Reported Intake of Selected Micronutrients and Risk of Colorectal Cancer: Results from a Large Population-based Case–control Study in Newfoundland, Labrador and Ontario, Canada

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Abstract. *Aim: The impact of micronutrient intake and colorectal cancer (CRC) risk is poorly understood. The objective of this study was to evaluate the associations of selected micronutrients with risk of incident CRC in study participants from Newfoundland, Labrador (NL) and Ontario (ON), Canada. Materials and Methods: We conducted a population-based study among 1760 case participants and 2481 age- and sex-matched control participants. Information on diet and other lifestyle factors were measured using a food frequency questionnaire and a personal history questionnaire. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression, controlling for covariables. Results: Highest compared to lowest quartile intakes of certain micronutrients were associated with lower risk of CRC, including: calcium (from food and supplements (FS), OR=0.59; 95% CI=0.45-0.77, and from food only (FO): OR=0.76, 95% CI=0.59-0.97), vitamin C (FS:OR=0.67; 95%CI:0.51-0.88), vitamin D (FS: OR=0.73; 95% CI: 0.57-0.94, FO: OR=0.79, 95% CI=0.62-1.00), riboflavin (FS: OR=0.61; 95% CI=0.47-0.78, and folate (FS: OR=0.72; 95% CI=0.56-0.92). Higher risk of CRC was observed for iron intake (highest versus lowest quintiles: OR=1.34, 95% CI=1.01-1.78). Conclusion: This study presents evidence that dietary intake of calcium, vitamin D, vitamin C, riboflavin and folate are associated with a lower risk of incident CRC and that dietary intake of iron may be associated with a higher risk of the disease.*

Diet and lifestyle factors play an important role in the aetiology of colorectal cancer (CRC) (1). The large Newfoundland and Ontario Colorectal Cancer Study (NOCS) has linked a wide range of these factors to CRC (2-4). Among dietary factors, calcium, vitamin D, folate, vitamin B6, and some antioxidants (*e.g.* vitamins C and E, and certain carotenoids) have been hypothesized to lower risk of colon cancer (5-7), whereas iron may increase risk (8). Calcium and vitamin D may protect against colorectal carcinogenesis by binding free fatty acids and secondary bile acids in the small intestine, thereby protecting colonic epithelial cells from mutagens (9). Folate and other B-vitamins may exert a protective effect against colorectal carcinogenesis by providing co-factors for the methylation of thymidylate for DNA synthesis and the production of S-adenosylmethionine, the primary methyl donor in the body (7, 10). Beta-carotene and vitamins A, C, and E may reduce the risk of CRC through antioxidant mechanisms (11, 12). It has been suggested that iron may increase the risk of CRC by generating free radicals that attack DNA and damage chromosomes (13, 14).

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Key Words: Colorectal cancer, case–control study, micronutrients, Canada, iron intake, vitamin C intake, vitamin D intake, riboflavin intake, folate intake.

Despite the biological plausibility, the associations between intakes of micronutrients and risk of CRC have not been universally defined, and the results for each micronutrient have been inconsistent. The recently updated World Cancer Research Fund/American Institute for Cancer Research Second Expert Report (SER) on CRC (15), identified 6 new cohort studies that investigated dietary vitamin D intake (16-21). Out of these, only one (18), but not others (19-21), has reported that dietary vitamin D is associated with a significantly lower risk of CRC. Some studies have suggested an increased risk with an increased intake of iron (22-24), but the amount of evidence is limited. Additionally, results from SER meta-analysis for dietary folate showing a reduced risk for CRC were primarily based on folate from dietary sources rather than supplements; thus no effect could be specifically attributed to folate (15, 25, 26).

The epidemiological evidence for the role of dietary antioxidants is also inconsistent. Results from a pooled analysis from five cohorts found a modestly reduced risk of CRC among those in the highest quartiles of alpha-tocopherol intake compared to those in the lowest (27). Another pooled analysis from 13 prospective cohorts suggested no association between dietary intakes of vitamin A, C, and alpha-tocopherol and CRC risk, whereas a strong inverse association for the vitamins was detected when looking at both dietary and supplementary multivitamin intake (28). On the other hand, some studies found no significant beneficial effects on the occurrence of CRC for supplementation with these nutrients (7, 29-32).

It is of considerable importance to examine the associations of micronutrients with CRC risk because of the inconsistent findings from previous studies. Bearing in mind the increasing frequency of dietary supplement use in the Canadian population, it is imperative to understand the health implications of these supplements, including potential associations with CRC.

Materials and Methods

Selection of cases and controls. Data for this study were obtained from the Ontario Familial Colorectal Cancer Registry (OFCCR) and the Newfoundland Familial Colorectal Cancer Registry (NFCCR). In ON, eligible cases were residents of the province, aged 20-74 years, with newly diagnosed colorectal adenomas identified in the Ontario Cancer Registry (OCR) during 1997-2000 (phase 1) or 2003-2006. In NL, eligible cases were incident patients aged 20-74 years, identified from the Newfoundland Cancer Registry during 1999-2003. Pathology reports were then used to verify the diagnosis (International Classification of Diseases (ICD) -9 codes: 153.0-153.9, 154.0-154.3 and 154.8, or ICD-10 codes: 18.0-18.9, 19.9, and 20.9).

Controls were a random sample of residents in each province aged 20-74 years, without a diagnosis of previous CRC. In ON, controls were randomly selected from population-based property assessment rolls (owners and occupants). In NL, controls were identified through random digit dialing using a list of residential telephone numbers provided by Aliant (the local telephone company in NL) (33). Controls were frequency matched to cases on sex and five-year age strata in both provinces. A detailed description of the recruitment of controls is reported elsewhere (34).

All cases and controls who consented to participate were sent a written consent form, family history questionnaire (FHQ), personal history questionnaire (PHQ), and food frequency questionnaire (FFQ). Those who did not respond were sent postcard reminders and were telephoned several weeks after initial contact to remind them of the mailing. The overall response rates for cases and controls were 65.0% and 53.5%, respectively

Dietary and epidemiologic data collection. Dietary intakes were gathered using a self-administrated FFQ. The FFQ administered in ON was the validated Hawaii FFQ, developed by the Epidemiology Program, Cancer Research Centre of Hawaii (35, 36). The NL FFQ was a modified version of the questionnaire used in ON which had been adapted to include foods regional in NL (*e.g.* salted/pickled meat and smoked/pickled fish). The FFQ was used to assess diet over one to two years prior to diagnosis or interview in each province. Participants were asked to estimate the frequency of food intake and their usual portion size from 'regular', 'small' or 'large' of almost 170 food items based on food photographs that provided examples of portion sizes. Questions were also assigned to gather information on the use of dietary supplements, including the usual brand name, the amounts taken and the duration of consumption. Quantities of each micronutrient were calculated by multiplying the reported frequency of each food item by the nutrient content of portion size on the basis of the US Department of Agriculture Nutrient Database (37) in ON and the 2005 Canadian Nutrient file (38) in NL.

Sociodemographic data, such as sex, age, date of birth, and marital status, were obtained by a self-administered PHQ. The PHQ also included close-ended questions about medical history, bowel screening history, diet, medication use, physical activity, reproductive factors, alcohol and tobacco use. For female participants, there were additional questions relating to reproductive factors.

For the analyses, we excluded those who did not provide sufficient information on diet and potential risk factors at baseline and those who had familial adenomatous polyposis (n=34). Another 225 individuals who reported implausible energy intake in the upper or lower 2.5% of intake were further excluded (the upper and lower cutoffs: in NL, 925 and 4700 kcal for men, 1100 and 4900 kcal for women, respectively; in ON, 1040 and 5200 kcal for men, 835 and 4100 kcal for women, respectively). After these exclusions, based on those who completed both the PHQ and FFQ, 3102 individuals (1272 cases and 1830 controls) from ON and 1139 (488 cases and 651 controls) from NL remained for this analysis.

Statistical analyses. Descriptive statistics, stratified by case–control status were utilized to show the distribution of sociodemographic characteristics in the study population. The baseline characteristics were analyzed as categorical variables using chi-squared tests. Intakes of micronutrients were adjusted for total energy intake by use of the nutrient residual method described by Willett and Stampfer (39). Micronutrient exposure included food sources and supplements. Intakes of micronutrients were calculated by summing energy-adjusted micronutrients from food and unadjusted micronutrients from supplements. Micronutrient intakes were categorized into quintiles based on the distribution among the study population without omitting endpoints and entered into models as indicator variables with the lowest quintile as the referent group.

Age-adjusted unconditional logistic regression models were calculated as stratified by province. Pooled analyses were conducted since odds ratios (OR) between provinces were similar. OR and the corresponding 95% confidence intervals (CI) used to interpret the association of selected micronutrients with CRC risk were calculated in two unconditional logistic regression models. Initial models were only adjusted for age and total energy intake. The multivariate models were further adjusted for sex, body mass index, physical activity, family history of CRC, history of polyps, diabetes, history of colon screening procedure, cigarette smoking , alcohol drinking, education attainment, household income, marital status, regular use of non-steroid anti-inflammatory drug (NSAID), multivitamin supplements, reported hormone replacement therapy (HRT, females only), and intakes of fruits, vegetables, red meat, and province of residence (NL, ON). Potential confounders were included in the model based on: (i) literature review, (ii) biological plausibility, (iii) whether the regression coefficient of the primary predictors changed by 10% or more after addition of the potential confounder, or (iv) whether the covariate entered the model at $p<0.05$. A backwards-stepwise procedure was performed to obtain the final model. Tests for trend were used to assess dose-response relationships based on the median of each category of micronutrient intake. Statistical tests were two sided, and p-values less than 0.05 were considered statistically significant.

Ethical considerations. Ethics approval for this study was obtained from the Human Investigation Committee, Faculty of Medicine, Memorial University of Newfoundland.

Results

The distribution of CRC cases and controls according to age, sex, dietary habits and other selected variables are shown in Table I. The study participants included 1760 cases and 2481 controls. By design, cases and controls had similar distributions of sex and province of residence. Compared to controls, cases tended to be younger, more overweight or obese, either physically inactive or extremely physically active, more likely to have a positive family history of CRC, and consume more red meat and have a higher total energy intake. Intakes of fruit and vegetables did not vary significantly between cases and controls.

Table II shows mean intakes of micronutrients from food sources alone, and from both food and supplements among cases and controls. For most micronutrients (except for iron and retinol), controls consistently reported significantly higher intakes of micronutrients from food sources or from food and supplement sources as compared to cases (all $p<0.05$). Furthermore, higher intakes of micronutrient in controls were primarily due to larger contributions from supplement sources. For example, no differences were found in retinol intake (from food), however, after including supplements in the intake, significant differences were found between cases and controls. Cases had higher intakes of iron, largely due to dietary supplements use ($p<0.0001$).

The OR and 95% CI of CRC according to intakes of selected micronutrients from both food and supplements are presented in Table III. After adjusting for potential covariates, risk of CRC was found to be significantly inversely associated with intakes of total calcium (highest *vs*. the lowest quintiles: OR=0.59; 95% CI=0.45-0.77), vitamin

Table I. *Selected characteristics of participants from the colorectal cancer case–control study in Newfoundland, Labrador, and Ontario*.

Characteristic	Cases (n=1760) No. (%)	Controls (n=2481) No. (%)
Age (years)*		
18-49	368 (20.9)	265 (10.7)
50-59	412 (23.4)	690 (27.8)
60-69	646 (36.7)	998 (40.2)
70+	334 (19.0)	528 (21.3)
Gender		
Males	935 (53.1)	1357 (54.7)
Females	825 (46.9)	1124 (45.3)
Province of residence		
Newfoundland and Labrador	488 (27.7)	651 (26.2)
Ontario	1272 (72.3)	1830 (73.8)
BMI (kg/m^2)*		
Underweight (<18.5)	23 (1.3)	22 (0.9)
Normal (18.5-24.9)	595 (33.8)	930 (37.5)
Overweight (25-29.9)	748 (42.5)	1069 (43.1)
Obese (≥30)	394 (22.4)	460 (18.5)
Physical activity (METs/week)*		
0-7.4	465 (26.4)	595 (24.0)
7.4-22.4	348 (19.8)	633 (25.5)
22.4-53.0	429 (24.4)	633 (25.5)
>53.0	518 (29.4)	620 (25.0)
Family history of CRC*		
No	1582 (89.9)	2337 (94.2)
Yes	178 (10.1)	144 (5.8)
Fruit intake (X servings/week)		
0≤X≤6	475 (27.0)	625 (25.2)
6<X≤7	502 (28.5)	754 (30.4)
7<X≤14	459 (26.1)	653 (26.3)
X>14	324 (18.4)	449 (18.1)
Vegetable intake (X servings/week)		
0≤X≤6	260 (14.8)	367 (14.8)
6<X≤7	549 (31.2)	796 (32.1)
7<X≤14	505 (28.7)	707 (28.5)
X>14	445 (25.3)	610 (24.6)
Red meat intake* (X servings/week)		
0≤X≤2	269 (15.3)	486 (19.6)
2<X≤3	702 (39.9)	987 (39.8)
3<X≤5	398 (22.6)	526 (21.2)
X>5	392 (22.3)	481 (19.4)
Total energy intake (kcal/day)*		
Quintile 1 (≤1580)	313 (17.8)	536 (21.6)
Quintile 2 (1580-1943)	341 (19.4)	506 (20.4)
Quintile 3 (1943-2314)	343 (19.5)	506 (20.4)
Quintile 4 (2314-2866)	359 (20.4)	489 (19.7)
Quintile 5 (>2866)	404 (22.9)	444 (17.9)

BMI, Body mass index; METs/week, metabolic equivalent hours per week; 'servings' according to the United States Department of Agriculture (USDA) Food Pyramid. *Significant difference between cases and controls ($p<0.05$).

Table II. *Comparison of mean±standard deviation (SD) intakes of selected micronutrients between cases and controls in the colorectal cancer case–control study in Newfoundland, Labrador, and Ontario.*

Micronutrient intake	Cases (n=1760)	Controls (n=2481)	Difference (Controls–Cases)	*P*-value[a]
Calcium (mg/d)				
From food	948.8±316.4	1003.8±337.7	55	<0.0001
From food and supplements	1095.7±489.9	1199.8±537.0	104.1	<0.0001
Iron (mg/day)				
From food	17.4±5.9	17.8±6.2	0.4	0.014
From food and supplements	30.1±58.6	24.5±25.4	–5.6	<0.0001
Retinol (μg/d)				
From food	890.9±454.1	914.8±442.0	23.9	0.087
From food and supplements	1351.9±1052.6	1490.5±1182.8	138.6	<0.0001
Vitamin C (mg/d)				
From food	160.5±89.5	168.2±88.5	7.7	0.006
From food and supplements	403.7±940.3	473.0±1058.2	69.3	0.03
Vitamin D (μg/d)				
From food	5.4±2.8	5.7±2.9	0.3	<0.0001
From food and supplements	8.1±5.6	9.1±6.3	1.0	<0.0001
Alpha-tocopherol (mg/d)				
From food	6.1±2.4	6.3±2.5	0.2	0.028
From food and supplements	44.2±85.5	57.6±97.0	13.4	<0.0001
Thiamin (mg/d)				
From food	1.9±0.5	2.0±0.5	0.1	0.029
From food and supplements	2.8±3.1	3.3±4.5	0.5	<0.0001
Riboflavin (mg/d)				
From food	2.5±0.7	2.6±0.7	0.1	<0.0001
From food and supplements	3.6±3.4	4.1±4.7	0.5	<0.0001
Vitamin B6 (mg/d)				
From food	2.4±0.8	2.5±0.8	0.1	0.025
From food and supplements	3.6±3.9	4.3±5.9	0.7	<0.0001
Vitamin B12 (μg/d)				
From food	7.1±3.9	7.1±3.4	0	0.81
From food and supplements	12.1±13.8	14.2±18.6	2.1	<0.0001
Folate (μg/d)				
From food	343.5±123.9	360.2±125.7	16.7	<0.0001
From food and supplements	534.9±404.3	598.1±452.2	63.2	<0.0001

[a]Differences between cases and controls based on *t*-test.

C (OR=0.67; 95% CI=0.51-0.88), vitamin D (OR=0.73; 95% CI=0.57-0.94), riboflavin (OR=0.61; 95% CI=0.47-0.78), and folate (OR=0.72; 95% CI=0.56-0.92). A direct relation emerged regarding the iron intake (OR=1.34, 95% CI=1.01-1.78). No links were found to retinol, alpha-tocopherol, thiamin, vitamin B6, and vitamin B12.

We also evaluated the associations of CRC risk with selected micronutrients from food sources only (Table IV). After adjusting for potential confounders, CRC risk was inversely associated with dietary calcium (OR=0.76, 95% CI=0.59-0.97) and dietary vitamin D (OR=0.79, 95% CI=0.62-1.00) intakes, and non-significantly inversely related to intakes of vitamin C (OR=0.87, 95% CI=0.67-1.13), riboflavin (OR=0.86, 95% CI=0.68-1.09), and folate (OR=0.83, 95% CI=0.65-1.05).

We additionally examined CRC risk according to individual supplement use and levels of micronutrient intakes from foods (Table V). After adjusting for multivitamin supplement use and other covariates, significantly reduced risks were observed among users of individual calcium, vitamin C, and folate supplements, and weak or nonexistent relationships were found among non-supplement users. The lowest risk was observed among calcium supplement users with higher dietary calcium intake. The use of vitamin C supplement or folate supplement appeared to have further benefit among those with relative lower dietary intakes of these nutrients. Among iron supplement users, however, OR reached 1.70 with a higher intake of iron from food compared with a relatively lower intake.

Discussion

Our data, from a large population-based case–control study, suggest inverse associations of CRC risk with intake of certain micronutrients from both food sources and supplement

Table III. *Adjusted odds ratio (OR), 95% confidence interval (CI) of colorectal cancer risk according to selected micronutrients intakes from both food and supplement sources in the case–control study in Newfoundland, Labrador, and Ontario.*

Micronutrients from food and supplements	Quintiles of intake					*P*-value for trend[c]
	Q1	Q2	Q3	Q4	Q5	
Calcium						
No. of cases/controls	414/436	362/486	385/463	315/533	284/563	
Median intake (mg/d)	662.7	865.0	1049.7	1285.6	1786.7	
OR[a] (95% CI)	1.00	0.83 (0.68-1.01)	0.94 (0.77-1.14)	0.66* (0.54,0.80)	0.57* (0.47-0.69)	0.04
OR[b] (95% CI)	1.00	0.86 (0.67-1.09)	1.10 (0.86-1.40)	0.72* (0.56-0.92)	0.59* (0.45-0.77)	0.11
Iron						
No. of cases/controls	374/476	373/475	343/505	320/528	350/497	
Median intake (mg/d)	12.6	15.3	18.5	25.1	36.7	
OR[a] (95% CI)	1.00	1.07 (0.88-1.30)	0.94 (0.77-1.14)	0.83 (0.68-1.01)	0.95 (0.78-1.15)	0.42
OR[b] (95% CI)	1.00	0.99 (0.79-1.25)	0.97 (0.77-1.23)	1.09 (0.84-1.43)	1.34* (1.01-1.78)	0.02
Retinol						
No. of cases/controls	369/481	380/468	361/487	338/510	312/535	
Median intake (μg/d)	454.8	777.6	1033.8	1684.6	2766.2	
OR[a] (95% CI)	1.00	1.13 (0.93-1.37)	1.05 (0.86-1.28)	0.90 (0.74-1.09)	0.82* (0.67-1.00)	0.07
OR[b] (95% CI)	1.00	1.29 (0.99-1.62)	1.24 (0.98-1.57)	1.14 (0.89-1.46)	1.08 (0.79-1.47)	0.77
Vitamin C						
No. of cases/controls	407/443	359/489	354/494	333/515	307/540	
Median intake (mg/d)	82.6	142.0	203.3	310.6	776.3	
OR[a] (95% CI)	1.00	0.87 (0.72-1.06)	0.85 (0.70-1.03)	0.74* (0.61-0.90)	0.67* (0.55-0.82)	0.06
OR[b] (95% CI)	1.00	0.79 (0.62-1.01)	0.84 (0.66-1.07)	0.81 (0.63-1.05)	0.67* (0.51-0.88)	0.09
Vitamin D						
No. of cases/controls	394/456	346/502	394/454	320/528	306/541	
Median intake (μg/d)	2.77	4.62	6.64	11.5	16.7	
OR[a] (95% CI)	1.00	0.86 (0.71-1.05)	1.08 (0.89-1.31)	0.73* (0.60-0.89)	0.71* (0.59-0.87)	0.13
OR[b] (95% CI)	1.00	0.93 (0.73-1.19)	1.20 (0.94-1.53)	0.75* (0.59-0.96)	0.73* (0.57-0.94)	0.18
Alpha-tocopherol						
No. of cases/controls	388/462	372/476	373/475	325/523	302/545	
Median intake (mg/d)	4.3	5.5	7.6	19.7	174.1	
OR[a] (95% CI)	1.00	0.97 (0.80-1.18)	0.96 (0.80-1.17)	0.76* (0.63-0.93)	0.72* (0.60-0.88)	0.15
OR[b] (95% CI)	1.00	0.97 (0.76-1.22)	1.05 (0.83-1.33)	1.04 (0.76-1.43)	0.86 (0.65-1.13)	0.05
Thiamin						
No. of cases/controls	381/468	376/473	365/483	342/507	296/550	
Median intake (mg/d)	1.53	1.82	2.11	2.95	4.16	
OR[a] (95% CI)	1.00	1.07 (0.88-1.30)	1.02 (0.84-1.24)	0.89 (0.73-1.08)	0.70* (0.58-0.86)	0.01
OR[b] (95% CI)	1.00	0.96 (0.76-1.21)	1.10 (0.87-1.39)	0.99 (0.74-1.31)	0.79 (0.55-1.12)	0.14
Riboflavin						
No. of cases/controls	412/438	360/488	355/493	343/505	290/557	
Median intake (mg/d)	1.93	2.37	2.85	3.71	5.44	
OR[a] (95% CI)	1.00	0.83 (0.69-1.01)	0.81* (0.66-0.98)	0.76* (0.62-0.92)	0.57* (0.47-0.70)	0.01
OR[b] (95% CI)	1.00	0.87 (0.69-1.11)	0.86 (0.68-1.10)	0.82 (0.64-1.05)	0.61* (0.47-0.78)	0.01
Vitamin B6						
No. of cases/controls	386/464	369/479	359/489	348/500	298/549	
Median intake (mg/d)	1.71	2.15	2.58	4.01	5.82	
OR[a] (95% CI)	1.00	0.99 (0.82-1.21)	0.95 (0.78-1.15)	0.89 (0.73-1.08)	0.69* (0.57-0.84)	0.01
OR[b] (95% CI)	1.00	1.04 (0.83-1.32)	1.03 (0.82-1.31)	1.22 (0.91-1.63)	0.95 (0.66-1.36)	0.95
Folate						
No. of cases/controls	412/438	389/459	343/505	303/545	313/534	
Median intake (μg/d)	236.5	311.3	390.7	701.2	1069.8	
OR[a] (95% CI)	1.00	1.00 (0.83-1.22)	0.81* (0.67-0.99)	0.64* (0.52-0.77)	0.69* (0.56-0.84)	0.08
OR[b] (95% CI)	1.00	0.97 (0.76-1.23)	0.86 (0.67-1.09)	0.72* (0.56-0.92)	0.72* (0.56-0.92)	0.04
Vitamin B12						
No. of cases/controls	387/464	368/479	350/498	352/496	303/544	
Median intake (μg/d)	4.15	6.03	8.15	15.2	22.6	
OR[a] (95% CI)	1.00	0.98 (0.80-1.19)	0.90 (0.74-1.10)	0.89 (0.73-1.08)	0.71* (0.58-0.86)	0.01
OR[b] (95% CI)	1.00	0.95 (0.75-1.20)	0.94 (0.74-1.19)	1.10 (0.83-1.45)	0.93 (0.66-1.32)	0.97

[a]Adjusted for age and total energy intake. [b]Adjusted for total energy intake. Other potential confounders included age, sex, BMI, physical activity (metabolic equivalent hours/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of non-steroid anti-inflammatory drug, regular use of multivitamin supplements, reported hormone replacement therapy (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alternation in the parameter coefficient of interest. [c]Two-sided *p*-value for test of linear trend was calculated by using median values for each quintile of intake. *Significant difference from reference category (Q1), $p \leq 0.05$.

Table IV. *Adjusted odds ratio (OR), 95% confidence interval (CI) of colorectal cancer risk according to selected micronutrients intakes from food source only in the colorectal cancer case–control study in Newfoundland, Labrador, and Ontario.*

Micronutrients from food source only	Quintiles of intake					*P*-value for trend[c]
	Q1	Q2	Q3	Q4	Q5	
Calcium						
No. of cases/controls	398/452	374/474	339/509	343/505	306/541	
Median intake (mg/d)	631.0	805.2	936.6	1091.5	1391.9	
OR[a] (95% CI)	1.00	0.96 (0.79-1.17)	0.84 (0.69-1.03)	0.82* (0.67-0.99)	0.66* (0.54-0.81)	0.003
OR[b] (95% CI)	1.00	0.94 (0.74-1.18)	0.81 (0.64-1.04)	0.90 (0.71-1.15)	0.76* (0.59-0.97)	0.06
Vitamin C						
No. of cases/controls	386/464	372/476	333/515	343/505	326/521	
Median intake (mg/d)	71.2	116.3	152.4	194.1	271.3	
OR[a] (95% CI)	1.00	1.05 (0.86-1.27)	0.87 (0.71-1.06)	0.93 (0.76-1.13)	0.82* (0.67-1.00)	0.09
OR[b] (95% CI)	1.00	0.93 (0.73-1.18)	0.89 (0.69-1.14)	0.89 (0.69-1.15)	0.87 (0.67-1.13)	0.05
Vitamin D						
No. of cases/controls	382/468	356/491	358/491	364/484	300/547	
Median intake (μg/d)	2.49	3.95	5.17	6.57	9.25	
OR[a] (95% CI)	1.00	0.98 (0.81-1.20)	1.01 (0.83-1.23)	1.04 (0.85-1.26)	0.73* (0.60-0.89)	0.17
OR[b] (95% CI)	1.00	0.97 (0.76-1.24)	1.07 (0.84-1.37)	1.08 (0.84-1.37)	0.79* (0.62-1.00)	0.32
Riboflavin						
No. of cases/controls	390/458	357/492	365/482	328/522	320/527	
Median intake (mg/d)	1.85	2.21	2.50	2.86	3.45	
OR[a] (95% CI)	1.00	0.91 (0.75-1.10)	0.96 (0.79-1.16)	0.80* (0.66-0.97)	0.74* (0.61-0.90)	0.02
OR[b] (95% CI)	1.00	0.97 (0.77-1.21)	1.03 (0.82-1.30)	0.84 (0.67-1.06)	0.86 (0.68-1.09)	0.13
Folate						
No. of cases/controls	405/445	365/483	347/501	328/520	315/532	
Median intake (μg/d)	225.5	288.5	335.4	393.7	495.0	
OR[a] (95% CI)	1.00	0.92 (0.76-1.12)	0.86 (0.71-1.05)	0.79* (0.65-0.96)	0.72* (0.59-0.88)	0.001
OR[b] (95% CI)	1.00	0.85 (0.68-1.07)	0.91 (0.73-1.15)	0.86 (0.68-1.08)	0.83 (0.65-1.05)	0.06

[a]Adjusted for age and total energy intake. [b]Adjusted for total energy intake. Other potential confounders included age, sex, BMI, physical activity (metabolic equivalent hours/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of non-steroid anti-inflammatory drug, regular use of multivitamin supplements, reported hormone replacement therapy (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alternation in the parameter coefficient of interest. [c]Two-sided *p*-value for test of linear trend was calculated by using median values for each quintile of intake. *Significant different from reference category (Q1), $p \leq 0.05$.

consumption, including calcium, vitamin C, vitamin D, riboflavin, and folate. A higher risk of CRC was observed for greater intake of iron. No links were found with retinol, alpha-tocopherol, thiamin, vitamin B6, and vitamin B12. Inverse associations of calcium, vitamin C, and folate with CRC were most pronounced among users of individual supplements. The positive association between iron intake and CRC risk was most pronounced among iron supplement users.

When intake from diet only was considered for the micronutrients of interest, associations were diminished. The exclusion of regular supplement users resulted in a slight decrease in power in these analyses. In addition, we observed that inverse associations of calcium, vitamin C, folate and CRC were mostly pronounced among users of individual supplements. One possibility is that the intake of micronutrients from supplemental sources additionally contributes to the differences between cases and controls, and adds to the total amount of micronutrient intake, making the protective effects more likely to be detected. Thus, we presume that using individual supplements that provide compensatory micronutrients may reduce risk for CRC. Therefore, with the increasing prevalence of dietary supplement use in the Canadian population, our findings highlight the importance of collecting information on supplement use when studying associations of micronutrients with disease risk.

Our findings suggest a possible inverse relation of CRC risk with intake of calcium and vitamin D, consistent with results from a recent Multiethnic Cohort Study conducted in Hawaii and Los Angeles (18). After follow-up of 85,903 men and 105,108 women for 5-8 years, Park *et al*. found that total calcium intake (from foods and supplements) was inversely associated with CRC risk in both men and women (relative risk (RR)=0.70, 0.64). The inverse association was also seen for total vitamin D intake in men (RR=0.72) (18). As in our study, we observed inverse associations with intake of total calcium (OR=0.59) and total vitamin D (OR=0.73). Moreover, after exclusion of supplement users, inverse associations with calcium and vitamin D (from food source only) remained significant.

Table V. *Adjusted odds ratio (OR), 95% confidence interval (CI) of colorectal cancer risk according to individual supplement use and levels of micronutrient intakes from food in the colorectal cancer case–control study in Newfoundland, Labrador, and Ontario.*

Individual supplement use	Dietary nutrient intake from food (mg/d)		*P*-value for trend[b]
	Lower intake	Higher intake	
Iron supplement	Iron intake from foods		
Median intake	≤16.15 mg/d	>16.15 mg/d	
Non-users			
No. of cases/controls	675/875	571/825	
OR[a] (95% CI)	1.00	1.04 (0.87-1.25)	
Users			
No. of cases/controls	229/344	285/437	
OR[a] (95% CI)	1.55*(1.04-2.30)	1.70*(1.15-2.52)	0.05
Calcium supplement	Calcium intake from foods		
Median intake	≤936.56 mg/d	>936.56 mg/d	
Non-users			
No. of cases/controls	643/758	524/700	
OR[a] (95% CI)	1.00	1.02 (0.84-1.24)	
Users			
No. of cases/controls	285/435	308/588	
OR[a] (95% CI)	0.80*(0.63-1.00)	0.68*(0.54-0.85)	0.07
Vitamin C supplement	Vitamin C intake from foods		
Median intake	≤152.44 mg/d	>152.44 mg/d	
Non-users			
No. of cases/controls	622/717	462/644	
OR[a] (95% CI)	1.00	0.89 (0.73-1.09)	
Users			
No. of cases/controls	298/484	378/636	
OR[a] (95% CI)	0.68*(0.54-0.86)	0.78*(0.63-0.96)	0.19
Folate supplement	Folate intake from foods		
Median intake	≤335.36 μg/d	>335.36 μg/d	
Non-users			
No. of cases/controls	748/825	512/798	
OR[a] (95% CI)	1.00	0.87 (0.72-1.04)	
Users			
No. of cases/controls	209/340	291/518	
OR[a] (95% CI)	0.67*(0.52-0.86)	0.73*(0.58-0.92)	0.12

[a]Adjusted for total energy intake, age, sex, BMI, physical activity (metabolic equivalent hours/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of non-steroid anti-inflammatory drug, regular use of multivitamin supplements, reported hormone replacement therapy (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alternation in the parameter coefficient of interest. [b]Two-sided *p*-value for test of linear trend was calculated by using median values for each quintile of intake. *Significant different from reference category, $p \leq 0.05$.

In 2007, Ryan-Harshman and Aldoori reviewed several case–control and prospective cohort studies and some clinical trials, and concluded that evidence of calcium and vitamin D for reducing risk of CRC was strong. Multivitamin and mineral supplements can complement a healthy diet (40). Consistent results in this study found that the lowest risk was observed among calcium supplement users with a higher dietary calcium intake. This finding again suggests that the use of calcium supplements have further benefit in preventing CRC. However, the protective role of vitamin D should be concluded with caution because vitamin D was partially derived from multivitamin supplements; it is challenging to separate the effect of vitamin D from those of other vitamins.

The findings support the hypothesis that vitamin C is protective against CRC, possibly through antioxidant mechanisms. Antioxidants, such as carotene, retinol, alpha-tocopherol and vitamin C, may reduce risk by quenching free radicals and reducing oxidative damage to DNA (11, 12). These findings were in line with other studies (41-43). However, in this study, we observed an inverse association only between vitamin C and CRC, but no relationships were found with retinol or alpha-tocopherol intake. A possible explanation is that the intakes of these nutrients were too

low, even in the highest quintiles, to observe significant associations. Results also revealed that vitamin C supplement may have further benefit among those with a relatively low dietary intake of vitamin C. This may be explained by the threshold effect that low intake of vitamin C may increase risk, while incremental intake above the threshold level may add minimal benefits.

We observed that higher intakes of folate and riboflavin were inversely associated with CRC risk and a linear dose-response effect of increasing protection emerged for both nutrients. Our findings support the hypothesis that folate may affect colorectal carcinogenesis through its role in the synthesis of nucleic acid and DNA methylation (44). Riboflavin, as a flavin adenine dinucleotide, is the cofactor for methylenetetrahydrofolate reductase, the enzyme that influences homocysteine remethylation and DNA methylation (45). In this study, folate intake was derived from foods, folate supplement and multivitamin supplements. After controlling for multivitamin supplement use, an inverse association between folate and CRC was mostly pronounced among folate supplement users. Besides food sources, riboflavin was mainly derived from multivitamin supplements, thus caution should be taken in interpreting the protective role of riboflavin. It is worthwhile to further explore independent effects of riboflavin and vitamin D on the occurrence of CRC as individual supplements.

A remarkable finding in this study was the strong positive association between iron intake and CRC risk, with the risk strongly increased in the highest quintile of iron intake. After controlling for multivitamin supplements use, a positive association between iron and CRC was also most pronounced among iron supplement users, and a 70% increased risk was observed in the association with a higher level of dietary iron intake. Iron may increase the risk of CRC by generating free radicals that attack DNA and damage chromosomes (13, 14, 46). Interestingly, in this study, cases had a higher intake of iron than the controls, primarily due to a larger contribution of iron supplement. Iron plays an important role in helping red blood cells deliver oxygen to the rest of the body. Low iron levels can cause iron deficiency anemia. However, excess intake of iron or inadequately using iron supplement may have harmful effects of increasing CRC risk (47). The recommended daily allowance (RDA) for men and women 50 years old and older has been established at 8 mg daily (48). In our study, the average daily intake of iron (from food and supplements) was 30 mg for cases and 25 mg for controls, which is much higher than the RDA. Thus, attention should be paid to consumption of iron-containing foods and supplements.

This study had a number of strengths. Particularly, it simultaneously covered a series of micronutrients, which enabled easy comparison between roles of the different micronutrients in CRC carcinogenesis. The sample size of this study was relatively large, thus some associations that would be undetectable in smaller studies would be observed. More importantly, previous findings about the protective effects of micronutrients were confined to a specific study population, which makes it difficult to generalize the results. In this study, we conducted pooled analyses of the population of two Canadian provinces to investigate the associations of selected micronutrients and CRC risk, and hence to make conclusions about specific micronutrients having possible preventive effects on CRC. Furthermore, the use of calorie-adjusted nutrient intake computed by the nutrient residual method in multivariate models is often expected to overcome the problem of high co-linearity frequently observed between nutritional factors (39). This adjustment also reduces between-person variation due to over- or underreporting of food intakes (39). Although some random misclassification of diet is likely, non-differential misclassification generally tends to bias the risk estimates toward the null.

Potential limitations of this study exist. Firstly, the use of self-reported data reflecting dietary habits before diagnosis may result in recall bias. In particular, cases may recall dietary exposures differently from controls because of the raised awareness. Controls may have agreed to join this study due to an interest in health and may therefore have healthier dietary and physical activity habits, a pattern that may exaggerate differences with the cases beyond what might have been seen with truly comparable controls.

Secondly, by design, although cases and controls had a similar sex distribution, they were not well matched by age strata. Estimates of nutrient intakes from a FFQ are not precise and there is always the potential for measurement error (2). Although the original FFQ used in this study has been validated (35, 36), this questionnaire requires further evaluation because it was originally developed for the Hawaiian and Californian populations, which may differ from people residing in NL and ON. FFQ used in NL has been adapted to include regional foods in NL; however, the Ontario Familial Colorectal Cancer Registry (OFCCR) used the original FFQ that has not been adapted. Thus, a sub-study will be necessary to assess the level of agreement between the FFQ used by the OFCCR and the FFQ that was previously developed specifically for Canadian populations. Finally, these findings may reflect problems of co-linearity between various micronutrients, between selected foods (*e.g.* fruits and vegetables), and between multivitamin supplements; however, complete elimination of these problems is unlike.

In conclusion, this study presents evidence that dietary intake of calcium, vitamin D, vitamin C, riboflavin and folate are associated with a lower risk of incident CRC and that dietary intake of iron may be associated with higher risk of the disease. Moreover, iron, calcium, vitamin C and folate may exert independent protective effects against colorectal carcinogenesis, and using supplements with these nutrients

may have further benefit in preventing CRC; in contrast, iron supplementation may have the harmful effect of increasing CRC risk. Yet it is also likely that other physiological, behavioural, and dietary factors interact with micronutrients to affect risk. Attempts to reduce risk by selecting one or even several micronutrients for supplementation may not have the anticipated results until their interactions with other factors are better understood.

Conflict of Interest

None disclosed

Acknowledgements

This work was supported by the Canadian Institutes of Health Research Team Grant [CIHR-CPT79845] and Canadian Institutes of Health Research Team in Interdisciplinary Research on Colorectal Cancer Studentship [205835]. Both Zhuoyu Sun and Yun Zhu were supported by the Newfoundland and Labrador Centre for Applied Health Research through Master's fellowships. Jing Zhao was supported by a trainee award from the Beatrice Hunter Cancer Research Institute with funds provided by The Terry Fox Foundation Strategic Health Research Training Program in Cancer Research at Canadian Institutes of Health Research. All Authors of this paper have participated in the preparation and implementing of the research and the compilation of the manuscript. None of the Authors of this paper have declared any conflict of interest.

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Received November 12, 2011
Revised December 22, 2011
Accepted December 23, 2011