Calcium intake and breast cancer risk: meta-analysis of prospective cohort studies

Khemayanto Hidayat1,2†, Guo-Chong Chen1†, Ru Zhang1, Xuan Du2, Sheng-Yi Zou2, Bi-Min Shi2* and Li-Qiang Qin1,3*
1Department of Nutrition and Food Hygiene, School of Public Health, Soochow University, Suzhou 215123, People’s Republic of China
2Department of Endocrinology and Metabolism, the First Affiliated Hospital of Soochow University, Suzhou 215123, People’s Republic of China
3Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Disease, Soochow University, Suzhou 215123, People’s Republic of China

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Abstract
Findings from observational studies have suggested a possible relation between Ca and breast cancer risk. However, the results of these studies are inconclusive, and the dose-response relationship between Ca intake and risk of breast cancer remains to be determined. A meta-analysis of prospective studies was conducted to address these issues. PubMed and Embase databases were searched for relevant studies concerning the association between Ca intake and breast cancer up to March 2016. The summary relative risks (RR) with 95% CI were calculated with a random-effects model. The final analysis included eleven prospective cohort studies involving 26606 cases and 872895 participants. The overall RR of breast cancer for high vs. low intake of Ca was 0·92 (95% CI 0·85, 0·99), with moderate heterogeneity (P=0·026, I²=44·2%). In the subgroup analysis, the inverse association appeared stronger for premenopausal breast cancer (RR 0·75; 95% CI 0·59, 0·96) than for postmenopausal breast cancer (RR 0·94; 95% CI 0·87, 1·01). Dose-response analysis revealed that each 300 mg/d increase in Ca intake was associated with 2% (RR 0·98; 95% CI 0·96, 0·99), 8% (RR 0·92; 95% CI 0·87, 0·98) and 2% (RR 0·98; 95% CI 0·97, 0·99) reduction in the risk of total, premenopausal and postmenopausal breast cancer, respectively. Our findings suggest an inverse dose-response association between Ca intake and risk of breast cancer.

Key words: Breast cancer; Calcium; Dose–response associations; Meta-analyses

Globally, breast cancer ranks first for cancer incidence and fifth for cancer mortality in women(13). Dietary and lifestyle factors may have an important role in the development of breast cancer(22–24), among which Ca intake has been suggested as a potential protective factor in mounting experimental research(5–10) and several observational studies(11–15). A meta-analysis by Chen et al(14) involving six prospective cohorts and nine case–control studies suggested a significant inverse association between Ca intake and risk of breast cancer, with a summary relative risk (RR) of 0·81 (95% CI 0·72, 0·90) for the highest compared with the lowest intake of Ca, with a significant publication bias. Their results became statistically non-significant after correcting for publication bias. The meta-analysis was followed by several subsequent prospective studies(13–19) that also focused on the same topic, but their findings continued to be inconsistent. To clarify the association between Ca intake and risk of breast cancer, we performed an updated meta-analysis of prospective studies. Given the fact that high amounts of Ca, particularly from supplements, might increase risks of certain diseases, such as CVD(20–24) and kidney stones(25,26), we also attempted to explore the shape of the dose–response association between Ca intake and breast cancer that has not been investigated in the previous meta-analysis.

Methods
Search strategy
This meta-analysis was planned, conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendation(27). PubMed and Embase databases were searched for studies assessing the association between Ca intake and breast cancer up to March 2016. The following search terms were used to retrieve the relevant literature in the databases: (‘calcium’ OR ‘dairy products’ OR ‘dairy’ OR ‘milk’ OR ‘cheese’ OR ‘yogurt’ OR ‘butter’ OR ‘cream’) AND (‘breast cancer’ OR ‘mammary gland cancer’ OR ‘breast neoplasms’ OR ‘mammary gland neoplasms’ OR ‘neoplasm of the breast’ OR

Abbreviation: ER, oestrogen receptor; PR, progesterone receptor; RR, relative risk.
* Corresponding author: L.-Q. Qin, email qinliqiang@suda.edu.cn; B.-M. Shi, email shibimin@163.com
† These authors contributed equally to this work.
were performed to investigate potential publication bias (30). The rank correlation test and Egger statistic, $Q$ across studies was assessed using a DerSimonian & Laird random-effects model (29) was used for the RR was chosen as the common measure of association across studies. The degree of heterogeneity in the relationship between Ca intake and breast cancer risk was calculated by testing the null hypothesis that the coefficient of the second spline is equal to 0. All statistical analyses were performed using the STATA software, version 11.0 (StataCorp. LP). All $P$ values were two-sided, and the level of significance was at $<0.05$, unless explicitly stated.

Results

Study characteristics

A flow chart of study selection, including reasons for exclusion, is presented in Fig. 1. We included eleven studies (31–13,15–19,35–37) that fully met our inclusion criteria for this meta-analysis. The characteristics of the included studies are summarised in Table 1. These studies were published between 2002 and 2013, with a total of 26,606 breast cancer cases diagnosed among 872,895 participants. Ten studies were conducted in Western populations (five in the USA, one in Finland, one in France, one in Norway, one in Sweden and one in ten European countries), and one study consisted of Singaporean Chinese. The duration of follow-up ranged from 7 to 25 years. One study (36) was conducted among postmenopausal women only, six studies (12,15,16,17,19,35) reported results by menopausal status and four studies (31,15,18,37) combined premenopausal and postmenopausal breast cancer. One study (37) further reported results by oestrogen receptor (ER) and progesterone receptor.
Either FFQ or 24-h recall was used as a dietary assessment tool. Ca intakes in the highest categories across studies ranged from >345 to >1750 mg/d, and the intakes in the lowest categories ranged from <203 to <807 mg/d. Most individual studies adjusted for a wide range of potential confounding factors, such as age, BMI, family history of breast cancer, hormone replacement therapy use and total energy intake. The details of quality assessment according to the nine-star NOS are presented in the online Supplementary Table S1. Nine of these studies were given scores of ≥7.

Calcium intake and breast cancer risk, high v. low intake

The combined multivariable-adjusted RR for the highest v. lowest Ca intake was 0.92 (95% CI 0.86, 0.99) (online Supplementary Fig. S1), with evidence of moderate heterogeneity (P = 0.026, I² = 44.2%). Both the Begg's rank correlation test and Egger's linear regression test suggested the presence of publication bias (Begg, P = 0.029; Egger, P = 0.016).

However, because no missing studies were detected to be filled, the results remained unchanged despite the fact that the trim and fill method was performed to correct the bias.

Subgroup and sensitivity analyses

The results of subgroup analyses stratified by geographic area, duration of follow-up, type of Ca intake, source of Ca, menopausal status and quality scores are presented in Table 2. The inverse association between Ca intake and breast cancer risk was not significantly affected by these factors (P difference >0.130). By menopausal status, the summary RR were 0.75 (95% CI 0.59, 0.96) for premenopausal breast cancer and 0.94 (95% CI 0.87, 1.01) for postmenopausal breast cancer. By sources of Ca, the summary RR were 0.93 (95% CI 0.84, 1.03) for total Ca, 0.90 (95% CI 0.84, 0.97) for dietary Ca and 0.98 (95% CI 0.92, 1.03) for supplemental Ca. Few studies also reported results for dairy Ca intake, and the summary RR were 0.80 (95% CI 0.53, 1.21) for dairy Ca and 1.00 (95% CI 0.78, 1.29)

Fig. 1. Flow chart of study selection.
Table 1. Prospective cohort studies of calcium intake and breast cancer risk
(Adjusted relative risks (RR) and 95 % confidence intervals)

<table>
<thead>
<tr>
<th>References (country)</th>
<th>Study population</th>
<th>Duration of follow-up (years)</th>
<th>No. of cases</th>
<th>Dietary assessment</th>
<th>Type of intake</th>
<th>Comparison</th>
<th>Adjusted RR (95 % CI)</th>
<th>Adjustments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knekt et al. (11) (Finland)</td>
<td>4697 women aged 15–90 years</td>
<td>25</td>
<td>88</td>
<td>Questionnaire</td>
<td>Dietary Ca</td>
<td>High v. low</td>
<td>0.44 (0.24, 0.80)</td>
<td>Age, total energy intake and other potential confounding factors</td>
</tr>
<tr>
<td>Shin et al. (35) (USA)</td>
<td>86 691 women with a mean age of 46-7 years</td>
<td>16</td>
<td>3842</td>
<td>FFQ</td>
<td>Total Ca</td>
<td>&gt;1250 v. ≤500 mg/d PRM: 0.80 (0.58, 1.12) POM: 0.93 (0.77, 1.12)</td>
<td>Age, BMI, physical activity, history of BBD, family history of breast cancer, height change, age at menarche, parity, age at first birth, alcohol intake, total energy and fat intake, glycaemic index value and intake of β-carotene, vitamin E</td>
<td></td>
</tr>
<tr>
<td>McCullough et al. (36) (USA)</td>
<td>68 567 postmenopausal women aged 50–74 years</td>
<td>7.8</td>
<td>2855</td>
<td>FFQ</td>
<td>Total Ca</td>
<td>&gt;1750 v. ≤500 mg/d PRM: 0.91 (0.79, 1.06) POM: 0.80 (0.67, 0.95)</td>
<td>Age, energy, history of breast cyst, family history of breast cancer, race, height, weight gain, alcohol intake, age at first birth, parity, education, mammography history, and HRT use</td>
<td></td>
</tr>
<tr>
<td>Kesse-Guyot et al. (37) (France)</td>
<td>3627 women aged 35–60 years</td>
<td>7.7</td>
<td>92</td>
<td>24-h recall</td>
<td>Total Ca</td>
<td>&gt;1144 v. &lt;807 mg/d All women: 0.50 (0.27, 0.91) PRM: 0.26 (0.10, 0.71) POM: 0.76 (0.34, 1.70)</td>
<td>Age, BMI, educational level, parity, marital status, energy from fat and other sources, alcohol intake, family history of breast cancer, menopausal status, smoking status, supplementation, HRT use and consumption of SFA, vegetable, meat</td>
<td></td>
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<tr>
<td>Lin et al. (38) (USA)</td>
<td>31 487 women aged ≥45 years</td>
<td>10</td>
<td>1019</td>
<td>FFQ</td>
<td>Total Ca</td>
<td>≥1366 v. &lt;617 mg/d PRM: 0.91 (0.35, 1.98)</td>
<td>Age, BMI, physical activity, family history of breast cancer, history of benign breast disease, age at menarche, age at first birth, multivitamin, smoking, alcohol, total energy intake, age at menopause and baseline HRT use Race/ethnicity, BMI, age at first birth, number of children, age at menopause, education, marital status, family history of cancer, physical activity, HRT use, smoking, and intakes of red meat, alcohol, fat and total energy</td>
<td></td>
</tr>
<tr>
<td>Park et al. (39) (USA)</td>
<td>198 903 women</td>
<td>7</td>
<td>5856</td>
<td>FFQ</td>
<td>Total Ca</td>
<td>1500 v. 526 mg/d PRM: 0.83 (0.98, 1.06)</td>
<td>Age, education, BMI, height, parity, age at first birth, age at menarche, age at menopause, use of oral contraceptives, use of postmenopausal hormones, family history of breast cancer, history of benign breast disease and intakes of alcohol, dietary fibre and total energy</td>
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Larsson et al. (37) (Sweden) | 61 433 women | 17.4 | 2952 | FFQ | Total Ca | ≥1125 v. <727 mg/d Overall: 0.97 (0.87, 1.09) ER+/PR+ tumours: 1.01 (0.85, 1.21) ER+/PR− tumours: 0.97 (0.70, 1.34) ER−/PR− tumours: 0.66 (0.44, 0.99) | Age, education, BMI, height, parity, age at first birth, age at menarche, age at menopause, use of oral contraceptives, use of postmenopausal hormones, family history of breast cancer, history of benign breast disease and intakes of alcohol, dietary fibre and total energy |
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<th>Adjusted RR (95% CI)</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hjartåker et al. (Norway)</td>
<td>64,904 women</td>
<td>8.6</td>
<td>1407</td>
<td>FFQ</td>
<td>Dietary Ca</td>
<td>≥814.2 v. &lt;552.6 mg/d</td>
<td>PRM: 0.65 (0.39, 1.08)</td>
<td>POM: 0.85 (0.70, 1.04)</td>
</tr>
<tr>
<td>Li et al. (Singapore)</td>
<td>35,298 women aged 45–74 years</td>
<td>14.2</td>
<td>823</td>
<td>FFQ</td>
<td>Total Ca</td>
<td>&gt;345 v. &lt;203.2 mg/d</td>
<td>PRM: 0.87 (0.59, 1.28)</td>
<td>POM: 1.09 (0.86, 1.38)</td>
</tr>
<tr>
<td>Genkinger et al. (USA)</td>
<td>52,062 women aged 21–69 years</td>
<td>12</td>
<td>1268</td>
<td>FFQ</td>
<td>Dietary Ca</td>
<td>≥1000 v. &lt;200 mg/d</td>
<td>1.10 (0.79, 1.53)</td>
<td></td>
</tr>
<tr>
<td>Abbas et al. (ten European countries)</td>
<td>319,985 women</td>
<td>8.8</td>
<td>7760</td>
<td>Questionnaire</td>
<td>Dietary Ca</td>
<td>≥1231 v. &lt;635 mg/d</td>
<td>All women: 0.91 (0.83, 1.01)</td>
<td>PRM: 0.98 (0.80, 1.19)</td>
</tr>
</tbody>
</table>

PRM, premenopausal women; POM, postmenopausal women; BBD, benign breast disease; HRT, hormone replacement therapy; ER/PR, oestrogen and progesterone receptors.
for non-dairy Ca. Results of the sensitivity analysis indicated that the overall risk estimates were not dominated by any single study, with summary RR ranging from 0.90 (95% CI 0.83, 0.98) to 0.93 (95% CI 0.87, 0.99). The summary RR was 0.91 (95% CI 0.84, 0.98) after an exclusion of the only Asian study. In addition, one study(37) reported results by ER and PR status of the tumour, and the inverse association of Ca intake with breast cancer risk appeared to be restricted to women with ER-negative and PR-negative tumours (RR 0.66; 95% CI 0.44, 0.99).

Dose–response analysis

Two studies(11,18) that did not report sufficient data for the dose–response analysis were excluded, and the remaining nine studies were eligible to be included in this analysis. In the linear dose–response analysis (Fig. 2), the summary RR for every 300 mg/d increase in Ca intake was 0.98 (95% CI 0.96, 0.99), $P_{\text{heterogeneity}}=0.123, I^2=30.8\%$ for all women, without evidence of a non-linear relationship ($P_{\text{non-linearity}}=0.17$), although the reduction in breast cancer risk appeared somewhat steeper in the lower range of Ca intake (<800 mg/d) than in the higher range (online Supplementary Fig. S2). By menopausal status (Fig. 2), the summary RR were 0.92 (95% CI 0.87, 0.98) for premenopausal breast cancer and 0.98 (95% CI 0.97, 0.99) for postmenopausal breast cancer. By sources of Ca, the summary RR were 0.97 (95% CI 0.95, 0.98) for dietary Ca and 0.99 (95% CI 0.97, 1.01) for supplemental Ca (online Supplementary Fig. S3).

Discussion

The present meta-analysis of eleven prospective studies supports an inverse association between Ca intake and breast cancer. Dose–response analysis revealed that each 300 mg/d increase in Ca intake was significantly associated with 2, 8 and 2 % reduced risk of total, premenopausal and postmenopausal breast cancer, respectively.

Although the exact mechanisms by which Ca may reduce the risk of breast cancer remain unclear, the ability of Ca in regulating cell proliferation, differentiation and apoptosis makes it biologically plausible as a potential protective factor against breast cancer(4–7). Evidence from animal studies suggests that Ca has anti-proliferative and pro-differentiation actions on mammary gland cells of rats fed the high-fat diet, and can reduce the incidence of mammary tumours in rats(8,9). Much of the evidence indicates that the anti-carcinogenic potential of Ca relies on its interrelation and correlation with vitamin D. However, experimental evidence suggests that an increased level of Ca alone is sufficient to trigger apoptosis(10). Increased risk of breast cancer has been linked with several chronic diseases such as diabetes, obesity and the metabolic syndrome(38–41), all of which have been suggested to be inversely associated with Ca intake(42–44). Therefore, Ca intake may indirectly be associated with lower breast cancer risk through its association with these disorders.

With additional five large prospective studies(45–50) included and comprehensive analyses conducted, our findings are generally consistent with those from the previous meta-analyses(14), and thereby further support a potentially beneficial role of Ca in the development of breast cancer. Indeed, a possible U-shaped association between Ca intake and health outcomes has been widely considered(45,46). Numerous studies also suggested that a high intake of Ca, particularly from supplements, may be associated with increased risks of CVD(49–53) and kidney stones(24,25). Given concerns about adverse risks of ingesting a high dose of Ca on health, the maximum daily Ca intake from
diet and/or dietary supplement should be carefully considered\(^{47}\). Results of our dose–response analysis showed that the inverse association of Ca intake with breast cancer risk remained when the intake was up to 1900 mg/d.

The inverse relation between Ca intake and breast cancer appeared to be stronger in the dietary Ca group (RR 0.90; 95% CI 0.84, 0.97; \(n = 9\)) than in the supplemental Ca group (RR 0.98; 95% CI 0.92, 1.03; \(n = 6\)). There were several explanations for these findings. First, it is possible that the interactions between Ca and other nutrient components in diets, such as vitamin D, conjugated linoleic acids and SFA, are necessary for Ca to exert its protection on breast cancer\(^{48}\). Second, it is possible that the benefits of Ca may be restricted to the individuals with Ca deficiencies, and Ca supplementation may not bring additional benefits for those who have consumed enough Ca from foods. Third, it is also possible that the observed inverse association may be partly or completely explained by other beneficial nutrients (potential confounders) that share similar food sources with Ca.

Furthermore, the findings from our meta-analysis were consistent with previous meta-analysis showing that inverse association of Ca intake with breast cancer risk is limited to premenopausal women\(^{14}\). To date, convincing explanations for the menopause-related difference in the association of Ca intake with the risk of breast cancer have not yet been established. We considered several possible explanations for this difference. First, the complex interactions among Ca, vitamin D and insulin-like growth factors may promote growth inhibition in breast cancer cells\(^{13,49}\). Second, Ca may serve as a potential regulator in oestrogen-driven cell proliferation\(^{50}\).

Third, as Ca inadequacy is more prominent in postmenopausal women\(^{51,52}\), it is also possible that the beneficial effects of Ca in postmenopausal women might only occur in higher doses.

### Fig. 2. Dose–response meta-analysis of calcium intake (per 300 mg/d) and breast cancer risk. RR, relative risks.
Strengths and limitations

The present study has several strengths, including incorporated evidence and relevant studies to the data. The enlarged sample size enhanced the power to detect a significant difference and provide more precise estimates of the effects. Most of the original studies included are of long follow-up durations, and all studies used a prospective design, which thereby reduced the likelihood of potential biases (e.g. recall and selection biases). We quantified the association between intake of Ca and risk of breast cancer by carrying out linear and non-linear dose–response analyses. Given the considerably distinct levels of the intake among different populations, a dose–response meta-analysis is necessary in addition to the comparison of the highest v. lowest categories of intake.

There are several potential limitations that are worthy of consideration in this meta-analysis. First, there was evidence of publication bias. Although the results did not change after using statistical methods to correct the bias, findings based on evidence of published data should always be interpreted with caution. Second, the strong interrelationship between Ca intake and vitamin D intake makes it difficult to identify the true effects of Ca intake on breast cancer risk as an independent variable in observational studies. Third, the present meta-analysis was unable to assess breast cancer subtypes by hormone receptor status because of the limited studies available. In clinical course of breast cancer, hormonal status is very important for predicting efficacy of chemotherapy; thereby, it may also be important to assess whether Ca intake and risk of breast cancer is modified by ER/PR status of the tumour. Fourth, there was moderate heterogeneity across studies. The heterogeneity may be because of the variation in exposure definitions, exposure ranges, dietary assessment methods or population characteristics among studies. Our further analyses indicated that menopausal status was a major potential contributor to the variation in the strength of the association. Finally, although individual studies have considered a wide range of potential confounders in their analyses, the potential impacts of residual/unknown confounding factors on our findings cannot be completely excluded.

Conclusion

In summary, results from this meta-analysis of eleven prospective cohort studies suggest an inverse dose–response association between Ca intake and breast cancer. Additional large prospective studies focusing on the influence of hormone receptor status on this association are necessary to confirm our findings, and such studies would also be helpful for exploring potential mechanisms whereby Ca may reduce breast cancer.

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K. H. and G.-C. C. contributed to the study design, literature search, data extraction and data analyses. K. H. wrote the paper. R. Z. assisted with literature selection. R. Z., X. D. and S.-Y. Z. provided statistical support and created all tables and figures. L.-Q. Q. and B.-M. S. critically reviewed the manuscript for important intellectual content. All authors read and approved the final manuscript.

There are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit http://dx.doi.org/10.1017/S0007114516001768

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