, quintiles of BMI, physical activity, potassium intake, fiber intake</td>
</tr>
<tr>
<td>Iso H, et al. 1999 [7]</td>
<td>USA</td>
<td>85,764</td>
<td>Women</td>
<td>690</td>
<td>14.0</td>
<td>FFQ</td>
<td>Yes</td>
<td>Quintile (V vs. I) 1145 vs 395</td>
<td>Age, smoking, time interval, and a history of hypertension, BMI, alcohol intake, menopausal status and postmenopausal hormone use, vigorous exercise, usual aspirin use, multivitamin use, vitamin E use, n-3 fatty acid intake, histories of diabetes, high cholesterol levels</td>
</tr>
<tr>
<td>Weng LC, et al. 2008 [8]</td>
<td>Taiwan</td>
<td>1,772</td>
<td>Both</td>
<td>132#</td>
<td>10.6</td>
<td>FFQ</td>
<td>No</td>
<td>Quintile (IV+III vs. I) (&gt;591.5) vs (&lt;451.2)</td>
<td>Age, sex, hypertension, use of antihypertensive drugs, diabetes mellitus, area, central obesity, alcohol consumption, smoking, sex-smoking habit interaction, BMI, self-report heart disease, high cholesterol levels, physical activity, fibrinogen, apolipoprotein B, plasminogen</td>
</tr>
<tr>
<td>Larsson SC, et al. 2008 [9]</td>
<td>Finland</td>
<td>26,556</td>
<td>Men</td>
<td>3281</td>
<td>13.6</td>
<td>FFQ</td>
<td>No</td>
<td>Quintile (V vs. I) 1916 vs 876</td>
<td>Age, supplementation group, number of cigarettes smoked daily, BMI, systolic and diastolic blood pressures, serum total cholesterol, serum high-density lipoprotein cholesterol, histories of diabetes, coronary heart disease, leisure-time physical activity, and intake of alcohol, total energy</td>
</tr>
<tr>
<td>Umesawa M, et al. 2008 [10]</td>
<td>Japan</td>
<td>41,526</td>
<td>Both</td>
<td>1321</td>
<td>12.9</td>
<td>FFQ</td>
<td>Yes</td>
<td>Quintile (V vs. I) 753 vs 233</td>
<td>Age, sex, BMI, history of diabetes, medication for hypercholesterolemia, menopause, smoking status, ethanol intake, sodium intake, potassium intake, n-3 fatty acid intake, public health center, history of hypertension</td>
</tr>
<tr>
<td>Larsson SC, et al. 2011 [11]</td>
<td>Sweden</td>
<td>34,670</td>
<td>Women</td>
<td>1680</td>
<td>10.4</td>
<td>FFQ</td>
<td>No</td>
<td>Quintile (V vs. I) 1422 vs 698</td>
<td>Age, smoking status, pack-years of smoking, educational level, body mass index, total physical activity level, history of diabetes, history of hypertension, aspirin use, family history of myocardial infarction, and intakes of total energy, alcohol, protein, cholesterol, total fiber, and folate</td>
</tr>
</tbody>
</table>

FFQ: food frequency questionnaire, BMI: body mass index, #: Only ischemic stroke number was available in this study.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Location</th>
<th>Total Participants</th>
<th>Sex</th>
<th>Stroke Cases</th>
<th>Follow up (years)</th>
<th>Exposure Assessment</th>
<th>Dairy calcium reported</th>
<th>Comparison Range/median (mg/day)</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li KR, et al. 2012 [12]</td>
<td>German</td>
<td>23,980</td>
<td>Both</td>
<td>260</td>
<td>11</td>
<td>FFQ,</td>
<td>Yes</td>
<td>Quintile (IV vs. I) 1130 vs 513</td>
<td>Age, sex, educational level, physical activity, BMI, smoking categories, lifetime alcohol intake, energy-adjusted dietary vitamin D, saturated fatty acid and total protein intake, total energy intake, self-reported diabetes mellitus at recruitment and use of calcium supplements</td>
</tr>
<tr>
<td>Ivonne Sluijs et al. 2014[13]</td>
<td>Netherlands</td>
<td>36094</td>
<td>Both</td>
<td>631</td>
<td>12</td>
<td>FFQ</td>
<td>No</td>
<td>Quintile (IV vs. I) 1316 vs 797</td>
<td>Age, sex, body mass index, education, physical activity, smoking status, intakes of alcohol, total energy, magnesium</td>
</tr>
<tr>
<td>J. M. Paik &amp; G. C et al. 2014[14]</td>
<td>American</td>
<td>74245</td>
<td>Women</td>
<td>1856</td>
<td>24</td>
<td>FFQ</td>
<td>No</td>
<td>Quintile (V vs. I) &gt;1000 vs 0</td>
<td>Diet calcium intake, total vitamin D intake, vitamin E intake, magnesium intake, multivitamin use, BMI, family history of heart disease, smoking status, alcohol intake, postmenopausal hormone use, physical activity, race, aspirin use, history of hypertension, diabetes, or high cholesterol, glycemic load, trans fat intake, polyunsaturated fat/saturated fat ratio, fiber intake, total energy intake, recent physical examination, and recent mammogram</td>
</tr>
</tbody>
</table>

FFQ: food frequency questionnaire, BMI: body mass index, #: Only ischemic stroke number was available in this study.