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Dietary folate, vitamin B_6, vitamin B_{12} and methionine intake and the risk of breast cancer by oestrogen and progesterone receptor status

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Abstract

Few studies have evaluated the relationship between the consumption of dietary folate and one-carbon metabolism-related nutrients and breast cancer risk defined by oestrogen receptor (ER) and progesterone receptor (PR) status. The objective of the present study was to examine the associations between dietary folate, vitamin B_6, vitamin B_{12}, and methionine intake and the risk of breast cancer by ER and PR status among Chinese women in Guangdong. A hospital-based case–control study was conducted from June 2007 to August 2008, with 438 cases and 438 age (5-year interval)- and residence (rural/urban)-matched controls. Dietary intake information was assessed using a validated FFQ administered through a face-to-face interview. Unconditional logistic regression models were used to calculate multivariate-adjusted OR and 95 % CI. A significant inverse association was found between dietary folate and vitamin B_6 intake and breast cancer risk. The adjusted OR of the highest *v.* the lowest quartile were 0·32 (95 % CI 0·21, 0·49; $P_{trend} < 0·001$) for dietary folate and 0·46 (95 % CI 0·30, 0·69; $P_{trend} < 0·001$) for vitamin B_6. No associations were observed for vitamin B_{12} and methionine intake. A significant inverse association between dietary folate intake and breast cancer risk was observed in all subtypes of ER and PR status. These findings suggest that dietary folate and vitamin B_6 intakes were inversely associated with breast cancer risk. The inverse association did not differ by ER and/or PR status.

Key words: Folate: Vitamin B_6: Vitamin B_{12}: Breast cancer: Hormone receptor status

One-carbon metabolism is a complex metabolic network. Dietary factors involved in this pathway include folate, methionine, vitamin B_6 and vitamin B_{12}. Folate is thought to play an important role in cancer prevention[1]. As a carbon donor in one-carbon metabolism, folate is essential for normal DNA synthesis and repair. Adequate folate status is also important for the production of *S*-adenosylmethionine for DNA methylation. Moreover, methionine is the precursor for the endogenous production of *S*-adenosylmethionine. Additionally, one-carbon metabolism may involve as many as twenty-five enzymes, some of which require the presence of vitamin B_6 and vitamin B_{12} as co-enzymes[2]. For example, vitamin B_{12} acts as a cofactor for the enzyme methionine synthase, and vitamin B_6 serves as a cofactor for folate-dependent enzymes in the one-carbon metabolism pathway.

Folate digestion and absorption are affected by many factors such as diseases or medications. Alcohol is a known folate antagonist. It can impair folate absorption and metabolism[3], and plausibly increase an individual's requirement for folate intake. Some studies, but not all, have found that alcohol consumption modifies the effect of folate intake on breast cancer risk[4].

Several epidemiological studies have examined the association of dietary folate with breast cancer, but the results are inconsistent. While some studies have reported a negative association between dietary folate intake and breast cancer risk[5–13], other studies have not observed a significant relationship[14–24]. In addition to folate, a few studies have investigated the relationships between other one-carbon metabolism-related nutrients (such as vitamin B_6, vitamin B_{12} and methionine) and breast cancer risk[8,9,11,16,23,25–31]. But the results remain inconclusive. Therefore, further investigation of these issues is warranted.

Abbreviations: ER, oestrogen receptor; PR, progesterone receptor.

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Oestrogen receptor (ER) and progesterone receptor (PR) are the most widely studied markers in breast tissue. Recent studies have raised the hypothesis that the association of dietary factors with breast cancer may differ by ER or PR status[32–34]. Folate may influence the methylation of ER or PR genes and thereby affecting the silencing of these genes[35,36]. The associations between folate intake and breast cancer risk may therefore differ according to ER or PR status of tumours. So far, only a few studies have examined folate intake in relation to breast cancer risk according to ER and/or PR status[22,23,28,34,37,38], and the results are inconsistent.

Moreover, the majority of these studies have been performed in Western populations. Unlike their Western counterparts, most Chinese women consume natural (unfortified and unprocessed) foods, and they seldom take vitamin supplements or drink regularly. Thus, Chinese dietary habits may allow a better assessment of nutrient intake and minimise potential misclassifications in epidemiological studies. The present study aimed to evaluate the association between dietary folate and other one-carbon metabolism-related nutrient intake and the risk of breast cancer characterised by ER and PR status among Chinese women in Guangdong Province.

Materials and methods

Study subjects

A hospital-based case–control study was conducted in Guangdong Province, China, from June 2007 to August 2008. The selection of cases and controls has been described in detail elsewhere[39,40]. In brief, potential case subjects were recruited from patients admitted to the surgical units of two affiliated hospitals of Sun Yat-sen University, Guangzhou, China. Inclusion criteria were female subjects aged 25–70 years and natives of Guangdong Province or having lived in Guangdong for at least 5 years, with incident, primary, histologically confirmed breast cancer diagnosed no more than 3 months before the interview. Women were excluded if they could not understand or speak Mandarin/Cantonese or with a prior history of breast cancer or other cancers. In total, 438 (96 %) cases out of 455 eligible cases were successfully interviewed.

As well as the 438 eligible patients, 438 control subjects with no history of cancer and admitted to the same hospitals during the same time period as the case subjects were interviewed. They were frequency-matched by age (5-year interval) and residence (rural/urban) to the case patients. These patients presented with a wide spectrum of non-neoplastic conditions including eye disorders (glaucoma, uveitis, keratitis, pterygium, dacryocystitis and optic neuritis), ear, nose and throat diseases (sudden deafness, acute bacterial/viral otitis media, sinusitis, deviation of nasal septum, tonsillitis), trifacial neuralgia, varicose veins, traumatic skeletal disorders, osteoarthritis, degenerate joint disease, orthopaedics and acute appendicitis. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Ethical Committee of the Chinese University of Hong Kong. Written informed consent was obtained from all participants.

Data collection

Data on sociodemographic characteristics, anthropometrics, menstrual and reproductive history, family history of breast cancer, physical activity, smoking habits, alcohol use and prior disease history were collected from each subject by in-person interviews using a structured questionnaire. Regular drinking was defined as alcohol drinking at least once per week over the past year. Relevant medical information, medical diagnosis, histological findings and ER or PR status were abstracted from the hospital medical records. Information on the ER and PR status of the tumour was available for 399 (91·1 %) cases.

Dietary assessment

Dietary information was obtained from an eighty-one-item interviewer-administered FFQ covering the habitual diet of participants during the previous year. Food photographs were used to help participants quantify the portions consumed. Daily dietary nutrient intakes were estimated using the China Food Composition Table[41,42]. Information on frequency of intake and portion size was used to calculate the amount of each food item consumed on average (g/d). Total dietary intakes of folate, vitamin B_6, vitamin B_{12} and methionine were calculated by summing the product of the frequency of consumption, usual portion consumed and micronutrient content of each food item. Information on cooking method was not collected in the present study, as stir-frying and boiling are the two most common methods of cooking green leafy vegetables and animal foods in China.

The validity and reproducibility of the FFQ has been described in detail elsewhere[43]. A total of sixty-one female subjects completed 3 d dietary records at intervals of 2 months during a 12-month period and two FFQ administered 1 year apart. The correlation coefficients comparing the second FFQ and 18 d dietary records were 0·35 for folate, 0·26 for vitamin B_6, 0·50 for vitamin B_{12} and 0·36 for methionine. The correlation coefficients between the two FFQ were 0·60 for folate, 0·57 for vitamin B_6, 0·60 for vitamin B_{12} and 0·49 for methionine. These results showed that the reproducibility and validity of our FFQ seem to be comparable with the values reported by others[44,45].

Statistical analysis

All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Dietary folate, vitamin B_6, vitamin B_{12} and methionine intakes were adjusted for total energy intake using the residual method[46] and then categorised into quartiles based on the distribution among the control subjects. Unconditional logistic regression models were used to estimate the OR and 95 % CI of each quartile, using the lowest quartile group as the reference after adjusting for the various potential confounding factors. Risk factors identified by comparison of baseline characteristics between cases and controls as independently associated with breast cancer risk were adjusted in the multivariate models. These included

Table 1. Comparison of breast cancer cases and controls on sociodemographic and selected characteristics among Chinese women

(Mean values, standard deviations, number of patients and percentages)

	Cases (*n* 438)		Controls (*n* 438)		
	n	%	*n*	%	*P*
Age (years)					0·875
Mean	47·04		47·14		
SD	9·53		9·58		
Marital status					0·574
Married	409	93·4	413	94·3	
Unmarried/divorced/widowed	29	6·6	25	5·7	
Educational level					0·401
Primary school or below	106	24·2	127	29·0	
Junior high school	127	29·0	116	26·5	
Senior high school/secondary technical school	109	24·9	109	24·9	
College or above	96	21·9	86	19·6	
Occupation					0·157
Administrator/other white-collar worker	190	43·4	166	37·9	
Blue collar worker	97	22·1	118	26·9	
Farmer/other	151	34·5	154	35·2	
Household income (yuan/month)					0·751
<2000	157	35·8	161	36·8	
2001–5000	152	34·7	149	34·0	
5001–8000	68	15·5	59	13·5	
>8001	61	13·9	69	15·8	
BMI (kg/m^2)					0·038
Mean	22·92		22·46		
SD	3·33		3·05		
Regular smoker	7	1·6	2	0·4	0·094
Passive smoker from a husband	202	46·1	170	38·8	0·029
Regular drinker	12	2·7	10	2·3	0·666
Physical activity (exercise for health)					0·016
Never	170	38·8	139	31·7	
Occasional	46	10·5	35	8·0	
≥ 1 time/week	222	50·7	264	60·3	
Age at menarche (years)					0·019
Mean	14·82		15·11		
SD	1·88		1·84		
Nulliparous	17	3·9	19	4·3	0·734
Number of live births*					0·532
Mean	1·98		2·03		
SD	1·12		1·20		
Age at first live birth (years)*					0·050
≤ 19	11	2·6	11	2·6	
20–24	139	33·0	170	40·6	
25–29	227	53·9	201	48·0	
≥ 30	44	10·5	37	8·8	
Months of breast-feeding*					0·034
Never	49	11·6	26	6·2	
1–3	21	5·0	22	5·3	
4–11	95	22·6	108	25·8	
12–23	118	28·0	102	24·3	
≥ 24	138	32·8	161	38·4	
Age at menopause (years)†					0·579
Mean	49·33		49·06		
SD	3·96		3·93		
Menopausal status					0·423
Premenopausal	306	69·9	295	67·4	
Postmenopausal	132	30·1	143	32·6	
Mother/sister/daughter with breast cancer	17	3·9	4	0·9	0·004
Ever had benign breast disease	177	40·4	84	19·2	$<0·001$
Ever used oral contraceptives	27	6·2	21	4·8	0·373
Ever used nutritional supplements	70	16·0	80	18·3	0·370

* Among parous women.
† Among menopausal women.

age at menarche (continuous), live births and age at first live birth (≤19, 20–24, 25–29, ≥30 years and nulliparous), months of breast-feeding (nulliparous and never breast-feeding, 1–3, 4–11, 12–23 and ≥24 months), BMI (continuous), family history of breast cancer in a first-degree relative (yes/no), history of benign breast disease (yes/no), passive smoking from a husband (yes/no) and physical activity (categorical, never, occasional and ≥1 time per week). Tests for trend were performed by entering the categorical variables as continuous variables in the models. Analyses stratified by ER/PR status and menopausal status were conducted to evaluate whether these factors modified the associations of dietary folate intake with breast cancer risk. The potential interactions (each quartile of folate × menopausal status) between folate intake and breast cancer risk by menopausal status were also examined. Our sample of 230 cases and 218 controls in two quartiles (Q1 and Q4) gave us 99% power to detect the OR of 0·32 for the association between folate intake and breast cancer risk at $P < 0·05$ (two-tailed). We had greater than 98% power to detect OR of 0·31 and 0·29 for the association between folate intake and ER+ and PR+ breast cancer. However, the power was lower than 65% among ER− and PR− tumours.

Results

Table 1 presents the sociodemographic and established breast cancer risk factors of the study populations. Compared with controls, cases had an earlier age at menarche, older age at first live birth, fewer months of breast-feeding and higher BMI. Cases were more likely to have a history of breast cancer in a first-degree relative, history of benign breast disease and history of passive smoking from a husband, and were less likely to be physically active than controls. All of the aforementioned variables were considered to be potential confounding factors and controlled for in subsequent analyses. No significant differences were found between the case and control subjects in sociodemographic factors, including educational level, occupational status, marital status and household income, or in reproductive factors, including nulliparity, number of live births, age at menopause and use of an oral contraceptive.

The median intake of folate was 228·3 μg/d, vitamin B_6 was 0·86 mg/d, vitamin B_{12} was 1·54 μg/d and methionine was 1·14 μg/d in the control group (Table 2). Compared with controls, the consumption of dietary folate and vitamin B_6 was significantly lower in the case subjects. No significant differences between cases and controls were observed for vitamin B_{12} and methionine intake.

The associations between the intake of dietary folate, vitamin B_6, vitamin B_{12} and methionine and the risk of breast cancer are shown in Table 3. After adjustment for the various potential confounders, a significant inverse association was observed between the intake of dietary folate and vitamin B_6 and the risk of breast cancer. The OR for the highest quartile of intake compared with the lowest were 0·32 (95% CI 0·21, 0·49; $P_{trend} < 0·001$) for folate intake and 0·46 (95% CI 0·30, 0·69; $P_{trend} < 0·001$) for vitamin B_6 intake. Associations for vitamin B_{12} and methionine were not statistically significant.

The associations between folate intake and breast cancer risk according to sources of folate are shown in Table 4. There was a consistent inverse association between folate intake from different food sources (e.g. vegetables, fruits, soya, grains and animal foods) and breast cancer risk.

Among the case subjects with information on hormone receptor status, 292 (73·2%) and 348 (87·2%) were for ER+ and PR+, respectively; 275 (68·9%) were ER+/PR+; seventeen (4·3%) were ER+/PR−; seventy-three (18·3%) were ER−/PR+; and thirty-four (8·5%) were ER−/PR−. Table 5 shows the impact of dietary folate consumption on the risk of breast cancer characterised by ER and PR status. The inverse association between folate intake and breast cancer risk was observed in all subtypes of ER and/or PR status, although the association was statistically non-significant among women with PR−, ER+PR− and ER−PR− breast cancer tumours due to the relatively small numbers.

Because pre- and postmenopausal breast cancers probably have a separate disease aetiology, a stratified analysis by menopausal status was conducted. The inverse association of folate intake with breast cancer risk did not vary by menopausal status. The multivariate-adjusted OR were 0·34 (95% CI 0·21, 0·58) among premenopausal women and 0·28 (95% CI 0·12, 0·65) among postmenopausal women, comparing the

Table 2. Comparison of dietary folate, vitamin B_6, vitamin B_{12} and methionine intake between breast cancer cases and controls (Mean values, standard deviations, 25th (P_{25}), 50th (P_{50}) and 75th (P_{75}) percentiles)

	Cases					Controls					
	Mean	SD	P_{25}	P_{50}	P_{75}	Mean	SD	P_{25}	P_{50}	P_{75}	*P**
Energy (kJ/d)	6118	1798	4830	5887	7099	6289	1795	4977	6043	7249	0·142
Total fat (g/d)	29·4	16·3	18·9	26·0	36·5	30·6	16·1	19·2	26·7	37·9	0·207
Folate (μg/d)	207·4	72·5	155·7	196·7	242·8	240·1	85·0	181·5	228·3	284·3	<0·001
Folate from vegetables	62·9	41·5	34·6	53·0	79·2	82·5	52·5	45·6	71·9	104·7	<0·001
Folate from fruits	18·1	18·1	7·0	12·9	23·5	21·9	19·3	8·9	16·7	29·2	0·001
Folate from grains	65·0	24·7	48·0	60·2	76·7	66·6	25·1	51·0	60·9	79·3	0·195
Folate from soya foods	18·0	21·3	5·1	11·2	23·9	25·6	29·9	6·1	15·9	34·3	<0·001
Folate from animal foods	41·7	26·4	21·4	36·3	53·5	45·0	27·7	24·9	39·6	59·7	0·043
Vitamin B_6 (mg/d)	0·79	0·27	0·60	0·76	0·93	0·90	0·32	0·67	0·86	1·04	<0·001
Vitamin B_{12} (μg/d)	1·73	1·08	0·93	1·57	2·29	1·79	1·17	1·01	1·54	2·27	0·648
Methionine (g/d)	1·16	0·41	0·84	1·10	1·38	1·19	0·42	0·90	1·14	1·39	0·196

* Wilcoxon's rank-sum test comparing the median consumption levels between cases and controls.

Table 3. Folate, vitamin B_6, vitamin B_{12} and methionine intake and breast cancer risk among Chinese women
(Odds ratios and 95 % confidence intervals)

	Q1*	Q2*		Q3*		Q4*		
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
Folate								
No. of cases/controls	174/109	114/110		94/110		56/109		
Crude	1	0·65	0·46, 0·93	0·54	0·37, 0·77	0·32	0·22, 0·48	<0·001
Adjusted†	1	0·68	0·47, 1·00	0·51	0·34, 0·75	0·32	0·21, 0·49	<0·001
Vitamin B_6								
No. of cases/controls	159/105	132/112		72/113		75/108		
Crude	1	0·82	0·58, 1·17	0·45	0·31, 0·66	0·47	0·32, 0·69	<0·001
Adjusted†	1	0·83	0·57, 1·21	0·42	0·28, 0·64	0·46	0·30, 0·69	<0·001
Vitamin B_{12}								
No. of cases/controls	122/108	106/113		106/106		104/111		
Crude	1	0·86	0·59, 1·25	0·86	0·59, 1·25	0·85	0·59, 1·24	0·417
Adjusted†	1	0·74	0·49, 1·09	0·74	0·50, 1·11	0·83	0·56, 1·24	0·383
Methionine								
No. of cases/controls	127/109	103/110		105/110		103/109		
Crude	1	0·80	0·56, 1·16	0·82	0·57, 1·19	0·81	0·56, 1·18	0·294
Adjusted†	1	0·83	0·56, 1·24	0·79	0·53, 1·18	0·79	0·53, 1·17	0·225

Q, quartile.
* Quartile cut-off points were based on residual energy-adjusted intake among the control subjects.
† OR were adjusted for age at menarche, live births and age at first live birth, months of breast-feeding, BMI, history of benign breast disease, mother/sister/daughter with breast cancer, physical activity, passive smoking and total energy intake.

fourth quartile with the first quartile. No interaction was observed between menopausal status and folate intake ($P_{interaction} = 0·570$, data not shown).

A number of sensitivity analyses were performed to examine the association between dietary folate intake and breast cancer risk. Ductal carcinoma was the most frequent histological type (415, 94·7 %), and restricting the analysis to these cases yielded very similar results. It was observed that seventy cases (16·1 %) and eighty controls (18·3 %) reported ever taking nutritional supplements. Sensitivity analysis that excluded women with nutritional supplement use revealed similar results as compared with the analyses that included nutritional supplement users. In the present study, only twelve (2·7 %) cases and ten (2·3 %) controls were regular drinkers. The results of sensitivity analyses excluding women with alcohol intake were essentially the same. Further analyses restricted to subjects reporting no dietary change showed no substantial change in the observed folate intake and breast cancer association.

Table 4. Folate intake from different food sources and breast cancer risk among Chinese women
(Odds ratios and 95 % confidence intervals)

	Q1*	Q2*		Q3*		Q4*		
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
Folate from vegetables								
No. of cases/controls	150/109	141/110		98/110		49/109		
Crude	1	0·93	0·66, 1·32	0·65	0·45, 0·94	0·33	0·22, 0·50	<0·001
Adjusted†	1	0·98	0·67, 1·42	0·63	0·42, 0·93	0·35	0·22, 0·54	<0·001
Folate from fruits								
No. of cases/controls	130/109	134/110		89/110		83/109		
Crude	1	1·02	0·71, 1·46	0·68	0·46, 0·99	0·64	0·44, 0·94	0·004
Adjusted†	1	1·12	0·76, 1·64	0·66	0·44, 0·99	0·63	0·42, 0·95	0·004
Folate from soya								
No. of cases/controls	152/107	92/108		120/107		71/107		
Crude	1	1·16	0·82, 1·66	0·86	0·59, 1·24	0·44	0·29, 0·66	<0·001
Adjusted†	1	0·64	0·43, 0·95	0·88	0·60, 1·28	0·46	0·31, 0·70	0·004
Folate from grains								
No. of cases/controls	130/109	128/110		100/110		80/109		
Crude	1	1·02	0·71, 1·47	0·78	0·54, 1·13	0·66	0·45, 0·97	0·134
Adjusted†	1	0·91	0·61, 1·34	0·71	0·48, 1·07	0·54	0·36, 0·82	0·022
Folate from other foods								
No. of cases/controls	131/109	114/110		102/110		91/109		
Crude	1	0·86	0·60, 1·24	0·78	0·54, 1·13	0·69	0·47, 1·00	0·043
Adjusted†	1	0·73	0·49, 1·08	0·70	0·47, 1·04	0·57	0·38, 0·86	0·009

Q, quartile.
* Quartile cut-off points were based on residual energy-adjusted intake among the control subjects.
† OR were adjusted for age at menarche, live births and age at first live birth, months of breast-feeding, BMI, history of benign breast disease, mother/sister/daughter with breast cancer, physical activity, passive smoking and total energy intake.

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Table 5. Dietary folate intake and breast cancer risk stratified by oestrogen receptor (ER)/progesterone receptor (PR) status (Odds ratios and 95 % confidence intervals)

	Q1*	Q2*		Q3*		Q4*		
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
ER+								
No. of cases/controls	115/109	85/110		58/110		34/109		
Adjusted†	1	0·76	0·50, 1·16	0·48	0·31, 0·75	0·31	0·19, 0·51	<0·001
ER−								
No. of cases/controls	42/109	20/110		27/110		18/109		
Adjusted†	1	0·50	0·27, 0·94	0·60	0·34, 1·07	0·40	0·21, 0·76	0·008
PR+								
No. of cases/controls	143/109	91/110		72/110		42/109		
Adjusted†	1	0·67	0·45, 0·99	0·46	0·31, 0·70	0·29	0·18, 0·47	<0·001
PR−								
No. of cases/controls	14/109	14/110		13/110		10/109		
Adjusted†	1	0·98	0·43, 2·24	0·82	0·35, 1·92	0·76	0·31, 1·87	0·490
ER+PR+								
No. of cases/controls	111/109	78/110		55/110		31/109		
Adjusted†	1	0·74	0·48, 1·13	0·46	0·30, 0·73	0·29	0·17, 0·48	<0·001
ER+PR−								
No. of cases/controls	4/109	7/110		3/110		3/109		
Adjusted†	1	1·67	0·43, 6·56	0·97	0·19, 4·87	0·72	0·14, 3·58	0·701
ER−PR+								
No. of cases/controls	32/109	13/110		17/110		11/109		
Adjusted†	1	0·43	0·21, 0·89	0·51	0·26, 1·00	0·31	0·14, 0·67	0·003
ER−PR−								
No. of cases/controls	10/109	7/110		10/110		7/109		
Adjusted†	1	0·74	0·26, 2·10	0·84	0·32, 2·23	0·68	0·24, 1·95	0·528

Q, quartile.
* Quartile cut-off points were based on residual energy-adjusted intake among the control subjects.
† OR were adjusted for age at menarche, live birth and age at first live birth, months of breast-feeding, BMI, history of benign breast disease, mother/sister/daughter with breast cancer, physical activity, passive smoking and total energy intake.

Discussion

In the present hospital-based case–control study, we observed a statistically significant inverse association between dietary folate, vitamin B_6 intake and the risk of breast cancer after adjusting for various confounding factors. The inverse association of folate intake with breast cancer risk was observed in all subtypes of ER and/or PR status. No association was found with vitamin B_{12} and methionine intake.

The association of dietary folate intake with breast cancer risk has been examined in many epidemiological studies. Of the fifteen case–control studies[5–13,27,29,36,38,47,48], nine have[5–13] reported the protective effect of dietary folate intake on breast cancer risk. A case–control study conducted in Shanghai, China, showed that participants in the highest quintile of dietary folate had a 38 % decrease in breast cancer risk compared with those in the lowest quintile[11]. The findings of the present study are consistent with these studies. No differences were observed in the inverse association between folate intake and breast cancer risk according to folate intake from different food sources. However, some prospective cohort studies[14–24] did not find a significant inverse association of dietary folate intake with breast cancer risk. A meta-analysis including nine prospective studies and fourteen case–control studies has shown a negative association between dietary folate intake and breast cancer risk in case–control studies but not in prospective studies[4]. Methodological differences may partially explain the inconsistent findings.

Relatively few epidemiological studies have evaluated the association of vitamin B_6, vitamin B_{12} and methionine intake with breast cancer. Most of the studies have not found a significant association of methionine intake[11,16,23,25–28], vitamin B_6 or vitamin B_{12} intake[8,9,11,23,26,29–31] with breast cancer risk. In agreement with these results, neither vitamin B_{12} nor methionine was associated with breast cancer in the present study. However, higher vitamin B_6 intake was found to be inversely associated with breast cancer risk. Since plant foods are the major sources of vitamin B_6 and folate and their intakes are highly correlated, the effect of confounding may exist. Thus, further investigation into the effect of vitamin B_6 on breast cancer risk is warranted.

In one case–control study[38] and five prospective cohort studies[22,23,28,34,37] that have investigated the association of folate with breast cancer according to the ER and/or PR status of breast tumour, the results remained inconclusive. In the Nurses' Health Study and the Vitamins and Lifestyle Cohort study[23,34], folate intake was inversely associated with the risk of developing ER− but not ER+ tumours. In the Swedish Mammography Cohort Study[22], high folate intake was associated with the decreased risk of developing ER+/PR− breast cancer but not ER+/PR+ and ER−/PR− tumours. However, no overall association was found between folate intake and ER− or ER+ breast cancer tumours in the Nurses' Health Study[28], the Iowa Women's Health Study[37] and one case–control study conducted in Japan[38]. In the present study, the protective effect of folate intake on breast

cancer was observed on all subtypes of the ER/PR status of breast cancer. However, in the present study, the power to detect the interactions between dietary folate intake and ER− and PR− status was low, as the number of ER− and PR− tumour subtypes was small. Further studies with larger sample sizes are thus needed to confirm this result.

Some[9,10,17,21], but not all[15,24,30], epidemiological studies have found a statistically significant reduction in breast cancer risk for high *v.* low folate intake among women with high alcohol intake. In the present study, however, the inverse association of dietary folate intake with breast cancer risk remained significant after excluding women with alcohol intake. Therefore, the present study did not support the evidence of the moderating effect of alcohol on folate intake. The reasons for the differences between the present study and others are unclear. The prevalence of alcohol intake is low in Chinese women, and only twelve (2·7 %) cases and ten (2·3 %) controls were regular drinkers in the present study. Further investigation in populations with a low prevalence of alcohol intake is warranted to help clarify this issue.

Folate intake is strongly influenced by various cooking methods. For example, the method of cooking of green vegetables has been found to have marked effects on folate retention[49], and green vegetables are the major food sources for folate in our population. Therefore, the 68 % reduction in risk associated with a high intake of folate that we observed in the present study may be a conservative estimate. It has been suggested that genetic polymorphisms in folate metabolic enzyme genes could influence breast cancer risk[26]. It is biologically plausible that folate-related gene–nutrient interactions might play a role in breast cancer risk. Therefore, further studies with polymorphisms relevant to folate metabolism will help clarify the mechanism of breast caner risk. In China, the recommended nutrient intakes for folate, vitamin B_6 and vitamin B_{12} are 400 μg/d, 1·2 mg/d and 2·4 μg/d, respectively[42]. The median intake of dietary folate, vitamin B_6 and vitamin B_{12} in the control group of the present study was 228·3 μg/d, 0·86 mg/d and 1·54 μg/d, respectively. These results showed the potential deficiency of one-carbon metabolism nutrients in the study population.

The present study has some limitations. Selection bias is a potential limitation in hospital-based case–control studies. We recruited controls from several conditions with no apparent association with a dietary cause to reduce this bias. Moreover, the high participation rate (96 and 98 % for cases and controls, respectively) and high comparability in sociodemographic factors between the case and control subjects also decreased the potential influence of selection bias on the present results. Recall bias is also of concern in case–control studies. To minimise this bias, we tried to interview the patients as soon as diagnosis was made. We also provided photographs with usual intake portions of foods to help participants quantify the amount of food consumed. The possible non-differential misclassification bias due to dietary folate intake may attenuate the estimated association between dietary intake and breast cancer risk. Therefore, some of the null associations observed in the present study may be due to random measurement error in dietary assessment.

In summary, the present study found that intakes of dietary folate and vitamin B_6 were inversely associated with breast cancer risk. The inverse association was similar by ER and/or PR status. No associations were observed for vitamin B_{12} and methionine intake and breast cancer risk.

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