Folate, vitamin B$_6$, vitamin B$_{12}$ and methionine intakes and risk for nasopharyngeal carcinoma in Chinese adults: a matched case–control study

Fang-fang Zeng[1,2]†, Yuan-ting Liu[1,3]†, Xiao-ling Lin[4], Yu-ying Fan[4], Xing-lan Zhang[4], Chun-hua Xu[1,5] and Yu-ming Chen[1]*

[1]*Guangdong Provincial Key Laboratory of Food, Nutrition and Health, School of Public Health, Sun Yat-sen University, Guangzhou 510080, People's Republic of China*
[2]*Epidemiology Research Unit, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, People's Republic of China*
[3]*Central Hospital of Panyu District, Guangzhou 511400, People's Republic of China*
[4]*Ophthalmic Center, Yat-sen University, Guangzhou 510060, People's Republic of China*
[5]*Guangdong Provincial Security Department, Guangzhou 510050, People's Republic of China*

Abstract

Many studies have suggested that folate-related one-carbon metabolism-related nutrients may play a role in certain cancer risks, but few studies have assessed their associations with the risk for nasopharyngeal carcinoma (NPC). In this study, we investigated the association between four folate-related one-carbon metabolism-related nutrients (folate, vitamin B$_6$, vitamin B$_{12}$ and methionine) and NPC risk in Chinese adults. A total of 600 patients newly diagnosed (within 3 months) with NPC were individually matched with 600 hospital-based controls by age, sex and household type (urban *v.* rural). Folate, vitamin B$_6$, vitamin B$_{12}$ and methionine intakes were measured using a validated seventy-eight-item FFQ. A higher dietary folate or vitamin B$_6$ intake was associated with a lower NPC risk after adjusting for potential confounders. The adjusted OR of NPC for quartiles 2–4 (*v.* 1) were 0·66 (95 % CI 0·48, 0·91), 0·52 (95 % CI 0·37, 0·74) and 0·34 (95 % CI 0·23, 0·50) ($P_{trend} < 0·001$) for folate and 0·72 (95 % CI 0·52, 1·00), 0·55 (95 % CI 0·39, 0·78) and 0·44 (95 % CI 0·30, 0·63) ($P_{trend} < 0·001$) for vitamin B$_6$. No significant association with NPC risk was observed for dietary vitamin B$_{12}$ or methionine intake. The risk for NPC with dietary folate intake was more evident in the participants who were not exposed to toxic substances than in those who were exposed ($P_{interaction} = 0·014$). This study suggests that dietary folate and vitamin B$_6$ may be protective for NPC in a high-risk population.

Key words: Nasopharyngeal carcinoma: Folate: B vitamins: Methionine: Case–control studies

Nasopharyngeal carcinoma (NPC) is a rare neoplasm in most countries ($<1 \times 10^{-5}$ person-years), but a much higher incidence has been observed in southern China ($20–50 \times 10^{-5}$ person-years)[1–3]. Infection with the Epstein–Barr virus (EBV) is considered a key cause of NPC[1], but the ubiquity of EBV suggests that other cofactors, such as diet, may have a role in NPC aetiology and may contribute to the remarkable geographic variation[4,5]. However, the role of diet has scarcely been investigated.

There is much evidence suggesting that DNA hypomethylation may interfere with DNA replication and repair and activate numerous cancer-related genes, each of which can predispose an individual to develop neoplasia (e.g. NPC)[6,7]. The one-carbon metabolism pathway plays an important role in regulating DNA methylation[8] and mediating nucleotide synthesis[9]. Thus, a low methyl supply may induce global DNA

hypomethylation and aberrant DNA synthesis, thus promoting carcinogenesis[8].

Dietary factors involved in the one-carbon metabolism pathway mainly include carbon donors (e.g. folate, choline and betaine), intermediate-product methionine, and enzymatic cofactors (e.g. vitamins B$_6$ and B$_{12}$). Recently, we reported protective associations between NPC risk and dietary intake of choline and betaine[10]. Like choline and betaine, folate is an important methyl-group donor. Epidemiological studies have noted inverse associations between dietary intake or circulating levels of folate and the risk for cancer in the breasts[11], colon[12], ovaries[13], oesophagus and stomach[14] and pancreas[15]. Thus, it is reasonable to speculate that folate may reduce NPC risk. However, only one case–control study of 198 NPC Italian cases focused on NPC risk and found a null association[16]. Although many other studies have found an inverse association between

dietary[17–23] or blood[24–27] folate and the risk for head and neck cancer, the numbers of NPC cases in these studies were generally <100. In addition, all of these studies were conducted among populations with dietary habits and NPC risks different from those of the Chinese in south China[1–3]. Thus, further study of the folate–NPC association in high-risk Chinese adults is necessary. In addition, only a few studies of limited numbers of NPC cases have assessed the effects of vitamin B_6[16,19,20], B_{12}[23–25,27] and methionine[28] on the risk for head and neck cancer, and their results have been inconsistent.

Hence, the primary purpose of this study was to evaluate the associations between four key dietary nutrients in the folate-mediated one-carbon cycle (folate, vitamins B_{12} and B_6 and methionine) and NPC risk among the Chinese in south China. We explored the modifying effects of some potential risk factors, such as smoking and alcohol consumption, which may interact with folate to alter methylation levels[29].

Methods

Study population

We conducted a 1:1 matched case–control study in Guangdong Province, China[30]. Between July 2009 and March 2011, patients newly diagnosed (within 3 months) with histologically confirmed epithelial cell carcinoma of the nasopharynx hospitalised at the Sun Yat-sen University Cancer Center were identified. Patients aged 30–75 years and resident in the Guangdong Province for over 10 years were eligible. We excluded those with a family history of NPC, with substantial self-reported changes in dietary habits over the previous 5 years, with chronic diseases (e.g. diabetes, other cancers) that may lead to dietary habit change, and with cognitive impairment. Potential eligible patients from the Department of Nasopharyngeal Carcinoma and Department of Radiotherapy were tracked through electronic medical records and made an appointment with for face-to-face interviews within 1 week.

Controls matched by age (±3 years), sex and household type (urban/rural) were identified from the following four departments of Sun Yat-sen University Ophthalmic Center – Department of Ocular Trauma, Department of Glaucoma, Department of Corneal Disease and Department of Fundus – where they had been concurrently hospitalised within 1 week for ocular disease. The recruitment flow of the controls was similar to that of cases. The same selection criteria for the NPC cases were applied to the control subjects, except for history of NPC.

Written consent was obtained from each participant, and the Ethics Committee of the School of Public Health of Sun Yat-sen University approved the study protocol.

Data collection

The participants were interviewed in person by experienced interviewers with relevant medical knowledge using a structured questionnaire[30]. Demographic and socio-economic status (SES) were collected, along with occupational and domestic exposure to toxic substances, smoking history, alcohol consumption, history of chronic disease(s), medical history, physical activity over the past year and habitual dietary consumption. For obtaining data on exposure to toxic substances, we interviewed participants if they had never- or ever-exposure to one of the following factors over 1 year: loud noise, heat, dust environment, radiation, pesticides, heavy metals, organic solvents and cooking fumes. Height and weight were measured, and BMI (in kg/m²) was calculated. Each interviewer completed an equal number of case and control subject interviews. Additional NPC-related information was obtained from the medical records, such as histological type, tumour node metastasis (TNM) stage and EBV viral capsid antigen (VCA)-IgA and early antigen (EA)-IgA antibody titres.

Assessment of dietary intake

Dietary intake was evaluated using a validated FFQ containing seventy-eight items of commonly eaten foods in Guangdong[30]. The participants were asked the frequency (never, per year, month, week or day) and amount (in bowls, grams, boxes, cups, etc.) of food intake for each food item during the year before diagnosis (for cases) or before the interview (for controls). Coloured pictures of different portion sizes of food were provided to help quantify the food consumed. Daily intakes of dietary energy and nutrients (including folate, vitamin B_6, vitamin B_{12} and methionine) were estimated using the *China Food Composition Table*[31]. The reproducibility and validity of the FFQ have been reported previously[32,33]. The correlation coefficients for dietary folate, vitamin B_6, vitamin B_{12} and methionine intakes were 0·35, 0·26, 0·50 and 0·36 when comparing the second FFQ and 18-d dietary records, and were 0·60, 0·57, 0·60 and 0·49 when comparing the two FFQ[11,32].

Statistical analysis

The analysis was performed using SPSS (version 17.0; SPSS Inc.). The differences between cases and controls were determined using the *t* test for continuous data and the χ^2 test for categorical data. All of the statistical tests were two-sided and considered statistically significant when $P < 0.05$.

The dietary intake of nutrients was adjusted for total energy using the residual method[34]. Energy-adjusted intakes of folate-mediated one-carbon metabolism-related nutrients were grouped into quartile 1 to quartile 4 (Q1–Q4) based on control subjects by sex. Sex-specific cutoffs were then applied to the cases. The lowest quartile (Q1) served as the reference group. Univariate (crude) and multivariate (adjusted) conditional logistic regression models were used to assess the associations between the quartiles of intake and NPC risk, and OR and 95 % CI were calculated. Crude adjusted OR (95 % CI) were obtained without further adjustment of covariates; adjusted OR (95 % CI) were obtained after covariates had been adjusted for age, BMI, occupation, marital status, educational level, household income, smoking status, drinking status, exposure to potentially toxic substances, multivitamin supplementation, chronic rhinitis history, physical activity and daily energy intake (log-transformed) for each nutrient. The multivariate-adjusted model was treated as the final model for conclusion. The criteria for entry and non-entry of these confounders were $P < 0.05$ and $P > 0.10$, respectively, using the forward stepwise method.

Stratified analyses were conducted to determine whether the folate–NPC associations were modified according to potential risk factors, including sex (women/men), smoking (yes/no), alcohol drinking (yes/no) and exposure to toxic substances (yes/no). We used unconditional logistic regression models to assess the associations because these factors were not matched between NPC patients and control subjects. The potential interactions were also examined via multiplicative interaction terms in the multivariate unconditional logistic regression models. We also conducted sensitivity analyses to explore whether the results were significantly changed after excluding those who used multivitamins using unconditional logistic regression models.

Results

Study participants

Among 1063 cases and 800 controls, 461 cases (mean age: 47·2 years; 23·9 % women) and 186 controls (mean age: 48·5 years; 24·7 % women) were excluded because of the following reasons: discomfort with completing the questionnaire (twenty cases and seven controls); refusal to participate (260 cases and forty-one controls); change of dietary habits (forty-nine cases and sixty-eight controls); language difficulties (twelve cases and thirteen controls); and having left the hospital (120 cases and fifty-seven controls). We further excluded two cases who reported implausible energy intake (<2928 or >17 572 kJ/d for men; <2092 or >14 644 kJ/d for women (<700 or >4200 kcal/d for men; <500 or >3500 kcal/d for women)) in the data analysis. In addition, fourteen controls were excluded because of 1:1 match to cases. Finally, 600 (56·4 %) cases and 600 (75 %) controls were included in the analyses. The majority of the 600 cases were in late-stage NPC (stages III and IV) (85 %), had undifferentiated carcinoma (95 %) and were seropositive for EBV VCA-IgA (93 %) and EA-IgA (75 %). The controls included 249 (41·3 %) cases of ocular fundus disease, 111 (18·5 %) cases of glaucoma, 107 (17·8 %) cases of ocular trauma and 133 (22·2 %) cases of other ocular diseases.

The demographics, lifestyle characteristics and selected NPC risk factors of the cases and controls are presented in Table 1. Compared with the controls, the cases were more likely to have higher BMI, education level, meat and fish intake, be married, and have a history of chronic rhinitis, but to have a lower activity level.

The NPC cases consumed less folate (268 $v.$ 295 µg/d; $P < 0.001$) and vitamin B_6 (1·06 $v.$ 1·14 mg/d; $P < 0.001$), but more vitamin B_{12} (2·51 $v.$ 2·30 µg/d; $P = 0.004$) and methionine (1·63 $v.$ 1·57 g/d; $P = 0.013$), compared with controls (Table 2). In addition, cases consumed higher meat and fish, but lower energy, vegetable and fruit, compared with controls (Table 2).

Both vegetables and cereal were the main source of folate (33·3 and 24·0 %) and vitamin B_6 (23·3 and 40·6 %); meat (52·9 %) and fish (23·5 %) were the major source of vitamin B_{12}; and cereal (34·6 %) and meat (29·5 %) were the major source of methionine.

Correlations between folate-mediated one-carbon metabolism-related nutrients

The intakes of folate, vitamin B_6, vitamin B_{12} and methionine by the controls were all significantly correlated with each other,

except between vitamin B_{12} and folate or vitamin B_6 among women. The correlations were highest between folate and vitamin B_6 and lowest between folate and vitamin B_{12} for both men and women (Table 3).

Folate-mediated one-carbon metabolism-related nutrients and risk for nasopharyngeal carcinoma

Univariate analyses showed dose-dependent and inverse associations between NPC risk and dietary intake of folate and vitamin B_6 (both $P_{trends} < 0.001$), but a positive relationship with methionine intake ($P_{trend} = 0.031$). After adjustment for covariates, significant associations remained for dietary folate and vitamin B_6 intake (Table 4). Compared with the lowest quartile of intake, the OR for quartiles 2–4 were 0·66, 0·52 and 0·34 ($P_{trend} < 0.001$) for folate, 0·72, 0·55 and 0·44 ($P_{trend} < 0.001$) for vitamin B_6, 0·76, 1·01 and 1·03 ($P_{trend} > 0.05$) for B_{12}, and 1·10, 1·38 and 1·33 ($P_{trend} > 0.05$) for methionine.

We also examined whether the folate–NPC relationship varied according to sex, smoking, alcohol consumption and exposure to toxic substances (Table 5). The favourable association between folate intake and NPC risk was more substantial among the participants without ($v.$ with) toxic substance exposure ($P_{interaction} = 0.014$). There were no significant interactions after stratification by sex, smoking and alcohol consumption (all $P_{interactions} > 0.50$). In addition, sensitivity analyses indicated that the results were not considerably changed after excluding those who used multivitamins (online Supplementary Table S1).

Discussion

This case–control study investigated the association between dietary nutrients involving the one-carbon metabolism pathway (e.g. folate, vitamins B_6 and B_{12} and methionine intake) and the risk for NPC in a high-risk population in southern China. Dietary folate and vitamin B_6 intakes were inversely related to NPC risk. A null association was observed for vitamin B_{12} and methionine.

Many studies have found protective associations between the intake of folate and vitamin B_6 and the risk for cancer in the breasts[11], colon[12], ovaries[13] and other organs[14,15]. For example, from a case–control study with 581 pairs of breast cancer cases and controls conducted in the same geographic area as our study, Zhang et al.[11] reported that folate and vitamin B_6 intakes were inversely associated with breast cancer risk. A combined analysis of the data from the Nurses' Health Study and the Health Professional Follow-up cohort showed an inverse association between the intake of folate and vitamin B_6 and colon cancer risk[12]. Consistent with these findings, our study, which had a relatively larger number of NPC cases (600 cases), showed that both folate and vitamin B_6 intake were inversely associated with NPC risk. To our knowledge, only one previous hospital-based case–control study has focused on the relationship between dietary folate or vitamin B_6 intake and NPC risk. It showed a non-significant association, possibly due to the limited study size of only 198 NPC cases[16]. Our findings and other related studies support a protective association

Table 1. Comparison of the demographics, lifestyle characteristics and selected nasopharyngeal carcinoma risk factors of cases and controls
(Mean values and standard deviations; numbers and percentages)

	Cases (n 600)		Controls (n 600)		
Characteristics	Mean	SD	Mean	SD	P
Continuous variables					
Age (years)	47·4	9·0	47·4	9·0	0·992
BMI (kg/m^2)	23·2	3·1	22·7	2·8	0·002
Physical activity (MET-h/d)*	38·4	9·4	39·5	11·8	0·073
	n	%	n	%	P
Categorical variables					
Region of residence					0·185
Pearl River Delta region	424	70·7	420	70·0	
Western Guangdong	74	12·3	67	11·2	
Northern Guangdong	44	7·3	64	10·7	
Eastern Guangdong	58	9·7	49	8·2	
Household type					1·000
Urban	399	66·5	399	66·5	
Rural	201	33·5	201	33·5	
Occupation					<0·001
Light intensity of activity	228	38·0	192	32·0	
Moderate intensity of activity	188	31·3	148	24·7	
Heavy intensity of activity	184	30·7	250	41·7	
Marital status					0·007
Married	590	98·3	574	95·7	
Unmarried/divorced/widowed	10	1·7	26	4·3	
Education level					<0·001
Primary school or below	109	18·2	130	21·7	
Secondary school	190	31·7	229	38·2	
High school	170	28·3	158	26·3	
College or above	131	21·8	83	13·8	
Household income (yuan/month per person)					0·052
≤500	54	9·0	68	11·3	
501–1500	202	33·7	236	39·3	
1501–3000	158	26·3	153	25·5	
>3000	186	31·0	143	23·8	
Smoking status†					0·549
Smoker	221	36·8	213	35·5	
Ex-smoker	73	12·2	64	10·7	
Non-smoker	306	51·0	323	53·8	
Drinking status‡					0·283
Drinker	118	19·7	121	20·2	
Ex-drinker	36	6·0	24	4·0	
Non-drinker	446	74·3	455	75·8	
Exposure to toxic substances§	274	45·7	269	44·8	0·814
Chronic rhinitis history	164	27·3	108	18·0	<0·001
Multivitamin supplement user	32	5·3	35	5·8	0·798

MET, metabolic equivalent.
* Physical activity included daily occupational, leisure time, and household chores activities, evaluated by MET-h/d.
† Smoker included current smoker and ex-smoker who had smoked at least 1 cigarette/d for at least 6 months earlier.
‡ Alcohol drinker included current drinker and ex-drinker who had consumed wine at least once a week for at least 6 consecutive months.
§ Exposure to potential toxic substances included ever being exposed to one of the following substances over the past year: heat, organic solvents, pesticides, heavy metals, smoke from burning incense, anti-mosquito coils, new furniture or decoration and radiation.

between folate and vitamin B$_6$ and the risk for NPC and certain other cancers. However, because of the scarcity of available evidence, further prospective studies are needed to confirm these protective associations.

A possible mechanism linking nutrients in the one-carbon metabolism pathway with cancer risk is their effect on the maintenance of nuclear and mitochondrial genome integrity[8,9]. In this pathway, carbon donors (e.g. folate, choline and betaine) can convert a one-carbon moiety to homocysteine to generate methionine, and then produce S-adenosyl methionine (SAM). SAM is the universal methyl donor required to prevent uracil

misincorporation into DNA and hypomethylation of DNA, and subsequently to maintain the methylation patterns in DNA that determine gene expression and chromosome conformation[9]. Diets deficient in methyl donors (e.g. folate) may lead to decreased tissue SAM, global DNA hypomethylation and ultimately hepatic tumourigenesis in rodents[35].

Vitamin B$_6$ and B$_{12}$ play an important role as co-enzymes in one-carbon metabolism[8]. Vitamin B$_6$ mediates the conversion of uracil to thymidylate to regenerate 5,10-methylenetetrahydrofolate[8]. In addition, vitamin B$_6$ may influence NPC risk through its antioxidant properties, as studies have reported that vitamin B$_6$

Table 2. Comparison of dietary intake between nasopharyngeal carcinoma cases and controls
(Means, medians and interquartile ranges (IQR))

	Cases (n 600)					Controls (n 600)					
	Mean	Median	IQR	Minimum	Maximum	Mean	Median	IQR	Minimum	Maximum	P
Energy intake (kJ/d)	7836	7447	6079, 9309	2912	17 568	8171	7665	6330, 9497	2552	17 543	0·029
Energy intake (kcal/d)	1873	1780	1453, 2225	696	4199	1953	1832	1513, 2270	610	4193	0·029
Folate intake (g/d)*	268	261	221, 303	118	752	295	285	239, 340	100	622	<0·001
Vitamin B₆ intake (g/d)*	1·06	1·03	0·88, 1·20	0·47	2·23	1·14	1·1	0·94, 1·31	0·41	2·41	<0·001
Vitamin B₁₂ intake (g/d)*	2·51	2·14	1·47, 3·12	0·21	9·38	2·3	2·03	1·43, 2·90	0·18	9·97	0·004
Met intake (g/d)*	1·63	1·56	1·28, 1·86	0·66	4·21	1·57	1·51	1·27, 1·80	0·68	3·69	0·013
Cereal intake (g/d)*	500	451	369, 595	144	1688	496	439	365, 551	58	3926	0·75
Meat intake (g/d)*	240	219	163, 294	27	802	216	199	150, 268	14	583	<0·001
Vegetable intake (g/d)*	356	329	221, 460	32	1410	429	393	286, 542	44	1269	<0·001
Fruit intake (g/d)*	142·6	115·6	62·9, 191·7	0·1	751	172·9	137·5	71·0, 230·4	0·1	831·6	<0·001
Fish intake (g/d)*	62·4	44·2	22·4, 84·1	0·7	290·3	51·2	35·3	15·0, 67·3	0·3	373	0·003

Table 3. Spearman's rank correlation coefficients (r_S) of the association between dietary energy-adjusted folate, vitamin B₆, vitamin B₁₂ and methionine intake among controls, divided by sex†

	Vitamin B₆		Vitamin B₁₂		Met	
	Men	Women	Men	Women	Men	Women
Folate	0·721***	0·739***	0·148**	0·047	0·398***	0·361***
Vitamin B₆	1·000	1·000	0·307***	0·069	0·561***	0·519***
Vitamin B₁₂			1·000	1·000	0·608***	0·532***
Methionine					1·000	1·000

** $P < 0.01$, *** $P < 0.001$.
† Adjusted for age and household type.

Table 4. Risk for nasopharyngeal carcinoma for quartiles (Q) of dietary folate, vitamin B₆, vitamin B₁₂ and methionine intake in Guangzhou, China
(Medians and numbers; odds ratios and 95 % confidence intervals)

	Cases (n 600)		Controls (n 600)				Crude		Adjusted*	
	Median	n	Median	Cutoffs for women	Cutoffs for men	n	OR	95 % CI	OR	95 % CI
Q of folate (µg/d)										
Q1	209	222	211	215	250	150	1		1	
Q2	266	171	264	255	294	150	0·75	0·55, 1·02	0·66	0·48, 0·91
Q3	304	127	313	310	346	150	0·57	0·41, 0·78	0·52	0·37, 0·74
Q4	384	80	384	–	–	150	0·37	0·26, 0·52	0·34	0·23, 0·50
P_{trend}								<0·001		<0·001
Q of vitamin B₆ (mg/d)										
Q1	0·84	210	0·84	0·82	0·99	150	1		1	
Q2	1·04	169	1·05	0·97	1·14	150	0·78	0·58, 1·06	0·72	0·52, 1·00
Q3	1·19	129	1·21	1·15	1·33	150	0·6	0·43, 0·83	0·55	0·39, 0·78
Q4	1·43	92	1·46	–	2·41	150	0·44	0·31, 0·61	0·44	0·30, 0·63
P_{trend}								<0·001		<0·001
Q of vitamin B₁₂ (µg/d)										
Q1	1·1	151	1·06	1·15	1·60	150	1		1	
Q2	1·78	124	1·79	1·62	2·19	150	0·81	0·57, 1·13	0·76	0·53, 1·10
Q3	2·48	163	2·46	2·17	3·18	150	1·1	0·80, 1·51	1·01	0·72, 1·42
Q4	3·97	162	3·65	–	–	150	1·08	0·77, 1·50	1·03	0·72, 1·47
P_{trend}								0·339		0·835
Q of methionine (g/d)										
Q1	1·14	119	1·14	1·05	1·37	150	1		1	
Q2	1·43	146	1·45	1·25	1·59	150	1·23	0·88, 1·70	1·1	0·78, 1·57
Q3	1·69	171	1·66	1·45	1·90	150	1·46	1·05, 2·03	1·38	0·96, 1·97
Q4	2·14	164	2·04	–	–	150	1·4	1·00, 1·96	1·33	0·92, 1·90
P_{trend}								0·031		0·119
Multivitamin supplement use										
No	–	568	–	–	–	565	1		1	
Yes	–	32	–	–	–	35	0·72	0·43, 1·20	0·76	0·46, 1·26

* Crude adjusted OR (95 % CI): without further adjustment; adjusted OR (95 % CI): covariates adjusted for age, BMI, occupation, marital status, educational level, household income, smoking status, drinking status, exposure to potentially toxic substances, multivitamin supplementation, chronic rhinitis history, physical activity and daily energy intake (log-transformed) for each nutrient. Multivitamin supplementation was not adjusted for multivitamin supplement use.

F.-F. Zeng et al.

Table 5. Risk for nasopharyngeal carcinoma for quartiles (Q) of dietary energy-adjusted folate intake by sex and potential risk factors in Guangzhou, China* (Numbers, odds ratios and 95% confidence intervals)

Q of folate (μg/d)	Q1			Q2				Q3				Q4				P_{trend}	$P_{interaction}$
	n (cases)	n (controls)	OR	n (cases)	n (controls)	OR	95 % CI	n (cases)	n (controls)	OR	95 % CI	n (cases)	n (controls)	OR	95 % CI		
Sex																	0.979
Men	174	112	1.00	126	112	0.67	0.46, 0.96	84	112	0.48	0.33, 0.71	64	112	0.35	0.24, 0.53	<0.001	
Women	48	38	1.00	45	38	0.82	0.44, 1.54	43	38	0.73	0.39, 1.37	16	38	0.26	0.12, 0.56	0.001	
Smoking status																	0.247
Smoker	98	60	1.00	53	49	0.66	0.40, 1.10	37	42	0.54	0.31, 0.93	33	62	0.33	0.19, 0.55	<0.001	
Ex-smoker	22	16	1.00	20	17	0.9	0.36, 2.24	18	17	0.81	0.32, 2.04	13	14	0.71	0.26, 1.92	0.316	
Non-smoker	102	74	1.00	98	84	0.77	0.51, 1.19	72	91	0.50	0.32, 0.78	34	74	0.26	0.15, 0.44	<0.001	
Drinking status																	0.555
Drinker	39	26	1.00	34	27	0.88	0.42, 1.87	22	25	0.59	0.27, 1.30	23	43	0.32	0.15, 0.67	0.002	
Ex-drinker	15	7	1.00	10	1	4.67	0.50, 43.96	5	7	0.33	0.08, 1.43	6	9	0.31	0.08, 1.22	0.182	
Non-drinker	168	117	1.00	127	122	0.73	0.52, 1.03	100	118	0.59	0.42, 0.85	51	98	0.37	0.24, 0.55	<0.001	
Exposure to toxic substances																	0.014
Yes	97	84	1.00	88	72	1.04	0.67, 1.60	52	60	0.72	0.45, 1.17	37	53	0.55	0.32, 0.92	0.014	
No	125	66	1.00	83	78	0.51	0.32, 0.79	75	90	0.41	0.26, 0.65	43	97	0.19	0.12, 0.32	<0.001	

* See Table 3 for the covariates.

is effective at scavenging free radicals that can promote carcinogenesis[36]. Vitamin B_{12} is needed for methionine synthase and is responsible for catalysing the methylation of homocysteine to methionine[8]. However, we failed to find any significant association for vitamin B_{12} in our study, probably because of the significantly lower levels of vitamin B_{12} (2·30 μg/d) compared with other nutrients (e.g. 294·6 μg/d (folate), 1·14 mg/d (vitamin B_6) and 1·57 g/d (methionine)). The resulting larger random measurement error for vitamin B_{12} may have led to greater variation and probably attenuated the associations to the null[37].

As mentioned above, methionine is also an intermediate methyl donor in one-carbon metabolism. However, a null association was observed between methionine intake and NPC risk in our study, similar to the results found in some studies on breast cancer[38], oral and pharyngeal cancer[28] and lung cancer[39], but not in other studies on ovarian cancer[13] and breast cancer[40]. The inconsistent findings across studies may be partly due to differences in the type of cancer, study design and population, sample size, method of measuring dietary nutrients and adjustments for covariates. In addition, two sources of methionine can be used in vivo: dietary and biotransformational. Pelucchi et al.[28] suggested that if methionine levels are low more folate is used as methyltetrahydrofolate to form methionine.

As reported in our previous article[10], we observed that the folate–NPC association was more significant among those without exposure to toxic substances (e.g. organic solvents, pesticides, heavy metals, smoke from burning incense and new furniture or decor) than among those with exposure ($P_{interaction} = 0·014$). Our findings provided evidence that exposure to toxic substances might mitigate the favourable effects of folate intake. The biological rationale for this finding is unclear. It is possible that toxic substances decrease DNA methylation[41], and hence more folate is needed to achieve a favourable effect. Further studies are warranted to confirm these findings and to elucidate the pathophysiological mechanisms.

Our study has important strengths, including a relatively large sample size providing higher power to detect associations and allowing potential confounders to be taken into account; the evaluation of dietary habits using a reproducible and validated FFQ; portion size assessment using visual aids; the completion by each interviewer of an equal proportion of interviews for both cases and controls to minimise information bias; and matching on key confounders to minimise confounding.

Potential limitations of our study should also be considered. First, we could not rule out the possibility of selection bias due to the hospital-based case–control study design, although we reduced the potential by selecting participants from the two comparable hospitals with the same catchment area in southern China. In addition, the dietary habits of hospital-based controls may differ from those of the source population. However, we included only those control subjects with ocular diseases that might not change their dietary habits. Further, the use of hospital control subjects tends to reduce the differential report on diet[42]. Second, a case–control design is unlikely to establish temporal links, although we minimised possible reverse causation by excluding participants with essential changes in dietary habits over the past 5 years and including only newly diagnosed cases (within 3 months). Third, the small number of participants among

cases (56·4 %) and controls (75·0 %) might reduce the generalisibility of the findings. We found that the age and sex of included cases and controls were comparable to those who did not participate. However, we could not further compare other characteristics because of lack of information for non-participants. Fourth, recall bias in dietary assessment using an FFQ is difficult to avoid in a case–control study. However, misclassification in dietary evaluation is unlikely to differ between cases and controls because of minimal public awareness of the cancer-preventing effects of these nutrients. Fifth, we did not determine the internal levels of these nutrients in the body, and the relatively less precise assessment by the FFQ may have attenuated any associations. Sixth, in the present study, we only assessed dietary nutrient intakes and each multivitamin supplement, but no data were available for individual supplements, and most common Chinese multivitamins contained folate, vitamin B_6 and B_{12}. Thus, we could not exclude the possibility of potential misclassifications of the intakes of some nutrients. Seventh, the differences in SES between the cases and controls suggested that they might have different lifestyles that might affect the NPC risk or modify the studied associations. However, after adjustment for SES, the associations were not considerably changed (Table 5), and stratified analyses indicated no significant interactions between folate or vitamin B_6 and SES (occupation, marital status, education level and household income) ($P_{interaction}$ range: 0·236–0·988, data not shown), suggesting that these SES factors did not significantly influence the associations found. Finally, we could not exclude the potentially confounding effects of other nutrients or food components coexisting with vitamin B_6 and folate.

In conclusion, our findings suggest protective associations between the risk for NPC and dietary folate and vitamin B_6 intake, especially among patients who have not been exposed to toxic substances. Considering the pitfalls of a hospital-based case–control study, confirmation of our results by other large well-designed studies, especially large-scale cohort studies, is warranted.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114515004146

References

1. Yu MC & Yuan JM (2002) Epidemiology of nasopharyngeal carcinoma. *Semin Cancer Biol* **12**, 421–429.
2. Bray F, Haugen M, Moger TA, *et al.* (2008) Age-incidence curves of nasopharyngeal carcinoma worldwide: bimodality in low-risk populations and aetiologic implications. *Cancer Epidemiol Biomarkers Prev* **17**, 2356–2365.
3. Jia WH, Huang QH, Liao J, *et al.* (2006) Trends in incidence and mortality of nasopharyngeal carcinoma over a 20–25 year period (1978/1983–2002) in Sihui and Cangwu counties in southern China. *BMC Cancer* **6**, 178.
4. Macsween KF & Crawford DH (2003) Epstein-Barr virus-recent advances. *Lancet Infect Dis* **3**, 131–140.
5. Yoshizaki T, Kondo S, Wakisaka N, *et al.* (2013) Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma. *Cancer Lett* **337**, 1–7.
6. Suzuki H, Maruyama R, Yamamoto E, *et al.* (2012) DNA methylation and microRNA dysregulation in cancer. *Mol Oncol* **6**, 567–578.
7. Challouf S, Ziadi S, Zaghdoudi R, *et al.* (2012) Patterns of aberrant DNA hypermethylation in nasopharyngeal carcinoma in Tunisian patients. *Clin Chim Acta* **413**, 795–802.
8. Christman JK, Sheikhnejad G, Dizik M, *et al.* (1993) Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis* **14**, 551–557.
9. Choi SW & Mason JB (2000) Folate and carcinogenesis: an integrated scheme. *J Nutr* **130**, 129–132.
10. Zeng FF, Xu CH, Liu YT, *et al.* (2014) Choline and betaine intakes are associated with reduced risk of nasopharyngeal carcinoma in adults: a case-control study. *Br J Cancer* **110**, 808–816.
11. Zhang CX, Ho SC, Chen YM, *et al.* (2011) Dietary folate, vitamin B_6, vitamin B_{12} and methionine intake and the risk of breast cancer by oestrogen and progesterone receptor status. *Br J Nutr* **106**, 936–943.
12. Schernhammer ES, Giovannuccci E, Fuchs CS, *et al.* (2008) A prospective study of dietary folate and vitamin B and colon cancer according to microsatellite instability and KRAS mutational status. *Cancer Epidemiol Biomarkers Prev* **17**, 2895–2898.
13. Harris HR, Cramer DW, Vitonis AF, *et al.* (2012) Folate, vitamin B(6) , vitamin B(12) , methionine and alcohol intake in relation to ovarian cancer risk. *Int J Cancer* **131**, E518–E529.
14. Xiao Q, Freedman ND, Ren J, *et al.* (2014) Intakes of folate, methionine, vitamin B_6, and vitamin B_{12} with risk of esophageal and gastric cancer in a large cohort study. *Br J Cancer* **110**, 1328–1333.
15. Lin HL, An QZ, Wang QZ, *et al.* (2013) Folate intake and pancreatic cancer risk: an overall and dose-response meta-analysis. *Public Health* **127**, 607–613.
16. Polesel J, Negri E, Serraino D, *et al.* (2012) Dietary intakes of carotenoids and other nutrients in the risk of nasopharyngeal carcinoma: a case-control study in Italy. *Br J Cancer* **107**, 1580–1583.
17. Matsuo K, Rossi M, Negri E, *et al.* (2012) Folate, alcohol, and aldehyde dehydrogenase 2 polymorphism and the risk of oral and pharyngeal cancer in Japanese. *Eur J Cancer Prev* **21**, 193–198.
18. Suzuki T, Matsuo K, Hasegawa Y, *et al.* (2007) One-carbon metabolism-related gene polymorphisms and risk of head and neck squamous cell carcinoma: case-control study. *Cancer Sci* **98**, 1439–1446.
19. Negri E, Franceschi S, Bosetti C, *et al.* (2000) Selected micronutrients and oral and pharyngeal cancer. *Int J Cancer* **86**, 122–127.

20. Bravi F, Bosetti C, Filomeno M, *et al.* (2013) Foods, nutrients and the risk of oral and pharyngeal cancer. *Br J Cancer* **109**, 2904–2910.

21. Aune D, Deneo-Pellegrini H, Ronco AL, *et al.* (2011) Dietary folate intake and the risk of 11 types of cancer: a case-control study in Uruguay. *Ann Oncol* **22**, 444–451.

22. Galeone C, Edefonti V, Parpinel M, *et al.* (2015) Folate intake and the risk of oral cavity and pharyngeal cancer: a pooled analysis within the International Head and Neck Cancer Epidemiology Consortium. *Int J Cancer* **136**, 904–914.

23. Arthur AE, Duffy SA, Sanchez GI, *et al.* (2011) Higher micro-nutrient intake is associated with human papillomavirus-positive head and neck cancer: a case-only analysis. *Nutr Cancer* **63**, 734–742.

24. Almadori G, Bussu F, Galli J, *et al.* (2005) Serum levels of folate, homocysteine, and vitamin B_{12} in head and neck squamous cell carcinoma and in laryngeal leukoplakia. *Cancer* **103**, 284–292.

25. Raval GN, Sainger RN, Rawal RM, *et al.* (2002) Vitamin B(12) and folate status in head and neck cancer. *Asian Pac J Cancer Prev* **3**, 155–162.

26. Eleftheriadou A, Chalastras T, Ferekidou E, *et al.* (2006) Association between squamous cell carcinoma of the head and neck and serum folate and homocysteine. *Anticancer Res* **26**, 2345–2348.

27. Almadori G, Bussu F, Galli J, *et al.* (2002) Serum folate and homocysteine levels in head and neck squamous cell carcinoma. *Cancer* **94**, 1006–1011.

28. Pelucchi C, Talamini R, Negri E, *et al.* (2003) Folate intake and risk of oral and pharyngeal cancer. *Ann Oncol* **14**, 1677–1681.

29. Halsted CH, Villanueva JA, Devlin AM, *et al.* (2002) Metabolic interactions of alcohol and folate. *J Nutr* **132**, 2367S–2372S.

30. Liu YT, Dai JJ, Xu CH, *et al.* (2012) Greater intake of fruit and vegetables is associated with lower risk of nasopharyngeal carcinoma in Chinese adults: a case-control study. *Cancer Causes Control* **23**, 589–599.

31. Yang YX, Wang GY & Pan XC (2004) *China Food Composition Table*. Beijing: Peking University Medical Press.

32. Zhang CX & Ho SC (2009) Validity and reproducibility of a food frequency Questionnaire among Chinese women in Guangdong province. *Asia Pac J Clin Nutr* **18**, 240–250.

33. Zhang CX, Pan MX, Li B, *et al.* (2013) Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case-control study in China. *Cancer Sci* **104**, 250–258.

34. Willett WC, Howe GR & Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* **65**, 1220S–1228S discussion 1229S–1231S.

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

36. Matxain JM, Padro D, Ristila M, *et al.* (2009) Evidence of high *OH radical quenching efficiency by vitamin B_6. *J Phys Chem B* **113**, 9629–9632.

37. Beaton GH (1994) Approaches to analysis of dietary data: relationship between planned analyses and choice of methodology. *Am J Clin Nutr* **59**, 253S–261S.

38. Shrubsole MJ, Jin F, Dai Q, *et al.* (2001) Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Res* **61**, 7136–7141.

39. Takata Y, Cai Q, Beeghly-Fadiel A, *et al.* (2012) Dietary B vitamin and methionine intakes and lung cancer risk among female never smokers in China. *Cancer Causes Control* **23**, 1965–1975.

40. Stevens VL, McCullough ML, Sun J, *et al.* (2010) Folate and other one-carbon metabolism-related nutrients and risk of postmenopausal breast cancer in the Cancer Prevention Study II Nutrition Cohort. *Am J Clin Nutr* **91**, 1708–1715.

41. Fonnum F & Lock EA (2000) Cerebellum as a target for toxic substances. *Toxicol Lett* **112–113**, 9–16.

42. D'Avanzo B, La Vecchia C, Katsouyanni K, *et al.* (1996) Reliability of information on cigarette smoking and beverage consumption provided by hospital controls. *Epidemiology* **7**, 312–315.