

Dietary Vitamin B6 Intake and the Risk of Colorectal Cancer

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Abstract

Vitamin B6, a coenzyme in the folate metabolism pathway, may have anticarcinogenic effects. Laboratory and epidemiologic studies support the hypothesis of its protective effect against colorectal cancer (CRC). The aim of this large Scottish case-control study, including 2,028 hospital-based cases and 2,722 population-based controls, was to investigate the associations between dietary and supplementary intake of vitamin B6 and CRC. Three logistic regression models adjusted for several confounding factors, including energy, folate, and fiber intake, were applied in the whole sample and after age, sex, cancer site, folate, *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087), and *MTRR* A66G (rs1801394) stratification (analysis on genotypes on 1,001 cases and 1,010 controls ≤ 55 years old). Moderately strong inverse and dose-dependent associations in the whole sample were

found between CRC risk and the intake of dietary and total vitamin B6 in all three models [model III: odds ratio (OR), 0.77; 95% confidence interval (95% CI), 0.61-0.98; *P* for trend = 0.03; OR, 0.86; 95% CI, 0.69-1.07; *P* for trend = 0.12]. In addition, meta-analyses of published studies showed inverse associations between vitamin B6 and CRC (combined relative risk, 0.81; 95% CI, 0.68-0.96; test for overall effect *P* = 0.01; combined odds ratio, 0.67; 95% CI, 0.60-0.75; test for overall effect *P* < 0.00001). Analysis within the stratified subgroups showed similar associations apart from a stronger effect among ≤ 55 -year-old individuals. Evidence from larger cohort and experimental studies is now required to confirm and define the anticarcinogenic actions of vitamin B6 and to explore the mechanisms by which this effect is mediated. (Cancer Epidemiol Biomarkers Prev 2008;17(1):171-82)

Introduction

Vitamin B6 is a water-soluble vitamin present in the human body as pyridoxine, pyridoxal, and pyridoxamine (1, 2). The main dietary sources of vitamin B6 include poultry, fish, meat, legumes, nuts, potatoes, and whole grains (3). The current reference daily intake is 1.4 mg/d for men and 1.2 mg/d for women (4). Vitamin B6 is involved in the folate metabolism pathway, which has been reported to be associated with colorectal carcinogenesis. Vitamin B6 acts as a coenzyme in two different steps in this pathway: in the synthesis of 5,10-methylenetetrahydrofolate (MTHF) and in catabolism of homocysteine to glutathione (5-7). Alcohol intake has also been found to affect several steps of the folate metabolism pathway, resulting possibly in interruption of DNA methylation and influencing the absorption and degradation of vitamin B6 and folate (7, 8).

Few observational studies have investigated the association between vitamin B6 intake and colorectal cancer (CRC; refs. 7, 9-19) and only five of them considered supplement intake in parallel to dietary intake (Table 1A and B; refs. 9, 10, 14, 16, 19). Additionally, three studies have investigated the modification effect of alcohol on vitamin B6 (7, 10, 20), three studies have investigated the modification effect of folate on vitamin B6 (10, 19, 21), and five studies have investigated the interaction effects between polymorphisms of the genes involved in the folate metabolism pathway and vitamin B6 (13, 14, 16, 21, 22).

The objective of this large case-control study was to evaluate the effect of dietary and supplementary intake of vitamin B6 on CRC in the whole sample and after age, sex, and CRC site stratification and to investigate whether vitamin B6 effects are modified by folate and alcohol intakes. Additionally, the genotypic effect of three polymorphic genes [*MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087), and the *MTRR* A66G (rs1801394)] involved in the folate metabolism pathway on vitamin B6 and CRC associations was examined. The first two polymorphisms are associated with a lower activity enzyme, whereas the functions of the other two are not clearly established (23). Vitamin B6 does not directly interact with *MTHFR*, *MTR*, and *MTRR* enzymes in the one-carbon metabolism pathway. However, this is a multistep and complicated pathway and different levels of vitamin B6 intake and polymorphisms that

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Table 1. Colorectal cancer risk and vitamin B6 intake; results from published studies

A. Risk of CRC from vitamin B6: cohort studies				
Study	Type of study	Country	Population	Vitamin B6 assessment
Zhang et al. (9)	Cohort (Women's Health Study)	USA	37,916 F	FFQ diet + supplements (quintiles)
Wei et al. (10)	Nested case-control (Nurses' Health Study)	USA	32,826 F	FFQ diet + supplements (quartiles) Plasma PLP concentration (quartiles)
Larsson et al. (7)	Cohort (Swedish Mammography Cohort)	Sweden	61,433 F	FFQ diet (quintiles)
Harnack et al. (19)	Cohort (Iowa Women's Health Study)	USA	32,215 F	FFQ diet + supplements (quintiles for colon, tertiles for rectal cancer)
B. Risk of CRC from vitamin B6: case-control studies				
Study	Type of study	Country	Population	Vitamin B6 assessment
Murtaugh et al. (14)	Case-control	USA	1,730 FM	Dietary history (CARDIA) + supplements (tertiles)
Kune and Watson (18)	Case-control	Australia	1,442 FM	FFQ diet (quintiles)
Otani et al. (13)	Case-control	Japan	331 FM	FFQ diet (tertiles)
Le Marchand et al. (16)	Case-control	USA	1,454 FM	FFQ diet + supplements (quintiles)
La Vecchia et al. (17)	Case-control	Italy	6,107 FM	FFQ diet (quartiles)
Slattery et al. (12)	Case-control	USA	4,403 FM	Dietary history (CARDIA; B6 intakes only from plant foods; quartiles)
Tuyns et al. (11)	Case-control	Belgium	3,669 FM	Dietary history
Macquart-Moulin et al. (15)	Case-control	France	798 FM	Dietary history

*Adjusted for age and randomized treatment assignment.

† Adjusted for age, randomized treatment assignment, BMI, family history of CRC, history of colon polyps, physical activity, smoking status, red meat intake, alcohol consumption, total energy intake, menopausal status, baseline postmenopausal hormone use, and baseline aspirin use.

‡ Matched on year of birth, month and year of blood collection, and fasting status; adjusted for BMI, physical activity, smoking, menopausal status, postmenopausal hormone use, duration of regular aspirin use, family history of CRC, intake of alcohol and red meat, plasma vitamin D, and history of endoscopy.

§ Matched on year of birth, month and year of blood collection, and fasting status; adjusted for BMI, physical activity, smoking, menopausal status, postmenopausal hormone use, duration of regular aspirin use, family history of CRC, intake of alcohol and red meat, plasma vitamin D, history of endoscopy, intake of folate and methionine, vitamin B supplement use, and multivitamin supplement use.

|| Adjusted for age.

¶ Adjusted for age, BMI, education, total energy intake, intake of red meat, saturated fat, calcium, folate, β -carotene, cereal fiber.

**Adjusted for age, pack-years of cigarettes, BMI, estrogen use, and intakes of calcium, vitamin E, and energy.

†† Adjusted for age, sex, BMI, activity, energy, fiber, calcium, ibuprofen use, and smoking (pack-years).

Table 1. Colorectal cancer risk and vitamin B6 intake; results from published studies (Cont'd)

A. Risk of CRC from vitamin B6: cohort studies				
Comparison (high vs low)	Outcome	No. cases	Relative risk (95% CI)	P for trend
≥4.00 vs ≤1.78 mg/d (total)	Colorectal	220	1.14 (0.78-1.67)*	0.06
≥2.40 vs ≤1.69 mg/d (diet)		220	0.88 (0.59-1.31)*	0.29
≥2.40 vs ≤1.69 mg/d (diet, excluding supplement users)		139	0.72 (0.44-1.20)*	0.07
≥4.00 vs ≤1.78 mg/d (total)	Colorectal	220	1.14 (0.77-1.69) [†]	0.07
≥2.40 vs ≤1.69 mg/d (diet)		220	0.84 (0.56-1.27) [†]	0.18
≥2.40 vs ≤1.69 mg/d (diet, excluding supplement users)		139	0.69 (0.41-1.15) [†]	0.047
8.6 vs 1.6 mg/d	Colorectal	194	0.60 (0.34-1.06) [‡]	0.3
	Colon	148	0.51 (0.27-0.97) [‡]	0.007
8.6 vs 1.6 mg/d	Colorectal	194	0.39 (0.18-0.84) [§]	0.01
	Colon	148	0.32 (0.14-0.75) [§]	0.003
131.2 vs 23.9 pmol/mL	Colorectal	188	0.56 (0.31-1.01) [‡]	0.07
	Colon	142	0.42 (0.21-0.85) [‡]	0.02
131.2 vs 23.9 pmol/mL	Colorectal	188	0.48 (0.25-0.92) [§]	0.03
	Colon	142	0.38 (0.18-0.80) [§]	0.01
≥2.05 vs <1.53 mg/d	Colorectal	805	0.77 (0.62-0.97)	0.01
	Colon	547	0.87 (0.66-1.14)	0.16
	Rectal	252	0.63 (0.42-0.95)	0.04
≥2.05 vs <1.53 mg/d	Colorectal	805	0.66 (0.50-0.86) [¶]	0.002
	Colon	547	0.75 (0.54-1.04) [¶]	0.04
	Rectal	252	0.50 (0.3, 0.82) [¶]	0.02
>4.35 vs <1.59 mg/d	Colon	598	0.69 (0.53-0.89)	0.01
>3.27 vs <1.93 mg/d	Rectal	123	0.88 (0.57-1.35)	0.62
>4.35 vs <1.59 mg/d	Colon	598	0.95 (0.67-1.36) ^{**}	0.88
>3.27 vs <1.93 mg/d	Rectal	123	1.97 (1.08-3.62) ^{**}	0.03
B. Risk of CRC from vitamin B6: case-control studies				
Comparison (high vs low)	Outcome	No. cases	OR (95% CI)	P for trend
Diet: ≤1.79 vs >2.6	Rectal	751	0.92 (0.72-1.17) ^{††}	0.59
Total: ≤2.44 vs >4.08			0.92 (0.72-1.17) ^{††}	0.46
>3.4 vs <1.7 mg/d	Colorectal	715	0.66 (0.46-0.94) ^{‡‡}	—
	Colon	392	0.60 (0.38-0.91) ^{‡‡}	—
	Rectal	323	0.73 (0.46-1.14) ^{‡‡}	—
>3.4 vs <1.7 mg/d	Colorectal	715	0.52 (0.34-0.80) ^{§§}	—
	Colon	392	0.47 (0.28-0.77) ^{§§}	—
	Rectal	323	0.57 (0.34-0.96) ^{§§}	—
>1.74 vs <1.46 mg/d	Colorectal	107	0.88 (0.41-1.9)	0.77
>2.46 vs ≤1.69 mg/d	Colorectal	727	1.0 (0.7-1.4) ^{¶¶}	0.74
≥2.78 vs ≤2.04 mg/d	Colorectal	1,953	0.53 (0.4-0.7) ^{**}	<0.001
	Colon	1,225	0.80 (0.6-1.0) ^{**}	—
	Rectal	728	0.99 (0.7-1.3) ^{**}	—
M: ≥1.18 vs ≤0.75 mg/1,000 kcal	All	1,099	0.7 (0.6-1.0) ^{†††}	<0.01
	Distal	542	0.7 (0.5-0.9) ^{†††}	0.02
	Proximal	526	0.8 (0.6-1.1) ^{†††}	0.10
F: ≥1.28 vs ≤0.82 mg/1,000kcal	All	849	0.6 (0.5-0.8) ^{†††}	<0.01
	Distal	429	0.6 (0.4-0.9) ^{†††}	<0.01
	Proximal	446	0.6 (0.4-0.9) ^{†††}	0.08
≥1.75 vs ≤1.16 mg/d	Colon	453	0.75 ^{†††}	0.10
	Rectal	365	0.81 ^{†††}	0.21
≥1.75 vs ≤1.16 mg/d	Colon	453	0.53	0.013
	Rectal	365	0.40	0.0001
Highest quartile vs lowest	Colorectal	399	0.46 ^{¶¶¶}	0.002

†† Adjusted for age and sex.

§§ Adjusted for age, sex, alcohol, BMI, energy intake, family history of CRC, oral contraceptive pill use, cigarette pack-years, and aspirin use.

||| Matched on sex, age, and residence area; adjusted for smoking, alcohol consumption, BMI, and total dietary fiber intake.

¶¶ Matched on sex, age, and ethnicity; adjusted for energy (residual method), pack-years of cigarette smoking, lifetime recreational physical activity, lifetime aspirin use, BMI, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements.

** Adjusted for age, center, sex, education, physical activity, total energy intake, and fiber intake; ORs and 95% CI for colon and rectal derived from continuous models.

††† Adjusted for age, BMI, lifetime vigorous leisure time physical activity, use of aspirin/NSAIDs, presence or absence of a first-degree relative with CRC, total energy intake, and calcium.

†††† ORs by sex and province; adjusted for age.

§§§ Adjusted for age, sex, province, and total calorie intake.

|||| Adjusted for age, sex, calories, and weight.

affect the enzymatic activity might change the relationship with CRC.

Materials and Methods

Study Population. We studied 2,028 cases and 2,722 controls from a case-control study of CRC (Study of Colorectal Cancer in Scotland). We aimed to recruit prospectively all incident cases of adenocarcinoma of colorectum in patients ages 16 to 79 years presenting to surgical units in Scotland. Exclusions were patient death before ascertainment, patient too ill to participate, recurrent cases, or patient unable to give informed consent due to learning difficulties or other medical conditions. We recruited ~40% of all incident cases in Scotland over the study period. During the same period, controls were drawn at random from a population-based register (community health index) and invited to participate. Participation rates among those approached were ~58% for cases and ~57% for controls. Questionnaire completion was sufficient for valid analysis in 82% of cases and 97% of controls recruited. More than 99% of the study participants were white Caucasian (see ref. 24 for further recruitment details). Ethical approval was obtained from the Multicentre Research Ethics Committee for Scotland and relevant Local Research Ethics committees, and all participants provided written informed consent.

Lifestyle and Dietary Data. Subjects completed one questionnaire with lifestyle and cancer information reporting their status 1 year before diagnosis or recruitment (reference period; ref. 24). Participants also completed a semiquantitative food frequency questionnaire (FFQ) consisted of 150 food items (Scottish Collaborative Group FFQ, version 6.41).⁵ Its validity for ranking macronutrients and micronutrients in younger adults (25) and its main characteristics have been described.⁵ The main sources of vitamin B6 that were included in the questionnaire were beans, legumes, nuts, eggs, meats, fish, breads, cereals, potatoes, and bananas. Participants were also asked to give full details of dietary supplement taken in the reference period. Frequencies of consumption of the specified measures of each food were converted into nutrients using an in-house calculation program based on the weights of these measures and the nutrient composition of representative foods derived from the UK food composition tables (26-32). Nutrient information on supplements was collected from the manufacturer's product information.

Genotyping Data. Genotyping was undertaken for *MTHFR*, *MTR*, and *MTRR* single nucleotide polymorphisms (rs1801133, rs1801131, rs1805087, and rs1801394) as part of an array-based candidate gene approach. Genotyping of patients ages ≤55 years along with matched controls was undertaken together using the Illumina Infinium I Custom array platform and done by Illumina in San Diego. DNA samples were accurately quantified by PicoGreen and quality controlled before dispatch to San Diego. To avoid potential systematic

batch-to-batch variation or bias, samples were anonymized as to affection status and were randomly distributed within plates. Only analysis of the *MTHFR*, *MTR*, and *MTRR* genotypes is considered here as this was a hypothesis-driven project at the outset to test individual candidate genes and enzymatic systems. Data were subject to Illumina quality control procedures and genotypes were discarded if call rates were <99.5%. Genotypes were available for 1,001 cases and 1,010 controls.

Statistical Analysis. The statistical package used was STATA version 10.0 (Stata Corp.). Spearman rank

Table 2. Demographic characteristics and lifestyle factors for the study population

Variables	Cases* (n = 2,028)	Controls* (n = 2,722)	P [†]
Age (y)	62.3 (10.68)	62.7 (10.51)	0.17
BMI (kg/m ²)	26.80 (7.88)	26.82 (6.31)	0.90
Energy intake [‡] (MJ/d)	11.25 (4.39)	10.65 (3.95)	<0.00005
Folate intake [‡] (μg/d)	330.97 (68.92)	339.87 (72.95)	<0.00005
Fiber intake [‡] (g/d)	21.36 (5.87)	22.22 (6.15)	<0.00005
Alcohol intake [§] (g/d)	2.89 (2.12)	2.95 (2.09)	0.31
Sex			
Men	1,160 (57.3)	1,549 (56.9)	0.83
Women	866 (43.7)	1,171 (43.1)	
Family history risk			
Low	1,592	2,639	<0.0005
Moderate or high	320	30	
Smoking			
No	828 (42.3)	1,146 (43.0)	0.24
Former	804 (41.1)	1,036 (38.9)	
Current	326 (16.6)	483 (16.3)	
Frequent NSAIDs intake			
No	1,379 (69.9)	1,671 (63.2)	0.005
Yes [¶]	593 (30.1)	972 (36.8)	
Physical activity (h/d)			
0	1,124 (58.5)	1,414 (53.9)	0.018
0-3.5	449 (23.3)	655 (25.0)	
3.5-7	194 (10.1)	314 (12.0)	
≥7	156 (8.1)	239 (9.1)	
Supplement intake			
No	1,388 (68.4)	1,735 (63.6)	0.0007
Yes	642 (31.6)	991 (36.4)	
Type of cancer			
Colon cancer	1,191 (58.9)		
Rectal cancer	830 (41.1)		
Deprivation**			
1	188	257	0.99
2	429	560	
3	531	737	
4	474	635	
5	218	288	
6	132	171	
7	54	70	

*Mean values and, in parentheses, SDs for quantitative variables; number of subjects and, in parenthesis, percentages for categorical variables.

[†]P values from the Pearson χ^2 for categorical variables; from t test for continuous variables.

[‡]Energy adjusted.

[§]Energy-adjusted and square root-transformed variable.

^{||}Smokers were defined as individuals who have smoked at least one cigarette per day.

[¶]Frequent use was defined as an intake of at least 4 d/wk for at least 1 mo.

**Locally based deprivation index (Carstairs deprivation index) based on the 2001 census data; seven categories ranging from very low deprivation (deprivation category = 1) to very high deprivation (deprivation category = 7).

⁵ <http://www.foodfrequency.org>

Table 3. Logistic regression models of the effect of vitamin B6 intake from dietary sources and of total vitamin B6 intake (diet and supplement intake) on CRC in the whole population and stratified by site of cancer, subsite of colon cancer, gender, age, and daily folate intake

Vitamin B6 intake (mg/d)	Cases	Controls	Model I*		Model I [†]		Model III [‡]	
			OR	95% CI	OR	95% CI	OR	95% CI
Whole sample								
Dietary intake								
≤2.55	551	637	1.00		1.00		1.00	
2.56-2.89	530	658	0.93	0.79-1.09	0.94	0.79-1.11	0.96	0.80-1.16
2.90-3.25	499	688	0.84	0.71-0.99	0.85	0.70-1.02	0.90	0.74-1.11
≥3.26	448	739	0.70	0.60-0.83	0.71	0.57-0.89	0.77	0.61-0.98
			<i>P</i> for trend < 0.0005		<i>P</i> for trend = 0.002		<i>P</i> for trend = 0.03	
Total intake								
≤2.58	549	639	1.00		1.00		1.00	
2.59-2.95	528	660	0.93	0.79-1.09	0.96	0.81-1.14	1.01	0.84-1.21
2.96-3.38	491	697	0.82	0.70-0.96	0.86	0.71-1.04	0.92	0.75-1.13
≥3.39	461	726	0.74	0.63-0.87	0.79	0.64-0.96	0.86	0.69-1.07
			<i>P</i> for trend < 0.0005		<i>P</i> for trend = 0.013		<i>P</i> for trend = 0.12	
Colon cancer								
Dietary intake								
≤2.55	318	637	1.00		1.00		1.00	
2.56-2.89	318	658	0.96	0.79-1.16	0.95	0.78-1.16	0.98	0.79-1.21
2.90-3.25	282	688	0.82	0.67-0.99	0.81	0.64-1.01	0.87	0.68-1.11
≥3.26	273	739	0.74	0.61-0.90	0.73	0.56-0.94	0.77	0.58-1.02
			<i>P</i> for trend = 0.001		<i>P</i> for trend = 0.009		<i>P</i> for trend = 0.05	
Total intake								
≤2.58	315	639	1.00		1.00		1.00	
2.59-2.95	319	660	0.97	0.80-1.18	0.98	0.80-1.20	1.03	0.83-1.27
2.96-3.38	280	697	0.82	0.67-0.99	0.83	0.66-1.05	0.87	0.68-1.11
≥3.39	277	726	0.78	0.63-0.94	0.79	0.62-1.01	0.85	0.66-1.11
			<i>P</i> for trend = 0.002		<i>P</i> for trend = 0.03		<i>P</i> for trend = 0.39	
Proximal colon cancer								
Dietary intake								
≤2.55	156	637	1.00		1.00		1.00	
2.56-2.89	135	658	0.84	0.65-1.08	0.80	0.61-1.05	0.82	0.61-1.10
2.90-3.25	142	688	0.85	0.66-1.10	0.78	0.58-1.05	0.83	0.61-1.15
≥3.26	137	739	0.77	0.60-0.99	0.67	0.47-0.96	0.68	0.47-0.99
			<i>P</i> for trend = 0.06		<i>P</i> for trend = 0.04		<i>P</i> for trend = 0.07	
Total intake								
≤2.58	152	639	1.00		1.00		1.00	
2.59-2.95	136	660	0.86	0.66-1.11	0.84	0.64-1.11	0.89	0.66-1.19
2.96-3.38	136	697	0.84	0.65-1.08	0.81	0.60-1.10	0.85	0.62-1.18
≥3.39	146	726	0.85	0.66-1.09	0.82	0.59-1.12	0.86	0.61-1.21
			<i>P</i> for trend = 0.20		<i>P</i> for trend = 0.25		<i>P</i> for trend = 0.80	
Distal colon cancer								
Dietary intake								
≤2.55	114	637	1.00		1.00		1.00	
2.56-2.89	140	658	1.20	0.91-1.57	1.20	0.90-1.59	1.22	0.91-1.65
2.90-3.25	116	688	0.94	0.71-1.25	0.95	0.68-1.31	0.99	0.70-1.39
≥3.26	108	739	0.83	0.62-1.10	0.83	0.57-1.21	0.88	0.59-1.32
			<i>P</i> for trend = 0.07		<i>P</i> for trend = 0.17		<i>P</i> for trend = 0.34	
Total intake								
≤2.58	115	639	1.00		1.00		1.00	
2.59-2.95	142	660	1.20	0.91-1.56	1.21	0.91-1.61	1.24	0.92-1.67
2.96-3.38	117	697	0.94	0.71-1.24	0.96	0.69-1.33	0.97	0.69-1.37
≥3.39	104	726	0.81	0.61-1.08	0.83	0.59-1.18	0.86	0.59-1.24
			<i>P</i> for trend = 0.05		<i>P</i> for trend = 0.13		<i>P</i> for trend = 0.39	
Rectal cancer								
Dietary intake								
≤2.55	224	637	1.00		1.00		1.00	
2.56-2.89	214	658	0.93	0.75-1.16	0.96	0.76-1.21	1.01	0.79-1.29
2.90-3.25	218	688	0.90	0.73-1.12	0.95	0.73-1.22	0.98	0.75-1.29
≥3.26	174	739	0.68	0.54-0.85	0.73	0.54-0.98	0.80	0.58-1.10
			<i>P</i> for trend = 0.001		<i>P</i> for trend = 0.05		<i>P</i> for trend = 0.19	
Total intake								
≤2.58	224	639	1.00		1.00		1.00	
2.59-2.95	213	660	0.93	0.75-1.15	0.98	0.78-1.23	1.06	0.83-1.36
2.96-3.38	209	697	0.86	0.69-1.07	0.95	0.73-1.22	1.03	0.79-1.36
≥3.39	184	726	0.73	0.59-0.92	0.82	0.63-1.08	0.90	0.67-1.21
			<i>P</i> for trend = 0.005		<i>P</i> for trend = 0.16		<i>P</i> for trend = 0.45	

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Table 3. Logistic regression models of the effect of vitamin B6 intake from dietary sources and of total vitamin B6 intake (diet and supplement intake) on CRC in the whole population and stratified by site of cancer, subsite of colon cancer, gender, age, and daily folate intake (Cont'd)

Vitamin B6 intake (mg/d)	Cases	Controls	Model I*		Model I [†]		Model III [‡]	
			OR	95% CI	OR	95% CI	OR	95% CI
Males								
Dietary intake								
≤2.55	350	387	1.00		1.00		1.00	
2.56-2.89	278	377	0.82	0.67-1.02	0.81	0.65-1.02	0.82	0.65-1.04
2.90-3.25	277	393	0.78	0.63-0.97	0.76	0.60-0.98	0.81	0.62-1.05
≥3.26	255	392	0.73	0.59-0.90	0.70	0.53-0.93	0.74	0.55-1.01
			<i>P</i> for trend = 0.003		<i>P</i> for trend = 0.02		<i>P</i> for trend = 0.07	
Total intake								
≤2.58	343	393	1.00		1.00		1.00	
2.59-2.95	294	389	0.87	0.71-1.08	0.88	0.71-1.10	0.90	0.71-1.14
2.96-3.38	280	413	0.78	0.63-0.96	0.79	0.62-1.01	0.82	0.64-1.07
≥3.39	243	354	0.79	0.64-0.98	0.80	0.61-1.05	0.89	0.67-1.19
			<i>P</i> for trend = 0.02		<i>P</i> for trend = 0.09		<i>P</i> for trend = 0.38	
Females								
Dietary intake								
≤2.55	201	250	1.00		1.00		1.00	
2.56-2.89	250	281	1.11	0.86-1.42	1.15	0.88-1.50	1.23	0.92-1.65
2.90-3.25	222	295	0.94	0.73-1.21	1.00	0.74-1.35	1.09	0.79-1.52
≥3.26	193	345	0.70	0.54-0.90	0.76	0.54-1.09	0.85	0.57-1.25
			<i>P</i> for trend = 0.001		<i>P</i> for trend = 0.07		<i>P</i> for trend = 0.24	
Total intake								
≤2.58	206	246	1.00		1.00		1.00	
2.59-2.95	232	271	1.02	0.79-1.32	1.08	0.83-1.42	1.23	0.91-1.65
2.96-3.38	210	283	0.89	0.69-1.15	0.98	0.73-1.33	1.12	0.81-1.56
≥3.39	218	371	0.70	0.55-0.90	0.79	0.58-1.08	0.87	0.62-1.22
			<i>P</i> for trend = 0.002		<i>P</i> for trend = 0.07		<i>P</i> for trend = 0.18	
≤55 y old								
Dietary intake								
≤2.55	177	169	1.00		1.00		1.00	
2.56-2.89	170	170	0.96	0.71-1.30	1.00	0.73-1.37	0.98	0.69-1.39
2.90-3.25	125	186	0.65	0.47-0.88	0.70	0.48-1.00	0.73	0.49-1.09
≥3.26	120	183	0.63	0.46-0.87	0.70	0.76-1.08	0.74	0.47-1.18
			<i>P</i> for trend < 0.0005		<i>P</i> for trend = 0.04		<i>P</i> for trend = 0.12	
Total intake								
≤2.58	183	168	1.00		1.00		1.00	
2.59-2.95	154	168	0.84	0.62-1.14	0.88	0.64-1.22	0.87	0.61-1.24
2.96-3.38	132	185	0.66	0.49-0.90	0.73	0.50-1.05	0.79	0.53-1.17
≥3.39	123	187	0.61	0.45-0.83	0.68	0.46-1.00	0.71	0.47-1.09
			<i>P</i> for trend = 0.001		<i>P</i> for trend = 0.04		<i>P</i> for trend = 0.11	
>55 y old								
Dietary intake								
≤2.55	374	468	1.00		1.00		1.00	
2.56-2.89	360	488	0.92	0.76-1.11	0.91	0.74-1.12	0.97	0.78-1.20
2.90-3.25	375	502	0.93	0.77-1.13	0.92	0.73-1.15	0.98	0.78-1.25
≥3.26	328	556	0.74	0.61-0.90	0.72	0.56-0.94	0.79	0.60-1.04
			<i>P</i> for trend = 0.004		<i>P</i> for trend = 0.03		<i>P</i> for trend = 0.13	
Total intake								
≤2.58	366	471	1.00		1.00		1.00	
2.59-2.95	374	492	0.97	0.80-1.18	0.99	0.81-1.22	1.08	0.87-1.34
2.96-3.38	358	512	0.90	0.74-1.09	0.93	0.74-1.17	1.00	0.7-1.26
≥3.39	338	539	0.81	0.66-0.98	0.84	0.66-1.07	0.94	0.73-1.21
			<i>P</i> for trend = 0.02		<i>P</i> for trend = 0.12		<i>P</i> for trend = 0.45	
Low folate intake (≤0.33 mg/d)								
Dietary intake								
≤2.55	499	583	1.00				1.00	
2.56-2.89	343	413	0.96	0.80-1.16			0.98	0.80-1.20
2.90-3.25	178	232	0.89	0.71-1.12			0.97	0.76-1.24
≥3.26	47	81	0.67	0.46-0.99			0.71	0.47-1.07
			<i>P</i> for trend = 0.06				<i>P</i> for trend = 0.25	
Total intake								
≤2.58	504	578	1.00				1.00	
2.59-2.95	322	388	0.94	0.78-1.14			0.97	0.79-1.20
2.96-3.38	145	191	0.87	0.68-1.11			0.96	0.73-1.25
≥3.39	96	152	0.72	0.54-0.95			0.77	0.57-1.05
			<i>P</i> for trend = 0.02				<i>P</i> for trend = 0.15	

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Table 3. Logistic regression models of the effect of vitamin B6 intake from dietary sources and of total vitamin B6 intake (diet and supplement intake) on CRC in the whole population and stratified by site of cancer, subsite of colon cancer, gender, age, and daily folate intake (Cont'd)

Vitamin B6 intake (mg/d)	Cases	Controls	Model I*		Model I [†]		Model III [‡]	
			OR	95% CI	OR	95% CI	OR	95% CI
High folate intake (>0.34 mg/d)								
Dietary intake								
≤2.55	52	54	1.00				1.00	
2.56-2.89	187	245	0.80	0.52-1.23			0.85	0.54-1.34
2.90-3.25	321	456	0.74	0.49-1.11			0.80	0.52-1.24
≥3.26	401	658	0.64	0.43-0.96			0.74	0.48-1.15
			P for trend = 0.007				P for trend = 0.13	
Total intake								
≤2.58	45	61	1.00				1.00	
2.59-2.95	206	272	1.04	0.68-1.59			1.17	0.74-1.86
2.96-3.38	345	506	0.93	0.62-1.40			1.05	0.67-1.64
≥3.39	365	574	0.87	0.58-1.32			1.06	0.68-1.66
			P for trend = 0.15				P for trend = 0.67	

*Adjusted for energy (residual method), age, and sex.

[†] Adjusted for energy (residual method), age, sex, and folate intake (energy adjusted, quartiles).

[‡] Adjusted for energy (residual method), age, sex, folate intake (energy adjusted, quartiles), fiber intake (energy adjusted, quartiles), alcohol intake (energy adjusted), smoking (nonsmoker, former smoker, and current smoker), BMI, physical activity (total hours of sports and cycling, four categories), NSAID intake, and family history of cancer (low and medium/high risk).

correlation coefficients were calculated to test the correlation between vitamin B6 and other selected micronutrients, individual foods, and food groups. The Pearson χ^2 test and the *t* test were used to test the difference between cases and controls in terms of categorical and continuous confounding variables. The Wilcoxon rank-sum test was used to test for differences in energy-adjusted vitamin B6 intake.

Logistic regression models were used to estimate the strength of association between CRC risk and vitamin B6 intake and its main food sources. Participants were divided into quartiles based on the combined distributions of cases and controls (to have the same measure of exposure in both groups). Vitamin B6 intake was adjusted for total energy intake by using the residual method (33). The core statistical model (model I) was corrected for age and sex. Model II was further corrected for folate intake ($\mu\text{g}/\text{d}$, energy adjusted, quartiles). Model III was further adjusted for fiber intake (g/d , energy adjusted, quartiles), alcohol intake (g/d , energy adjusted, continuously), smoking (nonsmoker, current smoker, and former smoker), body mass index (BMI; kg/m^2 , continuously), regular nonsteroidal anti-inflammatory drug (NSAID) intake (yes versus no), family history of cancer (low and medium/high risk), and physical activity (total hours of sports and cycling, four categories). In addition to the whole sample analysis, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated in stratified subgroups according to cancer site (colon cancer and rectal cancer), colon cancer subsite (proximal and distal colon cancer), sex, age (≤ 55 and > 55 years), folate intake (low and high intake), alcohol intake (data not shown), *MTHFR* C677T genotype (CC, CT, TT), *MTHFR* A1298C (AA, AC, CC), *MTR* A2756G (AA, AG/GG), and *MTRR* A66G (AA, AG, GG). The associations between CRC and each of the four genotypes were tested in unadjusted logistic regression models. Interaction associations were examined by investigating the combined effects of *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G, and *MTRR* A66G genotypes and vitamin B6 intake in the

whole sample and stratified by site of cancer. Interaction was tested by deviance of two different nested models: an interactive model and its nested multiplicative one. The referent category used was homozygotes of the wild-type allele being at greatest risk (low dietary vitamin B6 intake).

Meta-analysis of Published Studies. We identified published cohort and case-control studies [keywords: colorectal neoplasms (MeSH), colon cancer, rectal cancer, colon, rectum, vitamin B6 (MeSH), vitamin B6, and B6] searching MEDLINE. References from these publications were also examined to identify previous studies. The inclusion criteria were as follows: (a) cohort or case-control studies examining the associations between CRC (primary end point) and vitamin B6 intake (providing at least three categories of the exposure), (b) limited to humans and publications in English published until February 2007, and (c) providing relative risks (ORs for the case-control studies) and 95% CI or information allowing us to calculate them. Review Manager software (4.2) was used to do meta-analyses of three published cohort studies (including one nested case-control study) on CRC, three cohort studies on colon cancer, two cohort studies on rectal cancer, six case-control studies (including this current study), three case-control studies on colon cancer, and three case-control studies on rectal cancer to compare high versus low dietary intakes of vitamin B6. Fixed-effect models were adopted when there was no evidence for heterogeneity, which was quantified using a χ^2 test and *I*² score.

Results

There were no significant differences between the cases and the controls in terms of age, sex, BMI, daily alcohol intake (energy adjusted), smoking, and area deprivation index. Control individuals had a lower family history risk ($P < 0.0005$) and reported a significant lower total daily energy, fiber (energy adjusted), and folate intake

($P < 0.00005$, 0.00005 , and 0.00005 , respectively), taking NSAIDs ($P = 0.005$) and supplements ($P = 0.0007$) regularly more often, and being more physically active than cases ($P = 0.018$; Table 2). The variant allele frequencies of the four polymorphisms in the control sample were under Hardy-Weinberg equilibrium (*MTHFR* 677T, 11.6%; *MTHFR* 1298C, 10.0%; *MTR* 66G, 19.6%; *MTRR* 2756G, 3.0%).

The five main food sources of vitamin B6 were boiled or baked potatoes (14.4% of dietary vitamin B6 intake), bananas (5.0%), mixed vegetable dishes (4.3%), breakfast cereals (4.0%), and semiskimmed milk (3.5%). Spearman's correlation coefficients for correlation with vitamin B6 intake were for dietary energy-adjusted nutrient intake (folate, 0.75; fiber, 0.53; vitamin B2, 0.41; and vitamin B12, 0.28) and for food group intake (potatoes, 0.44; mixed vegetable dishes, 0.21; bananas, 0.31; and alcohol, 0.16). All the above correlations were statistically significant ($P < 0.00005$). Results from the Wilcoxon rank test showed that the consumption of both dietary and total (including supplement intake) vitamin B6 intake significantly differed between cases and controls ($P < 0.00005$ and 0.0001 , respectively).

Table 3 presents three multiple logistic regression models on the relationship between quartiles of dietary and of total vitamin B6 intake and the risk of CRC as OR, 95% CIs, and P for trend for CRC. Intakes of dietary and of total vitamin B6 showed a significant, inverse, and dose-dependent effect on CRC risk in all models (model I: P for trend < 0.0005 and 0.0005 ; model II: P for trend = 0.002 and 0.013 ; model III: P for trend = 0.03 and 0.12) with approximately 20% to 30% reduction in risk for those of high versus those of low intake (model I: OR, 0.70; 95% CI, 0.60-0.83; OR, 0.74; 95% CI, 0.63-0.87; model II: OR, 0.71; 95% CI, 0.57-0.89; OR, 0.79; 95% CI, 0.64-0.96; model III: OR, 0.77; 95% CI, 0.61-0.98; OR, 0.86; 95% CI, 0.69-1.07). ORs, 95% CIs, and P for trend for CRC

risk were estimated as before for groups stratified by sex, cancer site (colon or rectal), age (≤ 55 and > 55 years), folate intake (≤ 0.33 and > 0.33 mg/d), and alcohol intake (data not shown) for model I, II, and III analysis (Table 3). In general, vitamin B6 (dietary and total) had a similar strong inverse association with both colon, rectal, proximal, and distal cancer (Table 3). In addition, its effect did not vary by level of daily folate intake or daily alcohol intake (data not shown). In contrast, the associations were nonsignificantly stronger among those ages ≤ 55 years (model III, high versus low quartile of total intake, OR, 0.71; 95% CI, 0.47-1.09; P for trend = 0.11) than among those ages > 55 years (model III, high versus low quartile of total intake, OR, 0.94; 95% CI, 0.73-1.21; P for trend = 0.45; Table 3). Furthermore, we explored the associations between CRC and the three main vitamin B6 food sources in our population. ORs (model I) for CRC risk of highest versus lowest quartile intakes were 1.08 (95% CI, 0.91-1.28; P for trend = 0.78) for boiled or baked potatoes, 0.69 (95% CI, 0.59-0.82; P for trend < 0.0005) for bananas, and 0.80 (95% CI, 0.68-0.93; P for trend = 0.018) for mixed vegetable dishes.

None of the polymorphisms was significantly associated with CRC (Table 4). However, we observed a nonsignificant association between CRC and *MTR* 2756GG (OR, 1.30; 95% CI, 0.79-2.12; P for trend = 0.19; Table 4). Vitamin B6 intake (both dietary and total) was inversely associated in a dose-dependent fashion with CRC independently of the three genotypes of *MTHFR* C677T, *MTHFR* A1298C, *MTR* A2756G, and *MTRR* A66G (Table 5; Supplementary Table S1). Furthermore, there was no significant evidence for interaction between each polymorphism and vitamin B6 greater than the one expected from a multiplicative model. Results in groups stratified by cancer site (colon and rectal; Supplementary Table S2) were similar. There was an indication of a significant interaction effect between *MTHFR* A1298C

Table 4. Unadjusted genotype effect of the *MTHFR* A677C (AA, AC, CC), *MTHFR* A1298G (AA, AG, GG), *MTR* A2756G (AA, AG/GG), and *MTRR* A66G (AA, AG, GG) on CRC (participants ≤ 55 y old)

SNP	Cases	Controls	Unadjusted		Simple adjusted*	
			OR	95% CI	OR	95% CI
<i>MTHFR</i> 677C>T						
CC	447	439	1.00		1.00	
CT	441	455	0.95	0.79-1.15	0.93	0.75-1.15
TT	111	116	0.94	0.70-1.26	1.01	0.72-1.41
			P for trend = 0.58		P for trend = 0.78	
<i>MTHFR</i> 1298A>C						
AA	465	462	1.00		1.00	
AC	425	445	0.95	0.79-1.14	0.83	0.67-1.02
CC	106	102	1.03	0.76-1.39	0.94	0.66-1.33
			P for trend = 0.90		P for trend = 0.26	
<i>MTR</i> 2756A>G						
AA	630	662	1.00		1.00	
AG	332	318	1.10	0.91-1.32	1.10	0.88-1.37
GG	37	30	1.30	0.79-2.12	1.21	0.69-2.11
			P for trend = 0.19		P for trend = 0.30	
<i>MTRR</i> 66A>G						
AA	200	198	1.00		1.00	
AG	456	482	0.94	0.74-1.18	0.92	0.70-1.21
GG	339	329	1.02	0.80-1.31	0.93	0.70-1.24
			P for trend = 0.76		P for trend = 0.69	

Abbreviation: SNP, single nucleotide polymorphism.

*Adjusted for age, sex, deprivation score, and family history risk.

Table 5. Multiplicative logistic regression model of the effect of total vitamin B6 intake (diet and supplement intake) on CRC stratified by *MTHFR* A677C (AA, AC, CC), *MTHFR* A1298G (AA, AG, GG), *MTR* A2756G (AA, AG/GG), and *MTRR* A66G (AA, AG, GG) in the whole population (participants ≤55 y old)

Whole sample	<i>MTHFR</i> 677 CC		<i>MTHFR</i> 677 CT		<i>MTHFR</i> 677 TT	
	OR	95% CI	OR	95% CI	OR	95% CI
Total intake						
≤2.58	1.00		1.00		1.00	
2.59-2.95	1.02	0.61-1.69	0.62	0.37-1.06	1.20	0.38-3.80
2.96-3.38	0.81	0.48-1.36	0.73	0.42-1.27	0.67	0.21-2.18
≥3.39	0.68	0.40-1.17	0.41	0.22-0.75	0.55	0.16-1.85
	<i>P</i> for trend = 0.11		<i>P</i> for trend = 0.01 <i>P</i> for interaction = 0.67		<i>P</i> for trend = 0.20	
Whole sample	<i>MTHFR</i> 1298AA		<i>MTHFR</i> 1298AC		<i>MTHFR</i> 1298CC	
	OR	95% CI	OR	95% CI	OR	95% CI
Total intake						
≤2.58	1.00		1.00		1.00	
2.59-2.95	1.10	0.66-1.83	0.68	0.40-1.18	0.73	0.24-2.20
2.96-3.38	0.87	0.51-1.48	0.85	0.48-1.49	0.38	0.12-1.19
≥3.39	0.70	0.40-1.24	0.48	0.26-0.87	0.39	0.12-1.27
	<i>P</i> for trend = 0.15		<i>P</i> for trend = 0.04 <i>P</i> for interaction = 0.36		<i>P</i> for trend = 0.06	
Whole sample	<i>MTR</i> 2756AA		<i>MTR</i> 2756AG or <i>MTR</i> 2756GG			
	OR	95% CI	OR	95% CI	OR	95% CI
Total intake						
≤2.58	1.00		1.00			
2.59-2.95	0.85	0.55-1.30	0.79	0.44-1.44		
2.96-3.38	0.85	0.55-1.31	0.65	0.35-1.20		
≥3.39	0.59	0.37-0.95	0.47	0.25-0.90		
	<i>P</i> for trend = 0.04		<i>P</i> for trend = 0.02 <i>P</i> for interaction = 0.97			
Whole sample	<i>MTRR</i> 66AA		<i>MTRR</i> 66AG		<i>MTRR</i> 66GG	
	OR	95% CI	OR	95% CI	OR	95% CI
Total intake						
≤2.58	1.00		1.00		1.00	
2.59-2.95	0.43	0.19-0.98	0.97	0.58-1.62	0.95	0.53-1.74
2.96-3.38	0.71	0.30-1.70	0.76	0.45-1.27	0.86	0.46-1.60
≥3.39	0.32	0.12-0.81	0.60	0.35-1.03	0.66	0.34-1.31
	<i>P</i> for trend = 0.06		<i>P</i> for trend = 0.04 <i>P</i> for interaction = 0.96		<i>P</i> for trend = 0.22	

NOTE: Adjusted for energy (residual method), energy (included as a covariate) age, sex, deprivation score, fiber intake (energy adjusted, quartiles), alcohol intake (energy adjusted), smoking (nonsmoker, current smoker, and former smoker), BMI, NSAID intake, and family history of cancer (low and moderate/high risk).

polymorphism and total vitamin B6 intake among rectal cases for both models (*P* for interaction = 0.04 and 0.02, respectively; Supplementary Table S2). However, this was not an a priori hypothesis and is likely to be a chance finding due to multiple testing.

Supplement intake was significantly associated with the disease status. In addition, intake of >2 mg/d of supplementary vitamin B6 versus no supplementary vitamin B6 intake was nonsignificantly inversely associated with CRC (OR, 0.81; 95% CI, 0.54-1.22). We identified the exact nutrient composition of these dietary supplements and added the supplement nutrients to the estimate of dietary nutrient intake. However, we also examined vitamin B6 effects after excluding the supplement takers (data not shown) and found no difference in the direction and strength of the associations.

The combined effect of high intake of vitamin B6 on CRC in the data set of three cohort studies (including one nested case-control study; refs. 7, 9, 10) and of five case-control studies (13, 15-18) showed a significant inverse association for the cohort studies and a strong significant inverse association for the case-control studies (combined relative risk, 0.81; 95% CI, 0.68-0.96 and combined OR, 0.65; 95% CI, 0.55-0.76, respectively; Fig. 1A; Supplementary Fig. S1A). The combined effect of case-control studies after additionally including the current study (13, 15-18) showed the same decreased risk (combined OR, 0.67; 95% CI, 0.60-0.75; Fig. 1B). For different cancer sites, colon cancer was significantly inversely associated with vitamin B6 intake after combining the results of three cohort (7, 10, 19) and three case-control studies [combined relative risk, 0.73; 95% CI, 0.62-0.87 (Supplementary Fig. S1B) and combined OR,

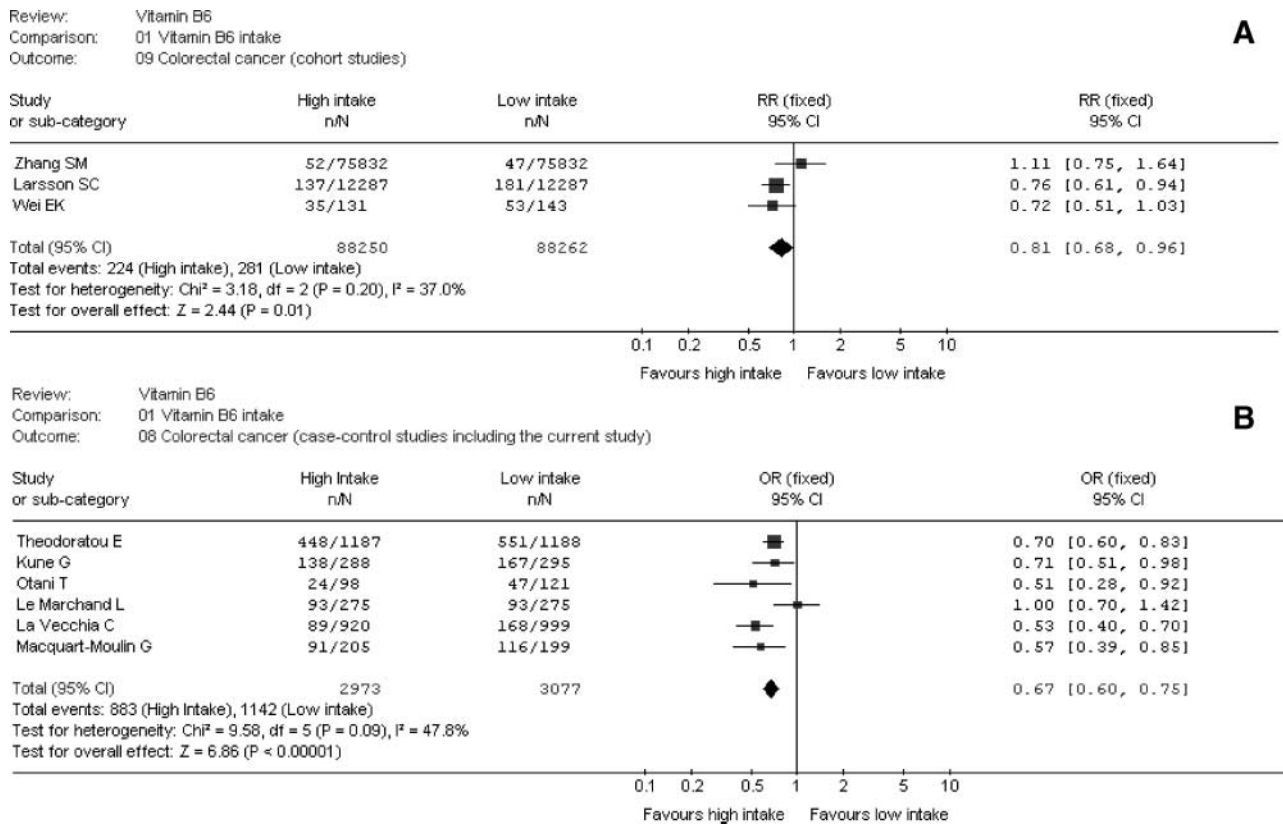


Figure 1. Risk ratios (ORs for case-control studies) of colorectal with high compared with low intake of vitamin B6 in cohort and case-control studies. The number of cases and controls for high and low category was calculated from OR and 95% CI for the following studies: La Vecchia et al. (17), Le Marchand et al. (16), and Slattery et al. (12). **A**, combined risk ratio of three cohort studies (CRC). Number of vitamin B6 intake categories: Zhang et al. (9), quintiles (mg/d); Larsson et al. (7), quintiles (mg/d); Wei et al. (10), quartiles (mg/d). Zhang et al. (9) vitamin B6 intake includes only dietary intake. **B**, combined OR of six case-control studies (CRC). Number of vitamin B6 intake categories: Theodoratou et al. (24), quartiles (mg/d); Kune and Watson (18), quintiles (mg/d); Otani et al. (13), tertiles (mg/d); Le Marchand et al. (16), quintiles (mg/d); La Vecchia et al. (17), quartiles (mg/d); Tuyns et al. (11); Macquart-Moulin et al. (15), quartiles (mg/d).

0.68; 95% CI, 0.60-0.78 (Supplementary Fig. S1D), respectively; refs. 12, 18]. Rectal cancer was significantly inversely associated with vitamin B6 intake after combining the results of two cohort (7, 19) and three case-control studies [combined relative risk, 0.72; 95% CI, 0.54-0.96 (Supplementary Fig. S1C) and combined OR, 0.81; 95% CI, 0.70-0.94 (Supplementary Fig. S1E), respectively; refs. 11, 18].

Discussion

We found in this large case-control study that patients with CRC reported lower dietary and total intakes of vitamin B6 than controls. These associations persisted after controlling for several confounding factors, including energy, fiber, and folate intake. There was a dose-response relationship with trend tests being highly significant. However, due to the high correlation and the common food sources of vitamin B6 and folate, this inverse association might be triggered by the folate effect on CRC. We tried to separate the vitamin B6 and folate effects by adjusting for folate intake and by examining the vitamin B6 and CRC associations among subjects

with low folate intake. The results suggested that the vitamin B6 inverse association was of similar strength when adjusting for folate and among subjects with low and high folate intake (Table 3). In addition, the folate and CRC associations in our population were less strong than the vitamin B6 ones and were further attenuated after fiber intake adjustment (Supplementary Table S3). The vitamin B6 effect was of same direction and magnitude after cancer site stratification but was stronger among females and those ages ≤ 55 years. After exploring the associations between CRC and the three main vitamin B6 food sources in our population, there was some evidence in favor of an inverse association with intake of bananas and mixed vegetables but less defined than the association of vitamin B6 and CRC. The lack of association with the intake of baked and boiled potatoes (one of the main vitamin B6 sources) might be because potatoes contribute to the glycemic load and this could possibly counter a vitamin B6 benefit.

When we examined the effect of high intake versus low intake of vitamin B6 in combined data sets of cohort and case-control studies, we found statistically significant decreased risks for colorectal (Fig. 1), colon, and

rectal cancer (Supplementary Fig. S1). Additionally, individual results from most of the published cohort and case-control studies showed an inverse effect of vitamin B6 intake on CRC risk (Table 1A and B). In the Nurses' Health Study (10), both plasma vitamin B6 and dietary (including supplement) intake were inversely associated with CRC. In the Women's Health Study (9), dietary but not total vitamin B6 intake was associated with a decreased risk of CRC. The two other cohort studies (7, 19) did not include vitamin B6 intake from supplements. In the Swedish Mammography Cohort (7), both colon and rectal cancer were inversely associated with vitamin B6 intake, whereas in the Iowa Women's Health Study (19) only colon cancer was inversely associated with vitamin B6 intake and this relationship was diluted after further adjustment. Of the eight case-control studies (11-18), only two included vitamin B6 intake from supplements (14, 16). Five of them showed an inverse association (11, 12, 15, 17, 18) and three of them showed no association with CRC (Table 1B; refs. 13, 14, 16). However, because most studies of dietary factors of one-carbon metabolism were focused on folate, nonsignificant findings for vitamin B6 could have been omitted from publications. Therefore, the results of the meta-analyses might be subject to publication bias.

It has been proposed that vitamin B6 effects might be modified by the intake of other nutrients, such as alcohol and folate (7), but our data showed no evidence of this. From the three published studies that investigated alcohol and B6 (7, 10, 20), only one found a clear interaction especially among women with high alcohol intake (>30 g/wk; ref. 7). (The mean intakes of daily alcohol intake of the studies that have studied the interaction between vitamin B6 and alcohol are as follows. Slattery et al. (20): men: cases, 37.5 g/d; controls, 29.1 g/d; women: cases, 18.2 g/d; controls, 17.9 g/d; Larsson et al. (7): 21.5 g/d; Wei et al. (10): cases, 7.6 g/d; controls, 6.3 g/d.) All three studies that investigated plasma or dietary folate and B6 (10, 19, 20) failed to show significant interaction.

Two previous meta-analyses (34, 35) have reported an inverse association of the *MTHFR* 677TT and the lack of statistical significance in our study might be due to limited power. Our data did not support the hypothesis that the vitamin B6 association with CRC is modified by polymorphisms of genes in the folate metabolism pathway (7). Three previous studies reported a lower risk of CRC (16, 21) and adenomas (22) in subjects carrying the *MTHFR* 677TT genotype and reporting high vitamin B6 intake. Otani et al. (13) also studied the effect of *MTHFR* A1298G and *MTRR* A66G and reported an interaction between the *MTRR* polymorphism and vitamin B6 intake.

There is a substantial body of data supporting the biological plausibility of a protective effect of vitamin B6 on CRC risk. Vitamin B6 plays a key role in the folate metabolism pathway as a coenzyme of the cystathionine β -synthase, which converts homocysteine into cystathionine (23). In addition, its role as a coenzyme in the synthesis of MTHF might be critical for synthesis, repair, and methylation of DNA and inhibition of single and double DNA breaks (7, 36). However, results from a HuGE review on folate-metabolizing polymorphisms and CRC (23) as well as the inconsistent results of the folate effect (37) indicate that the roles of both the

polymorphic genes and the involved dietary factors are complicated and need further research. Laboratory studies on mice suggest that high intake of pyridoxine has other anticarcinogenic effects by reducing cell proliferation, oxidative stress, nitric oxide production, and angiogenesis (38, 39) and a cultured human lymphocyte study reported a protective action against chromosomal damage (40). It has been proposed that the inhibition of DNA polymerases and steroid receptors of vitamin B6 may be useful as an adjuvant in cancer chemotherapy (41).

The strengths of our study include a very large sample size, use of a validated FFQ (25), and the identification of vitamin B6 intake from dietary supplements. Many different foods contributed to the intake of the vitamin B6 and so results are not determined by one major food category. Limitations of our study have been previously described (24). Briefly, underrepresentation of cases that were very ill when at presentation might limit external validity of results. Validity studies on nutrient estimates of this FFQ were carried out in younger subjects and we cannot be certain of the degree of validity in this older age group (25, 42). In addition, the FFQ was not validated against biomarkers, which is also a limitation of the study. However, any measurement error would most likely be nondifferential and thus underestimate true relationships. Limitations of case-control studies using FFQs include recall bias, misclassification bias due to imprecise measures of dietary intake, and residual confounding after attempts to control for confounders. However, we attempted to limit these problems by careful adjustment (e.g., fiber intake), adoption of identical study procedures in cases and controls, use of a FFQ that had been validated (25, 43), use of images of portion sizes and careful instructions to improve accuracy of reporting diet, and adoption of a recall period 1 year before diagnosis or recruitment date to reduce recall bias.

In conclusion, we report the findings of the largest case-control study to date investigating the association between CRC and dietary and total vitamin B6 intake. Given the evidence of anticarcinogenic effects from *in vitro*, animal *in vivo* studies, and some published epidemiologic studies, we gave this analysis high priority to minimize problems with multiple testing. Our finding of a moderately strong inverse association in models I and II between vitamin B6 intake and CRC risk with a dose-response relationship remained constant and statistically significant after further energy and fiber adjustment and stratification. This association was found for both colon and rectal cancer risk and was stronger in the younger age group of cases. Lack of any observed interaction with folate or alcohol intake or folate pathway polymorphisms may, despite the large sample size, be due to inadequate power to detect these effects. In addition, possible mechanism of action of vitamin B6 outwith its coenzyme function in the folate metabolism pathway needs further investigation. This inverse association is supported by our meta-analysis of all published case-control and cohort studies, which shows consistent evidence. Evidence of association is further strengthened by the report of a similar inverse association between CRC risk and plasma vitamin B6 levels (10). Confirmatory evidence from larger cohort studies and experimental studies is now required to confirm these associations,

to explore the mechanisms by which this effect, and to define the anticarcinogenic actions of vitamin B6.

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