



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

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2012-002270
Article Type:	Research
Date Submitted by the Author:	26-Oct-2012
Complete List of Authors:	Zhu, Yun; Memorial University of Newfoundland, Wu, Hao; Memorial University of Newfoundland, Wang, Peizhong; Memorial University of Newfoundland, Faculty of Medicine ; Savas, Sevtap; Memorial University of Newfoundland, Woodrow, Jennifer; Memorial University of Newfoundland, Wish, Tyler; Memorial University of Newfoundland, Green, Roger; Memorial University of Newfoundland, Woods, Michael; Memorial University of Newfoundland, Roebbothan, Barbara; Memorial University of Newfoundland, Buehler, Sharon; Memorial University of Newfoundland, Dicks, Elizabeth; Memorial University of Newfoundland, Mclaughlin, John; Mount Sinai Hospital, Campbell, Patrick; American Cancer Society, Parfrey, Patrick; Memorial University of Newfoundland,
<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Oncology
Keywords:	Epidemiology < ONCOLOGY, DIET, Gastrointestinal tumours < ONCOLOGY

SCHOLARONE™  
Manuscripts

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

	Item No	Recommendation
<b>Title and abstract</b>	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
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
<b>Methods</b>		
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
<b>Results</b>		
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
<b>Discussion</b>		
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
<b>Other information</b>		
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>.

1  
2  
3 **Dietary Patterns and Colorectal Cancer Recurrence and Survival: Overall and**  
4  
5 **by *BRAF* Mutation Status**  
6

7 Yun Zhu<sup>1,2\*</sup>, Hao Wu<sup>1\*</sup>, Peizhong Peter Wang<sup>1,2,§</sup>, Sevtap Savas<sup>3,4</sup>, Jennifer Woodrow<sup>1</sup>,  
8 Tyler Wish<sup>3</sup>, Roger Green<sup>3</sup>, Michael Woods<sup>3</sup>, Barbara Roebbothan<sup>1</sup>, Sharon Buehler<sup>1</sup>,  
9 Elizabeth Dicks<sup>5</sup>, John R. McLaughlin<sup>6</sup>, Peter T. Campbell<sup>7</sup>, and Patrick S. Parfrey<sup>5</sup>  
10

11 <sup>1</sup> Division of Community Health and Humanities, Faculty of Medicine, Memorial  
12 University of Newfoundland, St. John's, Newfoundland, Canada

13 <sup>2</sup> School of Public Health, Tianjin Medical University, Tianjin, China

14 <sup>3</sup> Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland,  
15 Canada

16 <sup>4</sup> Discipline of Oncology, Faculty of Medicine, Memorial University of  
17 Newfoundland, Canada

18 <sup>5</sup> Clinical Epidemiology Unit, Faculty of Medicine, Memorial University of  
19 Newfoundland, Canada

20 <sup>6</sup> Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario,  
21 Canada

22 <sup>7</sup> Epidemiology Research Program, American Cancer Society, Atlanta, GA, USA  
23

24 \* These authors contributed equally to this study.  
25  
26  
27

28 §Corresponding author

29 Division of Community Health and Humanities

30 Faculty of Medicine

31 Memorial University of Newfoundland

32 Health Sciences Centre

33 300 Prince Philip Drive

34 St. John's, NL

35 A1B 3V6

36 Email: [pwang@mun.ca](mailto:pwang@mun.ca)

37 Telephone: 709-777-8571

38 Fax: 709-777-7382  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

colorectal cancer.

For peer review only

## 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.

## 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



1  
2  
3 activity and tumor molecular phenotype.  
4  
5  
6

## 7 8 **SUBJECTS AND METHODS**

### 9 10 **Study Participants**

11 Patients in this prospective cohort study were enrolled through the  
12 Newfoundland Familial Colorectal Cancer Registry (NFCCR), described in detail  
13 elsewhere.[14,15] In brief, during the time period from 1999 to 2003, patients aged  
14 20-75 years, newly diagnosed with pathologically confirmed, invasive CRC were  
15 eligible for inclusion in the study (ICD-9 codes: 153.0-153.9, 154.0-154.3, and 154.8  
16 or ICD-10 codes: 18.0-18.9, 19.9, and 20.9).  
17  
18  
19  
20  
21  
22  
23

24 Written, informed consent was required from each study participant to access  
25 their archived tumor tissue and medical records. If patients died before they could  
26 give consent, a close relative/proxy was invited to participate. Enrolling deceased  
27 cases through proxies could remove the potential bias of eliminating patients at a late  
28 distant stage.[14] Thus, the inception cohort consisted of 750 eligible patients  
29 (64%).  
30  
31  
32  
33  
34  
35  
36  
37

38 Consenting participants completed and returned a detailed food frequency  
39 questionnaire (FFQ), personal history questionnaire (PHQ) and family history  
40 questionnaire (FHQ). To capture additional cancer diagnosis or recurrence in the  
41 family after enrollment, the FHQ was distributed to participants for the second time  
42 midway through the follow-up. To be included in this analysis, patients had to have  
43 completed at least the FFQ, provided informative lifestyle and medical data from the  
44 PHQ, and had known vital status information by the end of the follow-up period  
45 (April, 2010). For patients who died prior to enrollment, the designated relative/proxy  
46 completed the aforementioned questionnaires. The final analytical cohort comprised  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

6

1  
2  
3 529 eligible participants. The study protocol was approved by the Human  
4  
5 Investigation Committee of Memorial University of Newfoundland.  
6  
7  
8

### 9 10 **Dietary Assessment and Food Grouping**

11  
12 Diet was assessed using a semi-quantitative FFQ, developed from the well-known  
13  
14 Hawaii FFQ,[16] on the basis of a validated instrument adapted for the Canadian  
15  
16 population.[17,18] The FFQ included 170 foods, beverages, and vitamin- and  
17  
18 dietary-supplements.[19] Foods indigenous to the Newfoundland population (e.g.,  
19  
20 salted/pickled meat and smoked/pickled fish) were also included. For each food item  
21  
22 or beverage, participants were asked to estimate their frequency of consumption and  
23  
24 usual portion size as ‘Small’, ‘Regular’ or ‘Large’ one year prior to their colon or  
25  
26 rectal cancer diagnosis. Portion sizes for specific food were depicted in photographs.  
27  
28 Nutrient and total energy intakes were calculated by multiplying the frequency of  
29  
30 consumption of each food by the nutrient content of the portion size based on the  
31  
32 composition values from the 2005 Canadian Nutrient file. [20] Taking a similar  
33  
34 grouping scheme to that used elsewhere,[3] we collapsed individual food items on the  
35  
36 FFQ into 39 predefined food groups based on the roles of food in diet and cancer  
37  
38 etiology. Distinct food items were reserved as individual categories if it was deemed  
39  
40 inappropriate to combine them (e.g., jam, pies, beer, and wine).  
41  
42  
43  
44  
45  
46

### 47 **Covariates**

48  
49 Sociodemographic data, such as age, sex, marital status, and education attainment,  
50  
51 were gathered by the self-administered PHQ. The PHQ also included items regarding  
52  
53 medical history, bowel screening history, physical activity, reproductive factors  
54  
55 (female only), and alcohol and tobacco use. Family history of cancer was assessed by  
56  
57  
58  
59  
60

7

1  
2  
3 the FHQ.  
4  
5  
6

### 7 8 **Study Outcomes**

9  
10 Study outcomes were ascertained from follow-up questionnaires, local  
11 newspapers, death certificates, autopsy, pathology, radiology, surgical reports, as well  
12 as physician's notes. Additional data were gathered from the Dr. H. Bliss Murphy  
13 Cancer Care Foundation and Statistics Canada.[21] The cause of death was obtained  
14 for 93 of 168 deceased patients in this cohort, classified according to the International  
15 Classification of Disease (ICD) codes for underlying or contributing cause of  
16 death;[22] the majority (91%) of these had died from CRC. Since specific cause of  
17 death was not available for all deceased participants, all-cause mortality was used for  
18 analysis. In this study, two end points were considered: the first was disease-free  
19 survival (DFS), defined as time from cancer diagnosis to the first confirmed tumor  
20 recurrence, metastasis, or death from all causes occurring up to April, 2010; the  
21 second end point was overall survival (OS), measured from the date of cancer  
22 diagnosis to the date of death from all causes. Patients who did not have an event by  
23 the end of the follow-up were censored at the date of last contact.  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42

### 43 **Molecular Assessment**

44  
45 The *p.V600E BRAF* mutation and MSI status for the tumor DNA have been  
46 determined in previous studies using standard protocols.[23-25] Briefly, the  
47 mutational hotspot c.1799T>A. (p.Val600Glu) in the *BRAF* gene was detected using  
48 *BRAF V600E* allele-specific primers, with controls amplifying the GAPDH gene.[25]  
49 Positive mutations were then verified by direct automatic sequencing.[25] For MSI  
50 analyses, a panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4,  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

8

1  
2  
3 D5S346, D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify  
4  
5 both tumor and normal DNA.[23,24] MSI status was defined as MSI-High if 30% or  
6  
7 more of the markers were unstable and MS-Stable/MSI-Low if less than 30% of the  
8  
9 markers showed instability.[26] The primer sequences and PCR conditions are  
10  
11 provided in detail in earlier studies from this cohort.[14,23-25]  
12  
13

### 14 15 16 **Statistical Analysis**

17  
18 Exploratory principal component factor analysis[27] was used to identify major  
19  
20 dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation  
21  
22 (orthogonal) procedure was applied to rotate these factors, meaning that it produces  
23  
24 uncorrelated, easy interpreted components that explain the greatest amount of  
25  
26 variance in the original food groups.[28] We determined the number of factors to  
27  
28 retain for interpretation on the basis of criteria as follows: factor eigenvalue greater  
29  
30 than 1.15, the scree plot, the proportion of variance explained, and factor  
31  
32 interpretability.[9] Patterns were labeled based on food groups with absolute rotated  
33  
34 factor loading matrix greater than or equal to 0.50. Each participant was assigned a  
35  
36 factor score for each pattern (factor) by summing the intakes from each food group  
37  
38 multiplied by optimal weights (factor loadings).[5] Individuals with a higher factor  
39  
40 score had a closer adherence to that pattern.[5]  
41  
42  
43  
44

45  
46 Comparisons for baseline characteristics across quartiles of dietary patterns were  
47  
48 performed using ANOVA test for continuous variables and Chi-Square test for  
49  
50 categorical variables. Cox proportional hazards models, each adjusting for energy  
51  
52 intake and critical covariates, were used to evaluate the association between  
53  
54 individual dietary pattern and CRC recurrence and mortality, represented by hazard  
55  
56 ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed  
57  
58  
59  
60

1  
2  
3 by the log-rank test in a univariate setting; those with the p-value less than 0.1 were  
4 considered for inclusion. The final models only retained the items that entered the  
5 models at  $p < 0.1$  or altered the effect estimates by 10% or more; these include energy  
6 intake, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history,  
7 reported screening procedure, reported chemoradiotherapy, and MSI status. The  
8 assumption of proportional hazard rates was verified by checking the parallelism of  
9 the Kaplan-Meier curves and by including time-dependent covariates in the models to  
10 test for statistical significance.[29] Statistical linear trend was examined by modeling  
11 the median value of each quartile as an ordinal variable in a linear regression.[5]  
12 Potential interactions were evaluated by comparing estimates from stratified analyses  
13 and testing significance of interaction terms with a Wald test.[5]

14  
15 A sensitivity analysis was implemented by eliminating stage-advanced patients  
16 enrolled through proxies and re-calculating survival time from the completion of the  
17 first questionnaire to a predefined event, in order to determine whether associations  
18 might vary with the exclusion of stage-advanced cancer. Statistical significance was  
19 accepted for two-sided  $p < 0.05$ . All data management and analyses were performed  
20 with SAS software version 9.2 (SAS Institute Inc, Cary, NC).

## 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 **RESULTS**

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

### 48 49 50 51 52 53 54 **Dietary Patterns**

55 Three distinct dietary patterns, labeled “processed meat pattern”, “prudent  
56  
57  
58

59  
60

1  
2  
3 vegetable pattern” and “high sugar pattern”, were extracted using the aforementioned  
4  
5 factor analysis procedure. These patterns explained 73.82% of total variance in the  
6  
7 original 39 food groups (Table 1). A higher factor loading matrix of a given food  
8  
9 group is representative of a greater contribution of that food group on that specific  
10  
11 pattern. Therefore, the first pattern, termed “processed meat”, was characterized by  
12  
13 higher loadings and thus higher consumptions of cured/processed meat,  
14  
15 cured/processed red meat, red meat, fish, and processed fish; the second pattern,  
16  
17 labeled “prudent vegetable”, displayed higher loadings on other greens, other fruit,  
18  
19 other vegetables, and tomato sauce; and the third pattern, named “high sugar”, showed  
20  
21 higher loadings on desserts and sweets, pies and tarts.  
22  
23  
24  
25  
26  
27

### 28 **Baseline Characteristics by quartiles of dietary patterns**

29  
30 Higher processed meat pattern scores at baseline were detected in men, ever  
31  
32 smokers, patients who were single and individuals who had higher BMI at the time of  
33  
34 diagnosis (Table 2). Higher prudent vegetable pattern scores were observed in women,  
35  
36 never smokers, those with a slightly later age of diagnosis and with patients who had a  
37  
38 tumor harboring the *p.V600E BRAF* mutation. None of these characteristics varied  
39  
40 significantly by quartiles of high sugar pattern scores.  
41  
42  
43  
44

### 45 **Dietary Patterns and Cancer Recurrence or Death**

46  
47 The highest quartile of processed meat pattern was significantly associated with  
48  
49 poorer DFS after the adjustment for other predictors of CRC recurrence and death  
50  
51 (HR: 1.82, 95% CI: 1.07-3.09), although no overall trend was observed in the HRs  
52  
53 across the whole distribution of factor scores (p for trend=0.09) (Table 3).  
54

55  
56 Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was  
57  
58

1  
2  
3 observed to be significantly associated with predefined patient outcomes (i.e., DFS  
4 and OS).  
5  
6

7  
8 When stratified by tumor site, however, the association between processed meat  
9 pattern and DFS remained statistically significant only for patients who had tumors  
10 located in the colon (the highest versus the lowest quartile, HR: 2.29, 95% CI:  
11 1.19-4.40) and not the rectum (HR: 0.97, 95% CI: 0.38-2.45). Similarly, when OS was  
12 the outcome, the positive association between increasing consumption of the  
13 processed meat pattern and mortality was restricted to patients whose tumors were  
14 diagnosed in the colon (the fourth versus first quartiles: HR: 2.13, 95% CI: 1.03-4.43).  
15  
16  
17  
18  
19  
20  
21  
22

23 In the stratified analyses for dietary patterns by sex, physical activity, and *BRAF*  
24 mutation status, there was evidence for effect modification by sex ( $p=0.04$ ) for the  
25 association of processed meat pattern with DFS (HR: 3.85 for women and 1.22 for  
26 men) (Table 4). However, no evidence was observed to suggest that the effects of other  
27 dietary patterns on cancer recurrence or death were modified by physical activity, or  
28 *BRAF* mutation status.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38

## 39 DISCUSSION

40  
41 Three dietary patterns, termed “processed meat pattern”, “prudent vegetable  
42 pattern” and “high sugar pattern”, were generated in this cohort study. We found that  
43 high conformity with the processed meat pattern, characterized by high intakes of  
44 processed meat, red meat, fish, and processed fish, is associated with decreased DFS.  
45  
46  
47  
48 On the contrary, increasing consumption of the prudent vegetable pattern and the high  
49 sugar pattern displayed no clear relationships with mortality or recurrence.  
50  
51  
52  
53

54 The processed meat pattern in the present study shares most characteristics of the  
55 Western diet referred to in previous studies on CRC risk, which indicates a positive  
56  
57  
58  
59  
60

1  
2  
3 association between the Western dietary pattern and CRC risk.[7,9] However, there  
4  
5 has been minimal research examining the association between dietary factors (e.g.,  
6  
7 nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients;[30,31]  
8  
9  
10 moreover, our literature review identified only one study that investigated the  
11  
12 relationship between dietary patterns and survival among CRC patients. Consistent  
13  
14 with our results, that prospective cohort study of 1009 stage III colon cancer  
15  
16 patients[9] reported a deleterious disease-free colon cancer prognosis for patients  
17  
18 reporting high levels of the Western dietary pattern intake.  
19

20  
21 The mechanisms explaining the impact of red and processed meat on CRC  
22  
23 mortality are still unclear; however, some biologic mechanisms that link diet factors  
24  
25 to CRC risk may continue after diagnosis and subsequently impact cancer progression  
26  
27 and survival.[32] For example, strong carcinogens such as *N*-nitroso compounds  
28  
29 (NOCs) and probable carcinogenic mutagens like heterocyclic amines (HCA) and  
30  
31 polycyclic aromatic hydrocarbons (PAH), which have been suggested as significant  
32  
33 contributors for CRC development,[33,34] are found in smoked, fried or  
34  
35 high-temperature cooked meat. Sandhu et al [35] reported a Western dietary pattern is  
36  
37 related to high levels of serum insulin and insulin-like growth factors (IGF), and these  
38  
39 hormones are found to be associated with tumor growth and the inhibition of  
40  
41 apoptosis. In addition, a growing body of evidence suggests that disruption of the  
42  
43 normal gut microflora is associated with human disease, including the pathogenesis of  
44  
45 the intestinal tract (e.g. inflammatory bowel disease) and other diseases such as  
46  
47 obesity, cardiovascular disease, and autoimmune conditions.[36,37] Alterations in  
48  
49 intestinal microbiota are also strongly associated with colonic polyp formation and  
50  
51 with the risk of developing CRC.[38] Given the major role of diet on the intestinal  
52  
53 microbiome,[39] our findings between dietary patterns and CRC survival may also be  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 explained by the impact of dietary patterns on gut microflora and health outcomes.  
4

5 The influence of processed meat pattern on survival was evident among women  
6 rather than men in our study. Previous studies revealed that the higher colon pH and  
7 longer intestine transit time in women compared to men can influence the production  
8 of secondary bile acid or NOCs,[40] resulting in sex differences in the CRC  
9 development. This is the first study that considered effect modifications between  
10 dietary patterns and tumor molecular phenotype (i.e. *BRAF* mutation) on CRC  
11 survival. Although stratified analyses demonstrated a processed meat diet to  
12 significantly decrease survival time only in patients with *BRAF* wide type tumor, no  
13 evident interactions were detected. Further research is clearly warranted to verify  
14 these findings and to determine the biologic pathways that rationalize the underlying  
15 interactions between diet and tumor molecular features.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28

29 A reasonably large sample size with detailed information of patients is a merit of  
30 our study. These data not only includes demographic and personal lifestyle  
31 information, but also some molecular characteristics obtained from genetic testing.  
32 The ample information enables us to perform stratification analysis to control and  
33 assess effect modifiers and confounders.  
34  
35  
36  
37  
38  
39

40 Several limitations of this study should be recognized. Firstly, the results may be  
41 skewed by recall bias since the participants recalled their food consumption from one  
42 year prior to CRC diagnosis; however, this non-differential misclassification is only  
43 expected to bias the results towards the null. Secondly, dietary patterns in this study  
44 only reflect food consumption before diagnosis; it is unknown whether participants  
45 modified their diet post diagnosis. Since previous research has shown minimal change  
46 in diet between pre- and post- diagnosis among cancer patients,[31] the current study  
47 did not examine dietary changes before and after diagnosis. Moreover, immortal  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58

1  
2  
3 person-time bias may impact results. However, this is minimized by using proxies to  
4  
5 enroll deceased patients.  
6

7  
8 In summary, we found that high conformity to the processed meat pattern is  
9  
10 significantly associated with an increased risk of all-cause mortality and recurrence of  
11  
12 CRC. Though our study did not find a difference in effect by tumor molecular  
13  
14 phenotype, larger molecular studies should be conducted to examine if such  
15  
16 differences exist. Ultimately, confirmation of these findings and the underlying  
17  
18 mechanisms await further studies. Our observation not only underlines the importance  
19  
20 of maintaining a healthy diet, but also provides guidance to efficacious dietary  
21  
22 interventions;<sup>[8]</sup> that is, people may lower their risk of CRC mortality by reducing  
23  
24 consumption of a processed meat pattern diet.  
25  
26  
27  
28

29  
30 **Contributors** PPW, YZ and HW contributed to the conception and design of this  
31  
32 manuscript. YZ and HW analyzed the data. YZ, HW, PPW, JW, TW, ED, PTC  
33  
34 drafted and revised the manuscript. SS, RG, MW, BR, SB, JRM and PSP were  
35  
36 responsible for the data collection. All the authors provided final approval.  
37

38  
39 **Acknowledgments** This work was supported by the Canadian Institutes of Health  
40  
41 Research Team Grant [CIHR-CPT79845] and Canadian Institutes of Health Research  
42  
43 Team in Interdisciplinary Research on Colorectal Cancer Studentship [205835].  
44  
45 Yun Zhu was awarded by the Newfoundland and Labrador Centre for Applied Health  
46  
47 Research through a Master's fellowship.  
48

49  
50 **Competing interest** None.

51  
52 **Ethics approval** Human Investigation Committee in Memorial University of  
53  
54 Newfoundland.

55  
56 **Data sharing statement** No additional data available.  
57  
58

**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	<b>0.69</b>	-	0.17
Cured/processed red meat	<b>0.73</b>	-	0.21
Cured/processed meat	<b>0.93</b>	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	<b>0.58</b>	0.32	-0.22
Processed fish	<b>0.50</b>	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	0.54	-
Other fruit	-	<b>0.59</b>	-
Other greens	-	<b>0.60</b>	-0.22
Tomato sauce	-	<b>0.50</b>	-
Other vegetables	0.22	<b>0.54</b>	-
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	-	<b>0.63</b>
Pies, tarts	0.15	-	<b>0.54</b>
Canned fruit	-	0.21	0.23
Jam, jelly	-	-	0.26
Proportion of VAR explained (%)	39.79	22.93	11.10

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

---

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.

For peer review only

**Table2. Baseline Characteristics of 529 CRC Patients by Quartiles of the Three Major Dietary Patterns <sup>a</sup>**

	Processed Meat Pattern				P Value <sup>c</sup>	Prudent Vegetable Pattern				P Value <sup>c</sup>	High Sugar Pattern				P Value <sup>c</sup>
	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 <sup>b</sup>	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 <sup>b</sup>															
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 <sup>2</sup> )															
<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

<sup>a</sup> 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

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

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

**Table 3. Hazard Rate Ratios Associated with Disease-Free and Overall Colorectal Cancer Survival for Quartiles of Dietary Patterns<sup>a</sup>**

	Disease-Free Survival				Overall Survival			
	No. of Events <sup>b</sup> / No. at Risk	Overall CRC HR (95% CI) <sup>c</sup>	Colon cancer HR (95% CI) <sup>c</sup>	Rectal cancer HR (95% CI) <sup>c</sup>	No. of Events <sup>b</sup> / No. at Risk	Overall CRC HR (95% CI) <sup>c</sup>	Colon cancer HR (95% CI) <sup>c</sup>	Rectal cancer HR (95% CI) <sup>c</sup>
<b>Processed meat pattern</b>								
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 <sup>d</sup>		0.09	0.12	0.91		0.25	0.40	0.59
<b>Prudent vegetable pattern</b>								
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 <sup>d</sup>		0.62	0.83	0.19		0.90	0.60	0.92
<b>High sugar pattern</b>								
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 <sup>d</sup>		0.89	0.90	0.11		0.52	0.56	0.64

<sup>a</sup> Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; CI, confidence interval;

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

<sup>c</sup> 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.

<sup>d</sup> 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.

**Table 4. Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics<sup>a</sup>**

	No. of Events <sup>b</sup> /No. at Risk	Quartiles HR (95% CI) <sup>c</sup>				P for Trend <sup>d</sup>	P for Interaction <sup>e</sup>
		Q1	Q2 <sup>c</sup>	Q3	Q4		
<b>Processed meat pattern</b>							
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	0.04
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	
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	0.64
≥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	
<i>BRAF</i> 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	0.80
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	
<b>Prudent vegetables pattern</b>							
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	0.65
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	
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	0.83
≥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	
<i>BRAF</i> 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	0.80
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	
<b>High sugar pattern</b>							
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	0.72
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	
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	0.26
≥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	
<i>BRAF</i> 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	0.33
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	

<sup>a</sup> Abbreviations are as follows: CI, confidence interval; METs/week, metabolic equivalent hours per week;

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

20

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

<sup>c</sup> 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.

<sup>d</sup> 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.

<sup>e</sup> P for interaction is the significance of interaction term between smoking and respective stratification variable, calculated from Wald test.

For peer review only



## 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

- 1  
2  
3  
4 women. Arch Intern Med 2003;163(3):309-14.  
5  
6 11. Squires J, Roebathan B, Buehler S, et al. Pickled meat consumption and colorectal cancer  
7  
8 (CRC): a case-control study in Newfoundland and Labrador, Canada. Cancer Causes Control  
9  
10 2010;21(9):1513-21.  
11  
12  
13  
14 12. Sun Z, Zhu Y, Wang PP, et al. Reported intake of selected micronutrients and risk of  
15  
16 colorectal cancer: results from a large population-based case-control study in Newfoundland,  
17  
18 Labrador and Ontario, Canada. Anticancer Res 2012;32(2):687-96.  
19  
20  
21 13. Sun Z, Liu L, Wang PP, et al. Association of total energy intake and macronutrient  
22  
23 consumption with colorectal cancer risk: results from a large population-based case-control  
24  
25 study in Newfoundland and Labrador and Ontario, Canada. Nutr J 2012;11(1):18.  
26  
27  
28  
29 14. Woods MO, Youngusband HB, Parfrey PS, et al. The genetic basis of colorectal cancer in a  
30  
31 population-based incident cohort with a high rate of familial disease. Gut  
32  
33 2010;59(10):1369-77.  
34  
35  
36 15. Green RC, Green JS, Buehler SK, et al. Very high incidence of familial colorectal cancer in  
37  
38 Newfoundland: a comparison with Ontario and 13 other population-based studies. Fam  
39  
40 Cancer 2007;6(1):53-62.  
41  
42  
43 16. Stram DO, Hankin JH, Wilkens LR, et al. Calibration of the dietary questionnaire for a  
44  
45 multiethnic cohort in Hawaii and Los Angeles. Am J Epidemiol 2000;151(4):358-70.  
46  
47  
48  
49 17. Sharma S, Iwasaki M, Kunieda C, et al. Development of a quantitative food frequency  
50  
51 questionnaire for assessing food, nutrient, and heterocyclic aromatic amines intake in  
52  
53 Japanese Brazilians for a colorectal adenoma case-control study. Int J Food Sci Nutr 2009;60  
54  
55 Suppl 7:128-39.  
56  
57  
58

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*

- 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](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

- 1  
2  
3  
4 94(13):972-80.  
5  
6 36. Rabizadeh S, Sears C. New horizons for the infectious diseases specialist: how gut microflora  
7  
8 promote health and disease. *Curr Infect Dis Rep* 2008;10(2):92-8.  
9  
10 37. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and  
11  
12 disease. *Genome Med* 2011;3(3):14.  
13  
14 38. Tjalsma H, Boleij A, Marchesi JR, et al. A bacterial driver-passenger model for colorectal  
15  
16 cancer: beyond the usual suspects. *Nat Rev Microbiol* 2012.  
17  
18 39. Backhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine.  
19  
20 *Science* 2005;307(5717):1915-20.  
21  
22 40. Takachi R TY, Baba K, Inoue M, et al. Japan Public Health Center-Based Prospective Study  
23  
24 Group. Red meat intake may increase the risk of colon cancer in Japanese, a population with  
25  
26 relatively low red meat consumption. *Asia Pac J Clin Nutr.* 2011;20(4):603-12.  
27  
28 41. Bingham SA PB, Pollock JR, Ellul A, et al. Does increased endogenous formation of  
29  
30 N-nitroso compounds in the human colon explain the association between red meat and colon  
31  
32 cancer? *Carcinogenesis* 1996;17(no.3):515-523.  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



## Dietary Patterns and Colorectal Cancer Recurrence and Survival

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2012-002270.R1
Article Type:	Research
Date Submitted by the Author:	17-Dec-2012
Complete List of Authors:	Zhu, Yun; Memorial University of Newfoundland, Wu, Hao; Memorial University of Newfoundland, Wang, Peizhong; Memorial University of Newfoundland, Faculty of Medicine ; Savas, Sevtap; Memorial University of Newfoundland, Woodrow, Jennifer; Memorial University of Newfoundland, Wish, Tyler; Memorial University of Newfoundland, Jin, Rong; The First Affiliated Hospital of Wenzhou Medical College Green, Roger; Memorial University of Newfoundland, Woods, Michael; Memorial University of Newfoundland, Roebothan, Barbara; Memorial University of Newfoundland, Buehler, Sharon; Memorial University of Newfoundland, Dicks, Elizabeth; Memorial University of Newfoundland, Mclaughlin, John; Mount Sinai Hospital, Campbell, Patrick; American Cancer Society, Parfrey, Patrick; Memorial University of Newfoundland,
<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Oncology, Gastroenterology and hepatology, Nutrition and metabolism
Keywords:	Epidemiology < ONCOLOGY, DIET, Gastrointestinal tumours < ONCOLOGY

SCHOLARONE™  
Manuscripts

## Dietary Patterns and Colorectal Cancer Recurrence and Survival

Yun Zhu<sup>1,2\*</sup>, Hao Wu<sup>1\*</sup>, Peizhong Peter Wang<sup>1,2,§</sup>, Sevtap Savas<sup>3,4</sup>, Jennifer Woodrow<sup>1</sup>, Tyler Wish<sup>3</sup>, Rong Jin<sup>5</sup>, Roger Green<sup>3</sup>, Michael Woods<sup>3</sup>, Barbara Roebathan<sup>1</sup>, Sharon Buehler<sup>1</sup>, Elizabeth Dicks<sup>6</sup>, John R. Mclaughlin<sup>7</sup>, Peter T. Campbell<sup>8</sup>, and Patrick S. Parfrey<sup>6</sup>

<sup>1</sup> Division of Community Health and Humanities, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

<sup>2</sup> School of Public Health, Tianjin Medical University, Tianjin, China

<sup>3</sup> Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, Canada

<sup>4</sup> Discipline of Oncology, Faculty of Medicine, Memorial University of Newfoundland, Canada

<sup>5</sup> Department of Epidemiology, The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China

<sup>6</sup> Clinical Epidemiology Unit, Faculty of Medicine, Memorial University of Newfoundland, Canada

<sup>7</sup> Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

<sup>8</sup> Epidemiology Research Program, American Cancer Society, Atlanta, GA, USA

\* These authors contributed equally to this study.

§Corresponding author

Division of Community Health and Humanities

Faculty of Medicine

Memorial University of Newfoundland

Health Sciences Centre

300 Prince Philip Drive

St. John's, NL

A1B 3V6

Email: [pwang@mun.ca](mailto:pwang@mun.ca)

Telephone: 709-777-8571

Fax: 709-777-7382

## 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.



## 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.

## INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of cancer death in Canada.<sup>1</sup> 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,<sup>2</sup> although most studies have focused primarily on individual foods or nutrients. Since foods and nutrients act synergistically rather than in isolation,<sup>3-6</sup> 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.<sup>5-9</sup> Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse<sup>7,8</sup> or null<sup>5,6,10</sup> association with CRC risk.

The highest CRC incidence and mortality rates in Canada are observed in the province of Newfoundland and Labrador (NL).<sup>1</sup> 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.<sup>11</sup> Several studies have partially attributed the high CRC incidence rate in NL to its unique diet,<sup>11-13</sup> 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.

## 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.<sup>14 15</sup> 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.<sup>14</sup> 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

1  
2  
3 questionnaires. The final analytical cohort comprised 529 eligible participants. The  
4  
5 study protocol was approved by the Human Investigation Committee of Memorial  
6  
7 University of Newfoundland.  
8  
9

### 10 11 **Dietary assessment and food grouping**

12  
13  
14 Diet was assessed using a semi-quantitative FFQ, developed from the well-known  
15  
16 Hawaii FFQ,<sup>16</sup> on the basis of a validated instrument adapted for the Canadian  
17  
18 population.<sup>17 18</sup> The FFQ included 170 foods, beverages, and vitamin- and  
19  
20 dietary-supplements.<sup>19</sup> Foods indigenous to the Newfoundland population (e.g.,  
21  
22 salted/pickled meat and smoked/pickled fish) were also included. For each food item  
23  
24 or beverage, participants were asked to estimate their frequency of consumption and  
25  
26 usual portion size as ‘Small’, ‘Regular’ or ‘Large’ one year prior to their colon or  
27  
28 rectal cancer diagnosis. Portion sizes for specific food were depicted in photographs.  
29  
30 Nutrient and total energy intakes were calculated by multiplying the frequency of  
31  
32 consumption of each food by the nutrient content of the portion size based on the  
33  
34 composition values from the 2005 Canadian Nutrient file.<sup>20</sup> Taking a similar grouping  
35  
36 scheme to that used elsewhere,<sup>3</sup> we collapsed individual food items on the FFQ into  
37  
38 39 predefined food groups based on the roles of food in diet and cancer etiology.  
39  
40  
41 Distinct food items were reserved as individual categories if it was deemed  
42  
43 inappropriate to combine them (e.g., jam, pies, beer, and wine).  
44  
45  
46  
47  
48

### 49 **Covariates**

50  
51 Sociodemographic data, such as age, sex, marital status, and education attainment,  
52  
53 were gathered by the self-administered PHQ. The PHQ also included items regarding  
54  
55 medical history, bowel screening history, physical activity, reproductive factors  
56  
57  
58  
59  
60

(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.<sup>21</sup> 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,<sup>22</sup> 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.<sup>23-25</sup> 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.<sup>25</sup> Positive mutations were then verified by direct automatic sequencing.<sup>25</sup> For MSI analyses, a

1  
2  
3 panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4, D5S346,  
4  
5 D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify both tumor  
6  
7 and normal DNA.<sup>23 24</sup> MSI status was defined as MSI-High if 30% or more of the  
8  
9 markers were unstable and MS-Stable/MSI-Low if less than 30% of the markers  
10  
11 showed instability.<sup>26 27</sup> The primer sequences and PCR conditions are provided in  
12  
13 detail in earlier studies from this cohort.<sup>14 23-25</sup>  
14  
15  
16  
17  
18

### 19 **Statistical analysis**

20  
21 Exploratory principal component factor analysis<sup>28</sup> was used to identify major  
22  
23 dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation  
24  
25 (orthogonal) procedure was applied to rotate these factors, meaning that it produces  
26  
27 uncorrelated, easy interpreted components that explain the greatest amount of  
28  
29 variance in the original food groups.<sup>29</sup> We determined the number of factors to retain  
30  
31 for interpretation on the basis of criteria as follows: factor eigenvalue greater than  
32  
33 1.15, the scree plot, the proportion of variance explained, and factor interpretability.<sup>9</sup>  
34  
35 Patterns were labeled based on food groups with absolute rotated factor loading  
36  
37 matrix greater than or equal to 0.50. Each participant was assigned a factor score for  
38  
39 each pattern (factor) by summing the intakes from each food group multiplied by  
40  
41 optimal weights (factor loadings).<sup>5</sup> Individuals with a higher factor score had a closer  
42  
43 adherence to that pattern.<sup>5</sup>  
44  
45  
46

47  
48 Comparisons for baseline characteristics across quartiles of dietary patterns were  
49  
50 performed using ANOVA test for continuous variables and Chi-Square test for  
51  
52 categorical variables. Cox proportional hazards models, each adjusting for energy  
53  
54 intake and critical covariates, were used to evaluate the association between  
55  
56 individual dietary pattern and CRC recurrence and mortality, represented by hazard  
57  
58  
59  
60

1  
2  
3 ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed  
4  
5 by the log-rank test in a univariate setting; those with the p-value less than 0.1 were  
6  
7 considered for inclusion. The final models only retained the items that entered the  
8  
9 models at  $p < 0.1$  or altered the effect estimates by 10% or more; these include sex, age  
10  
11 at diagnosis, stage at diagnosis, BMI, marital status, family history, reported screening  
12  
13 procedure, reported chemoradiotherapy, and MSI status. All models were run with the  
14  
15 adjustment for total energy intake by including total calories in the model. The  
16  
17 assumption of proportional hazard rates was verified by checking the parallelism of  
18  
19 the Kaplan-Meier curves and by including time-dependent covariates in the models to  
20  
21 test for statistical significance.<sup>30</sup> Statistical linear trend was examined by modeling the  
22  
23 median value of each quartile as an ordinal variable in a linear regression.<sup>5</sup> Potential  
24  
25 interactions were evaluated by comparing estimates from stratified analyses and  
26  
27 testing significance of interaction terms with a Wald test.<sup>5</sup>  
28  
29  
30  
31

32 A sensitivity analysis was implemented by eliminating stage-advanced patients  
33  
34 enrolled through proxies and re-calculating survival time from the completion of the  
35  
36 first questionnaire to a predefined event, in order to determine whether associations  
37  
38 might vary with the exclusion of stage-advanced cancer. Statistical significance was  
39  
40 accepted for two-sided  $p < 0.05$ . All data management and analyses were performed  
41  
42 with SAS software version 9.2 (SAS Institute Inc, Cary, NC).  
43  
44  
45  
46  
47

## 48 RESULTS

49 The cohort was followed for a median of 6.4 years (minimum: 1.3 years;  
50  
51 maximum: 10.9 years). A total of 168 patients died from all causes and 30 had a  
52  
53 cancer recurrence or metastasis by the end of study follow-up (April, 2010).  
54  
55  
56  
57  
58  
59  
60

## 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



1  
2  
3 across the whole distribution of factor scores ( $p$  for trend=0.09) (Table 3).

4  
5 Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was  
6  
7 observed to be significantly associated with predefined patient outcomes (i.e., DFS  
8  
9 and OS).

10  
11 When stratified by tumor site, however, the association between processed meat  
12  
13 pattern and DFS remained statistically significant only for patients who had tumors  
14  
15 located in the colon (the highest versus the lowest quartile, HR: 2.29, 95% CI:  
16  
17 1.19-4.40) and not the rectum (HR: 0.97, 95% CI: 0.38-2.45). Similarly, when OS was  
18  
19 the outcome, the positive association between increasing consumption of the  
20  
21 processed meat pattern and mortality was restricted to patients whose tumors were  
22  
23 diagnosed in the colon (the fourth versus first quartiles: HR: 2.13, 95% CI: 1.03-4.43).

24  
25 In the stratified analyses for dietary patterns, there was evidence for effect  
26  
27 modification by sex ( $p=0.04$ ) for the association of processed meat pattern with DFS  
28  
29 (HR: 3.85 for women and 1.22 for men) (Table 4). However, no evidence was  
30  
31 observed to suggest that the effects of other dietary patterns on cancer recurrence or  
32  
33 death were modified by physical activity, *BRAF* mutation status and MSI (data not  
34  
35 shown).

36  
37 In the sensitivity analysis, when advanced-stage patients who died before  
38  
39 admittance were excluded, the association between processed meat pattern and  
40  
41 survival among CRC patients remained significant.

## 42 43 44 45 46 47 48 49 **DISCUSSION**

50  
51 Three dietary patterns, termed “processed meat pattern”, “prudent vegetable  
52  
53 pattern” and “high sugar pattern”, were generated in this cohort study. We found that  
54  
55 high conformity with the processed meat pattern, characterized by high intakes of  
56  
57

58  
59 11

1  
2  
3 processed meat, red meat, fish, and processed fish, is associated with decreased DFS  
4 of CRC, specifically of colon cancer. The differential associations by subsite indicate  
5 disease heterogeneity. On the contrary, increasing consumption of the prudent  
6 vegetable pattern and the high sugar pattern displayed no clear relationships with  
7 mortality or recurrence.  
8  
9

10  
11  
12  
13  
14 The processed meat pattern in the present study shares most characteristics of the  
15 Western diet referred to in previous studies on CRC risk, which indicates a positive  
16 association between the Western dietary pattern and CRC risk.<sup>7,9</sup> However, there has  
17 been minimal research examining the association between dietary factors (e.g.,  
18 nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients;<sup>31,32</sup>  
19 moreover, our literature review identified only one study that investigated the  
20 relationship between dietary patterns and survival among CRC patients. Consistent  
21 with our results, that prospective cohort study of 1009 stage III colon cancer patients<sup>9</sup>  
22 reported a deleterious disease-free colon cancer prognosis for patients reporting high  
23 levels of the Western dietary pattern intake.  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35

36 The mechanisms explaining the impact of red and processed meat on CRC  
37 mortality are still unclear; however, some biologic mechanisms that link diet factors  
38 to CRC risk may continue after diagnosis and subsequently impact cancer progression  
39 and survival.<sup>33</sup> For example, strong carcinogens such as *N*-nitroso compounds (NOCs)  
40 and probable carcinogenic mutagens like heterocyclic amines (HCA) and polycyclic  
41 aromatic hydrocarbons (PAH), which have been suggested as significant contributors  
42 for CRC development,<sup>34,35</sup> are found in smoked, fried or high-temperature cooked  
43 meat. Sandhu et al<sup>36</sup> reported a Western dietary pattern is related to high levels of  
44 serum insulin and insulin-like growth factors (IGF), and these hormones are found to  
45 be associated with tumor growth and the inhibition of apoptosis. In addition, a  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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.<sup>37 38</sup> Alterations in intestinal microbiota are also strongly associated with colonic polyp formation and with the risk of developing CRC.<sup>39</sup> Given the major role of diet on the intestinal microbiome,<sup>40</sup> 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,<sup>41</sup> 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;<sup>42</sup> 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

1  
2  
3 assess effect modifiers and confounders. Several limitations of this study should be  
4 recognized. Firstly, the results may be skewed by recall bias since the participants  
5 recalled their food consumption from one year prior to CRC diagnosis; however, this  
6 non-differential misclassification is only expected to bias the results towards the null.  
7  
8 Secondly, dietary patterns in this study only reflect food consumption before  
9 diagnosis; it is unknown whether participants modified their diet post diagnosis. Since  
10 previous research has shown minimal change in diet between pre- and post- diagnosis  
11 among cancer patients,<sup>32</sup> the current study did not examine dietary changes before and  
12 after diagnosis. Moreover, immortal person-time bias may impact results. However,  
13 this is minimized by using proxies to enroll deceased patients.  
14  
15

16  
17  
18 In summary, we found that high conformity to the processed meat pattern is  
19 significantly associated with an increased risk of all-cause mortality and recurrence of  
20 CRC. Though our study did not find a difference in effect by tumor molecular  
21 phenotype, larger molecular studies should be conducted to examine if such  
22 differences exist. Ultimately, confirmation of these findings and the underlying  
23 mechanisms await further studies. Our observation not only underlines the importance  
24 of maintaining a healthy diet, but also provides guidance to efficacious dietary  
25 interventions;<sup>8</sup> that is, people may lower their risk of CRC mortality by reducing  
26 consumption of a processed meat pattern diet.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 **Contributors** PPW, YZ and HW contributed to the conception and design of this  
4 manuscript. YZ and HW analyzed the data. YZ, HW, PPW, JW, TW, RJ, ED, PTC  
5  
6  
7 drafted and revised the manuscript. SS, RG, MW, BR, SB, JRM and PSP were  
8  
9  
10 responsible for the data collection. All the authors provided final approval.

11 **Acknowledgments** This work was supported by the Canadian Institutes of Health  
12  
13  
14 Research Team Grant [CIHR-CPT79845] and Canadian Institutes of Health Research  
15  
16  
17 Team in Interdisciplinary Research on Colorectal Cancer Studentship [205835].  
18  
19 Yun Zhu was awarded by the Newfoundland and Labrador Centre for Applied Health  
20  
21 Research through a Master's fellowship.

22  
23 **Competing interest** None.

24  
25 **Ethics approval** Human Investigation Committee in Memorial University of  
26  
27 Newfoundland.

28  
29  
30 **Data sharing statement** No additional data available.  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**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	<b>0.69</b>	-	0.17
Cured/processed red meat	<b>0.73</b>	-	0.21
Cured/processed meat	<b>0.93</b>	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	<b>0.58</b>	0.32	-0.22
Processed fish	<b>0.50</b>	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	<b>0.54</b>	-
Other fruit	-	<b>0.59</b>	-
Other greens	-	<b>0.60</b>	-0.22
Tomato sauce	-	<b>0.50</b>	-
Other vegetables	0.22	<b>0.54</b>	-
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	-	<b>0.63</b>
Pies, tarts	0.15	-	<b>0.54</b>
Canned fruit	-	0.21	0.23
Jam, jelly	-	-	0.26
Proportion of VAR explained (%)	39.79	22.93	11.10

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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.

For peer review only

Statistics of 529 CRC Patients by Quartiles of the Three Major Dietary Patterns <sup>a</sup>

1	Processed Meat Pattern				P Value <sup>c</sup>	Prudent Vegetable Pattern				P Value <sup>c</sup>	High Sugar Pattern				P Value <sup>c</sup>
	2	3	4			1	2	3	4		1	2	3	4	
3	Q2	Q3	Q4		Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4		
4	(n=132)	(n=133)	(n=132)		(n=132)	(n=132)	(n=133)	(n=132)		(n=132)	(n=132)	(n=133)	(n=132)		
5	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	
6	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)		
7	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	
8	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)		
9	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	
10	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)		
11	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)		
12	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	
13	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)		
14	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	
15	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)		
16	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	
17	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)		
18	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	
19	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)		
20	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	
21	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)		
22	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	
23	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)		
24	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	
25	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)		
26	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	

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

instability-high; Data are presented as mean±SD (standard deviation); categorical variables presented as number<sup>43</sup>

and P values are given in parentheses. Statistical significance was determined by the ANOVA test for continuous variables and of the Chi-Square test for categorical variables

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>



**Table 3. Hazard Rate Ratios Associated with Disease-Free and Overall Colorectal Cancer Survival for Quartiles of Dietary Patterns<sup>a</sup>**

	Disease-Free Survival				Overall Survival			
	No. of Events <sup>b</sup> / No. at Risk	Overall CRC HR (95% CI) <sup>c</sup>	Colon cancer HR (95% CI) <sup>c</sup>	Rectal cancer HR (95% CI) <sup>c</sup>	No. of Events <sup>b</sup> / No. at Risk	Overall CRC HR (95% CI) <sup>c</sup>	Colon cancer HR (95% CI) <sup>c</sup>	Rectal cancer HR (95% CI) <sup>c</sup>
<b>Processed meat pattern</b>								
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 <sup>d</sup>		0.09	0.12	0.91		0.25	0.40	0.59
<b>Prudent vegetable pattern</b>								
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 <sup>d</sup>		0.62	0.83	0.19		0.90	0.60	0.92
<b>High sugar pattern</b>								
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 <sup>d</sup>		0.89	0.90	0.11		0.52	0.56	0.64

<sup>a</sup> Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; CI, confidence interval;

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

<sup>c</sup> 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.

<sup>d</sup> 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.

**Table 4. Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics<sup>a</sup>**

	No. of Events <sup>b</sup> /No. at Risk	Quartiles HR (95% CI) <sup>c</sup>				P for Trend <sup>d</sup>	P for Interaction <sup>e</sup>
		Q1	Q2 <sup>c</sup>	Q3	Q4		
<b>Processed meat pattern</b>							
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	0.04
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	
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	0.64
≥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	
<i>BRAF</i> 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	0.80
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	
<b>Prudent vegetables pattern</b>							
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	0.65
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	
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	0.83
≥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	
<i>BRAF</i> 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	0.80
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	
<b>High sugar pattern</b>							
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	0.72
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	
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	0.26
≥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	
<i>BRAF</i> 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	0.33
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	

<sup>a</sup> Abbreviations are as follows: CI, confidence interval; METs/week, metabolic equivalent hours per week;

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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

<sup>c</sup> 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.  
<sup>d</sup> 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.  
<sup>e</sup> P for interaction is the significance of interaction term between smoking and respective stratification variable, calculated from Wald test.

For peer review only

## 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, Roebathan 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, Roebathan 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, Roebathan 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

- 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 EMAS 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](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.

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.

## Dietary Patterns and Colorectal Cancer Recurrence and Survival

Yun Zhu<sup>1,2\*</sup>, Hao Wu<sup>1\*</sup>, Peizhong Peter Wang<sup>1,2,§</sup>, Sevta Savas<sup>3,4</sup>, Jennifer Woodrow<sup>1</sup>, Tyler Wish<sup>3</sup>, Rong Jin<sup>5</sup>, Roger Green<sup>3</sup>, Michael Woods<sup>3</sup>, Barbara Roebathan<sup>1</sup>, Sharon Buehler<sup>1</sup>, Elizabeth Dicks<sup>6</sup>, John R. McLaughlin<sup>7</sup>, Peter T. Campbell<sup>8</sup>, and Patrick S. Parfrey<sup>6</sup>

<sup>1</sup> Division of Community Health and Humanities, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

<sup>2</sup> School of Public Health, Tianjin Medical University, Tianjin, China

<sup>3</sup> Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, Canada

<sup>4</sup> Discipline of Oncology, Faculty of Medicine, Memorial University of Newfoundland, Canada

<sup>5</sup> Department of Epidemiology, The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China

<sup>6</sup> Clinical Epidemiology Unit, Faculty of Medicine, Memorial University of Newfoundland, Canada

<sup>7</sup> Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

<sup>8</sup> Epidemiology Research Program, American Cancer Society, Atlanta, GA, USA

\* These authors contributed equally to this study.

§Corresponding author  
Division of Community Health and Humanities  
Faculty of Medicine  
Memorial University of Newfoundland  
Health Sciences Centre  
300 Prince Philip Drive  
St. John's, NL  
A1B 3V6  
Email: [pwang@mun.ca](mailto:pwang@mun.ca)  
Telephone: 709-777-8571  
Fax: 709-777-7382

## 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

2



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

For peer review only

## 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.

## INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of cancer death in Canada.<sup>1</sup> 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,<sup>2</sup> although most studies have focused primarily on individual foods or nutrients. Since foods and nutrients act synergistically rather than in isolation,<sup>3-6</sup> 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.<sup>5-9</sup> Whereas strong adherence to the prudent pattern, characterized by high intakes of fruit, vegetable, fish and poultry, often shows an inverse<sup>7,8</sup> or null<sup>5,6,10</sup> 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).<sup>1</sup> 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.<sup>11</sup> Several studies have partially attributed the high CRC incidence rate in NL to its unique diet,<sup>11-13</sup> 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,

5

1  
2  
3  
4  
5  
6 physical activity and tumor molecular phenotype.  
7  
8  
9

## 10 SUBJECTS AND METHODS

### 11 Study participants

12 Patients in this prospective cohort study were enrolled through the  
13  
14 Newfoundland Familial Colorectal Cancer Registry (NFCCR), described in detail  
15 elsewhere.<sup>14 15</sup> In brief, during the time period from 1999 to 2003, patients aged 20-75  
16 years, newly diagnosed with pathologically confirmed, invasive CRC were eligible  
17 for inclusion in the study (ICD-9 codes: 153.0-153.9, 154.0-154.3, and 154.8 or  
18 ICD-10 codes: 18.0-18.9, 19.9, and 20.9).  
19  
20  
21  
22  
23  
24

25 Written, informed consent was required from each study participant to access  
26 their archived tumor tissue and medical records. If patients died before they could  
27 give consent ([the median time from date of diagnosis to date of consent was 1.8 years](#)),  
28 a close relative/proxy, [who has lived with the patient](#), was invited to participate.  
29  
30  
31 Enrolling deceased cases through proxies could remove the potential bias of  
32 eliminating patients at a late distant stage.<sup>14</sup> Thus, the inception cohort consisted of  
33 750 eligible patients (64%).  
34  
35  
36  
37  
38

39 Consenting participants completed and returned a detailed food frequency  
40 questionnaire (FFQ), personal history questionnaire (PHQ) and family history  
41 questionnaire (FHQ). [All questionnaires were self-completed. Assistance from study  
42 staff was available to help with understanding items on the questionnaires.](#) To capture  
43 additional cancer diagnosis or recurrence in the family after enrollment, the FHQ was  
44 distributed to participants for the second time midway through the follow-up. To be  
45 included in this analysis, patients had to have completed at least the FFQ, provided  
46 informative lifestyle and medical data from the PHQ, and had known vital status  
47  
48  
49  
50  
51  
52  
53  
54

55  
56 6  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 information by the end of the follow-up period (April, 2010). For patients who died  
7  
8 prior to enrollment, the designated relative/proxy completed the aforementioned  
9  
10 questionnaires. The final analytical cohort comprised 529 eligible participants. The  
11  
12 study protocol was approved by the Human Investigation Committee of Memorial  
13  
14 University of Newfoundland.

### 15 16 17 18 **Dietary Assessment and Food Grouping**

19  
20 Diet was assessed using a semi-quantitative FFQ, developed from the well-known  
21  
22 Hawaii FFQ,<sup>16</sup> on the basis of a validated instrument adapted for the Canadian  
23  
24 population.<sup>17 18</sup> The FFQ included 170 foods, beverages, and vitamin- and  
25  
26 dietary-supplements.<sup>19</sup> Foods indigenous to the Newfoundland population (e.g.,  
27  
28 salted/pickled meat and smoked/pickled fish) were also included. For each food item  
29  
30 or beverage, participants were asked to estimate their frequency of consumption and  
31  
32 usual portion size as 'Small', 'Regular' or 'Large' one year prior to their colon or  
33  
34 rectal cancer diagnosis. Portion sizes for specific food were depicted in photographs.  
35  
36 Nutrient and total energy intakes were calculated by multiplying the frequency of  
37  
38 consumption of each food by the nutrient content of the portion size based on the  
39  
40 composition values from the 2005 Canadian Nutrient file.<sup>20</sup> Taking a similar grouping  
41  
42 scheme to that used elsewhere,<sup>3</sup> we collapsed individual food items on the FFQ into  
43  
44 39 predefined food groups based on the roles of food in diet and cancer etiology.  
45  
46 Distinct food items were reserved as individual categories if it was deemed  
47  
48 inappropriate to combine them (e.g., jam, pies, beer, and wine).

### 49 50 51 **Covariates**

52 Sociodemographic data, such as age, sex, marital status, and education attainment,  
53  
54

55  
56 7  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 were gathered by the self-administered PHQ. The PHQ also included items regarding  
7  
8 medical history, bowel screening history, physical activity, reproductive factors  
9  
10 (female only), and alcohol and tobacco use. Family history of cancer was assessed by  
11  
12 the FHQ.  
13

### 14 15 16 **Study Outcomes**

17  
18 Study outcomes were ascertained from follow-up questionnaires, local  
19  
20 newspapers ([e.g., death notices](#)), death certificates, autopsy, pathology, radiology,  
21  
22 surgical reports, as well as physician's notes. Additional data were gathered from the  
23  
24 Dr. H. Bliss Murphy Cancer Care Foundation and Statistics Canada.<sup>21</sup> The cause of  
25  
26 death was obtained for 93 of 168 deceased patients in this cohort, classified according  
27  
28 to the International Classification of Disease (ICD) codes for underlying or  
29  
30 contributing cause of death;<sup>22</sup> the majority (91%) of these had died from CRC. Since  
31  
32 specific cause of death was not available for all deceased participants, all-cause  
33  
34 mortality was used for analysis. In this study, two end points were considered: the first  
35  
36 was disease-free survival (DFS), defined as time from cancer diagnosis to the first  
37  
38 confirmed tumor recurrence, metastasis, or death from all causes occurring up to April,  
39  
40 2010; the second end point was overall survival (OS), measured from the date of  
41  
42 cancer diagnosis to the date of death from all causes. Patients who did not have an  
43  
44 event by the end of the follow-up were censored at the date of last contact.  
45  
46

### 47 48 **Molecular Assessment**

49  
50 The *p.V600E BRAF* mutation and MSI status for the tumor DNA have been  
51  
52 determined in previous studies using standard protocols.<sup>23-25</sup> Briefly, the mutational  
53  
54 hotspot c.1799T>A. (p.Val600Glu) in the *BRAF* gene was detected using *BRAF*  
55

56  
57  
58  
59  
60  
8

1  
2  
3  
4  
5  
6 *V600E* allele-specific primers, with controls amplifying the GAPDH gene.<sup>25</sup> Positive  
7  
8 mutations were then verified by direct automatic sequencing.<sup>25</sup> For MSI analyses, a  
9  
10 panel of 10 microsatellite repeats (BAT25, BAT26, BAT40, BAT34C4, D5S346,  
11  
12 D17S250, ACTC, D18S55, D10S197, and MYCL) were used to amplify both tumor  
13  
14 and normal DNA.<sup>23 24</sup> MSI status was defined as MSI-High if 30% or more of the  
15  
16 markers were unstable and MS-Stable/MSI-Low if less than 30% of the markers  
17  
18 showed instability.<sup>26 27</sup> The primer sequences and PCR conditions are provided in  
19  
20 detail in earlier studies from this cohort.<sup>14 23-25</sup>  
21  
22  
23

### 24 **Statistical Analysis**

25  
26 Exploratory principal component factor analysis<sup>28</sup> was used to identify major  
27  
28 dietary patterns based on 39 predefined food groups from the FFQ. A varimax rotation  
29  
30 (orthogonal) procedure was applied to rotate these factors, meaning that it produces  
31  
32 uncorrelated, easy interpreted components that explain the greatest amount of  
33  
34 variance in the original food groups.<sup>29</sup> We determined the number of factors to retain  
35  
36 for interpretation on the basis of criteria as follows: factor eigenvalue greater than  
37  
38 1.15, the scree plot, the proportion of variance explained, and factor interpretability.<sup>9</sup>  
39  
40 Patterns were labeled based on food groups with absolute rotated factor loading  
41  
42 matrix greater than or equal to 0.50. Each participant was assigned a factor score for  
43  
44 each pattern (factor) by summing the intakes from each food group multiplied by  
45  
46 optimal weights (factor loadings).<sup>5</sup> Individuals with a higher factor score had a closer  
47  
48 adherence to that pattern.<sup>5</sup>  
49

50 Comparisons for baseline characteristics across quartiles of dietary patterns were  
51  
52 performed using ANOVA test for continuous variables and Chi-Square test for  
53  
54 categorical variables. Cox proportional hazards models, each adjusting for energy  
55

56  
57  
58  
59  
60  
9

1  
2  
3  
4  
5  
6 intake and critical covariates, were used to evaluate the association between  
7  
8 individual dietary pattern and CRC recurrence and mortality, represented by hazard  
9  
10 ratios (HR) and 95% confidence intervals (CI). Potential confounders were assessed  
11  
12 by the log-rank test in a univariate setting; those with the p-value less than 0.1 were  
13  
14 considered for inclusion. The final models only retained the items that entered the  
15  
16 models at  $p < 0.1$  or altered the effect estimates by 10% or more; these include ~~energy-~~  
17  
18 ~~intake~~, sex, age at diagnosis, stage at diagnosis, BMI, marital status, family history,  
19  
20 reported screening procedure, reported chemoradiotherapy, and MSI status. [All](#)  
21  
22 [models were run with the adjustment for total energy intake by including total calories](#)  
23  
24 [in the model](#). The assumption of proportional hazard rates was verified by checking  
25  
26 the parallelism of the Kaplan-Meier curves and by including time-dependent  
27  
28 covariates in the models to test for statistical significance.<sup>30</sup> Statistical linear trend was  
29  
30 examined by modeling the median value of each quartile as an ordinal variable in a  
31  
32 linear regression.<sup>5</sup> Potential interactions were evaluated by comparing estimates from  
33  
34 stratified analyses and testing significance of interaction terms with a Wald test.<sup>5</sup>

35  
36 A sensitivity analysis was implemented by eliminating stage-advanced patients  
37  
38 enrolled through proxies and re-calculating survival time from the completion of the  
39  
40 first questionnaire to a predefined event, in order to determine whether associations  
41  
42 might vary with the exclusion of stage-advanced cancer. Statistical significance was  
43  
44 accepted for two-sided  $p < 0.05$ . All data management and analyses were performed  
45  
46 with SAS software version 9.2 (SAS Institute Inc, Cary, NC).

## 47 48 49 **RESULTS**

50  
51 The cohort was followed for a median of 6.4 years (minimum: 1.3 years;  
52  
53 maximum: 10.9 years). A total of 168 patients died from all causes and 30 had a

54  
55  
56 10  
57  
58  
59  
60



1  
2  
3  
4  
5  
6 cancer recurrence or metastasis by the end of study follow-up (April, 2010).  
7  
8

### 9 10 **Dietary Patterns**

11  
12 Three distinct dietary patterns, labeled “processed meat pattern”, “prudent  
13 vegetable pattern” and “high sugar pattern”, were extracted using the aforementioned  
14 factor analysis procedure. These patterns explained 73.82% of total variance in the  
15 original 39 food groups (Table 1). A higher factor loading matrix of a given food  
16 group is representative of a greater contribution of that food group on that specific  
17 pattern. Therefore, the first pattern, termed “processed meat”, was characterized by  
18 higher loadings and thus higher consumptions of cured/processed meat,  
19 cured/processed red meat, red meat, fish, and processed fish; the second pattern,  
20 labeled “prudent vegetable”, displayed higher loadings on other greens, other fruit,  
21 other vegetables, and tomato sauce; and the third pattern, named “high sugar”, showed  
22 higher loadings on desserts and sweets, pies and tarts.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35

### 36 **Baseline Characteristics by quartiles of dietary patterns**

37  
38 Higher processed meat pattern scores at baseline were detected in men, ever  
39 smokers, patients who were single and individuals who had higher BMI at the time of  
40 diagnosis (Table 2). Higher prudent vegetable pattern scores were observed in women,  
41 never smokers, those with a slightly later age of diagnosis and with patients who had a  
42 tumor harboring the *p.V600E BRAF* mutation. None of these characteristics varied  
43 significantly by quartiles of high sugar pattern scores.  
44  
45  
46  
47  
48  
49  
50

### 51 **Dietary Patterns and Cancer Recurrence or Death**

52  
53 The highest quartile of processed meat pattern was significantly associated with  
54  
55

56 11  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 poorer DFS after the adjustment for other predictors of CRC recurrence and death  
7  
8 (HR: 1.82, 95% CI: 1.07-3.09), although no overall trend was observed in the HRs  
9  
10 across the whole distribution of factor scores (p for trend=0.09) (Table 3).  
11  
12 Nevertheless, neither the prudent vegetable pattern nor the high sugar pattern was  
13  
14 observed to be significantly associated with predefined patient outcomes (i.e., DFS  
15  
16 and OS).

17  
18 When stratified by tumor site, however, the association between processed meat  
19  
20 pattern and DFS remained statistically significant only for patients who had tumors  
21  
22 located in the colon (the highest versus the lowest quartile, HR: 2.29, 95% CI:  
23  
24 1.19-4.40) and not the rectum (HR: 0.97, 95% CI: 0.38-2.45). Similarly, when OS was  
25  
26 the outcome, the positive association between increasing consumption of the  
27  
28 processed meat pattern and mortality was restricted to patients whose tumors were  
29  
30 diagnosed in the colon (the forth versus first quartiles: HR: 2.13, 95% CI: 1.03-4.43).

31  
32 In the stratified analyses for dietary patterns ~~by sex, physical activity, and BRAF~~  
33  
34 ~~mutation status~~, there was evidence for effect modification by sex (p=0.04) for the  
35  
36 association of processed meat pattern with DFS (HR: 3.85 for women and 1.22 for  
37  
38 men) (Table 4). However, no evidence was observed to suggest that the effects of other  
39  
40 dietary patterns on cancer recurrence or death were modified by ~~sex~~, physical activity,  
41  
42 ~~or BRAF~~ mutation status and MSI (data not shown).

43  
44 In the sensitivity analysis, when advanced-stage ~~cases~~ patients who died before  
45  
46 admittance were excluded, the association between processed meat pattern and  
47  
48 survival among CRC patients remained significant.

Formatted: Indent: First line: 0.29"

## 51 DISCUSSION

52  
53 Three dietary patterns, termed “processed meat pattern”, “prudent vegetable  
54  
55

56 12  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 pattern” and “high sugar pattern”, were generated in this cohort study. We found that  
7  
8 high conformity with the processed meat pattern, characterized by high intakes of  
9  
10 processed meat, red meat, fish, and processed fish, is associated with decreased DFS  
11  
12 [of CRC, specifically of colon cancer. The differential associations by subsite indicate](#)  
13  
14 [disease heterogeneity](#). On the contrary, increasing consumption of the prudent  
15  
16 vegetable pattern and the high sugar pattern displayed no clear relationships with  
17  
18 mortality or recurrence.

19  
20 The processed meat pattern in the present study shares most characteristics of the  
21  
22 Western diet referred to in previous studies on CRC risk, which indicates a positive  
23  
24 association between the Western dietary pattern and CRC risk.<sup>7,9</sup> However, there has  
25  
26 been minimal research examining the association between dietary factors (e.g.,  
27  
28 nutrient, carbohydrate, protein and lipid intake) and survival of CRC patients,<sup>31,32</sup>  
29  
30 moreover, our literature review identified only one study that investigated the  
31  
32 relationship between dietary patterns and survival among CRC patients. Consistent  
33  
34 with our results, that prospective cohort study of 1009 stage III colon cancer patients<sup>9</sup>  
35  
36 reported a deleterious disease-free colon cancer prognosis for patients reporting high  
37  
38 levels of the Western dietary pattern intake.

39  
40 The mechanisms explaining the impact of red and processed meat on CRC  
41  
42 mortality are still unclear; however, some biologic mechanisms that link diet factors  
43  
44 to CRC risk may continue after diagnosis and subsequently impact cancer progression  
45  
46 and survival.<sup>33</sup> For example, strong carcinogens such as *N*-nitroso compounds (NOCs)  
47  
48 and probable carcinogenic mutagens like heterocyclic amines (HCA) and polycyclic  
49  
50 aromatic hydrocarbons (PAH), which have been suggested as significant contributors  
51  
52 for CRC development,<sup>34,35</sup> are found in smoked, fried or high-temperature cooked  
53  
54 meat. Sandhu et al<sup>36</sup> reported a Western dietary pattern is related to high levels of

55  
56 13  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 serum insulin and insulin-like growth factors (IGF), and these hormones are found to  
7  
8 be associated with tumor growth and the inhibition of apoptosis. In addition, a  
9  
10 growing body of evidence suggests that disruption of the normal gut microflora is  
11  
12 associated with human disease, including the pathogenesis of the intestinal tract (e.g.  
13  
14 inflammatory bowel disease) and other diseases such as obesity, cardiovascular  
15  
16 disease, and autoimmune conditions.<sup>37,38</sup> Alterations in intestinal microbiota are also  
17  
18 strongly associated with colonic polyp formation and with the risk of developing  
19  
20 CRC.<sup>39</sup> Given the major role of diet on the intestinal microbiome,<sup>40</sup> our findings  
21  
22 between dietary patterns and CRC survival may also be explained by the impact of  
23  
24 dietary patterns on gut microflora and health outcomes. \_

25  
26 The influence of processed meat pattern on survival was evident among women  
27  
28 rather than men in our study. Previous studies revealed that the higher colon pH and  
29  
30 longer intestine transit time in women compared to men can influence the production  
31  
32 of secondary bile acid or NOCs,<sup>41</sup> resulting in gender differences in the CRC  
33  
34 development. This is the first study that considered effect modifications between  
35  
36 dietary patterns and tumor molecular phenotype (i.e. *BRAF* mutation) on CRC  
37  
38 survival. *BRAF* mutation is found to be significantly associated with poor CRC  
39  
40 survival,<sup>42</sup> however, whether it can modify the impacts of dietary factors on CRC  
41  
42 survival is not known. Although stratified analyses in our study demonstrated a  
43  
44 processed meat diet to significantly decrease survival time only in patients with *BRAF*  
45  
46 wildtype type tumor, no evident interactions were detected. Further research is clearly  
47  
48 warranted to verify these findings and to determine the biologic pathways that  
49  
50 rationalize the underlying interactions between diet and tumor molecular features.

51  
52 A reasonably large sample size with detailed information of patients is a merit of  
53  
54 our study. These data not only includes demographic and personal lifestyle

1  
2  
3  
4  
5  
6 information, but also some molecular characteristics obtained from genetic testing.

7  
8 The ample information enables us to perform stratification analysis to control and  
9  
10 assess effect modifiers and confounders.

11  
12 Several limitations of this study should be recognized. Firstly, the results may be  
13  
14 skewed by recall bias since the participants recalled their food consumption from one  
15  
16 year prior to CRC diagnosis; however, this non-differential misclassification is only  
17  
18 expected to bias the results towards the null. Secondly, dietary patterns in this study  
19  
20 only reflect food consumption before diagnosis; it is unknown whether participants  
21  
22 modified their diet post diagnosis. Since previous research has shown minimal change  
23  
24 in diet between pre- and post- diagnosis among cancer patients,<sup>32</sup> the current study did  
25  
26 not examine dietary changes before and after diagnosis. Moreover, immortal  
27  
28 person-time bias may impact results. However, this is minimized by using proxies to  
29  
30 enroll deceased patients.

31  
32 In summary, we found that high conformity to the processed meat pattern is  
33  
34 significantly associated with an increased risk of all-cause mortality and recurrence of  
35  
36 CRC. Though our study did not find a difference in effect by tumor molecular  
37  
38 phenotype, larger molecular studies should be conducted to examine if such  
39  
40 differences exist. Ultimately, confirmation of these findings and the underlying  
41  
42 mechanisms await further studies. Our observation not only underlines the importance  
43  
44 of maintaining a healthy diet, but also provides guidance to efficacious dietary  
45  
46 interventions;<sup>8</sup> that is, people may lower their risk of CRC mortality by reducing  
47  
48 consumption of a processed meat pattern diet.

49  
50  
51  
52  
53  
54  
55  
56 15  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 **Contributors** PPW, YZ and HW contributed to the conception and design of this  
7 manuscript. YZ and HW analyzed the data. YZ, HW, PPW, JW, TW, RJ, ED, PTC  
8 drafted and revised the manuscript. SS, RG, MW, BR, SB, JRM and PSP were  
9 responsible for the data collection. All the authors provided final approval.  
10

11 **Acknowledgments** This work was supported by the Canadian Institutes of Health  
12 Research Team Grant [CIHR-CPT79845] and Canadian Institutes of Health Research  
13 Team in Interdisciplinary Research on Colorectal Cancer Studentship [205835].  
14 Yun Zhu was awarded by the Newfoundland and Labrador Centre for Applied Health  
15 Research through a Master's fellowship.  
16

17 **Competing interest** None.  
18

19 **Ethics approval** Human Investigation Committee in Memorial University of  
20 Newfoundland.  
21

22 **Data sharing statement** No additional data available.  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

**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	<b>0.69</b>	-	0.17
Cured/processed red meat	<b>0.73</b>	-	0.21
Cured/processed meat	<b>0.93</b>	-	-
Game	0.23	-	-
Poultry	0.22	0.27	-
Fish	<b>0.58</b>	0.32	-0.22
Processed fish	<b>0.50</b>	0.25	-
Fruit juice	-	0.24	0.23
Root vegetables	0.28	-	0.15
Cruciferous vegetables	-	<b>0.54</b>	-
Other fruit	-	<b>0.59</b>	-
Other greens	-	<b>0.60</b>	-0.22
Tomato sauce	-	<b>0.50</b>	-
Other vegetables	0.22	<b>0.54</b>	-
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	-	-

Formatted: Font: Bold

Formatted: Font: 10.5 pt

17

White wine	-	-	-
Red wine	-	-	-
Liquor	-	-	-
Desserts and sweets	0.31	-	<b>0.63</b>
Pies, tarts	0.15	-	<b>0.54</b>
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.



**Table 2. Baseline Characteristics of 529 CRC Patients by Quartiles of the Three Major Dietary Patterns<sup>a</sup>**

	Processed Meat Pattern				P Value <sup>c</sup>	Prudent Vegetable Pattern				P Value <sup>c</sup>	High Sugar Pattern				P Value <sup>c</sup>
	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 <sup>b</sup>	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 <sup>b</sup>															
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 <sup>2</sup> )															
<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

<sup>a</sup> 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

<sup>b</sup> Continuous variables presented as mean±SD (standard deviation); categorical variables presented as number<sup>43</sup>

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

**Table 3. Hazard Rate Ratios Associated with Disease-Free and Overall Colorectal Cancer Survival for Quartiles of Dietary Patterns<sup>a</sup>**

	Disease-Free Survival				Overall Survival			
	No. of Events <sup>b</sup> /No. at Risk	Overall CRC HR (95% CI) <sup>c</sup>	Colon cancer HR (95% CI) <sup>c</sup>	Rectal cancer HR (95% CI) <sup>c</sup>	No. of Events <sup>b</sup> /No. at Risk	Overall CRC HR (95% CI) <sup>c</sup>	Colon cancer HR (95% CI) <sup>c</sup>	Rectal cancer HR (95% CI) <sup>c</sup>
<b>Processed meat pattern</b>								
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 <sup>d</sup>		0.09	0.12	0.91		0.25	0.40	0.59
<b>Prudent vegetable pattern</b>								
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 <sup>d</sup>		0.62	0.83	0.19		0.90	0.60	0.92
<b>High sugar pattern</b>								
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 <sup>d</sup>		0.89	0.90	0.11		0.52	0.56	0.64

<sup>a</sup> Abbreviations are as follows: CRC, colorectal cancer; HR, hazard rate ratios; CI, confidence interval;

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

<sup>c</sup> 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.

<sup>d</sup> 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.

**Table 4. Disease-Free Colorectal Cancer Survival in Relation to Quartiles of Dietary Patterns by Selected Lifestyle and Tumor Characteristics<sup>a</sup>**

	No. of Events <sup>b</sup> /No. at Risk	Quartiles HR (95% CI) <sup>c</sup>				P for Trend <sup>d</sup>	P for Interaction <sup>e</sup>
		Q1	Q2 <sup>c</sup>	Q3	Q4		
<b>Processed meat pattern</b>							
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
<b>BRAF mutation status</b>							
WideWild 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
<b>Prudent vegetables pattern</b>							
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
<b>BRAF mutation status</b>							
WideWild 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
<b>High sugar pattern</b>							
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
<b>BRAF mutation status</b>							
WideWild 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

<sup>a</sup> Abbreviations are as follows: CI, confidence interval; METs/week, metabolic equivalent hours per week;

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

<sup>c</sup> 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.

<sup>d</sup> 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.

<sup>e</sup> *P* for interaction is the significance of interaction term between smoking and respective stratification variable, calculated from Wald test.

For peer review only

## 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, Roebathan B, Buehler S, Sun Z, Cotterchio M, Youngusband 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, Roebathan 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, Roebathan 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, Youngusband 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, Roebotian 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 EMAS 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](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

- 1  
2  
3  
4  
5  
6 32. Dray X, Boutron-Ruault MC, Bertrais S, Sapinho D, Benhamiche-Bouvier AM, Faivre J. Influence  
7 of dietary factors on colorectal cancer survival. *Gut* 2003;52(6):868-73.  
8  
9 33. Dolecek TA, McCarthy BJ, Joslin CE, Peterson CE, Kim S, Freels SA, et al. Prediagnosis food  
10 patterns are associated with length of survival from epithelial ovarian cancer. *J Am Diet Assoc*  
11 2010;110(3):369-82.  
12  
13 34. Bingham SA, Pignatelli B, J.R. P, A. E, C. M, G. G, et al. Does increased endogenous formation of  
14 N-nitroso compounds in the human colon explain the association between red meat and colon  
15 cancer? *Carcinogenesis* 1996;17(no.3):515-523.  
16  
17 35. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ*  
18 *Mol Mutagen* 2004;44(1):44-55.  
19  
20 36. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF  
21 binding proteins, their biologic interactions, and colorectal cancer. *J Natl Cancer Inst.* 2002  
22 94(13):972-80.  
23  
24 37. Rabizadeh S, Sears C. New horizons for the infectious diseases specialist: how gut microflora  
25 promote health and disease. *Curr Infect Dis Rep* 2008;10(2):92-8.  
26  
27 38. Kinross JM, Darzi AW, Nicholson JK. Gut microbiome-host interactions in health and disease.  
28 *Genome Med* 2011;3(3):14.  
29  
30 39. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal  
31 cancer: beyond the usual suspects. *Nat Rev Microbiol* 2012.  
32  
33 40. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the  
34 human intestine. *Science* 2005;307(5717):1915-20.  
35  
36 41. Takachi R TY, Baba K, Inoue M, Sasazuki S, Iwasaki M, Tsugane S, Japan Public Health  
37 Center-Based Prospective Study Group. Red meat intake may increase the risk of colon cancer  
38 in Japanese, a population with relatively low red meat consumption. *Asia Pac J Clin Nutr.*  
39 2011;20(4):603-12.  
40  
41 42. Safaee Ardekani G, Jafarnejad SM, Tan L, Saeedi A, Li G. The Prognostic Value of BRAF  
42 Mutation in Colorectal Cancer and Melanoma: A Systematic Review and Meta-Analysis.  
43 *PLoS One* 2012;7(10):e47054.  
44  
45 43. Bingham SA PB, Pollock JR, Ellul A, Malaveille C, Gross G, Runswick S, Cummings JH, O'Neill  
46 IK. Does increased endogenous formation of N-nitroso compounds in the human colon  
47 explain the association between red meat and colon cancer? *Carcinogenesis*  
48 1996;17(no.3):515-523.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

	Item No	Recommendation
<b>Title and abstract</b>	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
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
<b>Methods</b>		
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
<b>Results</b>		
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
<b>Discussion</b>		
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
<b>Other information</b>		
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>.