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Dietary B vitamin and methionine intakes and risk for colorectal cancer: a case–control study in China

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Abstract

B vitamins (including folate, vitamin B_2, vitamin B_6 and vitamin B_{12}) and methionine are essential for methylation reactions, nucleotide synthesis, DNA stability and DNA repair. However, epidemiological evidence among Chinese populations is limited. The objective of this study was to evaluate B vitamins and methionine in relation to colorectal cancer risk in a Chinese population. A case–control study was conducted from July 2010 to April 2019. A total of 2502 patients with colorectal cancer were recruited along with 2538 age- (5-year interval) and sex-matched controls. Dietary data were collected using a validated FFQ. Multivariable logistic regression was used to assess OR and 95 % CI. The intake of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} was inversely associated with colorectal cancer risk. The multivariable OR for the highest quartile *v.* the lowest quartile were 0·62 (95 % CI 0·51, 0·74; $P_{trend} < 0·001$) for folate, 0·46 (95 % CI 0·38, 0·55; $P_{trend} < 0·001$) for vitamin B_2, 0·55 (95 % CI 0·46, 0·76; $P_{trend} < 0·001$) for vitamin B_6 and 0·72 (95 % CI 0·60, 0·86; $P_{trend} < 0·001$) for vitamin B_{12}. No statistically significant association was found between methionine intake and colorectal cancer risk. Stratified analysis by sex showed that the inverse associations between vitamin B_{12} and methionine intake and colorectal cancer risk were found only among women. This study indicated that higher intake of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} was associated with decreased risk of colorectal cancer in a Chinese population.

Key words: **Folate: Vitamin B_2: Vitamin B_6: Methionine: Colorectal cancer risk**

Colorectal cancer is the third most common cancer in men and the second in women, as well as the second most common cause of death from cancer worldwide, with an estimated 1·8 million new colorectal cancer cases and 881 000 deaths occurring in 2018[1]. Genetic alterations, epigenetic modifications and environmental factors cause the development of colorectal cancer in the normal colorectal epithelium[2]. Dietary factors are thought to have a major influence on colorectal cancer risk[3].

B vitamins (including folate, vitamin B_2, vitamin B_6 and vitamin B_{12}) and methionine are essential for methylation reactions, nucleotide synthesis, DNA stability and DNA repair through their role in regulating one-carbon metabolism[4]. One-carbon metabolism is a series of biochemical reactions that transfer single methyl groups from one site to another. Folate is present in cells as a family of enzyme cofactor that carries and chemically activates one-carbons[5]. Folate-activated one-carbons are required for the *de novo* synthesis of purines and thymidylate and for the remethylation of homocysteine to methionine. Vitamin B_2 acts as a cofactor for methylenetetrahydrofolate reductase, a crucial enzyme enhancing DNA integrity, and is involved in epigenetic alteration by promoting the production of methyl donors required for DNA methylation[6]. Vitamin B_6 is an important coenzyme of serine hydroxymethyltransferase for the synthesis of 5, 10-methylenetetrahydrofolate, involving the transfer of one-carbon groups for DNA synthesis and DNA methylation[7]. Vitamin B_{12} also serves as a cofactor for methionine synthase, which remethylates homocysteine to methionine. Methionine is an essential amino acid that is used for protein synthesis or can be adenosylated to *S*-adenosylmethionine, a methyl donor for DNA methylation[8]. Deficiency of B vitamins

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and methionine, leading to the disruption of one-carbon metabolism, can interfere with DNA replication, DNA repair and regulation of gene expression through methylation, each of which could promote colorectal carcinogenesis[9].

Considering one-carbon metabolism nutrients need to be obtained from food, several epidemiological studies have assessed the association between dietary folate intake and the risk of colorectal cancer; however, the results remained inconclusive. Some[10–15], but not all[16–22], epidemiological studies reported an inverse association between folate intake and colorectal cancer risk. Similarly, inverse associations between vitamin B_2, vitamin B_6 and vitamin B_{12} and colorectal risk have been observed in some studies[23–27]. The evidence that methionine affects colorectal cancer is limited. A 2013 meta-analysis of eight prospective cohort studies found no significant association of dietary methionine intake with colorectal cancer risk, but with a decreased colon cancer risk[28]. To our knowledge, only one relevant study has been conducted among Chinese women[29], where dietary pattern was different from Western countries[30]. Moreover, China did not have a mandatory folic acid fortification policy which was common in some Western countries. This may provide a good opportunity to examine the association between folate in natural foods and colorectal cancer risk.

The objective of this study was to assess the associations between dietary intake of folate, vitamin B_2, vitamin B_6, vitamin B_{12} and methionine and colorectal cancer risk in a Chinese population. We hypothesised that nutrients mentioned above were inversely associated with colorectal cancer risk.

Material and methods

Study subjects

A detailed description of this ongoing case–control study which began in July 2010 has been reported elsewhere[31]. Briefly, potential case participants aged 30–75 years were continuously recruited from Sun Yat-sen University Cancer Center, Guangzhou, China. Eligible criteria for cases included that patients were Guangdong natives or those who had lived in Guangdong for at least 5 years as well as had a firstly, histologically confirmed colorectal cancer diagnosed no more than 3 months before the interview. Subjects who could not understand or speak Mandarin/Cantonese or had a history of any cancer were excluded. Moreover, subjects with a too low or too high energy intake (<2510 or >14 644 kJ/d (<600 or >3500 kcal/d) for women; <3347 or >17 573 kJ/d (<800 or >4200 kcal/d) for men)[32] were not included in the analysis. From July 2010 to April 2019, a total of 2817 eligible cases were identified and 2528 were successfully interviewed, with a response rate of 89·74 %. Of them, 303 patients did not complete the investigation mainly because of fatigue, communication barriers and refusal. Twenty-six subjects with an energy intake which did not meet the criteria were excluded. Finally, 2502 cases were included in the analysis.

The inclusion criteria for the controls were the same as those for the cases except that they had no prior history of any cancers and they were frequency-matched to cases by 5-year age group and sex. Control subjects came from two control groups. The first group was hospital-derived controls who were recruited from the inpatients admitted to the Departments of Otorhinolarygology, Plastic and Reconstructive Surgery and Vascular Surgery in the First-affiliated Hospital of Sun Yat-sen University during the same period. Hospital-derived controls mainly suffered from chronic otitis media, chronic sinusitis, sudden deafness, vocal cord polyp, trigeminal neuralgia, varicose veins, orthopaedics and facial paralysis. Totally, 1533 hospital-derived controls were identified, and 1357 were successfully interviewed, yielding a participation rate of 88·51 %. The second control group of 1181 community-derived controls was recruited from the apparently healthy community residents in the same cities invited through a variety of strategies such as written invitations, community advertisements, flyers or subjects' referrals. Therefore, the total number of controls was 2538.

We assumed that there were 25 % people with higher fotale, vitamin B_2, vitamin B_6, vitamin B_{12} and methionine among the general population, and the estimated OR between colorectal cancer risk and nutrients mentioned above were 0·64[11], 0·61[12], 0·52[33],0·49[33], 0·72[18], respectively, the type I error rate was less than 0·05 ($\alpha = 0{\cdot}05$), the power of test was 90 % ($\beta = 0{\cdot}10$) and the response rate was 90 %. Based on these assumptions, we required a sample size of 638 cases for folate, 529 cases for vitamin B_2, 319 cases for vitamin B_6, 273 cases for vitamin B_{12} and 1137 cases for methionine.

The present study was conducted according to the guidelines of Declaration of Helsinki. All study procedures were approved by the Ethical Committee of School of Public Health, Sun Yat-sen University. Informed consent was obtained from all study subjects.

Data collection

All study subjects were interviewed face-to-face by trained interviewers using a structured questionnaire that collected information on socio-demographic characteristics, family history of cancer, lifestyle (e.g. active and passive smoking, alcohol consumption and physical activity) and body measurements (weight and height). For women, menstrual and reproductive histories were also obtained. Relevant medical diagnosis and histological findings were extracted from the hospital medical records. BMI was calculated as the ratio of weight (kg):squared height (m^2). In this study, the definition of smoker was someone who had smoked at least one cigarette/d for more than 6 months consecutively or accumulatively in their lifetime. Passive smokers were non-smokers who reported being exposed to the smoke exhaled by smokers for at least 15 min/d over a week. Regular drinking was defined as the consumption of alcohol at least once per week during the past year. In addition, the level of physical activity was evaluated on the basis of self-reported occupational, household and leisure-time activities. According to labour intensity, occupational activity was categorised as follows: (1) not working or being retired, (2) mainly sitting, (3) light intensity, (4) moderate intensity or (5) vigorous intensity. Household and leisure-time activities were also classified into light, moderate and vigorous physical activities, and data were collected on their frequency (d/week) and typical duration

(h/d). The mean metabolic equivalent task-h value of each activity was obtained by estimating the average of all comparable activities in the Compendium of Physical Activities[34,35]. Metabolic equivalent task-h/week was calculated using the following equation: (how many d/week × how many h/d × metabolic equivalent task for a specific type of activity = metabolic equivalent task-h/week). Postmenopausal status was defined as at least 12 months since the last menstrual cycle.

Dietary assessment

Habitual dietary intake of participants during the previous year was assessed by an eighty-one-item FFQ. The food group mainly included cereal products, soya and soya products, vegetables, fruit, red and processed meat, poultry, fish and other seafood, egg, dairy products and nuts. Information on portion size and frequency of dietary intake was collected. Food photographs were used to help participants to quantify their dietary intakes. The amount of each food item consumed on average (g/d) was measured based on frequency of intake and portion size. Dietary intake of folate (μg/d), vitamin B_2 (mg/d), vitamin B_6 (mg/d), vitamin B_{12} (μg/d) and methionine (mg/d) was calculated according to the 2002 Chinese Food Composition Table[36].

The validity and reproducibility of the FFQ have been confirmed elsewhere[37]. The energy-adjusted Pearson's correlation coefficients comparing the second FFQ and 18-d dietary records were 0·35 for folate, 0·49 for vitamin B_2, 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, 0·62 for vitamin B_2, 0·57 for vitamin B_6, 0·60 for vitamin B_{12} and 0·49 for methionine.

Statistical analysis

All analyses were performed using SPSS 22.0 (SPSS, Inc.). Differences of demographic characteristics and dietary data between cases and controls were evaluated by the *t* test or Wilcoxon signed-rank test for the continuous variables, and the χ^2 test method for the categorical variables. Nutrient intakes were adjusted for total energy intake by the regression residual method[38]. Quartiles (Q1–Q4) of dietary B vitamin and methionine intakes were categorised based on the distribution among the controls for men and women separately. OR and 95 % CI for the associations between dietary B vitamin and methionine intakes and colorectal cancer risk were computed using multivariable logistic regression models, with the lowest quartile as the reference group. Tests for trend were performed by entering the categorical variables (Q1–Q4) as continuous variables in the regression models. Based on the characteristics comparison between cases and controls, or previous-reported confounders, the following variables were evaluated as potential confounders: age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, alcohol drinking, family history of cancer, occupational physical activity, household and leisure-time activities and energy intake. The intakes of red and processed meat were also included in the final multivariable regression models for the association between folate, vitamin B_2 and vitamin B_6 intake and colorectal cancer risk. As for the associations between vitamin B_{12} and methionine and colorectal cancer risk, vegetable and fruit intakes were additionally adjusted in the final models. All confounders were included as categorical variables except for age, BMI, household and leisure activities, intakes of energy, red and processed meat, vegetables and fruits, which were regarded as continuous variables. Stratified analysis by sex and sub-group analysis by cancer site (colon or rectal cancer) and by sources of controls (community-derived controls and hospital-derived controls) were conducted. Moreover, alcohol consumption is known to alter metabolism of B vitamins[39]. Hence, stratified analysis by alcohol consumption was also conducted. The interaction between sex, alcohol consumption and dietary B vitamin and methionine intakes in relation to colorectal cancer risk was assessed by creating cross-product terms and including the cross-product terms in multivariable regression, respectively. In addition, sensitivity analysis was conducted by using only hospital-derived controls. In this study, all *P* values were two-sided and statistical significance was determined at the $P < 0{\cdot}05$ level.

Results

There were 2502 cases in total, of which 1425 were men and 1077 were women. Among the cases, 1565 were diagnosed with colon cancer and 937 were diagnosed with rectal cancer. Cases and controls were matched well with age and sex (Table 1). Compared with control subjects, more cases were married, lived in rural area and had less education, a larger proportion of farmers, lower income, lower BMI, heavier occupational activity and fewer household and leisure activities. Case subjects also tended to have a higher frequency of regular smoking, regular alcohol consumption and have family history of cancer. No statistically significant differences were found in passive smoking, age at menarche and menopausal status.

Compared with the controls, the intake of energy-adjusted folate, vitamin B_2, vitamin B_6 and vitamin B_{12} was significantly lower among cases. No significant differences were observed for methionine intake (Table 2).

As shown in Table 3, vegetables were the richest source of folate (31·06 %), followed by grains (29·33 %) and eggs (10·86 %). Vitamin B_2 and vitamin B_6 were rich in vegetables, grains and red and processed meat. Red and processed meat, dairy products, seafood and eggs were the main food sources of vitamin B_{12}. Methionine was rich in grains, red and processed meat and seafood.

As presented in Table 4, the intakes of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} were inversely associated with colorectal cancer risk. After controlling for the potential confounders, the OR for the highest quartile compared with the lowest quartile intake were 0·62 (95 % CI 0·51, 0·74; $P_{trend} < 0{\cdot}001$) for folate, 0·46 (95 % CI 0·38, 0·55; $P_{trend} < 0{\cdot}001$) for vitamin B_2, 0·55 (95 % CI 0·46, 0·76; $P_{trend} < 0{\cdot}001$) for vitamin B_6 and 0·72 (95 % CI 0·60, 0·86; $P_{trend} < 0{\cdot}001$) for vitamin B_{12}. No significant association was found between methionine intake and colorectal cancer risk

Stratified analysis by sex showed that intakes of folate, vitamin B_2 and vitamin B_6 were inversely associated with colorectal cancer both in men and women (Table 5). However, vitamin B_{12}

Table 1. Demographic and selected risk factors of colorectal cancer cases and controls in the Chinese population*
(Mean values and standard deviations; medians and 25th, 75th percentiles; numbers and percentages)

	Cases (*n* 2502)		Controls (*n* 2538)		
	n	%	*n*	%	*P*
Age (years)					0·261
Mean		57·0		56·7	
SD		10·3		10·2	
Sex					0·944
Men	1425	57·0	1443	56·9	
Women	1077	43·0	1095	43·1	
Marital status					<0·001
Married	2383	95·2	2335	92·0	
Unmarried/divorced/widowed	119	4·8	203	8·0	
Residence					<0·001
Urban	1612	64·4	1997	78·7	
Rural	890	35·6	541	21·3	
Educational level					<0·001
Primary school or below	789	31·5	567	22·3	
Secondary school	697	27·9	616	24·3	
High school	606	24·2	678	26·7	
College or above	410	16·4	677	26·7	
Occupation					<0·001
Administrator/other white-collar worker	347	13·9	463	18·2	
Blue-collar worker	547	21·9	553	21·8	
Farmer/other	1608	64·3	1522	60·0	
Income (yuan/month)					0·003
<2000	354	14·1	317	12·5	
2001–5000	836	33·4	935	36·8	
5001–8000	737	29·5	785	30·9	
>8001	575	23·0	501	19·7	
BMI (kg/m^2)					0·013
Mean		23·3		23·6	
SD		3·2		3·1	
Smokers	978	39·1	773	30·5	<0·001
Passive smoking	682	27·3	750	29·6	0·071
Regular drinker	450	18·0	359	14·1	<0·001
Family history of cancer	362	14·5	145	5·7	<0·001
Occupational activity					<0·001
Non-working	316	12·6	894	35·2	
Sedentary	697	27·9	533	21·0	
Light occupation	675	27·0	600	23·6	
Moderate occupation	390	15·6	238	9·4	
Heavy activity occupation	424	16·9	273	10·8	
Household and leisure-time activities (MET-h/week)					<0·001
Median		28·9		34·3	
25th, 75th percentiles		9·0, 52·5		16·3, 55·9	
Age at menarche (years)†					0·801
Mean		15·0		15·0	
SD		2·4		2·0	
Menopausal status†					0·923
Premenopausal	301	27·9	304	27·8	
Postmenopausal	776	72·1	791	72·2	
Stage of disease					
I	373	14·91			
II	834	33·33			
III	806	32·31			
IV	450	17·99			
Unknown	39	1·55			

MET, metabolic equivalent task.
* Continuous variables were evaluated using *t* tests or Wilcoxon rank-sum tests. Categorical variables were evaluated using χ^2 tests.
† Among women sub-group.

and methionine intakes were found to be related to the decreased risk of colorectal cancer only among women. Compared with the lowest quartile, the adjusted OR in the highest quartile was 0·58 (95 % CI 0·44, 0·76; $P_{trend} < 0·001$) for vitamin B_{12} and 0·66 (95 % CI 0·49, 0·88; $P_{trend} = 0·003$) for methionine, respectively. Stratified analyses by alcohol consumption showed no significant interaction in the associations between different B vitamins and methionine and colorectal cancer risk ($P_{interaction} > 0·05$) (Table 6). Intakes of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} but not methionine were inversely

Table 2. Intakes of energy, B vitamins and methionine among case and control subjects in Guangdong, China*
(Mean values and standard deviations; medians (P_{50}) and 25th, 75th percentiles)

	Cases					Controls					
	Mean	SD	P_{25}	P_{50}	P_{75}	Mean	SD	P_{25}	P_{50}	P_{75}	*P*
Energy (kJ/d)	1557·90	481·89	1211·70	1492·32	1832·34	1647·55	526·75	1267·10	1548·42	1943·72	<0·001
Vegetables (g/d)	410·26	198·77	278·99	384·16	508·32	426·63	188·44	296·59	400·96	525·40	<0·001
Fruits (g/d)	111·35	91·03	45·71	89·61	152·29	146·24	110·99	68·09	125·61	193·17	<0·001
Red and processed meat (g/d)	123·01	61·92	79·83	114·99	155·13	100·67	55·32	60·40	93·39	132·51	<0·001
Folate (μg/d)†	216·00	56·16	178·06	209·45	245·55	229·40	55·37	191·77	223·27	259·75	<0·001
Vitamin B_2 (mg/d)†	0·87	0·23	0·71	0·84	1·00	0·93	0·24	0·77	0·91	1·07	<0·001
Vitamin B_6 (mg/d)†	0·84	0·20	0·70	0·82	0·96	0·88	0·20	0·74	0·86	1·00	<0·001
Vitamin B_{12} (μg/d)†	1·89	0·99	1·19	1·70	2·35	1·97	0·93	1·33	1·84	2·42	<0·001
Methionine (mg/d)†	1243·58	308·53	1032·61	1207·69	1397·38	1238·85	287·97	1038·32	1207·98	1399·23	0·626

* Wilcoxon rank-sum test comparing the median consumption levels between cases and controls.
† Consumption was adjusted for total energy intake by the regression residual method.

Table 3. Main food sources of dietary B vitamins and methionine among control subjects
(Percentages)

Food sources	Proportion (%)
Folate	
Vegetables	31·06
Grains	29·33
Eggs	10·86
Soya foods	9·30
Fruits	6·44
Seafood	4·83
Red and processed meat	3·76
Dairy products	1·42
Poultry	0·52
Vitamin B_2	
Vegetables	25·25
Grains	17·17
Red and processed meat	14·14
Dairy products	12·12
Seafood	10·10
Eggs	8·08
Poultry	4·04
Fruits	3·03
Soya foods	2·02
Vitamin B_6	
Vegetables	37·63
Grains	22·58
Red and processed meat	16·13
Fruits	6·45
Soya foods	3·23
Seafood	3·23
Poultry	3·23
Dairy products	2·15
Eggs	1·08
Vitamin B_{12}	
Red and processed meat	44·98
Dairy products	15·79
Seafood	15·31
Eggs	15·31
Grains	4·78
Poultry	4·31
Methionine	
Grains	33·67
Red and processed meat	25·27
Seafood	15·70
Eggs	6·17
Poultry	5·97
Vegetables	5·12
Dairy products	2·79
Soya foods	2·54
Fruits	0·96

associated with colorectal cancer both in non-drinkers and regular drinkers.

Sub-group analysis by cancer site showed that intakes of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} were inversely associated with the risk of both colon cancer and rectal cancer. However, methionine intake was inversely associated with rectal cancer only, with an adjusted OR of 0·82 (95 % CI 0·64, 1·04; $P_{trend} = 0·031$) comparing the highest with the lowest quartile (Table 7). Sub-group analysis by community-derived controls and hospital-derived controls showed no significant differences when using either group ($P_{interaction} > 0·05$) (online Supplementary Table S1). Sensitivity analysis by using only hospital-derived controls did not find a significant difference except that the association between methionine intake and colorectal cancer risk became statistically significant. Compared with the lowest quartile, the adjusted OR in the highest quartile was 0·76 (95 % CI 0·62, 0·94; $P_{trend} = 0·001$) (online Supplementary Table S2).

Discussion

In this case–control study, a significant inverse association was found between dietary intake of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} and the risk of colorectal cancer.

Folate is a critical cofactor in both biological methylation and nucleotide synthesis, necessary for DNA synthesis, replication, and repair and playing a role in cancer prevention. Aberrancies of each of these two processes are thought to be among the most common mechanisms leading to cancer[5]. The present study provided the evidence for the decreased risk of colorectal cancer with higher intake of folate. In agreement with our results, the inverse association between dietary folate and colorectal cancer risk was observed in some case–control studies[11,12] and cohort studies[13–15]. Moreover, a 2017 meta-analysis of fourteen cohort studies and nineteen case–control studies showed that higher folate intake was associated with 29 and 23 % decreased risk of colorectal cancer risk in cohort studies and case–control studies, respectively[10]. However, no significant association was found in some epidemiological studies[16–22].

The following reasons might give rise to the inconsistent results. First, foods are the main sources of folate in the

Table 4. Colorectal cancer according to quartiles (Q) of B vitamin and methionine intakes (Odds ratios and 95 % confidence intervals)

	Q1	Q2		Q3		Q4		P_{trend}
		OR	95 % CI	OR	95 % CI	OR	95 % CI	
Folate								
Median intake (μg/d)	168·19	207·13		240·18		291·06		
No. of cases/controls	866/633	676/635		525/635		435/635		
Crude OR	1·00	0·78	0·67, 0·90	0·50	0·52, 0·71	0·50	0·43, 0·59	<0·001
Adjusted OR1*	1·00	0·76	0·65, 0·90	0·62	0·52, 0·74	0·58	0·49, 0·70	<0·001
Adjusted OR2†	1·00	0·78	0·66, 0·92	0·65	0·54, 0·77	0·62	0·51, 0·74	<0·001
Vitamin B_2								
Median intake (mg/d)	0·65	0·84		0·98		1·21		
No. of cases/controls	899/635	652/634		537/634		414/635		
Crude OR	1·00	0·73	0·63, 0·84	0·60	0·51, 0·70	0·46	0·39, 0·54	<0·001
Adjusted OR1*	1·00	0·79	0·56, 0·92	0·61	0·51, 0·72	0·53	0·44, 0·63	<0·001
Adjusted OR2†	1·00	0·70	0·59, 0·83	0·53	0·44, 0·63	0·46	0·38, 0·55	<0·001
Vitamin B_6								
Median intake (mg/d)	0·65	0·80		0·92		1·10		
No. of cases/controls	796/634	657/635		548/635		501/634		
Crude OR	1·00	0·82	0·71, 0·96	0·69	0·60, 0·81	0·63	0·54, 0·74	<0·001
Adjusted OR1*	1·00	0·80	0·64, 0·90	0·69	0·58, 0·81	0·68	0·57, 0·82	<0·001
Adjusted OR2†	1·00	0·74	0·62, 0·87	0·59	0·49, 0·70	0·55	0·46, 0·76	<0·001
Vitamin B_{12}								
Median intake (μg/d)	1·02	1·59		2·10		2·97		
No. of cases/controls	811/633	565/635		547/635		579/635		
Crude OR	1·00	0·69	0·60, 0·81	0·67	0·58, 0·79	0·71	0·61, 0·83	<0·001
Adjusted OR1*	1·00	0·71	0·60, 0·84	0·66	0·56, 0·78	0·70	0·59, 0·83	<0·001
Adjusted OR2†	1·00	0·71	0·60, 0·85	0·69	0·58, 0·82	0·72	0·60, 0·86	<0·001
Methionine								
Median intake (mg/d)	944·78	1130·04		1295·94		1544·10		
No. of cases/controls	689/633	573/635		601/635		639/635		
Crude OR	1·00	0·83	0·71, 0·97	0·87	0·75, 1·02	0·93	0·79, 1·08	0·423
Adjusted OR1*	1·00	0·89	0·74, 1·06	0·84	0·70, 0·99	0·88	0·74, 1·04	0·104
Adjusted OR2†	1·00	0·93	0·78, 1·11	0·90	0·76, 1·08	0·94	0·79, 1·13	0·454

* OR1 was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, alcohol drinking, family history of cancer, occupational activity, household and leisure-time activities and total energy intake.
† OR2 was adjusted for the above confounders. Folate, vitamin B_2 and vitamin B_6 were additionally adjusted for red and processed meat intake. Vitamin B_{12} and methionine were additionally adjusted for vegetable and fruit intakes.

Chinese population, but mandatory food fortification exists in other countries, such as Canada and America, and the effect of folic supplements and fortification is questionable[40,41]. It is possible that different biological properties of dietary and other folate sources may have a different effect on carcinogenesis, and the true beneficial effect may be due to other components that coexist with folate in the diet[5,15]. Second, green vegetables are the major sources for folate in our population. Cooking methods of vegetables may cause the variation of folate retention[42]. It is well known that Chinese populations have different eating patterns of vegetables from Western countries[43]. This may result in the variation of the bioavailability and the effect of dietary folate intake. Third, different studies were adjusted for different confounding factors. In these six studies with null results[16,18,19,22,26,44], Ca or fibre was included in the multivariable regression models. It was reported that intake of Ca was highly correlated with folate[18], and fibre and folate were mainly derived from vegetables. Thus, Ca and fibre intake included in the multivariable regression models might cause over-adjustment and bias the risk estimates. Fourth, different genetic backgrounds could alter the association between folate and carcinogenesis[45].

The inverse associations between intakes of vitamin B_2, vitamin B_6 and vitamin B_{12} and colorectal cancer risk observed in the present study were consistent with some previous studies. Two[12,17] of the eight[12,16,17,19,20,46–48] observational studies reported an inverse association of vitamin B_2 intake with colorectal cancer risk. Results from one cohort study[17] and one case–control study[48] suggested an inverse association between dietary intake of vitamin B_6 and colorectal cancer risk, while four cohort studies found no significant association between vitamin B_6 intake and colorectal cancer risk[16,18,19,47]. Similarly, one[26] of the three[12,26,48] case–control studies reported an inverse association between vitamin B_{12} intake and colorectal cancer risk. Vitamin B_2, vitamin B_6 and vitamin B_{12} are interrelated since they all serve as cofactors in the reactions of one-carbon metabolism, and therefore low dietary intake of these nutrients may result in colorectal carcinogenesis via the induction of aberrations in DNA methylation and synthesis[9].

Methionine is necessary for the synthesis of *S*-adenosylmethionine, which is the primary methyl donor for methylation process[8]. A 2013 meta-analysis of eight prospective studies assessing methionine intake and colorectal cancer risk found a 23 % decreased risk for colon cancer when

Table 5. Colorectal cancer according to quartiles (Q) of B vitamin and methionine intakes stratified by sex (Odds ratios and 95 % confidence intervals)

	Men (*n* 1425/1443)					Women (*n* 1077/1095)					
	Q1	Q2	Q3	Q4	P_{trend}	Q1	Q2	Q3	Q4	P_{trend}	$P_{interaction}$
Folate											0·122
Median intake (μg/d)	167·45	205·21	238·48	288·27		169·30	211·14	243·66	295·03		
No.of cases/controls	505/360	394/361	306/361	220/361		361/274	282/273	219/275	215/273		
Crude OR (95 % CI)	1·00	0·78 (0·64, 0·94)	0·61 (0·49, 0·74)	0·43 (0·35, 0·54)	<0·001	1·00	0·78 (0·62, 0·99)	0·60 (0·48, 0·77)	0·60 (0·47, 0·76)	<0·001	
Adjusted OR1 (95 % CI)*	1·00	0·77 (0·62, 0·97)	0·62 (0·49, 0·79)	0·46 (0·36, 0·59)	<0·001	1·00	0·79 (0·61, 1·01)	0·67 (0·52, 0·89)	0·77 (0·58, 1·01)	0·023	
Adjusted OR2 (95 % CI)†	1·00	0·80 (0·64, 1·01)	0·65 (0·51, 0·83)	0·50 (0·39, 0·65)	<0·001	1·00	0·78 (0·60, 1·01)	0·69 (0·51, 0·91)	0·79 (0·61, 0·99)	0·034	
Vitamin B_2											0·446
Median intake (mg/d)	0·66	0·81	0·95	1·16		0·66	0·87	1·03	1·31		
No. of cases/controls	488/361	362/361	332/360	243/361		411/273	290/275	206/274	170/273		
Crude OR (95 % CI)	1·00	0·74 (0·61, 0·91)	0·68 (0·56, 0·83)	0·50 (0·40, 0·62)	<0·001	1·00	0·70 (0·56, 0·88)	0·50 (0·39, 0·63)	0·41 (0·32, 0·53)	<0·001	
Adjusted OR1 (95 % CI)*	1·00	0·76 (0·60, 0·95)	0·62 (0·49, 0·79)	0·48 (0·37, 0·62)	<0·001	1·00	0·78 (0·61, 1·00)	0·61 (0·46, 0·80)	0·58 (0·44, 0·78)	<0·001	
Adjusted OR2 (95 % CI)†	1·00	0·65 (0·52, 0·83)	0·50 (0·39, 0·64)	0·38 (0·29, 0·49)	<0·001	1·00	0·75 (0·59, 0·97)	0·56 (0·42, 0·74)	0·56 (0·42, 0·74)	<0·001	
Vitamin B_6											0·498
Median intake (mg/d)	0·64	0·78	0·90	1·08		0·66	0·83	0·95	1·17		
No. of cases/controls	423/361	402/361	315/360	285/361		373/255	255/275	232/273	217/274		
Crude OR (95 % CI)	1·00	0·95 (0·78, 1·16)	0·75 (0·61, 0·92)	0·67 (0·55, 0·83)	<0·001	1·00	0·68 (0·54, 0·86)	0·62 (0·49, 0·79)	0·58 (0·46, 0·74)	<0·001	
Adjusted OR1 (95 % CI)*	1·00	0·94 (0·74, 1·18)	0·69 (0·54, 0·88)	0·68 (0·53, 0·87)	<0·001	1·00	0·65 (0·50, 0·84)	0·67 (0·51, 0·87)	0·66 (0·50, 0·87)	0·004	
Adjusted OR2 (95 % CI)†	1·00	0·84 (0·67, 1·06)	0·57 (0·45, 0·73)	0·51 (0·39, 0·66)	<0·001	1·00	0·63 (0·48, 0·81)	0·62 (0·47, 0·82)	0·60 (0·45, 0·80)	<0·001	
Vitamin B_{12}											0·002
Median intake (μg/d)	1·01	1·53	2·05	2·90		1·02	1·65	2·18	3·06		
No. of cases/controls	404/361	307/361	346/360	368/361		407/273	258/275	201/273	211/274		
Crude OR (95 % CI)	1·00	0·76 (0·62, 0·94)	0·86 (0·70, 1·05)	0·91 (0·74, 1·12)	0·574	1·00	0·63 (0·50, 0·79)	0·49 (0·39, 0·63)	0·52 (0·41, 0·65)	<0·001	
Adjusted OR1 (95 % CI)*	1·00	0·80 (0·63, 1·02)	0·78 (0·61, 0·99)	0·79 (0·62, 1·00)	0·052	1·00	0·61 (0·47, 0·79)	0·51 (0·39, 0·66)	0·59 (0·45, 0·77)	<0·001	
Adjusted OR2 (95 % CI)†	1·00	0·82 (0·65, 1·05)	0·81 (0·64, 1·03)	0·82 (0·65, 1·05)	0·125	1·00	0·60 (0·46, 0·78)	0·52 (0·39, 0·68)	0·58 (0·44, 0·76)	<0·001	
Methionine											<0·001
Median intake (mg/d)	946·30	1120·40	1280·29	1524·81		941·30	1143·01	1318·87	1566·96		
No. of cases/controls	371/360	298/360	330/360	426/361		318/274	275/274	271/273	213/274		
Crude OR (95 % CI)	1·00	0·80 (0·65, 0·99)	0·89 (0·72, 1·10)	1·15 (0·94, 1·40)	0·107	1·00	0·87 (0·69, 1·09)	0·86 (0·68, 1·08)	0·67 (0·53, 0·85)	0·002	
Adjusted OR1 (95 % CI)*	1·00	0·82 (0·64, 1·04)	0·81 (0·63, 1·03)	1·01 (0·79, 1·28)	0·951	1·00	0·96 (0·74, 1·24)	0·84 (0·65, 1·10)	0·69 (0·52, 0·92)	0·007	
Adjusted OR2 (95 % CI)†	1·00	0·89 (0·69, 1·14)	0·92 (0·72, 1·18)	1·18 (0·92, 1·51)	0·169	1·00	0·98 (0·75, 1·28)	0·84 (0·64, 1·10)	0·66 (0·49, 0·88)	0·003	

* OR1 was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, alcohol drinking, family history of cancer, occupational activity, household and leisure-time activities and total energy intake.
† OR2 was adjusted for the above confounders. Folate, vitamin B_2 and vitamin B_6 were additionally adjusted for red and processed meat intake. Vitamin B_{12} and methionine were additionally adjusted for vegetable and fruit intakes.

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Table 6. Colorectal cancer according to quartiles (Q) of B vitamin and methionine intakes stratified by alcohol consumption (Odds ratios and 95 % confidence intervals)

	Non-drinkers (*n* 2052/2179)					Regular drinkers (*n* 450/359)					
	Q1	Q2	Q3	Q4	P_{trend}	Q1	Q2	Q3	Q4	P_{trend}	$P_{interaction}$
Folate											0·310
Median intake (μg/d)	168·19	199·90	232·09	271·79		168·11	206·92	239·63	291·83		
No. of cases/controls	701/544	566/545	416/545	369/545		159/89	123/91	104/90	64/89		
Crude OR (95 % CI)	1·00	0·81 (0·69, 0·95)	0·59 (0·50, 0·70)	0·53 (0·44, 0·63)	<0·001	1·00	0·77 (0·53, 1·11)	0·65 (0·44, 0·95)	0·40 (0·26, 0·60)	<0·001	
Adjusted OR1 (95 % CI)*	1·00	0·76 (0·64, 0·91)	0·62 (0·51, 0·75)	0·61 (0·50, 0·74)	<0·001	1·00	0·75 (0·49, 1·15)	0·68 (0·44, 1·06)	0·47 (0·29, 0·76)	0·004	
Adjusted OR2 (95 % CI)†	1·00	0·82 (0·68, 0·98)	0·63 (0·52, 0·76)	0·66 (0·54, 0·80)	<0·001	1·00	0·74 (0·48, 1·14)	0·72 (0·46, 1·12)	0·46 (0·28, 0·75)	0·003	
Vitamin B_2											0·491
Median intake (mg/d)	0·60	0·80	0·94	1·13		0·69	0·84	0·98	1·20		
No. of cases/controls	728/545	554/544	417/545	343/545		168/89	97/91	110/89	75/90		
Crude OR (95 % CI)	1·00	0·75 (0·64, 0·88)	0·57 (0·48, 0·68)	0·47 (0·39, 0·55)	<0·001	1·00	0·57 (0·38, 0·83)	0·66 (0·45, 0·95)	0·43 (0·29, 0·64)	<0·001	
Adjusted OR1 (95 % CI)*	1·00	0·85 (0·60, 0·90)	0·61 (0·39, 0·63)	0·57 (0·47, 0·70)	<0·001	1·00	0·49 (0·32, 0·77)	0·63 (0·40, 0·97)	0·47 (0·29, 0·75)	0·005	
Adjusted OR2 (95 % CI)†	1·00	0·76 (0·64, 0·92)	0·52 (0·42, 0·63)	0·49 (0·40, 0·60)	<0·001	1·00	0·44 (0·28, 0·70)	0·55 (0·35, 0·87)	0·39 (0·24, 0·63)	<0·001	
Vitamin B_6											0·636
Median intake (mg/d)	0·65	0·80	0·92	1·10		0·65	0·79	0·91	1·10		
No. of cases/controls	650/545	532/545	468/544	402/545		149/89	125/91	87/90	89/89		
Crude OR (95 % CI)	1·00	0·82 (0·69, 0·97)	0·72 (0·61, 0·85)	0·62 (0·52, 0·74)	<0·001	1·00	0·82 (0·56, 1·20)	0·58 (0·39, 0·86)	0·60 (0·40, 0·89)	0·003	
Adjusted OR1 (95 % CI)*	1·00	0·75 (0·62, 0·90)	0·69 (0·57, 0·83)	0·69 (0·57, 0·84)	<0·001	1·00	0·74 (0·48, 1·15)	0·55 (0·34, 0·87)	0·64 (0·40, 1·02)	0·039	
Adjusted OR2 (95 % CI)†	1·00	0·74 (0·62, 0·89)	0·61 (0·51, 0·74)	0·55 (0·45, 0·67)	<0·001	1·00	0·69 (0·44, 1·07)	0·58 (0·30, 0·77)	0·52 (0·32, 0·85)	0·003	
Vitamin B_{12}											0·481
Median intake (μg/d)	0·99	1·56	2·07	2·91		1·18	1·77	2·30	3·31		
No. of cases/controls	672/544	474/545	435/545	471/545		138/89	117/90	96/90	99/90		
Crude OR (95 % CI)	1·00	0·70 (0·60, 0·83)	0·65 (0·54, 0·76)	0·71 (0·60, 0·84)	0·001	1·00	0·84 (0·57, 1·12)	0·68 (0·46, 1·01)	0·72 (0·49, 1·06)	0·051	
Adjusted OR1 (95 % CI)*	1·00	0·78 (0·65, 0·93)	0·64 (0·53, 0·77)	0·73 (0·61, 0·89)	<0·001	1·00	0·92 (0·59, 1·42)	0·76 (0·48, 1·20)	0·63 (0·40, 0·99)	0·023	
Adjusted OR2 (95 % CI)†	1·00	0·73 (0·60, 0·89)	0·68 (0·56, 0·82)	0·75 (0·62, 0·91)	0·002	1·00	0·82 (0·52, 1·29)	0·78 (0·48, 1·25)	0·60 (0·37, 0·96)	0·038	
Methionine											0·948
Median intake (mg/d)	944·78	1124·17	1288·59	1529·00		953·99	1169·34	1348·83	1608·44		
No. of cases/controls	540/544	476/545	493/545	513/545		135/89	98/90	99/90	118/90		
Crude OR (95 % CI)	1·00	0·83 (0·70, 0·99)	0·86 (0·73, 1·02)	0·90 (0·74, 1·06)	0·596	1·00	0·72 (0·49, 1·06)	0·73 (0·47, 1·07)	0·86 (0·59, 1·26)	0·462	
Adjusted OR1 (95 % CI)*	1·00	0·90 (0·75, 1·09)	0·85 (0·70, 1·02)	0·88 (0·73, 1·07)	0·214	1·00	0·70 (0·44, 1·10)	0·64 (0·40, 1·01)	0·86 (0·55, 1·37)	0·474	
Adjusted OR2 (95 % CI)†	1·00	0·95 (0·79, 1·15)	0·90 (0·74, 1·10)	0·93 (0·77, 1·14)	0·403	1·00	0·75 (0·47, 1·20)	0·77 (0·47, 1·24)	1·05 (0·65, 1·70)	0·815	

* OR1 was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, alcohol drinking, family history of cancer, occupational activity, household and leisure-time activities and total energy intake.
† OR2 was adjusted for the above confounders. Folate, vitamin B_2 and vitamin B_6 were additionally adjusted for red and processed meat intake. Vitamin B_{12} and methionine were additionally adjusted for vegetable and fruit intakes.

Table 7. Associations between B vitamin and methionine intakes and colon and rectal cancer (Odds ratios and 95 % confidence intervals)

	Colon cancer (*n* 1565)					Rectal cancer (*n* 937)				
	Q1	Q2	Q3	Q4	P_{trend}	Q1	Q2	Q3	Q4	P_{trend}
Folate										
Median intake (μg/d)	169·31	207·16	240·19	290·93		168·90	207·26	240·33	290·95	
No. of cases/controls	517/633	427/635	352/635	269/635		349/633	249/635	173/635	166/635	
Crude OR (95 % CI)	1·00	0·82 (0·69, 0·98)	0·68 (0·57, 0·81)	0·52 (0·43, 0·62)	<0·001	1·00	0·71 (0·58, 0·87)	0·49 (0·40, 0·61)	0·47 (0·38, 0·61)	<0·001
Adjusted OR1 (95 % CI)*	1·00	0·78 (0·65, 0·94)	0·66 (0·55, 0·81)	0·58 (0·47, 0·71)	<0·001	1·00	0·74 (0·59, 0·91)	0·56 (0·44, 0·70)	0·62 (0·49, 0·79)	<0·001
Adjusted OR2 (95 % CI)†	1·00	0·79 (0·65, 0·95)	0·70 (0·58, 0·85)	0·61 (0·49, 0·75)	<0·001	1·00	0·75 (0·60, 0·93)	0·57 (0·45, 0·72)	0·66 (0·52, 0·84)	<0·001
Vitamin B_2										
Median intake (mg/d)	0·67	0·84	0·98	1·21		0·67	0·84	0·98	1·21	
No. of cases/controls	528/635	423/634	338/634	276/635		371/635	229/634	199/634	138/635	
Crude OR (95 % CI)	1·00	0·80 (0·68, 0·95)	0·64 (0·54, 0·76)	0·52 (0·44, 0·63)	<0·001	1·00	0·62 (0·51, 0·75)	0·54 (0·44, 0·66)	0·37 (0·30, 0·47)	<0·001
Adjusted OR1 (95 % CI)*	1·00	0·75 (0·62, 0·90)	0·62 (0·51, 0·75)	0·57 (0·46, 0·70)	<0·001	1·00	0·61 (0·55, 0·85)	0·61 (0·49, 0·77)	0·49 (0·38, 0·64)	<0·001
Adjusted OR2 (95 % CI)†	1·00	0·78 (0·64, 0·94)	0·53 (0·43, 0·65)	0·50 (0·40, 0·62)	<0·001	1·00	0·63 (0·51, 0·79)	0·53 (0·42, 0·67)	0·43 (0·33, 0·56)	<0·001
Vitamin B_6										
Median intake (mg/d)	0·65	0·80	0·92	1·10		0·66	0·80	0·92	1·10	
No. of cases/controls	480/634	405/636	357/633	323/635		316/634	252/636	191/633	178/635	
Crude OR (95 % CI)	1·00	0·84 (0·71, 0·99)	0·75 (0·63, 0·89)	0·67 (0·56, 0·80)	<0·001	1·00	0·80 (0·65, 0·97)	0·61 (0·49, 0·75)	0·56 (0·45, 0·70)	<0·001
Adjusted OR1 (95 % CI)*	1·00	0·77 (0·64, 0·93)	0·70 (0·58, 0·85)	0·69 (0·56, 0·85)	<0·001	1·00	0·79 (0·63, 0·98)	0·65 (0·51, 0·82)	0·69 (0·30, 0·87)	0·002
Adjusted OR2 (95 % CI)†	1·00	0·73 (0·60, 0·89)	0·61 (0·49, 0·74)	0·55 (0·45, 0·68)	<0·001	1·00	0·72 (0·56, 0·91)	0·52 (0·39, 0·69)	0·45 (0·30, 0·66)	<0·001
Vitamin B_{12}										
Median intake (μg/d)	1·02	1·59	2·10	2·96		1·02	1·59	2·11	2·95	
No. of cases/controls	475/633	356/635	355/635	379/635		336/633	209/635	192/635	200/635	
Crude OR (95 % CI)	1·00	0·75 (0·63, 0·98)	0·75 (0·63, 0·89)	0·80 (0·67, 0·95)	0·010	1·00	0·62 (0·51, 0·76)	0·57 (0·46, 0·70)	0·59 (0·48, 0·73)	<0·001
Adjusted OR1 (95 % CI)*	1·00	0·72 (0·59, 0·87)	0·71 (0·58, 0·86)	0·75 (0·61, 0·91)	0·004	1·00	0·72 (0·57, 0·90)	0·58 (0·46, 0·74)	0·63 (0·50, 0·80)	<0·001
Adjusted OR2 (95 % CI)†	1·00	0·72 (0·59, 0·88)	0·74 (0·61, 0·91)	0·77 (0·63, 0·95)	0·018	1·00	0·68 (0·54, 0·85)	0·60 (0·48, 0·76)	0·61 (0·48, 0·77)	<0·001
Methionine										
Median intake (mg/d)	945·64	1130·04	1295·89	1541·57		941·03	1130·17	1296·30	1536·50	
No. of cases/controls	397/633	344/635	407/635	417/635		292/633	229/635	195/635	220/635	
Crude OR (95 % CI)	1·00	0·86 (0·72, 1·04)	1·02 (0·86, 1·22)	1·05 (0·88, 1·25)	0·292	1·00	0·78 (0·64, 0·96)	0·66 (0·54, 0·82)	0·76 (0·62, 0·93)	0·002
Adjusted OR1 (95 % CI)*	1·00	0·89 (0·73, 1·09)	0·94 (0·77, 1·14)	0·94 (0·77, 1·15)	0·683	1·00	0·90 (0·72, 1·13)	0·67 (0·53, 0·84)	0·78 (0·62, 0·99)	0·008
Adjusted OR2 (95 % CI)†	1·00	0·92 (0·75, 1·20)	1·02 (0·83, 1·25)	1·01 (0·82, 1·24)	0·719	1·00	0·91 (0·71, 1·16)	0·70 (0·50, 0·88)	0·82 (0·64, 1·04)	0·031

Q, quartile.
* OR1 was adjusted for age, sex, marital status, residence, education, occupation, income level, BMI, smoking status, alcohol drinking, family history of cancer, occupational activity, household and leisure-time activities and total energy intake.
† OR2 was adjusted for the above confounders. Folate, vitamin B_2 and vitamin B_6 were additionally adjusted for red and processed meat intake. Vitamin B_{12} and methionine were additionally adjusted for vegetable and fruit intakes.

comparing the highest with the lowest methionine intake[28]. However, contrary to our initial assumption, the present study found no significant association between methionine intake and colorectal cancer risk, which was in agreement with previous studies[44,47]. Lack of an association might partly be attributed to dietary red meat intake. Methionine is a component of animal proteins, and a considerable proportion of methionine intake comes from red meat. Greater red meat intake seems to play a role in the development of colorectal cancer[49]. Therefore, the association between methionine intake and colorectal cancer risk might be modified by the effect of red meat intake. Moreover, an *in vivo* study showed that methionine restriction could be a possible approach to reduce cancer development[50] and increase blood glutathione[51], which detoxifies carcinogenic compounds and protects cells from oxidative DNA damage. More studies, especially prospective cohort studies, are needed to clarify this issue.

The present study showed that intakes of folate, vitamin B_2 and vitamin B_6 were inversely associated with colorectal cancer both in men and women, whereas vitamin B_{12} and methionine intakes were found to be related to decreased risk of colorectal cancer only among women. These sex differences have been reported in previous studies; however, the results were inconclusive[13,21,28,46,52]. Consistent with our results, one cohort study conducted in the Netherlands found that methionine was associated with a decreased risk of rectal cancer among women[52], but a 2013 meta-analysis of eight prospective studies indicated that a significant inverse association was observed between dietary methionine intake and colorectal cancer risk only in men but not in women[28]. The reason for sex-specific association of vitamin B_{12} and methionine with colorectal cancer risk remained unclear, and there are some plausible explanations. It was reported that dietary patterns between men and women were different, and men were liable to consume more meat and relatively lower amount of vegetables than women[53]. Different combinations of food groups or nutrients among men's and women's diets may have distinct influence on the carcinogenesis of colorectal cancer. Moreover, the development of tumours harbouring promoter hypermethylation, which was observed more often among women[54,55], is more sensitive to these nutrients. Sex hormones may also be a factor that affects the mechanism and leads to the sex discrepancy. However, the evidence is still limited, and it may be a chance finding in the present study. Therefore, whether the association between vitamin B_{12} and methionine intakes and colorectal cancer risk is modified by sex requires further investigation.

Stratified analysis by alcohol consumption suggested that there was no indication of effect modification between B vitamins and methionine and colorectal cancer by alcohol consumption. Consistent with our results, some previous studies found no significant interaction in the associations between folate[16,52], vitamin B_2[46,47], vitamin B_6[56,57], vitamin B_{12}[47,56] and methionine[47,52] and colorectal cancer risk modified by alcohol consumption. However, the National Institutes of Health-American Association of Retired Persons Diet and Health Study showed total folate intake-associated colorectal cancer risk reductions among men and women drinkers (both >15 and ≤15 g/d) but not non-drinkers[13]. The Women's Health Initiative Observational Study also found that higher B vitamin intakes were significantly associated with lower risk of colorectal cancer among current drinkers who consumed <13 g of alcohol/week among postmenopausal women[17]. Folate absorption and the one-carbon cycle could be disturbed by alcohol consumption, so that the effect of B vitamin and methionine intakes may be modified by alcohol use[39]. Therefore, drinkers have a higher folate demand, and reduced colorectal cancer risks are more likely to be observed among those with high folate intake. Moreover, it was reported that Asian populations have a stronger alcohol–colorectal cancer association than do Western populations[58,59]. One explanation for null interactions in our study is that the prevalence of alcohol intake is low in Chinese populations. Only 16·05 % participants are drinkers in our study, whereas 89·24 % participants are drinkers in the Women's Health Initiative Observational Study[17]. Further investigation in populations with low prevalence of alcohol intake is needed.

Sub-group analysis by cancer site showed that intakes of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} were inversely associated with the risk of both colon cancer and rectal cancer. These results were consistent with previous studies[16–18]. However, an inverse association was observed between methionine intake and rectal cancer risk but not colon cancer. In agreement with our result, a case–control study suggested that methionine intake was associated with a decreased risk of rectal cancer[33]. The Iowa Women's Health study found the protective relationship between methionine intake and distal colorectal cancer risk[18]. Meanwhile, another cohort study detected null association between methionine intake and colorectal cancer risk[47]. It has been reported that the different pH levels and microbiota composition of the cancer site may affect their susceptibility to components of the diet[60,61]. Furthermore, different sub-sites tumours have different clinical features[62] and genetic characteristics[63]. For example, methylation levels in normal mucosa differ between the proximal and distal colon, which could contribute to distinct effect on these sub-sites[64]. However, few studies have explored the association between methionine intake and a specific tumour site. Further studies on whether methionine intake has different effect in specific cancer sub-sites are needed to confirm.

Sensitivity analysis by using only hospital-derived controls showed that the association between methionine intake and colorectal cancer risk became statistically significant. The following reasons might explain the changes of the result. The hospital-derived controls were younger than all controls in the present study (51·4 *v.* 56·7 years). The median intake of methionine among hospital-derived controls was higher than that among all controls (1275·77 *v.* 1238·85 mg/d). As we know, the incidence of cancer increases with age[65] and the elderly were less likely to have enough intake. Additionally, the proportion of women in hospital-derived controls (51·4 %) was higher than that in all controls (43·1 %). Consistent with the results of sensitively analysis, sub-group analysis by sex also showed that methionine intake was inversely associated with colorectal cancer risk only in women but not in men.

The present study has the following strengths. First, this is the first study to examine the association between B vitamins and methionine and colorectal cancer risk both in men and women in China. Second, various dietary and non-dietary factors were collected and adjusted in the analysis. In addition, the sample size in our study is larger than that in previous case–control studies. Therefore, we had adequate power to detect the associations between B vitamins and methionine and colorectal cancer risk.

Some limitations of this study should also be considered. First, selection bias may exist in hospital-based case–control studies. Although the colorectal cancer patients were only recruited from Sun Yat-sen University Cancer Center, it is the biggest cancer centre in Southern China, where colorectal cancer patients shared similar clinical characteristics with those from other big hospitals shared in Guangdong or in mainland China[66]. Besides, the high participation rate (89·3 % for cases and 86·6 % for hospital-derived controls) also helped to minimise the potential influence of selection bias. Second, recall bias is also difficult to rule out in case–control studies. To diminish this bias, we only investigated newly diagnosed cases and made great efforts to interview them as soon as possible. The average time interval between the diagnosis of colorectal cancer and study interview was 9·8 d for the case subjects. Photographs of foods with usual portion size were also provided to help participants accurately quantify dietary intake. Third, this study did not measure plasma levels of B vitamins and cannot test its correlation with dietary B vitamins estimated by FFQ. Some studies reported that intakes of B vitamins estimated by FFQ were correlated significantly with plasma levels[67–69]. Fourth, in the present study, B vitamins from dietary supplements were not included in the analysis. This might affect the evaluation of association between B vitamins and colorectal cancer risk. However, a previous study showed that only 2·4 % of adults took vitamin supplements in China[70] and the influence of B vitamin intakes might not be of concern.

In conclusion, the present study indicated that higher intake of folate, vitamin B_2, vitamin B_6 and vitamin B_{12} was inversely associated with the risk of colorectal cancer in a Chinese population.

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The authors declare that there are no conflicts of interest.

Supplementary material

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