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EPIDEMIOLOGY

Meta-analysis of vitamin D, calcium and the prevention of breast cancer

Peizhan Chen · Pingting Hu · Dong Xie · Ying Qin · Fudi Wang · Hui Wang

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Abstract Vitamin D and calcium intake have been suggested to have protective effects against breast cancer; however, the data have been inconclusive. The present meta-analysis examined the overall effects of vitamin D intake, circulating 25(OH)D and 1α,25$(OH)_2$D levels, and calcium intake on breast cancer risk. Data from 11 studies on vitamin D intake, 7 studies on circulating 25(OH)D levels, 3 studies of circulating 1α,25$(OH)_2$D levels, and 15 studies on calcium intake and breast cancer risk were included in this analysis. From the meta-analysis, there was a significant inverse relationship between vitamin D intake and breast cancer risk, with an overall relative risk (RR) of high versus low vitamin D intake for breast cancer of 0.91 (95% CI = 0.85–0.97). The highest quantile of circulating 25(OH)D was found to be associated with a 45% (OR = 0.55, 95% CI = 0.38–0.80) decrease in breast cancer when compared with the lowest quantile. No significant association for the circulating 1α,25$(OH)_2$D level and breast cancer was found (OR = 0.99, 95% CI = 0.68–1.44). For calcium, a 19% (RR = 0.81, 95% CI = 0.72–0.90) decrease in breast cancer risk was found for those with highest quantile of calcium intake compared to the lowest quantile. These results provide strong evidence that vitamin D and calcium have a chemopreventive effect against breast cancer.

Keywords Vitamin D · 25(OH)D · 1α,25$(OH)_2$D · Calcium · Breast cancer · Meta-analysis

Introduction

Vitamin D has been reported to have an anticancer activity against many cancer types including breast, colorectal and prostate cancers; however, the overall relationship between vitamin D and calcium intake, levels of circulating vitamin D metabolites, and cancer risk are not clear [1–5]. Ecological studies found an inverse relationship between solar radiation level and breast cancer morbidity and mortality rates [6–8]. It has also been demonstrated that high intake of vitamin D can reduce breast cancer risk; however, this association was not consistent between studies [3, 9].

There are two major forms of vitamin D, ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). In the skin, 7-dehydrocholesterol can be converted into vitamin D3 by the ultraviolet B radiation absorbed from sunlight. After oral intake or synthesis by skin, the vitamin D is converted into 25 hydroxyvitamin D (25(OH)D) by 25-hydroxylases in the liver. Circulating 25(OH)D is a well-accepted indicator of endogenous vitamin D status. The 25(OH)D is subsequently converted into 1α,25-Dihydroxyvitamin D (1α,25$(OH)_2$D), which is the active form of vitamin D, by 1α-hydroxylase in the kidney. It has been suggested that 1α,25$(OH)_2$D exerts its activity though VDR, a transcription factor that has been reported to decrease epithelial cell

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P. Chen · P. Hu · D. Xie · Y. Qin · F. Wang · H. Wang (✉)
Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Rm 427, 41# Building, 320 Yueyang Road, 200031 Shanghai, People's Republic of China
e-mail: huiwang@sibs.ac.cn

P. Chen · P. Hu · D. Xie · Y. Qin · F. Wang · H. Wang
Graduate School of the Chinese Academy of Sciences, 200031 Shanghai, People's Republic of China

proliferation and induce differentiation and apoptosis [9]. VDR is the key mediator of the vitamin D pathway and is expressed in both normal and malignant breast cells [9]. Various studies have also found that polymorphisms in VDR are associated with breast cancer risk [10, 11].

Calcium is required for optimal activity of vitamin D [12, 13] and has been found to participate in regulating apoptosis [13], cell proliferation [14–16], and differentiation [3, 17]. In rodent models, high calcium intake inhibits the hyperproliferation of the mammary gland induced by high-fat diets, and calcium can inhibit mammary carcinogenesis induced by 7,12-dimethylbenz(α)anthracene [3, 17, 18]. However, other studies have demonstrated an inverse relationship between dietary calcium intake and breast cancer risk [19–21]. Nevertheless, not all studies have found this to be the case [22, 23], and the association between calcium intake and breast cancer is still unclear.

As the existing data from epidemiological studies were inconclusive, we conducted a meta-analysis to estimate the effect of vitamin D intake, calcium intake, and circulating 25(OH)D and 1α,25$(OH)_2$D levels with breast cancer risk.

Materials and methods

Selection of studies

Both MEDLINE and PubMed were searched for articles published online before July 2009. We used the term "breast cancer" in combination with "vitamin D," "cholecalciferol," "ergocalciferol," "1α,25-dihydroxyvitamin D," "25-hydroxyvitamin D," and "calcium" to identify the studies related to vitamin D intake and breast cancer risk, circulating 25(OH)D and 1α,25-dihydroxyvitamin D levels and breast cancer risk, and calcium intake and breast cancer risk. References were checked to identify any missing studies in the database search.

Studies included were those that provided data about vitamin D intake (including dietary intake and/or supplements), the circulating 25(OH)D level and/or 1α,25$(OH)_2$D level, calcium intake (including dietary intake and/or supplements) in quantiles with the risk estimates and 95% confidential intervals (CI), or that provided the results for the highest quantile in contrast with the lowest quantile, or that provided data that could be used to calculate the risk estimate and its 95% CI. The eligible studies were case–control, cohort, and cross-sectional studies. In total, 11 studies of vitamin D intake (Supplementary Table 1), 7 studies of circulating 25(OH)D levels (Supplementary Table 2), 4 studies of circulating 1α,25(OH)2D levels (Supplementary Table 3), and 15 studies of calcium intake (Supplementary Table 4), and their associations with the risk of breast cancer were included.

Statistical analysis

The RRs (relative risk) and ORs (odds risk) with their 95% CIs were extracted from individual studies for the highest versus the lowest quantiles, and the OR from case–control studies were assumed as the estimate of the RR value in the vitamin D and calcium intake meta-analysis studies. In order to establish the appropriate weighting for each study, the SE for each logarithm RR was calculated and was recognized as the estimated variance of the log RR. The inverse variance weighting method was used for pooling. Both the fixed-effects and random-effects models were carried out to get the pooled estimate and its 95% CI. If more than one risk estimate was provided in a study that had been stratified by covariates, the estimates were pooled before data were entered into the final analysis.

The heterogeneity of the data was quantified by the Q statistic and in combination with the I^2 statistic, which represents the percentage of variability across studies that is attributable to heterogeneity rather than chance. Heterogeneity among studies was considered significant when $P < 0.05$ for the Q statistic or when the I^2 value was more than 25%. If there was significant heterogeneity among the studies, the random-effects model was used, otherwise, the fixed-effects model was acceptable. Publication bias was represented by funnel plots and was further assessed by the Egger test [24]. When there was a significant publication with regard to nutrient intake and breast cancer risk, the trim and fill method was applied to correct the publication bias [25, 26]. All the statistical analyses were performed with R software and the Meta package for R (www.r-project.org).

Results

Vitamin D and breast cancer risk

Five case–control [22, 27–30] and six cohort studies [23, 31–35] were identified that had examined the association between vitamin D intake and breast cancer risk (Supplementary Table 1). Of these reports, one study of pre-menopausal women reported a significant inverse association between vitamin D intake and breast cancer risk, with a P-trend value of 0.02 [22]. A second study also found a significant inverse association in the pre-menopausal subgroup ($P_{trend} = 0.01$), but no significant association was found in the post-menopausal women when the highest quantile for total dietary vitamin D intake was compared with the lowest quantile [23]. Another case–control study reported by Rossi et al. [30] found a marginally significant inverse association between dietary vitamin D intake and breast cancer risk ($P_{trend} = 0.056$). In this study, there was a significant association in the post-menopausal women

(OR = 0.70, 95% CI = 0.50–0.98), but not in the pre-/peri-menopausal women (OR = 0.78, 95% CI = 0.48–1.26). The other studies showed no significant association.

The pooled RR under the fixed-effects model showed a significant 9% decrease in breast cancer risk for women with high vitamin D intake compared to those with low vitamin D intake (RR = 0.91, 95% CI = 0.85–0.97; Fig. 1). The Q test showed no significant heterogeneity between the studies ($Q = 13.22$, df = 10, $P = 0.2117$), and the I^2 value was 24.3%. The random-effects model found a similar result for the pooled RR (RR = 0.91, 95% CI = 0.83–1.00; Fig. 1). No significant publication bias was found ($P = 0.5534$).

In order to explore the potential heterogeneity in the data, we conducted stratified studies of vitamin D intake and breast cancer risk. The pooled RR for the five case–control studies demonstrated no significant association between vitamin D intake and breast cancer risk (RR = 0.95, 95% CI = 0.69–1.32; Table 1), although there was significant heterogeneity between studies ($Q = 12.48$, df = 4, $P = 0.0141$; $I^2 = 67.9\%$). In the six cohort studies, there was a significant decrease in breast cancer risk for the patients with high total vitamin D intake compared to those with low vitamin D intake, yielding a pooled RR of 0.90 with a 95% CI of 0.83 to 0.98 (Table 1). No significant heterogeneity was found for the cohort studies ($Q = 0.63$, df = 5, $P = 0.9868$; $I^2 = 0\%$).

There were six studies reported that examined the total vitamin D intake and breast cancer risk in pre-/peri-menopausal women [22, 23, 28, 30, 32, 34] and five studies of postmenopausal women [23, 30, 33–35]. High vitamin D intake was found to be associated with a 17% decrease in breast cancer risk (RR = 0.83, 95% CI = 0.73–0.95; Table 1) in the pre-/peri-menopausal women. This inverse association was consistent among the studies ($Q = 7.63$, df = 5, $P = 0.1776$; $I^2 = 34.5\%$). There was also an inverse, but not statistically significant, association found for the post-menopausal women (RR = 0.94, 95% CI = 0.83–1.07; Table 1), although there was significant heterogeneity in these studies ($Q = 8.31$, df = 4, $P = 0.0809$; $I^2 = 51.9\%$). However, after excluding a study reported by Lin et al. [34] in the post-menopausal group with the strongest inverse finding by the sensitivity analysis, a significant inverse association between vitamin D intake and

Table 1 Summary of the results of the stratified studies in the meta-analysis

Study	Estimate (95% CI)
Vitamin D intake	RR (95% CI)
Study type	
Cohort studies	0.90 (0.83–0.98)
Case–control studies	0.95 (0.69–1.32)
Menopausal status	
Pre-/peri-menopausal	0.83 (0.73–0.95)
Post-menopausal	0.94 (0.83–1.07)
Type of intake	
Dietary only	0.93 (0.80–1.08)
Supplement	0.87 (0.76–0.99)
Circulating 25(OH)D	OR (95% CI)
Menopausal status	
Pre-/peri-menopausal	0.69 (0.42–1.11)
Post-menopausal	0.60 (0.35–1.03)
Calcium intake	RR (95% CI)
Study type	
Cohort studies	0.87 (0.75–1.00)
Case–control studies	0.77 (0.68–0.88)
Menopausal status	
Pre-/peri-menopausal	0.72 (0.55–0.95)
Post-menopausal	0.95 (0.79–1.14)
Type of intake	
Dietary only	0.79 (0.70–0.89)
Supplement	0.97 (0.87–1.08)

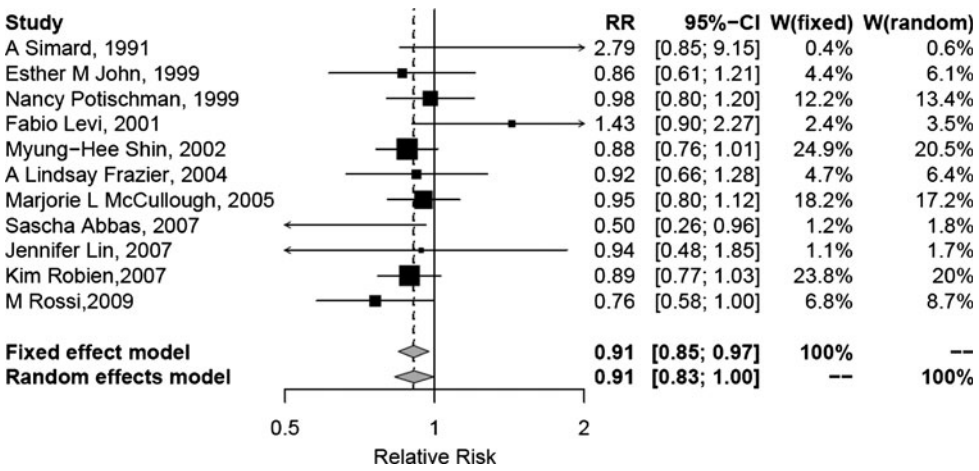

Fig. 1 Forest plot of total vitamin D intake and breast cancer risk for the highest versus the lowest quantiles of vitamin D intake

breast cancer risk (RR = 0.91, 95% CI = 0.83–0.99) was observed, and the I^2 was decreased to 0%.

In order to explore whether the source of vitamin D intake affects breast cancer risk, we conducted a stratified analysis of the type of vitamin D intake. Ten studies [22, 23, 27, 29–35] examined only the dietary vitamin D intake and three studies [31, 34, 35] examined the vitamin D intake via supplements, and the relationship of these types of vitamin D intake with breast cancer risk was included in the analysis. Of the studies examining vitamin D obtained only from the diet, an inverse (but not significant) association between vitamin D intake and breast cancer was found (RR = 0.93, 95% CI = 0.80–1.08; Table 1), although there was significant heterogeneity in the study ($Q = 18.67$, df = 9, $P = 0.0282$; $I^2 = 51.8\%$). Supplemental vitamin D intake was shown to have a significant inverse association with breast cancer risk (RR = 0.87, 95% CI = 0.76–0.99; Table 1), without any significant heterogeneity within the data ($Q = 0.17$, df = 2, $P = 0.9171$; $I^2 = 0\%$). However, given the small number of studies, the conclusion needs to be confirmed. No significant publication bias was found in any of the stratified studies.

Circulating 25(OH)D and breast cancer risk

Four case–control studies [36–39] and three nested case–control studies [40–42] examined the relationship between the circulating of 25(OH)D level and breast cancer (Supplementary Table 2). Among these studies, four studies reported a significant inverse association between the circulating 25(OH)D level and breast cancer risk [36–39]. The other three studies found a marginal or not statistically significant association [40–42]. The odds ratios for the higher compared with the lowest quantile of circulating 25(OH)D were recalculated in two studies reported by Lowe et al. [36] and Chlebowski et al. [41] based on the original data provided. The pooled odds ratio (OR) for the highest level of circulating 25(OH)D contrasted with the lowest quantile was 0.55 (95% CI = 0.38–0.80; Fig. 2) using the random-effects model. The fixed-effects model showed similar results (OR = 0.58, 95% CI = 0.50–0.66; Fig. 2). However, significant heterogeneity among the studies was found ($Q = 42.95$, df = 6, $P < 0.0001$; $I^2 = 86\%$).

Of these seven studies, three examined the circulating 25(OH)D level in pre-/peri-menopausal women [38–40] and five studied the circulating 25(OH)D level and breast cancer risk in post-menopausal women [37, 39–42]. A significant inverse relationship between circulating 25(OH)D levels and breast cancer risk was found based on the fixed-effects model (OR = 0.66, 95% CI = 0.50–0.88) but not under the random-effects model (OR = 0.69, 95% CI = 0.42–1.11; Table 1), with moderate heterogeneity among the pooled studies ($Q = 5.25$, df = 2, $P = 0.0723$; $I^2 = 61.9\%$) in the pre-/peri-menopausal women. A marginal inverse association for circulating 25(OH)D levels was found for the post-menopausal women (OR = 0.60, 95% CI = 0.35–1.03; Table 1), although there was significant heterogeneity among the pooled studies ($Q = 35.7$, df = 4, $P < 0.0001$; $I^2 = 88.8\%$). No significant publication bias was found.

Circulating 1α,25(OH)$_2$D and breast cancer risk

Circulating 1α,25(OH)$_2$D is the biologically active form of vitamin D, and its level is highly regulated in the body. Four studies have examined the relationship between circulating 1α,25(OH)$_2$D and breast cancer [40, 42–44]. One study reported by Janowsky et al. [44] was excluded, because the estimated risk for the higher level of circulating 1α,25(OH)$_2$D versus the lowest level of circulating 1α,25(OH)$_2$D could not be calculated based on the data provided (Supplementary Table 3). The pooled odds ratio for the highest circulating 1α,25(OH)$_2$D level compared with the lowest level for the remaining three studies was 1.02 (95% CI = 0.80–1.29; Fig. 3) based on the fixed-effects model. The random-effects model also indicated that the circulating 1α,25(OH)$_2$D level had no relationship with breast cancer risk, with an OR of 0.99 (95% CI = 0.68–1.44; Fig. 3). Moderate heterogeneity was found for the studies ($Q = 3.75$, df = 2, $P = 0.1536$; $I^2 = 46.6\%$). It appears that the level of circulating 1α,25(OH)$_2$D is not a good indicator of vitamin D status, likely because it is influenced by many factors such as PTH, calcium, and

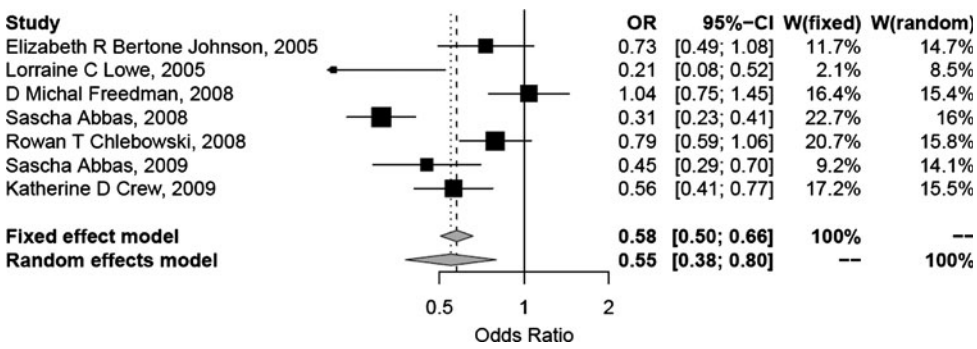

Fig. 2 Forest plot for the highest compared with the lowest quantiles of circulating 25(OH)D and their association with breast cancer risk

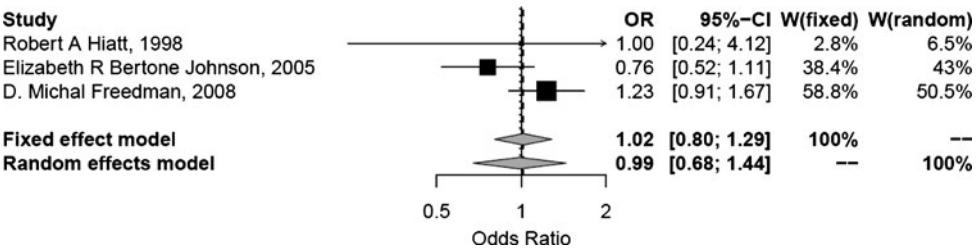

Fig. 3 Comparison of the highest and lowest quantiles of circulating $1\alpha,25(OH)_2D$ and the risk of breast cancer

phosphate feedback. No publication bias was found in the pooled study.

Calcium intake and breast cancer risk

Given the interaction between calcium and vitamin D, and the association between vitamin D and breast cancer risk, we conducted a meta-analysis to evaluate the association between calcium intake from dietary and/or supplements with breast cancer risk. A total of 15 studies have examined the relationship between calcium intake and breast cancer risk [19–23, 28, 29, 33, 34, 45–50], including 6 cohort studies [19, 20, 23, 33, 34, 45] and 9 case–control studies [21, 22, 28, 29, 46–50] (Supplementary Table 4). Among the studies, five reported a significant inverse relationship between total calcium intake and breast cancer [19–21, 48, 49]. A study reported by Lin et al. [34] found that high calcium intake was associated with decreased breast cancer in pre-menopausal women, but not in post-menopausal women. The other studies found a marginal or not statistically significant association.

The pooled estimate using the random-effects model found a significant 19% (RR = 0.81, 95% CI = 0.72–0.90; Fig. 4) decrease in breast cancer risk for those with highest quantile calcium intake compared to those with lowest calcium intake, however, significant heterogeneity among the studies was found ($Q = 25.2$, df = 14, $P = 0.0327$; $I^2 = 44.4\%$). The fixed-effects model yielded similar findings (RR = 0.88, 95% CI = 0.82–0.93; Fig. 4). However, the test of asymmetry of the funnel plot found significant publication bias among the studies (Egger's test $P = 4.43 \times 10^{-5}$). After introducing the trim and fill method to correct the publication bias, mirror images for seven studies were added in the meta-analysis, and a marginally significant 11% decreased risk of breast cancer was found (RR = 0.89, 95% CI = 0.78–1.01; Fig. 5) based on the random-effects model. Under the fixed-effects model, the overall estimate showed a significant (RR = 0.91, 95% CI = 0.85–0.97; Fig. 5) inverse relationship between calcium intake and breast cancer risk. However, significant heterogeneity among the studies was observed ($Q = 48.27$, df = 21, $P = 0.0006$; $I^2 = 56.5\%$).

For the six cohort studies, a significant inverse relationship between calcium intake and breast cancer was found, with a pooled RR of 0.87 (95% CI = 0.75–1.00; Table 1). The fixed-effects model yielded similar findings (RR = 0.91, 95% CI = 0.85–0.99). Moderate heterogeneity among the studies was found ($Q = 10.6$, df = 5,

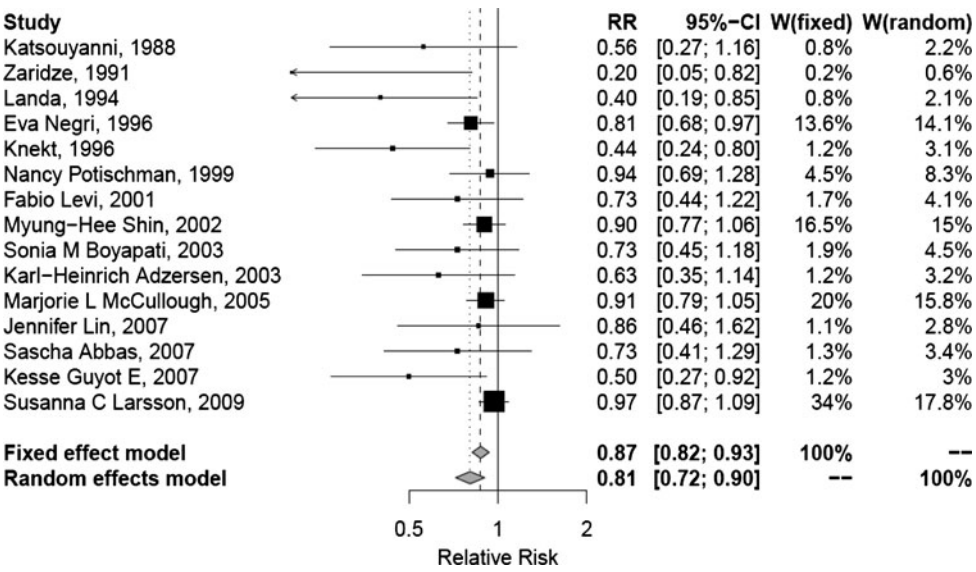

Fig. 4 Calcium intake and the risk of breast cancer for the highest versus the lowest quantiles of calcium intake

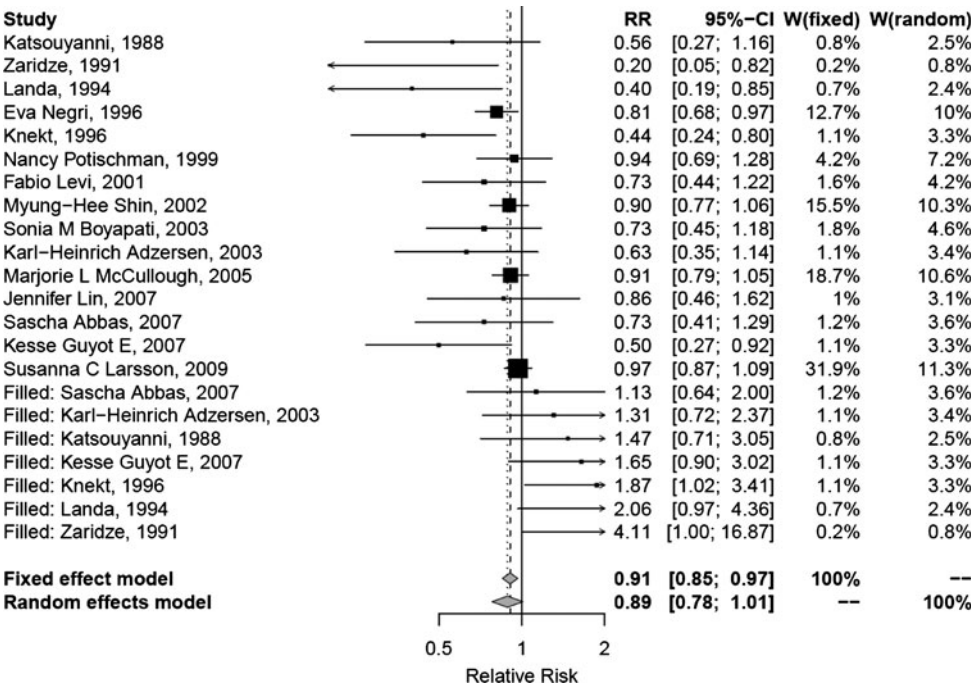

Fig. 5 Forest plot of calcium intake and breast cancer risk after correction for publication bias using the trim and fill method

$P = 0.06$; $I^2 = 52.8\%$). In the publication bias test, a slight asymmetry of the funnel plot was found using the Egger's test ($P = 0.03$). The pooled RR for the nine case–control studies was 0.75 (95% CI = 0.63–0.88) under the random-effects model, with a pooled RR of 0.77 (95% CI = 0.68–0.88; Table 1) under the fixed-effects model. No significant heterogeneity was found among the case–control studies ($Q = 9.62$, df = 8, $P = 0.2927$; $I^2 = 16.8\%$). There was also a slight deviation of symmetry for the funnel plot of the case–control pooled study (Egger's test $P = 0.01$) (Fig. 5).

The overall estimate for impact of calcium intake from the diet only for the 15 studies showed that high calcium intake led to a significant decrease of breast cancer under the random-effects model (RR = 0.79, 95% CI = 0.70–0.89; Table 1). The fixed-effects model gave a pooled RR of 0.87 (95% CI = 0.81–0.93). Significant heterogeneity was found among the studies ($Q = 28.42$, df = 14, $P = 0.0125$; $I^2 = 50.7\%$). Since the test for publication bias showed a significant deviation of symmetry of the funnel plot (Egger's test $P = 9.60 \times 10^{-5}$), the trim and fill method was used. Simulated mirror images of seven studies were included, and the pooled RR showed a significant inverse relationship between dietary calcium intake and breast cancer risk (RR = 0.88, 95% CI = 0.77–1.00) under the random-effects model, with a pooled RR of 0.90 (95% CI = 0.84–0.96) based on the fixed-effects model. The test of homogeneity found significant heterogeneity among the studies ($Q = 49.88$, df = 21, $P = 0.0004$; $I^2 = 57.9\%$). Three cohort studies have examined the relationship between supplemental calcium intake and breast cancer risk, however, no significant inverse association was found [19, 33, 34]. The pooled RR of these three studies was 0.97 (95% CI = 0.87–1.08; Table 1) under both the fixed- and random-effects models. No significant heterogeneity was found between the three studies ($Q = 0.69$, df = 2, $P = 0.71$; $I^2 = 0\%$). The lack of any significant effect may be due to poor absorption of supplemental calcium by the body or sub-optimal supplement use by the patient.

In the analysis of calcium intake and breast cancer risk stratified by menopausal status, there were six studies that evaluated the relationship between high calcium intake and breast cancer risk in pre-menopausal women [19, 22, 23, 28, 34, 49], but one study (by Zaridze et al. [49]) was excluded because the OR and its 95% CI were not provided. The pooled RR for pre-menopausal women with high calcium intake compared with those with low calcium intake was 0.76 (95% CI = 0.64–0.92) under the fixed-effects model and the RR was 0.72 (95% CI = 0.55–0.95; Table 1) based on the random-effects model. Significant heterogeneity was found among the studies ($Q = 7.59$, df = 4, $P = 0.1077$; $I^2 = 47.3\%$). Of the five studies [19, 23, 33, 34, 49] that assessed the effects of calcium intake on breast cancer risk in post-menopausal women, no significant relationship was found under either the fixed-effects model (RR = 0.95, 95% CI = 0.85–1.05) or the random-effects model (RR = 0.95, 95% CI = 0.79–1.14; Table 1). Moderate heterogeneity was found among the studies ($Q = 8.15$, df = 4, $P = 0.0863$; $I^2 = 50.9\%$). No significant publication bias was found for the studies of pre- or post-menopausal women (Egger's test $P = 0.0562$ and 0.3861, respectively).

Discussion

A previous meta-analysis of vitamin D data was conducted by Gissel et al. in 2008 [51]. This study found no significant association between vitamin D intake and breast cancer risk (RR = 0.98, 95% CI = 0.93–1.03), but when analysis was restricted to patients taking ≥400 IU/day of vitamin D, a significant inverse association with breast cancer risk was found (RR = 0.92, 95% CI = 0.87–0.97). However, only six studies were included in this previous meta-analysis. In the present study, 11 studies that examined the relationship between vitamin D intake and breast cancer risk were included, and a significant (RR = 0.91, 95% CI = 0.85–0.97) decrease in breast cancer risk was found for those with highest quantile of vitamin D intake compared with the lowest intake. Additionally, the combined results of seven studies examined the circulating 25(OH)D level and three studies examined the circulating $1\alpha,25(OH)_2D$ levels, and their association with breast cancer risk. We found a 45% (RR = 0.55, 95% CI = 0.38–0.80) decrease in breast cancer for those with the highest quantile of circulating 25(OH)D. No relationship was found for the level of circulating $1\alpha,25(OH)_2D$ and breast cancer, possibly because $1\alpha,25(OH)_2D$ is not a good indicator of vitamin D status. We examined 15 studies that evaluated the relationship between calcium intake and breast cancer risk and found that the overall estimate showed a statistically significant (RR = 0.81, 95% CI = 0.72–0.90) decrease in breast cancer risk when the highest and lowest calcium intake quantiles were compared. After correction of the publication bias using the trim and fill method, a marginally significant decrease in breast cancer risk was still present (RR = 0.89, 95% CI = 0.78–1.01).

In the stratified studies, the inverse association between dietary vitamin D intake and breast cancer risk was not significant (RR = 0.93, 95% CI = 0.80–1.08), perhaps because dietary vitamin D is not usually a major source of the vitamin (<400 IU/day). Solar exposure and vitamin supplementation are usually the major sources of vitamin D. Three studies reported a statistically significant inverse relationship between high vitamin D supplement intake and breast cancer risk (RR = 0.87, 95% CI = 0.76–0.99). Moreover, the effect size (13%) was slightly higher for vitamin D supplements than for total vitamin D intake (9%), which may be because supplemental sources of vitamin D also usually contain other types of micronutrients with anticancer activity [3].

The circulating 25(OH)D level has been demonstrated to be a good indicator of the vitamin D status in the body. A previously published pooled study found that individuals with serum 25(OH)D of approximately 52 ng/ml had a 50% lower risk of breast cancer than those with a serum level less than 13 ng/ml [52]. In the present analysis, evidence from seven studies indicated that there was a 45% (OR = 0.55, 95% CI = 0.38–0.80) decrease in breast cancer risk for women with the highest quantile (60 nmol/l) of circulating 25(OH)D compared to those with the lowest level. In order to achieve this level, at least 1000 IU/day of vitamin D intake would be necessary. However, it is usually hard to meet this standard only by food intake alone, thus supplementation of vitamin D and increased sun exposure would be necessary. No relationship was found for the circulating $1\alpha,25(OH)_2D$ level and breast cancer after the estimates of the three studies were pooled, which is consistent with previous reports showing that $1\alpha,25(OH)_2D$ is under tight regulation and is not a good indicator of vitamin D status [4, 53].

A randomized trial that evaluated the relationship between vitamin D and calcium supplementation and breast cancer risk was reported by Chlebowski et al. [41]. However, no significant reduction in breast cancer risk was found for those with vitamin D (400 IU/day) combined with calcium (1,000 mg/day) supplementation compared with those provided with placebo. This study also demonstrated that the baseline 25-hydroxyvitamin D levels were not associated with breast cancer risk. However, the dose of vitamin D administered in this study might have been too low to produce a protective effect. Additional randomized trials using higher doses of vitamin D supplements are needed to confirm the association between vitamin D intake and breast cancer.

It was also observed that high dietary calcium was associated with a decrease in the risk of breast cancer (RR = 19%, 95% CI = 0.72–0.90). However, publication bias was found for the pooled study of calcium intake (Egger's test $P = 4.431 \times 10^{-5}$). After the trim and fill method was applied to correct the bias, a marginally significant (RR = 0.89, 95% CI = 0.78–1.01) decrease in breast cancer risk was found. When only dietary calcium intake was examined, the pooled RR was 0.88 (95% CI = 0.77–1.00) for the highest versus lowest quantiles of calcium intake after correction for the publication bias. When the three cohort studies examining the effect of supplemental calcium on breast cancer risk were pooled, no significant relationship between calcium intake and breast cancer was found (RR = 0.97, 95% CI = 0.87–1.08). This suggests that the dose or form of supplemental calcium was not optimal, or that patients were not compliant with taking the supplements.

Although vitamin D and calcium intake and circulating plasma levels are two independent factors for breast cancer risk, most of the studies identified did not examine the relationship between vitamin D and calcium adjusted or stratified by the other. Of the studies that did examine this relationship, Abbas et al. [22] found a higher estimated effect for those with high levels of both vitamin D and calcium (OR = 0.38, 95% CI = 0.19–0.76) compared to

those with high calcium intake and low vitamin D intake (OR = 0.69, 95% CI = 0.46–1.02). However, the same protective effect was found for those with high vitamin D intake and high calcium intake (OR = 0.38, 95% CI = 0.19–0.76) and high vitamin D intake and low calcium intake (OR = 0.31, 95% CI = 0.10–0.96). This suggests that although there is an interaction between calcium and vitamin D intake, vitamin D may play the major role in preventing breast cancer. Nevertheless, because many sources of vitamin D also provide high levels of calcium and regulation of vitamin D is dependent upon the calcium levels, it is difficult to estimate the individual effects of vitamin D and calcium. Thus, at present, the decrease in breast cancer risk found for patients with a high intake of vitamin D and calcium cannot be conclusively attributed to either factor.

Other investigations have confirmed the association between vitamin D and calcium intake and breast cancer risk. Many studies have suggested that high vitamin D and calcium intake were related to a decrease of breast mammographic density, which suggests a decrease in the risk of developing breast cancer, since women with benign proliferation disorders are at an increased risk of developing the disease [54, 55]. The anti-proliferation and pro-differentiation activities of vitamin D and calcium may lead to a decrease in benign epithelial proliferation disorders, and this may underlie the decrease in breast cancer risk associated with high intake of these nutrients. However, a randomized controlled trial of calcium (1000 mg/d) and vitamin D supplementation (400 IU/day) reported by Rohan et al. [56] found no significant decrease in the risk of benign proliferative breast disease. Nevertheless, these doses were relatively low, so additional epidemiological studies are needed to confirm the relationship between vitamin D and calcium intake and breast density.

In conclusion, the overall results of the present study suggest that high vitamin D and calcium intake are associated with a decreased risk of breast cancer, and that the circulating 25(OH)D level has a significant inverse relationship with breast cancer. These findings provide support for the use of vitamin D and calcium as chemopreventive agents for breast cancer. However, more well-designed clinical trials are needed to determine the protective effect of vitamin D and calcium against breast cancer, and to optimize the doses of these nutrients needed to optimize their cancer preventive effects.

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