Association of vitamin B$_6$, vitamin B$_{12}$ and methionine with risk of breast cancer: a dose–response meta-analysis

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**Background:** Epidemiological studies evaluating the association of vitamin B$_6$, vitamin B$_{12}$ and methionine with breast cancer risk have produced inconsistent results.

**Methods:** Pertinent studies were identified by a search in PubMed and Web of Knowledge. Random-effect model was used. Dose–response relationship was assessed by restricted cubic spline.

**Results:** The combined relative risk (95% confidence interval) of breast cancer for the highest vs lowest category of serum pyridoxal 5'-phosphate (PLP, active form of vitamin B$_6$) levels and dietary methionine intake was 0.80 (0.66–0.98, $P = 0.03$) and 0.94 (0.89–0.99, $P = 0.03$), respectively, and the associations of breast cancer with higher serum PLP levels and dietary methionine intake were significant among post-menopausal women, but not among pre-menopausal women. The inverse association between breast cancer risk and dietary vitamin B$_{12}$ intake, serum vitamin B$_{12}$ levels and dietary vitamin B$_{12}$ intake was not significant overall. Linear dose–response relationship was found, and the risk of breast cancer decreased by 23% ($P < 0.001$) for every 100 pmol ml$^{-1}$ increment in PLP levels and 4% ($P = 0.05$) for every 1 g per day increment in dietary methionine intake, respectively.

**Conclusion:** Serum PLP levels and methionine intake might be inversely associated with breast cancer risk, especially among postmenopausal women, which need to be confirmed.

Breast cancer is the most frequently diagnosed types of cancer and the leading cause of cancer death among women worldwide, accounting for 23% of the total cancer cases and 14% of the cancer deaths, and about half the breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries (Jemal et al., 2011). Although incidence rates of breast cancer in some western countries decreased since the beginning of 2000, the incidence rates have been rising in many African and Asian countries (Jemal et al., 2011). According to the American Cancer Society Guidelines, the nutrition- and physical activity-related advice (Wu et al., 2013) is necessary to reduce the risk of breast cancer (Kushi et al., 2012). One-carbon metabolism nutrients, such as folate, vitamin B$_6$, vitamin B$_{12}$ and methionine, may protect against cancer through DNA synthesis and methylation, upholding DNA integrity and regulating gene expression (Ames, 2001; Selhub, 2002), and pyridoxal 5'-phosphate (PLP; the active form of vitamin B$_6$) is involved in almost 100 enzymatic reactions (Bairoch, 2000). Although no significant effect of folate on breast cancer was found in the previous meta-analysis overall (Larsson et al., 2007), high alcohol (the known antagonist for these B-vitamins) consumption was shown to be associated with increased risk of breast cancer (Suzuki et al., 2008). However, results from observational studies on vitamin B$_6$, vitamin B$_{12}$ and methionine and breast cancer risk are not consistent, and no meta-analysis is available. In addition, folate, vitamin B6 and vitamin B12 act in concert to affect the pathways of one-carbon metabolism.
(Kim, 2007), and these nutrient interactions were also proposed (Davis and Uthus, 2004). Therefore, we conducted a meta-analysis to (1) first assess the breast cancer risk for the highest vs lowest categories of vitamin B6, vitamin B12, and methionine; (2) then evaluate the possible dose–response relationship of vitamin B6, vitamin B12, and methionine with breast cancer risk; (3) investigate the joint association between folate intake with vitamin B6, vitamin B12, and methionine intake, and the risk of breast cancer; (4) evaluate the modification of key covariates to the association of vitamin B6, vitamin B12, and methionine with breast cancer risk; (5) and assess the heterogeneity among studies and publication bias.

MATERIALS AND METHODS

Literature search and selection. We performed a literature search up to 18 June 2013 using the databases of Pubmed and Web of Knowledge, using the following search terms vitamin B6 or pyridoxal 5'-phosphate (PLP; the active form of vitamin B6), or vitamin B12 or methionine, and breast cancer without restrictions. Moreover, we reviewed the reference lists from retrieved articles to search for further relevant studies.

Two investigators independently reviewed all identified studies, and studies were included if they met the following criteria: (1) the exposure of interest was vitamin B6 or PLP, or vitamin B12 or methionine; (2) the outcome of interest was breast cancer; (3) relative risk (RR) or odds ratio with 95% confidence interval (CI) was provided; (4) for dose–response analysis, the number of cases and participants or person-years for each category of vitamin B6 or PLP, or vitamin B12 or methionine, must also be provided (or data available to calculate them). If data were duplicated in more than one study, we included the study with the largest number of cases.

Data extraction. The following data were extracted from each study by two investigators: the design type (case–control or prospective study), the first author’s last name, publication year, location where the study was performed, sample size and number of cases, variables adjusted for in the analysis, RR estimates with corresponding 95% CI for the highest versus lowest categories of vitamin B6, vitamin B12, and methionine, respectively. The result for dietary intake of these nutrients was extracted if the result for both dietary intake and total intake (dietary intake plus supplement) were provided. To investigate the joint association between folate intake with vitamin B6, vitamin B12, and methionine intake, and risk of breast cancer, RR was extracted from the group with both the highest folate intake and highest vitamin B6, vitamin B12, or methionine intake group versus the group with both the lowest folate intake and lowest vitamin B6, vitamin B12, or methionine intake.

For dose–response analysis, the number of cases and participants (person-years), and RR (95% CI) for each category of vitamin B6, vitamin B12, and methionine were also extracted. The median or mean level of vitamin B6, vitamin B12, and methionine for each category was assigned to the corresponding RR for every study. If the upper boundary of the highest category was not provided, we assumed that the boundary had the same amplitude as the adjacent category (Larsson et al, 2010; Hong et al, 2012). We extracted the RR that reflected the greatest degree of control for potential confounders.

Statistical analysis. Pooled measure was calculated as the inverse variance-weighted mean of the logarithm of RR with 95% CI, to assess the strength of association between vitamin B6, vitamin B12, and methionine and the risk of breast cancer. Random-effects model was used to combine study-specific RR (95%CI), which considers both within-study and between-study variation (DerSimonian and Laird, 1986). The I² was used to assess heterogeneity, and I² values of 0, 25, 50 and 75% represent no, low, moderate and high heterogeneity (Higgins et al, 2003), respectively. Meta-regression with restricted maximum likelihood estimation was performed to assess the potentially important covariates that might exert substantial impact on between-study heterogeneity (Higgins and Thompson, 2004). A sensitivity analysis was performed with one study removed at a time to assess whether the results could have been affected markedly by a single study. Publication bias was evaluated using the Egger regression asymmetry test (Egger et al, 1997).

RESULTS

Literature search and study characteristics. The search strategy identified 552 articles from Pubmed and 1144 articles from the Web of Knowledge, and 28 articles were reviewed in full after reviewing the abstract. Two studies that assessed the MTHFR C677T (Maruti et al, 2009a,b), MTR and MTRR (Shrubsole et al, 2006) polymorphisms and breast cancer by intakes of one-carbon metabolism nutrients were further excluded because of duplicate reports from the same study population. Three studies that did not report the risk estimate were also excluded (Potera et al, 1997; Goodman et al, 2001; Schroen et al, 2003). Finally, 23 articles (Thorand et al, 1998; Wu et al, 1999a,b; Levi et al, 2001; Shrubsole et al, 2001; Feigelson et al, 2003; Zhang et al, 2003; Zhu et al, 2003; Lajous et al, 2006a; Lajous et al, 2006b; Cho et al, 2007; Kabat et al, 2008; Lin et al, 2008; Xue et al, 2008; Ma et al, 2009a; Ma et al, 2009b; Maruti et al, 2009a,b; Stevens et al, 2010; Chou et al, 2011; Shrubsole et al, 2011; Zhang et al, 2011; Lurie et al, 2012; Bassett et al, 2013; Yang et al, 2013) were included in this meta-analysis. The detailed steps of our literature search are shown in Supplementary Material.

For PLP levels, data from four nested case–control articles with five studies were used, including 2509 breast cancer cases, and all studies were carried out in the United States. For vitamin B6 intake, data from 14 articles (6 prospective studies and 8 case–control studies) were used, including 14260 breast cancer cases. Five studies were carried out in the United States, four in China, one in Japan, one in Mexico, one in Switzerland, one in Brazilia and one in Australia. For serum vitamin B12 levels, data from three nested case–control articles with four studies were used, including 1803 breast cancer cases, and all studies were carried out in the United States. For vitamin B12 intake, data from 14 articles (7 prospective studies and 7 case–control studies) were used, including 15783 breast cancer cases. Five studies were carried out in the United States, four in China, one in Japan, one in Mexico, one in France, one in Brazilia and one in Australia. For methionine, data from 13
articles (7 prospective studies and 6 case–control studies) were used, including 17 060 breast cancer cases. Seven studies were carried out in the United States, three China, one in Canada, one in Germany and one in Australia. The detailed characteristics of the studies are shown in Tables 1–3.

Quantitative synthesis. The main results are summarised in Table 4 (the result by adjustment of selected covariates is not included).

Serum PLP levels and risk of breast cancer. High serum PLP levels vs low levels were significantly associated with the risk of breast cancer (0.80 (0.66–0.98), P = 0.03, \( I^2 = 30.3\% \), Figure 1). The association was significant for post-menopausal women (0.71 (0.57–0.88), P < 0.00, \( I^2 = 0.00\% \)) but not for pre-menopausal women; however, the difference between the two groups was not significant (P = 0.26). No significant association was found by oestrogen receptor (ER) and progesterone receptor (PR) status. Among the five nested case–control studies, two studies in one article (Wu et al, 1999a,b) only adjusted for age, menopausal status and year of blood donation, and a positive but not significant association of serum PLP levels with the risk of breast cancer was found. Three studies (Zhang et al, 2003; Lin et al, 2008; Lurie et al, 2012) adjusted for the most known risk factors of breast cancer, and a significantly combined effect (Zhang et al, 2003; Lin et al, 2008; Lurie et al, 2012) was found (0.76 (0.62–0.94), P = 0.01, \( I^2 = 0.00\% \)).

For dose–response analysis, data from three studies (Zhang et al, 2003; Lin et al, 2008; Lurie et al, 2012) were used, including 2266 breast cancer cases. We found no evidence of statistically significant departure from linearity (P = 0.85). A 100 pmol \( \text{ml}^{-1} \) increment in serum PLP level conferred an RR of 0.77 (95% CI 0.69–0.86, P < 0.00, Figure 2).

Vitamin \( B_6 \) intake and risk of breast cancer. High vitamin \( B_6 \) intake vs low intake was not significantly associated with the risk of breast cancer (0.95 (0.83–1.08), P = 0.45, \( I^2 = 56.2\% \), Figure 1). And no significant association was found in subgroup analysis by study design (prospective and case–control), menopausal status (pre-menopausal and post-menopausal), geographic region where the study was conducted (America and Asia), ER status and PR status. No significant association was found in the subgroup analysis by adjustment (yes or no) of the known risk factors of breast cancer, including alcohol, smoking, BMI, FHBC and reproductive factors (≥3), physical activity, energy intake and use of exogenous hormones.

For dose–response analysis, data from eight studies (Lajous et al, 2006a,b; Lin et al, 2008; Ma et al, 2009a; Ma et al, 2009b; Stevens et al, 2010; Zhang et al, 2011; Bassett et al, 2013; Yang et al, 2013) were used, including 9429 breast cancer cases. We found no evidence of statistically significant departure from linearity (P = 0.08). A 1 mg per day increment in vitamin \( B_6 \) intake conferred an RR of 0.96 (0.89–1.02, P = 0.19).

Serum vitamin \( B_{12} \) levels and risk of breast cancer. High serum vitamin \( B_{12} \) levels vs low levels were not significantly associated with the risk of breast cancer (0.73 (0.44–1.22), P = 0.23, \( I^2 = 72.5\% \), Figure 3), and no significant association was found in the subgroup analysis by menopausal status. Only one study (Lin et al, 2008) provided the association of serum vitamin \( B_{12} \) levels with the risk of breast cancer by ER status and PR status; thus, the subgroup analysis by ER status and PR status was not conducted. Among the four studies included, one study (Lin et al, 2008) adjusted for the most known risk factors of breast cancer, and women in the highest quintile relative to those in the lowest quintile had multivariate RR of 1.29 (0.92–1.82). The result from the other three studies indicated an obvious protection of serum vitamin \( B_{12} \) levels on risk of breast cancer (0.61 (0.41–0.92), P = 0.02, \( I^2 = 22.3\% \)).

For the dose–response analysis, data from two studies (Zhang et al, 2003; Lin et al, 2008) were used, including 1560 breast cancer cases. We found no evidence of statistically significant departure from linearity (P = 0.51). A 100 pmol \( \text{ml}^{-1} \) increment in serum vitamin \( B_{12} \) levels conferred an RR of 0.99 (0.92–1.08, P = 0.88).

Vitamin \( B_{12} \) intake and risk of breast cancer. High vitamin \( B_{12} \) intake vs low intake was not significantly associated with the risk of breast cancer (0.88 (0.77–1.00), P = 0.05, \( I^2 = 68.9\% \), Figure 3). However, significant association was found in case–control studies (0.74 (0.56–0.98), P = 0.04, \( I^2 = 74.5\% \)) but not in prospective studies. No significant association was found in other subgroup analysis.

For dose–response analysis, data from nine studies (Lajous et al, 2006a,b; Lin et al, 2008; Ma et al, 2009a; Ma et al, 2009b; Stevens et al, 2010; Chou et al, 2011; Zhang et al, 2011; Bassett et al, 2013; Yang et al, 2013) were used, including 9832 breast cancer cases. We found no evidence of statistically significant departure from linearity (P = 0.14). A 1-\( \mu \)g per day increment in vitamin \( B_{12} \) intake conferred an RR of 0.98 (0.95–1.00, P = 0.12).

Methionine intake and risk of breast cancer. High methionine intake vs low intake was significantly associated with the risk of breast cancer (0.94 (0.89–0.99), P = 0.03, \( I^2 = 0.00\% \), Figure 4). Marginally significant association was found in prospective studies (0.94 (0.87–1.00), P = 0.06, \( I^2 = 0.00\% \)) but not in case–control studies (Figure 4). Significant association was found for post-menopausal women (0.89 (0.82–0.97), P = 0.01, \( I^2 = 0.01\% \)) but not for pre-menopausal women; however, the difference between the two groups was not significant (P = 0.24). No significant association was found by ER status and PR status. Marginally significant association was found for studies that adjusted for alcohol and use of exogenous hormones, and significant association was also found for studies that adjusted for physical activity, energy intake, BMI, FHBC and reproductive factors (≥3).

For dose–response analysis, data from six studies (Feigelson et al, 2003; Xu et al, 2008; Stevens et al, 2010; Zhang et al, 2011; Bassett et al, 2013; Yang et al, 2013) were used, including 10 316 breast cancer cases. We found no evidence of statistically significant departure from linearity (P = 0.82). A 1-g per day increment in methionine intake conferred an RR of 0.96 (0.92–1.00, P = 0.05).

Effect of combining folate with vitamin \( B_6 \), vitamin \( B_{12} \) and methionine on risk of breast cancer. For the joint association between breast cancer risk and folate intake with vitamin \( B_6 \) intake, data from four studies (Marutti et al, 2009a,b; Stevens et al, 2010; Chou et al, 2011; Shrubsole et al, 2011) were used, and the risk of breast cancer for the subjects with both highest intake of folate and vitamin \( B_6 \) was 0.91 (0.79–1.04), P = 0.17, \( I^2 = 0.00\% \). For the joint association between breast cancer risk and folate intake with vitamin \( B_{12} \) intake, data from three studies (Marutti et al, 2009a,b; Stevens et al, 2010; Shrubsole et al, 2011) were used, and the risk of breast cancer for the subjects with both highest intake of folate and methionine was 0.81 (0.62–1.05), P = 0.11, \( I^2 = 49.2\% \).

Sources of heterogeneity and meta-regression. In order to explore the moderate to high between-study heterogeneity found in several analysis, univariate meta-regression with the covariates of publication year, location where the study was conducted, study design (case–control or prospective), number of cases and degree of adjustments of covariates was performed. Degree of adjustments of covariates ranged from 0 to 9 based on adjustment (yes: 1, no: 0)
| Study | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates |
|-------|----------------------|--------------|------------------|-------------|-----------|-------------|--------------------------|
| Potera et al (1977)* | USA | Case–control | 130 (94) | 56 | PLP (ng ml$^{-1}$) | None |
| Wu et al (1999a), the 1974 cohort | USA (MD) serum bank | Prospective (NCC) | 266 (133) | 18–90 | PLP | Age, menopausal status and year of blood donation |
| Wu et al (1999b), the 1989 cohort | USA, (MD) serum bank | Prospective (NCC) | 220 (110) | 18–90 | PLP | Age, menopausal status and year of blood donation |
| Levi et al (2001) | Switzerland | Case–control (HCC) | 731 (289) | Cases: 57 | Vitamin B$_6$ | Age, education, parity, menopausal status, BMI, total energy intake and alcohol |
| Shrubsole et al (2001) | China, The Shanghai Breast Cancer Study | Case–control (PCC) | 2703 (1321) | 25–64 | Vitamin B$_6$ | Total energy, age, education, FHBC, personal history of fibroadenoma, age at menarche, parity, age at first live birth and menopause, menopausal status, physical activity, waist:hip ratio, total fruit and vegetable intake, and total animal food intake |
| Goodman et al (2001)* | USA | Prospective (NCC) | 225 (112) | Cases: 60.4 | PLP (pmol/ml$^{-1}$) | None |

* COMT$^{3}$

** COMT$^{4}$, COMT$^{5}$
| Study | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates |
|-------|---------------------|--------------|-----------------|-------------|----------|-------------|---------------------------|
| Zhang et al (2003) | USA, The Nurses' Health Study | Prospective (NCC) | 1424 (712) | 43–69 | PLP | 0.86 (0.60–1.22) | Age at menarche, parity, age at first birth, age at menopause, FHBC in mother or a sister, HBBC, alcohol intake, BMI and duration of post-menopausal hormone use |
| | | | | | | 28.5–40.2 | 28.5–40.2 | 0.86 (0.60–1.22) | |
| | | | | | 40.2–57.0 | 40.2–57.0 | 0.80 (0.56–1.14) | |
| | | | | | 57.1–94.1 | 57.1–94.1 | 0.79 (0.55–1.14) | |
| | | | | | > 95.3 | > 95.3 | 0.70 (0.48–1.02) | |
| Lajous et al (2006a,b) | Mexico | Case–control (PCC) | 1866 (475) | 18–82 | Vitamin B6 | 0.67 (0.49–0.92) | Age, socioeconomic status, FHBC, menopausal status, parity, BMI, total caloric intake, dietary fibre and carbohydrate intake, and polyunsaturated fat intake |
| | | | | | 1.06 (mg per day) | 1.06 (mg per day) | 1.06 (0.49–0.92) | |
| | | | | | 1.26 | 1.26 | 0.67 (0.49–0.92) | |
| | | | | | 1.40 | 1.40 | 0.76 (0.55–1.05) | |
| | | | | | 1.60 | 1.60 | 0.84 (0.61–1.13) | |
| Cho et al (2007) | USA, The Nurses' Health Study II | Prospective | 90,663 (1032) | 26–46 | Vitamin B6 | 1.16 (0.95–1.41) | Age, year of the current questionnaire cycle, smoking, height, parity and age at first birth, BMI, age at menarche, FHBC, HBBD, oral contraceptive use, alcohol, energy and animal fat |
| | | | | | 1.6 (mg per day) | 1.6 (mg per day) | 1.16 (0.95–1.41) | |
| | | | | | 1.9 | 1.9 | 1.16 (0.95–1.41) | |
| | | | | | 2.1 | 2.1 | 1.09 (0.89–1.33) | |
| | | | | | 2.3 | 2.3 | 0.96 (0.78–1.18) | |
| | | | | | 2.7 | 2.7 | 1.18 (0.96–1.44) | |
| Lin et al (2008) | USA, The Women's Health Study | Prospective (NCC) | 16,96 (848) | >45 | PLP | 1.02 (0.73–1.43) | Age, ethnicity, menopausal status, fasting status, month and year of blood return, post-menopausal hormone use and trial randomisation date, randomised treatment assignment, BMI, FHBC in a first-degree relative, HBBD, smoking, physical activity, alcohol, age at menarche, age at menopause, parity and age at first birth |
| | | | | | <38.9 (pmol/ml) | <38.9 (pmol/ml) | 1.02 (0.73–1.43) | |
| | | | | | 38.9–52.2 | 38.9–52.2 | 1.02 (0.73–1.43) | |
| | | | | | 52.2–66.3 | 52.2–66.3 | 0.91 (0.65–1.29) | |
| | | | | | 66.3–102.2 | 66.3–102.2 | 0.92 (0.65–1.32) | |
| | | | | | >102.2 | >102.2 | 0.91 (0.63–1.30) | |
| | | | | | Vitamin B12 | <1.7 (mg per day) | 1.7–1.9 | 1.16 (0.70–1.95) | |
| | | | | | | 1.9–2.1 | 1.9–2.1 | 1.00 (0.61–1.63) | |
| | | | | | | 2.1–2.3 | 2.1–2.3 | 1.02 (0.60–1.71) | |
| | | | | | | > 2.3 | > 2.3 | 1.02 (0.61–1.69) | |
| Study | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates |
|-------|---------------------|--------------|------------------|-------------|-----------|-------------|--------------------------|
| Ma et al (2009a) | Brazil | Case-control (HCC) | 916 (458) | 20–74 | Vitamin B₆ | <0.6 (mg per day) | 1 |
| | | | | | | 0.6–1.0 | 1.04 (0.74–1.46) |
| | | | | | | >1.0 | 1.18 (0.84–1.65) |
| Ma et al (2009b) | Japan | Case-control (HCC) | 776 (388) | 20–74 | Vitamin B₆ | <1.5 (mg per day) | 1 |
| | | | | | | 1.5–1.8 | 0.65 (0.41–1.03) |
| | | | | | | >1.8 | 0.85 (0.53–1.38) |
| Maruti et al (2009a,b) | USA, The VITAL cohort study | Prospective | 35 023 (743) | 50–76 | Vitamin B₆ | 0.96 (mg per day) | 1 |
| | | | | | | 1.39 | 1.04 (0.83–1.36) |
| | | | | | | 1.79 | 1.08 (0.84–1.38) |
| | | | | | | 2.45 | 0.90 (0.66–1.21) |
| Stevens et al (2010) | USA, CPS-II Nutrition Cohort | Prospective | 70 656 (3898) | 54–74 | Vitamin B₆ | <1.05 (mg per day) | 1 |
| | | | | | | 1.05–1.36 | 1.01 (0.90–1.12) |
| | | | | | | 1.36–2.29 | 0.93 (0.82–1.06) |
| | | | | | | 2.29–3.45 | 0.90 (0.75–1.09) |
| | | | | | | >3.45 | 0.95 (0.78–1.17) |
| Zhang et al (2011) | China | Case-control (HCC) | 876 (438) | 25–70 | Vitamin B₆ | <0.6 (mg per day) | 1 |
| | | | | | | 0.6–0.76 | 0.83 (0.57–1.21) |
| | | | | | | 0.76–0.93 | 0.62 (0.28–1.38) |
| | | | | | | >0.93 | 0.46 (0.30–0.69) |
| Shrubsole et al (2011) | China, Shanghai Women’s Health Study | Prospective | 7 2816 (718) | 40–70 | Vitamin B₆ | Age at menarche, live births and age at first live birth, months of breast feeding, BMI, HBBD, FHBC, physical activity, passive smoking and total energy intake |

Vitamin B₆, B₁₂ and methionine, and breast cancer
| Study            | Country, study name          | Study design    | Subjects (cases) | Age (years) | Category | RR (95% CI)     | Adjustment for covariates                                                                 |
|------------------|-----------------------------|-----------------|------------------|-------------|-----------|-----------------|--------------------------------------------------------------------------------------------|
| Chou et al (2011)| China                       | Case-control (HCC) | 782 (391)        | 24–72       | Vitamin B₆ | 1.23 1          | Date of enrolment and fasting status, age at enrolment, age at menarche, age at first full-term pregnancy, parity, menopausal status, age at menopause, post-menopausal hormone use, FHBC, use of multi-vitamin supplements and total energy intake |
|                  |                             |                 |                  |             |           | 1.50 (0.98–1.61) |                                                              |
|                  |                             |                 |                  |             |           | 1.67 (1.01–1.33) |                                                              |
|                  |                             |                 |                  |             |           | 1.88 (1.02–1.36) |                                                              |
|                  |                             |                 |                  |             |           | 2.23 (1.05–1.46) |                                                              |
| Lurie et al (2012)| USA, The Multiethnic Cohort| Prospective (NCC) | 1412 (706)       |             | PLP       | 0.58 mg per day | Date of birth, study site, ethnicity, date of blood draw, hours fasting before blood draw and HRT use of blood draw, education, FHBC, BMI, age at menarche, parity, age at first parity use of contraceptive hormones, oophorectomy, hysterectomy, age at natural menopause, current smoking status, ethanol, hours of daily moderate/vigorous physical activity |
|                  |                             |                 |                  |             |           | 0.78 (0.64–2.52) |                                                              |
|                  |                             |                 |                  |             |           | 0.70 (0.26–0.92) |                                                              |
| Yang et al (2013)| USA, The 4-Corners Breast   | Case-control (PCC) | 4850 (2325)      | 25–79       | Vitamin B₆ | 2.29 (mg per day) | Age, centre, vitamin B₁₂, vitamin B₆, vitamin B₉, folate, methionine (mutual adjustment), ethnicity, education, BMI, total MET hours per week, total energy intake per day, total daily fibre intake, cigarette status, alcohol, parity, FHBC, oral contraceptive use and menopausal status |
|                  | Cancer Study                |                 |                  |             |           | 2.93 (1.08–7.71) |                                                              |
|                  |                             |                 |                  |             |           | 3.67 (1.11–11.54)|                                                              |
|                  |                             |                 |                  |             |           | 4.90 (0.75–15.56)|                                                              |
| Bassett et al (2013)| Australia, The MCCS Study  | Prospective     | 20,756 (936)     | 27–80       | Vitamin B₆ | 1.25 (mg per day) | Ethnicity, menopausal status, age at menarche, parity and lactation, oral contraceptive use, HRT use, physical activity, alcohol, smoking status, education level and BMI |
|                  |                             |                 |                  |             |           | 1.56 (0.61–3.97) |                                                              |
|                  |                             |                 |                  |             |           | 1.88 (0.84–4.10) |                                                              |
|                  |                             |                 |                  |             |           | 2.60 (0.86–8.12)|                                                              |

Abbreviations: BMI = body mass index; CI = confidence interval; COMT = Catechol-O-methyltransferase; FHBC = family history of breast cancer; HBBD = history of benign breast disease; HCC = hospital-based case-control study; HRT = hormone replacement treatment; MET = metabolic equivalent; NCC = nested case-control study; PCC = population-based case-control study; PLP = pyridoxal 5’-phosphate; Q = quintile; RR = relative risk.

*The two studies were excluded in the final analysis, because RR was not available; the mean concentration of PLP was presented by COMT genotype (HH/HL/LL).
| Study | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates |
|-------|---------------------|--------------|-----------------|-------------|-----------|-------------|----------------------------|
| Wu et al (1999a); the 1974 cohort | USA (MD) serum bank | Prospective (NCC) | 266 (133) | 18–90 | SVB_{12} | | Age, menopausal status and year of blood donation |
| | | | | | | Q1 | 1 |
| | | | | | Q2 | 1.04 (0.47–2.33) |
| | | | | | Q3 | 1.06 (0.45–2.56) |
| | | | | | Q4 | 0.88 (0.38–2.04) |
| | | | | | Q5 | 0.39 (0.17–0.90) |
| Wu et al (1999a); the 1989 cohort | USA (MD) serum bank | Prospective (NCC) | 220 (110) | 18–90 | SVB_{12} | | Age, menopausal status and year of blood donation |
| | | | | | | Q1 | 1 |
| | | | | | | Q2 | 0.64 (0.25–1.64) |
| | | | | | | Q3 | 0.95 (0.38–2.38) |
| | | | | | | Q4 | 0.65 (0.27–1.56) |
| | | | | | | Q5 | 0.48 (0.20–1.15) |
| Goodman et al (2001)* | USA | Prospective (NCC) | 225 (112) | cases: SVB_{12} (pg ml^{-1}) | | None |
| | | | | | | 60.4 | COMT^{11} |
| | | | | | | Control: 60.2 | Cases: 450.97 |
| | | | | | | Controls: 500.56 |
| | | | | | | COMT^{11} |
| | | | | | | Cases: 428.64, Controls: 438.58 |
| | | | | | | COMT^{11} |
| | | | | | | Cases: 407.92, Controls: 486.07 |
| Shrubsole et al (2001) | China, The Shanghai Breast Cancer Study | Case-control (PCC) | (1321) | 25–64 | Vitamin B_{12} | | Total energy, age, education, FHBC, personal history of fibroadenoma, age at menarche, parity, age at first live birth, menopausal status, age at menopause, physical activity, waist:hip ratio, total fruit and vegetable intake, and total animal food intake. |
| | | | | | | Q1 | 1 |
| | | | | | | Q2 | 1.11 (0.85–1.44) |
| | | | | | | Q3 | 1.54 (0.87–1.64) |
| | | | | | | Q4 | 1.05 (0.50–1.36) |
| | | | | | | Q5 | 1.01 (0.77–1.33) |
| Zhang et al (2003) | USA, The Nurses' Health Study | Prospective (NCC) | 1424 (712) | 43–69 | SVB_{12} | | Age at menarche, parity, age at first birth, age at menopause, FHBC in mother or a sister; HBBC; alcohol intake, BMI and duration of post-menopausal hormone use |
| | | | | | | <320.5 (pg ml^{-1}) | 1 |
| | | | | | | 320.5–389.6 | 0.75 (0.51–1.10) |
| | | | | | | 389.8–469.4 | 0.98 (0.69–1.40) |
| | | | | | | 469.9–571.5 | 0.62 (0.42–0.89) |
| | | | | | | >572.7 | 0.76 (0.52–1.10) |
| Study          | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates                                                                 |
|---------------|---------------------|-------------|------------------|-------------|----------|-------------|-----------------------------------------------------------------------------------------|
| Lajous et al (2006a) | Mexico             | Case–control (PCC) | 1866 (475)       | 18–82       | Vitamin B₁₂ | 2.61 (0.99–6.59) | Age, socioeconomic status, FHBC, menopausal status, parity, BMI, total calorie intake, dietary fibre carbohydrate intake and polyunsaturated fat intake |
| Lajous et al (2006b) | France, the E3N cohort | Prospective | 62739 (1812)     | —           | Vitamin B₁₂ | 1.05 (0.90–1.20) | Unclear                                                                                   |
| Cho et al (2007)     | USA, The Nurses' Health Study II | Prospective | 90663 (1032)     | 26–46       | Vitamin B₁₂ | 1.09 (0.90–1.34) | Age at start of follow-up, calendar year of the current questionnaire cycle, smoking, height, parity and age at first birth, BMI, age at menarche, FHBC, HBBD, oral contraceptive use, alcohol, energy and animal fat |
| Lin et al (2008)      | USA, The Women's Health Study | Prospective (NCC) | 1696 (848)       | >45         | SVB₁₂     | 1.12 (0.80–1.56) | Age, ethnicity, menopausal status, fasting status, month and year of blood return, post-menopausal hormone use and trial randomisation date, randomised treatment assignment, BMI, FHBC in a first-degree relative, HBBD, smoking, physical activity, alcohol, age at menarche, age at menopause, parity and age at first birth |
| Ma et al (2009a)      | Brazilian           | Case–control (HCC) | 916 (458)        | 20–74       | Vitamin B₁₂ | 0.90 (0.64–1.25) | Smoking, alcohol, moderate physical activity in the preceding 5 years, and number of live births |
| Study                | Country, study name                      | Study design       | Subjects (cases) | Age (years) | Category     | RR (95% CI)     | Adjustments for covariates                                                                                                                                 |
|---------------------|-----------------------------------------|--------------------|------------------|-------------|--------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ma et al (2009b)    | Japan                                   | Case-control (HCC) | 776 (388)        | 20–74       | Vitamin B<sub>12</sub> | 7.4 (7.4–10.3) | 1.03 (0.66–1.63) 1.00 (0.50–1.24)                                                                                                                     |
|                     |                                         |                    |                  |             |              | 7.4–10.3       | 1.03 (0.66–1.63) |
|                     |                                         |                    |                  |             |              | >10.3          | 0.79 (0.50–1.24)  |
| Maruti et al (2009a,b) | USA, The VITAL cohort study              | Prospective        | 35023 (743)      | 50–76       | Vitamin B<sub>12</sub> | 2.71 (4.42)     | 0.99 (0.80–1.23)  |
|                     |                                         |                    |                  |             |              | 6.14           | 0.84 (0.66–1.06)  |
|                     |                                         |                    |                  |             |              | 9.33           | 0.91 (0.70–1.18)  |
| Stevens et al (2010)| USA, CPS-II Nutrition Cohort            | Prospective        | 70656 (3898)     | 54–74       | Vitamin B<sub>12</sub> | 1.94 (2.80)     | 0.94 (0.85–1.04)  |
|                     |                                         |                    |                  |             |              | 2.80–4.96      | 0.95 (0.86–1.06)  |
|                     |                                         |                    |                  |             |              | 4.96–9.07      | 0.96 (0.79–1.15)  |
| Shrubsole et al (2011)| China, Shanghai Women’s Health Study | Prospective        | 72816 (718)      | 40–70       | Vitamin B<sub>12</sub> | 1.00 (0.93–1.57) | 0.74 (0.49–1.09)  |
|                     |                                         |                    |                  |             |              | 0.93–1.57      | 0.74 (0.49–1.09)  |
|                     |                                         |                    |                  |             |              | 1.57–2.29      | 0.74 (0.50–1.11)  |
|                     |                                         |                    |                  |             |              | >2.29          | 0.83 (0.56–1.24)  |

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| Study                  | Country, study name | Study design    | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustments for covariates                                                                 |
|-----------------------|---------------------|-----------------|-----------------|-------------|-----------|-------------|------------------------------------------------------------------------------------------|
| Chou et al (2011)     | China               | Case-control (HCC) | 782 (391) | 24–72 | Vitamin B₁₂ | 5.28 (5.29–8.15) | Date of enrolment and fasting status, age at enrolment, age at menarche, age at first full-term pregnancy, parity, menopausal status, age at menopause, post-menopausal hormone use, use of multi-vitamin supplements and total energy intake |
|                       |                     |                 |                 |              |           | 0.89 (0.53–1.65) |                                                                                 |
|                       |                     |                 |                 |              |           | 0.83 (0.73–2.54) |                                                                                 |
| Yang et al (2013)     | USA, the 4-Corners Breast Cancer Study | Case-control (PCC) | 4850 (2325) | 25-79 | Vitamin B₁₂ | 5.32 (5.32–9.78) | Age, centre, vitamin B₂, vitamin B₆, vitamin B₁₂, folate, methionine (mutual adjustment), ethnicity, education, BMI, total MET hours per week, total energy intake per day, total daily fibre intake, cigarette status, alcohol, parity, FHBC, oral contraceptive use and menopausal status |
|                       |                     |                 |                 |              |           | 0.75 (0.60-0.93) |                                                                                 |
|                       |                     |                 |                 |              |           | 0.83 (0.62-1.11) |                                                                                 |
| Basset et al (2013)   | Australia, The MCCS study Prospective | | 20756 (936) | 27-80 | Vitamin B₁₂ | 1.66 (2.33) | Ethnicity, menopausal status, age at menarche, parity and lactation, oral contraceptive use, HRT use, physical activity, alcohol, smoking status, education level and BMI |
|                       |                     |                 |                 |              |           | 1.06 (3.04) |                                                                                 |
|                       |                     |                 |                 |              |           | 1.21 (4.61) |                                                                                 |

Abbreviations: BMI = body mass index; CI = confidence interval; COMT = Catechol-O-methyltransferase; FHBC = family history of breast cancer; HBBD = history of benign breast disease; HCC = hospital-based case–control study; HRT = hormone replacement treatment; MET = metabolic equivalent; NCC = nested case–control study; PCC = population-based case–control study; PLP = pyridoxal 5'-phosphate; Q = quintile; RR = relative risk.

*The study excluded in the final analysis, because RR was not available; the mean concentration of PLP was presented by COMT genotype (HH/HL/LL).
| Study                  | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates                                                                 |
|-----------------------|---------------------|--------------|------------------|-------------|-----------|-------------|------------------------------------------------------------------------------------------|
| Thorand et al (1998)  | German, The EURAMIC | Case-control (PCC) | 149 (43) | 38-80 | 1.73 vs 1.37 (g per day) | 1.29 (0.76-2.19) | Age, BMI, exogenous hormone use, age at menarche, nulliparity, smoking status, socioeconomic status |
| Shrubsole et al (2001)| China, The Shanghai Breast Cancer Study | Case-control (PCC) | 2703 (1321) | 25-64 | Q1 | 1.00 | Total energy, age, education, FHBC, personal history of fibroadenoma, age at menarche, parity, age at first live birth, menopausal status, age at menopause, physical activity, waist:hip ratio, total fruit and vegetable intake, and total animal food intake |
| Feigelson et al (2003)| USA, the CPS-II Nutrition Cohort | Prospective | 66561 (1303) | 50-74 | <0.64 (g per day) | 1.00 | Age, alcohol, dietary folate, multivitamin use, race, education, first-degree FHBC, history of breast lump, mammographic history, HRT use, parity and age at first birth, age at menopause, age at menarche, physical activity, BMI, adult weight gain and energy |
| Zhu et al (2003)      | USA                 | Case-control (PCC) | 609 (304) | > 20 | <0.54 (g per day) | 1.00 | Age, employment status, marital status, educational level, income, number of people household, religion, smoking, use of electric blanket/mattress pad, menopausal status, use of oestrogen, use of progesterone, HBBD, FHBC, weight, height, physical activity, number of pregnancies, number of miscarriages, age at menarche, age at first birth, on a diet to lose weight, having an infertility test, intake of vitamin B6, B12, B1 and C, and total energy intake per day |
| Cho et al (2007)      | USA, The Nurses’ Health Study II | Prospective | 90463 (1032) | 26-46 | 1.6 (g per day) | 1.00 | Age at start of follow-up, calendar year of the current questionnaire cycle, smoking, height, parity and age at first birth, BMI, age at menarche, FHBC, HBBD, oral contraceptive use, alcohol, energy and animal fat |
| Study | Country, study name | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates |
|-------|---------------------|-------------|-----------------|------------|----------|-------------|---------------------------|
| Kabat et al (2008) | Canada, CNBSS | Prospective | 89835 (2491) | 40–59 | <1.78 (g per day) | 1.00 | Age, BMI, years of education, menopausal status, FHBC, history of breast biopsy, age at menarche, parity, oral contraceptive use, HRT, intake of calories and alcohol |
| Xu et al (2008) | USA | Case-control (PCC) | 3064 (1508) | — | <0.65 (g per day) | 1.00 | Age and daily energy intake |
| Meruti et al (2009a,b) | USA, The VITAL cohort study | Prospective | 35023 (743) | 50–76 | 0.82 (g per day) | 1.00 | Age, race, FHBC, mammography within 2 years preceding baseline, history of breast biopsy, age at menarche, age at first birth, age at menopause, years of combined oestrogen and progestin post-menopausal hormone use, BMI, physical activity, alcohol intake in the past year and energy intake |
| Stevens et al (2010) | USA, CPS-II Nutrition Cohort | Prospective | 70656 (3898) | 54–74 | <0.64 (g per day) | 1.00 | Age, alcohol, multi-vitamin use, race, education, FHBC, history of breast lump, HRT, parity and age at first birth, age at menarche, age at menopause, physical activity, BMI and energy |
| Zhang et al (2011) | China | Case-control (HCC) | 876 (838) | 25–70 | <0.84 (g per day) | 1.00 | Age at menarche, live births and age at first live birth, months of breastfeeding, BMI, HBBD, FHBC, physical activity, passive smoking and total energy intake |
| Shrubsole et al (2011) | China, Shanghai Women’s Health Study | Prospective | 72816 (718) | 40–70 | 1.13 (g per day) | 1.00 | Age at baseline, age at menarche, parity, age at first live birth, educational attainment, physical activity, use of a B vitamin supplement, height, and total daily intakes of energy, vegetable and fat, and menopausal status |
of the following covariates: alcohol, smoking, BMI, FHBC, reproductive factors (≥3), physical activity, energy intake, dietary factors (≥2), and use of exogenous hormones. For the analysis between breast cancer risk and dietary vitamin B6 intake, study design was found contributing significantly to the between-study heterogeneity overall (P = 0.02) and among post-menopausal women (P = 0.02). No significant findings were found in the other analysis.

**Sensitivity analysis and publication bias.** Sensitivity analysis showed that no individual study had excessive influence on the above mentioned pooled effect. Egger test showed no evidence of significant publication bias for the analysis between breast cancer risk and serum PLP levels (P = 0.10), vitamin B6 intake (P = 0.14), serum vitamin B12 levels (P = 0.18), vitamin B12 intake (P = 0.12) and methionine (P = 0.49). The funnel plots were provided in the Supplementary Material.

**DISCUSSION**

The findings from this meta-analysis indicated that increased serum PLP levels and dietary methionine intake might be significantly associated with reduced risk of breast cancer, especially for post-menopausal women. No significant association was found between dietary vitamin B6 intake, serum vitamin B12, and dietary vitamin B12 intake and risk of breast cancer.

Several biological mechanisms for the inverse relationship of vitamin B6, vitamin B12 and methionine with the development of breast cancer have been proposed. First, vitamin B6, B12, and methionine participate in one-carbon metabolism, which is essential for DNA synthesis, repair and methylation (Ames, 2001), and vitamins B6 and B12 deficiencies also cause high uracil and chromosome breaks (Blount et al., 1997). Thus, deficiency in these nutrients may interfere with DNA methylation and synthesis, leading to aberrant gene expression and DNA instability, and eventually the development of cancer (Davis and Uthus, 2004). In addition, low B-group vitamin concentrations are associated with inflammation and higher oxidative stress (Shen et al., 2010), and antioxidants supplementation with B-group vitamins could enhance antioxidant capacity. Although the mechanism for the stronger association found for post-menopausal women remains unclear, previous meta-analysis also suggested that post-menopausal women are more susceptible to a wide range of dietary factors, including dietary fibre, vitamin A, β-carotene, retinol, vitamin C, calories, fat (Howe et al., 1990; Wu et al., 1999a,b), folate acid (Larsson et al., 2007), fatty acids (Saadatian-Elahi et al., 2004), and coffee and caffeine (Jiang et al., 2013), regarding their association with breast cancer risk. In addition, although these nutrient interactions were also proposed, no apparent joint association with high folate intake was found in this meta-analysis, which is consistent with the previous study (Zhang et al., 2008).

As a meta-analysis of published observational studies, our findings have several limitations. First, disparate results were found on dietary vitamin B6 intake and PLP with the risk of breast cancer. The multivariable Pearson’s correlation coefficients between plasma levels of vitamin B6 and vitamin B12, and the average intakes of vitamin B6 and vitamin B12 from food was 0.25 and 0.08, respectively (Zhang et al., 2003). In this respect, the serum biomarker of vitamin B6 (PLP) and vitamin B12 was able to examine these associations with higher precision. Disparate results between serum biomarkers of vitamins and dietary intake of vitamins and breast cancer risk were also found in other meta-analysis, such as vitamin B6 (Larsson et al., 2007) and vitamin D (Chen et al., 2010). Second, a meta-analysis of observational studies is susceptible to potential bias inherent in the original studies, especially for case–control studies. Stronger association was found

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**Table 3. (Continued)**

| Study    | Country, study name                  | Study design | Subjects (cases) | Age (years) | Category | RR (95% CI) | Adjustment for covariates                                                                 |
|----------|-------------------------------------|--------------|------------------|-------------|-----------|-------------|-----------------------------------------------------------------------------------------|
| Yang et al (2013) | USA, the 4-Corners Breast Cancer Study | Case control (PCC) | 4850 (2325) | 25–79      | < 56 (g per day) | 1.05 (0.86–1.26) | Age, centre, vitamin B6, vitamin B12, vitamin B12, fat intake, BMI, energy intake per day, total daily fiber intake, cigarette status, alcohol, parity, FHBC, oral contraceptive use and menopausal status. |
| Bassett et al (2013) | Australia, The MCCS study | Prospective | 20 756 (936) | 27–80 | 0.98 (0.82–1.17) | 1.39 (1.04–1.84) | Ethnicity, menopausal status, age at menarche, parity and lactation, oral contraceptive use, HRT use, physical activity, alcohol, smoking status, education level and BMI. |
### Table 4. Summary risk estimates of the association between vitamin B₆, vitamin B₁₂ and methionine and breast cancer risk

| Nutrients | Study | No. (cases) | No. (studies) | REM     | P-value | $I^2$ (%) | P-value |
|-----------|-------|-------------|---------------|---------|---------|----------|---------|
| Vitamin B₆ | PLP*  | 2509        | 5             | 0.80 (0.66–0.98) | 0.03    | 0.30     | 0.40    |
| **Menopausal status** | | | | | | | |
| | Pre-menopausal | 622 | 4 | 1.16 (0.67–2.02) | 0.59 | 16.0 | 0.31 |
| | Post-menopausal | 1871 | 5 | 0.71 (0.57–0.88) | 0.00 | 0.00 | 0.49 |
| **ER and PR status (positive: +, negative: –)** | | | | | | | |
| | ER + | 1577 | 3 | 0.69 (0.47–1.02) | 0.06 | 61.3 | 0.08 |
| | ER – | 348 | 3 | 0.90 (0.50–1.62) | 0.73 | 0.00 | 0.89 |
| | PR + | 1414 | 3 | 0.70 (0.48–1.01) | 0.06 | 53.6 | 0.12 |
| | PR – | 482 | 3 | 0.87 (0.55–1.39) | 0.56 | 0.00 | 0.89 |
| | Dietary intake | 14260 | 14 | 0.95 (0.83–1.08) | 0.45 | 56.2 | 0.01 |
| **Study design** | | | | | | | |
| | Prospective | 8175 | 6 | 1.03 (0.94–1.14) | 0.55 | 0.00 | 0.54 |
| | Case–control | 6085 | 8 | 0.85 (0.65–1.12) | 0.25 | 71.1 | 0.00 |
| **Geographic region where the study was conducted** | | | | | | | |
| | America | 9779 | 7 | 1.02 (0.92–1.14) | 0.69 | 0.00 | 0.49 |
| | Asia | 3256 | 5 | 0.84 (0.56–1.27) | 0.41 | 78.3 | 0.00 |
| **ER and PR status (positive: +, negative: –)** | | | | | | | |
| | ER + | 2684 | 6 | 1.02 (0.88–1.19) | 0.80 | 0.00 | 0.48 |
| | ER – | 1089 | 6 | 0.91 (0.71–1.18) | 0.49 | 25.2 | 0.25 |
| | PR + | 1493 | 4 | 1.06 (0.89–1.23) | 0.56 | 0.00 | 0.76 |
| | PR – | 579 | 5 | 0.83 (0.64–1.08) | 0.16 | 2.30 | 0.39 |
| | Vitamin B₁₂ | 1803 | 4 | 0.73 (0.44–1.22) | 0.23 | 72.5 | 0.01 |
| **Menopausal status** | | | | | | | |
| | Pre-menopausal | 622 | 4 | 0.78 (0.34–1.81) | 0.57 | 60.2 | 0.06 |
| | Post-menopausal | 1165 | 4 | 0.79 (0.47–1.31) | 0.35 | 59.0 | 0.06 |
| | Dietary intake | 15783 | 14 | 0.88 (0.77–1.00) | 0.05 | 68.9 | 0.00 |
| **Study design** | | | | | | | |
| | Prospective | 9987 | 7 | 1.01 (0.93–1.10) | 0.83 | 10.3 | 0.35 |
| | Case–control | 5796 | 7 | 0.74 (0.56–0.98) | 0.04 | 74.5 | 0.00 |
| **Geographic region where the study was conducted** | | | | | | | |
| | America | 9779 | 7 | 0.79 (0.63–1.00) | 0.05 | 78.4 | 0.00 |
| | Asia | 3256 | 5 | 0.89 (0.75–1.04) | 0.14 | 0.00 | 0.83 |
| **ER and PR status (positive: +, negative: –)** | | | | | | | |
| | ER + | 2416 | 5 | 0.83 (0.62–1.12) | 0.22 | 64.0 | 0.03 |
| | ER – | 986 | 5 | 1.02 (0.81–1.29) | 0.85 | 0.00 | 0.54 |
| | PR + | 1198 | 3 | 0.96 (0.70–1.33) | 0.25 | 58.2 | 0.09 |
| | PR – | 503 | 4 | 0.95 (0.71–1.26) | 0.38 | 4.50 | 0.37 |
| | Methionine | 17060 | 13 | 0.94 (0.89–0.99) | 0.03 | 0.00 | 0.52 |
Table 4. (Continued)

| Nutrients | Study | No. (cases) | No. (studies) | REM | P-value | $I^2$ (%) | P-value |
|-----------|-------|-------------|---------------|-----|---------|-----------|---------|
| Risk estimate (95% CI) | Heterogeneity test |

Study design

- Prospective: 11121, 7, 0.94 (0.88–1.00), 0.06, 0.00, 0.45
- Case-control: 5939, 6, 0.92 (0.80–1.06), 0.26, 6.00, 0.38

Menopausal status

- Pre-menopausal: 1377, 2, 1.06 (0.87–1.30), 0.56, 0.00, 0.34
- Post-menopausal: 6060, 5, 0.89 (0.82–0.97), 0.01, 0.00, 0.62

Geographic region where the study was conducted

- America: 13604, 8, 0.94 (0.88–1.01), 0.10, 14.0, 0.32
- Asia: 2477, 3, 0.84 (0.68–1.04), 0.11, 0.00, 0.79

ER and PR status (positive: +, negative: –)

- ER –: 900, 4, 1.12 (0.90–1.40), 0.32, 0.00, 0.56
- ER +: 2150, 3, 0.88 (0.76–1.02), 0.10, 0.00, 0.74
- PR +: 1000, 2, 0.93 (0.76–1.13), 0.45, 0.00, 0.76
- PR –: 349, 2, 1.13 (0.79–1.62), 0.50, 0.00, 0.78

Folate + Vitamin B6

- 5461, 4, 0.91 (0.79–1.04), 0.17, 0.00, 0.41

Folate + Vitamin B12

- 5275, 3, 0.99 (0.77–1.29), 0.97, 54.2, 0.11

Folate + Methionine

- 5318, 4, 0.81 (0.62–1.05), 0.11, 49.2, 0.12

Abbreviations: CI = confidence interval; ER = oestrogen receptor; PLP = pyridoxal 5’-phosphate; PR = progesterone receptor; REM = random effect model.

All studies are prospective design.

**Figure 1.** The multivariate-adjusted risk of breast cancer for the highest vs lowest categories of serum PLP levels and dietary vitamin B6 intake in random-effects model. The size of the grey box is positively proportional to the weight assigned to each study, which is inversely proportional to the s.e. of the RR, and horizontal lines represent the 95% CIs.
in the combined results from case–control studies in this meta-
analysis. Overstated association could be expected from the case–
control studies because of recall or selection bias, and early
symptoms in patients may have resulted in a change in dietary
habits. Thus, the results from prospective studies might provide a
more robust estimation of the associations.

Third, although we extracted the RRs that reflected the greatest
degree of control for potential confounders, the extent to which
they were adjusted and the possibility that the observed association
was due to unmeasured or residual confounding should be
considered. Furthermore, vitamin B6 intake tends to be associated
with healthy behaviours that may be protective against breast
cancer (Larsson et al, 2010). However, significant association
was also found (0.76 (0.62–0.94)) on serum PLP levels and breast
cancer risk for the three studies that adjusted for the most known
risk factors of breast cancer. In addition, no significant interactions
were found between MTHFR and MTR polymorphisms and B
vitamins (Ma et al, 2009a; Ma et al, 2009b), and result from the
study by Lin et al (2008) suggested a much stronger and significant
association of serum PLP and vitamin B12 levels with breast cancer
risk for never user of post-menopausal hormones. However, the
limited data in the reported articles precluded a more robust
assessment of the association by the above-mentioned risk factors.

Fourth, although significant association of PLP levels and
methionine intake with risk of breast cancer was found among
post-menopausal women, while not among pre-menopausal
women, the difference between the two groups was not significant,
and the apparent differences could simply be by chance,
considering relatively small number of studies included, especially
for pre-menopausal women. And subgroup analysis by tumour
stage (in situ or invasive) were not conducted because of limited
data availability (Zhang et al, 2003; Chou et al, 2011). Fifth,
between-study heterogeneity was found in some analysis in this
meta-analysis, but the between-study heterogeneity was not
successfully explained by the subgroup analysis and meta-
regression. However, other genetic and environment variables, as
well as their possible interaction, non-comparable measurement of
nutrients and variation of the covariates and so on, may well be

Figure 2. The dose–response analysis between serum PLP and breast
cancer risk, with restricted cubic splines in a multivariate random-
effects dose–response model. The solid line and the long dash line
represent the estimated RR and its 95% CI. Short, dash line represents
the linear relationship.

| Author      | Year | RR (95% CI)   |
|-------------|------|---------------|
| Serum vitamin B12 |
| Wu          | 1999a| 0.39 (0.17, 0.90) |
| Wu          | 1999b| 0.48 (0.20, 1.15) |
| Zhang       | 2003 | 0.76 (0.52, 1.10) |
| Lin         | 2008 | 1.29 (0.92, 1.82) |
| Dietary vitamin B12 |
| Shrubsole   | 2001 | 1.01 (0.77, 1.32) |
| Lajous      | 2006a| 0.32 (0.22, 0.49) |
| Lajous      | 2006b| 1.05 (0.90, 1.20) |
| Cho         | 2007 | 0.96 (0.78, 1.12) |
| Lin         | 2008 | 0.88 (0.54, 1.44) |
| Ma          | 2009a| 0.79 (0.50, 1.24) |
| Ma          | 2009b| 0.90 (0.65, 1.26) |
| Manuti      | 2009a,b| 0.91 (0.70, 1.18) |
| Stevens     | 2010 | 0.98 (0.80, 1.19) |
| Chou        | 2011 | 0.83 (0.73, 2.54) |
| Shrubsole   | 2011 | 0.83 (0.61, 1.12) |
| Zhang       | 2011 | 0.83 (0.56, 1.24) |
| Yang        | 2013 | 0.73 (0.53, 1.00) |
| Bassett     | 2013 | 1.21 (1.00, 1.46) |
| Subtotal    |      | 0.88 (0.77, 1.00) |

Figure 3. The multivariate-adjusted risk of breast cancer for the highest vs lowest categories of serum vitamin B12 levels and dietary vitamin B12 intake in random-effects model. The size of the grey box is positively proportional to the weight assigned to each study, which is inversely proportional to the s.e. of the RR, and horizontal lines represent the 95% CIs.
potential contributors to this disease–effect unconformity (Higgins et al, 2003). In this respect, the lack of relevant study-level covariates in the reported articles precluded a more robust assessment of sources of this heterogeneity. Finally, although no significant publication bias was detected in this meta-analysis, validity of publication bias test should be questioned because of small number of studies included (Sterne et al, 2000), especially for PLP and serum vitamin B12.

In summary, results from this meta-analysis suggested that serum PLP levels and dietary methionine intake might be significantly associated with reduced risk of breast cancer, especially for post-menopausal women. The finding needs to be confirmed further by a well-conducted randomised trial.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ames BN (2001) DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 475: 7–20.

Bairoch A (2000) The ENZYME database in 2000. Nucleic Acids Res 28: 304–305.

Bassett JK, Baglietto L, Hodge AM, Severi G, Hopper JL, English DR, Giles GG (2013) Dietary intake of B vitamins and methionine and breast cancer risk. Cancer Causes Control 24(8): 1555–1563.

Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN (1997) Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA 94: 3290–3295.

Chen P, Hu P, Xie D, Qin Y, Wang F, Wang H (2010) Meta-analysis of vitamin D, calcium and the prevention of breast cancer. Breast Cancer Res Treat 121: 469–477.

Cho E, Holmes M, Hankinson SE, Willett WC (2007) Nutrients involved in one-carbon metabolism and risk of breast cancer among premenopausal women. Cancer Epidemiol Biomarkers Prev 16: 2787–2790.

Chou YC, Chu CH, Wu MH, Hsu GC, Yang T, Chou WY, Huang HP, Lee MS, Yu CP, Yu JC, Sun CA (2011) Dietary intake of vitamin B(6) and risk of breast cancer in Taiwanese women. J Epidemiol 21: 329–336.

Davis CD, Uthus EO (2004) DNA methylation, cancer susceptibility, and nutrient interactions. Exp Biol Med (Maywood) 229: 988–995.

DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7: 177–188.

Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634.

Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE (2003) Alcohol, folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. Cancer Epidemiol Biomarkers Prev 12: 161–164.

Goodman JE, Lavigne JA, Wu K, Helzlsouer KJ, Strickland PT, Selhub J, Yager JD (2001) COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. Carcinogenesis 22: 1661–1665.

Harrel Jr FE, Lee KL, Pollock BG (1988) Regression models in clinical studies: determining relationships between predictors and response. J Natl Cancer Inst 80: 1198–1202.

Higgins JP, Thompson SG (2004) Controlling the risk of spurious findings from meta-regression. Stat Med 23: 1663–1682.

Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557–560.

Hong Z, Tian C, Zhang X (2012) Dietary calcium intake, vitamin D levels, and breast cancer risk: a dose-response analysis of observational studies. Breast Cancer Res Treat 136: 309–312.

Howe GR, Hirohata T, Hislop TG, Iscovich JM, Yuan JM, Katsouyanni K, Lubin F, Marubini E, Modan B, Rohan TE et al (1990) Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 82: 561–569.
Jackson D, White IR, Thompson SG (2010) Extending DerSimonian and Laird’s methodology to perform multivariate random effects meta-analyses. Stat Med 29: 1282–1297.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90.

Jiang W, Wu Y, Jiang X (2013) Coffee and caffeine intake and breast cancer risk: an updated dose-response meta-analysis of 37 published studies. Gynecol Oncol 129(3): 620–629.

Kabat GC, Miller AB, Jain M, Rohan TE (2008) Dietary intake of selected B vitamins in relation to risk of major cancers in women. Br J Cancer 99: 816–821.

Kim YI (2007) Folate and colorectal cancer: an evidence-based critical review. Mol Nutr Food Res 51: 267–292.

Kushi LH, Doyle C, McCullough M, Rock CL, Demark-Wahnefried W, Bandera EV, Gaziano J, Patel AV, Andrews K, Gansler T (2012) American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin 62: 30–67.

Lajous M, Lazo-Ponce E, Hernandez-Avila M, Willett W, Romieu I (2006a) Folate, vitamin B(6), and vitamin B(12) intake and the risk of breast cancer among Mexican women. Cancer Epidemiol Biomarkers Prev 15: 443–448.

Lajous M, Romieu I, Sabia S, Bouteron-Ruault MC, Clavel-Chapelon F (2006b) Folate, vitamin B12 and postmenopausal breast cancer in a prospective study of French women. Cancer Causes Control 17: 1209–1213.

Larsson SC, Giovannucci E, Wolk A (2007) Folate and risk of breast cancer: a meta-analysis. J Natl Cancer Inst 99: 64–76.

Larsson SC, Orsini N, Wolk A (2010) Vitamin B6 and risk of colorectal cancer: a meta-analysis of prospective studies. JAMA 303: 1077–1083.

Levi F, Pasche C, Lucchini F, La Vecchia C (2001) Dietary intake of selected micronutrients and breast-cancer risk. Int J Cancer 91: 260–263.

Lin J, Lee IM, Cook NR, Selhub J, Manson JE, Buring JE, Zhang SM (2008) Plasma folate, vitamin B-6, vitamin B-12, and risk of breast cancer in women. Am J Clin Nutr 87: 734–743.

Lurie G, Wilkens LR, Shvetsov YB, Olbberding NJ, Franke AA, Henderson BE, Kolonel LN, Goodman MT (2012) Prediagnostic plasma pyridoxal 5'-phosphate (vitamin B6) levels and invasive breast carcinoma risk: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 21: 1942–1948.

Ma E, Iwasaki M, Junko I, Hamada GS, Nishimoto IN, Carvalho SM, Motola Jr J, Laginha FM, Tsugane S (2009a) Dietary intake of folate, vitamin B6, and vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Brazilian women. BMC Cancer 9: 122.

Ma E, Iwasaki M, Kobayashi M, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S (2009b) Dietary intake of folate, vitamin B2, vitamin B6, vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Japan. Nutr Cancer 61: 447–456.

Marutti SS, Ulrich CM, Jupe ER, White E (2009a) MTHFR C677T and postmenopausal breast cancer risk by intakes of one-carbon metabolism nutrients: a nested case-control study. Breast Cancer Res 11: R91.

Marutti SS, Ulrich CM, White E (2009b) Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. Am J Clin Nutr 89: 624–633.

Orsini N, Bellocco R (2006) Generalized least squares for trend estimation of nutrients: a nested case-control study. Stat Med 25: 40–57.

Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D (2012) Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. Am J Epidemiol 175: 66–73.

Potera C, Rose DP, Brown RR (1977) Vitamin B6 deficiency in cancer patients. Am J Clin Nutr 30: 1677–1679.

Saadatian-Elihi M, Norat T, Goudable J, Riboli E (2004) Biomarkers of dietary fatty acid intake and the risk of breast cancer: a meta-analysis. Int J Cancer 111: 584–591.

Schroocksadkel K, Frick B, Fuchs D (2003) Re: Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 95: 1091 author reply 1091.

Sellhub J (2002) Folate, vitamin B12 and vitamin B6 and one carbon metabolism. J Nutr Health Aging 6: 39–42.

Shen J, Lai CQ, Mattei J, Or dovas JM, Tucker KL (2010) Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. Am J Clin Nutr 91: 337–342.

Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Jin F, Zheng W (2006) MTR and MTRR polymorphisms, dietary intake, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 15: 586–588.

Shrubsole MJ, Jin F, Dai Q, Shu XO, Potter JD, Hebert JR, Gao YT, Zheng W (2001) Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Res 61: 7136–7141.

Shrubsole MJ, Shu XO, Li HL, Cai H, Yang G, Gao YT, Gao J, Zheng W (2011) Dietary vitamin and methionine intakes and breast cancer risk among Chinese women. Am J Epidemiol 173: 1171–1182.

Sterne JA, Gavaghan D, Egger M (2000) Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. J Clin Epidemiol 53: 1119–1129.

Stevens VL, McCullough ML, Sun J, Gaziano JM, Selhub J (2010) Folate and other one-carbon metabolism-related nutrients and risk of postmenopausal breast cancer in the Cancer Prevention Study II Nutrition Cohort. Am J Epidemiol 170: 1708–1715.

Suzuki R, Orsini N, Mignone L, Saji S, Wolk A (2008) Alcohol intake and risk of breast cancer defined by estrogen and progesterone receptor status—a meta-analysis of epidemiological studies. Int J Cancer 122: 1832–1841.

Thorand B, Kohlmeier L, Simonssen N, Croghan C, Thamm M (1998) Intake of fruits, vegetables, folate and related nutrients and risk of breast cancer in postmenopausal women. Public Health Nutr 1: 147–156.

Wu AH, Pike MC, Stram DO (1999a) Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. J Natl Cancer Inst 91: 529–534.

Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J (1999b) A prospective study on folate, B12, and pyridoxal 5'-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev 8: 209–217.

Wu Y, Zhang D, Kang S (2013) Physical activity and risk of breast cancer: a meta-analysis of prospective studies. Breast Cancer Res Treat 137: 869–882.

Xu X, Gammon MD, Zeisel SH, Lee YL, Wmettur JG, Teitelbaum SL, Bradshaw PT, Neugut AI, Santella RM, Chen J (2008) Choline metabolism and risk of breast cancer in a population-based study. FASEB J 22: 2045–2052.

Yang D, Baumgartner RN, Slattery ML, Wang C, Giuliani AR, Murtaugh MA, Risendal BC, Byers T, Baumgartner KB (2013) Dietary intake of folate, B-vitamins and methionine and breast cancer risk among Hispanic and non-Hispanic white women. PLoS One 8: e54495.

Zhang CX, Ho SC, Chen YM, Lin FY, Fu JH, Cheng SZ (2011) Dietary folate, vitamin B6, vitamin B12 and methionine intake and the risk of breast cancer by oestrogen and progesterone receptor status. Br J Nutr 106: 936–943.

Zhang SM, Cook NR, Albert CM, Gaziano JM, Buring JE, Manson JE (2008) Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. JAMA 300: 2012–2021.

Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA, Hankinson SE (2003) Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 95: 373–380.

Zhu K, Davidson NE, Hunter S, Yang X, Payne-Wilks K, Roland CL, Phillips D, Bentley C, Dai M, Williams SM (2003) Methyl-group dietary intake and risk of breast cancer among African-American women: a case-control study by methylation status of the estrogen receptor alpha genes. Cancer Causes Control 14: 827–836.

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