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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_6 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% ($P<0.00$) 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

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(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 B_6, vitamin B_{12} and methionine; (2) then evaluate the possible dose–response relationship of vitamin B_6, vitamin B_{12} and methionine with breast cancer risk; (3) investigate the joint association between folate intake with vitamin B_6, vitamin B_{12} and methionine intake, and the risk of breast cancer; (4) evaluate the modification of key covariates to the association of vitamin B_6, vitamin B_{12} 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 B_6 or pyridoxal 5'-phosphate (PLP; the active form of vitamin B_6), or vitamin B_{12} 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 B_6 or PLP, or vitamin B_{12} or methionine; (2) the outcome of interest was breast cancer; (3) relative risk (RR) or odds ratio with 95% confidence interval (CI) was provided (we presented all results with RR for simplicity); (4) for dose–response analysis, the number of cases and participants or person-years for each category of vitamin B_6 or PLP, or vitamin B_{12} 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 B_6, vitamin B_{12} 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 B_6, vitamin B_{12} and methionine intake, and risk of breast cancer, RR was extracted from the group with both the highest folate intake and highest vitamin B_6, vitamin B_{12} or methionine intake group versus the group with both the lowest folate intake and lowest vitamin B_6, vitamin B_{12} or methionine intake.

For dose–response analysis, the number of cases and participants (person-years), and RR (95% CI) for each category of vitamin B_6, vitamin B_{12} and methionine were also extracted. The median or mean level of vitamin B_6, vitamin B_{12} 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 RRs 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 B_6, vitamin B_{12} 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 I^2 was used to assess heterogeneity, and I^2 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).

For dose–response analysis, a two-stage random-effects dose–response meta-analysis (Orsini *et al*, 2012) was performed to compute the trend from the correlated log RR estimates across levels of vitamin B_6, vitamin B_{12} and methionine, respectively, taking into account the between-study heterogeneity. In the first stage, a restricted cubic spline model with three knots at the 25th, 50th and 75th percentiles (Harrell *et al*, 1988) of the levels of vitamin B_6, vitamin B_{12} and methionine was estimated using generalised least-square regression, taking into account the correlation within each set of published RRs (Orsini and Bellocco, 2006). Then the study-specific estimates were combined using the restricted maximum likelihood method in a multivariate random-effects meta-analysis (Jackson *et al*, 2010). A *P*-value for non-linearity was calculated by testing the null hypothesis that the coefficient of the second spline is equal to 0. All statistical analyses were performed with STATA version 12.0 (Stata Corporation, College Station, TX, USA). All reported probabilities (*P*-values) were two-sided with $P<0.05$ considered statistically significant.

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*, 1977; Goodman *et al*, 2001; Schroecksnadel *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; Xu *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 B_6 intake, data from 14 articles (6 prospective studies and 8 case–control studies) were used, including 14 260 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 B_{12} 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 B_{12} intake, data from 14 articles (7 prospective studies and 7 case–control studies) were used, including 15 783 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), $P=0.03$, $I^2=0.30\%$, 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 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, reproductive factors ($\geqslant 3$), 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 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-μg 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 ($\geqslant 3$).

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 10316 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 (Maruti *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 (Maruti *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 vitamin B_{12} was 0.99 (0.77–1.29), $P=0.97$, $I^2=54.2\%$. For the joint association between breast cancer risk and folate intake with methionine intake, data from four studies (Thorand *et al*, 1998; Maruti *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)

Table 1. Characteristics of studies on serum PLP and vitamin B_6 intake and breast cancer risk

Study	Country, study name	Study design	Subjects (cases)	Age (years)	Category	RR (95% CI)	Adjustment for covariates
Potera *et al* (1977)[a]	USA	Case–control	130 (94)		PLP (ng ml^{-1})		None
				56	Control: 7.20		
				52	Early breast cancer: 5.71		
				56	Local recurrence: 4.43		
				53	Systemic metastases: 3.95		
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
					Q1	1	
					Q2	1.89 (0.88–4.17)	
					Q3	1.56 (0.71–3.45)	
					Q4	1.41 (0.70–2.86)	
					Q5	1.09 (0.49–2.44)	
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
					Q1	1	
					Q2	1.19 (0.50–2.86)	
					Q3	0.82 (0.37–1.82)	
					Q4	1.28 (0.53–3.03)	
					Q5	1.56 (0.59–4.00)	
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
				Control: 59	1.4 (mg per day)	1	
					1.9	0.58 (0.35–0.95)	
					2.6	0.54 (0.30–0.96)	
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 vegetableintake, and total animal food intake
					Q1	1	
					Q2	1.26 (0.97–1.65)	
					Q3	1.22 (0.92–1.63)	
					Q4	1.35 (0.99–1.84)	
					Q5	1.46 (1.01–2.13)	
Goodman *et al* (2001)[a]	USA	Prospective (NCC)	225 (112)	Cases: 60.4	PLP (pmol ml^{-1})[a]		None
				Control: 60.2	$COMT^{HH}$		
					Cases: 67.52, Controls: 38.72		
					$COMT^{HL}$ Cases: 42.24, Controls: 51.29		
					$COMT^{LL}$ Cases: 44.55, Controls: 40.99		

Table 1. (Continued)

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		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(pmol ml^{-1})	1	
					28.5–40.2	0.86 (0.60–1.22)	
					40.2–57.0	0.80 (0.56–1.14)	
					57.1–94.1	0.79 (0.55–1.14)	
					>95.3	0.70 (0.48–1.02)	
Lajous *et al* (2006a,b)	Mexico	Case–control (PCC)	1866 (475)	18–82	Vitamin B_6		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	
					1.26	0.67 (0.49–0.92)	
					1.40	0.76 (0.55–1.05)	
					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 B_6		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	
					1.9	1.16 (0.95–1.41)	
					2.1	1.09 (0.89–1.33)	
					2.3	0.96 (0.78–1.18)	
					2.7	1.18 (0.96–1.44)	
Lin *et al* (2008)	USA, The Women's Health Study	Prospective (NCC)	1696 (848)	>45	PLP:		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^{-1})	1	
					38.9–52.2	1.02 (0.73–1.43)	
					52.2–66.3	0.91 (0.65–1.29)	
					66.3–102.2	0.92 (0.65–1.32)	
					>102.2	0.91 (0.63–1.30)	
					Vitamin B_6:		
					⩽1.7 (mg per day)	1	
					1.7–1.9	1.16 (0.70–1.95)	
					1.9–2.1	1.00 (0.61–1.63)	
					2.1–2.3	1.02 (0.60–1.71)	
					>2.3	1.02 (0.61–1.69)	

Table 1. (Continued)

Study	Country, study name	Study design	Subjects (cases)	Age (years)	Category	RR (95% CI)	Adjustment for covariates
Ma *et al* (2009a)	Brazilian	Case–control (HCC)	916 (458)	20–74	Vitamin B_6		Smoking, alcohol, moderate physical activity in the preceding 5 years, and number of live births
					<0.6 (mg per day)	1	
					0.6–1.0	1.04 (0.74–1.46)	
					⩾1.0	1.18 (0.84–1.05)	
Ma *et al* (2009b)	Japan	Case–control (HCC)	776 (388)	20–74	Vitamin B_6		BMI, education, smoking, alcohol, age at menarche, age at first live birth in parous women, menstruation status, breast feeding, number of live births and moderate physical activity in the preceding 5 years
					<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	35023 (743)	50–76	Vitamin B_6		Age, race, FHBC, mammography within 2 y 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
					0.96(mg per day)	1	
					1.39	1.04 (0.83–1.30)	
					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	70656 (3898)	54–74	Vitamin B_6		Age, alcohol, multivitamin 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.
					<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 B_6		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
					<0.6 (mg per day)	1	
					0.6–0.76	0.83 (0.57–1.21)	
					0.76–0.93	0.42 (0.28–0.64)	
					>0.93	0.46 (0.30–0.69)	
Shrubsole *et al* (2011)	China, Shanghai Women's Health Study	Prospective	72816 (718)	40–70	Vitamin B_6		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

Table 1. (Continued)

Study	Country, study name	Study design	Subjects (cases)	Age (years)	Category	RR (95% CI)	Adjustment for covariates
					1.23	1	
					1.50	1.26 (0.98–1.61)	
					1.67	1.01 (0.77–1.33)	
					1.88	1.02 (0.76–1.36)	
					2.23	1.05 (0.76–1.46)	
Chou *et al* (2011)	China	Case-control (HCC)	782 (391)	24–72	Vitamin B_6		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
					⩽0.58 (mg per day)	1	
					0.58–0.70	0.78 (0.64–2.52)	
					>0.70	0.64 (0.26–0.92)	
Lurie *et al* (2012)	USA, The Multiethnic Cohort	Prospective (NCC)	1412 (706)		PLP		Date of birth, study site, ethnicity, date of blood draw, hours fasting before blood draw and HRT use at 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
					⩽41.1 (nmol l^{-1})	1	
					41.2–66.0	0.91 (0.66–1.25)	
					66.1–116.6	0.77 (0.55–1.08)	
					>116.6	0.70 (0.50–0.98)	
Yang *et al* (2013)	USA, The 4-Corners Breast Cancer Study	Case–control (PCC)	4850 (2325)	25–79	Vitamin B_6		Age, centre, vitamin B_2, vitamin B_6, vitamin B_{12}, 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
					⩽2.29 (mg per day)	1	
					2.29–3.87	1.10 (0.87–1.40)	
					3.87–4.90	1.11 (0.81–1.54)	
					>4.90	1.09 (0.75–1.58)	
Bassett *et al* (2013)	Australia, The MCCS Study	Prospective	20756 (936)	27–80	Vitamin B_6		Ethnicity, menopausal status, age at menarche, parity and lactation, oral contraceptive use, HRT use, physical activity, alcohol, smoking status, education level and BMI
					1.25 (mg per day)	1	
					1.56	0.81 (0.67–0.97)	
					1.88	0.84 (0.70–1.01)	
					2.80	1.03 (0.86–1.22)	

Abbreviations: BMI = body mass index; CI = confidence interval; COMT=Catechol-Omethyltransferase; 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.

[a]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).

Table 2. Characteristics of studies on serum vitamin B_{12} (SVB_{12}) and vitamin B_{12} intake and breast cancer risk

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)[a]	USA	Prospective (NCC)	225 (112)	cases:	SVB_{12} ($pg\,ml^{-1}$)[a]		None
				60.4	$COMT^{HH}$		
				Control:60.2	Cases: 450.97,		
					Controls: 500.56		
					$COMT^{HL}$		
					Cases: 428.64, Controls: 438.58		
					$COMT^{LL}$		
					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	1.14 (0.87–1.49)	
					Q4	1.05 (0.80–1.36)	
					Q5	1.01 (0.77–1.32)	
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 (0.51–1.08)	
					389.8–469.4	0.98 (0.69–1.38)	
					469.9–571.5	0.62 (0.42–0.89)	
					>572.7	0.76 (0.52–1.10)	

Table 2. (Continued)

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 B_{12}		Age, socioeconomic status, FHBC, menopausal status, parity, BMI, total calorie intake, dietary fibre carbohydrate intake and polyunsaturated fat intake
					2.61 (μg per day)	1	
					4.03	0.80 (0.59–1.10)	
					5.68	0.51 (0.36–0.73)	
					7.46	0.32 (0.22–0.49)	
Lajous *et al* (2006b)	France, the E3N cohort	Prospective	62739 (1812)	—	Vitamin B_{12}		Unclear
					Q5 *vs* Q1	1.05 (0.90–1.20)	
Cho *et al* (2007)	USA, The Nurses' Health Study II	Prospective	90663 (1032)	26–46	Vitamin B_{12}		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
					4 (μg per day)	1	
					5	1.09 (0.90–1.34)	
					6	1.08 (0.88–1.32)	
					7	1.13 (0.93–1.39)	
					9	0.96 (0.78–1.19)	
Lin *et al* (2008)	USA, The Women's Health Study	Prospective (NCC)	1696 (848)	>45	SVB_{12}		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
					$\leqslant$337 (pg ml^{-1})	1	
					337–414	1.12 (0.80–1.56)	
					414–512	1.45 (1.03–2.04)	
					512–686	1.23 (0.87–1.72)	
					>686	1.29 (0.92–1.82)	
					Vitamin B_{12}		
					$\leqslant$3.8 (μg per day)	1.00	
					3.8–4.8	0.66 (0.39–1.10)	
					4.8–5.6	0.78 (0.46–1.33)	
					5.6–7.1	1.19 (0.73–1.94)	
					>7.1	0.88 (0.54–1.44)	
Ma *et al* (2009a)	Brazilian	Case–control (HCC)	916 (458)	20–74	Vitamin B_{12}		Smoking, alcohol, moderate physical activity in the preceding 5 years, and number of live births.
					<3.9 (μg per day)	1	
					3.9–7.3	0.90 (0.64–1.25)	
					$\geqslant$7.3	0.90 (0.65–1.26)	

Table 2. (Continued)

Study	Country, study name	Study design	Subjects (cases)	Age (years)	Category	RR (95% CI)	Adjustment for covariates
Ma *et al* (2009b)	Japan	Case-control (HCC)	776 (388)	20–74	Vitamin B_{12}		BMI, education, smoking, alcohol, age at menarche, age at first live birth in parous women, menstruation status, breast feeding, number of live births and moderate physical activity in the preceding 5 years
					<7.4 (μg per day)	1.00	
					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	35 023 (743)	50–76	Vitamin B_{12}		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
					2.71 (μg per day)	1.00	
					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	70 656 (3898)	54–74	Vitamin B_{12}		Age, alcohol, multivitamin 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
					<1.94 (μg per day)	1.00	
					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 (0.79–1.16)	
					⩾9.07	0.98 (0.80–1.19)	
Zhang *et al* (2011)	China	Case–control (HCC)	876 (438)	25–70	Vitamin B_{12}		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
					<0.93 (μg per day)	1.00	
					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)	
Shrubsole *et al* (2011)	China, Shanghai Women's Health Study	Prospective	72 816 (718)	40–70	Vitamin B_{12}		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, vegetables and fat, and menopausal status
					1.00 (μg per day)	1.00	
					1.83	0.89 (0.69–1.14)	
					2.44	0.88 (0.68–1.15)	
					3.11	0.91 (0.69–1.19)	
					4.50	0.83 (0.61–1.12)	

Table 2. (Continued)

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 B_{12}		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
					⩽5.28 (μg per day)	1.00	
					5.29–8.15	0.89 (0.53–1.65)	
					>8.15	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 B_{12}		Age, centre, vitamin B_2, vitamin B_6, vitamin B_{12}, 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
					⩽5.32 (μg per day)	1.00	
					5.32–9.78	0.75 (0.60–0.93)	
					9.78–13.98	0.83 (0.62–1.11)	
					>13.98	0.73 (0.53–1.00)	
Bassett *et al* (2013)	Australia, The MCCS study	Prospective	20 756 (936)	27–80	Vitamin B_{12}		Ethnicity, menopausal status, age at menarche, parity and lactation, oral contraceptive use, HRT use, physical activity, alcohol, smoking status, education level and BMI
					1.66 (μg per day)	1.00	
					2.33	1.06 (0.89–1.27)	
					3.04	0.94 (0.78–1.14)	
					4.61	1.21 (1.00–1.46)	

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.

[a]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).

Table 3. Characteristics of studies on methionine intake and breast cancer risk

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
					Q2	0.95 (0.73–1.24)	
					Q3	0.84 (0.63–1.12)	
					Q4	1.00 (0.74–1.36)	
					Q5	0.79 (0.54–1.16)	
Feigelson *et al* (2003)	USA, the CPS-II Nutrition Cohort	Prospective	66 561 (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.
					0.64–0.76	1.01 (0.85–1.20)	
					0.76–0.88	1.08 (0.91–1.28)	
					0.88–1.04	1.01 (0.84–1.20)	
					>1.04	0.92 (0.77–1.11)	
Zhu *et al* (2003)	USA	Case–control (PCC)	609 (304)	>20	$\leqslant 0.54$ (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 B_2, B_6, B_{12} and C, and total energy intake per day
					0.55–0.78	0.69 (0.40–1.18)	
					0.79–1.06	0.67 (0.35–1.29)	
					>1.06	0.50 (0.22–1.15)	
Cho *et al* (2007)	USA, The Nurses' Health Study II	Prospective	90 663 (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
					1.8	0.92 (0.76–1.13)	
					2.0	1.03 (0.85–1.25)	
					2.2	0.86 (0.70–1.06)	
					2.5	1.10 (0.89–1.36)	

Table 3. (Continued)

Study	Country, study name	Study design	Subjects (cases)	Age (years)	Category	RR (95% CI)	Adjustment for covariates
Kabat *et al* (2008)	Canada, CNBSS	Prospective	89 835 (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
					1.78–2.00	1.04 (0.92–1.19)	
					2.00–2.20	1.08 (0.95–1.22)	
					2.20–2.48	0.98 (0.86–1.12)	
					>2.48	1.00 (0.88–1.14)	
Xu *et al* (2008)	USA	Case–control (PCC)	3064 (1508)	—	<0.65 (g per day)	1.00	Age and daily energy intake
					0.65–0.86	0.97 (0.77–1.23)	
					0.86–1.06	0.98 (0.77–1.26)	
					1.06–1.34	0.94 (0.69–1.30)	
Maruti *et al* (2009a,b)	USA, The VITAL cohort study	Prospective	35 023 (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
					1.22	0.98 (0.78–1.22)	
					1.59	0.84 (0.65–1.08)	
					2.17	0.78 (0.56–1.09)	
Stevens *et al* (2010)	USA, CPS-II Nutrition Cohort	Prospective	70 656 (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
					0.64–0.76	0.92 (0.83–1.01)	
					0.76–0.88	0.98 (0.88–1.08)	
					0.88–1.04	0.93 (0.84–1.03)	
					⩾1.04	0.88 (0.79–0.98)	
Zhang *et al* (2011)	China	Case–control (HCC)	876 (438)	25–70	<0.84 (g per day)	1.00	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
					0.84–1.10	0.83 (0.56–1.24)	
					1.10–1.38	0.79 (0.53–1.18)	
					>1.38	0.79 (0.53–1.17)	
Shrubsole *et al* (2011)	China, Shanghai Women's Health Study	Prospective	72 816 (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
					1.34	0.98 (0.76–1.26)	
					1.48	0.94 (0.71–1.23)	
					1.65	0.98 (0.73–1.32)	
					1.97	0.92 (0.66–1.28)	

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	⩽1.56 (g per day)	1.00	Age, centre, vitamin B_2, vitamin B_6, vitamin B_{12}, 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.
					1.56–1.82	1.05 (0.88–1.24)	
					1.82–2.10	1.00 (0.84–1.18)	
					>2.10	0.98 (0.82–1.17)	
Bassett *et al* (2013)	Australia, The MCCS study	Prospective	20 756 (936)	27–80	1.154 (g per day)	1.00	Ethnicity, menopausal status, age at menarche, parity and lactation, oral contraceptive use, HRT use, physical activity, alcohol, smoking status, education level and BMI
					1.398	1.04 (0.88–1.25)	
					1.616	0.90 (0.75–1.08)	
					1.969	0.97 (0.80–1.17)	

Abbreviations: BMI = body mass index; CI = confidence interval; 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.

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 B_{12} 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 B_6 intake ($P = 0.14$), serum vitamin B_{12} levels ($P = 0.18$), vitamin B_{12} 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 B_6 intake, serum vitamin B_{12} and dietary vitamin B_{12} intake and risk of breast cancer.

Several biological mechanisms for the inverse relationship of vitamin B_6, vitamin B_{12} and methionine with the development of breast cancer have been proposed. First, vitamin B_6, B_{12} and methionine participate in one-carbon metabolism, which is essential for DNA synthesis, repair and methylation (Ames, 2001), and vitamins B_6 and B_{12} 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 B_6 intake and PLP with the risk of breast cancer. The multivariable Pearson's correlation coefficients between plasma levels of vitamin B_6 and vitamin B_{12}, and the average intakes of vitamin B_6 and vitamin B_{12} from food was 0.25 and 0.08, respectively (Zhang *et al*, 2003). In this respect, the serum biomarker of vitamin B_6 (PLP) and vitamin B_{12} 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 B_6 (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

Table 4. Summary risk estimates of the association between vitamin B_6, vitamin B_{12} and methionine and breast cancer risk

				Risk estimate (95% CI)		Heterogeneity test	
Nutrients	**Study**	**No. (cases)**	**No. (studies)**	**REM**	***P*-value**	**I^2 (%)**	***P*- value**
Vitamin B_6	PLP[a]	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	14 260	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
Menopausal status							
	Pre-menopausal	1885	5	0.94 (0.66–1.33)	0.73	64.9	0.02
	Post-menopausal	5661	6	0.90 (0.78–1.04)	0.16	0.80	0.41
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	1.05 (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 B_{12}	Serum[a]	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	15 783	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
Menopausal status							
	Pre-menopausal	1567	3	0.87 (0.64–1.19)	0.38	37.1	0.20
	Post-menopausal	7111	5	0.74 (0.53–1.03)	0.07	87.3	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	Dietary intake	17 060	13	0.94 (0.89–0.99)	0.03	0.00	0.52

Table 4. (Continued)

				Risk estimate (95% CI)		Heterogeneity test	
Nutrients	**Study**	**No. (cases)**	**No. (studies)**	**REM**	***P*-value**	**I^2 (%)**	***P*- value**
Study design							
	Prospective	11 121	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	13 604	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 B_6		5461	4	0.91 (0.79–1.04)	0.17	0.00	0.41
Folate + Vitamin B_{12}		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.
[a]All studies are prospective design.

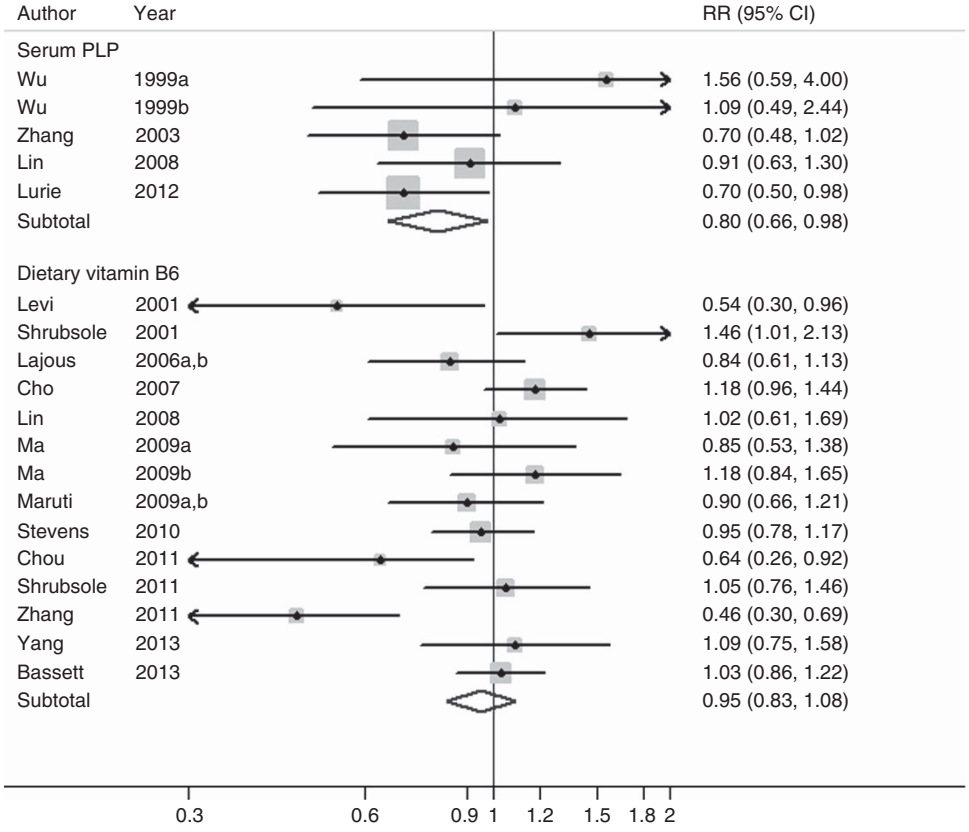

Figure 1. The multivariate-adjusted risk of breast cancer for the highest *vs* lowest categories of serum PLP levels and dietary vitamin B_6 intake in random-effects model. The size of the grey box is positively proportional to the weight assigned to each study, which is inversely proportional to the 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.

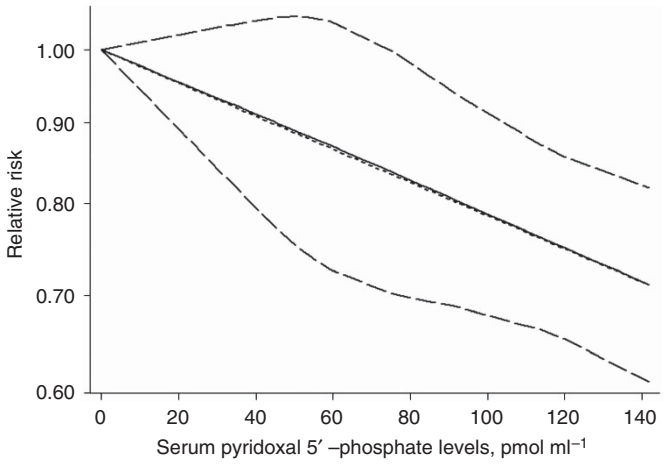

Figure 2. The dose–response analysis between serum PLP and breast cancer risk, with restricted cubic splines in a multivariate random-effects dose–response model. The solid line and the long dash line represent the estimated RR and its 95% CI. Short, dash line represents the linear relationship.

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 B_6 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 B_{12} 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

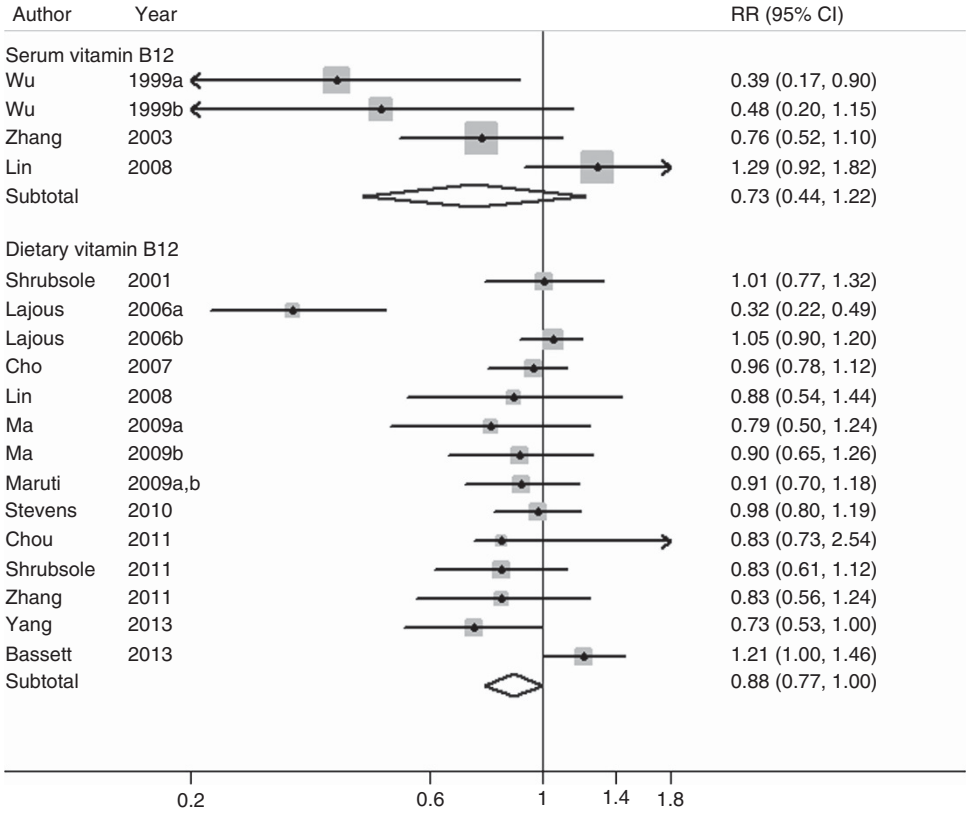

Figure 3. The multivariate-adjusted risk of breast cancer for the highest *vs* lowest categories of serum vitamin B_{12} levels and dietary vitamin B_{12} intake in random-effects model. The size of the grey box is positively proportional to the weight assigned to each study, which is inversely proportional to the s.e. of the RR, and horizontal lines represent the 95% CIs.

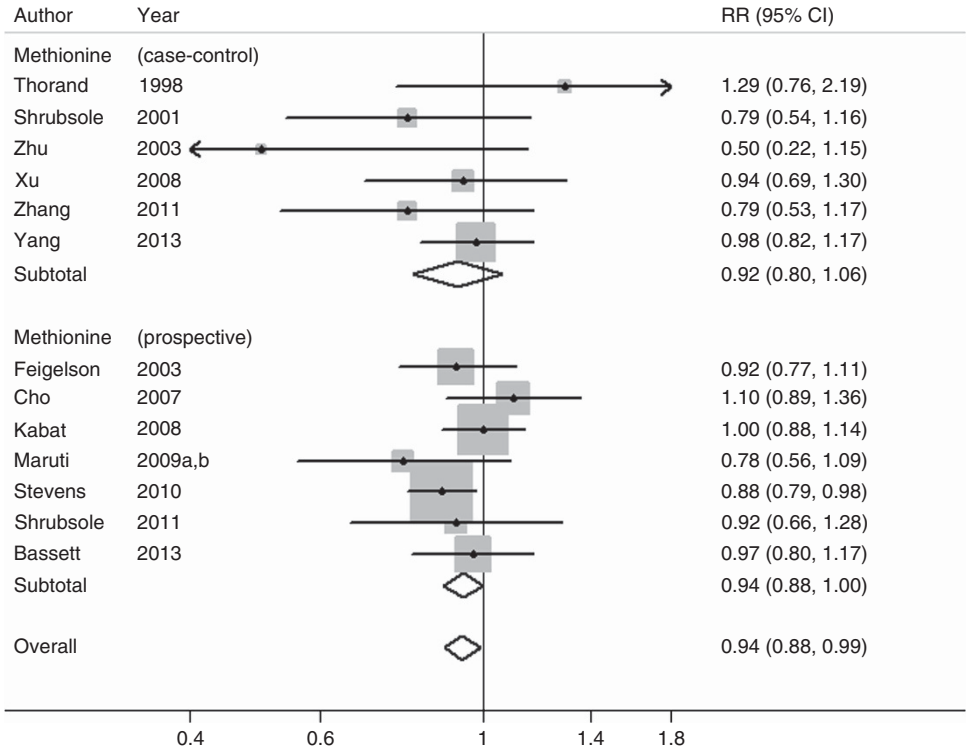

Figure 4. The multivariate-adjusted risk of breast cancer for the highest *vs* lowest categories of dietary methionine intake in random-effects model. The size of the grey box is positively proportional to the weight assigned to each study, which is inversely proportional to the s.e. of the RR, and horizontal 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 B_{12}.

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.

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