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Dietary *N*-nitroso compounds and risk of colorectal cancer: a case–control study in Newfoundland and Labrador and Ontario, Canada

Yun Zhu[1,2], Peizhon Peter Wang[1,2]*, Jing Zhao[1], Roger Green[3], Zhuoyu Sun[1], Barbara Roebothan[1], Josh Squires[1], Sharon Buehler[1], Elizabeth Dicks[4], Jinhui Zhao[1], Michelle Cotterchio[5], Peter T. Campbell[6], Meera Jain[7], Patrick S. Parfrey[4] and John R. Mclaughlin[8]

[1]*Division of Community Health and Humanities, Faculty of Medicine, Memorial University of Newfoundland, St John's, NL A1B 3V6, Canada*
[2]*School of Public Health, Tianjin Medical University, Tianjin, People's Republic of China*
[3]*Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, St John's, NL, Canada*
[4]*Clinical Epidemiology Unit, Faculty of Medicine, Memorial University of Newfoundland, St John's, NL, Canada*
[5]*Population Studies and Surveillance, Cancer Care Ontario, Toronto, ON, Canada*
[6]*Epidemiology Research Program, American Cancer Society, Atlanta, GA, USA*
[7]*Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada*
[8]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*

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Abstract

Several *N*-nitroso compounds (NOC) have been shown to be carcinogenic in a variety of laboratory animals, but evidence of their carcinogenicity in humans is lacking. We aimed to examine the association between NOC intake and colorectal cancer (CRC) risk and possible effect modification by vitamins C and E and protein in a large case–control study carried out in Newfoundland and Labrador and Ontario, Canada. A total of 1760 case patients with pathologically confirmed adenocarcinoma and 2481 population controls were asked to complete a self-administered FFQ to evaluate their dietary intakes 1 year before diagnosis (for cases) or interview (for controls). Adjusted OR and 95 % CI were calculated across the quintiles of NOC (measured by *N*-nitrosodimethylamine (NDMA)) intake and relevant food items using unconditional logistic regression. NDMA intake was found to be associated with a higher risk of CRC (highest *v.* lowest quintiles: OR 1·42, 95 % CI 1·03, 1·96; *P* for trend=0·005), specifically for rectal carcinoma (OR 1·61, 95 % CI 1·11, 2·35; *P* for trend=0·01). CRC risk also increased with the consumption of NDMA-containing meats when the highest tertile was compared with the lowest tertile (OR 1·47, 95 % CI 1·03, 2·10; *P* for trend=0·20). There was evidence of effect modification between dietary vitamin E and NDMA. Individuals with high NDMA and low vitamin E intakes had a significantly increased risk than those with both low NDMA and low vitamin E intakes (OR 3·01, 95 % CI 1·43, 6·51; *P* for interaction=0·017). The present results support the hypothesis that NOC intake may be positively associated with CRC risk in humans. Vitamin E, which inhibits nitrosation, could modify the effect of NDMA on CRC risk.

Key words: ***N*-nitroso compounds: Colorectal cancer: Vitamin C: Vitamin E: Effect modification: Case–control studies**

Colorectal cancer (CRC) is one of the most serious types of colorectal health problems in North America, but its incidence rates vary geographically[1,2]. A high incidence rate has been observed in Newfoundland and Labrador (NL), Canada, where people frequently consume pickled/processed meats[3]. In recent years, several *N*-nitroso compounds (NOC) have been detected in pickled/processed meats, including volatile *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine, which have been shown to be carcinogenic in a variety of laboratory animals[4,5]; yet, evidence of the carcinogenicity of NOC in humans is lacking.

Humans are exposed to NOC through both exogenous and endogenous pathways[6]. Preformed NOC have been found in processed/cured meats and smoked/salted fish and in foods subjected to additives in the production process, such as beer and preserved products[4,7]. Endogenously, nitrates and nitrites in the diet could act as precursors for the production of nitrosamines when reacting with the nitrosatable amines

Abbreviations: CRC, colorectal cancer; ICD, International Classification of Disease; NDMA, *N*-nitrosodimethylamine; NFCCR, Newfoundland Familial Colorectal Cancer Registry; NL, Newfoundland and Labrador; NOC, *N*-nitroso compounds; OFCCR, Ontario Familial Colorectal Cancer Registry; ON, Ontario.

* **Corresponding author:** P. P. Wang, fax +1 709 777 7382, email pwang@mun.ca

(generally present in protein-rich foods) in the gastrointestinal tract[8,9]. Thus, many studies investigating the relationship between NOC and cancers[8] do consider nitrates and nitrites.

NOC have been suspected to play an important role in colorectal carcinogenesis through the induction of DNA-damaging metabolites, such as aldehydes and alkyldiazonium ions, which could consequently lead to cancerous lesions in cells[10]. Many antioxidants, such as vitamin C and vitamin E, have been shown to inhibit the synthesis of NOC[11,12]. It is biologically plausible that NOC in the diet may act as risk factors for CRC; yet epidemiological investigations carried out in population-based studies have been inconclusive. A previous review of dietary nitrate, nitrite, and NOC and risk of cancers has found that most studies had concentrated on cancers of the stomach[13–17] and oesophagus[8,18,19]. Only minimal studies have specifically reported results in relation to CRC and shown a positive association with NOC intake[6,8]. However, current evidence is not enough to warrant this association. Additionally, studies had seldom included the estimates of possible effect modification by antioxidants and proteins (a rich source of amines).

Since NDMA is one of the major nitrosamines present in the diet and is more widely estimated than other volatile NOC reported to be present in food stuffs[20,21], we selected NDMA as the measure of NOC to investigate whether the consumption of NDMA or foods high in NOC is associated with CRC risk in a case–control study carried out in NL and Ontario (ON) and whether this association varied by vitamin C, vitamin E or protein intakes.

Subjects and methods

Study participants

In the present study, we used data collected as part of a large Newfoundland and Ontario colorectal cancer study. The details of the multicentre colorectal cancer project have been reported previously[2,22,23]. Cases for the study were recruited through the Newfoundland Familial Colorectal Cancer Registry (NFCCR) and the Ontario Familial Colorectal Cancer Registry (OFCCR). From the NFCCR, we identified 1159 pathologically confirmed incident CRC cases on the basis of International Classification of Disease (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). Case patients were diagnosed from 1999 to 2003 and aged 20–74 years. In the OFCCR, case patients were enrolled from 1997 to 2000 (phase 1) and from 2003 to 2006 (phase 2) with pathological confirmation and were aged between 20 and 74 years.

Controls for the present study consisted of a random sample of each provincial population aged between 20 and 74 years and were selected using random digit dialling (NL) and through a list of residential phone numbers or from population-based property assessment rolls (ON). Detailed descriptions of the selection process followed for controls in each province have been reported in our previous studies[24,25]. Potential control participants who had been diagnosed previously with CRC were unqualified for inclusion. Controls were frequency-matched with cases on sex and 5-year age strata.

All the participants gave written informed consent, and the study protocol was approved at each provincial site. All the individuals who were selected were sent a written consent form, a FFQ, a personal history questionnaire and a family history questionnaire (FHQ). Those who did not respond were sent a reminder card, and a follow-up call was made if needed. The median time from the date of diagnosis to the date of questionnaire completion was 1·8 years for the NL participants, and it was slightly shorter for the ON participants. The overall response rates for the study were 65·0 % for cases and 53·5 % for controls.

Data collection

The FFQ was used to assess dietary intakes 1 year before enrolment in the study (controls) or 1 year before CRC diagnosis (cases). In ON, we utilised the validated Hawaii semi-quantitative FFQ that included 170 foods and beverages plus vitamin and dietary supplements[26]. The FFQ used in NL was very similar to the one used in ON, but it was based on a validated instrument that had been adapted for the Canadian population[27–29], and for the present study, it was modified slightly to include foods indigenous to the Newfoundland population (e.g. salted/pickled meats and smoked/pickled fish). For each food item, examples of portion sizes were specified. The intakes of nutrients from the diet including vitamin C and vitamin E and those of energy were computed by multiplying the frequency of the consumption of each unit food by the nutrient content of the portion size[23]. The intakes of total vitamin C and total vitamin D were also calculated by adding the intakes of nutrients from the diet and those of nutrients from supplements. Dietary exposures to NDMA, nitrite and nitrate were calculated for the NL population using the instrument developed by Howe *et al.*[30]. Briefly, the estimation algorithm identified thirty-one food items/groups in the questionnaires that made the greatest individual contribution to the consumption of NOC and then linked them to the National Cancer Institute of Canada nutrient data bank[30]. In ON, approximated intakes of NOC were determined based on the report of the United States Department of Agriculture.

The personal history questionnaire was used to collect information on demographics (e.g. age, sex and marital status), medical history, bowel screening history, aspirin use, physical activity, and alcohol and tobacco consumption. For female participants, there were additional questions related to reproductive factors. Finally, the family history questionnaire was used to assess family history of cancer.

In the present analysis, we excluded individuals who reported a history of familial adenomatous polyposis and those who provided insufficient information on diet and related risk factors[23]. Individuals with extreme scores for energy intake in the upper or lower 2·5 % of total energy intake of each province (NL: men <3870 or $>19\,665$ kJ and women <4602 or $>20\,502$ kJ; ON: men <4351 or $>21\,757$ kJ and women <3494 or $>17\,154$ kJ) were considered unreliable and were further excluded[23]. As a result, a total of 896 subjects in ON and 281 subjects in NL were excluded, resulting in a final sample size of 1760 cases and 2481 controls for this pooled analysis.

Statistical analysis

Comparisons of continuous variables (i.e. age and BMI) between cases and controls were made with Student's *t* test, and categorical variables were analysed using Pearson's χ^2 test. Unconditional logistic regression models were used to examine the association between dietary NDMA/nitrite/nitrate intakes and CRC risk. OR and the corresponding 95 % CI were computed for quintiles of intake, using the lowest quintile as the reference. The initial model was adjusted only for age, sex and province of residence. In the selection method of the multivariate model, all the potential confounders based on the literature and previous studies were entered in a stepwise fashion. Only terms that entered the model at $P<0{\cdot}1$, altered the effect estimates by 10 % or more, or improved the fit of the model[31] were retained for the final models. These included total energy intake, BMI, cigarette smoking, alcohol consumption, physical activity (metabolic equivalent hours/week), education attainment, household income, reported colon screening procedure, non-steroidal anti-inflammatory drug use, multivitamin supplement use, folate supplement use and province of residence. All the covariates were entered into the models as categorical variables. Statistical hypotheses of trend were tested based on the median of each category of intake. Adjusted OR and 95 % CI for various subsites were calculated using unconditional logistic regression by comparing cases having each of the three tumour subsites independently with the controls in relation to dietary NDMA/nitrite/nitrate intakes.

Effect modification of NDMA by vitamin C, vitamin E and protein was examined by testing the significance of multiplicative interaction terms in the models with a Wald test and by stratifying the study population into high *v.* low categories of intake for each nutrient, with cut points set at the 80th and 20th percentiles of intakes. The associations of beer, pickled vegetable and NDMA-containing meat consumption with CRC risk were examined using multivariate logistic regression for the tertiles of intake of each.

A sensitivity analysis was carried out to determine whether associations varied with the exclusion of cases aged less than 50 years who tend not to be sporadic. Statistical significance was considered at $P<0{\cdot}05$. All calculations were carried out with the SAS software (version 9.2).

Ethical considerations

The Newfoundland and Ontario colorectal cancer study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Investigation Committee of Memorial University of Newfoundland and the Office of Research Services of University of Toronto. Written informed consent was obtained from all the participants at recruitment.

Results

The percent distributions of cases and controls were similar for sex, province of residence and cigarette smoking status (Table 1). Cases were more likely to have a lower education level and lower household income, more hormone replacement therapy (women) use, and higher total energy consumption than controls, while controls were more likely to be non-steroidal anti-inflammatory drug users and to have had colon screening.

As expected, dietary NDMA intake was significantly associated with a higher risk of CRC, even in the multivariate-adjusted model (OR 1·42, 95 % CI 1·03, 1·96; Table 2). The positive linear trend was consistent and significant (*P* for trend=0·005). An increase in the risk of CRC was also observed for the fourth (OR 1·32, 95 % CI 1·08, 1·61) and fifth (OR 1·50, 95 % CI 1·22, 1·83) quintiles of nitrite intake after adjustment only for age, sex and province of residence; however, this deleterious association was not significant after multivariate adjustment. No significant relationship was found between gradients of nitrate intake and CRC risk in the multivariate-adjusted model. We also assessed the effects of NOC stratified by province; however, a significant NDMA–CRC association was observed in NL than in ON (data not shown).

When case groups with various tumour subsites were compared independently with controls, differences in the association between NDMA intake and CRC risk emerged (Table 3). A significant rising trend in risk with increasing consumption of NDMA was observed for the rectum (*P* for trend=0·01) and proximal colon (*P* for trend=0·003) cancers, but the risk estimate of being in the highest quintile of NDMA intake was statistically significant for cases with tumours located in the rectum only (OR 1·61, 95 % CI 1·11, 2·35).

We examined the effect modification of NDMA by vitamin C, vitamin E and protein from food on CRC risk (Table 4). CRC risk became more pronounced (OR 3·05, 95 % CI 1·43, 6·51) than would be expected if NDMA exposure was high and vitamin E intake was low (*P* for interaction=0·017). Similarly, the risk was higher in individuals with high protein and high NDMA intakes (OR 2·16, 95 % CI 1·12, 4·15) than in those with low protein and low NDMA intakes. However, the test for interaction was not statistically significant (*P* for interaction=0·46). There was no indicated interaction between NDMA and vitamin C intakes and CRC risk (*P* for interaction=0·95). We also evaluated the potential effect modification of dietary nitrate by dietary vitamin C. A borderline significant interaction between dietary nitrate and vitamin C was observed (*P* for interaction=0·04), with a greater risk being observed among those with high nitrate and low vitamin C intakes (data not shown). When we included information on the intakes of vitamins C and E from supplements, similar patterns were observed; yet none of the interaction terms of total vitamins (diet plus supplement) with NDMA was statistically significant (data not shown).

Table 5 summarises the OR of CRC for NDMA-containing food items. All the foods were divided into tertiles and entered as categorical variables into the model that included potential confounders. After adjustment, no statistically significant associations were observed between consumption of beer or pickled vegetables examined in the present study and CRC risk. However, subjects who consumed NDMA-containing meats at the highest tertile were 1·47 times as likely to have

Table 1. Distribution of selected characteristics of the study population by case and control status (Mean values and standard deviations; number of cases and controls and percentages)

	Cases (*n* 1760)		Controls (*n* 2481)		
Characteristics	*n*	%	*n*	%	*P**
Age (years)					
Mean	59·6		61·2		<0·0001
SD	10·6		9·6		
BMI (kg/m^2)					
Mean	27·0		26·6		0·004
SD	4·8		4·5		
Sex					
Males	934	53·1	1357	54·7	
Females	826	46·9	1124	45·3	0·29
Province of residence					
NL	488	27·7	650	26·2	
ON	1272	72·3	1831	73·8	0·27
Physical activity (MET-h/week)					
0–7·4	454	26·3	590	24·0	
7·4–22·4	341	19·8	628	25·5	
22·4–53·0	420	24·4	627	25·5	
> 53·0	508	29·5	614	25·0	<0·0001
Level of education					
Lower than high school	568	32·6	632	25·7	
High-school graduate	307	17·6	402	16·3	
College	536	30·7	861	34·9	
Bachelor or higher	333	19·1	569	23·1	<0·0001
Level of income ($/year)					
<12 000	82	6·2	115	6·2	
12 000–29 999	382	28·8	431	23·1	
30 000–49 999	412	31·1	583	31·3	
≥50 000	450	33·9	735	39·4	0·001
Cigarette smoking status					
Current	306	17·5	369	15·0	
Former	768	43·9	1096	44·6	
Never	674	38·6	995	40·5	0·08
Reported any colon screening					
Yes	260	14·8	619	25·0	
No	1500	85·2	1862	75·1	<0·0001
NSAID use					
Yes	595	33·9	1039	42·0	
No	1158	66·1	1437	58·0	<0·0001
Hormone replacement therapy use (women)					
Yes	545	67·1	622	56·2	
No	267	32·9	485	43·8	<0·0001
Total energy intake (kJ/d)					
Q1 (≤6611)	313	17·8	537	21·6	
Q2 (6611–8130)	341	19·4	507	20·4	
Q3 (8130–9682)	343	19·5	505	20·4	
Q4 (9682–11 991)	359	20·4	489	19·7	
Q5 (>11 991)	404	22·9	443	17·9	0·0002

NL, Newfoundland and Labrador; ON, Ontario; MET-h/week, metabolic equivalent hours per week; NSAID, non-steroidal anti-inflammatory drug; Q, quintile.
* *P* values are for the significance of the *t* test for continuous variables and of the χ^2 test for categorical variables.

been diagnosed with CRC as individuals who consumed NDMA-containing meats at the lowest tertile, although the overall trend was not significant.

Results remained largely unchanged in the sensitivity analysis of NOC intake and CRC risk when we restricted the analysis to cases aged above 50 years.

Discussion

The present study examined the association between dietary NDMA intake and CRC risk in 1760 cases and 2481 controls. The exposure assessments were based on the assumptions that information provided by the participants reflected their actual dietary intakes in the recent past and each food contained the same amount of nutrients/chemicals as the values assigned for in the data bank[30,32]. Our findings are broadly in agreement with those reported by prospective studies showing a positive association between increased intake of NDMA and CRC occurrence[6,8]. Loh *et al.*[8] found an increased risk of gastrointestinal cancers (hazard ratio: 1·13, 95 % CI 1·00, 1·28), specifically of rectal cancer (hazard ratio 1·46, 95 % CI 1·16, 1·84), with per 1 SD (0·05 μg) increase of dietary NDMA intake in Norfolk, UK, although no significant

Table 2. Risk of colorectal cancer for dietary *N*-nitrosodimethylamine (NDMA), nitrite and nitrate exposures (Number of cases and controls, odds ratios and 95 % confidence intervals, and median values)

	Quintiles (Q) of intake					
	Q1	Q2	Q3	Q4	Q5	*P* for trend*
NDMA						
Cases (*n*)	328	335	354	336	407	
Controls (*n*)	532	502	493	513	441	
Median intake†	0·03	0·07	0·20	0·77	2·29	
OR‡	1·00	1·09	1·22	1·14	1·68	0·01
95 % CI		0·89, 1·32	0·99, 1·49	0·92, 1·41	1·33, 2·12	
Multivariate OR§	1·00	1·06	1·13	1·22	1·42	0·005
95 % CI		0·83, 1·37	0·87, 1·47	0·92, 1·63	1·03, 1·96	
Nitrite						
Cases (*n*)	312	352	328	371	397	
Controls (*n*)	536	496	520	479	450	
Median intake†	0·65	0·89	1·12	1·40	1·92	
OR‡	1·00	1·21	1·08	1·32	1·50	0·03
95 % CI		0·99, 1·47	0·88, 1·31	1·08, 1·61	1·22, 1·83	
Multivariate OR§	1·00	1·07	0·99	1·05	1·09	0·66
95 % CI		0·83, 1·38	0·75, 1·30	0·77, 1·43	0·77, 1·54	
Nitrate						
Cases (*n*)	331	371	361	367	330	
Controls (*n*)	517	477	488	481	518	
Median intake†	56·94	91·45	124·81	169·59	264·14	
OR‡	1·00	1·21	1·18	1·22	1·01	0·79
95 % CI		0·99, 1·47	0·97, 1·43	1·00, 1·48	0·83, 1·23	
Multivariate OR§	1·00	1·27	1·19	1·17	0·89	0·43
95 % CI		0·99, 1·60	0·93, 1·52	0·91, 1·51	0·68, 1·16	

* Test for linear trend was based on the median of each category of dietary intake.
† NDMA expressed as μg/d and nitrite and nitrate as mg/d.
‡ Logistic regression model adjusted for age, sex and province of residence.
§ Logistic regression model adjusted for age, sex, energy intake, BMI, cigarette smoking status, alcohol consumption, physical activity (metabolic equivalent hours/week), education attainment, household income, reported colon screening procedure, non-steroidal anti-inflammatory drug use, multivitamin supplement use, folate supplement use and province of residence.

associations were observed between dietary nitrite intake and cancer risk. Knekt *et al.*[6] also reported a significant positive association between NDMA intake and subsequent occurrence of CRC in a large cohort of Finnish men and women, with the relative risk being 2·12 (95 % CI 1·04, 4·33) when the highest quartile of intake was compared with the lowest quartile. A potential mechanism underlying this association might be the formation of alkylating agents, resulting in DNA modification[4,21].

In the present study, rectum cancer, rather than colon cancer, was found to be associated with dietary exposures to NDMA. A possible reason for the observed heterogeneity is that the NOC-carrying faeces that are of a higher concentration when reaching the mucosa of the rectum than the colon would lead to a stronger relationship with rectal cancer[8]. A significant NDMA–CRC association was observed in NL than in ON. The reason for this interprovincial discrepancy in results is unclear, but the differences in dietary habits and dietary assessment methods used in the provinces may account for this variation. We initially hypothesised that there may be a positive association between nitrate/nitrite intakes and CRC risk, as they are precursors of NDMA, but this was not confirmed when cancers in different subsites were analysed together. The lack of an association between nitrate/nitrite intakes and CRC risk is in line with the results of previous prospective studies[6,8]. In the stratified analysis, however, there was some evidence that supported a relationship between nitrite intake and rectal carcinoma.

Analyses of the combined effects showed that CRC risk is highly pronounced in individuals with high NDMA and low vitamin E intakes and individuals with both high NDMA and high protein intakes. There was some indication of increased CRC incidence being associated with dietary nitrate intake among subgroups with low vitamin C intake. To our knowledge, this is the first study to simultaneously examine the interrelationships among vitamin C, vitamin E, protein and NOC intakes in relation to CRC risk. Given that the interaction term for NDMA and protein is not significant, it should be taken as merely indicative of a possible biological interaction. A mechanism has been postulated that colorectal carcinogenesis may involve the endogenous formation of NOC including nitrosamines in the stomach if nitrite combines readily with secondary and tertiary amines[32]. Protein has been suggested to be the main source of secondary and tertiary amines[32,33], the indispensable agents for the nitrosation reaction. However, vitamins C and E may exert an inhibitory effect in cancer carcinogenesis by blocking the nitrosation process by quenching free radicals in their anaerobic reaction with nitrite[34], thus reducing the endogenous synthesis of NOC[8,11]. Similar patterns have been reported in other investigations[20,32,35,36]. For instance, a population-based case–control study carried out in Italy has shown that dietary vitamins C and E could neutralise the elevated risk caused

Table 3. Dietary *N*-nitrosodimethylamine (NDMA), nitrite and nitrate intakes and colorectal cancer by tumour subsite (Number of cases and controls; odds ratios and 95 % confidence intervals)

	Proximal colon				Distal colon				Rectum			
	Cases (*n*)	Controls (*n*)	OR*	95 % CI	Cases (*n*)	Controls (*n*)	OR*	95 % CI	Cases (*n*)	Controls (*n*)	OR*	95 % CI
NDMA												
Q1	139	518	1·00		109	518	1·00		93	518	1·00	
Q2	138	510	0·96	0·72, 1·28	117	510	1·06	0·77, 1·45	117	510	1·06	0·77, 1·45
Q3	123	488	1·08	0·79, 1·46	131	488	1·24	0·89, 1·72	126	488	1·19	0·86, 1·66
Q4	132	519	1·11	0·80, 1·53	101	519	0·97	0·68, 1·39	121	519	1·15	0·81, 1·63
Q5	129	446	1·58	0·80, 1·67	128	446	1·37	0·93, 2·01	168	446	1·61	1·11, 2·35
P for trend†			0·003				0·20				0·01	
Nitrite												
Q1	131	536	1·00		107	536	1·00		95	536	1·00	
Q2	145	496	1·15	0·86, 1·54	112	496	0·97	0·70, 1·34	120	496	1·26	0·91, 1·73
Q3	126	520	0·91	0·66, 1·26	101	520	0·93	0·65, 1·32	124	520	1·20	0·84, 1·71
Q4	120	474	0·81	0·56, 1·18	132	474	1·21	0·82, 1·78	145	474	1·51	1·02, 2·22
Q5	139	455	0·95	0·63, 1·43	134	455	1·32	0·85, 2·04	141	455	1·45	0·94, 2·24
P for trend†			0·43				0·06				0·08	
Nitrate												
Q1	127	517	1·00		109	517	1·00		118	517	1·00	
Q2	153	480	1·25	0·93, 1·66	113	480	1·07	0·78, 1·48	126	480	1·12	0·83, 1·53
Q3	122	489	0·90	0·66, 1·23	128	489	1·24	0·90, 1·71	130	489	1·23	0·90, 1·69
Q4	137	479	1·06	0·78, 1·46	122	479	1·31	0·94, 1·83	133	479	1·34	0·96, 1·85
Q5	122	516	0·75	0·54, 1·05	114	516	1·01	0·71, 1·45	118	516	1·03	0·73, 1·46
P for trend†			0·22				0·93				0·90	

Q, quintile.
*Logistic regression model adjusted for age, sex, energy intake, BMI, cigarette smoking status, education attainment, reported colon screening procedure, non-steroidal anti-inflammatory drug use, multivitamin supplement use, folate supplement use, vegetable intakes and province of residence.
†Test for linear trend was based on the median of each category of dietary intake.

by the simultaneous intake of NOC[8,36]. Another study carried out by de Roos *et al.*[37] has shown nitrate exposure from drinking-water to be associated with increased colon cancer risk among individuals with low vitamin C intakes (>10 years with average nitrate intake >5 mg/l and vitamin C intake <131·8 mg/d *v.* nitrate intake ≤5 mg/l and vitamin C intake ≥131·8 mg/d: OR 2·0, 95 % CI 1·2, 3·3). These patterns suggest that some antioxidants, such as vitamins C and E, may account in part for the previously observed protective effects of vegetables in CRC carcinogenesis, while both NDMA and protein may contribute to the increased risk related to the consumption of red/processed meats[22,32].

The diet followed in NL is unique in that it contains an abundance of foods that are high in nitrite, nitrate and preformed NOC, such as pickled meats. A previous case–control study carried out by Squires *et al.*[22] has found that

Table 4. Effect modification of *N*-nitrosodimethylamine (NDMA) by vitamin C, vitamin E and protein in the diet (Number of cases and controls; odds ratios and 95 % confidence intervals)

NDMA		Cases (*n*)	Controls (*n*)	OR*	95 % CI	*P* for interaction†
	Vitamin C					
Low‡	Low	72	142	1·00		
Low	High	70	95	0·76	0·42, 1·40	
High‡	High	44	56	1·07	0·47, 2·43	
High	Low	115	103	1·34	0·67, 2·69	0·95
	Vitamin E					
Low	Low	69	108	1·00		
Low	High	60	124	1·86	0·97, 3·58	
High	High	73	87	1·75	0·69, 4·45	
High	Low	98	97	3·05	1·43, 6·51	0·017
	Protein					
Low	Low	139	227	1·00		
Low	High	46	43	1·17	0·56, 2·45	
High	Low	26	31	1·72	0·82, 3·61	
High	High	159	132	2·16	1·12, 4·15	0·46

*Logistic regression model adjusted for age, sex, BMI, cigarette smoking status, alcohol consumption, physical activity (metabolic equivalent hours/week), education attainment, household income, reported colon screening procedure, non-steroidal anti-inflammatory drug use, multivitamin supplement use, folate supplement use, province of residence and energy intake (not adjusted for in the NDMA–protein model because of Pearson's correlation coefficient with protein over 0·80).
†*P* for interaction is the significance of the multiplicative interaction term between NDMA and respective variable, calculated from a Wald test.
‡Low, below the 20th percentile of intake of each nutrient; high, above the 80th percentile of intake of each nutrient.

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Table 5. Risk of colorectal cancer associated with the consumption of beer, pickled vegetables and *N*-nitrosodimethylamine (NDMA)-containing meats, Newfoundland

(Number of cases and controls, odds ratios and 95 % confidence intervals, and median values)

	Cases (*n*)	Controls (*n*)	Median intake*	OR†	95 % CI
Beer					
Q1	305	409	0	1·00	
Q2	22	35	17·51	1·02	0·55, 1·90
Q3	161	206	175·07	1·09	0·78, 1·52
P for trend‡				0·08	
Pickled vegetables					
Q1	141	241	0	1·00	
Q2	167	208	8·55	1·35	0·97, 1·89
Q3	180	201	27·92	1·26	0·89, 1·77
P for trend‡				0·63	
NDMA-containing meats§					
Q1	145	234	4·27	1·00	
Q2	159	221	12·11	1·03	0·73, 1·44
Q3	184	195	25·59	1·47	1·03, 2·10
P for trend‡				0·20	

Q, quintile.

* Beer, pickled vegetables and NDMA-containing meats expressed as g/d.

† Logistic regression model adjusted for age, sex, energy intake, BMI, cigarette smoking status, alcohol consumption, physical activity (metabolic equivalent hours/week), education attainment, household income, reported colon screening procedure, non-steroidal anti-inflammatory drug use, multivitamin supplement use, folate supplement use and province of residence.

‡ Test for linear trend was based on the median of each category of dietary intake.

§ NDMA-containing meats were bacon, hot dogs, wieners, sausage, corned beef, cold cuts, canned fish, and smoked fish or lox.

pickled meats significantly increase the risk of CRC in NL. As an outgrowth, the present study further examined the associations between other foods with potentially high contents of nitrosamines and CRC risk in this population and consequently found a dose-dependent increase with the increased consumption of meats rich in NDMA; however, pickled vegetables were observed to non-significantly elevate the risk of developing CRC. Possible reasons for the associations include that meats such as bacon, hot dogs, wieners and sausage, which contain NDMA, are naturally high in amines that are derived from protein. The synergistic effect between NDMA and protein could explain the significant positive association. Although pickled vegetables may contain NDMA, they are also rich in vitamin E, which may neutralise the deleterious effect of NDMA or, alternatively, vegetables may also contain other anti-carcinogens that reduce the cancer risk[21]. These findings further demonstrate the potential joint effects of NDMA with vitamin E and NDMA with protein.

We did not observe a statistically significant association between beer consumption and CRC risk. A possible explanation for this is that the amount of NDMA has been greatly reduced in beer production during the last 20 years[38], and consequently the amount of NDMA in beer is too small to either have an adverse effect or be detected.

The present study has both strengths and limitations. First, case–control studies of dietary factors in relation to cancers are mostly subject to recall and selection biases[39]. The relatively long duration from the point of reference could also adversely affect recall. Although both differential and non-differential reporting of dietary exposures may result in biased risk estimates, non-differential misclassification is expected to bias the results towards the null. Nevertheless, the direction and magnitude of possible differential misclassification cannot be easily determined beyond speculation, and this underscores the importance of future cohort studies to further confirm our findings. Second, the measurement of dietary intakes is complex[39]. The different exposure estimation methods used in NL and ON may have led to a measurement error, although similar FFQ were used. Some uncertainties in the dietary assessment may exist, not only because dietary NDMA values were derived from a selected number of foods, but also because several foods have been reported with a wide range of NDMA values[16,40,41]. Therefore, more efforts are needed to develop a comprehensive and high-quality food composition database of NOC for humans, which would allow more precise identification of dietary NOC values. Besides, the reported association could be affected by the endogenous formation of NOC in the gastrointestinal tract. However, it is unlikely that the differential misclassification of NOC exposure has led to such consistent patterns and strong dose–response effects observed in the present study[40,42].

On the other hand, the strength of the present study is the relatively large sample size with 1760 cases and 2481 controls. The availability of the large amount of information on personal history and family history from the participants allowed for a comprehensive assessment of potentially relevant confounders.

Conclusion

The findings give support to the 'NOC hypothesis' and suggest possible mechanisms underlying the association between consumption of red/processed meats and CRC risk. The patterns that we observed suggest that vitamin E may modify

the effect of NDMA on CRC risk. These results have implications for the prevention of CRC by encouraging people to avoid a diet rich in red/processed meats and to increase the intake of vegetables high in vitamin E. Future directions would involve the development of nutritional assessment methodologies that are more robust and discriminating, research resources that enable dietary intake data to be calibrated against biological measures, and more comprehensive food composition databases of NOC, as they are challenges that remain for epidemiological research on cancer risk in relation to dietary NOC[6].

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None of the authors has any conflicts of interest to declare.

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