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Dietary and supplemental folate and the risk of left- and right-sided colorectal cancer

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Abstract

Epidemiological evidence suggests that folate may lower the risk of colorectal cancer (CRC) although studies have been inconsistent and some have indicated differences in the effects of naturally occurring dietary folate and the synthetic form of this vitamin, folic acid. Most studies to date have considered CRC as a single disease; however, cancers that develop on the left and right sides of the colorectum display important phenotypic differences, suggesting they may also have different risk factors.

A population-based case-control study was conducted in Western Australia to examine the relationship between intake of both natural dietary folate and supplements containing folic acid and the risk of left- and right-sided CRC. Data were available for 850 cases (575 left-sided and 275 right-sided) and 958 controls. Odds ratios were calculated using multinomial logistic regression models.

There was no association between natural dietary folate intake and risk of either left-or right-sided CRC. Supplement use similarly had no significant effect on right-sided CRC. However, long-term supplement users (4+ years) were at lower risk of left-sided CRC than those who had not taken supplements (OR = 0.65, 95% CI 0.50-0.86) and there was a significant trend in risk reduction as duration of use increased ($p < 0.01$).

Keywords: folate, folic acid, colorectal cancer, left-sided, right-sided

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, accounting for around 10% of all new cancer cases (1). Epidemiologic studies have indicated that environmental factors, and in particular diet, are important risk factors for CRC. There has been considerable interest in the possible protective effect of folate, an important factor in the maintenance of normal DNA synthesis and methylation. However, studies have been inconsistent in their findings and there is limited evidence to support the hypothesis that folate reduces the risk of CRC (2).

Research into the relationship between folate and CRC risk has generally focused on cancer of the colon, the rectum or both together. However, there are considerable epidemiologic, genetic and molecular differences between cancers that develop proximal to the splenic flexure (right-sided) and those that develop distal to this site (left-sided) (3-7). If the two categories of CRC have distinct etiologies, then the inclusion of CRC as a single outcome in the studies undertaken may fail to identify risk factors that apply differentially to the two sides of the colorectum. The few studies that have reported dietary folate intake and the risk of subsite-specific CRC have been very heterogeneous, with no consistent findings (8-11).

Folic acid, the synthetic, monoglutamyl form of folate that is used in supplements and fortification, is more stable and has greater bioavailability than naturally occurring folates (12, 13). Some authors have raised concerns regarding intake of folic acid, which may have a promoting effect on the progression of established colorectal neoplasms (14, 15). Other studies have found that intake of multivitamins containing folic acid is associated with a reduction in CRC risk, especially when taken over a longer period of time (16, 17). Studies which have examined the effect separately by site of cancer have reported contradictory and sex-specific findings (18).

Collectively, the evidence from epidemiological studies is inconsistent. This study aims to provide further input regarding the relationship between folate intake, both from natural dietary sources and in the form of supplements, and the risk of left- and right-sided CRC.

Methods

Subjects

Cases were eligible if they were histologically-confirmed first incident cases of CRC (ICD-10 C18-C20) diagnosed during the period 1st June 2005 to 31st August 2007 and reported to the Western Australian Cancer Registry, resident in Western Australia and aged between 40 and 79 years old. Registration of all cancers except non-melanoma skin cancer is mandatory in Western Australia. There were 1,770 CRC cases notified to the Cancer Registry of which 226 were ineligible for inclusion for the following reasons: living outside of Western Australia (n=13), not adenocarcinoma or doubtful diagnosis (n=52), did not speak English (n=8), not well enough to participate (n=5), Hereditary Non-Polyposis Colorectal Cancer (n=2), incorrect address or age (n=8), clinician declined permission (n = 33) or died within 3 months of diagnosis (n = 105). Of the remaining 1,544 cases who were invited to participate, 1009 consented and 918 (59.5%) returned the questionnaires. Non-participating cases were more likely to be female.

Information on site for each cancer was obtained from the diagnostic reports stored at the WA Cancer Registry. Right-sided (proximal) CRC was defined as carcinoma arising in the caecum, ascending colon, hepatic flexure and transverse colon. Left-sided (distal) CRC was defined as occurring in the splenic flexure, descending colon, sigmoid colon, rectosigmoid junction and rectum.

Controls were randomly selected from the WA electoral roll, being frequency matched to cases for age and sex based on the approximate distribution of CRC in WA in 2002. All controls had no previous diagnosis of CRC, were able to speak adequate English and were sufficiently healthy to complete the questionnaires. Of the 2,198 controls approached, 1,021 (46.5%) participated. Non-participating controls were more likely to be aged 60 years or older.

Ethics approval for the WABOHS study was obtained from the Human Research Ethics Committee at The University of Western Australia and the Confidentiality of Health Information Committee, WA Department of Health. Written informed consent was obtained from all subjects.

Collection of data

Lifestyle and dietary data were collected using self-administered questionnaires. In addition, buccal DNA was collected from consenting participants using the FTA card method and used to genotype the C677T polymorphism in methylenetetrahydrofolate reductase (MTHFR) as previously described (7).

Dietary intakes were gathered using a 74-item food frequency questionnaire (FFQ) developed by the Cancer Council of Victoria (CCV, http://www.cancervic.org.au/about-our-research/cancer_epidemiology_centre/nutritional_assessment_services) and modified to gather information about intakes ten years earlier. Each food item had 10 frequency response options, ranging from 'never' to '3 or more times per day'. The FFQ has previously been validated against seven day weighed food records for young to middle-aged women (19). Folate was not one of the nutrients measured but the correlation coefficient for fibre, which correlates well with folate, was 0.49. The modified FFQ has additionally been

validated for 10-year dietary recall against 28 day diet records completed ten years earlier, with no difference reported for most nutrients, including fibre (20). All nutritional data gathered were processed by the CCV using food composition tables to provide an estimate of daily intake of specific food nutrients as well as total energy (21).

Estimates for intake of dietary folate, calcium and fibre were adjusted for total energy intake by calculating the residual nutrient densities according the method described by Willett and Stampfer (22). Energy-adjusted natural dietary folate was categorized into quintiles of intake based on distribution in the control group.

The FFQ included questions about alcohol, incorporating number of glasses per day of all alcohol and frequency of consumption for a range of different alcohols. Alcohol in grams per day was determined from total alcohol consumption, adjusted for energy intake using the same technique as described above. Subjects were classified as non-drinkers, low alcohol (<30g/day) or high alcohol ($\geq$ 30g/day) based on previous studies that have shown an increase in CRC risk at levels of more than 30g/day (23).

The lifestyle questionnaire was used to obtain data on area of residence, sex, age, country of birth, education level, smoking history, cholecystectomy, recreational physical activity and BMI. Socioeconomic status was determined from a Social-Economic Index for Area (SEIFA) score based on the participant's postcode. The SEIFA Index of Relative Socio-economic Advantage and Disadvantage is a continuous scale which summarises a set of social and economic information derived from census variables such as income and education (24). Quintiles of SEIFA disadvantage were based upon distribution in the control group.

Respondents were asked if they had taken multivitamins, multivitamins with minerals, B-complex, vitamin B or folic acid supplements at least once per week on a regular basis at any

time during the previous ten year period. The options available were 'Never', '1-3 years', '4-6 years' or '7+ years'. Because numbers in the '4-6 years' category were small (n=138), the upper two categories were combined for the analysis.

Information on recreational physical activity was gathered for two separate periods: between the ages 19-34 and 35-50 years. A calculated total was used to categorize physical activity according to Australian Institute of Health and Welfare guidelines with ≤ 150 min per week (insufficient) or > 150 min per week (sufficient) (25).

BMI at ages 20 and 40 years was calculated using height and weight at corresponding ages and categorized according to WHO guidelines as 'underweight', 'normal', 'overweight' or 'obese' (26).

Missing values

A high number of missing values for weight resulted in 8.6% missing data for BMI at both ages. Using multiple imputation to generate missing values for weight made no significant difference to the models developed so a non-imputed dataset was used.

There were also data missing for energy adjusted fibre intake (2.4%) and energy adjusted calcium intake (2.3%). For all other covariates considered for inclusion in the final model, less than 1% of values were missing.

Data analysis

Statistical analyses were performed using Stata version 11.1 (StataCorp LP, College Station, TX). Odds ratios were estimated for each exposure using maximum likelihood multinomial logistic regression and the p-values for differences in the beta estimates for each risk factor across left- and right-sided CRC were estimated using the post-estimation commands written

by Long and Freese (27) (spost9_ado from <http://www.indiana.edu/~jslsoc/stata>). Trend tests were performed by treating the variables of interest (dietary folate intake quintiles and supplement intake duration) as continuous variables. Statistical significance was defined as a p-value of less than 0.05.

The effects of the main exposure variables of interest (natural dietary folate intake and supplement intake duration) were firstly assessed in models adjusted only for the frequency-matching variables of age group and sex. All other variables in **Error! Reference source not found.** were investigated as potential confounders if they were differently distributed between cases and controls (Pearson chi-square, $p < 0.2$), by removing them one at a time from multivariate models containing the main exposure variable of interest. A resultant change of at least 10% in odds ratios for the exposure variable was considered to be evidence of confounding. Interactions between the main exposure variables of interest, the MTHFR genotype and alcohol consumption group were also considered by adding each possible interaction term to the model in turn. A p-value of 0.10 or lower for the interaction term was regarded as significant. There was no evidence of either confounding or interaction for any variables investigated, so multivariable models were additionally adjusted only for those variables hypothesized to be important in the metabolism of folate (alcohol and MTHFR genotype).

In the case of natural dietary folate intake, models were rerun excluding supplement users to investigate whether this would impact risk.

Odds ratios for CRC risk associated with each different type of supplement were generated using models for duration of use for each of the five types individually.

Results

A total of 918 cases and 1,021 controls consented to participate and completed the questionnaires. Of these, 16 (0.8%) did not consent to DNA testing, five participants were excluded from the analysis because tumor subsite was not recorded and 34 were excluded because of extreme energy values ($<3,000$ kJ/day or $>21,000$ kJ/day). A further 76 participants were excluded because genotyping for the MTHFR polymorphism failed, leaving a total of 1,808 participants (850 cases and 958 controls) included in these analyses.

Of the 850 cases, 575 (67.6%) were classified as left-sided and 275 (32.3%) as right-sided (Table 1). Cases were more likely than controls to be in the lowest socioeconomic group, to be former smokers, to have had a cholecystectomy, to be either overweight or obese at both 20 and 40 years of age and to be in the lower physical activity group (35-50 years). Dietary calcium and dietary fibre intake did not differ between cases and controls.

A larger proportion of CRCs arose on the left side in males (72%) than in females (62%) and in 40-49 year olds (75%) than in the 70-79 year olds (60%). Left-sided cases consumed more alcohol than right-sided and they were more likely to be genotype MTHFR 677CC and to be either overweight or obese at both 20 and 40 years of age. Right-sided cases were more likely than left-sided to have had a cholecystectomy.

There was no difference in the distribution of natural dietary folate intake between cases and controls or between left- and right-sided CRC cases. By contrast, controls were more likely than cases to have taken supplements with 24% having taken supplements for four years or more compared with 18% of cases. Left-sided cases were more likely never to have taken supplements (68%) than right-sided cases (61%).

In the regression analyses, natural dietary folate intake was not associated with risk of CRC overall (OR for highest vs lowest quintile of intake = 1.12, 95% CI 0.83-1.52) nor with risk of either left- or right-sided CRC (Table 2). There was no difference in risk between the two sides of the colorectum ($p=0.83$).

Excluding supplement users did not affect the findings and considering men and women separately revealed no association between natural dietary folate intake and risk of CRC in either (results not shown).

For supplement intake, there was a reduction in risk of CRC overall and of left-sided CRC as duration of supplement use increased (p for trend <0.01 ; Table 2). Taking supplements for four years or longer was inversely associated with risk of CRC (OR = 0.69, 95% CI 0.54-0.87) and with risk of left-sided CRC (OR = 0.65, 95% CI 0.50-0.86). There was no association between supplement intake duration and risk of right-sided CRC. The difference in risk for the two sides of the colorectum was significant ($p<0.01$).

Considering CRC overall, the risk reduction associated with taking supplements for four years or longer compared with not taking supplements was similar in men (OR = 0.64, 95% CI 0.46-0.88) and women (OR = 0.75, 95% CI 0.52-1.08). Men taking supplements for four or more years were at 41% lower risk of developing left-sided CRC than men who had not used supplements (OR = 0.59, 95% CI 0.41-0.85) while in women there was also a reduced risk observed but this was not statistically significant (OR = 0.72, 95% CI 0.48-1.09). There was no relationship between supplement intake duration and risk of right-sided CRC in either sex.

Risk of CRC was reduced for exclusive users of folic acid, B-complex and multivitamins with minerals compared with those who took no supplements although the results were non-

significant for all supplement types (Table 3). Non-exclusive supplement users as a group were at reduced risk of CRC compared with those who took no supplements (OR=0.75, 95% CI 0.59-0.95). Considering all users of each supplement type, both exclusive and non-exclusive, there was a reduced risk of CRC associated with increasing duration of use for each type, the effect being strongest for supplements reported as folic acid (p for trend = 0.05). Amongst non-exclusive users, 58% of supplement users reported taking more than one type of supplement and 6% reported taking all 5 types.

Discussion

Our study found that intake of natural dietary folate had no significant effect on the overall risk of CRC. This is not consistent with a meta-analysis of cohort studies which found a significant 25% reduced risk of CRC among those in the highest dietary folate intake group compared with those in the lowest (28). The same meta-analysis concluded that although case-control studies were consistent with a protective effect, there was considerable heterogeneity between studies and pooled results should be interpreted cautiously.

We also found no association between dietary folate intake and subsite-specific risk of CRC in men or in women. The few previous studies that have examined this relationship have returned inconsistent results, most of which similarly suggest no relationship (8-11). Slattery et al (8) reported non-significant increases in risk with increasing consumption of dietary folate for right-sided CRC and for left-sided CRC in men in their 1997 US-based case-control study. In contrast, studies of two European cohorts predominantly reported a reduction in risk on both sides of the colorectum with increasing dietary folate intake (9-11) although it was significant only for right-sided CRC in one cohort (11).

Given the differences between studies in terms of the population characteristics and the levels of folate consumed, some inconsistency in findings is not surprising. The levels of dietary folate intake were relatively low in the European cohort studies, with a mean daily intake below 225µg in both (9, 11). This compares with mean daily intake levels for controls of 352µg (women) and 422µg (men) in the US-based case-control study (8) and 314µg in the current case-control study.

The accuracy with which dietary folate intake can be measured is in itself a limitation in the current study and in others that have investigated this variable. The possible error in measurement of nutrient intake using FFQs, compounded by the impact of cooking methods on folate availability and possible inaccuracies in folate value estimations, make modest effects more difficult to detect in studies of diet and cancer. This has prompted some investigators to analyse the relationship between CRC risk and red blood cell or plasma folate levels but these studies have been no more consistent in their findings (reviewed in (29)). These measures may permit a more accurate assessment of folate status at one point in time but they vary with short term fluctuations in folate intake and cannot be used to estimate dietary folate intake over an extended period.

In contrast to dietary folate, we found that supplement intake duration had a significantly different effect on risk of CRC on the two sides of the colorectum (p for difference < 0.01), reducing risk on the left (OR for 4+ years of supplement intake vs no supplement use 0.65, 95% CI 0.50-0.86, p for trend < 0.01) and having no consistent impact on risk of right-sided CRC.

Information gathered for supplement intake related only to the duration of use of supplements belonging to five broad categories, all of which were likely to contain folic acid. Consumption of any of the different categories of supplement was associated with a reduced

risk of CRC, particularly if they were taken for four years or more (Table 3). This concurs with a 2007 Canadian study which reported that taking B-complex vitamin supplements (containing folic acid) for more than 5 years reduced the risk of both left- and right-sided colon cancer in women (18). It is also consistent with the findings of the Nurses' Health Study which reported a reduced risk of CRC for multivitamin use of five years or more (16). It is possible that supplement components other than folic acid contribute to the lower risk of CRC.

The possibility that individuals who use supplements tend to lead a "healthier" lifestyle than those who do not also warrants consideration. This study found no relationship between the duration of supplement intake and any of the lifestyle variables considered (alcohol intake, socioeconomic status, education level, smoking status, fibre intake, natural dietary folate intake, BMI and physical activity). It remains possible that other factors that were not investigated differentiate supplement users from non-users.

The inverse relationship between supplement use duration and CRC risk observed in this study was limited to left-sided CRC. Although not significant, risk of right-sided CRC was also reduced for long term users of supplements but it was increased for short-term users. The underlying mechanism for the differential protective effect of supplements according to tumor site in the large bowel is currently unclear, but it may be related to the different distribution of the CpG island methylator phenotype (CIMP+) and chromosomal instability (CIN) tumor phenotypes (7). CIMP+ tumors are characterized by frequent hypermethylation of gene promoter regions, but the effect of folate intake on methylation levels in normal colonic mucosa has not yet been established. Similarly, the effect of folate intake on DNA repair mechanisms involved in the development of CIN tumors in humans remains to be determined.

Individuals with the MTHFR 677TT genotype have been reported to be at lower risk of CRC than those with genotype MTHFR 677CC, particularly if on a diet high in folate (30, 31). The current study, however, found no significant interaction between MTHFR genotype and either natural dietary folate intake or the duration of supplement intake.

Alcohol is a known antagonist of folate metabolism (32) and a synergistic effect between excess alcohol intake and low folate intake has been proposed as a risk factor for cancer, including CRC (33). Giovannucci et al (34) reported that high alcohol consumption combined with inadequate dietary intakes of folate and methionine may increase the risk of colon cancer. However, the current study, which did not consider methionine intake, found no interaction between alcohol consumption and dietary folate intake. Similarly, no interaction was observed between alcohol consumption and supplement intake duration. Since folic acid intake itself was not measured, no definitive conclusions can be drawn from this observation or that concerning MTHFR genotype.

Strengths of this study include the fact that it was population-based and that for all cases, the anatomical site of origin of the CRC was accurately determined from pathology reports. Selection bias may have occurred in this study because of the lower response fraction of controls (46%) compared with cases (64%). Participants in the control group may have been more concerned about their health than the general population and therefore more likely to have taken supplements. Recall bias may also have been an issue, particularly as the FFQ required 10-year dietary recall. However, this questionnaire has been assessed and found to be as reliable as those which measure recent dietary intake (20). Additionally, the comparison of left- and right-sided CRC cases is less likely to be affected by bias, particularly recall bias, than comparison of cases and controls.

A number of variables that are frequently included as confounders in analyses of the relationship between dietary folate intake and CRC were not measured in this study, with the major one being family history of CRC. This may be important as it is possible that knowledge of a family predisposition to CRC may influence dietary and lifestyle choices. In addition, data on methionine intake were not available and as this micronutrient also plays an important role in DNA synthesis and methylation and may be related to risk of CRC (34, 35), it could be an important consideration.

In conclusion, the present study suggests that intake of dietary folate and intake of supplements containing folic acid may have different effects on the risk of CRC. Supplements appear to be protective against left-sided CRC, particularly when taken for extended periods of time. This study provides further evidence that left- and right-sided CRC may have different risk factors, highlighting the importance of considering tumor subsite in the design of future epidemiological studies involving CRC.

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References

- 1 World Cancer Research Fund: Cancer Facts - Bowel Cancer Rates. Available from: http://www.wcrf.org/cancer_facts/bowel_cancer_rates.php. Accessed Jan 27, 2011.
- 2 World Cancer Research Fund/American Institute for Cancer Research: Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective. Washington DC: AICR, 2007.
- 3 Distler P and Holt PR: Are right- and left-sided colon neoplasms distinct tumors? *Dig Dis* **15**, 302-311, 1997.
- 4 Lindblom A: Different mechanisms in the tumorigenesis of proximal and distal colon cancers. *Curr Opin Oncol* **13**, 63-69, 2001.
- 5 Iacopetta B: Are there two sides to colorectal cancer? *Int J Cancer* **101**, 403-408, 2002.
- 6 Sugai T, Habano W, Jiao Y, Tsukahara M, Takeda Y, et al.: Analysis of molecular alterations in left- and right-sided colorectal carcinomas reveals distinct pathways of carcinogenesis. *J Mol Diagn* **8**(2), 193-201, 2006.
- 7 Iacopetta B, Heyworth J, Girschik J, Grieu F, Clayforth C, et al.: The MTHFR C677T and DNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. *Int J Cancer* **125**, 84-90, 2009.
- 8 Slattery ML, Potter JD, Coates A, Ma KN, Berry TD, et al.: Plant foods and colon cancer: an assessment of specific foods and their related nutrients (United States). *Cancer Causes Control* **8**, 575-590, 1997.
- 9 Konings EJ, Goldbohm RA, Brants HA, Saris WHM, and van den Brandt PA: Intake of dietary folate vitamers and risk of colorectal carcinoma. *Cancer* **95**(7), 1421-1433, 2002.
- 10 de Vogel S, Dindore V, van Engeland M, Goldbohm RA, van den Brandt PA, et al.: Dietary folate, methionine, riboflavin, and vitamin B-6 and risk of sporadic colorectal cancer. *J Nutr* **138**(12), 2372-2378, 2008.
- 11 Larsson SC, Giovannucci E, and Wolk A: A prospective study of dietary folate intake and risk of colorectal cancer: modification by caffeine intake and cigarette smoking. *Cancer Epidemiol Biomarkers Prev* **14**(3), 740-743, 2005.
- 12 Caudill M: Folate bioavailability: implications for establishing dietary recommendations and optimizing status. *Am J Clin Nutr* **91**(suppl), S1455-S1460, 2010.
- 13 McNulty H and Pentieva K: Folate bioavailability. *Proc Nutr Soc* **63**(4), 529-536, 2004.
- 14 Kim YI: Role of folate in colon cancer development and progression. *J Nutr* **133**(suppl), S3731-S3739, 2003.

- 15 Kim YI: Folate and colorectal cancer: an evidence-based critical review. *Mol Nutr Food Res* **51**, 267-292, 2007.
- 16 Giovannucci E, Stampfer MJ, Colditz GA, and et al: Multivitamin use, folate and colon cancer in women in the Nurses' Health Study. *Ann Intern Med* **129**, 517-524, 1998.
- 17 Jacobs EJ, Connell CJ, Chao A, McCullough ML, Rodriguez C, et al.: Multivitamin use and colorectal cancer incidence in a US cohort: does timing matter? *Am J Epidemiol* **158**, 621-628, 2003.
- 18 Hu J, Morrison H, Mery L, DesMeules M, and Macleod M: Diet and vitamin or mineral supplementation and risk of colon cancer by subsite in Canada. *Eur J Cancer Prev* **16**(4), 275-291, 2007.
- 19 Hodge A, Patterson AJ, Brown WJ, Ireland P, and Giles G: The Anti Cancer Council of Victoria FFQ: Relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation. *Aust N Z J Public Health* **24**(6), 576-583, 2000.
- 20 Ambrosini GL, van Roosbroeck SAH, Mackerras D, Fritschi L, de Klerk NH, et al.: The reliability of ten-year dietary recall: implications for cancer research. *J Nutr* **133**(8), 2663-2668, 2003.
- 21 National Food Authority: NUTTAB95. Canberra, Australia: Commonwealth Government of Australia, 1995.
- 22 Willett WC and Stampfer MJ: Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* **124**, 17-27, 1986.
- 23 Cho E, Smith-Warner SA, Ritz J, van den Brandt PA, Colditz GA, et al.: Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Ann Intern Med* **140**, 603-613, 2004.
- 24 ABS and Australian Bureau of Statistics: Information paper: An Introduction to Socio-Economic Indexes for Areas (SEIFA). Canberra: Catalogue nr 2039.0. 2006.
- 25 Australian Institute of Health and Welfare: Risk factors: physical inactivity. Available from: <http://www.aihw.gov.au/risk-factors-physical-inactivity/>. Accessed Jan 17, 2012.
- 26 World Health Organisation: BMI classification. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. Accessed Feb 2, 2011.
- 27 Long J and Freese J. *Regression models for categorical outcomes using Stata*: 2nd ed. College Station TX, Stata Press, 2005.
- 28 Sanjoaquin MA, Allen N, Couto E, Roddam AW, and Key TJ: Folate intake and colorectal cancer risk: a meta-analytical approach. *Int J Cancer* **113**(5), 825-828, 2005.
- 29 Hubner RA and Houlston RS: Folate and colorectal cancer prevention. *Br J Cancer* **100**(2), 233-239, 2009.
- 30 Slattery ML, Potter JD, Samowitz W, Schaffer D, and Leppert M: Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* **8**(6), 513-518, 1999.

- 31 Sharp L and Little J: Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol* **159**, 423-443, 2004.
- 32 Hillman RS and Steinberg MD: The effects of alcohol on folate metabolism. *Annu Rev Med* **33**, 345-354, 1982.
- 33 Mason JB and Choi SW: Effects of alcohol on folate metabolism: implications for carcinogenesis. *Alcohol* **35**, 235-241, 2005.
- 34 Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, et al.: Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* **87**(4), 265-273, 1995.
- 35 Kune G and Watson L: Colorectal cancer protective effects and the dietary micronutrients folate, methionine, vitamins B6, B12, C, E, selenium, and lycopene. *Nutr Cancer* **56**(1), 11-21, 2006.

Tables

Table 1 Distribution of variables for CRC cases and population controls in the Western Australian Bowel Health Study

Table 2 Associations between folate intake and left-sided, right-sided and all CRC

Table 3 Associations between supplement intake and risk of CRC for each supplement type

Table 1: Distribution of variables for CRC cases and population controls in the Western Australian Bowel Health Study

Demographic variables and potential confounders		% of left- sided cases (n=575)	% of right- sided cases (n=275)	% of all CRC cases (n=850)	% of controls (n=958)
Sex ¹	Male	64.0	53.4	60.6	58.7
	Female	36.0	46.6	39.4	41.3
Age Group ¹	40-49 years	5.7	4.0	5.2	7.4
	50-59 years	28.5	16.4	24.6	22.3
	60-69 years	36.6	38.2	37.1	35.2
	70-79 years	29.2	41.4	33.1	35.1
Alcohol intake	None	20.4	19.6	20.1	21.6
	Low (<30g/day)	53.7	59.6	55.7	57.7
	High (≥30g/day)	25.9	20.7	24.2	20.7
		$p^2 = 0.195$		$p^3 = 0.184$	
MTHFR genotype	CC	47.8	38.9	44.9	44.7
	CT	43.3	49.8	45.4	44.8
	TT	8.9	11.3	9.7	10.5
		$p^2 = 0.046$		$p^3 = 0.817$	
Socioeconomic status	Quintile 1 (low)	25.9	21.5	24.5	19.7
	Quintile 2	16.9	16.7	16.8	20.3
	Quintile 3	19.7	16.7	18.7	19.4
	Quintile 4	18.6	24.4	20.5	20.7
	Quintile 5 (high)	17.9	19.3	18.3	19.2
	Unknown	1.0	1.4	1.2	0.6
		$p^2 = 0.323$		$p^3 = 0.092$	
Country of birth	Australia/NZ	61.7	65.5	63.0	66.5
	UK/Ireland	23.1	22.2	22.8	21.8
	Europe	8.2	7.6	8.0	6.6
	Asia	3.6	1.1	2.8	3.0
	Other	3.1	3.6	3.3	1.7
	Unknown	0.2	0.0	0.1	0.4
		$p^2 = 0.359$		$p^3 = 0.119$	
Smoker	Never	38.4	42.2	39.7	45.5
	Former	51.5	49.4	50.8	45.1
	Current	10.1	8.4	9.5	9.4

		$p^2 = 0.499$		$p^3 = 0.035$	
BMI at 20 years	Underweight	5.6	3.6	4.9	7.5
	Normal	63.3	73.4	66.6	69.3
	Overweight	18.1	13.4	16.6	13.4
	Obese	2.8	1.4	2.4	2.0
	Unknown	10.2	8.0	9.5	7.8
		$p^2 = 0.059$		$p^3 = 0.038$	
BMI at 40 years	Underweight	1.4	1.1	1.3	1.0
	Normal	45.4	49.1	46.6	52.5
	Overweight	30.1	30.9	30.3	29.9
	Obese	13.9	10.2	12.7	8.3
	Unknown	9.2	8.7	9.1	8.3
		$p^2 = 0.591$		$p^3 = 0.015$	
Cholecystectomy	Yes	11.1	14.6	12.2	10.0
	No	88.5	83.6	87.0	89.9
	Unknown	0.4	1.8	0.8	0.1
		$p^2 = 0.028$		$p^3 = 0.021$	
Physical activity	≤ 150 mins/week	51.3	47.3	50.0	50.0
19-34 years	> 150 mins/week	47.8	51.6	49.1	49.8
	Unknown	0.9	1.1	0.9	0.2
		$p^2 = 0.535$		$p^3 = 0.110$	
Physical activity	≤ 150 mins/week	44.9	42.2	44.0	41.5
35-50 years	> 150 mins/week	54.2	56.4	54.9	58.3
	Unknown	0.9	1.4	1.1	0.2
		$p^2 = 0.590$		$p^3 = 0.033$	
Main exposure variables of interest		% of left-sided cases (n=575)	% of right-sided cases (n=275)	% of all CRC cases (n=850)	% of controls (n=958)
Natural dietary	Q1 (< 254.6)	19.8	18.6	19.4	20.2
folate intake	Q2 (254.6-287.8)	17.4	17.1	17.3	19.8
quintile ($\mu\text{g/day}$)	Q3 (287.9-322.7)	21.1	21.1	21.1	20.0
	Q4 (322.8-374.5)	18.6	22.5	19.9	19.9
	Q5 (> 374.5)	23.1	20.7	22.3	20.1
		$p^2 = 0.718$		$p^3 = 0.550$	
Supplement intake	Never	68.5	61.4	66.2	60.6

duration	1–3 years	14.1	20.0	16.0	15.7
	4–6 years	6.4	4.4	5.8	9.3
	7+ years	11.0	14.2	12.0	14.4
		$p^2 = 0.037$		$p^3 = 0.009$	

¹cases and controls frequency matched on these variables

²p-value (left- v right-sided), excludes controls

³p-value (cases v controls)

Table 2: Associations between folate intake and left-sided, right-sided and all CRC

Natural dietary folate intake quintile (µg/day)	Left-sided cases n=575			Right-sided cases n=275			p ⁴	All CRC cases	
	OR ¹ (95% CI)	AOR ² (95% CI)	p ³	OR ¹ (95% CI)	AOR ² (95% CI)	p ³		AOR ² (95% CI)	p ³
Q1 (<254.6)	1.00	1.00	0.45	1.00	1.00	0.39	0.83		0.34
Q2 (254.6-287.8)	0.92 (0.65-1.29)	0.93 (0.66-1.30)		0.88 (0.56-1.38)	0.92 (0.58-1.44)			0.92 (0.68-1.24)	
Q3 (287.9-322.7)	1.06 (0.76-1.47)	1.07 (0.77-1.49)		1.11 (0.72-1.71)	1.11 (0.72-1.72)			1.08 (0.81-1.45)	
Q4 (322.8-374.5)	0.94 (0.67-1.31)	0.93 (0.66-1.31)		1.18 (0.77-1.81)	1.20 (0.78-1.85)			1.01 (0.75-1.37)	
Q5 (>374.5)	1.16 (0.84-1.61)	1.15 (0.82-1.61)		1.09 (0.70-1.68)	1.09 (0.70-1.69)			1.12 (0.83-1.52)	
Supplement intake duration									
Never	1.00	1.00	<0.01	1.00	1.00	0.29	<0.01		<0.01
1-3 years	0.79 (0.58-1.07)	0.78 (0.58-1.06)		1.32 (0.92-1.90)	1.30 (0.90-1.86)			0.93 (0.72-1.22)	
4+ years	0.65 (0.50-0.86)	0.65 (0.50-0.86)		0.78 (0.55-1.12)	0.77 (0.54-1.10)			0.69 (0.54-0.87)	

¹Odds Ratio, adjusted for age group and sex²Adjusted Odds Ratio, adjusted for MTHFR genotype, alcohol intake category, supplement intake, age group and sex³p for trend ⁴p value for difference between left-sided and right-sided CRC

Table 3: Associations between supplement intake and risk of CRC for each supplement type

Supplement type	All CRC cases n=850		Controls n=958	
	n	OR ¹ (95% CI)		n
No supplements	563	1.00		581
Folic acid only	11	0.52 (0.25-1.08)		22
B-complex only	10	0.71 (0.31-1.59)		15
Multivitamins only	59	1.10 (0.74-1.62)		54
Multivit/minerals only	31	0.68 (0.42-1.09)		46
Vitamin B only	14	1.13 (0.52-2.43)		13
2+ types in combination	162	0.75 (0.59-0.95)		227
Specific supplement use, including exclusive and non-exclusive users²				
Folic acid				
Never	776	1.00	0.05 ³	850
1-3 years	46	0.95 (0.63-1.44)		57
4+ years	28	0.60 (0.38-0.97)		51
B-complex				
Never	749	1.00	0.11 ³	820
1-3 years	50	0.89 (0.60-1.31)		63
4+ years	51	0.74 (0.51-1.08)		75
Multivitamins				
Never	660	1.00	0.07 ³	714
1-3 years	100	0.97 (0.72-1.31)		113
4+ years	90	0.75 (0.56-1.00)		131
Multivit/minerals				
Never	698	1.00	0.11 ³	756
1-3 years	66	0.86 (0.61-1.21)		85
4+ years	86	0.80 (0.59-1.08)		117
Vitamin B				
Never	761	1.00	0.08 ³	839

1-3 years	54	1.04 (0.70-1.54)	58
4+ years	35	0.63 (0.41-0.98)	61

¹Odds Ratio, adjusted for age group and sex

²Participants may have taken one or more supplement types

³p for trend