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Dietary Patterns and Colorectal Cancer Recurrence and Survival: Overall and by BRAF Mutation Status

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Dietary Patterns and Colorectal Cancer Recurrence and Survival: Overall and

by *BRAF* Mutation Status

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ABSTRACT

Objective: To examine the association between dietary patterns and colorectal cancer (CRC) survival.

Design: Cohort study

Setting and participants: Five hundred and twenty nine newly diagnosed CRC patients from the Newfoundland Familial Colorectal Cancer Registry (NFCCR) were recruited and followed until April, 2010.

Outcome measure: Participants reported their dietary intake using a food frequency questionnaire. Dietary patterns were identified with factor analysis. Multivariable Cox proportional hazards models were employed to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association of dietary patterns with CRC recurrence and death from all-causes, after controlling for covariates.

Results: Disease-free survival (DFS) among CRC patients was significantly worsened among patients with a high processed meat dietary pattern (the highest versus the lowest quartile HR: 1.82, 95% CI: 1.07-3.09). No associations were observed with the prudent vegetable or the high sugar patterns and DFS. The association between the processed meat pattern and DFS was restricted to patients diagnosed with colon cancer (the highest versus the lowest quartile: HR: 2.29, 95% CI: 1.19-4.40) while the relationship between overall survival (OS) and this pattern was observed among patients with colon cancer only (the highest versus the lowest quartile: HR: 2.13, 95%CI: 1.03-4.43). Potential effect modification was noted for sex (p for interaction=0.04, HR: 3.85 for women and 1.22 for men).

Conclusion

The processed meat dietary pattern prior to diagnosis is associated with higher risk of tumor recurrence, metastasis, and death from any cause among patients with

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Article Summary

Article Focus

- We used the data of 529 colorectal cancer patients in Newfoundland and Labrador to investigate the association of dietary patterns and colorectal cancer survival.

- We further explored if the relationship between dietary pattern and colorectal cancer survival is modified by sex, physical activity and *BRAF* mutation.

Key Messages

- The processed meat dietary pattern is associated with a worsened colorectal cancer disease-free survival.

- The prudent vegetable or the high sugar patterns show no association with disease-free survival.

- The relationship between processed meat pattern and colorectal cancer survival is modified by sex.

Strengths and limitations of this study

- The sample size is reasonably large with detailed information on diet, lifestyle and molecular characteristics.

- Recall bias remains a problem since the food consumption was collected from one year prior to CRC diagnosis. In addition, dietary patterns only reflect food consumption before diagnosis which might be modified after diagnosis.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of cancer death in Canada.[1] Epidemiological studies have established a strong link between few dietary factors, such as fiber (inversely) and red/processed meat (increases risk), and the risk of developing CRC,[2] although most studies have focused primarily on individual foods or nutrients. Since foods and nutrients act synergistically rather than in isolation,[3-6] recent research has investigated the role of dietary patterns on CRC incidence. Dietary patterns identified in prior research often include the "Western" and "prudent" patterns. Adherence to the Western diet pattern, characterized by high intakes of meat, fat, sweets and desserts, is often associated with increased risk of CRC.[5-9] Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse[7,8] or null[5,6,10] association with CRC risk. However, the impact of dietary patterns on CRC survival remains largely unknown.

The highest CRC incidence and mortality rates in Canada are observed in the province of Newfoundland and Labrador (NL).[1] Geographically isolated in the Atlantic Ocean, NL has long maintained its traditional foods, a Western-style diet consisting of a large proportion of processed meat, red meat and insufficient vegetables.[11] Several studies have partially attributed the high CRC incidence rate in NL to its unique diet,[11-13] but no study has explored the association between the NL diet and its impact on survival among CRC patients.

This prospective cohort study investigated the influence of dietary patterns, identified by factor analysis, on survival and recurrence or metastasis among an incident case series of 529 CRC patients from NL. Additionally, the present study evaluated the possible effect modification among dietary patterns with sex, physical

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activity and tumor molecular phenotype.

SUBJECTS AND METHODS

Study Participants

Patients in this prospective cohort study were enrolled through the Newfoundland Familial Colorectal Cancer Registry (NFCCR), described in detail elsewhere.[14,15] In brief, during the time period from 1999 to 2003, patients aged 20-75 years, newly diagnosed with pathologically confirmed, invasive CRC were eligible for inclusion in the study (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).

Written, informed consent was required from each study participant to access their archived tumor tissue and medical records. If patients died before they could give consent, a close relative/proxy was invited to participate. Enrolling deceased cases through proxies could remove the potential bias of eliminating patients at a late distant stage.[14] Thus, the inception cohort consisted of 750 eligible patients (64%).

Consenting participants completed and returned a detailed food frequency questionnaire (FFQ), personal history questionnaire (PHQ) and family history questionnaire (FHQ). To capture additional cancer diagnosis or recurrence in the family after enrollment, the FHQ was distributed to participants for the second time midway through the follow-up. To be included in this analysis, patients had to have completed at least the FFQ, provided informative lifestyle and medical data from the PHQ, and had known vital status information by the end of the follow-up period (April, 2010). For patients who died prior to enrollment, the designated relative/proxy completed the aforementioned questionnaires. The final analytical cohort comprised

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529 eligible participants. The study protocol was approved by the Human

Investigation Committee of Memorial University of Newfoundland.

Dietary Assessment and Food Grouping

Diet was assessed using a semi-quantitative FFQ, developed from the well-known

Hawaii FFQ,[16] on the basis of a validated instrument adapted for the Canadian

population.[17,18] The FFQ included 170 foods, beverages, and vitamin- and

dietary-supplements.[19] Foods indigenous to the Newfoundland population (e.g.,

salted/pickled meat and smoked/pickled fish) were also included. For each food item

or beverage, participants were asked to estimate their frequency of consumption and

usual portion size as 'Small', 'Regular' or 'Large' one year prior to their colon or

rectal cancer diagnosis. Portion sizes for specific food were depicted in photographs.

Nutrient and total energy intakes were calculated by multiplying the frequency of

consumption of each food by the nutrient content of the portion size based on the

composition values from the 2005 Canadian Nutrient file. [20] Taking a similar

grouping scheme to that used elsewhere,[3] we collapsed individual food items on the

FFQ into 39 predefined food groups based on the roles of food in diet and cancer

etiology. Distinct food items were reserved as individual categories if it was deemed

inappropriate to combine them (e.g., jam, pies, beer, and wine).

Covariates

Sociodemographic data, such as age, sex, marital status, and education attainment,

were gathered by the self-administered PHQ. The PHQ also included items regarding

medical history, bowel screening history, physical activity, reproductive factors

(female only), and alcohol and tobacco use. Family history of cancer was assessed by

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the FHQ.

Study Outcomes

Study outcomes were ascertained from follow-up questionnaires, local newspapers, death certificates, autopsy, pathology, radiology, surgical reports, as well as physician's notes. Additional data were gathered from the Dr. H. Bliss Murphy Cancer Care Foundation and Statistics Canada.[21] The cause of death was obtained for 93 of 168 deceased patients in this cohort, classified according to the International Classification of Disease (ICD) codes for underlying or contributing cause of death;[22] the majority (91%) of these had died from CRC. Since specific cause of death was not available for all deceased participants, all-cause mortality was used for analysis. In this study, two end points were considered: the first was disease-free survival (DFS), defined as time from cancer diagnosis to the first confirmed tumor recurrence, metastasis, or death from all causes occurring up to April, 2010; the second end point was overall survival (OS), measured from the date of cancer diagnosis to the date of death from all causes. Patients who did not have an event by the end of the follow-up were censored at the date of last contact.

Molecular Assessment

The *p.V600E BRAF* mutation and MSI status for the tumor DNA have been determined in previous studies using standard protocols.[23-25] Briefly, the mutational hotspot c.1799T>A. (p.Val600Glu) in the *BRAF* gene was detected using *BRAF V600E* allele-specific primers, with controls amplifying the GAPDH gene.[25] Positive mutations were then verified by direct automatic sequencing.[25]   For MSI analyses, a panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4,

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D5S346, D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify

both tumor and normal DNA.[23,24] MSI status was defined as MSI-High if 30% or

more of the markers were unstable and MS-Stable/MSI-Low if less than 30% of the

markers showed instability.[26] The primer sequences and PCR conditions are

provided in detail in earlier studies from this cohort.[14,23-25]

Statistical Analysis

Exploratory principal component factor analysis[27] was used to identify major

dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation

(orthogonal) procedure was applied to rotate these factors, meaning that it produces

uncorrelated, easy interpreted components that explain the greatest amount of

variance in the original food groups.[28] We determined the number of factors to

retain for interpretation on the basis of criteria as follows: factor eigenvalue greater

than 1.15, the scree plot, the proportion of variance explained, and factor

interpretability.[9] Patterns were labeled based on food groups with absolute rotated

factor loading matrix greater than or equal to 0.50. Each participant was assigned a

factor score for each pattern (factor) by summing the intakes from each food group

multiplied by optimal weights (factor loadings).[5] Individuals with a higher factor

score had a closer adherence to that pattern.[5]

Comparisons for baseline characteristics across quartiles of dietary patterns were

performed using ANOVA test for continuous variables and Chi-Square test for

categorical variables. Cox proportional hazards models, each adjusting for energy

intake and critical covariates, were used to evaluate the association between

individual dietary pattern and CRC recurrence and mortality, represented by hazard

ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed

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by the log-rank test in a univariate setting; those with the p-value less than 0.1 were

considered for inclusion. The final models only retained the items that entered the

models at p<0.1 or altered the effect estimates by 10% or more; these include energy

intake, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history,

reported screening procedure, reported chemoradiotherapy, and MSI status. The

assumption of proportional hazard rates was verified by checking the parallelism of

the Kaplan-Meier curves and by including time-dependent covariates in the models to

test for statistical significance.[29] Statistical linear trend was examined by modeling

the median value of each quartile as an ordinal variable in a linear regression.[5]

Potential interactions were evaluated by comparing estimates from stratified analyses

and testing significance of interaction terms with a Wald test.[5]

A sensitivity analysis was implemented by eliminating stage-advanced patients

enrolled through proxies and re-calculating survival time from the completion of the

first questionnaire to a predefined event, in order to determine whether associations

might vary with the exclusion of stage-advanced cancer. Statistical significance was

accepted for two-sided p < 0.05. All data management and analyses were performed

with SAS software version 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

The cohort was followed for a median of 6.4 years (minimum: 1.3 years;

maximum: 10.9 years). A total of 168 patients died from all causes and 30 had a

cancer recurrence or metastasis by the end of study follow-up (April, 2010).

Dietary Patterns

Three distinct dietary patterns, labeled "processed meat pattern", "prudent

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vegetable pattern" and "high sugar pattern", were extracted using the aforementioned factor analysis procedure. These patterns explained 73.82% of total variance in the original 39 food groups (Table 1). A higher factor loading matrix of a given food group is representative of a greater contribution of that food group on that specific pattern. Therefore, the first pattern, termed "processed meat", was characterized by higher loadings and thus higher consumptions of cured/processed meat, cured/processed red meat, red meat, fish, and processed fish; the second pattern, labeled "prudent vegetable", displayed higher loadings on other greens, other fruit, other vegetables, and tomato sauce; and the third pattern, named "high sugar", showed higher loadings on desserts and sweets, pies and tarts.

Baseline Characteristics by quartiles of dietary patterns

Higher processed meat pattern scores at baseline were detected in men, ever smokers, patients who were single and individuals who had higher BMI at the time of diagnosis (Table 2). Higher prudent vegetable pattern scores were observed in women, never smokers, those with a slightly later age of diagnosis and with patients who had a tumor harboring the *p.V600E BRAF* mutation. None of these characteristics varied significantly by quartiles of high sugar pattern scores.

Dietary Patterns and Cancer Recurrence or Death

The highest quartile of processed meat pattern was significantly associated with poorer DFS after the adjustment for other predictors of CRC recurrence and death (HR: 1.82, 95% CI: 1.07-3.09), although no overall trend was observed in the HRs across the whole distribution of factor scores (p for trend=0.09) (Table 3). Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was

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observed to be significantly associated with predefined patient outcomes (i.e., DFS and OS).

When stratified by tumor site, however, the association between processed meat pattern and DFS remained statistically significant only for patients who had tumors located in the colon (the highest versus the lowest quartile, HR: 2.29, 95% CI: 1.19-4.40) and not the rectum (HR: 0.97, 95% CI: 0.38-2.45). Similarly, when OS was the outcome, the positive association between increasing consumption of the processed meat pattern and mortality was restricted to patients whose tumors were diagnosed in the colon (the forth versus first quartiles: HR: 2.13, 95% CI: 1.03-4.43).

In the stratified analyses for dietary patterns by sex, physical activity, and *BRAF* mutation status, there was evidence for effect modification by sex (p=0.04) for the association of processed meat pattern with DFS (HR: 3.85 for women and 1.22 for men) (Table 4). However, no evidence was observed to suggest that the effects of other dietary patterns on cancer recurrence or death were modified by physical activity, or *BRAF* mutation status.

DISCUSSION

Three dietary patterns, termed "processed meat pattern", "prudent vegetable pattern" and "high sugar pattern", were generated in this cohort study. We found that high conformity with the processed meat pattern, characterized by high intakes of processed meat, red meat, fish, and processed fish, is associated with decreased DFS. On the contrary, increasing consumption of the prudent vegetable pattern and the high sugar pattern displayed no clear relationships with mortality or recurrence.

The processed meat pattern in the present study shares most characteristics of the Western diet referred to in previous studies on CRC risk, which indicates a positive

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association between the Western dietary pattern and CRC risk.[7,9] However, there has been minimal research examining the association between dietary factors (e.g., nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients;[30,31] moreover, our literature review identified only one study that investigated the relationship between dietary patterns and survival among CRC patients. Consistent with our results, that prospective cohort study of 1009 stage III colon cancer patients[9] reported a deleterious disease-free colon cancer prognosis for patients reporting high levels of the Western dietary pattern intake.

The mechanisms explaining the impact of red and processed meat on CRC mortality are still unclear; however, some biologic mechanisms that link diet factors to CRC risk may continue after diagnosis and subsequently impact cancer progression and survival.[32] For example, strong carcinogens such as *N*-nitroso compounds (NOCs) and probable carcinogenic mutagens like heterocyclic amines (HCA) and polycylic aromatic hydrocarbons (PAH), which have been suggested as significant contributors for CRC development,[33,34] are found in smoked, fried or high-temperature cooked meat. Sandhu et al [35] reported a Western dietary pattern is related to high levels of serum insulin and insulin-like growth factors (IGF), and these hormones are found to be associated with tumor growth and the inhibition of apoptosis. In addition, a growing body of evidence suggests that disruption of the normal gut microflora is associated with human disease, including the pathogenesis of the intestinal tract (e.g. inflammatory bowel disease) and other diseases such as obesity, cardiovascular disease, and autoimmune conditions.[36,37] Alterations in intestinal microbiota are also strongly associated with colonic polyp formation and with the risk of developing CRC.[38] Given the major role of diet on the intestinal microbiome,[39] our findings between dietary patterns and CRC survival may also be

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explained by the impact of dietary patterns on gut microflora and health outcomes.

The influence of processed meat pattern on survival was evident among women rather than men in our study. Previous studies revealed that the higher colon pH and longer intestine transit time in women compared to men can influence the production of secondary bile acid or NOCs,[40] resulting in sex differences in the CRC development. This is the first study that considered effect modifications between dietary patterns and tumor molecular phenotype (i.e. *BRAF* mutation) on CRC survival. Although stratified analyses demonstrated a processed meat diet to significantly decrease survival time only in patients with *BRAF* wide type tumor, no evident interactions were detected. Further research is clearly warranted to verify these findings and to determine the biologic pathways that rationalize the underlying interactions between diet and tumor molecular features.

A reasonably large sample size with detailed information of patients is a merit of our study. These data not only includes demographic and personal lifestyle information, but also some molecular characteristics obtained from genetic testing. The ample information enables us to perform stratification analysis to control and assess effect modifiers and confounders.

Several limitations of this study should be recognized. Firstly, the results may be skewed by recall bias since the participants recalled their food consumption from one year prior to CRC diagnosis; however, this non-differential misclassification is only expected to bias the results towards the null. Secondly, dietary patterns in this study only reflect food consumption before diagnosis; it is unknown whether participants modified their diet post diagnosis. Since previous research has shown minimal change in diet between pre- and post- diagnosis among cancer patients,[31] the current study did not examine dietary changes before and after diagnosis. Moreover, immortal

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person-time bias may impact results. However, this is minimized by using proxies to enroll deceased patients.

In summary, we found that high conformity to the processed meat pattern is significantly associated with an increased risk of all-cause mortality and recurrence of CRC. Though our study did not find a difference in effect by tumor molecular phenotype, larger molecular studies should be conducted to examine if such differences exist. Ultimately, confirmation of these findings and the underlying mechanisms await further studies. Our observation not only underlines the importance of maintaining a healthy diet, but also provides guidance to efficacious dietary interventions;[8] that is, people may lower their risk of CRC mortality by reducing consumption of a processed meat pattern diet.

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Table 1. Factor Loadings and Explained Variances (VAR) for the Three Major Dietary Patterns Identified from the Food Frequency Questionnaire at baseline using a Principal Component Factor Analysis, Newfoundland

Food Groups	Processed Meat Pattern	Prudent Vegetable Pattern	High Sugar Pattern
Milk	-	0.19	-
Yogurt	-	0.31	-
Sugar	-	-0.19	0.20
Tea	-	-	0.17
Coffee	0.17	-	-
Soft drinks	0.19	-	-
Cheese	0.15	0.21	-
Egg	0.21	-	0.16
Mixed dishes	0.31	0.17	0.23
Red meat	**0.69**	-	0.17
Cured/processed red meat	**0.73**	-	0.21
Cured/processed meat	**0.93**	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	**0.58**	0.32	-0.22
Processed fish	**0.50**	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	0.54	-
Other fruit	-	**0.59**	-
Other greens	-	**0.60**	-0.22
Tomato sauce	-	**0.50**	-
Other vegetables	0.22	**0.54**	-
Beans, peas	0.15	0.25	-
Pickled vegetables	0.15	0.26	0.15
Total cereals and grains	0.23	0.38	0.28
Whole grains	-	0.33	-
Citrus	-	0.34	-
Berries	-	0.45	-
Dried fruit	-	0.39	-
Vegetable juice	-	0.17	-
Beer	0.19	-	-
White wine	-	-	-
Red wine	-	-	-
Liquor	-	-	-
Desserts and sweets	0.31	-	**0.63**
Pies, tarts	0.15	-	**0.54**
Canned fruit	-	0.21	0.23
Jam, jelly	-	-	0.26
Proportion of VAR explained (%)	39.79	22.93	11.10

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| Cumulative VAR explained (%) | 39.79 | 62.72 | 73.82 |

Absolute loading values <0.15 were not listed for simplicity.
Those with loadings of 0.50 or greater are in bold.

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Table2. Baseline Characteristics of 529 CRC Patients by Quartiles of the Three Major Dietary Patterns [a]

	Processed Meat Pattern				P Value[c]	Prudent Vegetable Pattern				P Value[c]	High Sugar Pattern				P Value[c]
	Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)	
Age at diagnosis[b]	61.4±8.7	60.6±9.0	60.2±8.8	59.3±9.3	0.29	57.4±10.3	60.1±7.9	61.0±9.0	62.1±8.0	<.0001	59.5±9.3	60.2±9.1	60.0±8.8	61.7±8.6	0.21
Sex[b]															
Female	67(50.8)	66(50.0)	39(29.3)	39(29.6)		38(28.8)	39(29.5)	58(43.6)	76(57.6)		60(45.5)	49(37.1)	51(38.3)	51(38.6)	
Male	65(49.2)	66(50.0)	94(70.7)	93(70.5)	<.0001	94(71.2)	93(70.5)	75(56.4)	56(42.4)	<.0001	72(54.5)	83(62.9)	82(61.7)	81(61.4)	0.50
Stage at diagnosis															
I/II	87(65.9)	81(61.4)	70(52.6)	71(53.8)		72(54.5)	71(53.8)	83(62.4)	83(62.9)		79(59.8)	77(58.3)	77(57.9)	76(57.6)	
III/IV	45(34.1)	51(38.6)	63(47.4)	61(46.2)	0.09	60(45.5)	61(46.2)	50(37.6)	49(37.1)	0.27	53(40.2)	55(41.7)	56(42.1)	56(42.4)	0.98
BMI (kg/m^2)															
<25.0	38(30.6)	47(36.1)	35(26.5)	27(21.1)		42(33.6)	32(24.8)	34(26.4)	38(29.7)		33(25.6)	40(31.0)	36(28.1)	38(29.7)	
25.0-29.9	57(46.0)	52(40.0)	53(40.2)	53(41.4)		45(35.2)	57(44.2)	55(42.6)	58(45.3)		55(42.6)	47(36.4)	58(45.3)	55(43.0)	
≥30	29(23.4)	31(23.9)	44(33.3)	48(37.5)	0.03	40(31.2)	40(31.0)	40(31.0)	32(25.0)	0.78	41(31.8)	42(32.6)	34(26.6)	35(27.3)	0.63
Physical activity															
<24.9 MET h/wk	73(55.3)	71(53.4)	56(42.1)	65(49.2)		68(51.5)	60(45.4)	69(51.9)	68(51.5)		68(51.5)	71(53.8)	69(51.9)	57(43.2)	
≥24.9 MET h/wk	59(44.7)	61(46.6)	77(57.9)	67(50.8)	0.13	64(48.5)	72(54.6)	64(48.1)	64(48.5)	0.67	64(48.5)	61(46.2)	64(48.1)	75(56.8)	0.32
Marital status															
Single	31(23.5)	29(22.0)	18(13.5)	37(28.0)		26(19.7)	27(20.4)	27(20.3)	35(26.5)		26(19.7)	30(22.7)	30(22.6)	29(22.0)	
Married or living as married	101(76.5)	103(78.0)	115(86.5)	95(72.0)	0.04	106(80.3)	105(79.6)	106(79.7)	97(73.5)	0.50	106(80.3)	102(77.3)	103(77.4)	103(78.0)	0.93
Smoking status															
Ever	77(58.3)	94(71.2)	113(85.0)	104(78.8)		108(81.8)	97(73.5)	100(75.2)	83(62.9)		101(76.5)	95(72.0)	95(71.4)	97(73.5)	
Never	55(41.7)	38(28.8)	20(15.0)	28(21.2)	<.0001	24(18.2)	35(26.5)	33(24.8)	49(37.1)	0.006	31(23.5)	37(28.0)	38(28.6)	35(26.5)	0.79
Tumor location															
Colon	91(69.5)	90(68.2)	85(63.9)	79(59.9)		75(56.8)	91(69.5)	87(65.4)	92(69.7)		82(62.1)	85(64.9)	87(65.4)	91(68.9)	
Rectum	40(30.5)	42(31.8)	48(36.1)	53(40.1)	0.34	57(43.2)	40(30.5)	46(34.6)	40(30.3)	0.10	50(37.9)	46(35.1)	46(34.6)	41(31.1)	0.71
Reported chemoradiotherapy															
Yes	36(27.3)	31(23.5)	20(15.0)	21(15.9)		24(18.2)	23(17.4)	24(18.1)	37(28.0)		30(22.7)	28(21.2)	25(18.8)	25(18.9)	
No	96(72.7)	101(76.5)	113(85.0)	111(84.1)	0.04	108(81.8)	109(82.6)	109(81.9)	95(72.0)	0.10	102(77.3)	104(78.8)	108(81.2)	107(81.1)	0.83
MSI status															
MSS /MSI-L	108(86.4)	110(86.6)	113(91.9)	106(86.9)		107(85.6)	104(86.7)	113(91.1)	113(88.3)		107(84.9)	106(87.6)	110(88.0)	114(91.2)	
MSI-H	17(13.6)	17(13.4)	10(8.1)	16(13.1)	0.49	18(14.4)	16(13.3)	11(8.9)	15(11.7)	0.57	19(15.1)	15(12.4)	15(12.0)	11(8.8)	0.50
BRAF mutation status															
Wide type	104(85.2)	107(89.9)	109(90.8)	106(93.0)		108(91.5)	103(87.3)	112(95.7)	103(84.4)		103(88.8)	110(91.7)	106(89.1)	107(89.2)	
V600E mutant	18(14.8)	12(10.1)	11(9.2)	8(7.0)	0.25	10(8.5)	15(12.7)	5(4.3)	19(15.6)	0.02	13(11.2)	10(8.3)	13(10.9)	13(10.8)	0.88

a   Abbreviations are as follows: BMI, Body mass index; CRC, colorectal cancer; MSI, microsatellite instability; MSS/MSI-L, microsatellite stable/ microsatellite instability-low; MSI-H, microsatellite instability-high

b   Continuous variables presented as mean±SD (standard deviation); categorical variables presented as number[41]

c   P values are for the significance of the ANOVA test for continuous variables and of the Chi-Square test for categorical variables

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Table3. Hazard Rate Ratios Associated with Disease-Free and Overall Colorectal Cancer Survival for Quartiles of Dietary Patterns [a]

	Disease-Free Survival				Overall Survival			
	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]
Processed meat pattern								
Q1	38/132	1.00	1.00	1.00	33/132	1.00	1.00	1.00
Q2	45/132	1.51(0.95-2.41)	1.69(0.97-2.96)	0.91(0.39-2.14)	40/132	1.47(0.89-2.44)	2.18*(1.16-4.09)	0.75(0.28-2.03)
Q3	58/132	1.56(0.97-2.49)	1.37(0.76-2.48)	1.72(0.85-3.95)	49/133	1.32(0.78-2.22)	1.44(0.74-2.79)	1.54(0.57-4.13)
Q4	57/132	1.82*(1.07-3.09)	2.29*(1.19-4.40)	0.97(0.38-2.45)	46/132	1.53(0.85-2.74)	2.13*(1.03-4.43)	1.17(0.41-3.36)
P for trend [d]		0.09	0.12	0.91		0.25	0.40	0.59
Prudent vegetable pattern								
Q1	46/132	1.00	1.00	1.00	41/132	1.00	1.00	1.00
Q2	54/132	1.21(0.79-1.85)	1.35(0.78-2.34)	0.97(0.47-2.01)	45/132	1.09(0.69-1.73)	1.18(0.65-2.14)	0.90(0.41-1.98)
Q3	50/133	1.18(0.75-1.86)	1.16(0.63-2.13)	1.30(0.65-2.60)	40/133	0.82(0.49-1.36)	1.04(0.55-1.97)	0.59(0.25-1.42)
Q4	48/131	1.12(0.69-1.84)	1.02(0.52-1.99)	1.28(0.58-2.83)	42/132	1.03(0.61-1.75)	0.96(0.47-1.96)	1.00(0.42-2.40)
P for trend [d]		0.62	0.83	0.19		0.90	0.60	0.92
High sugar pattern								
Q1	42/131	1.00	1.00	1.00	30.132	1.00	1.00	1.00
Q2	54/132	1.07(0.70-1.63)	0.96(0.54-1.68)	1.30(0.64-2.65)	48/132	1.25(0.77-2.04)	1.21(0.62-2.36)	2.12(0.87-5.14)
Q3	54/133	1.09(0.69-1.73)	0.94(0.51-1.73)	1.44(0.67-3.07)	50/133	1.64(0.98-2.75)	1.35(0.66-2.78)	2.49*(1.02-6.10)
Q4	48/132	1.02(0.62-1.69)	0.99(0.52-1.89)	1.49(0.61-3.63)	40/132	1.27(0.72-2.25)	1.16(0.54-2.47)	1.68(0.55-5.08)
P for trend [d]		0.89	0.90	0.11		0.52	0.56	0.64

[a] Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; CI, confidence interval;

[b] Events are defined as death/recurrence/metastasis (which occurred earliest) for disease-free survival and deaths for overall survival.

[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, marital status, family history, reported screening procedure, reported chemoradiotherapy and MSI status, where appropriate.
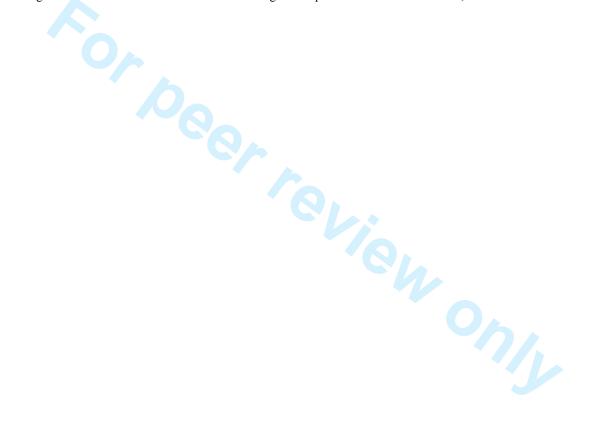
[d] Two-sided *p* value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

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Table 4. Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics [a]

	No. of Events[b] /No. at Risk	Quartiles HR (95% CI)[c]				P for Trend[d]	P for Interaction[e]
		Q1	Q2 [c]	Q3	Q4		
Processed meat pattern							
Sex							
Female	65/210	1.00	2.20(0.99-4.91)	2.38(0.97-5.85)	3.85*(1.49-9.99)	0.03	
Male	133/318	1.00	1.20(0.66-2.18)	1.23(0.69-2.17)	1.22(0.64-2.32)	0.27	0.04
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.96*(1.05-3.67)	2.13*(1.11-4.11)	2.03(0.96-4.30)	0.42	
≥24.9 MET h/wk	101/264	1.00	1.22(0.59-2.55)	1.27(0.62-2.62)	1.64(0.74-3.62)	0.01	0.64
BRAF mutation status							
Wide type	163/425	1.00	1.28(0.77-2.12)	1.41(0.80-2.34)	1.80*(1.01-3.21)	0.009	
V600E mutant	17/49	1.00	1.82(0.40-8.34)	0.54(0.10-2.83)	0.79(0.09-7.01)	0.50	0.80
Prudent vegetables pattern							
Sex							
Female	65/210	1.00	1.57(0.59-4.20)	1.55(0.63-3.85)	1.22(0.46-3.24)	0.71	
Male	133/318	1.00	1.25(0.76-2.04)	1.08(0.62-1.88)	1.14(0.62-2.09)	0.67	0.65
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.48(0.80-2.76)	1.52(0.81-2.87)	1.22(0.56-2.69)	0.66	
≥24.9 MET h/wk	101/264	1.00	1.02(0.55-1.89)	1.02(0.53-1.96)	1.05(0.55-2.04)	0.03	0.83
BRAF mutation status							
Wide type	163/425	1.00	1.32(0.83-2.10)	1.29(0.80-2.08)	1.19(0.70-2.02)	0.58	
V600E mutant	17/49	1.00	2.50(0.38-16.59)	0.88(0.06-12.99)	1.24(0.12-13.20)	0.73	0.80
High sugar pattern							
Sex							
Female	65/210	1.00	1.41(0.63-3.16)	0.88(0.36-2.15)	0.82(0.30-2.27)	0.42	
Male	133/318	1.00	1.14(0.67-1.97)	1.34(0.75-2.39)	1.39(0.73-2.66)	0.06	0.72
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.01(0.55-1.86)	1.10(0.56-2.16)	1.19(0.56-2.54)	0.06	
≥24.9 MET h/wk	101/264	1.00	1.36(0.70-2.65)	1.21(0.60-2.45)	1.04(0.49-2.22)	0.86	0.26
BRAF mutation status							
Wide type	163/425	1.00	0.99(0.61-1.59)	1.20(0.71-2.01)	1.03(0.59-1.82)	0.70	
V600E mutant	17/49	1.00	0.53(0.07-4.25)	0.27(0.04-1.66)	0.32(0.04-2.64)	0.09	0.33

[a] Abbreviations are as follows: CI, confidence interval; METs/week, metabolic equivalent hours per week;

[b] Events are defined as death/recurrence/metastasis (which occurred earliest) for disease-free survival and deaths for overall survival.

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[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening procedure, reported chemoradiotherapy, and MSI status, where appropriate.

[d] Two-sided p value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

[e] P for interaction is the significance of interaction term between smoking and respective stratification variable, calculated from Wald test.

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REFERENCES

1. Canadian Cancer Statistics 2012. Toronto, ON: Canadian Cancer Society's Steering Committee on Cancer Statistics. Canadian Cancer Society, 2012.

2. Norat T, Chan D, Lau R, et al. WCRF/AICR Systematic Literature Review Continuous Update Project Report. The Associations between Food, Nutrition and Physical Activity and the Risk of Colorectal Cancer: American Institute for Cancer Research, 2010.

3. Dixon LB, Balder HF, Virtanen MJ, et al. Dietary patterns associated with colon and rectal cancer: results from the Dietary Patterns and Cancer (DIETSCAN) Project. Am J Clin Nutr 2004;80(4):1003-11.

4. Terry P, Hu FB, Hansen H, et al. Prospective study of major dietary patterns and colorectal cancer risk in women. Am J Epidemiol 2001;154(12):1143-9.

5. Kwan ML, Weltzien E, Kushi LH, et al. Dietary patterns and breast cancer recurrence and survival among women with early-stage breast cancer. J Clin Oncol 2009;27(6):919-26.

6. Kim MK, Sasaki S, Otani T, et al. Dietary patterns and subsequent colorectal cancer risk by subsite: a prospective cohort study. Int J Cancer 2005;115(5):790-8.

7. Slattery ML, Boucher KM, Caan BJ, et al. Eating patterns and risk of colon cancer. Am J Epidemiol 1998;148(1):4-16.

8. Williams CD, Satia JA, Adair LS, et al. Dietary patterns, food groups, and rectal cancer risk in Whites and African-Americans. Cancer Epidemiol Biomarkers Prev 2009;18(5):1552-61.

9. Meyerhardt JA, Niedzwiecki D, Hollis D, et al. Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. Jama 2007;298(7):754-64.

10. Fung T, Hu FB, Fuchs C, et al. Major dietary patterns and the risk of colorectal cancer in

22

women. Arch Intern Med 2003;163(3):309-14.

11. Squires J, Roebothan B, Buehler S, et al. Pickled meat consumption and colorectal cancer (CRC): a case-control study in Newfoundland and Labrador, Canada. Cancer Causes Control 2010;21(9):1513-21.

12. Sun Z, Zhu Y, Wang PP, et al. 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. Anticancer Res 2012;32(2):687-96.

13. Sun Z, Liu L, Wang PP, et al. Association of total energy intake and macronutrient consumption with colorectal cancer risk: results from a large population-based case-control study in Newfoundland and Labrador and Ontario, Canada. Nutr J 2012;11(1):18.

14. Woods MO, Younghusband HB, Parfrey PS, et al. The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. Gut 2010;59(10):1369-77.

15. Green RC, Green JS, Buehler SK, et al. Very high incidence of familial colorectal cancer in Newfoundland: a comparison with Ontario and 13 other population-based studies. Fam Cancer 2007;6(1):53-62.

16. Stram DO, Hankin JH, Wilkens LR, et al. Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. Am J Epidemiol 2000;151(4):358-70.

17. Sharma S, Iwasaki M, Kunieda C, et al. Development of a quantitative food frequency questionnaire for assessing food, nutrient, and heterocyclic aromatic amines intake in Japanese Brazilians for a colorectal adenoma case-control study. Int J Food Sci Nutr 2009;60 Suppl 7:128-39.

23

18. Jain MG, Rohan TE, Soskolne CL, et al. Calibration of the dietary questionnaire for the Canadian Study of Diet, Lifestyle and Health cohort. Public Health Nutr 2003;6(1):79-86.

19. Hankin JH, Wilkens LR, Kolonel LN, et al. Validation of a quantitative diet history method in Hawaii. Am J Epidemiol 1991;133(6):616-28.

20. Sun Z, Zhu Y, Wang PP, et al. 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. Anticancer Res 2012;32(2):687-96.

21. Dr. H. Bliss Murphy Cancer Care Foundation. From http://www.cancercarefoundation.nl.ca/ (accessed July 18, 2012).

22. MANUAL of the international statistical classification of diseases, injuries, and causes of death. Addendum 1. Supplementary interpretations and instructions for coding causes of death. Bull World Health Org Suppl 1953;7(Suppl 6):1-55.

23. Raptis S, Mrkonjic M, Green RC, et al. MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. J Natl Cancer Inst 2007;99(6):463-74.

24. Campbell PT, Jacobs ET, Ulrich CM, et al. Case-Control Study of Overweight, Obesity, and Colorectal Cancer Risk, Overall and by Tumor Microsatellite Instability Status. J Natl Cancer Inst 2010;102(6):391-400.

25. Loughrey MB, Waring PM, Tan A, et al. Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer. Fam Cancer 2007;6(3):301-10.

26. Phipps AI, Baron J, Newcomb PA. Prediagnostic smoking history, alcohol consumption, and colorectal cancer survival: the Seattle Colon Cancer Family Registry. Cancer

24

2010;117(21):4948-57.

27. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. Stat Methods Med Res 1992;1(1):69-95.

28. Heidemann C, Schulze MB, Franco OH, et al. Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. Circulation 2008;118(3):230-7.

29. Introduction to SAS, UCLA: Academic Technology Services, Statistical Consulting Group. From http://www.ats.ucla.edu/stat/examples/asa/test_proportionality.htm (accessed June 4, 2012).

30. Slattery ML, French TK, Egger MJ, et al. Diet and survival of patients with colon cancer in Utah: is there an association? Int J Epidemiol 1989;18(4):792-7.

31. Dray X, Boutron-Ruault MC, Bertrais S, et al. Influence of dietary factors on colorectal cancer survival. Gut 2003;52(6):868-73.

32. Dolecek TA, McCarthy BJ, Joslin CE, et al. Prediagnosis food patterns are associated with length of survival from epithelial ovarian cancer. J Am Diet Assoc 2010;110(3):369-82.

33. Bingham SA, Pignatelli B, J.R. P, et al. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996;17(no.3):515-523.

34. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environ Mol Mutagen 2004;44(1):44-55.

35. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. J Natl Cancer Inst. 2002

25

94(13):972-80.

36. Rabizadeh S, Sears C. New horizons for the infectious diseases specialist: how gut microflora promote health and disease. Curr Infect Dis Rep 2008;10(2):92-8.

37. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. Genome Med 2011;3(3):14.

38. Tjalsma H, Boleij A, Marchesi JR, et al. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. Nat Rev Microbiol 2012.

39. Backhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine. Science 2005;307(5717):1915-20.

40. Takachi R TY, Baba K, Inoue M, et al. Japan Public Health Center-Based Prospective Study Group. Red meat intake may increase the risk of colon cancer in Japanese, a population with relatively low red meat consumption. Asia Pac J Clin Nutr. 2011;20(4):603-12.

41. Bingham SA PB, Pollock JR, Ellul A, et al. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996;17(no.3):515-523.

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Dietary Patterns and Colorectal Cancer Recurrence and Survival

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Dietary Patterns and Colorectal Cancer Recurrence and Survival

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ABSTRACT

Objective: To examine the association between dietary patterns and colorectal cancer (CRC) survival.

Design: Cohort study

Setting and participants: Five hundred and twenty nine newly diagnosed CRC patients from the Newfoundland Familial Colorectal Cancer Registry (NFCCR) were recruited and followed until April, 2010.

Outcome measure: Participants reported their dietary intake using a food frequency questionnaire. Dietary patterns were identified with factor analysis. Multivariable Cox proportional hazards models were employed to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association of dietary patterns with CRC recurrence and death from all-causes, after controlling for covariates.

Results: Disease-free survival (DFS) among CRC patients was significantly worsened among patients with a high processed meat dietary pattern (the highest versus the lowest quartile HR: 1.82, 95% CI: 1.07-3.09). No associations were observed with the prudent vegetable or the high sugar patterns and DFS. The association between the processed meat pattern and DFS was restricted to patients diagnosed with colon cancer (the highest versus the lowest quartile: HR: 2.29, 95% CI: 1.19-4.40) while the relationship between overall survival (OS) and this pattern was observed among patients with colon cancer only (the highest versus the lowest quartile: HR: 2.13, 95%CI: 1.03-4.43). Potential effect modification was noted for sex (p for interaction=0.04, HR: 3.85 for women and 1.22 for men).

Conclusion

The processed meat dietary pattern prior to diagnosis is associated with higher risk of tumor recurrence, metastasis, and death among patients with colorectal cancer.

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ARTICLE SUMMARY

Article focus

- We used the data of 529 colorectal cancer patients in Newfoundland and Labrador to investigate the association of dietary patterns and colorectal cancer survival.

- We further explored if the relationship between dietary pattern and colorectal cancer survival is modified by sex, physical activity and *BRAF* mutation.

Key messages

- The processed meat dietary pattern is associated with a worsened colorectal cancer disease-free survival.

- The prudent vegetable or the high sugar patterns show no association with disease-free survival.

- The relationship between processed meat pattern and colorectal cancer survival is modified by sex.

Strengths and limitations of this study

- The sample size is reasonably large with detailed information on diet, lifestyle and molecular characteristics.

- Recall bias remains a problem since the food consumption was collected from one year prior to CRC diagnosis. In addition, dietary patterns only reflect food consumption before diagnosis which might be modified after diagnosis.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of cancer death in Canada.[1] Epidemiological studies have established a strong link between few dietary factors, such as fiber (inversely) and red/processed meat (increases risk), and the risk of developing CRC, [2] although most studies have focused primarily on individual foods or nutrients. Since foods and nutrients act synergistically rather than in isolation,[3-6] recent research has investigated the role of dietary patterns on CRC incidence. Dietary patterns identified in prior research often include the "Western" and "prudent" patterns. Adherence to the Western diet pattern, characterized by high intakes of meat, fat, sweets and desserts, is often associated with increased risk of CRC.[5-9] Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse[7 8] or null[5 6 10] association with CRC risk.

The highest CRC incidence and mortality rates in Canada are observed in the province of Newfoundland and Labrador (NL).[1] Geographically isolated in the Atlantic Ocean, NL has long maintained its traditional foods, a Western-style diet consisting of a large proportion of processed meat, red meat and insufficient vegetables.[11] Several studies have partially attributed the high CRC incidence rate in NL to its unique diet,[11-13] but no study has explored the association between the NL diet and its impact on survival among CRC patients.

This prospective cohort study investigated the influence of dietary patterns, identified by factor analysis, on survival and recurrence or metastasis among an incident case series of 529 CRC patients from NL. Additionally, the present study evaluated the possible effect modification among dietary patterns with gender, physical activity and tumor molecular phenotype.

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SUBJECTS AND METHODS

Study participants

Patients in this prospective cohort study were enrolled through the Newfoundland Familial Colorectal Cancer Registry (NFCCR), described in detail elsewhere.[14] [15] In brief, during the time period from 1999 to 2003, patients aged 20-75 years, newly diagnosed with pathologically confirmed, invasive CRC were eligible for inclusion in the study (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).

Written, informed consent was required from each study participant to access their archived tumor tissue and medical records. If patients died before they could give consent (the median time from date of diagnosis to date of consent was 1.8 years), a close relative/proxy, who has lived with the patient, was invited to participate. Enrolling deceased cases through proxies could remove the potential bias of eliminating patients at a late distant stage.[14] Thus, the inception cohort consisted of 750 eligible patients (64%).

Consenting participants completed and returned a detailed food frequency questionnaire (FFQ), personal history questionnaire (PHQ) and family history questionnaire (FHQ). All questionnaires were self-completed. Assistance from study staff was available to help with understanding items on the questionnaires. To capture additional cancer diagnosis or recurrence in the family after enrollment, the FHQ was distributed to participants for the second time midway through the follow-up. To be included in this analysis, patients had to have completed at least the FFQ, provided informative lifestyle and medical data from the PHQ, and had known vital status information by the end of the follow-up period (April, 2010). For patients who died prior to enrollment, the designated relative/proxy completed the aforementioned

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questionnaires. The final analytical cohort comprised 529 eligible participants. The study protocol was approved by the Human Investigation Committee of Memorial University of Newfoundland.

Dietary assessment and food grouping

Diet was assessed using a semi-quantitative FFQ, developed from the well-known Hawaii FFQ,[16] on the basis of a validated instrument adapted for the Canadian population. [17] [18] The FFQ included 170 foods, beverages, and vitamin- and dietary-supplements.[19] Foods indigenous to the Newfoundland population (e.g., salted/pickled meat and smoked/pickled fish) were also included. For each food item or beverage, participants were asked to estimate their frequency of consumption and usual portion size as 'Small', 'Regular' or 'Large' one year prior to their colon or rectal cancer diagnosis. Portion sizes for specific food were depicted in photographs. Nutrient and total energy intakes were calculated by multiplying the frequency of consumption of each food by the nutrient content of the portion size based on the composition values from the 2005 Canadian Nutrient file.[20] Taking a similar grouping scheme to that used elsewhere,[3] we collapsed individual food items on the FFQ into 39 predefined food groups based on the roles of food in diet and cancer etiology. Distinct food items were reserved as individual categories if it was deemed inappropriate to combine them (e.g., jam, pies, beer, and wine).

Covariates

Sociodemographic data, such as age, sex, marital status, and education attainment, were gathered by the self-administered PHQ. The PHQ also included items regarding medical history, bowel screening history, physical activity, reproductive factors

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(female only), and alcohol and tobacco use. Family history of cancer was assessed by

the FHQ.

Study outcomes

Study outcomes were ascertained from follow-up questionnaires, local

newspapers (e.g.,death notices), death certificates, autopsy, pathology, radiology,

surgical reports, as well as physician's notes. Additional data were gathered from the

Dr. H. Bliss Murphy Cancer Care Foundation and Statistics Canada.[21] The cause of

death was obtained for 93 of 168 deceased patients in this cohort, classified according

to the International Classification of Disease (ICD) codes for underlying or

contributing cause of death;[22] the majority (91%) of these had died from CRC. Since

specific cause of death was not available for all deceased participants, all-cause

mortality was used for analysis. In this study, two end points were considered: the first

was disease-free survival (DFS), defined as time from cancer diagnosis to the first

confirmed tumor recurrence, metastasis, or death from all causes occurring up to April,

2010; the second end point was overall survival (OS), measured from the date of

cancer diagnosis to the date of death from all causes. Patients who did not have an

event by the end of the follow-up were censored at the date of last contact.

Molecular assessment

The *p.V600E BRAF* mutation and MSI status for the tumor DNA have been

determined in previous studies using standard protocols.[23-25] Briefly, the mutational

hotspot c.1799T>A. (p.Val600Glu) in the *BRAF* gene was detected using *BRAF*

V600E allele-specific primers, with controls amplifying the GAPDH gene.[25] Positive

mutations were then verified by direct automatic sequencing.[25]   For MSI analyses, a

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panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4, D5S346, D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify both tumor and normal DNA.[23 24] MSI status was defined as MSI-High if 30% or more of the markers were unstable and MS-Stable/MSI-Low if less than 30% of the markers showed instability.[26 27]The primer sequences and PCR conditions are provided in detail in earlier studies from this cohort.[14 23-25]

Statistical analysis

Exploratory principal component factor analysis [28] was used to identify major dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation (orthogonal) procedure was applied to rotate these factors, meaning that it produces uncorrelated, easy interpreted components that explain the greatest amount of variance in the original food groups.[29] We determined the number of factors to retain for interpretation on the basis of criteria as follows: factor eigenvalue greater than 1.15, the scree plot, the proportion of variance explained, and factor interpretability.[9] Patterns were labeled based on food groups with absolute rotated factor loading matrix greater than or equal to 0.50. Each participant was assigned a factor score for each pattern (factor) by summing the intakes from each food group multiplied by optimal weights (factor loadings).[5] Individuals with a higher factor score had a closer adherence to that pattern.[5]

Comparisons for baseline characteristics across quartiles of dietary patterns were performed using ANOVA test for continuous variables and Chi-Square test for categorical variables. Cox proportional hazards models, each adjusting for energy intake and critical covariates, were used to evaluate the association between individual dietary pattern and CRC recurrence and mortality, represented by hazard

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ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed by the log-rank test in a univariate setting; those with the p-value less than 0.1 were considered for inclusion. The final models only retained the items that entered the models at p<0.1 or altered the effect estimates by 10% or more; these include sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening procedure, reported chemoradiotherapy, and MSI status. All models were run with the adjustment for total energy intake by including total calories in the model. The assumption of proportional hazard rates was verified by checking the parallelism of the Kaplan-Meier curves and by including time-dependent covariates in the models to test for statistical significance.[30] Statistical linear trend was examined by modeling the median value of each quartile as an ordinal variable in a linear regression.[5] Potential interactions were evaluated by comparing estimates from stratified analyses and testing significance of interaction terms with a Wald test.[5]

A sensitivity analysis was implemented by eliminating stage-advanced patients enrolled through proxies and re-calculating survival time from the completion of the first questionnaire to a predefined event, in order to determine whether associations might vary with the exclusion of stage-advanced cancer. Statistical significance was accepted for two-sided p < 0.05. All data management and analyses were performed with SAS software version 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

The cohort was followed for a median of 6.4 years (minimum: 1.3 years; maximum: 10.9 years). A total of 168 patients died from all causes and 30 had a cancer recurrence or metastasis by the end of study follow-up (April, 2010).

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Dietary patterns

Three distinct dietary patterns, labeled "processed meat pattern", "prudent vegetable pattern" and "high sugar pattern", were extracted using the aforementioned factor analysis procedure. These patterns explained 73.82% of total variance in the original 39 food groups (Table 1). A higher factor loading matrix of a given food group is representative of a greater contribution of that food group on that specific pattern. Therefore, the first pattern, termed "processed meat", was characterized by higher loadings and thus higher consumptions of cured/processed meat, cured/processed red meat, red meat, fish, and processed fish; the second pattern, labeled "prudent vegetable", displayed higher loadings on other greens, other fruit, other vegetables, and tomato sauce; and the third pattern, named "high sugar", showed higher loadings on desserts and sweets, pies and tarts.

Baseline characteristics by quartiles of dietary patterns

Higher processed meat pattern scores at baseline were detected in men, ever smokers, patients who were single and individuals who had higher BMI at the time of diagnosis (Table 2). Higher prudent vegetable pattern scores were observed in women, never smokers, those with a slightly later age of diagnosis and with patients who had a tumor harboring the *p.V600E BRAF* mutation. None of these characteristics varied significantly by quartiles of high sugar pattern scores.

Dietary patterns and cancer recurrence or death

The highest quartile of processed meat pattern was significantly associated with poorer DFS after the adjustment for other predictors of CRC recurrence and death (HR: 1.82, 95% CI: 1.07-3.09), although no overall trend was observed in the HRs

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across the whole distribution of factor scores (p for trend=0.09) (Table 3).

Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was

observed to be significantly associated with predefined patient outcomes (i.e., DFS

and OS).

When stratified by tumor site, however, the association between processed meat

pattern and DFS remained statistically significant only for patients who had tumors

located in the colon (the highest versus the lowest quartile, HR: 2.29, 95% CI:

1.19-4.40) and not the rectum (HR: 0.97, 95% CI: 0.38-2.45). Similarly, when OS was

the outcome, the positive association between increasing consumption of the

processed meat pattern and mortality was restricted to patients whose tumors were

diagnosed in the colon (the forth versus first quartiles: HR: 2.13, 95% CI: 1.03-4.43).

In the stratified analyses for dietary patterns, there was evidence for effect

modification by sex (p=0.04) for the association of processed meat pattern with DFS

(HR: 3.85 for women and 1.22 for men) (Table 4). However, no evidence was

observed to suggest that the effects of other dietary patterns on cancer recurrence or

death were modified by physical activity, *BRAF* mutation status and MSI (data not

shown).

In the sensitivity analysis, when advanced-stage patients who died before

admittance were excluded, the association between processed meat pattern and

survival among CRC patients remained significant.

DISCUSSION

Three dietary patterns, termed "processed meat pattern", "prudent vegetable

pattern" and "high sugar pattern", were generated in this cohort study. We found that

high conformity with the processed meat pattern, characterized by high intakes of

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processed meat, red meat, fish, and processed fish, is associated with decreased DFS

of CRC, specifically of colon cancer. The differential associations by subsite indicate

disease heterogeneity. On the contrary, increasing consumption of the prudent

vegetable pattern and the high sugar pattern displayed no clear relationships with

mortality or recurrence.

The processed meat pattern in the present study shares most characteristics of the

Western diet referred to in previous studies on CRC risk, which indicates a positive

association between the Western dietary pattern and CRC risk.[7 9] However, there has

been minimal research examining the association between dietary factors (e.g.,

nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients;[31 32]

moreover, our literature review identified only one study that investigated the

relationship between dietary patterns and survival among CRC patients. Consistent

with our results, that prospective cohort study of 1009 stage III colon cancer patients [9]

reported a deleterious disease-free colon cancer prognosis for patients reporting high

levels of the Western dietary pattern intake.

The mechanisms explaining the impact of red and processed meat on CRC

mortality are still unclear; however, some biologic mechanisms that link diet factors

to CRC risk may continue after diagnosis and subsequently impact cancer progression

and survival.[33] For example, strong carcinogens such as *N*-nitroso compounds (NOCs)

and probable carcinogenic mutagens like heterocyclic amines (HCA) and polycyclic

aromatic hydrocarbons (PAH), which have been suggested as significant contributors

for CRC development,[34 35] are found in smoked, fried or high-temperature cooked

meat. Sandhu et al[36] reported a Western dietary pattern is related to high levels of

serum insulin and insulin-like growth factors (IGF), and these hormones are found to

be associated with tumor growth and the inhibition of apoptosis. In addition, a

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growing body of evidence suggests that disruption of the normal gut microflora is associated with human disease, including the pathogenesis of the intestinal tract (e.g. inflammatory bowel disease) and other diseases such as obesity, cardiovascular disease, and autoimmune conditions. [37][38] Alterations in intestinal microbiota are also strongly associated with colonic polyp formation and with the risk of developing CRC.[39] Given the major role of diet on the intestinal microbiome, [40] our findings between dietary patterns and CRC survival may also be explained by the impact of dietary patterns on gut microflora and health outcomes.

The influence of processed meat pattern on survival was evident among women rather than men in our study. Previous studies revealed that the higher colon pH and longer intestine transit time in women compared to men can influence the production of secondary bile acid or NOCs,[41] resulting in gender differences in the CRC development. This is the first study that considered effect modifications between dietary patterns and tumor molecular phenotype (i.e. *BRAF* mutation) on CRC survival. *BRAF* mutation is found to be significantly associated with poor CRC survival;[42] however, whether it can modify the impacts of dietary factors on CRC survival is not known. Although stratified analyses in our study demonstrated a processed meat diet to significantly decrease survival time only in patients with *BRAF* wild type tumor, no evident interactions were detected. Further research is clearly warranted to verify these findings and to determine the biologic pathways that rationalize the underlying interactions between diet and tumor molecular features.

A reasonably large sample size with detailed information of patients is a merit of our study. These data not only includes demographic and personal lifestyle information, but also some molecular characteristics obtained from genetic testing. The ample information enables us to perform stratification analysis to control and

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assess effect modifiers and confounders. Several limitations of this study should be recognized. Firstly, the results may be skewed by recall bias since the participants recalled their food consumption from one year prior to CRC diagnosis; however, this non-differential misclassification is only expected to bias the results towards the null. Secondly, dietary patterns in this study only reflect food consumption before diagnosis; it is unknown whether participants modified their diet post diagnosis. Since previous research has shown minimal change in diet between pre- and post- diagnosis among cancer patients,[32] the current study did not examine dietary changes before and after diagnosis. Moreover, immortal person-time bias may impact results. However, this is minimized by using proxies to enroll deceased patients.

In summary, we found that high conformity to the processed meat pattern is significantly associated with an increased risk of all-cause mortality and recurrence of CRC. Though our study did not find a difference in effect by tumor molecular phenotype, larger molecular studies should be conducted to examine if such differences exist. Ultimately, confirmation of these findings and the underlying mechanisms await further studies. Our observation not only underlines the importance of maintaining a healthy diet, but also provides guidance to efficacious dietary interventions;[8] that is, people may lower their risk of CRC mortality by reducing consumption of a processed meat pattern diet.

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Table 1. Factor Loadings and Explained Variances (VAR) for the Three Major Dietary Patterns Identified from the Food Frequency Questionnaire at baseline using a Principal Component Factor Analysis, Newfoundland

Food Groups	Processed Meat Pattern	Prudent Vegetable Pattern	High Sugar Pattern
Milk	-	0.19	-
Yogurt	-	0.31	-
Sugar	-	-0.19	0.20
Tea	-	-	0.17
Coffee	0.17	-	-
Soft drinks	0.19	-	-
Cheese	0.15	0.21	-
Egg	0.21	-	0.16
Mixed dishes	0.31	0.17	0.23
Red meat	**0.69**	-	0.17
Cured/processed red meat	**0.73**	-	0.21
Cured/processed meat	**0.93**	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	**0.58**	0.32	-0.22
Processed fish	**0.50**	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	**0.54**	-
Other fruit	-	**0.59**	-
Other greens	-	**0.60**	-0.22
Tomato sauce	-	**0.50**	-
Other vegetables	0.22	**0.54**	-
Beans, peas	0.15	0.25	-
Pickled vegetables	0.15	0.26	0.15
Total cereals and grains	0.23	0.38	0.28
Whole grains	-	0.33	-
Citrus	-	0.34	-
Berries	-	0.45	-
Dried fruit	-	0.39	-
Vegetable juice	-	0.17	-
Beer	0.19	-	-
White wine	-	-	-
Red wine	-	-	-
Liquor	-	-	-
Desserts and sweets	0.31	-	**0.63**
Pies, tarts	0.15	-	**0.54**
Canned fruit	-	0.21	0.23
Jam, jelly	-	-	0.26
Proportion of VAR explained (%)	39.79	22.93	11.10

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Cumulative VAR explained (%)	39.79	62.72	73.82

Absolute loading values <0.15 were not listed for simplicity.
Those with loadings of 0.50 or greater are in bold.

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istics of 529 CRC Patients by Quartiles of the Three Major Dietary Patterns [a]

	Processed Meat Pattern			P Value[c]	Prudent Vegetable Pattern				P Value[c]	High Sugar Pattern				P Value[c]
Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)	
	60.6±9.0	60.2±8.8	59.3±9.3	0.29	57.4±10.3	60.1±7.9	61.0±9.0	62.1±8.0	<.0001	59.5±9.3	60.2±9.1	60.0±8.8	61.7±8.6	0.21
	66(50.0)	39(29.3)	39(29.6)		38(28.8)	39(29.5)	58(43.6)	76(57.6)		60(45.5)	49(37.1)	51(38.3)	51(38.6)	
	66(50.0)	94(70.7)	93(70.5)	<.0001	94(71.2)	93(70.5)	75(56.4)	56(42.4)	<.0001	72(54.5)	83(62.9)	82(61.7)	81(61.4)	0.50
	81(61.4)	70(52.6)	71(53.8)		72(54.5)	71(53.8)	83(62.4)	83(62.9)		79(59.8)	77(58.3)	77(57.9)	76(57.6)	
	51(38.6)	63(47.4)	61(46.2)	0.09	60(45.5)	61(46.2)	50(37.6)	49(37.1)	0.27	53(40.2)	55(41.7)	56(42.1)	56(42.4)	0.98
	47(36.1)	35(26.5)	27(21.1)		42(33.6)	32(24.8)	34(26.4)	38(29.7)		33(25.6)	40(31.0)	36(28.1)	38(29.7)	
	52(40.0)	53(40.2)	53(41.4)		45(35.2)	57(44.2)	55(42.6)	58(45.3)		55(42.6)	47(36.4)	58(45.3)	55(43.0)	
	31(23.9)	44(33.3)	48(37.5)	0.03	40(31.2)	40(31.0)	40(31.0)	32(25.0)	0.78	41(31.8)	42(32.6)	34(26.6)	35(27.3)	0.63
	71(53.4)	56(42.1)	65(49.2)		68(51.5)	60(45.4)	69(51.9)	68(51.5)		68(51.5)	71(53.8)	69(51.9)	57(43.2)	
	61(46.6)	77(57.9)	67(50.8)	0.13	64(48.5)	72(54.6)	64(48.1)	64(48.5)	0.67	64(48.5)	61(46.2)	64(48.1)	75(56.8)	0.32
	29(22.0)	18(13.5)	37(28.0)		26(19.7)	27(20.4)	27(20.3)	35(26.5)		26(19.7)	30(22.7)	30(22.6)	29(22.0)	
	103(78.0)	115(86.5)	95(72.0)	0.04	106(80.3)	105(79.6)	106(79.7)	97(73.5)	0.50	106(80.3)	102(77.3)	103(77.4)	103(78.0)	0.93
	94(71.2)	113(85.0)	104(78.8)		108(81.8)	97(73.5)	100(75.2)	83(62.9)		101(76.5)	95(72.0)	95(71.4)	97(73.5)	
	38(28.8)	20(15.0)	28(21.2)	<.0001	24(18.2)	35(26.5)	33(24.8)	49(37.1)	0.006	31(23.5)	37(28.0)	38(28.6)	35(26.5)	0.79
	90(68.2)	85(63.9)	79(59.9)		75(56.8)	91(69.5)	87(65.4)	92(69.7)		82(62.1)	85(64.9)	87(65.4)	91(68.9)	
	42(31.8)	48(36.1)	53(40.1)	0.34	57(43.2)	40(30.5)	46(34.6)	40(30.3)	0.10	50(37.9)	46(35.1)	46(34.6)	41(31.1)	0.71
	31(23.5)	20(15.0)	21(15.9)		24(18.2)	23(17.4)	24(18.1)	37(28.0)		30(22.7)	28(21.2)	25(18.8)	25(18.9)	
	101(76.5)	113(85.0)	111(84.1)	0.04	108(81.8)	109(82.6)	109(81.9)	95(72.0)	0.10	102(77.3)	104(78.8)	108(81.2)	107(81.1)	0.83
	110(86.6)	113(91.9)	106(86.9)		107(85.6)	104(86.7)	113(91.1)	113(88.3)		107(84.9)	106(87.6)	110(88.0)	114(91.2)	
	17(13.4)	10(8.1)	16(13.1)	0.49	18(14.4)	16(13.3)	11(8.9)	15(11.7)	0.57	19(15.1)	15(12.4)	15(12.0)	11(8.8)	0.50
	107(89.9)	109(90.8)	106(93.0)		108(91.5)	103(87.3)	112(95.7)	103(84.4)		103(88.8)	110(91.7)	106(89.1)	107(89.2)	
	12(10.1)	11(9.2)	8(7.0)	0.25	10(8.5)	15(12.7)	5(4.3)	19(15.6)	0.02	13(11.2)	10(8.3)	13(10.9)	13(10.8)	0.88

s: BMI, Body mass index; CRC, colorectal cancer; MSI, microsatellite instability; MSS/MSI-L, microsatellite stable/ microsatellite instability-low; MSI-H, microsatellite

nted as mean±SD (standard deviation); categorical variables presented as number[43]

and of the ANOVA test for continuous variables and of the Chi-Square test for categorical variables

Table3. Hazard Rate Ratios Associated with Disease-Free and Overall Colorectal Cancer Survival for Quartiles of Dietary Patterns [a]

	Disease-Free Survival				Overall Survival			
	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]
Processed meat pattern								
Q1	38/132	1.00	1.00	1.00	33/132	1.00	1.00	1.00
Q2	45/132	1.51(0.95-2.41)	1.69(0.97-2.96)	0.91(0.39-2.14)	40/132	1.47(0.89-2.44)	2.18(1.16-4.09)	0.75(0.28-2.03)
Q3	58/132	1.56(0.97-2.49)	1.37(0.76-2.48)	1.72(0.85-3.95)	49/133	1.32(0.78-2.22)	1.44(0.74-2.79)	1.54(0.57-4.13)
Q4	57/132	1.82(1.07-3.09)	2.29(1.19-4.40)	0.97(0.38-2.45)	46/132	1.53(0.85-2.74)	2.13(1.03-4.43)	1.17(0.41-3.36)
P for trend [d]		0.09	0.12	0.91		0.25	0.40	0.59
Prudent vegetable pattern								
Q1	46/132	1.00	1.00	1.00	41/132	1.00	1.00	1.00
Q2	54/132	1.21(0.79-1.85)	1.35(0.78-2.34)	0.97(0.47-2.01)	45/132	1.09(0.69-1.73)	1.18(0.65-2.14)	0.90(0.41-1.98)
Q3	50/133	1.18(0.75-1.86)	1.16(0.63-2.13)	1.30(0.65-2.60)	40/133	0.82(0.49-1.36)	1.04(0.55-1.97)	0.59(0.25-1.42)
Q4	48/131	1.12(0.69-1.84)	1.02(0.52-1.99)	1.28(0.58-2.83)	42/132	1.03(0.61-1.75)	0.96(0.47-1.96)	1.00(0.42-2.40)
P for trend [d]		0.62	0.83	0.19		0.90	0.60	0.92
High sugar pattern								
Q1	42/131	1.00	1.00	1.00	30.132	1.00	1.00	1.00
Q2	54/132	1.07(0.70-1.63)	0.96(0.54-1.68)	1.30(0.64-2.65)	48/132	1.25(0.77-2.04)	1.21(0.62-2.36)	2.12(0.87-5.14)
Q3	54/133	1.09(0.69-1.73)	0.94(0.51-1.73)	1.44(0.67-3.07)	50/133	1.64(0.98-2.75)	1.35(0.66-2.78)	2.49(1.02-6.10)
Q4	48/132	1.02(0.62-1.69)	0.99(0.52-1.89)	1.49(0.61-3.63)	40/132	1.27(0.72-2.25)	1.16(0.54-2.47)	1.68(0.55-5.08)
P for trend [d]		0.89	0.90	0.11		0.52	0.56	0.64

[a] Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; CI, confidence interval;

[b] Events are defined as death/recurrence/metastasis (which occurred earliest) for disease-free survival and deaths for overall survival.

[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, marital status, family history, reported screening procedure, reported chemoradiotherapy and MSI status, where appropriate.

[d] Two-sided p value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

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Table 4. Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics [a]

	No. of Events[b] /No. at Risk	Q1	Q2 [c]	Q3	Q4	P for Trend [d]	P for Interaction [e]
Processed meat pattern							
Sex							
Female	65/210	1.00	2.20(0.99-4.91)	2.38(0.97-5.85)	3.85(1.49-9.99)	0.03	
Male	133/318	1.00	1.20(0.66-2.18)	1.23(0.69-2.17)	1.22(0.64-2.32)	0.27	0.04
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.96(1.05-3.67)	2.13(1.11-4.11)	2.03(0.96-4.30)	0.42	
≥24.9 MET h/wk	101/264	1.00	1.22(0.59-2.55)	1.27(0.62-2.62)	1.64(0.74-3.62)	0.01	0.64
BRAF mutation status							
Wild type	163/425	1.00	1.28(0.77-2.12)	1.41(0.80-2.34)	1.80(1.01-3.21)	0.009	
V600E mutant	17/49	1.00	1.82(0.40-8.34)	0.54(0.10-2.83)	0.79(0.09-7.01)	0.50	0.80
Prudent vegetables pattern							
Sex							
Female	65/210	1.00	1.57(0.59-4.20)	1.55(0.63-3.85)	1.22(0.46-3.24)	0.71	
Male	133/318	1.00	1.25(0.76-2.04)	1.08(0.62-1.88)	1.14(0.62-2.09)	0.67	0.65
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.48(0.80-2.76)	1.52(0.81-2.87)	1.22(0.56-2.69)	0.66	
≥24.9 MET h/wk	101/264	1.00	1.02(0.55-1.89)	1.02(0.53-1.96)	1.05(0.55-2.04)	0.03	0.83
BRAF mutation status							
Wild type	163/425	1.00	1.32(0.83-2.10)	1.29(0.80-2.08)	1.19(0.70-2.02)	0.58	
V600E mutant	17/49	1.00	2.50(0.38-16.59)	0.88(0.06-12.99)	1.24(0.12-13.20)	0.73	0.80
High sugar pattern							
Sex							
Female	65/210	1.00	1.41(0.63-3.16)	0.88(0.36-2.15)	0.82(0.30-2.27)	0.42	
Male	133/318	1.00	1.14(0.67-1.97)	1.34(0.75-2.39)	1.39(0.73-2.66)	0.06	0.72
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.01(0.55-1.86)	1.10(0.56-2.16)	1.19(0.56-2.54)	0.06	
≥24.9 MET h/wk	101/264	1.00	1.36(0.70-2.65)	1.21(0.60-2.45)	1.04(0.49-2.22)	0.86	0.26
BRAF mutation status							
Wild type	163/425	1.00	0.99(0.61-1.59)	1.20(0.71-2.01)	1.03(0.59-1.82)	0.70	
V600E mutant	17/49	1.00	0.53(0.07-4.25)	0.27(0.04-1.66)	0.32(0.04-2.64)	0.09	0.33

[a] Abbreviations are as follows: CI, confidence interval; METs/week, metabolic equivalent hours per week;

[b] Events are defined as death/recurrence/metastasis (which occurred earliest) for disease-free survival and deaths for overall survival.

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[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening procedure, reported chemoradiotherapy, and MSI status, where appropriate.

[d] Two-sided *p* value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

[e] P for interaction is the significance of interaction term between smoking and respective stratification variable, calculated from Wald test.

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REFERENCES

1. Canadian Cancer Statistics 2012. Toronto, ON: Canadian Cancer Society's Steering Committee on Cancer Statistics.

Canadian Cancer Society., 2012.

2. Norat T, Chan D, Lau R, Aune D, Vieira R, Greenwood D, et al. WCRF/AICR Systematic Literature Review Continuous Update Project Report. *The Associations between Food, Nutrition and Physical Activity and the Risk of Colorectal Cancer*: American Institute for Cancer Research, 2010.

3. Dixon LB, Balder HF, Virtanen MJ, Rashidkhani B, Mannisto S, Krogh V, et al. Dietary patterns associated with colon and rectal cancer: results from the Dietary Patterns and Cancer (DIETSCAN) Project. *Am J Clin Nutr* 2004;80(4):1003-11.

4. Terry P, Hu FB, Hansen H, Wolk A. Prospective study of major dietary patterns and colorectal cancer risk in women. *Am J Epidemiol* 2001;154(12):1143-9.

5. Kwan ML, Weltzien E, Kushi LH, Castillo A, Slattery ML, Caan BJ. Dietary patterns and breast cancer recurrence and survival among women with early-stage breast cancer. *J Clin Oncol* 2009;27(6):919-26.

6. Kim MK, Sasaki S, Otani T, Tsugane S. Dietary patterns and subsequent colorectal cancer risk by subsite: a prospective cohort study. *Int J Cancer* 2005;115(5):790-8.

7. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. Eating patterns and risk of colon cancer. *Am J Epidemiol* 1998;148(1):4-16.

8. Williams CD, Satia JA, Adair LS, Stevens J, Galanko J, Keku TO, et al. Dietary patterns, food groups, and rectal cancer risk in Whites and African-Americans. *Cancer Epidemiol Biomarkers Prev* 2009;18(5):1552-61.

9. Meyerhardt JA, Niedzwiecki D, Hollis D, Saltz LB, Hu FB, Mayer RJ, et al. Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *Jama* 2007;298(7):754-64.

10. Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, et al. Major dietary patterns and the risk of colorectal cancer in women. *Arch Intern Med* 2003;163(3):309-14.

11. Squires J, Roebothan B, Buehler S, Sun Z, Cotterchio M, Younghusband B, et al. Pickled meat consumption and colorectal cancer (CRC): a case-control study in Newfoundland and Labrador, Canada. *Cancer Causes Control* 2010;21(9):1513-21.

12. Sun Z, Zhu Y, Wang PP, Roebothan B, Zhao J, Zhao J, et al. 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. *Anticancer Res* 2012;32(2):687-96.

13. Sun Z, Liu L, Wang PP, Roebothan B, Zhao J, Dicks E, et al. Association of total energy intake and macronutrient consumption with colorectal cancer risk: results from a large population-based case-control study in Newfoundland and Labrador and Ontario, Canada. *Nutr J* 2012;11(1):18.

14. Woods MO, Younghusband HB, Parfrey PS, Gallinger S, McLaughlin J, Dicks E, et al. The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. *Gut* 2010;59(10):1369-77.

15. Green RC, Green JS, Buehler SK, Robb JD, Daftary D, Gallinger S, et al. Very high incidence of familial colorectal cancer in Newfoundland: a comparison with Ontario and 13 other

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population-based studies. *Fam Cancer* 2007;6(1):53-62.

16. Stram DO, Hankin JH, Wilkens LR, Pike MC, Monroe KR, Park S, et al. Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. *Am J Epidemiol* 2000;151(4):358-70.

17. Sharma S, Iwasaki M, Kunieda C, Cao X, Ishihara J, Hamada G, et al. Development of a quantitative food frequency questionnaire for assessing food, nutrient, and heterocyclic aromatic amines intake in Japanese Brazilians for a colorectal adenoma case-control study. *Int J Food Sci Nutr* 2009;60 Suppl 7:128-39.

18. Jain MG, Rohan TE, Soskolne CL, Kreiger N. Calibration of the dietary questionnaire for the Canadian Study of Diet, Lifestyle and Health cohort. *Public Health Nutr* 2003;6(1):79-86.

19. Hankin JH, Wilkens LR, Kolonel LN, Yoshizawa CN. Validation of a quantitative diet history method in Hawaii. *Am J Epidemiol* 1991;133(6):616-28.

20. Sun Z, Zhu Y, Wang PP, Roebothan B, Zhao J, Dicks E, et al. 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. *Anticancer Res* 2012;32(2):687-96.

21. Dr. H. Bliss Murphy Cancer Care Foundation. From http://www.cancercarefoundation.nl.ca/ (accessed July 18, 2012).

22. MANUAL of the international statistical classification of diseases, injuries, and causes of death. Addendum 1. Supplementary interpretations and instructions for coding causes of death. *Bull World Health Org Suppl* 1953;7(Suppl 6):1-55.

23. Raptis S, Mrkonjic M, Green RC, Pethe VV, Monga N, Chan YM, et al. MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. *J Natl Cancer Inst* 2007;99(6):463-74.

24. Campbell PT, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, et al. Case-Control Study of Overweight, Obesity, and Colorectal Cancer Risk, Overall and by Tumor Microsatellite Instability Status. *J Natl Cancer Inst* 2010;102(6):391-400.

25. Loughrey MB, Waring PM, Tan A, Trivett M, Kovalenko S, Beshay V, et al. Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer. *Fam Cancer* 2007;6(3):301-10.

26. Phipps AI, Baron J, Newcomb PA. Prediagnostic smoking history, alcohol consumption, and colorectal cancer survival: the Seattle Colon Cancer Family Registry. *Cancer* 2010;117(21):4948-57.

27. Hile SE, Shabashev S, Eckert KA. Tumor-Specific Microsatellite Instability: Do Distinct Mechanisms Underlie the MSI-L and EMAST Phenotypes? *Mutat Res* 2012.

28. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. *Stat Methods Med Res* 1992;1(1):69-95.

29. Heidemann C, Schulze MB, Franco OH, van Dam RM, Mantzoros CS, Hu FB. Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. *Circulation* 2008;118(3):230-7.

30. Introduction to SAS, UCLA: Academic Technology Services, Statistical Consulting Group. From http://www.ats.ucla.edu/stat/examples/asa/test_proportionality.htm (accessed June 4, 2012).

31. Slattery ML, French TK, Egger MJ, Lyon JL. Diet and survival of patients with colon cancer in Utah: is there an association? *Int J Epidemiol* 1989;18(4):792-7.

23

32. Dray X, Boutron-Ruault MC, Bertrais S, Sapinho D, Benhamiche-Bouvier AM, Faivre J. Influence of dietary factors on colorectal cancer survival. *Gut* 2003;52(6):868-73.

33. Dolecek TA, McCarthy BJ, Joslin CE, Peterson CE, Kim S, Freels SA, et al. Prediagnosis food patterns are associated with length of survival from epithelial ovarian cancer. *J Am Diet Assoc* 2010;110(3):369-82.

34. Bingham SA, Pignatelli B, J.R. P, A. E, C. M, G. G, et al. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 1996;17(no.3):515-523.

35. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen* 2004;44(1):44-55.

36. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. *J Natl Cancer Inst.* 2002 94(13):972-80.

37. Rabizadeh S, Sears C. New horizons for the infectious diseases specialist: how gut microflora promote health and disease. *Curr Infect Dis Rep* 2008;10(2):92-8.

38. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. *Genome Med* 2011;3(3):14.

39. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. *Nat Rev Microbiol* 2012.

40. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005;307(5717):1915-20.

41. Takachi R TY, Baba K, Inoue M, Sasazuki S, Iwasaki M, Tsugane S, Japan Public Health Center-Based Prospective Study Group. Red meat intake may increase the risk of colon cancer in Japanese, a population with relatively low red meat consumption. *Asia Pac J Clin Nutr.* 2011;20(4):603-12.

42. Safaee Ardekani G, Jafarnejad SM, Tan L, Saeedi A, Li G. The Prognostic Value of BRAF Mutation in Colorectal Cancer and Melanoma: A Systematic Review and Meta-Analysis. *PLoS One* 2012;7(10):e47054.

43. Bingham SA PB, Pollock JR, Ellul A, Malaveille C, Gross G, Runswick S, Cummings JH, O'Neill IK. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 1996;17(no.3):515-523.

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Dietary Patterns and Colorectal Cancer Recurrence and Survival

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ABSTRACT

Objective: To examine the association between dietary patterns and colorectal cancer (CRC) survival.

Design: Cohort study

Setting and participants: Five hundred and twenty nine newly diagnosed CRC patients from the Newfoundland Familial Colorectal Cancer Registry (NFCCR) were recruited and followed until April, 2010.

Outcome measure: Participants reported their dietary intake using a food frequency questionnaire. Dietary patterns were identified with factor analysis. Multivariable Cox proportional hazards models were employed to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association of dietary patterns with CRC recurrence and death from all-causes, after controlling for covariates.

Results: Disease-free survival (DFS) among CRC patients was significantly worsened among patients with a high processed meat dietary pattern (the highest versus the lowest quartile HR: 1.82, 95% CI: 1.07-3.09). No associations were observed with the prudent vegetable or the high sugar patterns and DFS. The association between the processed meat pattern and DFS was restricted to patients diagnosed with colon cancer (the highest versus the lowest quartile: HR: 2.29, 95% CI: 1.19-4.40) while the relationship between overall survival (OS) and this pattern was observed among patients with colon cancer only (the highest versus the lowest quartile: HR: 2.13, 95%CI: 1.03-4.43). Potential effect modification was noted for sex (p for interaction=0.04, HR: 3.85 for women and 1.22 for men).

Conclusion

The processed meat dietary pattern prior to diagnosis is associated with higher risk of

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tumor recurrence, metastasis, and death ~~from any cause~~ among patients with

colorectal cancer.

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ARTICLE SUMMARY

Article Focus

- We used the data of 529 colorectal cancer patients in Newfoundland and Labrador to investigate the association of dietary patterns and colorectal cancer survival.

- We further explored if the relationship between dietary pattern and colorectal cancer survival is modified by sex, physical activity and *BRAF* mutation.

Key Messages

- The processed meat dietary pattern is associated with a worsened colorectal cancer disease-free survival.

- The prudent vegetable or the high sugar patterns show no association with disease-free survival.

- The relationship between processed meat pattern and colorectal cancer survival is modified by sex.

Strengths and limitations of this study

- The sample size is reasonably large with detailed information on diet, lifestyle and molecular characteristics.

- Recall bias remains a problem since the food consumption was collected from one year prior to CRC diagnosis. In addition, dietary patterns only reflect food consumption before diagnosis which might be modified after diagnosis.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of cancer death in Canada.[1] Epidemiological studies have established a strong link between few dietary factors, such as fiber (inversely) and red/processed meat (increases risk), and the risk of developing CRC, [2] although most studies have focused primarily on individual foods or nutrients. Since foods and nutrients act synergistically rather than in isolation,[3-6] recent research has investigated the role of dietary patterns on CRC incidence. Dietary patterns identified in prior research often include the "Western" and "prudent" patterns. Adherence to the Western diet pattern, characterized by high intakes of meat, fat, sweets and desserts, is often associated with increased risk of CRC.[5-9] Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse[7 8] or null[5 6 10] association with CRC risk. ~~However, the impact of dietary patterns on CRC survival remains largely unknown.~~

The highest CRC incidence and mortality rates in Canada are observed in the province of Newfoundland and Labrador (NL).[1] Geographically isolated in the Atlantic Ocean, NL has long maintained its traditional foods, a Western-style diet consisting of a large proportion of processed meat, red meat and insufficient vegetables.[11] Several studies have partially attributed the high CRC incidence rate in NL to its unique diet,[11-13] but no study has explored the association between the NL diet and its impact on survival among CRC patients.

This prospective cohort study investigated the influence of dietary patterns, identified by factor analysis, on survival and recurrence or metastasis among an incident case series of 529 CRC patients from NL. Additionally, the present study evaluated the possible effect modification among dietary patterns with gender,

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physical activity and tumor molecular phenotype.

SUBJECTS AND METHODS

Study participants

Patients in this prospective cohort study were enrolled through the Newfoundland Familial Colorectal Cancer Registry (NFCCR), described in detail elsewhere.[14 15] In brief, during the time period from 1999 to 2003, patients aged 20-75 years, newly diagnosed with pathologically confirmed, invasive CRC were eligible for inclusion in the study (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).

Written, informed consent was required from each study participant to access their archived tumor tissue and medical records. If patients died before they could give consent (the median time from date of diagnosis to date of consent was 1.8 years), a close relative/proxy, who has lived with the patient, was invited to participate. Enrolling deceased cases through proxies could remove the potential bias of eliminating patients at a late distant stage.[14] Thus, the inception cohort consisted of 750 eligible patients (64%).

Consenting participants completed and returned a detailed food frequency questionnaire (FFQ), personal history questionnaire (PHQ) and family history questionnaire (FHQ). All questionnaires were self-completed. Assistance from study staff was available to help with understanding items on the questionnaires. To capture additional cancer diagnosis or recurrence in the family after enrollment, the FHQ was distributed to participants for the second time midway through the follow-up. To be included in this analysis, patients had to have completed at least the FFQ, provided informative lifestyle and medical data from the PHQ, and had known vital status

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information by the end of the follow-up period (April, 2010). For patients who died prior to enrollment, the designated relative/proxy completed the aforementioned questionnaires. The final analytical cohort comprised 529 eligible participants. The study protocol was approved by the Human Investigation Committee of Memorial University of Newfoundland.

Dietary Assessment and Food Grouping

Diet was assessed using a semi-quantitative FFQ, developed from the well-known Hawaii FFQ,[16] on the basis of a validated instrument adapted for the Canadian population. [17][18] The FFQ included 170 foods, beverages, and vitamin- and dietary-supplements.[19] Foods indigenous to the Newfoundland population (e.g., salted/pickled meat and smoked/pickled fish) were also included. For each food item or beverage, participants were asked to estimate their frequency of consumption and usual portion size as 'Small', 'Regular' or 'Large' one year prior to their colon or rectal cancer diagnosis. Portion sizes for specific food were depicted in photographs. Nutrient and total energy intakes were calculated by multiplying the frequency of consumption of each food by the nutrient content of the portion size based on the composition values from the 2005 Canadian Nutrient file.[20] Taking a similar grouping scheme to that used elsewhere,[3] we collapsed individual food items on the FFQ into 39 predefined food groups based on the roles of food in diet and cancer etiology. Distinct food items were reserved as individual categories if it was deemed inappropriate to combine them (e.g., jam, pies, beer, and wine).

Covariates

Sociodemographic data, such as age, sex, marital status, and education attainment,

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were gathered by the self-administered PHQ. The PHQ also included items regarding medical history, bowel screening history, physical activity, reproductive factors (female only), and alcohol and tobacco use. Family history of cancer was assessed by the FHQ.

Study Outcomes

Study outcomes were ascertained from follow-up questionnaires, local newspapers (e.g.,death notices), death certificates, autopsy, pathology, radiology, surgical reports, as well as physician's notes. Additional data were gathered from the Dr. H. Bliss Murphy Cancer Care Foundation and Statistics Canada.[21] The cause of death was obtained for 93 of 168 deceased patients in this cohort, classified according to the International Classification of Disease (ICD) codes for underlying or contributing cause of death;[22] the majority (91%) of these had died from CRC. Since specific cause of death was not available for all deceased participants, all-cause mortality was used for analysis. In this study, two end points were considered: the first was disease-free survival (DFS), defined as time from cancer diagnosis to the first confirmed tumor recurrence, metastasis, or death from all causes occurring up to April, 2010; the second end point was overall survival (OS), measured from the date of cancer diagnosis to the date of death from all causes. Patients who did not have an event by the end of the follow-up were censored at the date of last contact.

Molecular Assessment

The *p.V600E BRAF* mutation and MSI status for the tumor DNA have been determined in previous studies using standard protocols.[23-25] Briefly, the mutational hotspot c.1799T>A. (p.Val600Glu) in the *BRAF* gene was detected using *BRAF*

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V600E allele-specific primers, with controls amplifying the GAPDH gene.[25] Positive

mutations were then verified by direct automatic sequencing.[25]   For MSI analyses, a

panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4, D5S346,

D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify both tumor

and normal DNA.[23][24] MSI status was defined as MSI-High if 30% or more of the

markers were unstable and MS-Stable/MSI-Low if less than 30% of the markers

showed instability.[26][27]The primer sequences and PCR conditions are provided in

detail in earlier studies from this cohort.[14][23-25]

Statistical Analysis

    Exploratory principal component factor analysis [28] was used to identify major

dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation

(orthogonal) procedure was applied to rotate these factors, meaning that it produces

uncorrelated, easy interpreted components that explain the greatest amount of

variance in the original food groups.[29] We determined the number of factors to retain

for interpretation on the basis of criteria as follows: factor eigenvalue greater than

1.15, the scree plot, the proportion of variance explained, and factor interpretability.[9]

Patterns were labeled based on food groups with absolute rotated factor loading

matrix greater than or equal to 0.50. Each participant was assigned a factor score for

each pattern (factor) by summing the intakes from each food group multiplied by

optimal weights (factor loadings).[5] Individuals with a higher factor score had a closer

adherence to that pattern.[5]

    Comparisons for baseline characteristics across quartiles of dietary patterns were

performed using ANOVA test for continuous variables and Chi-Square test for

categorical variables. Cox proportional hazards models, each adjusting for energy

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intake and critical covariates, were used to evaluate the association between individual dietary pattern and CRC recurrence and mortality, represented by hazard ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed by the log-rank test in a univariate setting; those with the p-value less than 0.1 were considered for inclusion. The final models only retained the items that entered the models at p<0.1 or altered the effect estimates by 10% or more; these include ~~energy intake,~~ sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening procedure, reported chemoradiotherapy, and MSI status. <u>All models were run with the adjustment for total energy intake by including total calories in the model.</u> The assumption of proportional hazard rates was verified by checking the parallelism of the Kaplan-Meier curves and by including time-dependent covariates in the models to test for statistical significance.[30] Statistical linear trend was examined by modeling the median value of each quartile as an ordinal variable in a linear regression.[5] Potential interactions were evaluated by comparing estimates from stratified analyses and testing significance of interaction terms with a Wald test.[5]

A sensitivity analysis was implemented by eliminating stage-advanced patients enrolled through proxies and re-calculating survival time from the completion of the first questionnaire to a predefined event, in order to determine whether associations might vary with the exclusion of stage-advanced cancer. Statistical significance was accepted for two-sided p < 0.05. All data management and analyses were performed with SAS software version 9.2 (SAS Institute Inc, Cary, NC).

RESULTS

The cohort was followed for a median of 6.4 years (minimum: 1.3 years; maximum: 10.9 years). A total of 168 patients died from all causes and 30 had a

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cancer recurrence or metastasis by the end of study follow-up (April, 2010).

Dietary Patterns

Three distinct dietary patterns, labeled "processed meat pattern", "prudent vegetable pattern" and "high sugar pattern", were extracted using the aforementioned factor analysis procedure. These patterns explained 73.82% of total variance in the original 39 food groups (Table 1). A higher factor loading matrix of a given food group is representative of a greater contribution of that food group on that specific pattern. Therefore, the first pattern, termed "processed meat", was characterized by higher loadings and thus higher consumptions of cured/processed meat, cured/processed red meat, red meat, fish, and processed fish; the second pattern, labeled "prudent vegetable", displayed higher loadings on other greens, other fruit, other vegetables, and tomato sauce; and the third pattern, named "high sugar", showed higher loadings on desserts and sweets, pies and tarts.

Baseline Characteristics by quartiles of dietary patterns

Higher processed meat pattern scores at baseline were detected in men, ever smokers, patients who were single and individuals who had higher BMI at the time of diagnosis (Table 2). Higher prudent vegetable pattern scores were observed in women, never smokers, those with a slightly later age of diagnosis and with patients who had a tumor harboring the *p.V600E BRAF* mutation. None of these characteristics varied significantly by quartiles of high sugar pattern scores.

Dietary Patterns and Cancer Recurrence or Death

The highest quartile of processed meat pattern was significantly associated with

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poorer DFS after the adjustment for other predictors of CRC recurrence and death (HR: 1.82, 95% CI: 1.07-3.09), although no overall trend was observed in the HRs across the whole distribution of factor scores (p for trend=0.09) (Table 3). Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was observed to be significantly associated with predefined patient outcomes (i.e., DFS and OS).

When stratified by tumor site, however, the association between processed meat pattern and DFS remained statistically significant only for patients who had tumors located in the colon (the highest versus the lowest quartile, HR: 2.29, 95% CI: 1.19-4.40) and not the rectum (HR: 0.97, 95% CI: 0.38-2.45). Similarly, when OS was the outcome, the positive association between increasing consumption of the processed meat pattern and mortality was restricted to patients whose tumors were diagnosed in the colon (the forth versus first quartiles: HR: 2.13, 95% CI: 1.03-4.43).

In the stratified analyses for dietary patterns by sex, physical activity, and *BRAF* mutation status, there was evidence for effect modification by sex (p=0.04) for the association of processed meat pattern with DFS (HR: 3.85 for women and 1.22 for men) (Table 4). However, no evidence was observed to suggest that the effects of other dietary patterns on cancer recurrence or death were modified by sex, physical activity, or *BRAF* mutation status and MSI (data not shown).

In the sensitivity analysis, when advanced-stage casespatients who died before admittance were excluded, the association between processed meat pattern and survival among CRC patients remained significant.

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DISCUSSION

Three dietary patterns, termed "processed meat pattern", "prudent vegetable

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pattern" and "high sugar pattern", were generated in this cohort study. We found that high conformity with the processed meat pattern, characterized by high intakes of processed meat, red meat, fish, and processed fish, is associated with decreased DFS of CRC, specifically of colon cancer. The differential associations by subsite indicate disease heterogeneity. On the contrary, increasing consumption of the prudent vegetable pattern and the high sugar pattern displayed no clear relationships with mortality or recurrence.

The processed meat pattern in the present study shares most characteristics of the Western diet referred to in previous studies on CRC risk, which indicates a positive association between the Western dietary pattern and CRC risk.[7][9] However, there has been minimal research examining the association between dietary factors (e.g., nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients;[31][32] moreover, our literature review identified only one study that investigated the relationship between dietary patterns and survival among CRC patients. Consistent with our results, that prospective cohort study of 1009 stage III colon cancer patients [9] reported a deleterious disease-free colon cancer prognosis for patients reporting high levels of the Western dietary pattern intake.

The mechanisms explaining the impact of red and processed meat on CRC mortality are still unclear; however, some biologic mechanisms that link diet factors to CRC risk may continue after diagnosis and subsequently impact cancer progression and survival.[33] For example, strong carcinogens such as *N*-nitroso compounds (NOCs) and probable carcinogenic mutagens like heterocyclic amines (HCA) and polycylic aromatic hydrocarbons (PAH), which have been suggested as significant contributors for CRC development,[34][35] are found in smoked, fried or high-temperature cooked meat. Sandhu et al[36] reported a Western dietary pattern is related to high levels of

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serum insulin and insulin-like growth factors (IGF), and these hormones are found to be associated with tumor growth and the inhibition of apoptosis.   In addition, a growing body of evidence suggests that disruption of the normal gut microflora is associated with human disease, including the pathogenesis of the intestinal tract (e.g. inflammatory bowel disease) and other diseases such as obesity, cardiovascular disease, and autoimmune conditions. [37][38] Alterations in intestinal microbiota are also strongly associated with colonic polyp formation and with the risk of developing CRC.[39] Given the major role of diet on the intestinal microbiome, [40] our findings between dietary patterns and CRC survival may also be explained by the impact of dietary patterns on gut microflora and health outcomes.

The influence of processed meat pattern on survival was evident among women rather than men in our study. Previous studies revealed that the higher colon pH and longer intestine transit time in women compared to men can influence the production of secondary bile acid or NOCs,[41] resulting in gender differences in the CRC development. This is the first study that considered effect modifications between dietary patterns and tumor molecular phenotype (i.e. *BRAF* mutation) on CRC survival. *BRAF* mutation is found to be significantly associated with poor CRC survival;[42] however, whether it can modify the impacts of dietary factors on CRC survival is not known. Although stratified analyses in our study demonstrated a processed meat diet to significantly decrease survival time only in patients with *BRAF* widewild type tumor, no evident interactions were detected. Further research is clearly warranted to verify these findings and to determine the biologic pathways that rationalize the underlying interactions between diet and tumor molecular features.

A reasonably large sample size with detailed information of patients is a merit of our study. These data not only includes demographic and personal lifestyle

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information, but also some molecular characteristics obtained from genetic testing. The ample information enables us to perform stratification analysis to control and assess effect modifiers and confounders.

Several limitations of this study should be recognized. Firstly, the results may be skewed by recall bias since the participants recalled their food consumption from one year prior to CRC diagnosis; however, this non-differential misclassification is only expected to bias the results towards the null. Secondly, dietary patterns in this study only reflect food consumption before diagnosis; it is unknown whether participants modified their diet post diagnosis. Since previous research has shown minimal change in diet between pre- and post- diagnosis among cancer patients,[32] the current study did not examine dietary changes before and after diagnosis. Moreover, immortal person-time bias may impact results. However, this is minimized by using proxies to enroll deceased patients.

In summary, we found that high conformity to the processed meat pattern is significantly associated with an increased risk of all-cause mortality and recurrence of CRC. Though our study did not find a difference in effect by tumor molecular phenotype, larger molecular studies should be conducted to examine if such differences exist. Ultimately, confirmation of these findings and the underlying mechanisms await further studies. Our observation not only underlines the importance of maintaining a healthy diet, but also provides guidance to efficacious dietary interventions;[8] that is, people may lower their risk of CRC mortality by reducing consumption of a processed meat pattern diet.

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Ethics approval Human Investigation Committee in Memorial University of
Newfoundland.

Data sharing statement No additional data available.

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Table 1. Factor Loadings and Explained Variances (VAR) for the Three Major Dietary Patterns Identified from the Food Frequency Questionnaire at baseline using a Principal Component Factor Analysis, Newfoundland

Food Groups	Processed Meat Pattern	Prudent Vegetable Pattern	High Sugar Pattern
Milk	-	0.19	-
Yogurt	-	0.31	-
Sugar	-	-0.19	0.20
Tea	-	-	0.17
Coffee	0.17	-	-
Soft drinks	0.19	-	-
Cheese	0.15	0.21	-
Egg	0.21	-	0.16
Mixed dishes	0.31	0.17	0.23
Red meat	**0.69**	-	0.17
Cured/processed red meat	**0.73**	-	0.21
Cured/processed meat	**0.93**	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	**0.58**	0.32	-0.22
Processed fish	**0.50**	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	**0.54**	-
Other fruit	-	**0.59**	-
Other greens	-	**0.60**	-0.22
Tomato sauce	-	**0.50**	-
Other vegetables	0.22	**0.54**	-
Beans, peas	0.15	0.25	-
Pickled vegetables	0.15	0.26	0.15
Total cereals and grains	0.23	0.38	0.28
Whole grains	-	0.33	-
Citrus	-	0.34	-
Berries	-	0.45	-
Dried fruit	-	0.39	-
Vegetable juice	-	0.17	-
Beer	0.19	-	-

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White wine	-	-	-
Red wine	-	-	-
Liquor	-	-	-
Desserts and sweets	0.31	-	**0.63**
Pies, tarts	0.15	-	**0.54**
Canned fruit	-	0.21	0.23
Jam, jelly	-	-	0.26
Proportion of VAR explained (%)	39.79	22.93	11.10
Cumulative VAR explained (%)	39.79	62.72	73.82

Absolute loading values <0.15 were not listed for simplicity.
Those with loadings of 0.50 or greater are in bold.

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Table2. Baseline Characteristics of 529 CRC Patients by Quartiles of the Three Major Dietary Patterns [a]

	Processed Meat Pattern				P Value[c]	Prudent Vegetable Pattern				P Value[c]	High Sugar Pattern				P Value[c]
	Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)		Q1 (n=132)	Q2 (n=132)	Q3 (n=133)	Q4 (n=132)	
Age at diagnosis[b]	61.4±8.7	60.6±9.0	60.2±8.8	59.3±9.3	0.29	57.4±10.3	60.1±7.9	61.0±9.0	62.1±8.0	<.0001	59.5±9.3	60.2±9.1	60.0±8.8	61.7±8.6	0.21
Sex[b]															
Female	67(50.8)	66(50.0)	39(29.3)	39(29.6)		38(28.8)	39(29.5)	58(43.6)	76(57.6)		60(45.5)	49(37.1)	51(38.3)	51(38.6)	
Male	65(49.2)	66(50.0)	94(70.7)	93(70.5)	<.0001	94(71.2)	93(70.5)	75(56.4)	56(42.4)	<.0001	72(54.5)	83(62.9)	82(61.7)	81(61.4)	0.50
Stage at diagnosis															
I/II	87(65.9)	81(61.4)	70(52.6)	71(53.8)		72(54.5)	71(53.8)	83(62.4)	83(62.9)		79(59.8)	77(58.3)	77(57.9)	76(57.6)	
III/IV	45(34.1)	51(38.6)	63(47.4)	61(46.2)	0.09	60(45.5)	61(46.2)	50(37.6)	49(37.1)	0.27	53(40.2)	55(41.7)	56(42.1)	56(42.4)	0.98
BMI (kg/m²)															
<25.0	38(30.6)	47(36.1)	35(26.5)	27(21.1)		42(33.6)	32(24.8)	34(26.4)	38(29.7)		33(25.6)	40(31.0)	36(28.1)	38(29.7)	
25.0-29.9	57(46.0)	52(40.0)	53(40.2)	53(41.4)		45(35.2)	57(44.2)	55(42.6)	58(45.3)		55(42.6)	47(36.4)	58(45.3)	55(43.0)	
≥30	29(23.4)	31(23.9)	44(33.3)	48(37.5)	0.03	40(31.2)	40(31.0)	40(31.0)	32(25.0)	0.78	41(31.8)	42(32.6)	34(26.6)	35(27.3)	0.63
Physical activity															
<24.9 MET h/wk	73(55.3)	71(53.4)	56(42.1)	65(49.2)		68(51.5)	60(45.4)	69(51.9)	68(51.5)		68(51.5)	71(53.8)	69(51.9)	57(43.2)	
≥24.9 MET h/wk	59(44.7)	61(46.6)	77(57.9)	67(50.8)	0.13	64(48.5)	72(54.6)	64(48.1)	64(48.5)	0.67	64(48.5)	61(46.2)	64(48.1)	75(56.8)	0.32
Marital status															
Single	31(23.5)	29(22.0)	18(13.5)	37(28.0)		26(19.7)	27(20.4)	27(20.3)	35(26.5)		26(19.7)	30(22.7)	30(22.6)	29(22.0)	
Married or living as married	101(76.5)	103(78.0)	115(86.5)	95(72.0)	0.04	106(80.3)	105(79.6)	106(79.7)	97(73.5)	0.50	106(80.3)	102(77.3)	103(77.4)	103(78.0)	0.93
Smoking status															
Ever	77(58.3)	94(71.2)	113(85.0)	104(78.8)		108(81.8)	97(73.5)	100(75.2)	83(62.9)		101(76.5)	95(72.0)	95(71.4)	97(73.5)	
Never	55(41.7)	38(28.8)	20(15.0)	28(21.2)	<.0001	24(18.2)	35(26.5)	33(24.8)	49(37.1)	0.006	31(23.5)	37(28.0)	38(28.6)	35(26.5)	0.79
Tumor location															
Colon	91(69.5)	90(68.2)	85(63.9)	79(59.9)		75(56.8)	91(69.5)	87(65.4)	92(69.7)		82(62.1)	85(64.9)	87(65.4)	91(68.9)	
Rectum	40(30.5)	42(31.8)	48(36.1)	53(40.1)	0.34	57(43.2)	40(30.5)	46(34.6)	40(30.3)	0.10	50(37.9)	46(35.1)	46(34.6)	41(31.1)	0.71
Reported chemoradiotherapy															
Yes	36(27.3)	31(23.5)	20(15.0)	21(15.9)		24(18.2)	23(17.4)	24(18.1)	37(28.0)		30(22.7)	28(21.2)	25(18.8)	25(18.9)	
No	96(72.7)	101(76.5)	113(85.0)	111(84.1)	0.04	108(81.8)	109(82.6)	109(81.9)	95(72.0)	0.10	102(77.3)	104(78.8)	108(81.2)	107(81.1)	0.83
MSI status															
MSS /MSI-L	108(86.4)	110(86.6)	113(91.9)	106(86.9)		107(85.6)	104(86.7)	113(91.1)	113(88.3)		107(84.9)	106(87.6)	110(88.0)	114(91.2)	
MSI-H	17(13.6)	17(13.4)	10(8.1)	16(13.1)	0.49	18(14.4)	16(13.3)	11(8.9)	15(11.7)	0.57	19(15.1)	15(12.4)	15(12.0)	11(8.8)	0.50
BRAF mutation status															
~~Wide~~Wild type	104(85.2)	107(89.9)	109(90.8)	106(93.0)		108(91.5)	103(87.3)	112(95.7)	103(84.4)		103(88.8)	110(91.7)	106(89.1)	107(89.2)	
V600E mutant	18(14.8)	12(10.1)	11(9.2)	8(7.0)	0.25	10(8.5)	15(12.7)	5(4.3)	19(15.6)	0.02	13(11.2)	10(8.3)	13(10.9)	13(10.8)	0.88

[a] Abbreviations are as follows: BMI, Body mass index; CRC, colorectal cancer; MSI, microsatellite instability; MSS/MSI-L, microsatellite stable/ microsatellite instability-low; MSI-H, microsatellite instability-high

[b] Continuous variables presented as mean±SD (standard deviation); categorical variables presented as number[43]

[c] P values are for the significance of the ANOVA test for continuous variables and of the Chi-Square test for categorical variables

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Table3. Hazard Rate Ratios Associated with Disease-Free and Overall Colorectal Cancer Survival for Quartiles of Dietary Patterns [a]

	Disease-Free Survival				Overall Survival			
	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]	No. of Events[b] /No. at Risk	Overall CRC HR (95% CI) [c]	Colon cancer HR (95% CI) [c]	Rectal cancer HR (95% CI) [c]
Processed meat pattern								
Q1	38/132	1.00	1.00	1.00	33/132	1.00	1.00	1.00
Q2	45/132	1.51(0.95-2.41)	1.69(0.97-2.96)	0.91(0.39-2.14)	40/132	1.47(0.89-2.44)	2.18*(1.16-4.09)	0.75(0.28-2.03)
Q3	58/132	1.56(0.97-2.49)	1.37(0.76-2.48)	1.72(0.85-3.95)	49/133	1.32(0.78-2.22)	1.44(0.74-2.79)	1.54(0.57-4.13)
Q4	57/132	1.82*(1.07-3.09)	2.29*(1.19-4.40)	0.97(0.38-2.45)	46/132	1.53(0.85-2.74)	2.13*(1.03-4.43)	1.17(0.41-3.36)
P for trend [d]		0.09	0.12	0.91		0.25	0.40	0.59
Prudent vegetable pattern								
Q1	46/132	1.00	1.00	1.00	41/132	1.00	1.00	1.00
Q2	54/132	1.21(0.79-1.85)	1.35(0.78-2.34)	0.97(0.47-2.01)	45/132	1.09(0.69-1.73)	1.18(0.65-2.14)	0.90(0.41-1.98)
Q3	50/133	1.18(0.75-1.86)	1.16(0.63-2.13)	1.30(0.65-2.60)	40/133	0.82(0.49-1.36)	1.04(0.55-1.97)	0.59(0.25-1.42)
Q4	48/131	1.12(0.69-1.84)	1.02(0.52-1.99)	1.28(0.58-2.83)	42/132	1.03(0.61-1.75)	0.96(0.47-1.96)	1.00(0.42-2.40)
P for trend [d]		0.62	0.83	0.19		0.90	0.60	0.92
High sugar pattern								
Q1	42/131	1.00	1.00	1.00	30.132	1.00	1.00	1.00
Q2	54/132	1.07(0.70-1.63)	0.96(0.54-1.68)	1.30(0.64-2.65)	48/132	1.25(0.77-2.04)	1.21(0.62-2.36)	2.12(0.87-5.14)
Q3	54/133	1.09(0.69-1.73)	0.94(0.51-1.73)	1.44(0.67-3.07)	50/133	1.64(0.98-2.75)	1.35(0.66-2.78)	2.49*(1.02-6.10)
Q4	48/132	1.02(0.62-1.69)	0.99(0.52-1.89)	1.49(0.61-3.63)	40/132	1.27(0.72-2.25)	1.16(0.54-2.47)	1.68(0.55-5.08)
P for trend [d]		0.89	0.90	0.11		0.52	0.56	0.64

[a] Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; CI, confidence interval;

[b] Events are defined as death/recurrence/metastasis (which occurred earliest) for disease-free survival and deaths for overall survival.

[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, marital status, family history, reported screening procedure, reported chemoradiotherapy and MSI status, where appropriate.

[d] Two-sided *p* value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

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Table 4. Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics [a]

	No. of Events[b] /No. at Risk	Quartiles HR (95% CI)[c]				P for Trend [d]	P for Interaction [e]
		Q1	Q2 [c]	Q3	Q4		
Processed meat pattern							
Sex							
Female	65/210	1.00	2.20(0.99-4.91)	2.38(0.97-5.85)	3.85*(1.49-9.99)	0.03	
Male	133/318	1.00	1.20(0.66-2.18)	1.23(0.69-2.17)	1.22(0.64-2.32)	0.27	0.04
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.96*(1.05-3.67)	2.13*(1.11-4.11)	2.03(0.96-4.30)	0.42	
≥24.9 MET h/wk	101/264	1.00	1.22(0.59-2.55)	1.27(0.62-2.62)	1.64(0.74-3.62)	0.01	0.64
BRAF mutation status							
~~Wide~~Wild type	163/425	1.00	1.28(0.77-2.12)	1.41(0.80-2.34)	1.80*(1.01-3.21)	0.009	
V600E mutant	17/49	1.00	1.82(0.40-8.34)	0.54(0.10-2.83)	0.79(0.09-7.01)	0.50	0.80
Prudent vegetables pattern							
Sex							
Female	65/210	1.00	1.57(0.59-4.20)	1.55(0.63-3.85)	1.22(0.46-3.24)	0.71	
Male	133/318	1.00	1.25(0.76-2.04)	1.08(0.62-1.88)	1.14(0.62-2.09)	0.67	0.65
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.48(0.80-2.76)	1.52(0.81-2.87)	1.22(0.56-2.69)	0.66	
≥24.9 MET h/wk	101/264	1.00	1.02(0.55-1.89)	1.02(0.53-1.96)	1.05(0.55-2.04)	0.03	0.83
BRAF mutation status							
~~Wide~~Wild type	163/425	1.00	1.32(0.83-2.10)	1.29(0.80-2.08)	1.19(0.70-2.02)	0.58	
V600E mutant	17/49	1.00	2.50(0.38-16.59)	0.88(0.06-12.99)	1.24(0.12-13.20)	0.73	0.80
High sugar pattern							
Sex							
Female	65/210	1.00	1.41(0.63-3.16)	0.88(0.36-2.15)	0.82(0.30-2.27)	0.42	
Male	133/318	1.00	1.14(0.67-1.97)	1.34(0.75-2.39)	1.39(0.73-2.66)	0.06	0.72
Physical activity							
<24.9 MET h/wk	97/263	1.00	1.01(0.55-1.86)	1.10(0.56-2.16)	1.19(0.56-2.54)	0.06	
≥24.9 MET h/wk	101/264	1.00	1.36(0.70-2.65)	1.21(0.60-2.45)	1.04(0.49-2.22)	0.86	0.26
BRAF mutation status							
~~Wide~~Wild type	163/425	1.00	0.99(0.61-1.59)	1.20(0.71-2.01)	1.03(0.59-1.82)	0.70	
V600E mutant	17/49	1.00	0.53(0.07-4.25)	0.27(0.04-1.66)	0.32(0.04-2.64)	0.09	0.33

[a] Abbreviations are as follows: CI, confidence interval; METs/week, metabolic equivalent hours per week;

[b] Events are defined as death/recurrence/metastasis (which occurred earliest) for disease-free survival and deaths for overall survival.

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[c] Cox proportional hazard model adjusted for total energy intake, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening procedure, reported chemoradiotherapy, and MSI status, where appropriate.

[d] Two-sided *p* value for test of linear trend was calculated by modeling median values for each quartile of dietary pattern scores as an ordinal variable.

[e] P for interaction is the significance of interaction term between smoking and respective stratification variable, calculated from Wald test.

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REFERENCES

1. Canadian Cancer Statistics 2012. Toronto, ON: Canadian Cancer Society's Steering Committee on Cancer Statistics.
Canadian Cancer Society., 2012.

2. Norat T, Chan D, Lau R, Aune D, Vieira R, Greenwood D, et al. WCRF/AICR Systematic Literature Review Continuous Update Project Report. *The Associations between Food, Nutrition and Physical Activity and the Risk of Colorectal Cancer*: American Institute for Cancer Research, 2010.

3. Dixon LB, Balder HF, Virtanen MJ, Rashidkhani B, Mannisto S, Krogh V, et al. Dietary patterns associated with colon and rectal cancer: results from the Dietary Patterns and Cancer (DIETSCAN) Project. *Am J Clin Nutr* 2004;80(4):1003-11.

4. Terry P, Hu FB, Hansen H, Wolk A. Prospective study of major dietary patterns and colorectal cancer risk in women. *Am J Epidemiol* 2001;154(12):1143-9.

5. Kwan ML, Weltzien E, Kushi LH, Castillo A, Slattery ML, Caan BJ. Dietary patterns and breast cancer recurrence and survival among women with early-stage breast cancer. *J Clin Oncol* 2009;27(6):919-26.

6. Kim MK, Sasaki S, Otani T, Tsugane S. Dietary patterns and subsequent colorectal cancer risk by subsite: a prospective cohort study. *Int J Cancer* 2005;115(5):790-8.

7. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. Eating patterns and risk of colon cancer. *Am J Epidemiol* 1998;148(1):4-16.

8. Williams CD, Satia JA, Adair LS, Stevens J, Galanko J, Keku TO, et al. Dietary patterns, food groups, and rectal cancer risk in Whites and African-Americans. *Cancer Epidemiol Biomarkers Prev* 2009;18(5):1552-61.

9. Meyerhardt JA, Niedzwiecki D, Hollis D, Saltz LB, Hu FB, Mayer RJ, et al. Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer. *Jama* 2007;298(7):754-64.

10. Fung T, Hu FB, Fuchs C, Giovannucci E, Hunter DJ, Stampfer MJ, et al. Major dietary patterns and the risk of colorectal cancer in women. *Arch Intern Med* 2003;163(3):309-14.

11. Squires J, Roebothan B, Buehler S, Sun Z, Cotterchio M, Younghusband B, et al. Pickled meat consumption and colorectal cancer (CRC): a case-control study in Newfoundland and Labrador, Canada. *Cancer Causes Control* 2010;21(9):1513-21.

12. Sun Z, Zhu Y, Wang PP, Roebothan B, Zhao J, Zhao J, et al. 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. *Anticancer Res* 2012;32(2):687-96.

13. Sun Z, Liu L, Wang PP, Roebothan B, Zhao J, Dicks E, et al. Association of total energy intake and macronutrient consumption with colorectal cancer risk: results from a large population-based case-control study in Newfoundland and Labrador and Ontario, Canada. *Nutr J* 2012;11(1):18.

14. Woods MO, Younghusband HB, Parfrey PS, Gallinger S, McLaughlin J, Dicks E, et al. The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. *Gut* 2010;59(10):1369-77.

15. Green RC, Green JS, Buehler SK, Robb JD, Daftary D, Gallinger S, et al. Very high incidence of familial colorectal cancer in Newfoundland: a comparison with Ontario and 13 other

23

population-based studies. *Fam Cancer* 2007;6(1):53-62.

16. Stram DO, Hankin JH, Wilkens LR, Pike MC, Monroe KR, Park S, et al. Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. *Am J Epidemiol* 2000;151(4):358-70.

17. Sharma S, Iwasaki M, Kunieda C, Cao X, Ishihara J, Hamada G, et al. Development of a quantitative food frequency questionnaire for assessing food, nutrient, and heterocyclic aromatic amines intake in Japanese Brazilians for a colorectal adenoma case-control study. *Int J Food Sci Nutr* 2009;60 Suppl 7:128-39.

18. Jain MG, Rohan TE, Soskolne CL, Kreiger N. Calibration of the dietary questionnaire for the Canadian Study of Diet, Lifestyle and Health cohort. *Public Health Nutr* 2003;6(1):79-86.

19. Hankin JH, Wilkens LR, Kolonel LN, Yoshizawa CN. Validation of a quantitative diet history method in Hawaii. *Am J Epidemiol* 1991;133(6):616-28.

20. Sun Z, Zhu Y, Wang PP, Roebothan B, Zhao J, Dicks E, et al. 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. *Anticancer Res* 2012;32(2):687-96.

21. Dr. H. Bliss Murphy Cancer Care Foundation. From http://www.cancercarefoundation.nl.ca/ (accessed July 18, 2012).

22. MANUAL of the international statistical classification of diseases, injuries, and causes of death. Addendum 1. Supplementary interpretations and instructions for coding causes of death. *Bull World Health Org Suppl* 1953;7(Suppl 6):1-55.

23. Raptis S, Mrkonjic M, Green RC, Pethe VV, Monga N, Chan YM, et al. MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. *J Natl Cancer Inst* 2007;99(6):463-74.

24. Campbell PT, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, et al. Case-Control Study of Overweight, Obesity, and Colorectal Cancer Risk, Overall and by Tumor Microsatellite Instability Status. *J Natl Cancer Inst* 2010;102(6):391-400.

25. Loughrey MB, Waring PM, Tan A, Trivett M, Kovalenko S, Beshay V, et al. Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer. *Fam Cancer* 2007;6(3):301-10.

26. Phipps AI, Baron J, Newcomb PA. Prediagnostic smoking history, alcohol consumption, and colorectal cancer survival: the Seattle Colon Cancer Family Registry. *Cancer* 2010;117(21):4948-57.

27. Hile SE, Shabashev S, Eckert KA. Tumor-Specific Microsatellite Instability: Do Distinct Mechanisms Underlie the MSI-L and EMAST Phenotypes? *Mutat Res* 2012.

28. Joliffe IT, Morgan BJ. Principal component analysis and exploratory factor analysis. *Stat Methods Med Res* 1992;1(1):69-95.

29. Heidemann C, Schulze MB, Franco OH, van Dam RM, Mantzoros CS, Hu FB. Dietary patterns and risk of mortality from cardiovascular disease, cancer, and all causes in a prospective cohort of women. *Circulation* 2008;118(3):230-7.

30. Introduction to SAS, UCLA: Academic Technology Services, Statistical Consulting Group. From http://www.ats.ucla.edu/stat/examples/asa/test_proportionality.htm (accessed June 4, 2012).

31. Slattery ML, French TK, Egger MJ, Lyon JL. Diet and survival of patients with colon cancer in Utah: is there an association? *Int J Epidemiol* 1989;18(4):792-7.

24

32. Dray X, Boutron-Ruault MC, Bertrais S, Sapinho D, Benhamiche-Bouvier AM, Faivre J. Influence of dietary factors on colorectal cancer survival. *Gut* 2003;52(6):868-73.

33. Dolecek TA, McCarthy BJ, Joslin CE, Peterson CE, Kim S, Freels SA, et al. Prediagnosis food patterns are associated with length of survival from epithelial ovarian cancer. *J Am Diet Assoc* 2010;110(3):369-82.

34. Bingham SA, Pignatelli B, J.R. P, A. E, C. M, G. G, et al. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 1996;17(no.3):515-523.

35. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen* 2004;44(1):44-55.

36. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. *J Natl Cancer Inst.* 2002 94(13):972-80.

37. Rabizadeh S, Sears C. New horizons for the infectious diseases specialist: how gut microflora promote health and disease. *Curr Infect Dis Rep* 2008;10(2):92-8.

38. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease. *Genome Med* 2011;3(3):14.

39. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. *Nat Rev Microbiol* 2012.

40. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005;307(5717):1915-20.

41. Takachi R TY, Baba K, Inoue M, Sasazuki S, Iwasaki M, Tsugane S, Japan Public Health Center-Based Prospective Study Group. Red meat intake may increase the risk of colon cancer in Japanese, a population with relatively low red meat consumption. *Asia Pac J Clin Nutr.* 2011;20(4):603-12.

42. Safaee Ardekani G, Jafarnejad SM, Tan L, Saeedi A, Li G. The Prognostic Value of BRAF Mutation in Colorectal Cancer and Melanoma: A Systematic Review and Meta-Analysis. *PLoS One* 2012;7(10):e47054.

43. Bingham SA PB, Pollock JR, Ellul A, Malaveille C, Gross G, Runswick S, Cummings JH, O'Neill IK. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 1996;17(no.3):515-523.

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.