ASSOCIATION OF DIETARY INTAKES WITH RISK
OF COLORECTAL CANCER:
RESULTS FROM A POPULATION-BASED
CASE-CONTROL STUDY IN NEWFOUNDLAND
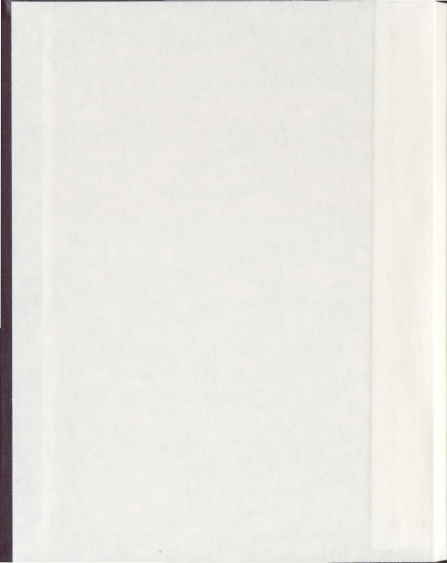## AND LABRADOR AND ONTARIO

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Association of dietary intakes with risk of colorectal cancer: results from a population-based case-control study in Newfoundland and Labrador and Ontario

by

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Abstract

Colorectal cancer (CRC) is the second leading cause of death from cancer in men and women combined in Canada. Research to date suggests that CRC is a result of complex genetic-environmental interactions and diet has long been regarded as the most important environmental factor. Yet epidemiologic studies have been inconclusive in demonstrating the role of dietary factors in the etiology of CRC. This thesis examined the effects of dietary factors on CRC risk using data from a large population-based case-control study conducted in Newfoundland and Labrador (NL) and Ontario (ON) through three coherent papers. The first two papers were based on pooled data from the two provinces assessing the effects of total energy/macronutrients and selected micronutrients on CRC, respectively. Overall, findings from these two parts of the thesis suggested that diets high in energy increased the risk, whereas diets high in fibre, carbohydrate and protein reduced the risk of CRC. Significant protective effects on CRC were also observed for calcium, vitamin D, vitamin C, riboflavin and folate. Stratified analyses by supplement intake status further indicated that the protective effects varied according to nutrients intake levels from foods. In addition, among individual supplement users, the protective effects were more pronounced in people with lower nutrients intake from foods. Based on the findings from the first two papers, this thesis further compared the effects of calcium, vitamin D and dairy products on the occurrence of CRC risk between two provinces. While the results were consistent in both provinces, statistical associations were only observed in the ON population. Additionally, this study also found significant synergistic effects between calcium and vitamin D intake. In summary, based on a large population

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case-control study, this thesis comprehensively evaluates various micronutrients and macronutrients in relation to CRC. This work corroborates and adds to the existing literature in many aspects. Findings from this thesis may have important public health implications, particularly for those with lower intakes for a number of selected nutrients, who can benefit greatly from supplement intakes.

Acknowledgments

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Abbreviations

CRC	colorectal cancer
NL	Newfoundland and Labrador
ON	Ontario
CTCC	Colorectal Cancer Interdisciplinary Health Research team in Colorectal Cancer
NFCCR	Newfoundland familial colorectal cancer registry
OFCCR	Ontario familial colorectal cancer registry
CCFR	Colon Cancer Familial Registries
ICD	international classification of disease
FHQ	family history questionnaire
PHQ	personal history questionnaire
FFQ	food frequency questionnaire
NSAID	nonsteroidal anti-inflammatory drug
BMI	body mass index
HRT	hormone replacement therapy
METs	metabolic equivalent hours
RDA	recommended dietary allowances
OR	odds ratios
95%CI	95% confidence interval

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Chapter 1: Introduction

Diet has long been regarded as one of the most important environmental factors associated with the cause of colorectal cancer (CRC)[1,2], particularly when an unbalanced diet is combined with weight gain, physical inactivity, and such unhealthy practices as smoking and consuming a great deal of alcohol[1,3,4]. Several case-control studies investigating the influence of diet on the risk for CRC have demonstrated a positive association between cancer risk and total energy intake[5-9]. The association between CRC and total energy intake has an important impact on the interpretation of dietary data.

Thus, excess intake of any of the important energy-supplying macronutrient components of the diet (eg, proteins, fats, carbohydrates) could contribute to a higher risk of CRC. However, whether or not individual energy-supplying macronutrients, independent of their contribution to energy intake, are related to CRC risk remains controversial. A comprehensive report from the World Cancer Research Fund and the American Institute for Cancer Research[10] concluded that total energy has no simple relationship with CRC risk, that the data were inconsistent for carbohydrates, cholesterol and proteins, and that diets high in total fat possibly increase risk. Therefore, the objective of the first component in this thesis is to identify the association between CRC risk and intakes of total energy, proteins, fats, carbohydrates (specifically including cholesterol, selected fatty acids and dietary fibre), and alcohol using data from a large case-control study conducted in Newfoundland and Labrador (NL) and Ontario (ON).

Consistent with the theme of dietary intake's influence on CRC, the second component of this thesis is to examine the effects of selected micronutrients on the

occurrence of CRC. Recent epidemiologic studies suggest that calcium, vitamin D, folate, and some antioxidants are protective against cancer[11-13], whereas iron increases risk[14]. Calcium and vitamin D may protect against colorectal carcinogenesis by binding free fatty acids and secondary bile acids in the small intestine, thereby protecting colonic epithelial cells from mutagens[15]. Folate may exert a protective effect on colorectal carcinogenesis as a co-factor in the methylation of thymidylate for DNA synthesis and the production of S-adenosylmethionine, the primary methyl donor in the body[13,16]. B6 and folate may function as coenzymes in the synthesis of purines and thymidylate for DNA[17-19]. Carotene and vitamins A, C, and E may decrease the risk through antioxidant mechanisms[20,21]. Finally, iron may increase the risk of CRC by generating free radicals that attack DNA and damage chromosomes[22,23].

Despite the biological plausibility, epidemiological studies have been inconclusive in demonstrating the role of the above micronutrients in the etiology of CRC. Prior to 1998, epidemiologic studies suggested that calcium intake was not associated with a lower risk of colorectal adenoma or cancer[24]. In contrast, Ryan-Harshman[25] recently reviewed several case-control and prospective cohort studies and some clinical trials and concluded that the evidence of calcium and vitamin D being protective against CRC was stronger. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group conducted a randomized, double-blind, placebo-controlled primary-prevention trial and found no interaction between alpha-tocopherol and beta carotene with respect to the incidence of CRC[26]. After four years, results from another double-blind three-year intervention with antioxidants (beta-carotene 15 mg, vitamin C 150 mg, vitamin E 75 mg,

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selenium 101 microg) suggested a protective role of antioxidants on new adenoma formation. No effect was detected on the growth of adenomas and polyps[27].

There may be a number of reasons for these inconsistencies between studies, such as the potential selection bias and possibly recall bias that could have played a larger role in retrospective studies. If some micronutrients are beneficial, whether or not diet alone can furnish enough micronutrients to have a substantially protective effect on CRC is a question. With the increasing frequency of using individual or multivitamin supplements by the Canadian population, whether or not supplement could exert a protective effect against CRC becomes a crucial question. Therefore, the second component of this study examines potential relationships between selected micronutrients and the risk of CRC in population residing in two Canadian provinces.

Based on the results from the second paper we found protective effects of calcium and vitamin D on CRC risk. Dairy products contains large amount of calcium and vitamin D through fortification. Whether or not dairy products are protective against CRC is a question. Again, if dairy product intakes are associated with a lower risk of CRC, whether these associations can be explained by components of calcium and vitamin D. Recently, a prospective study by Jarvinen *et al.* [28] indicated that individuals with high consumption of milk have a reduced risk of colon cancer; however, the association does not appear to be due to intakes of calcium and vitamin D. In contrast, it has been shown that calcium, especially in combinations as found in milk, effectively precipitates luminal cytotoxic surfactants and thus inhibits colonic cytotoxicity [29,30].

Given the high incidence rate of CRC in NL and ON, it is of great public health

importance to compare differences of these associations between the two provinces. To our knowledge, little has been done in this area. Therefore, the purpose of third component of this study is to assess the effects of calcium, vitamin D and dairy products on the occurrence of CRC and compare differences of these associations between two provinces.

1.1 Study objectives

1. To identify an association between CRC risk and intakes of total energy, proteins, fats, carbohydrates (specifically including cholesterol, selected fatty acids and dietary fibre), and alcohol using data from a large population-based case-control study conducted in NL and ON.

2. To examine the relationships between selected micronutrients (calcium, iron, retinol, vitamin C, vitamin D, alpha-tocopherol, thiamin, riboflavin, vitamin B6, vitamin B12, and folate) and the risk of CRC in population residing in two provinces.

3. To assess the effects of calcium, vitamin D and dairy products in the occurrence of CRC and compare differences of these associations between two provinces.

1.2 Involvement of author in thesis

The author was not involved in the conceptualization or implementation of the familial colorectal cancer registries used in this thesis. Drs. John McLaughlin and Steven Gallinger were the Principal Investigators of the Ontario component of this study; Drs. H. Ban Younghusband and Pat Parfrey were the Principal Investigators of the Newfoundland component. Dr. Peter Wang was the principal investigator for the component project of "Exploration of Risk Factors, Inter-provincial Differences and Risk

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Modifiers". Peter Todd Campbell cleaned and converted the previously unused epidemiologic questionnaire data from Newfoundland to conform to the Ontario data formats. The author played a substantial role in conceptualizing and conducting the studies presented in this thesis. The author was responsible for the statistical analyses and presentation of the findings from this thesis.

1.3 Organization of the thesis

This thesis is divided into five chapters. Chapter 1 is an overall introduction to the study. Chapter 2 reviews the incidence and mortality of CRC and associated factors. Chapter 3 reviews in detail the research methods employed in this study. Chapter 4 includes three results sections. Each section is written in a manuscript format, including its own *Introduction, Methods, Results,* and *Discussion* section. Some repetition of Methods was unavoidable. Chapter 5 summaries the key findings and discusses the implications of the study results and future research.

Chapter 2: Background

2.1 Colorectal cancer: the disease

Colorectal cancer (CRC) includes cancerous growths in the colon, rectum and appendix. The colon is the first four to five feet of the large intestine. The colon consists of the cecum, the ascending colon, the transverse colon, the descending colon, and the sigmoid colon (Figure 1). The main function of the colon is to absorb water and nutrients from food. The rectum is the last six to ten inches of the digestive tract. Its main function is to store waste material prior to excretion.

CRC is an age-related disease with half of all cases occurring in individuals aged over 60 years[31]. The disease is believed to arise from benign tumors called adenomatous polyps (adenomas). About 1-10% of adenomas go on to develop into invasive cancer[32]. In general, the diagnosis of CRC is through colonoscopy and therapy is usually through surgery which may be followed by chemotherapy.

Figure 1: Anatomy of the colon and rectum[33]

Source: http://www.fitcare.net/category/cancer/

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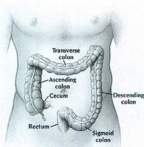
2.2 The incidence and mortality of CRC

With 655,000 deaths worldwide per year, CRC is the third most common form of cancer and the third leading cause of cancer-related death in the Western world[34,35]. Incidence and mortality rates vary widely throughout the world. High rates are characteristic of developed countries in North America, northern and western Europe, the United Kingdom, Australia, and New Zealand, where meat consumption is characteristically high and cereal consumption is low[36]. The lowest rates in the world are found in Asia, Africa, and most of Latin America, where there is a high per capita consumption of fibre-rich vegetables and starch carbohydrates[36]. This 30-fold difference in incidence underscores the importance of environmental factors in inducing this cancer.

In Canada, CRC is the second leading cause of death from cancer in men and women combined. In 2009, an estimated 22,000 Canadians were diagnosed with CRC and 9,100 died of it[37]. At current rates, one in 14 men is expected to develop CRC during his lifetime and one in 27 will die of it; one in 15 women is expected to develop CRC during her lifetime and one in 31 will die of it[38]. The incidence and mortality rates increase precipitously with age in both sexes. In 2009 it was estimated that more than half of all newly diagnosed CRC would occur among Canadians aged 70 years or older[38]. The estimated age-standardized incidence rates of CRC were 62 per 100,000 among men and 41 per 100,000 among women, and the estimated age-standardized mortality rates were 26 per 100,000 men and 16 per 100,000 women in 2009.

There are substantial inter-provincial incidence rate variations in Canada. The highest CRC incidence rates are seen among men in Newfoundland and Labrador (NL)

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with a rate of 87 per 100,000, and among women in Prince Edward Island, Nova Scotia and NL (incidence rates of 52 per 100,000 in NL females). The lowest rates for both sexes are in British Columbia [34]. Ontario (ON) ranks in the middle among Canadian provinces at rates of 60 per 100,000 among men and 41 per 100,000 among women[58].

2.3 Factors associated with CRC

Although high rates of the disease in NL may be partly explained by a higher prevalence of families with a predisposition to hereditary colon cancer[39], environmental factors may be an important component of CRC risk[1,40-43].

2.3.1 Hereditary factors

About 15% of people who develop CRC have disease that is familial[44]. Those with a family history of CRC or adenomatous polyps in any first-degree relative younger than age 60, or two or more first-degree relatives at any age are considered at increased risk for the disease. Among familial cases, a small proportion is attributed to the major CRC predisposition syndromes, familial adenomatous polyposis (FAP), and hereditary non-polyposis colorectal cancer (HNPCC).

FAP is a disease where people typically develop hundreds of polyps in their colon and rectum. Usually this occurs between the ages of five and 40. FAP is caused by mutations in the Adenomatous polyposis coli (APC) gene. In people with FAP, this gene is inoperative, leading to the growth of hundreds of polyps in the colon and rectum. Over time, gene mutations in the cells of the polyps cause the polyps to become cancerous[45]. FAP occurs in less than 1% of all CRC patients[44].

HNPCC accounts for 1% to 3% of all CRCs[46]. This syndrome also develops when

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people are relatively young. These people have polyps, but they only have a few, not hundreds as in FAP. This syndrome is characterized by early onset of CRC with microsatellite instability. Microsatellite instability is a molecular marker for DNA mismatch repair deficiency. Mutations in mismatch repair genes lead to a lifetime colon cancer risk of 85% in these patients. Carcinomas of the endometrium, ovary, and other organs also occur with increased frequency[45].

2.3.2 Environmental factors

Genetic predisposition plays a role in about 15% of CRC but most cases are sporadic[47]. About two decades ago, Doll and Peto[1], using international comparisons of exposure prevalences and disease rates, estimated that up to 90% of colon cancers may have a primary dietary contribution. A more recent study estimated that about 70% of CRC can be prevented by changes in diet and lifestyle[43]. Migrant studies of first- and second-generation Japanese immigrants to the United States show that their mortality from colon cancer is closer to that of the United States than to Japanese rates which are 2.5 times lower[40]. Similar variations are observed in European migrants to the United States and Polish migrants to Australia[41,42]. Immigrants rapidly acquire the incidence rates of the host country, suggesting that environmental factors play a crucial role in CRC development.

Epidemiologic population studies have shown that obesity, physical inactivity, tobacco and alcohol use, total caloric intake, dietary fat intake, meat consumption, ethanol and cholesterol intake may be related to an increased risk of CRC[48]. Similar studies have suggested that a decreased risk of CRC is associated with increased intake of dietary fibre and that a protective effect may be related to consumption of synthetic

antioxidants, such as vitamin A, beta-carotene and ascorbic acid [49]. Each of these potential risk factors will be considered individually.

2.3.2.1 Diet

Diet has long been regarded as the most important environmental factor for colon cancer[1], particularly when a poor diet is combined with inappropriate weight gain, physical inactivity, and such unhealthy practices as smoking and consuming a great deal of alcohol[1,3,4]. High intakes of animal fat and/or protein and low intakes of fruits and vegetables have repeatedly been shown to increase the risk of CRC[12]. However, although diet and colon cancer relationships have been studied extensively, the impact of many dietary factors on colon carcinogenesis remains unresolved[50-63].

2.3.2.1.1 Red meat and fat

Most studies have shown a positive association between red meat consumption and the risk of CRC[64]. One possible reason is that a diet high in meat tends to be low in vegetables, fruit, and fibre. Additionally, red meat is a major source of fat, especially saturated fat. It is thought that fat promotes large bowel cancer by increasing the levels of free ionized fatty acids and unconjugated bile acids in the bowel lumen, which are irritating and toxic to the surrounding epithelium [65,66]. Besides the role of fat itself, the carcinogenic effect of heterocyclic amines produced during cooking of red meat has been suggested as a link between red meat and CRC[67].

A Meta-analysis of 13 published cohort studies determined that a daily increase in consumption of all meat or red meat by 100 g was associated with a 12% to 17% increase in the risk of CRC[53]. However, in 2000 an expert panel workshop reviewed 30 case-

control and 15 cohort studies conducted over largely the same period as the above meta-analysis, and the report showed that red meat was not associated with CRC risk[32,54]. Together with more recent studies, these data indicate a modest positive association between red meat intake and CRC risk, while a significant association was found in intakes of processed meats[50,51].

As with red meat, total dietary fat intake is not strongly associated with CRC risk[56–58], despite what has been suggested in experimental studies on rats and mice[55,62]. Results from a prospective cohort study of 47,949 U.S. male health professionals between 1986 and 1992 showed that intakes of total fat, saturated fat, and animal fat were not related to the risk of colon cancer[58]. Howe *et al.* indicated no links between total dietary fat or saturated fat and CRC risk in pooled individual-level data from 13 case-control and cohort studies that included 5,287 CRC cases and 10,470 controls[56]. However, in a Swiss case-control study, Levi *et al.* found that different types of fat may play different roles in colorectal carcinogenesis; saturated fats were related to increased risk of CRC, while monounsaturated and polyunsaturated fats reduced the risk[6]. Results from an Italian case-control study found that only polyunsaturated fat intake was inversely associated with colon cancer risk, particularly in the right colon[7].

2.3.2.1.2 Vegetables, fruits and fibres

In 1971 Burkitt first proposed that high-fibre diets were associated with increased fecal bulk and faster rates of intestinal transit. These changes in colonic function would help to dilute out any carcinogens or tumor promoters present within the intestinal lumen while also reducing the time available for their interaction with the intestinal epithelium[63].

Fruits, vegetables and whole grains contain a high amount of fibre. Terry *et al.* examined fruit, vegetable, and fibre intakes and the risk of CRC among Swedish women, and concluded that higher consumption of fruit was associated with a 32% reduction in CRC risk, while higher intake of cereal fibre did not lower the risk[60]. Results from a case-control study in the Swiss Canton of Vaud found a significant inverse relationship between total fibre intake, intake of certain specific fibre and risk of CRC. Vegetable fibre appeared to be more protective than either fruit or grain fibre[59]. In contrast, in a comprehensive prospective study followed up over 16 years to examine the role of fibre and its components on the risk of colorectal neoplasms, Fuchs *et al.* found no protective effect of a high-fibre diet against CRC or adenoma. Furthermore, no significant associations were observed in intakes of cereal, fruit, or vegetable fibre[61].

Experimental data regarding the influence of vegetables, fruits and dietary fibre on colorectal carcinogenesis are inconsistent. This is likely due to a complex relationship. Grain fibre may have a different effect than fruit fibre or vegetable fibre. Moreover, other components of vegetables and fruits, such as vitamins and minerals, may be the factors responsible for any reduction in cancer risk[68].

2.3.2.1.3 Total energy and macronutrients

CRC rates are highly correlated with economic development or "Westernization." Although several factors related to the Western lifestyle may contribute to colon cancer, a large and growing body of evidence implicates energy balance. High energy expenditure seems to decrease the risk of CRC[69]. Case-control studies investigating the influence of diet on the risk for CRC have demonstrated a positive correlation between cancer risk and

total energy intake[9-10]. Energy intake can be responsible for glycaemic overload and a compensatory increase of serum insulin and the related insulin growth factor-1 (IGF-1). IGF-1 is a promoter of tumor cell growth in vitro[58,70], and it may expose colonic and rectal cells to a proliferative stimulus [71,72]. Thus, diabetes has also been related to increased CRC risk[71,73].

The correlation between CRC and total energy intake has an important impact on the interpretation of dietary data. Excess intake of any of the important energy-supplying macronutrient components of the diet (eg. proteins, fats, carbohydrates) could contribute to a higher risk of CRC. However, the question of whether or not individual energy-supplying macronutrients, independent of their contribution to energy intake, are related to CRC risk remains controversial. A report from the Nurses' Health Study published in 1990 showed that, after adjustment for total energy intake, consumption of animal fat was associated with increased risk of CRC, but no association was found with vegetable fat[74]. In contrast, reports from prospective cohort studies found that intakes of total fat, saturated fat, and animal fat were not related to risk of colon cancer[58]. Non-red meat sources of animal protein derived from low-fat dairy products, fish and poultry have been typically associated with a lower risk of CRC[58,75]. A comprehensive report from the World Cancer Research Fund and the American Institute for Cancer Research concluded that total energy has no simple relationship with CRC risk and that data are inconsistent for carbohydrates, cholesterol and proteins[10]. Thus, the epidemiological evidence on the relationship between CRC risk and intake of macronutrients remains largely unclear.

2.3.2.1.4 Micronutrients

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The role of various micronutrients has been considered in colorectal carcinogenesis. Recent epidemiologic studies suggest that calcium, vitamin D, folate, vitamin B6, and some antioxidants are protective against cancer[11-13], whereas iron increases risk[14]. Calcium has been hypothesized to protect against CRC by binding secondary bile acids and ionized fatty acids in the colon lumen to form insoluble calcium soaps, thereby reducing their proliferative effects on the colonic mucosa[15]. Furthermore, calcium may have effects on cell proliferation and differentiation, apoptosis, angiogenesis, and cell-cycle regulation[76,77]. The roles of dietary calcium and vitamin D are highly correlated because vitamin D regulates the absorption of calcium[78]. Folate may exert a protective effect on colorectal carcinogenesis as a co-factor in the methylation of thymidylate for DNA synthesis and the production of S-adenosylmethionine, the primary methyl donor in the body[13,16]. Antioxidants, including carotene and vitamins A, C, and E, may decrease the risk by quenching free radicals and reducing oxidative damage to DNA[20,21]. Vitamin B6 and folate function as coenzymes in the synthesis of purines and thymidylate for DNA. Low levels of these vitamins may result in misincorporation of uracil into DNA, leading to chromosome breaks and disruption of DNA repair[17-19]. Iron may increase the risk of CRC by generating free radicals that attack DNA and damage chromosomes[22,23].

Despite the plausibility of the biologic mechanisms proposed to explain the effects of these micronutrients on colorectal carcinogenesis, the epidemiological evidence on this issue is unclear. Prior to 1998, epidemiologic studies suggested that calcium intake was not associated with lower risk of colorectal adenoma or cancer[24]. However, after a follow-up of 61,463 women for 11.3 years, Terry *et al.* found an inverse association

between dietary calcium intake and CRC risk. Vitamin D intake was not clearly associated with risk[79]. Results from the Cancer Prevention Study II Nutrition Cohort indicated that calcium modestly reduces risk of CRC. Vitamin D was associated with reduced risk of CRC only in men[80]. Recent research indicates that calcium and vitamin D might act together, rather than separately, to reduce risk of CRC. Results from a multi-centre, placebo-controlled randomized clinical trial found that calcium supplementation was inversely associated with adenoma recurrence only when circulating vitamin D levels were above the median (29.1 ng/ml)[81].

Vegetable and fruits are generally accepted as having protective effects against CRC[59,60], however, researchers have recently found weak or nonexistent inverse associations for total fruits and vegetables[61,82,83]. Vegetables and fruits are major sources of dietary antioxidants, so the weak results in recent studies may reflect on the true strength of the association between consumption of dietary antioxidants and risk of colorectal neoplasia. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group conducted a randomized, double-blind, placebo-controlled primary-prevention trial and found no interaction between alpha-tocopherol and beta carotene with respect to the incidence of CRC[26]. In contrast, after four years, results from another double-blind three-year intervention with antioxidants (beta-carotene 15 mg, vitamin C 150 mg, vitamin E 75 mg, and selenium 101 microg) suggested a protective role of antioxidants on new adenoma formation. No effect was detected on the growth of adenomas and polyps[27].

In North America, multivitamins are major sources of folate. Recent evidence from

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Cancer Prevention Study II Nutrition Cohort Study of 145,260 men and women indicated that regular (≥4 times/wk) use of multivitamins 10 years before enrolment was associated with reduced risk of CRC (RR=0.71)[84]. In contrast, Zhang *et al.* followed up 37,916 US women for 10.1 years to examine the role of folate and vitamin B6 on colorectal carcinoma. They concluded that the use of multivitamin supplements was not related to CRC risk. Their findings suggested the protective role of dietary folate and vitamin B6 on CRC[85].

2.3.2.1.5 Dairy products

Dairy products are important components of the human diet. Many components of dairy foods have been shown experimentally to protect against CRC. These components include calcium and vitamin D (which have been discussed before), conjugated linoleic acid[86], sphingolipids[87], and butyric acid which can potentially be formed by colonic lactobacilli from milk products. Dairy products with probiotics may be beneficial in reducing CRC neoplasia[88]. However, whole milk and many types of cheese have a relatively high fat content, which may increase the risk of colorectal adenoma and cancer[89].

Many studies have found inverse relationships between dairy product consumption and CRC, although the most important components responsible for the apparent protection remain unclear[28-30,90]. Recently, a prospective study of Jarvinen *et al.* indicated that individuals with high consumption of milk have a potentially reduced risk of colon cancer. The association does not appear to be due to intakes of calcium, vitamin D, or to the specific effects of fermented milk[28]. In contrast, it has been shown that calcium,

especially in combinations as found in milk, effectively precipitates luminal cytolytic substances and reduces cytotoxicity of fecal water, an accepted risk marker for colon cancer[29,30]. Cho et al. conducted a pooled analysis of 10 cohort studies including 534,536 individuals followed up 6 to 16 years to examine a possible association between dairy products and calcium intakes with CRC risk. They found that intakes of milk and calcium were inversely related to CRC risk with a continued dose-response effect on CRC risk[90].

2.3.2.2 Smoking

Smoking has been implicated in many malignant diseases. More recent evidence from two meta-analyses of 28[91] and 36[92] prospective studies found that a consistent association exists between smoking and CRC. Both studies found that the associated risk was stronger for rectal cancers. The association of tobacco consumption and CRC risk appeared to be dose-related. All four dose-response variables examined-daily cigarette consumption (RR = 1.38 for an increase of 40 cigarettes/day), duration (RR = 1.20 for an increase of 40 years of duration), pack-years (RR = 1.51 for an increase of 60 pack-years) and age of initiation (RR = 0.96 for a delay of 10 years in smoking initiation)-were significantly associated with CRC incidence[92]. Based on estimates from various studies of the U.S. population attributing the risk of CRC to smoking, approximately 12% of CRC deaths were due to smoking[93].

2.3.2.3 Alcohol drinking

Alcohol drinking together with smoking may additively affect CRC risk. More recent results from a prospective study of the Singapore Chinese population indicated that subjects who drank seven or more alcoholic drinks per week had a 72% increased risk of

CRC, and suggested that alcohol drinking and smoking may share a common etiologic pathway in rectal carcinogenesis[94]. In addition, the National Health and Nutrition Examination Survey I Epidemiologic Follow-Up Study found that a 70% increased risk of colon cancer was observed in those with a history of 34 years alcohol drinking as compared to nondrinkers[95]. When folate intake is low, alcohol appears to increase the CRC risk. This interaction may be related to the antifolate properties of alcohol[96].

2.3.2.4 Obesity

Many studies have found that obesity, usually assessed by body mass index (BMI), is associated with an increased risk of colon cancer[12,97]. The following evidence also suggests that several indicators of adiposity and adipose tissue distribution are related to CRC risk. Results from a large cohort study of U.S. males showed that BMI ≥ 29 kg/m^2 relative to BMI < 23 kg/m^2 was linked with an approximate 1.5-fold increased risk of CRC; waist circumference ≥ 43 inches relative to waist circumference < 35 inches was linked with about a 2.5-fold increased risk of disease; and waist-to-hip ratio ≥ 0.99 relative to waist-to-hip ratio < 0.90 was linked with an almost 3.5-fold increased risk[98]. More recent cohort data indicated that BMI, waist circumference, and fat mass were similarly associated with increased CRC risk[99]. Not all studies support these associations[100,101].

2.3.2.5 Physical activity

Whereas obesity increases risk, physical activity is associated with a decreased risk. Over 50 studies in diverse populations showed that physical activity was associated with lower risk for colon cancer, though not for rectal cancer. As much as 50% of a reduction in

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incidence of colon cancer has been observed among individuals with high levels of physical activity[102]. It is estimated that two hours or more of physical activity per week can significantly reduce the risk of CRC in most people[36]. Although physical activity is often associated with other lifestyle factors that may be related with reduced colon cancer risk[38], the inverse association between colon cancer risk and physical activity appears to be independent. Thune *et al.* concluded that physical activity may shorten the fecal transit time and thereby reduce the period of contact between carcinogens and mucosal cells, inducing favourable effects on insulin, prostaglandin, and bile acid levels, which may otherwise influence the growth and proliferation of colonic cells[103].

Chapter 3: Research methods

3.1 Collaborative data sources

This study occurred within the *Colorectal Cancer Interdisciplinary Health Research team in Colorectal Cancer* (CTCC), which was established in 2001. In 1997, the Ontario Familial Colorectal Cancer Registry (OFCCR)[104,105] was established by the U.S. National Cancer Institute as one of six international sites in the consortium of Colon Cancer Familial Registries (CCFR)[106]. In 1999 the Newfoundland Familial Colorectal Cancer Registry (NFCCR) was established by CTCC modeled on the existing OFCCR. The methods of the OFCCR and NFCCR have been described previously[29,104,105,107] and are outlined below. Information of colorectal cancer cases and population control subjects participating in the OFCCR and NFCCR was used in this study. Ethics approval for this study was obtained from the Human Investigation Committee, Faculty of Medicine, Memorial University.

3.2 Case ascertainment

Provincial cancer registries (Ontario Cancer Registry and the Newfoundland Cancer Registry) were used to identify newly diagnosed cases of colon or rectal cancer and to recruit into the OFCCR and NFCCR. Inclusion criteria for cases were:

1) Incident primary invasive colon or rectal cancer [pathology confirmed International Classification of Diseases 9th revision codes: 153.0-153.9, 154.1-154.3 and 154.8 (ON & NL); or ICD-0 codes: 18.0-18.7, 19.9, 20.9 (NL only)].

2) Diagnosed between July 1997 and June 2000 (phase one) or January 2003 and April 2006 (phase two) in ON. Diagnosed between January 1999 and December 2003 in NL.

3) Diagnosed at ages between 20 and 74 years old (20 and 74 years old included)

4) Residents of ON and NL at time of diagnosis.

Pathology reports were reviewed by the study pathologist in each province. Initial contact was with the surgeon/physician identified on the pathology report. A letter was sent to the physician that described the study and requested permission to contact the patient. Once physician consent was obtained, individuals were then contacted to inform them of the study. Participants who indicated their willingness to participate the study were sent, in sequence, a written consent form, family history questionnaire (FHQ), personal history questionnaire (PHQ) and food frequency questionnaire (FFQ). In NL only, the original study package also contained a blood requisition form.

Non-responders were sent post-card reminders two to three weeks after the initial mailing, and phoned six to eight weeks after initial contact to remind them of the mailing. Subjects were given the opportunity to complete the required information by phone. Subjects were provided a toll-free telephone number to contact study staff if they had questions about any of the questionnaire items or procedures of the study. Telephone follow-up occurred to a maximum of two contacts for non-responders. If a subject made any indication of not wanting to participate in the study, the interviewer attempted to determine and record the reason. No further contact was made with these subjects. Blood samples were sent directly from the lab closest to the participant to a central laboratory

for investigation of genetic markers. Family history questionnaires were used to classify families as high, intermediate or low risk for genetic counseling.

3.3 Control ascertainment

Population controls were a random sample of residents in each province, aged 20-74 years. As a frequency matched case-control study, controls were 5-year age group and sex matched with the colorectal cancer cases. In ON during 1999 and 2000 controls were identified through a list of residential phone numbers provided by Bell Canada. Info-direct, a service from Bell Canada, provided information that included names, telephone numbers, and addresses of potential control subjects. Households were randomly selected from this list and telephoned to obtain a census of household members (age and sex) so as to identify eligible persons. One eligible person within each household was randomly selected and invited to participate in the OFCCR. To increase the sample size and approach a 1:2 case: control ratio, additional controls were identified from population-based assessment rolls (owners and occupants) provided by the provincial government during 2001 and 2002. A detailed description of selecting controls in ON can be found elsewhere[108].

In NL controls were identified through random digit dialing. In total, a batch of some 192,000 possible residential telephone numbers was generated and arranged in a random order for this study. These numbers served as the basis for recruiting controls. Trained interviewer with prior experience in telephone surveying made the initial contacts by dialing those numbers in a sequential order until the desired number of controls was reached. A detailed description of selecting controls in NL can be found

elsewhere[109].

A screening interview was conducted among potential control subjects to identify if any household member was eligible based on sex and age and whether that person was willing to take part in the study. Once verbal consent for participation was obtained during the phone contact, a survey package was then forwarded to each potential participant. The package included an information pamphlet with general information concerning the study, a consent form, a FHQ, a PHQ, and a FFQ, and a self addressed stamped envelope. As well, participants were asked to submit 30 ml of peripheral blood for DNA analysis. If a participant was unable to return finished questionnaires within three weeks, a follow-up telephone call was made to make ensure the study package had been received. A telephone interview or assistance was offered when illiteracy or physical disability was a concern.

3.4 Dietary information

Dietary information was collected using a self-administrated food frequency questionnaire (FFQ). In ON participants were questioned about their average consumption of 170 foods about two years before diagnosis or interview. This 19-page FFQ was originally developed for the Hawaiian and Californian populations by the Epidemiology Program, Cancer Research Centre of Hawaii and has been previously described and validated against 24-h recalls among a multi-ethnic Hawaiian/Southern Californian population[110,111]. The FFQ assessed average food consumption and cooking method for certain foods. Participants were asked to indicate the portion size of their usual serving for each listed food item from 'Regular', 'Small' or 'Large'. The frequency

of food consumption was assessed using eight options (never or hardly ever, once a month, 2-3 times a month, once a week, 2-3 times a week, 4-6 times a week, once a day, 2 or more times a day). Subjects were also questioned on their use of any individual or multivitamin supplements, including information of the usual brand, amount and the duration of consumption (Appendix C).

The FFQ used in NL was based on the ON FFQ with specific modifications to adapt for the usual food habits of NL residents (Appendix B). In NL participants were questioned about their intakes of 169 foods approximately one year before diagnosis or interview. For each food item, subjects were asked to estimate the frequency of food consumption (daily, weekly, monthly and never scales) and their usual portion size (average, smaller or larger) about one year before diagnosis/interview. A food photographs were provided that showed smaller, average and larger portion sizes for vegetables, meat and chicken. The NL FFQ also assessed vitamin and other dietary supplements that the participants may have used one year before diagnosis or interview.

Nutrient intakes were computed by multiplying the frequency of consumption of each food item by the nutrient content of portion size. In ON values for the amounts of nutrients in the foods were obtained from the USDA file. In NL nutrient contents were based on the 2005 Canadian Nutrient file.

3.5 Epidemiologic data collection

The ON and NL registries used the same personal history questionnaire (PHQ). The self-administered PHQ sought information on possible risk factors for CRC, including medical history, bowel screening history, diet, medication use, diet, physical activity,

reproductive factors, alcohol and tobacco use and socio-demographic measures such as education and income. As well, identifying information such as sex, age, date of birth, and marital status was collected. For female participants there were additional questions relating to reproductive factors (Appendix A).

3.6 Response rates and counts

During phase one of the OFCCR (1997-2000), 3776 patients with CRC were identified in ON. After we obtained their physicians' approval, the patients were asked to complete and return the family history questionnaire. Among 3776 case patients contacted, 1593 were willing and able to participate in the study with 1187 cases (75%) completing the PHQ and 1143 cases (72%) completing the FFQ (Figure 3).

Phase two of the OFCCR was initiated in January 2003 and was scheduled to continue to the end of 2006. Phase two data that were available up to April 2006 were used in this thesis. During this period, among 1263 eligible patients contacted, 727 cases were able to participate in the study. Personal history data were returned by 641 cases (88%), and FFQs were returned by 279 cases (38%) (Figure 3).

Population controls in ON were contacted via telephone. A total of 2736 control subjects from ON agreed to participate in the study with 1957 controls (72%) completing both PHQ and FFQ (Figure 3).

As of July 2006, 1,175 potentially eligible cases in NL were identified through NFCCR. 16 cases were determined ineligible after contact with their physician (too ill). Among 1,159 eligible cases contacted about the study, 1126 cases were willing and able to participate in the study. 705 cases (63%) returned the PHQ and 608 cases (54%)

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returned FFQ. Population controls in NL were contacted through random digit dialing. By July 2005, among 2168 controls contacted, 1603 controls had agreed to participate in the study. 720 controls (45%) returned the PHQ and 687 controls (43%) returned the FFQ (Figure 2).

For the analyses, we excluded those who did not provide sufficient dietary information at baseline, or failed to provide information on potential risk factors at baseline, those who reported energy intake in the upper or lower 2.5% of intake (lower and upper cutoff: In NL, 925 and 4700 kcal for men, 1100 and 4900 kcal for women, respectively; In ON, 1040 and 5200 kcal for men, 835 and 4100 kcal for women, respectively), and patients who had familial adenomatous polyposis (FAP) and an in-situ tumor were excluded. After these exclusions, based on those who completed both the PHQ and FFQ, 3102 subjects (1272 cases and 1830 controls) from ON and 1139 subjects (488 cases and 651 controls) from NL remained. Data collected from these subjects were used for the analysis.
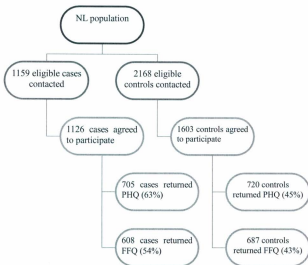
Figure 2. Sample size and response rates of NL population
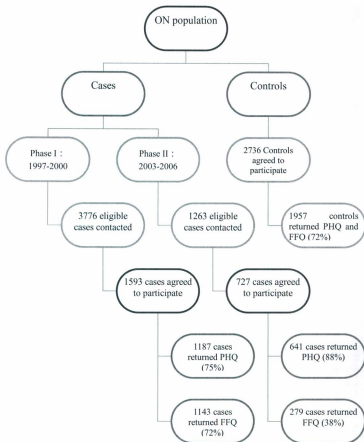
Figure 3. Sample size and response rates of ON population

3.7 Data analyses

3.7.1 Nutrient analyses—energy adjusted

It is possible that over- or under-eating (caloric excess or deficiency) is a primary cause of a disease. In this situation, nutrients that contribute to calories (proteins, fats, carbohydrates, and alcohol) might be considered as the primary exposures that lead to increased caloric intake, which in turn causes disease. In addition, larger, more active, and less metabolically efficient persons tend to eat more of everything so that even nutrients without caloric value, such as vitamins and minerals, are correlated with total energy intake. As a result, in epidemiologic settings, total caloric intake has implications for the interpretation of other nutrients, as well as being of intrinsic interest.

Before attributing causality to a specific nutrient, the burden is upon the epidemiologist to demonstrate that the effect of this nutrient is independent of caloric intake. A number of investigators have employed "nutrient densities" to control for the effect of total caloric intake. Nutrient densities are computed by dividing nutrient values by total caloric intake; they provide a convenient and practical way to describe foods or diets. Nutrient density has the appeal of apparent simplicity, but, unfortunately, this is a complex variable with a generally obscure meaning when used to address diet-disease relationships.

Willett and Stampfer [112] created "calorie-adjusted" nutrient intakes, which is a measure of nutrient intake that is independent of total caloric intake, particularly when caloric intake is associated with disease. Calorie-adjusted nutrient intakes are computed as the residuals from the regression model with total caloric intake as the independent

variable and absolute nutrient intake as the dependent variable. Since residuals have a mean of zero and include negative values, it may therefore be desirable to add a constant (see figure 1). If the usual assumptions for regression analysis are met, these calorie-adjusted nutrient intakes will be uncorrelated with caloric intake.
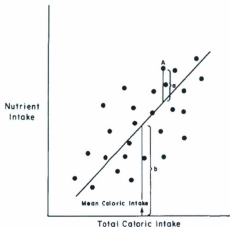

FIGURE 4 Calorie-adjusted nutrient intake = $a + b$, where a = residual for subject A from regression model with nutrient intake as the dependent variable and total caloric intake as the independent variable and b = the expected nutrient intake for a person with mean caloric intake.[112]

According to Willett theory, the formula of calorie-adjusted nutrient intake is as followed[112]:

$$N_E = a + b$$

N_E is calorie-adjusted nutrient intake; a is residual for subject A from regression model with nutrient intake as the dependent variable and total caloric intake as the independent

variable; b is the expected nutrient intake for a person with mean caloric intake.

Formula derivation:

$$N_E = N_i - (A \times K_i + B) + A \times K_m + B$$

N_E is calorie-adjusted nutrient intake; N_i is absolute nutrient intake; A and B are the slope coefficient and intercept of regression model with nutrient intake as the dependent variable and total caloric intake as the independent variable; K_i is individual caloric intake; K_m is mean caloric intake.

In this study, nutrient intakes were energy-adjusted by using the derived formula. Because men and women have different caloric intakes, so nutrients were adjusted by different mean caloric intake in each sex. In NL, the mean caloric intake is 2411.5 kcal/d for men and 2276.2 kcal/day for women; in ON, the mean caloric intake is 2404.8 kcal/d for men and 1987.9 kcal/day for women.

This approach of calorie adjustment is analogous to include both caloric intake and absolute nutrient intake as terms in multivariate models with disease outcome as the dependent variable. In addition, the use of calorie-adjusted values in multivariate models will often overcome the problem of high collinearity frequently observed between nutritional factors. To the extent that this adjustment also reduces between-person variation due to over- or underreporting of intake, a further gain in accuracy will be obtained.

3.7.2 Descriptive analyses

Statistical analyses were performed using SAS statistical software (version 9.1 SAS Institute, Cary, NC, USA). Box plots and histograms of variables were constructed to display distributions and to detect outliers. The characteristics of cases and controls were

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compared by t tests for continuous variables, and chi-square test for categorical variables. All tests of statistical inference employed a two-sided alpha level of 0.05. Correlations between continuous variables were assessed with Pearson correlation coefficients.

3.7.3 Odds ratios estimates

Nutrient intakes were adjusted for total energy intake via the residual method of Willett to reduce potential bias due to differential over- or under-reporting of food intakes[112]. Intakes of macronutrients(objective one), micronutrients(objective two), calcium, vitamin D and dairy products(objective three) were categorized into quintiles based on the distribution among the study population without missing endpoints and were entered into models as indicator variables with the lowest quintiles as the referent group.

Age-adjusted unconditional logistic regression models were calculated stratified by province. Pooled analyses were conducted since odds ratios between provinces were similar and tests of two-way interaction for province exposure were not significant according to the likelihood ratio test statistic. This test was used to assess all interactions in this thesis.

Age and total energy intake-adjusted odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated from maximum-likelihood estimates in unconditional logistic regression to assess the association of the outcome with macronutrient intakes(objective one), micronutrient intakes (objective two) and intakes of dairy products (objective three).

Multivariate unconditional logistic regression was used to evaluate the association between the macronutrient intakes(objective one), micronutrient intakes (objective two)

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and intakes of dairy products (objective three) with CRC risk after adjusting a set of potential confounders or covariates. Tests for linear trend in the log odds ratio of quintiles of intake for each micronutrient were conducted by fitting a logistic model including the same risk factors listed above plus a variable representing the scaled median value for each quintile of nutrient intake. We chose to include median values for each quintile rather than using the values 1.0, 2.0, 3.0, 4.0 and 5.0 to represent each quintile. The latter method assumes equidistance between quintile when intake amounts may actually be quite different between consecutive quintiles. The median values were scaled by dividing by the median for the lowest quintile so that the lowest quintile value would equal 1.0.

3.7.4 Potential covariates definition and selection

The basis for the assessment of confounding factors included: (1) literature and previous studies, (2) biological plausibility, (3) whether the regression coefficient of the primary dependent variable changed by 10% or more after addition of the potentially confounding variable, or (4) whether the covariate entered the model at P<0.10. A backwards-stepwise procedure was performed to obtain the final model.

Potential confounding factors include age, sex, body mass index(BMI), physical activity(metabolic equivalent hours/week, METs/week), education attainment, household income, marital status, and province of residence; medical history: family history of CRC, polyps, and diabetes; history of colon screening procedure; cigarette smoking and alcohol drinking; regular use of medications and supplements: non-steroidal anti-inflammatory drugs (NSAID), folate, calcium, and multivitamins; dietary intake: total energy, fruits, vegetables, red meat; reported hormone replacement therapy (HRT, females only)

(Appendix A).

Respondents were classified into four age groups (18-49, 50-59, 60-69 and 70+years). More than 95% participants were whites, so race was not a confounding factor. Total energy intake was classified into five groups based on quintiles. Respondents were classified into four groups regarding body mass index (BMI), i.e., underweight ($<18.5 kg/m^2$), normal weight ($18.5-24.9 kg/m^2$), overweight($25-29.9 kg/m^2$) and obese ($\geq 30 kg/m^2$). BMI was estimated based on self-reported height and weight.

Physical activity was categorized according to average weekly metabolic equivalent hours (METs) of physical activity. Physical activity includes walking, jogging, running, bicycling, swimming laps, playing tennis, squash racquetball, doing calisthenics, aerobics, vigorous dance, using a rowing machine, lifting weights, playing football, soccer rugby, basketball, doing heavy household work (such as using a non-power mower, shoveling, moving heavy loads, scrubbing floors), and doing any other strenuous activities (including skiing, skating, hockey, hunting, sledding or tobogganing, water-skiing) for a total of at least 30 minutes a week at ages 20-29 years, 30-49 years, 50+years, and lifetime. Average weekly total lifetime physical activity METs was calculated by sum of all applicable physical activity METs divided by the number of weeks since the participant turned 20yrs. Respondents were then classified into four groups regarding METs/week, i.e., <7.4, 7.4-22.4, 22.4-53.0, >53.0 METs/week (Appendix A).

Respondents were classified into two groups having family history of CRC and having no family history. A family CRC history indicates that one member of the

immediate family had suffered from CRC. Polyps were investigated with the question: "Has a doctor ever told you that you had polyps in your large bowel or colon or rectum?" with answers including yes, no and don't know. Diabetes was investigated by asking the question: "Has a doctor ever told you that you had diabetes, also known as diabetes mellitus? Please do not include diabetes which you had only during pregnancy." and answers including yes, no and don't know. History of colon screening procedure were derived from reports of a hemoccult test or sigmoidoscopy test or colonoscopy test for screening purpose. Participants were questioned: "Have you ever had a hemoccult test or sigmoidoscopy test or colonoscopy test?" and "What were the reasons for your first test?". Answers for each question were yes, no and don't know.

Subjects were classified as ever-smokers or non-smokers according to the question that "Have you ever smoke one cigarette per day for three months or more?" Alcohol users were defined based on consuming any alcoholic beverages at least once a week for six months or longer in their 20s, 30s-40s, or 50s and non-users were those who did not drink in their lifetime. Alcohol users were classified into heavy drinkers ($\geq$14drinks/week) and occasional drinkers (<14drinks/week) according to average drinks per week in their 20s, 30s, 40s, or 50s.

Education attainment was investigated through the question: "What is the highest level of education that you completed?" and respondents were classified into three education groups (high school graduate or less; technical school/ some college/ university; bachelor's degree/ graduate degree). Household income was investigated through the question: "Which of the following categories best describes your total annual household

income about 1-2 years before your recent diagnosis?" and respondents were classified into four annual household income groups (less than \$12,000, \$12,000-29,999, \$30,000-49,999, \$50,000 or more). Marital status was investigated by the question: "What is your marital status?" and respondents were classified into three groups: currently married/living as married, single or never married, and separated, divorced or widowed.

Medications were assessed with the question "Have you ever taken any of the following medications regularly (at least twice a week for more than a month)?". Answer choices included yes, no and don't know. Use of nonsteroid anti-inflammatory drugs (including ibuprofen and aspirin use), multivitamin supplements (such as One-A-Day, Theragram, Centrum, Unicap), folic acid, folate pills or tablets, and calcium pills or tablets was included in this analysis. Hormone replacement therapy (HRT) was investigated specially for females with the question: "Have you ever taken HRT prescribed by a doctor and in the form of a pill or a patch (i.e. progestin/estrogen)?". Answer choices included yes and no.

The dietary intake investigation focused on total energy, fruits, vegetables and red meat. Total energy intake was categorized into quintiles based on the distribution among the study population. Eating fruits was investigated through the question: "About 1-2 years before your recent cancer diagnosis, on average, how often did you eat a piece or serving of fruit? (One serving of fruit was interpreted as: 1 medium-sized fresh fruit; ½ cup of chopped, cooked or canned fruit; ¼ cup of dried fruit; 6 ounces of fruit juice (50%-100% pure juice).)" Respondents were classified into four groups by their responses: 0-6 servings/week, 6-7 servings/week, 7-14 servings/week and >14

servings/week. Eating vegetables was investigated by asking the question: "About 1-2 years before your recent cancer diagnosis, on average, how often did you eat a piece or serving of vegetables? Please include green salads, beans, lentils, etc., and potatoes (not packaged potato chips). (One serving of vegetables was interpreted as: 1 cup raw leafy vegetables; ½ cup of other vegetables, cooked or chopped raw; 6 ounces of vegetable juice.)" Respondents were classified into four groups: 0-6 servings/week, 6-7 servings/week, 7-14 servings/week and >14 servings/week. Eating red meat was investigated through the question: "About 1-2 years before your recent cancer diagnosis, on average, how often did you eat a serving of red meat (not chicken or fish)? (One serving of red meat was interpreted as: 2-3 ounces of red meat (a piece of meat about the size of a deck of cards) and includes beef, steak, hamburger, prime rib, beef hot dogs, beef-based processed meat, veal, pork, bacon, pork sausage, ham, lamb, venison.)" Respondents were classified into four groups by their responses: 0-2 servings/week, 2-3 servings/week, 3-5 servings/week and >5 servings/week.

Chapter 4: Results

4.1 Paper 1. 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

4.1.1 Introduction

In Canada, colorectal cancer (CRC) is the second leading cause of death from cancer in men and women combined[37]. In 2009, an estimated 22,000 Canadians were diagnosed with CRC and 9,100 will die of it[37]. Genetic predisposition plays a role in about 15% of CRC but most cases are sporadic[47]. Immigrants rapidly acquire the incidence rates of the host country, suggesting that environmental factors play a crucial role in CRC development[40-42].

Diet has long been regarded as one of the most important environmental factors for colon cancer[1,2]; particularly when an unbalanced diet is combined with inappropriate weight gain, physical inactivity, and such unhealthy practices as smoking and consuming a great deal of alcohol[1,3,4]. Several case-control studies investigating the influence of diet on the risk for CRC have demonstrated a positive correlation between cancer risk and total energy intake[5-10]. Thus, excess intake of any of the important energy-supplying macronutrient components of the diet (eg, proteins, fats, carbohydrates) could contribute to a higher risk of CRC. However, the question of whether or not individual energy-supplying macronutrients, independent of their contribution to energy intake, are related to CRC risk remains controversial.

In a recent Italian case-control study, the risk of CRC increased with total energy

intake, whereas it moderately decreased with an increase of protein intake. Consumption of monounsaturated fats appeared uninfluential, while saturated fats showed a modest positive association with rectal cancer[7]. In contrast, a report from prospective cohort studies found that intakes of total fat, saturated fat, and animal fat were not related to risk of colon cancer[39]. Non-red meat sources of animal protein derived from low-fat dairy products, fish and poultry have been typically associated with a lower risk of CRC[58,75]. A comprehensive report from the World Cancer Research Fund and the American Institute for Cancer Research concluded that total energy has no simple relationship with CRC risk, and that data were inconsistent for carbohydrates, cholesterol and proteins[10].

Given the high incidence rate of CRC in Newfoundland and Labrador (NL) and Ontario (ON), it is of great public health importance to identify possible associations between CRC risk and intakes of total energy, three primary macronutrients (specifically including selected fatty acids, cholesterol, and dietary fibre), and alcohol,. These will be investigated using data from a large population based case-control study conducted in NL and ON.

4.1.2 Materials and methods

4.1.2.1 Selection of cases and controls

Data for this case-control study were from the Ontario Familial Colorectal Cancer Registries (OFCCR) and Newfoundland Familial Colorectal Cancer Registries (NFCCR). In ON incident cases diagnosed during 1997-2000 were identified through the population-based Ontario Cancer Registry. In NL incident cases diagnosed during 1999-2003 were identified through the population tumor registry maintained by the

Newfoundland Cancer Registry. Both registries were used to identify newly diagnosed cases of colon or rectal cancer (pathology confirmed ICD 9th revision codes: 153.0-153.9, 154.1-154.3, and 154.8 or ICD-0 codes: 18.0-18.7, 19.9, 20.9), and aged 20-74 years. Phase two of the OFCCR was initiated to collect data for cases diagnosed in ON during 2003-2006. Initial contact was with the surgeon/physician identified on the pathology report. Once physician consent was obtained, individuals were then contacted to inform them of the study. Participants who indicated their willingness to participate the study were sent, in sequence, a written consent form, family history questionnaire (FHQ), personal history questionnaire (PHQ), and food frequency questionnaire (FFQ). Non-responders were sent post-card reminders and phoned several weeks after initial contact to remind them of the mailing.

Controls were a random sample of residents in each province aged 20-74 years. In ON controls were identified through a list of residential phone numbers or from population-based property assessment rolls (owners and occupants). In NL controls were identified through random digit dialing[109]. Both registries frequency matched controls to cases on sex and five-year age strata. Once verbal consent for participation was obtained during the phone contact, a survey package was forwarded to each potential participant. The package included an information pamphlet with general information concerning the study, a consent form, a FHQ, a PHQ, and a FFQ, and a self addressed stamped envelope.

4.1.2.2 Dietary and epidemiologic data collection

Information on dietary intake was collected using a self-administrated FFQ. This 19-page FFQ was originally developed for the Hawaiian and Californian populations by the

Epidemiology Program, Cancer Research Centre of Hawaii and has been previously described and validated against 24-h recalls among a multi-ethnic Hawaiian/Southern Californian population[110,111]. This previously validated food frequency questionnaire has been adapted to include regional foods in NL and was used to assess diet over 1-2 years prior to diagnosis or interview. Participants were questioned about their intake of almost 170 foods which were believed to be important contribution of calories and most macronutrients in the diet. For each food item, subjects were asked to estimate the frequency of food intake and their usual portion size from 'Regular', 'Small' or 'Large'. A food photographs were provided that showed regular, small and large portion sizes for vegetables, meat and chicken. Participants were also questioned on their use of any individual or multivitamin supplements, including the usual brand name, the amounts taken and the duration of consumption. Intakes of total energy, macronutrients and alcohol were computed by multiplying the frequency of consumption of each food item by the nutrient content of portion size. In ON values for the amounts of nutrients in the foods were obtained from the USDA file. In NL nutrient contents were based on the 2005 Canadian Nutrient file.

The self-administered personal history questionnaire included many close-ended questions about medical history, bowel screening history, diet, medication use, diet, physical activity, reproductive factors, alcohol and tobacco use and socio-demographic measures such as education and income. Identifying information such as sex, age, date of birth, and marital status was collected. For female participants there were additional questions relating to reproductive factors.

For the analyses, we excluded those who did not provide sufficient dietary information at baseline, those who failed to provide information on potential risk factors at baseline, those who reported energy intake in the upper or lower 2.5% of intake (lower and upper cutoff: In NL, 925 and 4700 kcal for men, 1100 and 4900 kcal for women, respectively; In ON, 1040 and 5200 kcal for men, 835 and 4100 kcal for women, respectively), and patients who had familial adenomatous polyposis (FAP) and an in-situ tumor were excluded. After these exclusions, based on those who completed both the PHQ and FFQ, 3102 subjects (1272 cases and 1830 controls) from ON and 1139 subjects (488 cases and 651 controls) from NL remained. Data collected from these subjects were used for the analysis.

4.1.2.3 Statistical analyses

Statistical analyses were performed using SAS statistical software (version 9.1 SAS Institute, Cary, NC, USA). Descriptive statistics stratified by case-control status were used to describe the demographic/health-related characteristics and dietary intakes of the study participants. Intakes of macronutrients and alcohol were adjusted for total energy intake via the residual method of Willett that were used to reduce potential bias due to differential over- or under-reporting of food intakes[112]. Nutrient intakes were categorized into quintiles based on the distribution among the study population without missing endpoints and were entered into models as indicator variables with the lowest quintile as the referent group.

Age-adjusted unconditional logistic regression models were calculated stratified by province. Pooled analyses were conducted since odds ratios between provinces were

similar. Age and total energy intake-adjusted odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated from maximum-likelihood estimates in unconditional logistic regression to assess the association of the outcome with macronutrient intakes. Multivariate unconditional logistic regression was used to evaluate the association of intakes of total energy, macronutrients and alcohol with CRC risk after adjusting a set of potential confounders or covariates. Tests for trend were used to assess dose-response relationships based on the median of each category of nutrient intake.

Potential confounding factors include age(18-49, 50-59, 60-69, and 70+years); sex; body mass index(BMI<18.5, 18.5-24.9, 25-29.9, and ≥30kg/m^2); physical activity(<7.4, 7.4-22.4, 22.4-53.0, and >53.0 METs/week); family history of CRC(yes, no); polyps (yes, no); diabetes(yes, no); history of colon screening procedure(yes, no); cigarette smoking (ever smoke, never smoke); alcohol drinking(<14, ≥14drinks/week); education attainment (high school graduate or less, technical school/ some college/ university, and bachelor's degree/ graduate degree); household income(less than $12,000, $12,000-29,999, $30,000-49,999, and $50,000); marital status(married, single/ never married, and separated/ divorced/widowed); regular use of medication and supplements: non-steroid anti-inflammatory drug (NSAID)(yes, no), multivitamin supplements(yes, no), folate supplement(yes, no), calcium supplement(yes, no); reported hormone replacement therapy (HRT, females only)(yes, no); and dietary intakes: total energy intake (quintiles), fruits(0-6, 6-7, 7-14, and >14 servings/week), vegetables(0-6, 6-7, 7-14, and >14 servings/week), red meat(0-2, 2-3, 3-5, and >5 servings/week); province of residence(NL, ON). The basis for the assessment of confounding factors included: (1) literature and

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previous studies, (2) biological plausibility, (3) whether the regression coefficient of the primary dependent variable changed by 10% or more after addition of the potentially confounding variable, or (4) whether the covariate entered the model at $p<0.10$. A backwards-stepwise procedure was performed to obtain the final model. Statistical tests were two sided, and p values less than 0.05 were considered statistically significant.

4.1.3 Results

Table 4.1.1 shows the distribution of CRC cases and controls according to age, sex, province of residence, BMI and other selected variables. By design, cases and controls had similar sex distribution. However, cases tended to be younger than controls. Cases were more likely to be obese, to be either physically inactive or extremely active, and to have a family history of CRC. Controls more often reported regular use of NSAID, a higher education, a higher income, and having had a colon screening procedure.

The mean daily intakes of total energy, macronutrients and alcohol among cases and controls are shown in Table 4.1.2. Mean daily intake of controls was 2195.1kcal for total energy, 87.2g for protein, 286.2g for carbohydrate, 80.4g for total fat, 25.2g for total dietary fibre, 26.8g for saturated fatty acids, 29.1g for monounsaturated fatty acids, 16.6g for polyunsaturated fatty acids, 277.1mg for cholesterol, 6.5g for alcohol. Results showed that 15% of calories from protein, 31% of calories from total fat, 50% of calories from carbohydrate, and 4% of calories from alcohol. Cases reported significantly higher intakes of total energy, percentage of calories from total fat, percentage of calories from saturated fat and cholesterol (all $p<0.05$) than controls. Controls had higher intakes of carbohydrate and total dietary fibre compared with cases (all $p<0.05$).

Table 4.1.3 gives the ORs and corresponding 95% CI of CRC according to quintile intakes of macronutrients associated food components. High total energy intake was significantly related to an increased risk of CRC (OR=1.56 in the highest versus the lowest quintile of intake; 95% CI: 1.21-2.01, p-trend=0.02), whereas inverse associations emerged for intakes of protein (OR=0.85, 95%CI: 0.69-1.00, p-trend=0.06), carbohydrate (OR=0.81, 95%CI: 0.63-1.00, p-trend=0.05) and total dietary fibre (OR=0.84, 95% CI:0.67-0.99, p-trend=0.04). Total fat, alcohol, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol appeared unrelated to CRC risk.

We additionally evaluated intakes of percentage of calories from macronutrients in relation to the risk of CRC (Table 4.1.4). A significant inverse trend was observed for percentage of calories from protein (OR=0.76, 95%CI: 0.61-0.96, p-trend=0.05), while a direct trend in risk of significance was observed for percentage of calories from alcohol (OR=1.34, 95%CI: 1.04-1.72, p-trend=0.05). No associations were observed for percentage of energy from other macronutrients.

The relationship between intakes of total energy, protein, carbohydrate and dietary fibre with CRC risk was further examined in strata of various covariates. No substantial heterogeneity was observed in separate strata of sex; age(≤60, >60years); BMI(<25, ≥25kg/m²); physical activity(<22.4, ≥22.4METs/week); family history of CRC(no, yes); reported colon screening procedure(no, yes); NSAID use(no, yes); education attainment(lower, higher); household income(lower, higher), and total energy intake(≤2109.3, >2109.3 kcal/day) (Data not shown).

4.1.4 Discussion

Our present case-control study, one of the largest investigations of diet and CRC to date, showed that intakes of total energy were significantly positively associated with risk of CRC, whereas inverse associations were seen with intakes of protein, carbohydrate, and dietary fibre. Intakes of total fat, fatty acids, cholesterol and alcohol were unrelated to the risk.

Our study observed a direct association between total energy intake and the risk of CRC, confirming results from several previous case-control studies of other populations[5-10], the evidence that caloric restriction reduces cancer incidence in rodents[113,114], and colorectal cell proliferation in humans[7,58,70,115]. Our study found that CRC risk was positively related with percentage of calories consumed as alcohol but was not related with energy-adjusted total alcohol intake. These results suggest that alcohol, independent of its contribution to energy, may not be associated with CRC risk. Energy intake can be responsible for glycaemic overload and a compensatory increase of serum insulin and related insulin growth factor-1 (IGF-1). IGF-1 is a promoter of tumor cell growth in vitro[58,70], and it may expose colonic and rectal cells to a proliferative stimulus[71,72]. Diabetes has also been related to increased CRC risk[71,73]. Additionally, higher energy intake is associated with the cause of obesity, which in turn is a possible risk factor for CRC.

Most previous studies found no excess risk for animal protein intake after adjusting for total energy intake[6,11,116,117]. However, a recent Italian case-control study[7] found that the risk of CRC moderately decreased with an increase of protein intake (OR=0.82 for

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colon cancer, OR=0.88 for rectal cancer). Consistent with the Italian study, findings of our study showed that CRC risk was inversely associated with protein intake (OR=0.85, 95%CI: 0.69-1.00) and percentage of calories from protein intake (OR=0.76, 95%CI: 0.61-0.96). In our study population, red meat intake was moderate (about 4 servings/week) and similar proportions (around 20%) of proteins derived from red meat, dairy products and the combination of white meat and fish. Thus, non-red meat sources of animal protein may have a beneficial influence[58]. Moreover, several previous studies have consistently found inverse associations with high protein foods (dairy products, white meat, fish and poultry) or with non-red meat protein [3,58,74,75,118,119]. In our study, a clearer inverse association with protein may have emerged if non-red meat protein sources were analyzed separately from red meat. A possible explanation for a protective effect of protein is that low intakes of methionine may contribute to DNA methylation abnormalities, which appear to be important in the initiation and progression of colon cancer[13].

Carbohydrate intake was shown to be inversely related to CRC risk in our study. Compared with participants in the lowest quintile of carbohydrate consumption, those in the highest quintile were 19% less likely to develop CRC. We also observed an inverse association with fibre intake, which was in agreement with several previous studies[59,60,117,120]. In our study, a 16% reduced risk was observed among participants with higher intake of fibre. The inverse relationships with carbohydrate and fibre may be related to the protection of fruits, vegetables and grains on CRC, because these foods contain large amount of carbohydrate and fibre.

Different types of fibre appear to have different effects. Terry *et al.* examined fruit, vegetable, and fibre intakes and risk of CRC among Swedish women, and concluded that a higher consumption of fruit was associated with a 32% reduction in CRC risk, while higher intake of cereal fibre did not lower the risk[60]. Results from a case-control study in the Swiss Canton of Vaud indicated that vegetable fibre appeared to be more protective than either fruit or grain fibre[59]. A beneficial effect from fibre may arise by several mechanisms, including increased fecal weight, greater frequency of defecation, decreased intestinal transit time, dilution of colonic contents, greater microbial growth, alteration of energy metabolism, decreased bile acid hydroxylation, and greater productions of hydrogen, methane, carbon dioxide, and short-chain fatty acids[21].

We found no evidence of any substantial effect of the intake of total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol intake on risk of CRC in the present study. These results are consistent with some previous studies [5,56-58], but not all[6,7]. Results from a prospective cohort study of 47,949 U.S. male health professionals between 1986 and 1992 showed that intakes of total fat, saturated fat, and animal fat were not related to risk of colon cancer[58]. Combined data from 13 case-control studies of 5,287 CRC cases and 10,470 controls provided substantive evidence of the lack of any meaningful or strong density-independent association between the intake of total fat or any of the fat components and risk of CRC[56]. These results again suggest that fat, independent of its contribution to energy, may not be associated with CRC risk. Although there exists a sensible biological rationale for the possible involvement of fat in colorectal carcinogenesis[12], it appears that if fat is indeed involved, the mechanism must

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be more complex than that which would be implied by a simple empirical association with daily fat intake per day. It could involve foods or some complex interaction amongst nutrients or other food components. Discussion of such potential biological mechanisms is beyond the scope of the present paper.

Consideration must be given to the potential limitations in the present study that may have influenced the observed associations. First, as in most case-control studies, potential recall and selection biases are possible. Since exposure information was collected after diagnosis, differential recall between cases and controls would bias results; in particular, cases may recall dietary exposures differently from controls because of the presence of illness or symptoms. Controls may have agreed to join this study because of an interest in health and may therefore have healthier dietary and physical activity habits, a pattern that may exaggerate differences with the cases beyond what might have been seen with truly comparable controls.

Second, by design, cases and controls had similar sex distribution, however, cases and controls were not well comparable according to age group. Estimates of nutrient intakes from a FFQ are not precise and there is always the potential for measurement error. Although the original FFQ used in this study has been validated[110,111], this questionnaire requires further evaluation because it was originally developed for the Hawaiian and Californian populations that may be different from people residing in NL and ON. FFQ used in NL has been adapted to include regional foods in NL, however, OFCCR used the original FFQ that has not been adapted. Thus, a sub-study will be necessary to assess the level of agreement between the FFQ used by the OFCCR and the

FFQ that was previously developed specifically for Canadian populations. Finally, it is also possible that the 1-2 year referent period on which dietary data were based is insufficient if more remote diet (eg. 5-10 yrs) has a stronger influence on CRC risk.

This study had a number of strengths. We had a large sample size, which allowed us to observe associations that would be undetectable in smaller studies. More importantly, the previous findings about the protective effects of macronutrients were confined to a specific study population, which makes it difficult to generalize the results. In this study, we conducted pooled analyses of the population of two Canadian provinces to investigate the associations of total energy, macronutrients, alcohol and CRC risk. Furthermore, nutrient intakes were adjusted for total energy intake. The use of calorie-adjusted values in multivariate models will often overcome the problem of high collinearity frequently observed between nutritional factors[112]. To the extent that, this adjustment also reduces between-person variation due to over- or underreporting of food intakes[112]. The relationships of total energy, macronutrients, alcohol and CRC risk may differ appreciably by several factors, so we controlled for a wide range of potential confounding factors using multivariate logistic regression models. Additionally, results of the consistent findings in separate strata for total energy, protein, carbohydrate and dietary fibre would argue against multiple comparisons as an explanation for these associations. Although some random misclassification of diet is likely, non-differential misclassification generally tends to bias the risk estimates toward the null.

In conclusion, our large population-based case-control study of CRC conducted in two Canadian provinces with high incidence of CRC, and findings of this study provides

further evidence that diets high in energy may increase the risk, whereas diets high in protein, fibre, and carbohydrate may reduce the risk of CRC. These results underline the importance of some aspects of total energy and macronutrients and consequently the potential for prevention through dietary changes.

Table 4.1.1 Selected characteristics of subjects from CRC case-control study in NL and ON

Characteristics[a]	Cases(n=1760) No. (%)	Controls(n=2481) No. (%)
Age (years)[*]		
18-49	368(20.9)	265(10.7)
50-59	412(23.4)	690(27.8)
60-69	646(36.7)	998(40.2)
70+	334(19.0)	528(21.3)
Sex		
Males	935(53.1)	1357(54.7)
Females	825(46.9)	1124(45.3)
Province of residence		
NL	488(27.7)	651(26.2)
ON	1272(72.3)	1830(73.8)
BMI[b] (kg/m²)[*]		
Underweight(<18.5)	23(1.3)	22(0.9)
Normal(18.5-24.9)	595(33.8)	930(37.5)
Overweight (25-29.9)	748(42.5)	1069(43.1)
Obese (≥30)	394(22.4)	460(18.5)
Physical activity (METs/week[b])[*]		
0 - 7.4	465(26.4)	595(24.0)
7.4 -22.4	348(19.8)	633(25.5)
22.4 - 53.0	429(24.4)	633(25.5)
> 53.0	518(29.4)	620(25.0)
Family history of CRC[*]		
No	1582(89.9)	2337(94.2)
Yes	178(10.1)	144(5.8)
Reported any colon screening procedure[*]		
No	1500(85.2)	1861(75.0)
Yes	260(14.8)	620(25.0)
Regular use of NSAID[b] [*]		
No	1163(66.1)	1439(58.0)
Yes	597(33.9)	1042(42.0)
Education attainment[*]		
High school graduate or less	884(50.2)	1042(42.0)
Technical school/ some college/ university	540(30.7)	866(34.9)
Bachelor's degree/ graduate degree	336(19.1)	573(23.1)
Household income ($CAN)[*]		
<12,000	109(6.2)	154(6.2)
12,000-29,999	507(28.8)	573(23.1)
30,000-49,999	547(31.1)	777(31.3)
≥50,000	597(33.9)	977(39.4)

[a] All characteristic variables presented as number(%).
[b] BMI, body mass index; METs/week, metabolic equivalent hours per week; NSAID, nonsteroid anti-inflammatory drug.   [*] Significant differences between cases and controls ($p \leq 0.05$)

Table 4.1.2 Mean intakes of total energy, macronutrients, and alcohol among subjects from CRC case-control study in NL and ON

Intakes of total energy and macronutrients[a]	Cases (n=1760)	Controls (n=2481)	Difference (Cases-Controls)
Total energy (kcal/day)[*]	2316.1±810.6	2195.1±750.8	121
Macronutrients			
Protein (g/day)	86.2±18.5	87.2±17.1	-1
% of Calories from Protein	15.2±2.8	15.4±2.9	-0.2
Carbohydrate (g/day)[*]	282.0±49.7	286.2±49.6	-4.2
% of Calories from Carbohydrates	49.6±7.7	50.0±8.0	-0.4
Total Fat (g/day)	81.3 ±18.4	80.4±18.0	0.9
% of Calories from Total Fat[*]	31.8±6.1	31.3±6.3	0.5
Dietary fibre (g/day)[*]	24.0±8.5	25.2±9.0	-1.2
Fatty Acids and Cholesterol			
Saturated Fatty Acids (g/day)	27.1±7.0	26.8±7.1	0.3
% of Calories from Saturated Fat[*]	10.6±2.4	10.4±2.6	0.2
Monounsaturated Fatty Acids (g/day)	29.6±7.4	29.1±7.2	0.5
Polyunsaturated Fatty Acids (g/day)	16.7±5.0	16.6±4.6	0.1
Cholesterol (mg/day)[*]	286.2±116.4	277.1±100.9	9.1
Alcohol (g/day)	7.4±49.4	6.5±36.8	0.9
% of Calories from Alcohol	3.9±6.3	3.8±5.9	0.1

[a] All continuous variables presented as mean ± SD (standard deviation).
[*] Significant differences between cases and controls ($p \leq 0.05$)

Table 4.1.3 Associations (adjusted OR[a], 95%CI[b]) of total energy, macronutrients, and alcohol intakes with CRC risk, CRC case-control study in NL and ON

Intakes of total energy, macronutrients, and alcohol	Quintiles of intakes			*p*-trend[d]
	Q1[c]	Q3[c]	Q5[c]	
Total energy				
No. of cases/controls	313/517	343/505	404/443	
Median intake (kcal/day)	1348.5	2109.3	3308.9	
OR[a] (95% CI)	1.00	1.16(0.95,1.41)	1.53*(1.26,1.86)	0.01
OR[c] (95% CI)	1.00	1.19(0.92,1.53)	1.56*(1.21,2.01)	0.02
Protein				
No. of cases/controls	372/479	334/513	335/512	
Median intake (g/day)	68.4	85.6	106.7	
OR[a] (95% CI)	1.00	0.88(0.72,1.07)	0.82*(0.67,0.99)	0.09
OR[c] (95% CI)	1.00	0.88(0.70,1.11)	0.85*(0.69,1.00)	0.06
Carbohydrate				
No. of cases/controls	392/458	332/516	334/513	
Median intake (g/day)	229.1	282.6	341.5	
OR[a] (95% CI)	1.00	0.83*(0.68,1.00)	0.80*(0.66,0.97)	0.08
OR[c] (95% CI)	1.00	0.84(0.67,1.02)	0.81*(0.63,1.00)	0.05
Total Fat				
No. of cases/controls	344/506	362/487	372/475	
Median intake (g/day)	60.1	80.5	102.8	
OR[a] (95% CI)	1.00	1.14(0.94,1.39)	1.07(0.88,1.30)	0.45
OR[c] (95% CI)	1.00	1.18(0.94,1.50)	0.96(0.75,1.22)	0.71
Total dietary fibre				
No. of cases/controls	388/462	355/493	308/539	
Median intake (g/day)	15.1	23.7	35.2	
OR[a] (95% CI)	1.00	0.98(0.80,1.19)	0.75*(0.62,0.91)	0.04
OR[c] (95% CI)	1.00	0.97(0.77,1.23)	0.84*(0.67,0.99)	0.04
Saturated Fatty Acids				
No. of cases/controls	346/504	331/517	378/469	
Median intake (g/day)	19.0	26.6	35.2	
OR[a] (95% CI)	1.00	0.99(0.81,1.20)	1.12(0.92,1.36)	0.10
OR[c] (95% CI)	1.00	1.03(0.81,1.31)	1.00(0.79,1.26)	0.80
Monounsaturated Fatty Acids				
No. of cases/controls	341/509	342/506	371/476	
Median intake (g/day)	21.2	29.1	38.2	
OR[a] (95% CI)	1.00	1.05(0.86,1.28)	1.09(0.89,1.32)	0.12
OR[c] (95% CI)	1.00	1.07(0.84,1.35)	0.99(0.78,1.26)	0.70
Polyunsaturated Fatty Acids				
No. of cases/controls	357/493	343/505	372/475	
Median intake (g/day)	11.6	16.4	22.4	
OR[a] (95% CI)	1.00	1.00(0.82,1.22)	1.04(0.86,1.27)	0.77
OR[c] (95% CI)	1.00	1.03(0.81,1.30)	0.98(0.77,1.23)	0.47

Intakes of total energy, macronutrients, and alcohol	Quintiles of intakes			p-trend[d]
	$Q1^e$	$Q3^e$	$Q5^e$	
Cholesterol				
No. of cases/controls	342/508	339/509	380/467	
Median intake (mg/day)	178.8	265.8	392.0	
OR^b (95% CI)	1.00	1.04(0.85,1.26)	1.21*(1.00,1.47)	0.02
OR^c (95% CI)	1.00	0.84(0.65,1.07)	1.00(0.79,1.28)	0.88
Alcohol				
No. of cases/controls	382/467	309/539	344/503	
Median intake (g/day)	0	13.6	182.8	
OR^b (95% CI)	1.00	0.86(0.69,1.06)	1.15(0.90,1.48)	0.35
OR^c (95% CI)	1.00	0.88(0.67,1.17)	1.17(0.85,1.61)	0.38

[a] OR, Odds ratio; 95%CI, 95% confidence interval.

[b] Adjusted for age and total energy intake.

[c] Adjusted for total energy intake, age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, regular use of folate supplement, regular use of calcium supplement, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on $n \geq 10\%$ alternation in the parameter coefficient of interest.

[d] Two-sided p value for test of linear trend was calculated by using median values for each quintile of intake.

[e] Q1 for quintile 1, Q3 for quintile 3, and Q5 for quintile 5.

* Significant different from reference category, p<0.05

Table 4.1.4 Associations (adjusted OR[a], 95%CI[a]) of percentage of energy from macronutrients and alcohol with CRC risk, CRC case-control study in NL and ON

Intakes of % energy from macronutrients and alcohol	Quintiles of intakes			p-trend[d]
	Q1[e]	Q3[e]	Q5[e]	
% of Calories from Protein				
No. of cases/controls	369/481	365/483	331/516	
Median intake per day	12.0	15.1	18.8	
OR† (95% CI)	1.00	0.94(0.78,1.14)	0.82*(0.67,1.00)	0.08
OR‡ (95% CI)	1.00	0.91(0.72,1.15)	0.76*(0.61,0.96)	0.05
% of Calories from Total Fat				
No. of cases/controls	327/523	344/504	365/482	
Median intake per day	23.8	31.7	39.2	
OR† (95% CI)	1.00	1.05(0.86,1.27)	1.12(0.92,1.37)	0.19
OR‡ (95% CI)	1.00	1.02(0.81,1.29)	0.99(0.79,1.26)	0.92
% of Calories from Saturated Fat				
No. of cases/controls	332/518	352/496	376/471	
Median intake per day	7.5	10.4	13.5	
OR† (95% CI)	1.00	1.06(0.87,1.29)	1.17(0.96,1.42)	0.03
OR‡ (95% CI)	1.00	1.07(0.85,1.36)	1.02(0.81,1.30)	0.47
% of Calories from Carbohydrates				
No. of cases/controls	376/474	356/492	340/507	
Median intake per day	40.2	49.9	59.7	
OR† (95% CI)	1.00	0.93(0.77,1.13)	0.90(0.74,1.09)	0.41
OR‡ (95% CI)	1.00	1.00(0.80,1.27)	0.90(0.71,1.14)	0.59
% of Calories from Alcohol				
No. of cases/controls	366/484	371/477	356/491	
Median intake per day	0	1.3	11.1	
OR† (95% CI)	1.00	0.97(0.80,1.18)	0.95(0.78,1.15)	0.94
OR‡ (95% CI)	1.00	1.31*(1.02,1.68)	1.34*(1.04,1.72)	0.05

[a] OR, Odds ratio; 95%CI, 95% confidence interval.
[b] Adjusted for age and total energy intake.
[c] Adjusted for total energy intake; age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, regular use of folate supplement, regular use of calcium supplement, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alternation in the parameter coefficient of interest.
[d] Two-sided p value for test of linear trend was calculated by using median values for each quintile of intake.
[e] Q1 for quintile 1, Q3 for quintile 3, and Q5 for quintile 5.
* Significant different from reference category, p≤0.05

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4.2 Paper 2. Association of selected micronutrient intakes with colorectal cancer risk: results from a large population-based case-control study in Newfoundland and Labrador and Ontario

4.2.1 Introduction

The role of various micronutrients have been considered in colorectal carcinogenesis. Recent epidemiologic studies suggest that calcium, vitamin D, folate, vitamin B6, and some antioxidants are protective against cancer[11-13], whereas iron may increase risk[14]. Calcium and vitamin D may protect against colorectal carcinogenesis by binding free fatty acids and secondary bile acids in the small intestine, thereby protecting colonic epithelial cells from mutagens[15]. Folate and other B-vitamins may exert a protective effect on colorectal carcinogenesis as co-factors in the methylation of thymidylate for DNA synthesis and the production of S-adenosylmethionine, the primary methyl donor in the body[15,16]. Beta-carotene and vitamins A, C, and E may decrease the risk through antioxidant mechanisms[20,21]. Finally, it has been suggested that iron may increase the risk of colorectal cancer (CRC) by generating free radicals that attack DNA and damage chromosomes[22,23].

Despite the plausibility of the biologic mechanisms proposed to explain the effects of these micronutrients on colorectal carcinogenesis, the epidemiological evidence on this issue is unclear. Prior to 1998, epidemiologic studies suggested that calcium intake was not associated with lower risk of colorectal adenoma or cancer[24]. However, after a follow-up of 61,463 women for 11.3 years, Terry *et al.* found an inverse association between dietary calcium intake and CRC risk. Vitamin D intake was not clearly

associated with risk[79]. A recently published review concludes that the available research suggests a protective effect of vitamin D on colon cancer risk[122].

Vegetable and fruits are generally accepted as having protective effects against CRC[59,60], however, researchers have recently found weak or nonexistent inverse associations for total fruits and vegetables[61,82,83]. Vegetables and fruits are major sources of dietary antioxidants, so the weak results in recent studies may reflect on the true strength of the association between consumption of dietary antioxidants and risk of colorectal neoplasia. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study group conducted a randomized, double-blind, placo-controlled primary-prevention trial and concluded that there was no interaction between alpha-tocopherol and beta carotene with respect to the incidence of CRC[26]. After four years, results from another double-blind three year intervention with antioxidants (beta-carotene 15 mg, vitamin C 150 mg, vitamin E 75 mg, selenium 101 microg) suggested a protective role of antioxidants on new adenoma formation. No effect was detected on the growth of adenomas and polyps[27].

There may be a number of reasons for inconsistencies between studies, such as the potential selection bias and possible recall bias that could played a larger role in retrospective studies. If some micronutrients are beneficial, whether or not diet alone can furnish enough micronutrient to have a substantially protective effect on CRC is a question. With the increasing frequency of using individual or multivitamin supplements by the Canadian population, whether or not supplement use could exert a protective effect against CRC becomes a crucial question. Therefore, this paper presents the results of a large case-control study conducted in two Canadian provinces that examines relationships

between selected micronutrients and the risk of CRC.

4.2.2 Materials and methods

(note to readers: this section mostly duplicates 4.1.2)

4.2.2.1 Selection of cases and controls

Data for this case-control study were from the Ontario Familial Colorectal Cancer Registries (OFCCR) and Newfoundland Familial Colorectal Cancer Registries (NFCCR). In Ontario (ON) incident cases diagnosed during 1997-2000 were identified through the population-based Ontario Cancer Registry. In Newfoundland and Labrador (NL) incident cases diagnosed during 1999-2003 were identified through the population tumor registry maintained by the Newfoundland Cancer Registry. Both registries were used to identify newly diagnosed cases of colon or rectal cancer (pathology confirmed ICD 9th revision codes: 153.0-153.9, 154.1-154.3, and 154.8 or ICD-0 codes: 18.0-18.7, 19.9, 20.9), and aged 20-74 years. Phase two of the OFCCR was initiated to collect data for cases diagnosed in ON during 2003-2006. Initial contact was with the surgeon/physician identified on the pathology report. Once physician consent was obtained, individuals were then contacted to inform them of the study. Participants who indicated their willingness to participate the study were sent, in sequence, a written consent form, family history questionnaire (FHQ), personal history questionnaire (PHQ), and food frequency questionnaire (FFQ). Non-responders were sent post-card reminders and phoned several weeks after initial contact to remind them of the mailing.

Controls were a random sample of residents in each province aged 20-74 years. In ON controls were identified through a list of residential phone numbers or from

population-based property assessment rolls (owners and occupants). In NL controls were identified through random digit dialing[109]. Both registries frequency matched controls to cases on sex and five-year age strata. Once verbal consent for participation was obtained during the phone contact, a survey package was forwarded to each potential participant. The package included an information pamphlet with general information concerning the study, a consent form, a FHQ, a PHQ, a FFQ, and a self addressed stamped envelope.

4.2.2.2 Dietary and epidemiologic data collection

Information on dietary intake was collected using a self-administered FFQ. This 19-page FFQ was originally developed for the Hawaiian and Californian populations by the Epidemiology Program, Cancer Research Centre of Hawaii and has been previously described and validated against 24-h recalls among a multi-ethnic Hawaiian/Southern Californian population[110,111]. This previously validated food frequency questionnaire has been adapted to include regional foods in NL and was used to assess diet over 1-2 years prior to diagnosis or interview in each province. Participants were questioned about their intake of almost 170 foods which were believed to be important to the contribution of most micronutrients in the diet. For each food item, subjects were asked to estimate the frequency of food intake and their usual portion size from 'Regular', 'Small' or 'Large'. A food photographs were provided that showed regular, small and large portion sizes for vegetables, meat and chicken. Participants were also questioned on their use of any individual or multivitamin supplements, including the usual brand name, the amounts taken and the duration of consumption. Intake of micronutrient was computed by multiplying the frequency of consumption of each food item by the nutrient content of

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portion size. In ON values for the amounts of micronutrients in the foods were obtained from the USDA file. In NL micronutrient contents were based on the 2005 Canadian Nutrient file.

The self-administered personal history questionnaire included many close-ended questions about medical history, bowel screening history, diet, medication use, diet, physical activity, reproductive factors, alcohol and tobacco use and socio-demographic measures such as education and income. Identifying information such as sex, age, date of birth, and marital status was also collected. For female participants there were additional questions relating to reproductive factors.

For the analyses, we excluded those who did not provide sufficient dietary information at baseline, those who failed to provide information on potential risk factors at baseline, those who reported energy intake in the upper or lower 2.5% of intake (lower and upper cutoff: In NL, 925 and 4700 kcal for men, 1100 and 4900 kcal for women, respectively; In ON, 500 and 5200 kcal for men, 835 and 4100 kcal for women, respectively), and patients who had familial adenomatous polyposis (FAP) and an in-situ tumor were excluded. After these exclusions, based on those who completed both the PHQ and FFQ, 3102 subjects (1272 cases and 1830 controls) from ON and 1139 subjects (488 cases and 651 controls) from NL remained. Data collected from these subjects were used for the analysis.

4.2.2.3 Statistical analyses

Statistical analyses were performed using SAS statistical software (version 9.1 SAS Institute, Cary, NC, USA). Descriptive statistics stratified by case-control status were

used to describe the demographic/health-related characteristics and dietary intakes of the study participants. Intakes of micronutrients were adjusted for total energy intake via the residual method of Willett that were used to reduce potential bias due to differential over- or under-reporting of food intakes[112]. Micronutrients exposure included food sources and supplements. Intakes of micronutrients were calculated by adding energy-adjusted micronutrients from food and unadjusted micronutrients from supplements. Micronutrient intakes were categorized into quintiles based on the distribution among the study population without missing endpoints and were entered into models as indicator variables with the lowest quintile as the referent group.

Age-adjusted unconditional logistic regression models were calculated stratified by province. Pooled analyses were conducted since odds ratios between provinces were similar. Age and total energy intake-adjusted odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated from maximum-likelihood estimates in unconditional logistic regression to assess the association of the outcome with micronutrients intakes. Multivariate unconditional logistic regression was used to evaluate the association of intakes of selected micronutrients with CRC risk after adjusting a set of potential confounders or covariates. Tests for trend were used to assess dose-response relationships based on the median of each category of micronutrient intake.

Potential confounding factors include age(18-49, 50-59, 60-69, and 70+years); sex; body mass index(BMI<18.5, 18.5-24.9, 25-29.9, and $\geq 30 \text{kg/m}^2$); physical activity(<7.4, 7.4-22.4, 22.4-53.0, and >53.0 METs/week); family history of CRC(yes, no); polyps (yes, no); diabetes(yes, no); history of colon screening procedure(yes, no); cigarette smoking

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(ever smoke, never smoke); alcohol drinking(<14, ≥14drinks/week); education attainment (high school graduate or less, technical school/ some college/ university, and bachelor's degree/ graduate degree); household income(less than $12,000, $12,000-29,999, $30,000-49,999, and $50,000); marital status(married, single/ never married, and separated/ divorced/widowed); regular use of medication and supplements: non-steroid anti-inflammatory drug (NSAID)(yes, no), multivitamin supplements(yes, no), folate supplement(yes, no), calcium supplement(yes, no); reported hormone replacement therapy (HRT, females only)(yes, no); and dietary intakes: total energy intake (quintiles), fruits(0-6, 6-7, 7-14, and >14 servings/week), vegetables(0-6, 6-7, 7-14, and >14 servings/week), red meat(0-2, 2-3, 3-5, and >5 servings/week); province of residence(NL, ON). The basis for the assessment of confounding factors included: (1) literature and previous studies, (2) biological plausibility, (3) whether the regression coefficient of the primary dependent variable changed by 10% or more after addition of the potentially confounding variable, or (4) whether the covariate entered the model at $p<0.10$. A backwards-stepwise procedure was performed to obtain the final model. Statistical tests were two sided, and p values less than 0.05 were considered statistically significant.

4.2.3 Results

Table 4.2.1 The distribution of CRC cases and controls according to age, sex, dietary habits and other selected variables are given in Table 4.2.1. By design, cases and controls had a similar distribution of sex and province of residence. Cases tended to be younger, obese, either physically inactive or physically extremely active, more likely to have positive family history of CRC, and consume more red meats and total energy than

controls. Intakes of fruits and vegetables did not vary significantly between cases and controls.

Table 4.2.2 shows mean intakes of micronutrients from food sources only or from both food and supplement sources among cases and controls. For most micronutrients (except iron), controls consistently reported significantly higher intakes of micronutrients from food sources or from food and supplement sources as compared to cases (all $p<0.05$). Furthermore, higher intakes of micronutrient in controls were primarily due to larger contributions from supplement sources. For example, no differences were found in retinol intake (from food), however, after adding supplements into intake, significant differences were found between cases and controls. Cases had higher intakes of iron, largely due to dietary supplements use($p<0.0001$).

The OR and 95%CI of CRC according to intakes of selected micronutrients from both food and supplements are shown in Table 4.2.3. After adjusting for potential covariates, risk of CRC was found to be significantly inversely associated with intakes of total calcium (highest *vs.* the lowest quintiles: OR=0.59; 95%CI: 0.45, 0.77), vitamin C(OR=0.67; 95%CI:0.51, 0.88), vitamin D(OR=0.73; 95%CI: 0.57, 0.94), riboflavin(OR=0.61; 95%CI: 0.47, 0.78), and folate(OR=0.72; 95%CI: 0.56, 0.92). A direct relation emerged for iron intake (OR=1.34, 95%CI:1.01-1.78). No links were found with retinol, alpha-tocopherol, thiamin, vitamin B6, vitamin B12.

We also evaluated the associations of CRC risk with selected micronutrients from food source only (Table 4.2.4). After adjusting for potential confounders, CRC risk were inversely significantly associated with dietary calcium intake (OR=0.76, 95%CI:

0.59,0.97), dietary vitamin D intake (OR=0.79, 95%CI: 0.62, 1.00), and non-significantly inversely related to intakes of vitamin C(OR=0.87, 95%CI: 0.67, 1.13), riboflavin(OR=0.86, 95%CI: 0.68, 1.09), folate(OR=0.83, 95%CI: 0.65, 1.05).

We additionally examined CRC risk according to individual supplement use and levels of micronutrient intakes from foods (Table 4.2.5). After adjusting for multivitamin supplements use and other covariates, significantly reduced risks were observed among individual supplements users, and weak or nonexistent relationships were found among non-supplements users. Stratified analyses by supplement intake status showed that the protective effects vary according to micronutrients intake levels from foods. In addition, the protective effects are more pronounced in people with lower nutrients intake from foods, such as vitamin C and folate.

4.2.4 Discussion

Our data from a large population based case-control study suggested that inverse associations with CRC emerged for certain micronutrients from both food sources and supplements consumption, including calcium, vitamin C, vitamin D, riboflavin, and folate. A positive association emerged for iron intake. No links were found with retinol, alpha-tocopherol, thiamin, vitamin B6, vitamin B12. Inverse associations of calcium, vitamin C, folate and CRC were most pronounced among these micronutrients-containing individual supplements users. The positive association between iron intake and CRC risk was most pronounced among iron supplement users.

When intake from diet only was considered for the micronutrients of interest, associations were diminished. The exclusion of regular supplement users resulted in a

slight decrease in power in these analyses. In addition, we observed that inverse associations of calcium, vitamin C, folate and CRC were most pronounced among individual supplement users. One possibility is that in this study micronutrients from supplemental sources are mainly contributable to the differences between cases and controls. Intakes of micronutrients from food sources did not vary significantly among cases and controls. Thus, we presume that diet alone might not furnish enough micronutrients and the levels of micronutrients in the Canadian diet may be insufficient to greatly reduce the risk of CRC. Instead, individual supplements provide sufficient micronutrients and exert protective effects. Therefore, with the increasing prevalence of dietary supplements use in Canadian population, our findings highlight the importance of collecting information on supplements use when studying associations of micronutrients with disease risk.

Our findings suggesting a possible inverse relation of CRC risk with intakes of calcium and vitamin D are consistent with results from a recent Multiethnic Cohort Study conducted in Hawaii and Los Angeles [123]. After a follow-up of 85,903 men and 105,108 women for 5-8 years, Park *et al.* found that total calcium intake (from foods and supplements) was inversely associated with CRC risk in both men and women (RR=0.70, 0.64, respectively). The inverse association was also seen for total vitamin D intake in men (RR=0.72). As in our study, we observed inverse associations with intakes of total calcium(OR=0.59) and total vitamin D(OR=0.73). Moreover, after exclusion of supplement users, inverse associations with calcium and vitamin D (from food source only) remained significant.

In 2007, Ryan-Harshman reviewed several case-control and prospective cohort studies and some clinical trials and concluded that evidence of calcium and vitamin D reducing risk of CRC was stronger. Multivitamin and mineral supplements can complement a healthy diet[29]. Consistent results in our study found that the lowest risk was observed among calcium supplements users with higher dietary calcium intake. This finding again suggests that the use of calcium supplements have further benefit in preventing CRC. However, the protective role of vitamin D should be concluded with caution, because vitamin D is partially derived from multivitamin supplements.

The findings support the hypothesis that vitamin C is protective against CRC, possibly through antioxidant mechanisms. Antioxidants, such as carotene, retinol, alpha-tocopherol and vitamin C, may decrease the risk by quenching free radicals and reducing oxidative damage to DNA[20,21]. These findings were in keeping with many other studies[124-126]. However, in this study, we observed an inverse association only between vitamin C and CRC, but no relationships were found with retinol or alpha-tocopherol intake. One possibility is that intakes of these nutrients were too low, even in the highest quintiles, to observe significant associations. Results also showed that vitamin C supplement may have further benefit among those with relative lower dietary intake of vitamin C. This may be explained by the threshold effect that low intake of vitamin C may increase risk but incremental intake above the threshold level may add minimal benefits.

We observed that higher intakes of folate and riboflavin were inversely associated with CRC risk and a linear dose-response effect of increasing protection emerged for

both nutrients. Our findings support the hypothesis that folate may affect colorectal carcinogenesis because of its role in the synthesis of nucleic acid and DNA methylation[127]. Riboflavin, as flavin adenine dinucleotide, is the cofactor for methylenetetrahydrofolate reductase (MTHFR), the enzyme that influences homocysteine remethylation and DNA methylation[128]. In our study, folate intake was derived from foods, folate supplement and multivitamin supplements. After controlling for multivitamin supplements use, an inverse association between folate and CRC was most pronounced among folate supplement users. Except for those from food sources, riboflavin was mainly derived from multivitamin supplements, thus caution should be taken in interpreting the protective role of riboflavin. It is worthwhile to further explore independent effects of riboflavin and vitamin D as individual supplementation on the occurrence of CRC.

An important finding in our study was the strong positive association between iron intake and CRC risk, with the risk strongly increased in the highest quintile of iron intake. Also, after controlling for multivitamin supplements use, an positive association between iron and CRC was most pronounced among iron supplement users, and a 70% increased risk was observed in the higher level of dietary iron intake. Iron may increase the risk of CRC by generating free radicals that attack DNA and damage chromosomes[22,23,129]. Interestingly, in this study, cases had higher intake of iron than controls, primarily due to larger contribution of iron supplement. Iron plays an important role in helping our red blood cells deliver oxygen to the rest of the body. Low iron levels can cause people to develop iron deficiency anemia. However, excess intake of iron or inadequately using

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iron supplement may have harmful effects of increasing CRC risk. The recommended daily allowance (RDA) for men and women 50 years old and older has been established at 8mg daily[130]. In our study, the average daily intake of iron (from food and supplements) was 30mg for cases and 25mg for controls, which is much higher than the RDA. Thus, attention should be taken in consuming iron containing foods and supplements.

This study had a number of strengths. We had a large sample size, which allowed us to observe associations that would be undetectable in smaller studies. More importantly, the previous findings about the protective effects of micronutrients were confined to a specific study population, which makes it difficult to generalize the results. In this study, we conducted pooled analyses of the population of two Canadian provinces to investigate the associations of selected micronutrients and CRC risk, and hence to make conclusions about specific micronutrients having possible chemo-preventive effects on CRC. Furthermore, nutrient intakes were adjusted for total energy intake. The use of calorie-adjusted values in multivariate models will often overcome the problem of high collinearity frequently observed between nutritional factors[112]. To the extent that, this adjustment also reduces between-person variation due to over- or underreporting of food intakes[112]. The relationships of selected micronutrients and CRC risk may differ appreciably by several factors, so we controlled for a wide range of potential confounding factors using multivariate logistic regression models. Although some random misclassification of diet is likely, non-differential misclassification generally tends to bias the risk estimates toward the null.

Consideration must be given to the potential limitations in the present study that

may have influenced the observed associations. First, as in most case-control studies, potential recall and selection biases are possible. Since exposure information was collected after diagnosis, differential recall between cases and controls would bias results; in particular, cases may recall dietary exposures differently from controls because of the presence of illness or symptoms. Controls may have agreed to join this study because of an interest in health and may therefore have healthier dietary and physical activity habits, a pattern that may exaggerate differences with the cases beyond what might have been seen with truly comparable controls.

Second, by design, cases and controls had similar sex distribution, however, cases and controls were not well comparable according to age group. Estimates of nutrient intakes from a FFQ are not precise and there is always the potential for measurement error. Although the original FFQ used in this study has been validated[110,111], this questionnaire requires further evaluation because it was originally developed for the Hawaiian and Californian populations that may be different from people residing in NL and ON. FFQ used in NL has been adapted to include regional foods in NL, however, OFCCR used the original FFQ that has not been adapted. Thus, a sub-study will be necessary to assess the level of agreement between the FFQ used by the OFCCR and the FFQ that was previously developed specifically for Canadian populations.

Third, in this study micronutrient intakes were categorized into quintiles based on the distribution among the study population. Although this method was often used in several studies[5,124,131], we may take individual recommended dietary allowances(RDA) into consideration in future study. For example, micronutrient intakes may be categorized

as ≤50% RDA, 50%-80%RDA, 80%-120%RDA, >120%RDA. However, this is likely not a major problem for most micronutrients, as many requirements are related to body size and physical activity level, and we have taken both BMI and physical activity into account in this study.

Fourth, these findings may reflect problems of collinearity between various micronutrients, between selected foods (such as fruits and vegetables), and between multivitamin supplements, thus this possibility cannot be completely eliminated. Finally, it is also possible that the 1-2 year referent period on which dietary data were based is insufficient if more remote diet (eg. 5-10 yrs) has a stronger influence on CRC risk.

In conclusion, our study presents evidence that certain micronutrients (calcium, vitamin D, vitamin C, riboflavin and folate) may protect against CRC, however, iron may increase the risk. Levels of micronutrients in the Canadian diet may be insufficient to greatly reduce the risk of CRC. Our findings suggest that specific micronutrients may exert an independent protective effect against colorectal carcinogenesis. Furthermore, calcium supplement, vitamin C supplement, and folate supplement may have further benefit in preventing CRC, whereas iron supplement may have harmful effect of increasing CRC risk. Yet it is also likely that other physiologic, behavioral, and dietary factors interact with micronutrients to affect risk. Attempts to reduce risk by selecting one or even several micronutrients for supplementation may not have the anticipated results until their interactions with other factors are better understood.

Table 4.2.1 Selected characteristics of subjects from CRC case-control study in NL and ON

Characteristics[a]	Cases(n=1760)		Controls(n=2481)	
	No. (%)		No. (%)	
Age (years)[*]				
18-49	368(20.9)		265(10.7)	
50-59	412(23.4)		690(27.8)	
60-69	646(36.7)		998(40.2)	
70+	334(19.0)		528(21.3)	
Sex				
Males	935(53.1)		1357(54.7)	
Females	825(46.9)		1124(45.3)	
Province of residence				
NL	488(27.7)		651(26.2)	
ON	1272(72.3)		1830(73.8)	
BMI[b] (kg/m²)[*]				
Underweight(<18.5)	23(1.3)		22(0.9)	
Normal(18.5-24.9)	595(33.8)		930(37.5)	
Overweight (25-29.9)	748(42.5)		1069(43.1)	
Obese (≥30)	394(22.4)		460(18.5)	
Physical activity (METs/week[b])[*]				
0 - 7.4	465(26.4)		595(24.0)	
7.4 -22.4	348(19.8)		633(25.5)	
22.4 - 53.0	429(24.4)		633(25.5)	
> 53.0	518(29.4)		620(25.0)	
Family history of CRC[*]				
No	1582(89.9)		2337(94.2)	
Yes	178(10.1)		144(5.8)	
Fruit intake (X servings/week)				
0 ≤ X ≤ 6	475(27.0)		625(25.2)	
6 < X ≤ 7	502(28.5)		754(30.4)	
7 < X ≤ 14	459(26.1)		653(26.3)	
X >14	324(18.4)		449(18.1)	
Vegetable intake (X servings/week)				
0 ≤ X ≤ 6	260(14.8)		367(14.8)	
6 < X ≤ 7	549(31.2)		796(32.1)	
7 < X ≤ 14	505(28.7)		707(28.5)	
X >14	445(25.3)		610(24.6)	
Red meat intake[*] (X servings/week)				
0 ≤ X ≤ 2	269(15.3)		486(19.6)	
2 < X ≤ 3	702(39.9)		987(39.8)	
3 < X ≤ 5	398(22.6)		526(21.2)	
X >5	392(22.3)		481(19.4)	

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Characteristics[a]	Cases(n=1760)	Controls(n=2481)
	No. (%)	No. (%)
Total energy intake (kcal/day)[*]		
Quintile 1 (≤1580)	313(17.8)	536(21.6)
Quintile 2 (1580-1943)	341(19.4)	506(20.4)
Quintile 3 (1943-2314)	343(19.5)	506(20.4)
Quintile 4 (2314-2866)	359(20.4)	489(19.7)
Quintile 5 (>2866)	404(22.9)	444(17.9)

[a] All characteristic variables presented as number(%).

[b] BMI, Body mass index; METs/week, metabolic equivalent hours per week

[*] Significant difference between cases and controls (P<0.05)

Table 4.2.2 Comparison of mean intakes of selected micronutrients between cases and controls, CRC case-control study in NL and ON

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

[a] All continuous variables presented as mean ± SD (standard deviation).
[b] For continuous variables, differences between cases and controls based on T-test.

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Table 4.2.3 Adjusted OR[a], 95%CI[a] of CRC risk according to selected micronutrients intakes from both food and supplement sources, CRC case-control study in NL and ON

Micronutrients from food and supplements	Quintiles of intakes					p-trend[d]
	Q1	Q2	Q3	Q4	Q5	
Calcium						
No. of cases/controls	414/436	362/486	385/463	315/533	284/563	
Median intake (mg/d)	662.7	865.0	1049.7	1285.6	1786.7	
OR[a] (95% CI)	1.00	0.83	0.94	0.66*	0.57*	0.04
		(0.68,1.01)	(0.77,1.14)	(0.54,0.80)	(0.47,0.69)	
OR[a] (95% CI)	1.00	0.86	1.10	0.72*	0.59*	0.11
		(0.67,1.09)	(0.86,1.40)	(0.56,0.92)	(0.45,0.77)	
Iron						
No. of cases/controls	374/476	373/475	343/505	320/528	350/497	
Median intake (mg/d)	12.6	15.3	18.5	25.1	36.7	
OR[a] (95% CI)	1.00	1.07	0.94	0.83	0.95	0.42
		(0.88,1.30)	(0.77,1.14)	(0.68,1.01)	(0.78,1.15)	
OR[a] (95% CI)	1.00	0.99	0.97	1.09	1.34*	0.02
		(0.79,1.25)	(0.77,1.23)	(0.84,1.41)	(1.01,1.78)	
Retinol						
No. of cases/controls	369/481	380/468	361/487	338/510	312/535	
Median intake (ug/d)	454.8	777.6	1033.8	1684.6	2766.2	
OR[a] (95% CI)	1.00	1.13	1.05	0.90	0.82*	0.07
		(0.93,1.37)	(0.86,1.28)	(0.74,1.09)	(0.67,1.00)	
OR[a] (95% CI)	1.00	1.29	1.24	1.14	1.08	0.77
		(0.99,1.62)	(0.98,1.57)	(0.89,1.46)	(0.79,1.47)	
Vitamin C						
No. of cases/controls	407/443	359/489	354/494	333/515	307/540	
Median intake (mg/d)	82.6	142.0	203.3	310.6	776.3	
OR[a] (95% CI)	1.00	0.87	0.85	0.74*	0.67*	0.06
		(0.72,1.06)	(0.70,1.03)	(0.61,0.90)	(0.55,0.82)	
OR[a] (95% CI)	1.00	0.79	0.84	0.81	0.67*	0.09
		(0.62,1.01)	(0.66,1.07)	(0.63,1.05)	(0.51,0.88)	
Vitamin D						
No. of cases/controls	394/456	346/502	394/454	320/528	306/541	
Median intake (ug/d)	2.77	4.62	6.64	11.5	16.7	
OR[a] (95% CI)	1.00	0.86	1.08	0.73*	0.71*	0.13
		(0.71,1.05)	(0.89,1.31)	(0.60,0.89)	(0.59,0.87)	
OR[a] (95% CI)	1.00	0.93	1.20	0.75*	0.75*	0.18
		(0.73,1.19)	(0.94,1.53)	(0.59,0.96)	(0.57,0.94)	
Alpha-tocopherol						
No. of cases/controls	388/462	372/476	373/475	325/523	302/545	
Median intake (mg/d)	4.3	5.5	7.6	19.7	174.1	
OR[a] (95% CI)	1.00	0.97	0.96	0.76*	0.72*	0.15
		(0.80,1.18)	(0.80,1.17)	(0.63,0.93)	(0.60,0.88)	
OR[a] (95% CI)	1.00	0.97	1.05	1.04	0.86	0.05
		(0.76,1.23)	(0.83,1.33)	(0.76,1.43)	(0.65,1.13)	
Thiamin						
No. of cases/controls	381/468	376/473	365/483	342/507	296/550	

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| Micronutrients from food | Quintiles of intakes | | | | | p- |
and supplements	Q1	Q2	Q3	Q4	Q5	trend[d]
Median intake (mg/d)	1.53	1.82	2.11	2.95	4.16	
OR[b] (95% CI)	1.00	1.07	1.02	0.89	0.70*	0.01
		(0.88,1.30)	(0.84,1.24)	(0.73,1.08)	(0.58,0.86)	
OR[c] (95% CI)	1.00	0.96	1.10	0.99	0.79	0.14
		(0.76,1.21)	(0.87,1.39)	(0.74,1.31)	(0.55,1.12)	
Riboflavin						
No. of cases/controls	412/438	360/488	355/493	343/505	290/557	
Median intake (mg/d)	1.93	2.37	2.85	3.71	5.44	
OR[b] (95% CI)	1.00	0.83	0.81*	0.76*	0.57*	0.01
		(0.69,1.01)	(0.66,0.98)	(0.62,0.92)	(0.47,0.70)	
OR[c] (95% CI)	1.00	0.87	0.86	0.82	0.61*	0.01
		(0.69,1.11)	(0.68,1.10)	(0.64,1.05)	(0.47,0.78)	
Vitamin B6						
No. of cases/controls	386/464	369/479	359/489	348/500	298/549	
Median intake (mg/d)	1.71	2.15	2.58	4.01	5.82	
OR[b] (95% CI)	1.00	0.99	0.95	0.89	0.69*	0.01
		(0.82,1.21)	(0.78,1.15)	(0.73,1.08)	(0.57,0.84)	
OR[c] (95% CI)	1.00	1.04	1.03	1.22	0.95	0.95
		(0.83,1.32)	(0.82,1.31)	(0.91,1.63)	(0.66,1.36)	
Folate						
No. of cases/controls	412/438	389/459	343/505	303/545	313/534	
Median intake (ug/d)	236.5	311.3	390.7	701.2	1069.8	
OR[b] (95% CI)	1.00	1.00	0.81*	0.64*	0.69*	0.08
		(0.83,1.22)	(0.67,0.99)	(0.52,0.77)	(0.56,0.84)	
OR[c] (95% CI)	1.00	0.97	0.86	0.72*	0.72*	0.04
		(0.76,1.23)	(0.67,1.09)	(0.56,0.92)	(0.56,0.92)	
Vitamin B12						
No. of cases/controls	387/464	368/479	350/498	352/496	303/544	
Median intake (ug/d)	4.15	6.03	8.15	15.2	22.6	
OR[b] (95% CI)	1.00	0.98	0.90	0.89	0.71*	0.01
		(0.80,1.19)	(0.74,1.10)	(0.73,1.08)	(0.58,0.86)	
OR[c] (95% CI)	1.00	0.95	0.94	1.10	0.93	0.97
		(0.75,1.20)	(0.74,1.19)	(0.83,1.45)	(0.66,1.32)	

[a] OR, Odds ratio; 95%CI, 95% confidence interval.

[b] Adjusted for age and total energy intake.

[c] Adjusted for total energy intake, age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alternation in the parameter coefficient of interest.

[d] Two-sided p value for test of linear trend was calculated by using median values for each quintile of intake.

* Significant different from reference category, $p \leq 0.05$

Table 4.2.4 Adjusted OR[a], 95%CI[b] of CRC risk according to selected micronutrients intakes from food source only, CRC case-control study in NL and ON

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

[a] OR, Odds ratio; 95%CI, 95% confidence interval.

[b] Adjusted for age and total energy intake.

[c] Adjusted for total energy intake, age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alternation in the parameter coefficient of interest.

[d] Two-sided p value for test of linear trend was calculated by using median values for each quintile of intake.

* Significant different from reference category, $p \leq 0.05$

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Table 4.2.5 Adjusted OR[a], 95%CI[b] of CRC risk according to individual supplement use and levels of micronutrient intakes from foods, CRC case-control study in NL and ON

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

[a] OR, Odds ratio; 95%CI, 95% confidence interval.
[b] Adjusted for total energy intake, age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat.
[c] Two-sided p value for test of linear trend was calculated by using median values for each quintile of intake.
* Significant different from reference category, p≥0.05

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4.3 Paper 3. Association of calcium, vitamin D and dairy products intakes with colorectal cancer risk: results from a large population-based case-control study in Newfoundland and Labrador and Ontario

4.3.1 Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer death in men and women combined in Canada[37]. In 2009, an estimated 22,000 Canadians were diagnosed with CRC and 9,100 died of it[37]. There are substantial inter-provincial incidence rate variations in Canada, and Newfoundland and Labrador (NL) has the highest incidence, while Ontario (ON) ranks in the middle. According to the Canadian Cancer Statistics 2009, the age-standardized incidence rates of CRC in NL were 87 per 100,000 for men and 52 per 100,000 for women, which are higher than ON rates (men: 60 per 100,000 and women: 41 per 100,000) and the national average rates(men: 62 per 100,000 and women: 41 per 100,000)[38].

The high rates of the disease in NL may be partly explained by a high prevalence of families with a predisposition to hereditary colon cancer[39], however environmental factors have been shown to be important components to CRC risks[1,40-43]. Diet has long been regarded as one of the most important environmental factors associated with the cause of CRC[1]. High intake of animal fat or protein and low intake of fruits and vegetables have repeatedly been shown to increase the risk of CRC[12]. It is thought that fat promotes large bowel cancer by increasing the levels of free ionized fatty acids and unconjugated bile acids in the bowel lumen. These are irritating and toxic to the surrounding epithelium[65,66].

Calcium has been hypothesized to protect against CRC by binding secondary bile acids and ionized fatty acids in the colon lumen to form insoluble calcium soaps, thereby reducing their proliferative effects on the colonic mucosa[15]. Furthermore, calcium may have effects on cell proliferation and differentiation, apoptosis, angiogenesis, and cell-cycle regulation[76,132-136]. The roles of dietary calcium and vitamin D are highly correlated because vitamin D regulates the absorption of calcium[76]. In addition to its indirect role in maintaining calcium homeostasis, the direct genomic action of vitamin D is linked to a multitude of biological responses, including DNA synthesis and preventing double-strand breaks by exogenous or endogenous sources, making vitamin D an important independent contributor to the calcium/colorectal carcinogenesis mechanistic pathway[137].

Despite the biological plausibility, epidemiological studies have been inconclusive in demonstrating the role of calcium and vitamin D in the etiology of CRC. Prior to 1998, reviewers of epidemiologic studies concluded that calcium intake was not associated with lower risk of colorectal adenoma or cancer [28,138-140]. Dairy products contain large amount of calcium and vitamin D through fortification. It has been shown that calcium, especially in combinations as found in milk, effectively precipitates luminal cytotoxic surfactants and thus inhibits colonic cytotoxicity [29,30]. In contrast, in a prospective study, Jarvinen *et al.* [28] indicated that individuals with a high consumption of milk have a potentially reduced risk of colon cancer, however, the association did not appear to be due to intake of calcium, vitamin D, or to specific effects of fermented milk. A recently published review concludes that the available research suggests a protective effect of vitamin D on colon cancer risk[122]. Recent research indicates that calcium and vitamin D might act

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together, rather than separately, to reduce risk of CRC. Results from a multi-centre, placebo-controlled randomized clinical trial found that calcium supplementation was associated with adenoma recurrence only when vitamin D levels were above the median (29.1 ng/ml)[81].

There may be a number of reasons for these inconsistencies between studies, such as the danger of selection bias and possibly recall bias that could have played a larger role in retrospective studies. In recent years, Canada population are taking more individual or multivitamin supplements, however, some studies do not take supplements use into account. Moreover, the effects of calcium and vitamin D on risk may differ appreciably by several factors including gender, total dietary fat intake, and use of nonsteroidal anti-inflammatory drugs (NSAIDs). Whether or not dairy products are protective against CRC is a question. Again, if dairy products intakes are associated with reduced risk of CRC, whether these associations can be explained by components of calcium and vitamin D.

In order to investigate these associations, we selected NL and ON residents as the target population. NL is geographically isolated, culturally distinct, relatively economically disadvantaged, and fresh fruits and vegetables are less often available. Consequently, people may consume more preserved and salted traditional foods. Whether or not the highest incidence of CRC in NL can be partly explained by the unique dietary habits of NL population is a question. Squire *et al.* recently found that in NL higher intake of red pickled meat was associated with increased risk of CRC[141]. In contrast, ON is a centrally located, culturally diverse, and economically advantaged province. It was hypothesized that if consistent results of the protective effects of calcium

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and vitamin D were found in two diverse provinces, we could provide support to the argument that calcium and vitamin D have chemo-preventive effects on CRC. To our knowledge, little has been done in this area. Therefore, the purpose of this report is to assess the effects of calcium, vitamin D, dairy products on the occurrence of CRC and compare differences of these associations between two provinces.

4.3.2 Materials and Methods

(note to readers: this section mostly duplicates 4.1.2)

4.3.2.1 Selection of cases and controls

Data for this case-control study were from the Ontario Familial Colorectal Cancer Registries (OFCCR) and Newfoundland Familial Colorectal Cancer Registries (NFCCR). In ON incident cases diagnosed during 1997-2000 were identified through the population-based Ontario Cancer Registry. In NL incident cases diagnosed during 1999-2003 were identified through the population tumor registry maintained by the Newfoundland Cancer Registry. Both registries were used to identify newly diagnosed cases of colon or rectal cancer (pathology confirmed ICD 9th revision codes: 153.0-153.9, 154.1-154.3, and 154.8 or ICD-0 codes: 18.0-18.7, 19.9, 20.9), and aged 20-74 years. Phase two of the OFCCR was initiated to collect data for cases diagnosed in ON during 2003-2006. Initial contact was with the surgeon/physician identified on the pathology report. Once physician consent was obtained, individuals were then contacted to inform them of the study. Participants who indicated their willingness to participate the study were sent, in sequence, a written consent form, family history questionnaire (FHQ), personal history questionnaire (PHQ), and food frequency questionnaire (FFQ). Non-

responders were sent post-card reminders and phoned several weeks after initial contact to remind them of the mailing.

Controls were a random sample of residents in each province aged 20-74 years. In ON controls were identified through a list of residential phone numbers or from population-based property assessment rolls (owners and occupants). In NL controls were identified through random digit dialing. Both registries frequency matched controls to cases on sex and five-year age strata. Once verbal consent for participation was obtained during the phone contact, a survey package was forwarded to each potential participant. The package included an information pamphlet with general information concerning the study, a consent form, a FHQ, a PHQ, and a FFQ, as well as self addressed stamped envelopes.

4.3.2.2 Dietary and epidemiologic data collection

Information on dietary intake was collected using a self-administrated FFQ. This 19-page FFQ was originally developed for the Hawaiian and Californian populations by the Epidemiology Program, Cancer Research Centre of Hawaii and has been previously described and validated against 24-h recalls among a multi-ethnic Hawaiian/Southern Californian population[110,111]. This previously validated food frequency questionnaire has been adapted to include regional foods in NL and was used to assess diet over 1-2 years prior to diagnosis or interview in each province. Participants were questioned about their intake of almost 170 foods which were believed to be important to the contribution of most nutrients in the diet. For each food item, subjects were asked to estimate the frequency of food intake and their usual portion size from 'Regular', 'Small' or 'Large'.

A food photographs were provided that showed regular, small and large portion sizes for vegetables, meat and chicken. Participants were also questioned on their use of any individual or multivitamin supplements, including the usual brand name, the amounts taken and the duration of consumption.

Nutrient intakes were computed by multiplying the frequency of consumption of each food item by the nutrient content of portion size. In ON values for the amounts of nutrients in the foods were obtained from the USDA file. In NL nutrient contents were based on the 2005 Canadian Nutrient file. Intakes of calcium and vitamin D were energy-adjusted by using residuals calculated from the linear regression of the log of nutrient intake versus the log of energy intake[112]. Residuals were adjusted to mean energy intake in men or women in each province. In NL, residuals were adjusted to 2411.5 kcal/day for men and 2276.2 kcal/day for women; in ON, residuals were adjusted to 2404.8 kcal/d for men and 1987.9 kcal/day for women. Intakes of total calcium and total vitamin D were calculated by adding energy-adjusted nutrients from food and unadjusted nutrients from supplements.

We also examined associations between CRC risk and the consumption of five groups of dairy foods (total dairy products, milk, non-milk dairy products, yogurt, and cheese), because these groups were measured in most of the studies. We computed total dairy food consumption by adding the daily servings of all foods in the dairy categories. Total milk consumption was calculated by adding the daily servings of nonfat milk or skim milk, low-fat milk, and whole milk. Non-milk dairy products consumption was calculated by adding the daily servings of yogurt, cheese and cream.

The self-administered personal history questionnaire included many close-ended questions about medical history, bowel screening history, diet, medication use, diet, physical activity, alcohol and tobacco use and socio-demographic measures such as education and income. Identifying information such as sex, age, date of birth, and marital status was collected. For female participants there were additional questions relating to reproductive factors.

For the analyses, we excluded those who did not provide sufficient dietary information at baseline, those who failed to provide information on potential risk factors at baseline, those who reported energy intake in the upper or lower 2.5% of intake (lower and upper cutoff: In NL, 925 and 4700 kcal for men, 1100 and 4900 kcal for women, respectively; In ON, 1040 and 5200 kcal for men, 835 and 4100 kcal for women, respectively), and patients who had familial adenomatous polyposis (FAP) and an in-situ tumor were excluded. After these exclusions, based on those who completed both the PHQ and FFQ, 3102 subjects (1272 cases and 1830 controls) from ON and 1139 subjects (488 cases and 651 controls) from NL remained. Data collected from these subjects were used for the analysis.

4.3.2.3 Statistical analyses.

Statistical analyses were performed using SAS statistical software (version 9.1 SAS Institute, Cary, NC, USA). Descriptive statistics stratified by case-control status were used to describe the demographic/health-related characteristics and dietary intakes of subjects. Intakes of calcium, vitamin D and dairy products were categorized into quintiles based on the distribution among the study population without missing endpoints and were

entered into models as indicator variables with the lowest quintile as the referent group.

Age and total energy intake-adjusted odds ratios (OR) and their corresponding 95% confidence intervals (95%CI) were calculated from maximum-likelihood estimates in unconditional logistic regression to assess the association of the outcome with dietary intakes. Multivariate unconditional logistic regression was used to evaluate the association of intakes of calcium, vitamin D and dairy products with CRC risk after adjusting for a set of potential confounders or covariates. Tests for trend were used to assess dose-response relationships based on the median of each category of dietary intake.

Potential confounding factors include age(18-49, 50-59, 60-69 and 70+years); sex; body mass index(BMI<18.5, 18.5-24.9, 25-29.9, and $\geq 30 kg/m^2$); physical activity(<7.4, 7.4-22.4, 22.4-53.0, and >53.0 metabolic equivalent hours/week, METs/week); family history of CRC(yes, no); polyps(yes, no); diabetes(yes, no); history of colon screening procedure(yes, no); cigarette smoking(ever smoke, never smoke); alcohol drinking(<14, ≥ 14drinks/week); education attainment(high school graduate or less, technical school/ some college/ university, and bachelor's degree/graduate degree); household income(less than \$12,000; \$12,000-29,999, \$30,000-49,999, and \$50,000); marital status(married, single/never married, and separated/divorced/widowed); regular use of medication and supplements: non-steroid anti-inflammatory drug (NSAID)(yes, no), multivitamin supplements (yes, no), folate supplement(yes, no); reported hormone replacement therapy (HRT, females only)(yes, no); and dietary intakes: total energy intake (quintiles), fruits(0-6, 6-7, 7-14, and >14 servings/week), vegetables(0-6, 6-7, 7-14, and >14 servings/week), red meat(0-2, 2-3, 3-5, and >5 servings/week); province of residence(NL, ON). The basis

for the assessment of confounding factors included: (1) literature and previous studies, (2) biological plausibility, (3) whether the regression coefficient of the primary dependent variable changed by 10% or more after addition of the potentially confounding variable, or (4) whether the covariate entered the model at $p<0.05$. A backwards-stepwise procedure was performed to obtain the final model. Statistical tests were two sided, and p values less than 0.05 were considered statistically significant.

4.3.3 Results

Demographic and lifestyle characteristics of the study participants, stratified by provinces and case-control status, are shown in Table 4.3.1. The study participants included 1760 cases (488 from NL, 1272 from ON) and 2481 controls (651 from NL, 1830 from ON). NL cases were slightly older than controls, while ON cases were slightly younger than controls. In both provinces, cases had higher BMI than controls, and were less likely to report any colon screening procedure, to report use of multivitamin supplements, and to have taken HRT over the previous 1-2 years (females only). Physical activity (METs/week), or heavy alcohol drinking history did not vary significantly between cases and controls in two provinces. NL cases tended to be smokers, and less likely to have acquired higher education or obtain a high income during the preceding year (all $p<0.05$). ON CRC cases more often had a family history of CRC, and they less often used NSAID during the past year (all $p<0.05$).

Table 4.3.2 gives the mean intakes of food, selected nutrients and dairy products by the cases and controls in both provinces. Both provinces' cases reported higher intakes of total energy than controls. There was a greater red meat consumption among ON cases,

but no marked differences in the fruit and vegetable consumption between cases and controls were found in either province. Controls generally reported higher levels of mean daily intake of calcium and vitamin D, however, the extent of the differences varied by province. Specifically, both provinces controls reported significantly higher intakes of total calcium, calcium from food, calcium from supplements, total vitamin D and vitamin D from supplements than did their respective cases (all $p<0.05$). In ON controls also reported significantly higher consumption of vitamin D from food, total dairy products and milk than did cases (all $p<0.05$).

The OR and 95%CI of CRC according to intakes of calcium and vitamin D from food and supplements, stratified by provinces, are shown in Table 4.3.3. Inverse associations with CRC risk were observed for high intakes of age-energy-adjusted total calcium, calcium from food and total vitamin D in both provinces, however, after other potential covariates were taken into account, the inverse associations were no longer significant in NL, but the protective effect of these nutrients remained significant only in ON. The multivariate-adjusted OR of CRC in ON for individuals in the highest quintile of intake compared with those in the lowest quintile was 0.57 for total calcium(95% CI: 0.42-0.77, p-trend=0.03), 0.76 for dietary calcium(95% CI: 0.60-0.97, p-trend=0.06), and 0.73 for total vitamin D(95% CI: 0.54-1.00, p-trend=0.18). In addition, higher intake of dietary vitamin D in ON subjects were also significantly inversely associated with CRC risk (OR=0.77, 95% CI: 0.61-0.99, p-trend=0.38). The observed reduction in risk among participants in both provinces with consuming calcium-containing supplements were 33% (NL) and 24% (ON), and 32% reduced risk in NL emerged for consuming vitamin

D-containing supplements.

We additionally evaluated the consumption of total dairy foods and specific dairy foods in relation to the risk of CRC (Table 4.3.4). In ON, the risk of CRC was significantly reduced for those who consumed total dairy food >25.5servings/week compared with those who consumed <3.1servings/week (OR=0.78, 95%CI: 0.60-1.00) in both age-energy adjusted models and multivariate-adjusted models. In particular, those who consumed ≥14.9cups/week of milk had a 22% lower risk of CRC than did those who consumed <0.6cups/week. A non-significant inverse association was found in yogurt intake. In NL, inverse associations were observed for age-energy adjusted total dairy foods and milk, however, after adjusting for multi-variables, the inverse relationships were no longer significant.

When the combined effect of total calcium and total vitamin D was considered, the inverse association was most pronounced among subjects reporting high calcium and high vitamin D intakes compared to those reporting a low intake of bot h nutrients (Table 4.3.5).

4.3.4 Discussion

In this large population case-control study conducted in two Canadian provinces, we found inverse associations of intakes of total calcium, dietary calcium, total vitamin D, dietary vitamin D, total dairy products and milk with CRC risk in two provinces. However, after adjusting for potential confounders and covariates, significant inverse associations are only observed in the ON population. Nonetheless, in NL the inverse associations of calcium, vitamin D with CRC risk were most pronounced among calcium-

or vitamin D- containing supplements users. In ON use of calcium-containing supplements was inversely related to CRC risk. These results add to support for synergistic effects of calcium and vitamin D in the prevention of colorectal carcinogenesis.

Results from this study support hypothesis that calcium may protect against CRC by binding secondary bile acids and ionized fatty acids in the colon lumen to form insoluble calcium soaps, thereby reducing their proliferative effects on the colonic mucosa[15] and vitamin D regulates the absorption of calcium[78], as well as their effects on cell proliferation and differentiation, apoptosis, angiogenesis, and cell-cycle regulation[78,132-136].

Our findings are consistent with a number of studies that have reported inverse associations between calcium, vitamin D and CRC risk[25,122]. In 2004 Grant, W. B.[122] found that the available research suggested a protective effect for vitamin D on colon cancer risk. In 2007, Ryan-Harshman reviewed several case-control and prospective cohort studies and some clinical trials and concluded that evidences of calcium, vitamin D reducing CRC risk were stronger. In particular, recent research indicates that calcium and vitamin D might act together, rather than separately, to reduce the risk of colorectal adenomas. People might be able to reduce their risk of CRC by increasing their vitamin and mineral levels through eating more vegetables and fruit. Multivitamin and mineral supplements can complement a healthy diet[25]. As in our study, significant inverse associations of CRC risk emerged with intakes of total calcium, dietary calcium(from food), total vitamin D, and dietary vitamin D(from food) in ON population. We also observed a combined effect of calcium and vitamin D that a significant decreased risk of

CRC was observed among those who had higher levels of calcium and vitamin D intake. Moreover, calcium or vitamin D supplement use was inversely related to CRC risk.

Nonetheless, it is rather surprising that we did not observe meaningful associations of calcium, vitamin D with CRC risk in NL after adjusting for multi-variables. We did observe these inverse associations in NL after adjusting for age and total energy intake only. One possibility is that intakes of these nutrients in NL were too low, even in the highest quintiles, for us to observe significant associations. This may be the case with calcium, for which intakes in ON subjects were considerably higher than in NL subjects (Table 2). It is also possible that the findings in this study may be due in part to colliearity between nutrients and foods of which they are constituents. For instance, dietary fat, phosphorous and dietary fibre may limit the intestinal absorption of calcium due to increased production of insoluble calcium complexes[142-144]. However, the inverse associations of calcium, vitamin D with CRC risk were most pronounced among calcium- or vitamin D-containing supplement users in NL subjects. NL controls were more likely than cases to consume calcium- or vitamin D-containing supplements (Table 2). Yet it is also likely that supplements users may be more conscious about health and therefore may have healthier dietary and physical activity habits. However, we attempted to control for the effects of other physiologic, behavioral, and dietary factors in these analyses. Another possibility that calcium- or vitamin D containing supplements use may have independent effects on cancer risk. Discussion of such potential biological mechanisms is beyond the scope of the present paper.

Consistent with results of relationships among calcium, vitamin D and CRC risk in

two provinces, results from this study found that inverse associations emerged for total dairy products and milk in ON, whereas these associations were not significant in NL. These findings suggest that these associations appeared to be largely due to intake of calcium and vitamin D. Our findings add to the evidence that dairy products, in particular milk, is an important contributor to dietary calcium and vitamin D. It has been shown that calcium, especially in combinations as found in milk, effectively precipitates luminal cytolytic substances and reduces cytotoxicity of fecal water, an accepted risk marker for colon cancer[29,30]. Besides calcium and vitamin D through fortification, many other components of dairy foods have been shown experimentally to protect against CRC. Dairy foods contain conjugated linoleic acid and lactoferrin, which inhibit colonic carcinogenesis in animal models[145,146], and the milk protein casein has antimutagenic activity on the digestive tract[147]. Certain microorganisms in fermented dairy foods have also been hypothesized to reduce the risk of CRC[148]. In this study, fermented dairy foods, such as cheese and yogurt, did not appear to be related to CRC risk. A possible reason is that cheese fats, particularly saturated fats, might increase risk[89]. In addition, the intakes of cheese and yogurt were too low to observe significant associations.

This study had a number of strengths. We had a large sample size, which allowed us to observe associations that would be undetectable in smaller studies. More importantly, the previous findings about the protective effects of macronutrients were confined to a specific study population, which makes it difficult to generalize the results. In this study, we examined the effects of dietary calcium, vitamin D and dairy products on the occurrence of CRC in two Canadian provinces with different rates of CRC incidence. We

compared differences of these associations between two provinces. Furthermore, nutrient intakes were adjusted for total energy intake. The use of calorie-adjusted values in multivariate models will often overcome the problem of high collinearity frequently observed between nutritional factors[112]. To the extent that, this adjustment also reduces between-person variation due to over- or underreporting of food intakes[112]. The relationships of calcium, vitamin D, dairy products and CRC risk may differ appreciably by several factors, so we controlled for a wide range of potential confounding factors using multivariate logistic regression models. Although some random misclassification of diet is likely, non-differential misclassification generally tends to bias the risk estimates toward the null.

Consideration must be given to the potential limitations in the present study that may have influenced the observed associations. First, as in most case-control studies, potential recall and selection biases are possible. Since exposure information was collected after diagnosis, differential recall between cases and controls may bias results; in particular, cases may recall dietary exposures differently from controls because of the presence of illness or symptoms. Controls may have agreed to join this study because of an interest in health and may therefore have healthier dietary and physical activity habits, a pattern that may exaggerate differences with the cases beyond what might have been seen with truly comparable controls.

Second, by design, cases and controls had similar sex distribution, however, cases and controls were not well comparable according to age group. Estimates of nutrient intakes from a FFQ are not precise and there is always the potential for measurement

error. Although the original FFQ used in this study has been validated[110,111], this questionnaire requires further evaluation because it was originally developed for the Hawaiian and Californian populations that may be different from people residing in NL and ON. FFQ used in NL has been adapted to include regional foods in NL, however, OFCCR used the original FFQ that has not been adapted. Thus, a sub-study will be necessary to assess the level of agreement between the FFQ used by the OFCCR and the FFQ that was previously developed specifically for Canadian populations. Third, these findings may reflect problems of collinearity between various nutrients, between selected foods, and between multivitamin supplements, thus this possibility cannot be completely eliminated.

Another potential limitation of this study may be the absence of information on sun exposure. As we know, it is difficult to accurately measure vitamin D exposure in humans[122]. We did not have information on sunshine exposure at baseline. Finally, it is also possible that the 1-2 year referent period on which dietary data were based is insufficient if more remote diet (eg. 5-10 yrs) has a stronger influence on CRC risk.

In conclusion, the results of our case-control study add to the evidence that total calcium, dietary calcium, total vitamin D and dietary vitamin D are associated with a lower risk of CRC. Total dairy products and milk intakes were inversely related with CRC risk, and these associations may be largely due to calcium and vitamin D. Furthermore, Calcium- or vitamin D-containing supplements may reduce risk of CRC. Finally, the present data support a joint action of calcium and vitamin D in the prevention of colorectal carcinogenesis.

Table 4.3.1 Selected characteristics of cases and controls, stratified by provinces, CRC case-control study in NL and ON

Characteristics	NL		ON	
	Cases (n=488)	Controls (n=651)	Cases (n=1272)	Controls (n=1830)
Age (years)[a]	62.7*±9.0	60.5±9.5	58.4*±10.9	61.5±9.7
BMI[c] (kg/m²)[a]	27.8*±4.8	27.2±4.4	26.7*±4.7	26.3±4.5
Physical activity (METs/week[c])[a]	58.0±74.7	50.2±73.2	43.8±58.6	41.4±59.2
Positive family history of CRC (%)[b]	49(10.0)	54(8.3)	127*(10.0)	92(5.0)
Reported any colon screening (%)[b]	60*(12.3)	145(22.3)	198*(15.6)	476(26.0)
Regular use of NSAID[c] (%)[b]	164(33.5)	252(38.7)	433*(34.0)	787(43.0)
Regular use of multivitamin supplements (%)[b]	66*(13.5)	145(22.3)	436*(34.3)	701(38.3)
Reported HRT[c] (%)[b]	132*(27.1)	251(38.6)	440*(34.6)	827(45.2)
Smokers, current and/or past (%)[b]	353*(72.3)	401(61.6)	733(57.6)	1078(58.9)
Heavy drinkers[c] (%)[b]	54(11.0)	68(10.4)	154(12.1)	205(11.2)
High level of education[c] (%)[b]	181*(37.0)	351(53.9)	700(55.0)	1087(59.4)
High household income[c] (%)[b]	102*(20.9)	229(35.1)	506(39.8)	758(41.4)

[a] Continuous variables presented as mean±SD(standard deviation), differences between cases and controls based on T-test.

[b] Categorical variables presented as number(%), differences between cases and controls based on chi-square test.

[c] BMI, body mass index; METs/week, metabolic equivalent hours per week; NSAID, nonsteroidal anti-inflammatory drugs; HRT, hormone replacement therapy, female only; Heavy drinkers, average drinks>14times/week; High level of education, included some college, university or post secondary school; High household income, average household income>$50,000/year.

* Significant difference between cases and controls ($p≤0.05$)

Table 4.3.2 Mean intakes of foods and nutrients among cases and controls, stratified by province, CRC case-control study

Intakes of foods and nutrients[a]	NL subjects		ON subjects	
	Cases (n=488)	Controls (n=651)	Cases (n=1272)	Controls (n=1830)
Fruit (servings/week)	9.6±8.1	10.5±8.2	11.3±8.1	11.0±8.2
Vegetable (servings /week)	11.1±7.6	11.9±8.3	13.8±9.0	13.2±8.5
Red meat (servings/week)	3.5±3.3	3.6±3.4	4.6*±4.4	4.0±3.8
Total energy (kcal/day)	2441.5*±838.2	2293.6±744.9	2266.0*±796.1	2161.5±757.7
Calcium (mg/day)				
Total calcium	989.6*±402.6	1108.3±500.9	1137.1*±509.2	1231.6±544.1
Calcium from food	933.4*±354.1	989.0±394.9	956.0*±302.0	1009.5±314.9
Calcium from supplements	56.2*±160.2	119.3±249.1	181.1*±404.7	222.1±440.6
Vitamin D (IU/day)				
Total vitamin D	332.2*±242.5	393.5±299.5	319.8*±218.4	352.7±236.4
Vitamin D from food	244.9±124.1	251.0±130.0	202.1*±104.5	220.8±111.0
Vitamin D from supplements	87.3*±201.0	142.5±260.3	117.7*±186.8	131.9±203.6
Dairy products (servings/week)				
Total dairy products	12.8±10.2	13.4±10.4	12.2*±8.6	13.0±9.9
Milk	8.2±8.0	8.3±8.0	7.2*±6.6	8.1±8.1
Non-milk products	5.0±5.7	5.2±5.9	5.6±5.1	5.5±5.2
Yogurt	2.0±3.7	2.2±3.9	1.3±1.9	1.3±1.7
Cheese	3.0±3.7	3.0±4.1	3.8±4.2	3.8±4.3

[a] Continuous variables presented as mean±SD (standard deviation), differences between cases and controls based on t-test.

* Significant difference between cases and controls ($p \leq 0.05$)

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Table 4.3.3 Associations (adjusted OR* 95%CI*) of Calcium and Vitamin D intakes with CRC risk among cases and controls, stratified by province, CRC case-control study

Index of calcium and vitamin D	NL subjects (n=1136)			ON subjects (n=1021)		
	No. of cases/controls	Median intake*	OR* (95% CI)	No. of cases/controls	Median intake*	OR* (95% CI)
Total Calcium						
Q1	109/119	580.0	1.00		708.5	1.00
Q2	107/121	798.1	1.040 (0.71, 1.51)		898.1	0.840 (0.47, 1.50)
Q3	106/121	963.5	0.710 (0.48, 1.03)		1077.1	0.840 (0.47, 1.49)
Q4	93/135	1196.6	0.770 (0.53, 1.12)		1308.4	0.680 (0.38, 1.21)
Q5	73/155	1653.4	0.500 (0.34, 0.74)		1834.0	1.670 (0.48, 0.77)
P for trend*			0.02			0.03
Calcium from food						
Q1	105/123	573.3	1.00		656.3	1.00
Q2	108/118	764.5	1.040 (0.71, 1.52)		816.7	0.970 (0.77, 1.22)
Q3	96/131	902.4	0.920 (0.63, 1.35)		946.5	0.980 (0.78, 1.23)
Q4	101/125	1089.7	1.020 (0.70, 1.49)		1094.5	0.790 (0.63, 1.00)
Q5	82/146	1405.7	0.690 (0.61, 1.45)		1382.9	0.710 (0.56, 0.90)
P for trend*			0.67			0.06
Total Vitamin D						
Non-users	407/471	0	1.00	761/1002	0	1.00
≥1 Users	81/180	>0	0.510 (0.38, 0.68)	511/828	>0	0.870 (0.75, 1.00)
Q1	102/126	124.2	1.00		107.7	1.00
Q2	106/122	199.4	1.280 (0.88, 1.86)		179.9	0.860 (0.68, 1.08)
Q3	115/112	261.4	1.110 (0.76, 1.63)		265.8	0.790 (0.63, 0.99)
Q4					464.0	0.790 (0.62, 1.00)
Q5					645.4	0.790 (0.59, 1.05)
P for trend*			0.39			0.18

Intakes of calcium and vitamin D	NL subjects (n=1139)				ON subjects (n=3102)			
	No. of cases/controls	Median intake[a]	OR[*] (95% CI)	OR[*] (95% CI)	No. of cases/controls	Median intake[a]	OR[*] (95% CI)	OR[*] (95% CI)
Vitamin D from food								
Q1	97/131	110.7	1.00	1.00	95.5	1.00	1.00	
Q2	101/127	179.8	1.17(0.80,1.71)	1.30(0.85,1.98)	148.5	0.91(0.72,1.14)	0.92(0.72,1.16)	
Q3	94/133	228.7	1.01(0.69,1.48)	1.26(0.82,1.92)	198.9	1.02(0.81,1.28)	1.09(0.86,1.38)	
Q4	108/120	285.3	1.24(0.85,1.81)	1.51(0.98,2.31)	253.9	1.03(0.82,1.30)	1.07(0.84,1.36)	
Q5	88/140	404.0	0.81(0.55,1.18)	0.95(0.62,1.47)	359.0	0.717*(0.56,0.89)	0.777*(0.61,0.99)	
P for trend[d]			0.49	0.90			0.22	0.38
Vitamin from supplements								
Non-users	402/674	0	1.00	1.00	0	1.00	1.00	
Users	867/77	>0	0.549*(0.41,0.73)	0.68*(0.48,0.97)	>0	0.91(0.78,1.06)	1.11(0.76,1.61)	

* OR, Odds ratio; 95%CI, 95% confidence interval

[a] Adjusted for age and total energy intake.

[b] Adjusted for total energy intake, age, sex, BMI, physical activity(MET,h/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a 10% alteration in the parameter coefficient of interest.

[c] Units of mcg/day for calcium, and IU/day for vitamin D.

[d] Two-sided p value for test of linear trend was calculated by using median values for each quintile of intake.

* Significant different from reference category, p<0.05

Table 4.3.4 Associations (adjusted OR*, 95%CI*) of dairy products intakes with CRC risk among cases and controls, stratified by province, CRC case-control study

Dairy products	NL subjects (n=1102)			ON subjects (n=3102)		
	No. of cases/controls	Median intake*	OR* (95%CI*)	No. of cases/controls	Median intake*	OR* (95%CI*)
Total dairy products						
Q1	110/133	2.4	1.00	271/385	3.1	1.00
Q2	87/127	7.2	0.85(0.59,1.23)	239/353	6.9	0.97(0.77,1.22)
Q3	103/126	10.5	0.79(0.54,1.14)	267/354	10.4	1.05(0.84,1.33)
Q4	97/129	16.1	0.75(0.51,1.09)	263/352	15.6	1.01(0.80,1.28)
Q5	91/136	25.9	0.60(0.40,1.01)	232/386	25.5	0.79(0.61,1.00)
P for trend*			0.03			0.21
Milk						
Q1	115/138	0	1.00	333/475	0.6	1.00
Q2	101/145	3.5	0.85(0.60,1.22)	240/345	3.0	1.00(0.83,1.31)
Q3	92/122	6.9	0.80(0.62,1.26)	296/402	7.9	1.09(0.81,1.39)
Q4	112/140	8.9	0.85(0.60,1.22)	254/346	7.9	1.06(0.85,1.32)
Q5	67/106	17.0	0.77(0.45,0.99)	149/272	14.9	0.79(0.56,0.94)
P for trend*			0.02			0.23
Non-milk						
Q1	97/143	0.3	1.00	255/402	1.1	1.00
Q2	98/129	2.0	1.35(0.94,1.95)	245/375	2.5	1.02(0.81,1.28)
Q3	105/117	3.6	0.94(0.64,1.38)	270/356	4.1	1.15(0.92,1.45)
Q4	103/124	6.0	1.16(0.80,1.69)	253/341	6.5	1.10(0.88,1.40)
Q5	85/138	11.4	0.91(0.61,1.37)	249/356	11.5	0.96(0.76,1.26)
P for trend*			0.51			0.79
Yogurt*						
Q1	157/229	0	1.00	553/766	0	1.00
Q2	165/214	1.1	1.15(0.86,1.53)	230/320	0.3	0.95(0.77,1.16)
Q3	112/71	5.3	0.91(0.64,1.32)	292/380	0.5	0.92(0.70,1.20)
Q4	-	-	-	168/266	1.3	0.81(0.65,1.07)
Q5	-	-	-	191/282	3.5	0.83(0.68,1.07)
P for trend*			0.56			0.06

Dairy products		NJ subjects (n=1139)			ON subjects (n=3102)		
	No. of cases/controls	Median intake[f]	OR[e] (95% CI)		No. of cases/controls	Median intake[f]	OR[e] (95% CI)
Cheese							
Q1	104/158	0	1.00		303/444	0.5	1.00
Q2	103/108	1.0	1.26(0.87,1.81)		230/359	1.3	0.94(0.75,1.18)
Q3	101/144	2.0	1.12(0.78,1.61)		244/349	2.5	0.90(0.70,1.24)
Q4	87/110	5.3	1.08(0.68,1.46)		259/322	5.0	1.10(0.87,1.37)
Q5	93/131	7.0	0.97(0.66,1.44)		236/356	10.0	0.87(0.69,1.10)
P for trend[d]			0.34				0.51				0.56

		OR[a] (95% CI)				OR[b] (95% CI)				OR[c] (95% CI)

[Last three rows with additional OR columns:]

Q5: 93/131 — 7.0 — 0.97(0.66,1.44) — 236/356 — 10.0 — 0.87(0.69,1.10)

1.00
0.94(0.75,1.18)
0.90(0.70,1.24)
1.10(0.87,1.37)
0.87(0.69,1.10)

1.00
0.98(0.78,1.23)
1.00(0.79,1.25)
1.12(0.89,1.42)
0.90(0.70,1.14)

[a] OR, Odds ratio; 95%CI, 95% confidence interval.
[b] Adjusted for age and total energy intake.
[c] Adjusted for total energy intake, age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamins, supplement, hormone replacement therapy(women), and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% change in the parameter coefficient of interest.
[d] Tests of servings/week for each dairy product.
[e] A two-sided p value for test of linear trend was calculated by using median values for each quartile of intake.
[f] Due to small sample size in NJ, yogurt intake were only divide into 3 groups.
* Significant different from reference category, p<0.05

Table 4.3.5 Adjusted OR[a], 95%CI[b] of CRC risk according to level of total calcium and total vitamin D intake

Total vitamin D intake	Total Calcium intake (mg/day)		
(IU/day)	T1[c] (<=835.2)	T2[c] (835.3-1064.2)	T3[c] (>1064.2)
T1[c](<=157.3)			
No. of cases/controls	343/420	99/127	15/30
OR[b] (95%CI)	1.00	0.94(0.69,1.29)	0.84(0.65,1.14)
T2[c] (157.4-241.5)			
No. of cases/controls	115/120	230/322	86/141
OR[b] (95%CI)	1.10(0.80,1.86)	1.06(0.84,1.34)	0.81(0.59,1.11)
T3[c] (>241.5)			
No. of cases/controls	15/20	94/162	275/488
OR[b] (95%CI)	1.00(0.50,2.02)	0.86(0.63,1.17)	0.75*(0.49,0.99)

[a] OR, Odds ratio; 95%CI, 95% confidence interval.

[b] Adjusted for total energy intake, age, sex, BMI, physical activity(METs/week), family history of CRC, polyps, diabetes, reported colon screening procedure, cigarette smoking, alcohol drinking, education attainment, household income, marital status, regular use of NSAID, regular use of multivitamin supplements, reported HRT (females only), province of residence, and intakes of fruits, vegetables, and red meat. Variables were included in the final model based on a ≥10% alteration in the parameter coefficient of interest.

[c] Intakes of total calcium and vitamin D were categorized into tertiles based on the distribution among subjects, T1 for tertile 1, T2 for tertile 2, and T3 for tertile 3.

* Significant different from reference category, $p \leq 0.05$

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Chapter 5: Summary

5.1 Associations between dietary factors and colorectal cancer risk

This thesis examined the possible association of dietary factors with colorectal cancer (CRC) risk in people residing in Newfoundland and Labrador (NL) and Ontario (ON) through three coherent papers. The first two papers were based on pooled data from two provinces assessing the effects of total energy/ macronutrients and selected micronutrients on CRC, respectively. Based on the findings from the first two papers, the third paper compared two provinces to further explore the relationships among calcium, vitamin D, dairy products and the risk of CRC.

Consistent with previous studies[5-10], this study observed a positive association between total energy intake and the risk of CRC. This finding implicates energy balance. Higher energy intake with lower energy expenditure may increase CRC risk. Additionally, higher energy intake is associated with the cause of obesity, which in turn is a possible risk factor for CRC. In this study, percentage of calorie from alcohol were associated with increased CRC risk. Given the high incidence of CRC in two provinces, it is necessary to encourage people to intake low-energy foods and engage in physical activities.

High intakes of protein, carbohydrate and fibre seemed to decrease CRC risk. In this study population, red meat intake was moderate (about 4 servings/week) and similar proportions (around 20%) of proteins derived from red meat, dairy products and the combination of white meat and fish. Thus, consistent with several previous studies[7,58,74,75,118,119], this study suggests that the protective effect of protein may be largely contributable to non-red meat protein. The inverse associations of CRC risk with

carbohydrate and fibre may be related to the protection of fruits, vegetables and grains, because these foods contain a large amount of carbohydrate and fibre. There is a great need to encourage people to modify their diets to prevent CRC, such as increasing vegetable and fruit intake, and the frequency of high fibre diet.

Certain micronutrients (e.g. calcium, vitamin D, vitamin C, riboflavin and folate) seemed to have protective effects on CRC. However, when intake from food source only was considered for the above micronutrients, associations were diminished (except for calcium and vitamin D). The exclusion of intake from supplement sources resulted in a slight decrease in power in these analyses. In addition, inverse associations of calcium, vitamin C, folate and CRC were most pronounced among individual supplement users. These findings suggest that diet alone might not furnish enough micronutrients and the levels of micronutrients in the Canadian diet may be insufficient to greatly reduce the risk of CRC. Instead, individual supplements provide sufficient micronutrients and exert protective effects. Stratified analyses by individual supplement use status further indicated that the use of vitamin C or folate supplement appeared to have further benefit among those with relative lower intakes of these nutrients from food. Therefore, these findings highlight the importance of dietary supplements use in prevention for CRC.

Another important finding in this study was the strong positive association between iron intake and CRC risk. Stratified analyses by individual supplement use status further indicated that a positive association between iron and CRC was most pronounced among iron supplement users. Interestingly, in this study, cases had higher intake of iron than controls, primarily due to larger contribution of iron supplement. In addition, the average

daily intake of total iron (from food and supplements) was much higher than the recommended dietary allowances (RDA). Thus, excess intake of iron or inadequately using iron supplement may have harmful effects of increasing CRC risk. Attention should be taken in consuming iron-containing foods and supplements.

Consistent results of calcium, vitamin D and dairy products with CRC risk were observed in two provinces; however, significant associations were only observed in the ON population. One possibility is that genetic predisposition for CRC is stronger in NL as compared to ON. The high rates of the disease in NL may be partly explained by a high prevalence of families with a predisposition to hereditary colon cancer[39]. However, in ON genetic factors is not as strong as in NL and dietary factors are relatively more important in the cause of CRC. The complex genetic-environmental interactions may be investigated in future research.

5.2 Implications of the study

Consideration should be given to the potential limitations in the present study that have been discussed previously in three papers. This study has a number of strengths and adds to the existing literature in many aspects. First, built on existing literature, this study underlines the importance of some aspects of total energy, macronutrients and micronutrients and consequently the potential for prevention through dietary changes in CRC. Second, to our knowledge, this is the first study that assessed relationships of nutrient intakes and CRC risk in NL and ON both with a high incidence of CRC. A large sample size allowed us to observe associations that would be undetectable in smaller studies. Third, this study highlights the importance of supplement use in prevention for

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CRC. Diet alone might not furnish enough micronutrients and levels of micronutrients in the Canadian diet may be insufficient to greatly reduce the risk of CRC. Findings from this study may have important public health implications, particularly for those with lower intakes of a number of micronutrients, who can benefit greatly from supplement intakes. With respect to other physiologic, behavioral, and dietary factors interacting with nutrients to affect risk, future research is warranted to investigate these complex causal relationships for more effective cancer prevention.

5.3 Future research

The results from this study indicate that vitamin and/or mineral containing supplements may have further benefits in preventing CRC. Multivitamin supplements contain large numbers of nutrients which may be correlated in influencing the likelihood of cancer. For instance, phosphorous and dietary fibre may limit the intestinal absorption of calcium due to increased production of insoluble calcium complexes[142-144]. Future research should carefully design clinical trials using multivitamin and individual supplements as intervention to assess supplement intake as cumulative dose over a long time frame (e.g. supplements use over a 10-year reference period).

The current study examines associations of individual dietary components with CRC risk, which is beneficial for gaining insight into possible mechanisms of each dietary component. However, it is not adequate for considering the synergistic effect of highly correlated nutrients and other compounds found in foods[149]. Future research will investigate dietary intakes in a more logical approach – dietary patterns. The analysis of dietary patterns will take into consideration the synergistic effect of foods and nutrients,

neither of which is consumed in isolation.

Dietary patterns include numerous dietary exposures and are often associated with other health behaviors such as physical activity, smoking, and cancer screening[150]. However, most existing cancer studies focus on examining the independent effect of a particular risk factor(s) and have paid little attention on how various factors are inter-related in affecting cancer occurrence. Knowledge around causal relationships of various risk factors and outcome is directly relevant to more effective cancer prevention. Future study will use structural equation modeling analysis to test a hypothesized conceptual model that specify complex causal relationships among various risk factors of CRC.

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Appendices

Appendix A. Personal history questionnaire used in Newfoundland and Labrador and Ontario

Ontario
Familial Colon Cancer Registry

Personal History Questionnaire

This questionnaire is about factors that may relate to a person's risk of developing cancer. Although it is important to have complete data for scientific reasons and we encourage you to answer all questions, if you come to a question that you do not want to answer, please write "prefer not to answer" beside it and then continue to answer the remaining questions.

Should you wish to talk to someone about this questionnaire, you may call (416) 946-4409 or 1-800-832-5949.

Please write in your answers where space is provided, or place tick marks in circles. ✔

What date are you filling out this questionnaire? _ _ / _ _ / _ _ _ _
day, month, year

Identifying Information

1. Are you male or female?
   - ○ male
   - ○ female

2. What is your age? _ _ _ _ years
   - ○ don't know

3. What is your date of birth?
   day _ _ _ _
   month _ _ _ _
   year _ _ _ _
   - ○ don't know day
   - ○ don't know month
   - ○ don't know year

4. Are you a twin or triplet?
   - ○ yes, a twin
   - ○ yes, other multiple (triplet, quadruplet, etc.) _ _ _ _ _ _ _ please, specify
   - ○ no
   - ○ don't know

   ➔ *If yes, please read the following statement and answer the question:*

   Non-identical twins are no more alike than ordinary brothers and sisters. Genetically identical twins, on the other hand, look so much alike that is, they have a strong resemblance to each other in height, colouring, features of the face, etc. (that people often mistake one for the other, especially during their childhood.

   Do you have a genetically identical twin or triplet?
   - ○ yes
   - ○ no
   - ○ don't know

5. What is your marital status?
   - ○ currently married or living as married
   - ○ separated
   - ○ divorced
   - ○ widowed
   - ○ single or never married
   - ○ don't know

6.

Bowel Screening and Health

5. Have you ever had a **test for blood in your stool, called a smear test or a hemoccult**? This test is frequently done as part of a routine physical examination, or it can be done at home.

    ○ yes

    ○ no ────► *Please go to #7*

    ○ don't know ────► *Please go to #7*

5a. When did you **first** have this test?

    age when **first** tested

    or

    year of **first** test

    ○ don't know

5b. What was the reason for your **first** test? *Please tick all that apply*

    ○ to investigate a new problem

    ○ family history of colorectal cancer

    ○ routine/yearly examination or check-up

    ○ follow-up of a previous problem

    ○ other

    _____ *please specify*

    ○ don't know

5c. How many times have you had a hemoccult test?

    _____ number of hemoccult tests

    ○ don't know

5d. If you have had a hemoccult test more than once, when did you last have this test?

    age when **last** tested

    or

    year of **last** test

    ○ don't know

7. Have you ever had a **sigmoidoscopy**? Sigmoidoscopy involves looking inside the lower bowel and rectum with a lighted instrument. The examination is usually done in a doctor's office without anesthesia.

    ○ yes

    ○ no ────► *Please go to #9*

    ○ don't know ────► *Please go to #9*

7a. When did you **first** have this test?

    age when **first** tested

    or

    year of **first** test

    ○ don't know

7b. What was the reason for your **first** sigmoidoscopy? *Please tick all that apply*

    ○ to investigate a new problem

    ○ family history of colorectal cancer

    ○ routine/yearly examination or check-up

    ○ follow-up of a previous problem

    ○ other

    _____ *please specify*

    ○ don't know

7c. How many times have you had a sigmoidoscopy?

    _____ number of sigmoidoscopies

    ○ don't know

7d. If you have had a sigmoidoscopy more than once, when did you last have this test?

    age when **last** tested

    or

    year of **last** test

    ○ don't know

129

5. Have you ever had a **colonoscopy**? Colonoscopy is an examination of the entire large bowel using a long flexible instrument. This examination is usually done under sedation.

   ○ yes

   ○ no   ← *Please go to 6*

   ○ don't know ← *Please go to 6*

5a. When did you **first** have this test?

   age when **first** tested

   or

   year of **first** test

   ○ don't know

5b. What were the reasons for your **first** colonoscopy? *Please tick all that apply.*

   ○ to investigate a new problem

   ○ family history of colorectal cancer

   ○ routine/yearly examination or check up

   ○ follow up of a previous problem

   ○ other:

        please specify

   ○ don't know

5c. How many times have you had a colonoscopy?

   number of colonoscopies

   ○ don't know

5d. If you have had a colonoscopy more than once, when did you **last** have this test?

   age when **last** tested

   or

   year of **last** test

   ○ don't know

6. Have a doctor ever told you that you had **polyps** in your large bowel or colon or rectum? Polyps are growths in the lining of the colon which vary in size from a tiny dot to several inches.

   ○ yes

   ○ no ← *Please go to 6b*

   ○ don't know ← *Please go to 6b*

6a. When did your doctor **first** tell you that you had polyps?

   age at **first** diagnosis

   or

   year of **first** diagnosis

   ○ don't know

6b. Have you been told more than once that you had polyps?

   ○ yes

   ○ no

   ○ don't know

6c. When did your doctor **last** tell you that you had polyps?

   age at **last** diagnosis

   or

   year of **last** diagnosis

   ○ don't know

6d. Do you know what kind of polyps they were? *Please include all the separate times you were told you had polyps. Please tick all that apply.*

   ○ benign

   ○ adenomatous pre-cancerous

   ○ hyperplastic

   ○ other:

        please specify

   ○ don't know

5

9e. Did you have the polyps removed (by a procedure called a polypectomy)? (This can be done during a sigmoidoscopy or colonoscopy.)

○ yes
○ no → Please go to #10
○ don't know → Please go to #10

9f. When did you first have polyps removed?

age at first polypectomy

or

year of first polypectomy
○ don't know

9g. Have you had polyps removed more than once?

○ yes
○ no
○ don't know

9h. If you have had polyps removed more than once, when did you last have polyps removed?

age at last polypectomy

or

year of last polypectomy
○ don't know

10. Has a doctor ever told you that you had familial adenomatous polyposis, known also as FAP? This is a condition, sometimes occurring in families, in which numerous polyps line the inside of the large bowel or colon.

○ yes
○ no → Please go to #11
○ don't know → Please go to #11

10a. When did your doctor first tell you that you had FAP?

age at diagnosis

or

year of diagnosis
○ don't know

11. Has a doctor ever told you that you had Crohn's disease? This is where you have an inflammation that extends into the deeper layers of the intestinal wall. It may also affect other parts of the digestive tract, including the mouth, esophagus, stomach, and small intestine.

○ yes
○ no → Please go to #12
○ don't know → Please go to #12

11a. When did your doctor first tell you that you had Crohn's disease?

age at diagnosis

or

year of diagnosis
○ don't know

11b. Has a doctor ever told you that you had ulcerative colitis? This is an inflammation and ulceration of the lining of the bowel (colon and rectum). It is not a stomach ulcer.

○ yes
○ no → Please go to #13
○ don't know → Please go to #13

12. When did your doctor first tell you that you had ulcerative colitis?

age at diagnosis

or

year of diagnosis
○ don't know

13. Has a doctor ever told you that you had irritable bowel syndrome? This is a disorder of the bowels leading to cramping, spasms, bloating, and alternating diarrhea and constipation. It is sometimes called IBS, or spastic colon.

○ yes
○ no → Please go to #14
○ don't know → Please go to #14

13a. When did your doctor **first** tell you that you had a bladder/bowel condition?

○ age at diagnosis

———

year of diagnosis

○ don't know

13b. Has a doctor ever told you that you had **diverticular disease**? This may also be called diverticulitis or diverticulosis. It's a condition in which the bowel lining becomes infected, and can lead to pain and chronic problems with bowel habits.

○ yes

○ no ———➤ *Please go to #15*

○ don't know ———➤ *Please go to #15*

14a. When did your doctor **first** tell you that you had diverticular disease?

○ age at diagnosis

———

year of diagnosis

○ don't know

15. Have you ever had any of your **large bowel or colon** removed?

○ yes

○ no ———➤ *Please go to #16*

○ don't know ———➤ *Please go to #16*

➤ Was it completely removed, or only part of it removed?

○ completely removed

○ partly removed

○ don't know

15a. When did you **first** have one of these surgeries/operations?

——— at first operation

year of first operation

○ don't know

16a. Have you had more than one surgery to remove your bowel or colon?

○ yes

○ no ———➤ *Please go to #16*

○ don't know ———➤ *Please go to #16*

16b. When did you last have all or part of your gallbladder removed?

——— at last operation

year of last operation

○ don't know

16. Have you had your **gallbladder** removed?

○ yes

○ no ———➤ *Please go to #17*

○ don't know ———➤ *Please go to #17*

16c. When did you have your gallbladder removed?

——— age at operation

year of operation

○ don't know

17. Has a doctor ever told you that you had **diabetes**, also known as **diabetes mellitus**? Please do not tell us about diabetes which you had only during pregnancy.

○ yes

○ no ———➤ *Please go to #18*

○ don't know ———➤ *Please go to #18*

17a. When did your doctor **first** tell you that you had diabetes?

——— age at diagnosis

———

year of diagnosis

○ don't know

17) Did your next door neighbour ever mention your diabetes?

　○ yes

　○ no — *Please go to #18.*

　○ don't know — *Please go to #18.*

17) What type of medication did you use, pills or insulin injections?

　○ pills

　○ insulin injections

　○ both

　○ don't know — *Please go to #18.*

17b) How often did you really take it?
Please choose the most appropriate answer.

	Pills	Insulin
once per day or		
twice per week or		
once per month		
once per year		
don't know	○	○

18) **Blood test years** ago, were you taking it?

	Pills	Insulin
yes	○	○
no	○	○
don't know	○	○

18) How long should have you taken this medication?

	Pills	Insulin
number of months or		
number of years		
don't know	○	○

18) Has a doctor ever told you that you had **high cholesterol**? If you do not know what was cholesterol, please ask us.

　○ yes

　○ no — *Please go to #19.*

　○ don't know — *Please go to #19.*

18a) When did you doctor **first** tell you that you had high cholesterol?

　　age at diagnosis

　　or

　　year of diagnosis

　○ don't know

18b) Did you ever take medication to control your high cholesterol?

　○ yes

　○ no — *Please go to #19.*

　○ don't know — *Please go to #19.*

18) How often did you really take it?
Please choose the most appropriate answer.

　　once per day or

　　twice per week or

　　once per month

　　once per year

　○ don't know

18a) **Blood test years** ago, were you taking it?

　○ yes

　○ no

　○ don't know

18b) How long, in total, have you taken this medication?

　　number of months or

　　number of years

　○ don't know

22. Has a doctor ever told you that you had any other cancer?

   ○ yes
   ○ no → *Please go to #24*
   ○ don't know → *Please go to #24*

22a. What type of cancer was it?

   _____ cancer

22b. When did your doctor first tell you that you had this type of cancer?

   age at diagnosis
   _____
   year at diagnosis
   ○ don't know

22c. Were you treated with radiation therapy (radiotherapy) for this cancer?

   ○ yes
   ○ no
   ○ don't know

23. Has a doctor ever told you that you had any other cancer?

   ○ yes
   ○ no → *Please go to #25*
   ○ don't know → *Please go to #25*

23a. What type of cancer was it?

   _____ cancer

23b. When did your doctor first tell you that you had this type of cancer?

   age at diagnosis
   _____
   year at diagnosis
   ○ don't know

23c. Were you treated with radiation therapy (radiotherapy) for this cancer?

   ○ yes
   ○ no
   ○ don't know

Medications

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)?

24. Aspirin (such as Anacin, Bufferin, Bayer, Excedrin, Ecotrin)

   ○ yes
   ○ no → *Please go to #25*
   ○ don't know → *Please go to #25*

24a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? (Please choose one of the following)

   _____ times per day, or
   _____ times per week
   ○ don't know

25. About how years have you taken it regularly?

   ○ yes
   ○ no
   ○ don't know

26. How long (total) have you taken this medication regularly? (If you started and stopped and restarted several times, please count only the time you were taking this medication)

   _____ number of months or
   _____ number of years
   ○ don't know

Have you ever taken any of the following medications regularly (at least twice a week for more than a month)? *(continued)*

75. Acetaminophen (such as Tylenol, Anacin-3, Panadol)

○ yes

○ no ——→ *Please go to #76*

○ don't know ——→ *Please go to #76*

75a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? *Please choose one of the following.*

○ three per day or

○ twice per week

○ don't know

75b. About two years ago, were you taking it regularly?

○ yes

○ no

○ don't know

75c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.

number of months or

number of years

○ don't know

76. Ibuprofen-based medications (such as Advil, Motrin, Nuprin, Medipren, Indocid, Naprosyn, NSAIDs (NSAIDS are non-steroidal anti-inflammatory drugs)

○ yes

○ no ——→ *Please go to #77*

○ don't know ——→ *Please go to #77*

76a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? *Please choose one of the following.*

○ three per day or

○ twice per week

○ don't know

76b. About two years ago, were you taking it regularly?

○ yes

○ no

○ don't know

76c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.

number of months or

number of years

○ don't know

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Have you ever taken one of the following medications regularly (at least twice a week for more than a month)? *(continued)*

27. **Body-forming laxatives (such as Metamucil, Citrucel, FiberCon, Serutan, psyllium)**

    ○ yes

    ○ no ➞ *Please go to #29*

    ○ don't know ➞ *Please go to #29*

27a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? *Please choose one of the following:*

    _____ times per day or

    _____ times per week

    ○ don't know

27b. **About two years ago, were you taking it regularly?**

    ○ yes

    ○ no

    ○ don't know

27c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking the medication.

    _____ number of months or

    _____ number of years

    ○ don't know

28. **Other laxatives (such as Ex-Lax, Correctol, Dulcolax, Senokot, Colace, castor oil, cod liver oil, mineral oil, milk of magnesia, lactulose, Epsom salts)**

    ○ yes

    ○ no ➞ *Please go to #29*

    ○ don't know ➞ *Please go to #29*

28a. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? *Please choose one of the following:*

    _____ times per day or

    _____ times per week

    ○ don't know

28b. **About two years ago, were you taking it regularly?**

    ○ yes

    ○ no

    ○ don't know

28c. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking the medication.

    _____ number of months or

    _____ number of years

    ○ don't know

10

137

Have you ever taken any of the following medications regularly
(at least twice a week for more than a month)? (continued)

75. **Multivitamin supplements (such as One-A-Day, Theragran, Centrum, Unicap)** (not individual vitamins)

○ yes

○ no   ➤ *Please go to #81*

○ don't know   ➤ *Please go to #81*

76. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
Please choose one of the following.

    _____ times per day, or

    _____ times per week

○ don't know

77. **About two years** ago, were you taking it regularly?

○ yes

○ no

○ don't know

78. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.

    _____ number of months or

    _____ number of years

○ don't know

81. **Folic acid or folate pills or tablets**

○ yes

○ no   ➤ *Please go to #85*

○ don't know   ➤ *Please go to #85*

82. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)?
Please choose one of the following.

    _____ times per day or

    _____ times per week

○ don't know

83. **About two years** ago, were you taking it regularly?

○ yes

○ no

○ don't know

84. How long, in total, have you taken this medication regularly? If you started and stopped and then started again, please count only the time you were taking this medication.

    _____ number of months or

    _____ number of years

○ don't know

Have you ever taken any of the following medication regularly (at least twice a week for more than a month)? *(continued)*

31. **Calcium pills or tablets**

    ○ yes

    ○ no → *Please go to F7*

    ○ don't know → *Please go to F7*

F1. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? *Please choose one of the following:*

    ____ times per day; or

    ____ times per week

    ○ don't know

F2. About two years ago, were you taking it regularly?

    ○ yes

    ○ no

    ○ don't know

F3. How long, in total, have you taken this medication regularly? *If you started and stopped and then started again, please count only the time you were taking this medication.*

    ____ number of months; or

    ____ number of years

    ○ don't know

32. **Calcium-based antacids (such as Tums, Rolaids, Extra-strength Rolaids, Alka-Mints, Chooz Antacid gum)**

    ○ yes

    ○ no → *If female, please go to #31 If male, please go to #34*

    ○ don't know → *If female, please go to #31 If male, please go to #34*

G1. How often did you usually take it when you were taking it regularly (that is, at least twice a week for more than a month)? *Please choose one of the following:*

    ____ times per day; or

    ____ times per week

    ○ don't know

G2. About two years ago, were you taking it regularly?

    ○ yes

    ○ no

    ○ don't know

G3. How long, in total, have you taken this medication regularly? *If you started and stopped and then started again, please count only the time you were taking this medication.*

    ____ number of months; or

    ____ number of years

    ○ don't know

Men: please go to #44 on page 17

Women: please continue with #33 on page 15

Menstruation, Pregnancy, and Menopause

71. How old were you when you had your first menstrual period?

_____ years of age

○ don't know

○ never had a menstrual period

72. Have you ever been pregnant?

○ yes

○ no ⟶ *Please go to #81*

○ don't know ⟶ *Please go to #81*

➤ How many times have you been pregnant? (Please include miscarriages, stillbirths, tubal pregnancies and abortions.)

_____ number of pregnancies

○ don't know

73. How many times were you pregnant with more than one baby (twins, triplets or more)? *If you are pregnant now, please do not include your current pregnancy.*

○ never

_____ number of pregnancies with more than one baby

○ don't know

74. How many of your pregnancies lasted 6 months or longer? (Pregnancy usually lasts 9 months, but trouble is about the end of a baby could survive if it was an immature one, please do not include your current pregnancy.)

○ all of them

_____ number of pregnancies lasting 6 months or longer

○ don't know

75. How many of your pregnancies resulted in live births?

○ all of them

_____ number of pregnancies with live births

○ don't know

76. How old were you at the time of your **first** live birth?

_____ age at **first** birth

○ year of **first** birth

○ don't know

77. How old were you at the time of your **last** live birth?

_____ age at **last** birth

○ year of **last** birth

○ don't know

78. Have you ever used birth control pills or other hormonal contraception (implant or injection) for at least one year?

○ yes

○ no ⟶ *Please go to #80*

○ don't know ⟶ *Please go to #80*

➤ How old were you when you **first** used any of these hormonal contraceptives?

_____ age at **first** use

○ year of **first** use

○ don't know

79. Were you still using hormonal contraceptives **about two years ago**?

○ yes

○ no

○ don't know

In total, how long did you take blood-
thinning medication? If you started and
stopped and then started again please count
only the time you were taking these
medications.

_____ number of years

○ don't know

14. Have you had a **menstrual period** in the last
12 months? Please include your menstrual
bleeding and bleeding that results from
hormone replacement therapy (HRT) or
progestogens, progestins or combined
therapies.

○ Yes ⟶ *Please go to #17*

○ no

○ don't know ⟶ *Please go to #16*

⟶ Have your periods stopped permanently,
or only temporarily due to pregnancy,
breast-feeding, or other conditions?

○ permanently

○ temporarily ⟶ *Please go to #16*

15. How old were you when your period
stopped permanently?

_____ age they stopped   or

_____ now they stopped

○ don't know

16. Why did your menstrual periods stop
permanently? Please tick all that apply.

○ natural menopause

○ surgery

○ radiation or chemotherapy

○ other reason
   Please specify: _____

○ don't know

**Please complete the next few questions which
ask about surgeries you may have had.**
Please answer all questions.

17. Hysterectomy (only the uterus or womb
removed):

○ yes

○ no

○ don't know

⟶ age when removed   or

_____ year when removed

○ don't know

17a. Hysterectomy with one ovary or part of an
ovary removed:

○ yes

○ no

○ don't know

⟶ age when removed   or

_____ year when removed

○ don't know

18. Hysterectomy with both ovaries removed:

○ yes

○ no

○ don't know

⟶ age when removed   or

_____ year when removed

○ don't know

19. One ovary removed, completely or partly,
without hysterectomy:

○ yes

○ no

○ don't know

⟶ age when removed   or

_____ year when removed

○ don't know

11

39) Did you remove surface in the last year?

- ○ yes
- ○ no
- ○ don't know

➤ *if yes, how much? ...* or

(year when removed)

- ○ don't know

---

40) **If you had radiation or chemotherapy**, when did you first have it?

- ○ had radiation or chemotherapy
- ➤ age when this was given ...

(year when this was given)

- ○ don't know
- ○ never had radiation or chemotherapy

---

41) **If your period stopped permanently for any reason other than surgery, radiation or chemotherapy**, when did this occur?

- ○ other reason
  Please specify...
- ➤ age when occurred ... or

(year when occurred)

- ○ don't know
- ○ not applicable

---

42) Did you ever take **hormone replacement therapy** for more reasons, including menopausal symptoms, surgical issues or the control, enjoyment, and health illnesses prevention of menopausal symptoms including hot flashes, sweating, and depression?

Because you take hormone replacement therapy prescribed by a doctor and in the form of a pill or a patch?

Please do not include hormone therapy that was prescribed for birth control, infertility therapies delivered by intra-uterine devices, creams or vaginal suppositories, or herbal or soy products.

- ○ yes
- ○ no ➤ *Please go to #45*
- ○ don't know ➤ *Please go to #45*

---

43) Were you still having **menstrual periods** when you first took these hormones?

- ○ yes
- ○ no
- ○ don't know

---

44) Were you prescribed either an estrogen-only pill or patch for this Hormone/or hormone replacement therapy?

- ○ yes
- ○ no
- ○ don't know

➤ How old were you when you first took estrogen-only medication?

- ○ age when first taken ... or

(year when first taken)

- ○ don't know

---

45) Were you still using estrogen-only medication for hormone replacement therapy about two years ago?

- ○ yes
- ○ no
- ○ don't know

---

46) In total, how long did you take estrogen-only medication for hormone replacement therapy? If you started and stopped then started again, please count only the time you were taking this medication.

- ○ number of months or
- ○ number of years
- ○ don't know

43. Prescription or proprietary (frequently prescribed by doctors together with category for treating or preventing mouth breast). One is often used as a Protective. Another one is Protectant. Have you ever taken prescription or proprietary (together with category for treating replace mouth the age?

○ yes

○ no ▸ *Please go to 44*

○ don't know ▸ *Please go to 44*

▸ How old were you when you first took proprietary or proprietary together with category?

age when **first** taken _____

year when **first** taken _____

○ don't know

44. Were you still taking proprietary or proprietary medication **about two years** ago?

○ yes

○ no

○ don't know

45. In total, how long did you take proprietary or proprietary together with category? If you started and stopped and then started again, please count only the time you were taking this medication.

number of months, or

number of years

○ don't know

47. Have you ever taken tamoxifen, raloxifene, or other anti-estrogen medication such as Evista or Depo-Provera?

○ yes

○ no ▸ *Please go to 48*

○ possibly – I have participated in a clinical trial for tamoxifen or other anti-estrogen medication

○ don't know ▸ *Please go to 48*

▸ What anti-estrogen medication did you take? *Please tick all that apply.*

○ tamoxifen

○ raloxifene

○ other

please specify _____

48. How old were you when you **first** took tamoxifen, raloxifene or other anti-estrogen medication?

age when **first** taken _____

year when **first** taken _____

○ don't know

49. Were you still taking tamoxifen, raloxifene or other anti-estrogen medication **about two years** ago?

○ yes

○ no

○ don't know

50. In total, how long did you take tamoxifen, raloxifene or other anti-estrogen medication? If you started and stopped and then started again, please count only the time you were taking this medication.

number of months, or

number of years

○ don't know

Diet

14. **About two years ago, on average, how often did you eat a piece or serving of fruit?**

A serving of fruit is: 1 medium sized fresh fruit, 1/2 cup of chopped, cooked or canned fruit, 3/4 cup of dried fruit, 6 ounces of fruit juice (50%–100% pure juice). Please choose one of the following:

    servings per day or

    servings per week or

    servings per month

  ○ don't know

15. **About two years ago, on average, how often did you eat a serving of vegetables?** *Please include any vegetable, beans, lentils, etc., and potatoes (not included french fries).*

A serving of vegetables is: 1 cup raw leafy vegetables, 1/2 cup of other vegetables, cooked or chopped raw, 6 ounces of vegetable juice. Please choose one of the following:

    servings per day or

    servings per week or

    servings per month

  ○ don't know

16. **About two years ago, on average, how often did you eat a serving of red meat** *such chicken or fish?*

A serving of red meat is: 2–3 ounces of red meat (a piece of meat about the size of a deck of cards). Red meats include: beef, steak, hamburger, prime rib, ribs, beef hot dogs, beef based processed meat, veal, pork, bacon, pork sausage, ham, lamb, venison. Please choose one of the following:

    servings per day or

    servings per week or

    servings per month

  ○ didn't eat red meat ⟶ *Please go to #17*

  ○ don't know

16a. **About two years ago, on average, how often did you eat a serving of red meat** that was cooked by broiling, grilling, barbecuing or pan frying (**not** stir fried or deep fried)? *Please choose one of the following:*

    servings per day or

    servings per week or

    servings per month

  ○ didn't eat red meat that was cooked by these methods ⟶ *Please go to #17*

  ○ don't know

46) On average, when you eat **red meat** cooked by these methods, which of the following best describes its appearance?

What was its **outside** appearance?

○ lightly browned

○ medium browned

○ heavily browned or blackened

○ don't know

What was its **inside** appearance, that is, how well done it was?

○ still rare

○ pink medium

○ brown well done

○ don't know

47) **About two years** ago, on average, how often did you eat a serving of **chicken**? Please do not include turkey or any other meat.

('A serving of chicken is: 2-3 ounces of chicken meat, 1 drumstick, 1 thigh, half a breast, 2 wings, 1 nugget) Please choose one of the following:

_____ servings per day or

_____ servings per week or

_____ servings per month

○ didn't eat chicken ──► *Please go to #48*

○ don't know

47a) **About two years** ago, on average, how often did you eat a serving of **chicken** that was cooked by broiling, grilling, barbecuing or pan-frying (**not** on time in deep fried)? Please choose one of the following:

_____ servings per day or

_____ servings per week or

_____ servings per month

○ didn't eat chicken that was cooked by these methods ──► *Please go to #48*

○ don't know

47b) On average, when you ate **chicken** cooked by these methods, which of the following best describes its appearance?

What was its **outside** appearance?

○ lightly browned

○ medium browned

○ heavily browned or blackened

○ don't know

45

Physical Activity

We would like you to think back to when you were **in your 20s** and remember the physical activities you participated in then.

28. **In your 20s**, did you participate **regularly** in physical activity **for a total of at least 30 minutes a week**? *(Please tick in the same activities below.)*

		For how many years?	During those years, for how many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Jogging *(slow running; distance of less than a mile in 10 minutes)*	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Running *(running faster than a mile in 10 minutes)*	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Bicycling *(including home exercise bicycle)*	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Swimming laps	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Tennis, squash, racquetball	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Calisthenics, aerobics, vigorous dance *(including ballet), using a rowing machine, lifting weights*	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Football, soccer rugby, basketball	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Heavy household work *(examples: scrubbing floors, washing windows, moving heavy loads, scrubbing floors)*	○ yes ➤ ○ no	____ years	____ months	____ minutes per week or ____ hours per week

In your 20s, did you do any other **strenuous activities?** Strenuous activity means something that really increased your heart rate, made you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, running, climbing or subsequently, a tree doing.

Activity (please specify)	For how many years?	During those years, for how many months per year?	During those months, on average, for how many minutes or hours per week?
⟶	years	months	minutes per week _or_ hours per week
⟶	years	months	minutes per week _or_ hours per week
⟶	years	months	minutes per week _or_ hours per week
⟶	years	months	minutes per week _or_ hours per week
⟶	years	months	minutes per week _or_ hours per week
⟶	years	months	minutes per week _or_ hours per week

49. When you were **in your 20s,** what was your usual occupation? (This means what you did for the longest time, includes any paid or unpaid employment, such as being a student or housewife or being unemployed.)

occupation

○ don't know

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Now, please think back to your 30s and 40s.

44. In your 30 and 40s, did you participate regularly in physical activity for a total of at least 30 minutes a week? Please describe your activities below.

	For how many years?	During those years, for how many months per year?	During those months, on average, for how many minutes or hours per week?
Working	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Jogging (running slowly, that is, only in 10 minutes)	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Running (running faster, that is, only in 10 minutes)	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Bicycling (including using stationary bicycle)	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Swimming laps	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Tennis, squash, racquetball	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Calisthenics, aerobics, vigorous dance (including belly, using a rowing machine, lifting weights)	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Football, soccer, rugby, basketball	○ yes ➝ ○ no	years	months	minutes per week or hours per week
Heavy household work (example: cleaning house, moving heavy loads, scrubbing floors)	○ yes ➝ ○ no	years	months	minutes per week or hours per week

In your **30s and 40s**, did you do any other **strenuous activities**? Strenuous activity means something that really increased your heart rate, made you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, chopping or tobogganing, water skiing.

Activity (please specify)	For how many years?	During these years, for how many months per year?	During these months, for how many minutes or hours per week?
	years	months	minutes per week or hours per week
	years	months	minutes per week or hours per week
	years	months	minutes per week or hours per week
	years	months	minutes per week or hours per week
	years	months	minutes per week or hours per week
	years	months	minutes per week or hours per week

95. When you were in your **30s and 40s**, what was your usual occupation? (We mean what you did for the longest time, including any paid or unpaid employment, such as being a student or housewife or being incarcerated.)

occupation

☐ don't know

Now, please think back to **since you turned 50**.

52. **Since you turned 50**, did you participate **regularly** in physical activity for a total of at least 30 minutes a week? *Please tick each activity below.*

		For how many years?	During those years, for how many months per year?	During those months, on average, for how many minutes or hours per week?
Walking	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Jogging (running slower than a mile in 10 minutes)	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Running (running faster than a mile in 10 minutes)	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Bicycling (including using an exercise bicycle)	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Swimming laps	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Tennis, squash, racquetball	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Calisthenics, aerobics, vigorous dance (including ballet), using a rowing machine, lifting weights	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Football, soccer, rugby, basketball	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week
Heavy household work (occupation, using a motorised mower, chopping wood, moving heavy loads, scrubbing floors)	○ yes ○ no	____ years	____ months	____ minutes per week or ____ hours per week

Since you turned 50, did you do any other **strenuous activities?** Strenuous activity means something that really increased your heart rate, made you hot, and caused you to sweat. Some examples are: skiing, skating, hockey, hunting, chopping or tobogganing, water-skiing.

Activity (Please specify)	For how many years?	During these years, for how many months per year?	During those months, on average, for how many minutes or hours per week?
→■	years	months	minutes per week _or_ hours per week
→■	years	months	minutes per week _or_ hours per week
→■	years	months	minutes per week _or_ hours per week
→■	years	months	minutes per week _or_ hours per week
→■	years	months	minutes per week _or_ hours per week
→■	years	months	minutes per week _or_ hours per week

[ ] Since you turned 50, what was your usual occupation? (We mean what you did for the longest time, including any paid or unpaid employment, such as being a student or homemaker or being unemployed.)

occupation

[ ] don't know

151

Alcohol Consumption

We would like you to think back to when you were in your 20s.

24. **In your 20s**, did you ever consume any alcoholic beverages at least **once a week for 6 months or longer**? Please describe your consumption below.

		Ever have many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	○ yes ──▶ ○ no ○ don't know	years consumed	_____ number of 12 ounce cans or bottles ○ per day ○ per week ○ don't know
Wine	○ yes ──▶ ○ no ○ don't know	years consumed	_____ number of 4 ounce glasses of wine ○ per day ○ per week ○ don't know
Soda, sherry, port	○ yes ──▶ ○ no ○ don't know	years consumed	_____ number of 3 ounce servings ○ per day ○ per week ○ don't know
Spirits, liquor (mixed drinks, brandy, liqueur)	○ yes ──▶ ○ no ○ don't know	years consumed	_____ number of 1 ounce shots (gin or spirit) ○ per day ○ per week ○ don't know

25. When you were in your 20s, how many years **in total** did you consume at least one alcoholic beverage (of any type) a week?

_____ years consumed

○ never consumed alcohol

26. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4 ounce glasses of wine or 12 ounce cans or bottles of beer or hard cider or 1 ounce servings of soda, sherry, port or spirits, mixed drinks and cocktails.

_____ number of alcoholic beverages a week

If you never consumed alcohol in your 20s, please go to the question. You may skip to page 29.
(However, please continue with#41)

25

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Next, please think back to **your 30s and 40s.**

37. **In your 30s and 40s**, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? *Please describe your consumption below.*

		For how many years?	During those years, how much did you typically consume?
Beer, hard cider (at least 3% alcohol)	○ yes ➤ ○ no ○ don't know	years consumed	____ number of 12-ounce cans or bottles ○ per day ○ per week ○ don't know
Wine	○ yes ➤ ○ no ○ don't know	years consumed	____ number of 4-ounce glasses of wine ○ per day ○ per week ○ don't know
Sake, sherry, port	○ yes ➤ ○ no ○ don't know	years consumed	____ number of 3-ounce servings ○ per day ○ per week ○ don't know
Spirits, liquor, mixed drinks, brandy, liqueurs	○ yes ➤ ○ no ○ don't know	years consumed	____ number of 1-ounce shots, liquor, or spirits ○ per day ○ per week ○ don't know

38. When you were in your 30s and 40s, how many years **in total** did you consume at least one alcoholic beverage of any type a week?

____ years consumed

○ never consumed alcohol

39. On average, how many alcoholic beverages a week did you consume during those years? That is, how many 4-ounce glasses of wine or 12-ounce cans or bottles of beer or hard cider, or 1-ounce servings of sake, sherry, port, or spirits, mixed drinks and cocktails.

____ number of alcoholic beverages a week

○ never consumed alcohol

If you are younger than age 51, please go to the next section (Smoking) on page 28. Otherwise, please continue and ask you.

153

Now, please think back to since you turned 50.

(a) Since you turned 50, did you ever consume any alcoholic beverages at least once a week for 6 months or longer? *Please describe your consumption below.*

		For how many years?	During these years, how much did you typically consume?
Beer, hard cider (3% alcohol)	○ yes → ○ no ○ don't know	_____ years consumed	_____ number of 12-ounce cans or bottles ○ per day ○ per week ○ don't know
Wine	○ yes → ○ no ○ don't know	_____ years consumed	_____ number of 4-ounce glasses of wine ○ per day ○ per week ○ don't know
Sake, sherry, port	○ yes → ○ no ○ don't know	_____ years consumed	_____ number of 3-ounce servings ○ per day ○ per week ○ don't know
Spirits, liquor, mixed drinks, brandy, liqueurs	○ yes → ○ no ○ don't know	_____ years consumed	_____ number of 1-ounce shots of liquor or spirits ○ per day ○ per week ○ don't know

(b) Since you turned 50, how many years in total did you consume at least one alcoholic beverage per day or per week?

_____ years consumed alcohol

○ never consumed alcohol

(c) On average, how many alcoholic beverages total did you consume during these years? That is, how many 4-ounce glasses of wine or 12-ounce cans or bottles of beer or hard cider, or 3-ounce servings of sake, sherry, port, or spirits, mixed drinks, and cocktails

_____ number of alcoholic beverages a week

○ never consumed alcohol

Smoking

Have you ever smoked at least one cigarette a day for 3 months or longer?
- ○ yes
- ○ no ⟶ Please go to #6d
- ○ don't know ⟶ Please go to #6d

6a. When did you first start smoking at least one cigarette a day?

age at first use _____

year of first use _____

- ○ don't know

6b. During periods when you smoked regularly, how many cigarettes did you typically smoke in a day?

cigarettes per day _____

- ○ don't know

6c. About how many years have you still smoking at least one cigarette a day?

- ○ yes
- ○ no
- ○ don't know

6d. Do you still smoke at least one cigarette a day?

- ○ yes ⟶ Please go to #6h
- ○ no ⟶ Please go to #6h
- ○ don't know

6e. When did you stop smoking at least one cigarette a day, even though you were smoking permanently?

age of last use _____

year of last use _____

- ○ don't know

6f. How many years, in total, did you smoke at least one cigarette a day for 3 months or longer? (If you have stopped and started at least once, count only the time when you were smoking.)

total number of years _____

- ○ don't know

6d. Have you ever smoked at least one cigar a month for at least 3 months?

- ○ yes
- ○ no ⟶ Please go to #6h
- ○ don't know ⟶ Please go to #6h

6i. When did you first start smoking at least one cigar a month?

age at first use _____

year of first use _____

- ○ don't know

6h. During periods when you smoked regularly, how many cigars did you typically smoke in a month?

cigars per month _____

- ○ don't know

6i. About how many years have you still smoking at least one cigar a month?

- ○ yes
- ○ no
- ○ don't know

6dd. Do you still smoke at least one cigar a month?

- ○ yes ⟶ Please go to #6hh
- ○ no ⟶ Please go to #6hh
- ○ don't know

6k. When did you stop smoking at least one cigar a month even though you were smoking permanently?

age of last use _____

year of last use _____

- ○ don't know

6l. How many years, in total, did you smoke at least one cigar a month for 3 months or longer? (If you have stopped and started at least once, count only the time when you were smoking.)

total number of years _____

- ○ don't know

Have you ever smoked at least one **pipe** a month for at least 3 months?

- ○ yes
- ○ no → Please go to #56
- ○ don't know → Please go to #56

53a. When did you **first** start smoking at least one pipe a month?

    age at **first** use _____ or

    year of **first** use _____

- ○ don't know

53b. During periods when you smoked regularly, how many pipes did you typically smoke in a month?

    _____ pipes per month

- ○ don't know

54a. **About two years** ago, were you still smoking at least one **pipe** a month?

- ○ yes
- ○ no
- ○ don't know

54b. Do you still smoke at least one pipe a month?

- ○ yes → Please go to #63
- ○ no → Please go to #63
- ○ don't know

54c. When did you stop smoking at least one pipe a month (smoking permanently)?

    age of last use _____ or

    year of last use _____

- ○ don't know

54d. Over these years, in total, did you smoke at least one pipe a month for 2 months or longer? (if you have stopped and restarted at different times, count all the time when you were smoking.)

    total number of years _____

- ○ don't know

Height and Weight

56. About how tall are you without your shoes on?

    _____ feet _____ inches

    or

    _____ centimeters

- ○ don't know

57. How much did you weigh **about two years ago**?

    _____ pounds

    or

    _____ kilograms

- ○ don't know

58. How much did you weigh when you were **about 20 years old**?

    _____ pounds

    or

    _____ kilograms

- ○ don't know

Additional Information

59. Pertinent to this study, have you and your relatives ever taken part in any family health studies?

- ○ yes
- ○ no
- ○ don't know

Background Information

1) Which is the highest level of education that you completed?

- ○ less than 8 years
- ○ 8 to 11 years
- ○ high school graduate
- ○ vocational or technical school
- ○ some college or university
- ○ bachelor's degree
- ○ graduate degree
- ○ don't know

2) Country of birth sometimes affects disease risk. Please fill in country of birth for **yourself, your parents and your grandparents.**

In addition, scientists have found that some genetic traits are more common in one common ancestry found in people of different ethnic backgrounds. Please answer the questions about Jewish descent for each person.

	Country of birth	Is this person of Jewish descent	Additional (fill-in European)	Sephardic	other	don't know
You		○ yes → ○ no ○ don't know	○	○	○	○
Your mother		○ yes → ○ no ○ don't know	○	○	○	○
Your father		○ yes → ○ no ○ don't know	○	○	○	○
Your mother's mother		○ yes → ○ no ○ don't know	○	○	○	○
Your mother's father		○ yes → ○ no ○ don't know	○	○	○	○
Your father's mother		○ yes → ○ no ○ don't know	○	○	○	○
Your father's father		○ yes → ○ no ○ don't know	○	○	○	○

72. How many years have you lived in Canada?

   ○ all my life

   ○ _____ number of years

   ○ don't know

73. Ethnicity and race sometimes affect disease risk. Scientists have found that some genetic traits are more common or less common among people of different backgrounds. We would like to know if this is true for genes associated with colorectal cancer.

Please fill in the background for yourself, your parents and your grandparents.
Please tick all that apply.

	You	Your mother	Your father	Your mother's mother	Your mother's father	Your father's mother	Your father's father
Black, from Africa	○	○	○	○	○	○	○
Black, from the Caribbean (e.g. Trinidad, Jamaica, Haiti)	○	○	○	○	○	○	○
Black, from North America	○	○	○	○	○	○	○
Black, other	○	○	○	○	○	○	○
White	○	○	○	○	○	○	○
First Nations (e.g. Indian, Inuit)	○	○	○	○	○	○	○
North African (e.g. Egyptian)	○	○	○	○	○	○	○
Middle Eastern (e.g. Iranian)	○	○	○	○	○	○	○
Filipino	○	○	○	○	○	○	○
Japanese	○	○	○	○	○	○	○
Korean	○	○	○	○	○	○	○
Chinese	○	○	○	○	○	○	○
Other South East Asian (e.g. Vietnamese)	○	○	○	○	○	○	○
South Asian (e.g. East Indian, Pakistani)	○	○	○	○	○	○	○
other, *please specify*							
don't know	○	○	○	○	○	○	○

73. Which of the following categories best describes your total annual **household** income about five years ago?

○ $0-$10,000
○ less than $6,000
○ $6,000 - $11,999
○ $12,000 - $19,999
○ $20,000 - $29,999
○ $30,000 - $39,000

○ $40,000 - $49,999
○ $50,000 - $59,999
○ $60,000 - $69,999
○ $70,000 - $79,999
○ $80,000 or more
○ don't know

74. In case we need to contact you in the future and you have moved, could we have the name of someone whom we could contact in order to call for your new address?

Name of relative or friend:

His or her address:

His or her telephone number: (   )

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Appendix B. Food frequency questionnaire used in Newfoundland and Labrador

Canadian Study of Diet and Health

Memorial University of Newfoundland

SAMUEL LUNENFELD RESEARCH INSTITUTE

Who this questionnaire is for and what it asks about:

This questionnaire is to be completed by the person taking part in this study.

Part I asks about the foods you ate about one year before your diagnosis.

Part II asks about vitamins and other dietary supplements that you may have used.

If possible, please return this questionnaire within two weeks.

The completed questionnaire should be sealed in the pre-paid envelope and mailed back to:
**CRC-IHRT,
Room 17598, Level 1, Health Science Centre,
300 Prince Phillip Drive,
St. John's, NL, Canada, A1B 5Z9.**

If you have any questions about this form or the study, call our toll-free number: **1-888-908-4988.**

The information given to us in this questionnaire will be kept confidential.

Thank you for your time and assistance.

HOW TO COMPLETE THIS QUESTIONNAIRE

We would like to know how often you ate certain foods **about one year before diagnosis**, and their amounts.

Section A (lists foods and portion sizes)
Amounts are described in various ways, including the number of:

| cups, | teaspoons (tsp), | ounces (oz), | inches ("), | pieces (e.g., 1 apple) |
| grams (gm), | tablespoons (tbsp), | milliliters (ml), | centimetres (cm) |

We want to know the **Portion Size** of your USUAL SERVING. We have given an example of an average portion size. If your portion size was different than the average, you can indicate this by putting an X or (✓) the circles for Smaller or Larger portion sizes. Smaller than average is about 25% or less than the average portion size, while Larger than average is about 25% or more than the average size. Leave the circle blank if your typical portion size was average.

Included with this questionnaire is a FOOD PHOTOGRAPH PAGE that shows small, medium and large portion sizes for vegetables, meat and chicken. Some questions ask you to refer to the photo page to help you choose your usual portion size.

Section B (asks about how often you ate certain foods one year before diagnosis)
For each food item listed, choose one column (Per Day, Per Week, Per Month, or Never / Rarely) that best describes HOW OFTEN you ate or drank that item. For example, if you ate CREAM CHEESE 3 times a month during the year of interest, you would write (3) in the PER MONTH column. If you ate SWEET POTATOES only 2 times during the year of interest, you can place a checkmark (✓) in the NEVER OR RARELY column.

Section C (To be completed only for seasonal foods)
Some foods (for example fresh fruit and vegetables) are not available throughout the year. For foods that you do not eat all year round (i.e. in season only), indicate the number of months of the year that you ate them.

Please complete each question as best you can. We know that it is difficult to recall exactly how often you ate something. If you are not certain, try to give your best estimate.

EXAMPLE	Section A			Section B					Section C
FOOD	Average Portion Size	Your Portion Size, if NOT Average		**HOW OFTEN?** (Complete one column only)					If Ate Food in Season Only
		Smaller	Larger	per Day (write number)	per week (write a number)	per MONTH (write a number)	NEVER or RARELY (check)		enter Months per Year
1	CREAM CHEESE	3 tsp 30 ml 1 oz	O	O				✓		
2	CANTELOUPE	1/8 or 1 slice	O	O						
3	SWEET POTATOES	1 medium/½ cup	O	O		1			✓	4

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Dietary Questionnaire Information Sheet

This information sheet will assist you with some of the questions that may be unclear in the questionnaire. This study is being done in Newfoundland and Labrador and in Ontario. We want to be sure that all of the questions are comfortable with the questionnaire and we know that the comments won't apply to everyone. We ask that you note the following questions.

- Question 86. Hotdog or wiener. This question refers to the meat portion only. If you eat wieners with a bun check the bun portion in the bread section.

- Question 89. Bologna. Be sure to check your usual portion size in the question on cold cuts. In Newfoundland we often eat much thicker slices so you would tick "larger" portion size.

- Question 95. Pickled Meat. Newfoundland and Labrador salt beef should be ticked here. The salted/dried meat in question 94 is about beef jerky and those kinds of meats. Pickled fish in question 101 is asking about things like bottled pickled herring.

- Question 154-155. Mayonnaise/Miracle Whip. Please consider both kinds as you complete this question. We know that most people use Miracle Whip as salad dressing and refer to it as mayonnaise but some people may use real mayonnaise.

- Question 158. Margarine and Butter. Be sure to keep separate the amount of margarine and butter you eat. Again we know that many people use margarine although they may refer to it as butter.

- Question 164. Gravy. This asks about an average serving of ⅛ cup. Most of us in Newfoundland and Labrador use much more than this so you would tick the larger portion here, too.

- You will also see that questions about fat or dairy products often separate out regular and light brands.

- Part Two If you take separate vitamins by themselves please look at the container to see what "dosage" you are taking. It will be given in either mg or iu.

- We are also interested in the amount of seasonal foods you eat. If you only eat certain foods at certain times of the year, please indicate how many months of the year you ate these foods.

Thank-you

	Section A			Section B YEAR BEFORE DIAGNOSIS				Section C
FOOD	Average Portion Size	Your Portion Size, if NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food, In Season Only, Enter Months per Year
		Smaller Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	
Beverages								
2	2% MILK, 2% Evaporated milk (any, if in cereal & drinks)	1 cup/ 250 ml	O O					
4	MILK SHAKE	1 cup/ 250 ml	O O					
6	COFFEE (not decaffeinated)	1 cup/ 250 ml	O O					
8	TEA (not herbal)	1 cup/ 250 ml	O O					
10	SUGAR (in tea and coffee)	1tsp or 1 cube	O O					
12	DIET SOFT DRINKS	1 can/ 250 ml	O O					
14	ORANGE or GRAPEFRUIT JUICE	¾ cup/ 175 ml	O O					
15	APPLE or GRAPE JUICE	¾ cup/ 175 ml	O O					
16	OTHER FRUIT JUICES (pineapple, cranberry, etc)	¾ cup/ 175 ml	O O					
17	FRUIT DRINKS, LEMONADE	¾ cup/ 175 ml	O O					
18	FRUIT DRINKS, ICED TEA	¾ cup/ 175 ml	O O					
19	VEGTABLE JUICES	¾ cup/ 175 ml	O O					
20	BEER or ALE	355 ml/ 1 bottle	O O					
21	WHITE WINE	150 ml/ 5 oz	O O					
22	RED WINE (sherry, PORT or other fortified wine)	150 ml/ 5 oz	O O					
23	LIQUOR (for example, whiskey, rum etc)	45 ml/ 1.5 oz	O O					

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	Section A			Section B				Section
FOOD	Average Portion Size	Your Portion Size, if NOT Average		**HOW OFTEN?** (Complete one column only)				If Ate For In Season Only
				per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER RARELY (check)	enter Months per Year
		Smaller	Larger					
Dairy Products								
26	EGG (fried, scrambled, omelette)	1 egg	O	O					
27	CHEESE, Regular fat (such as cheddar, Swiss, processed)	1 slice/ 30 g/ 1oz	O	O					
29	CHEESE, Ultra Light (3% fat or less, such as cheddar)	1 slice/ 30 g/ 1oz	O	O					
31	CREAM (coffee, whipping, sour or regular)	1 tbsp/ 15 ml	O	O					
33	COFFEE WHITENER (non-dairy)	1 tbsp/ 15 ml	O	O					
35	YOGURT, Light (plain, less than 2.4% fat)	½ cup/ 175 ml	O	O					
37	YOGURT, Light (fruit flavoured or frozen, less than 2.4% fat)	½ cup/ 175 ml	O	O					
Mixed Dishes								
39	SOUPS (cream based)	1 cup/ 250 ml	O	O					
41	PASTA with meat sauce (spaghetti, lasagna)	1 cup/ 250 ml	O	O					
43	MIXED DISHES with cheese or cheese sauce (macaroni and cheese, cannelloni)	1 cup/ 250 ml	O	O					
45	PIZZA with vegetable only	1 Medium slice	O	O					

	Section A			Section B				Section C
FOOD	Average Portion Size	Your Portion Size, If NOT Average		HOW OFTEN? (Complete one column only)				If Ate Food In Season Only
		Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months (per Year)
47 CHILI with meat or Con Carne	1 cup 250 ml	O	O					
Vegetables								
49 FRENCH FRIES or FRIED POTATOES	1 cup 250 ml	O	O					
51 BROCCOLI	1 cup 250 ml	O	O					
53 CAULIFLOWER	½ cup 125 ml	O	O					
55 PEAS or LIMA BEANS	½ cup 125 ml	O	O					
57 BEANS or LENTILS (baked or boiled beans, kidney beans, chickpeas)	½ cup 125 ml cooked	O	O					
59 GREEN SALAD (with lettuce)	1 cup 250 ml	O	O					
61 TOMATOES (fresh)	1 medium ½ cup 125 ml	O	O					
62 TOMATOES canned, pureed or stewed	½ cup 125 ml	O	O					
63 ONIONS (raw or cooked)	½ cup 125 ml	O	O					
64 CARROTS (cooked or pickled)	½ cup 125 ml	O	O					
65 TURNIPS or RUTABAGAS	1 medium ½ cup 125 ml	O	O					
66 OTHER ROOT VEGETABLES (sweet potatoes, yams, radish, etc)	½ cup 125 ml	O	O					
67 YELLOW SQUASH (winter type)		O	O					

Section A				Section B				Section C
FOOD	Average Portion Size	Your Portion Size, If NOT Average		YEAR BEFORE DIAGNOSIS HOW OFTEN? (Complete one column only)				If Ate For Its Season Only
		Smaller	Larger	per DAY (enter # times per day)	per WEEK (enter # times per week)	per MONTH (enter # times per month)	NEVER or RARELY (check)	enter Months per Year
69 SWEET PEPPER (green, red or yellow)	½ cup/125 ml	O	O					
71 BEAN SPROUTS, ALFALFA SPROUTS	½ cup/125 ml	O	O					
73 AVOCADO	½ Medium	O	O					

Meats and Fish

FOOD	Average Portion Size	Smaller	Larger	per DAY	per WEEK	per MONTH	NEVER or RARELY	Months per Year
78 GROUND BEEF, medium (hamburger, meat loaf, in casserole etc)	60 g/3 oz/3" patty	O 2 oz	O 4 oz					
ROAST BEEF	photo 9, medium	O	O					
79 PORK CHOP	photo 8, medium	O	O					
80 ROAST PORK	photo 8, medium	O	O					
82 BAKED HAM	photo 8, medium	O	O					
83 BACON	3 slices	O	O					
84 VEAL	photo 8, medium	O	O					
85 LAMB	photo 8, medium	O	O					
86 HOT DOG or WIENER (Enter bun/rolls under item 115)	1 hot dog	O	O					
87 SAUSAGE	1 sausage	O	O					
88 CORNED BEEF	1 slice	O	O					
89 COLDCUTS (ham, salami, bologna, etc)	1 med. Slice/30g/1 oz	O	O					

	FOOD	Average Portion Size	Your Portion Size, If NOT Average		HOW OFTEN (Complete one column only)				If Ate Fish In Season Only Enter Months per Year
			Smaller	Larger	per DAY (enter number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	
90	LIVER	85 g 3 oz	O	O					
92	CHICKEN TURKEY (roasted or stewed)	photo C, medium	O	O					
94	SALTED/DRIED MEAT	photo C, small	O	O					
96	SHELLFISH (shrimp, lobster, crab)	85 g 3 oz photo C, small	O	O					
98	FISH (baked or broiled)	170 g 6 oz photo B, medium	O	O					
100	SMOKED FISH or LOX	85 g 3 oz photo C, small	O	O					
102	PICKLED FISH	85 g 3 oz photo C, small	O	O					
104	CARIBOU, MOOSE	85 g 3 oz photo C, small	O	O					

Cereals and Grains

	FOOD	Average Portion Size	Smaller	Larger	per DAY	per WEEK	per MONTH	NEVER or RARELY	Months per Year
107	WHOLE WHEAT CEREALS (such as shredded wheat)	½ cup 125 ml 1 biscuit	O	O					
109	HOT CEREALS (for example oatmeal)	½ cup 125 ml	O	O					
110	SUGAR COATED CEREALS		O	O					
111	OTHER BREAKFAST CEREALS	½ cup 125 ml	O	O					
112	SUGAR ON CEREAL	1 tsp 15 ml	O	O					

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	Section A		Section B						Section C
	FOOD	Average Portion Size	**Your Portion Size, If NOT Average**		**HOW OFTEN?** (Complete one column only)				**# Meals per Year**
			Smaller	Larger	per DAY	per WEEK	per MONTH	NEVER or RARELY	
113	100% WHOLE GRAIN or DARK BREAD	1 slice	O	O					
115	WHITE BREAD	1 slice	O	O					
117	WHOLE WHEAT ROLLS	1 roll	O	O					
119	BRAN/OAT MUFFIN	1 medium, ½ extra large	O	O					
121	PANCAKES, WAFFLES	1	O	O					
123	RICE	½ cup cooked 125 ml	O	O					
	Fruits								
125	APPLE, PEAR	1	O	O					
126	CITRUS FRUITS (orange, grapefruit)	1 orange, ½ grapefruit	O	O					
127		½ cup 125 ml							
128	GRAPES	½ cup 125 ml	O	O					
129	BANANA	1							
130	PEACH PLUM, NECTARINE, APRICOT	1	O	O					
131	CANTELOUPE	1/8 or 1 slice	O	O					
132	WATERMELON	1 slice	O	O					
133	HONEYDEW MELON	1/8 or 1 slice	O	O					
134	MANGO	1	O	O					
135	PAPAYA	1	O	O					
136	APPLESAUCE	½ cup 125 ml	O	O					

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	Section A			Section B			Section C
FOOD	Average Portion Size	Your Portion Size, if NOT Average		YEAR BEFORE DIAGNOSIS HOW OFTEN? (Complete one column only)			If Ate Food In Season Only
		Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	enter Months per Year

FOOD	Average Portion Size	Smaller	Larger	per DAY	per WEEK	per MONTH	NEVER/RARELY	Months per Year
136 CANNED FRUIT (all kinds)	½ cup 125 ml	O	O					
Desserts and Sweets								
141 PIES and TARTS	1 slice	O	O					
143 COOKIES	1	O	O					
145 LIGHT or DIET ICE CREAM	½ cup 125 ml	O	O					
147 DIET or LIGHT PUDDING	½ cup 125 ml	O	O					
148 POPSICLES, FREEZIES	1	O	O					
149 CHOCOLATE BAR / CHOCOLATE CANDY	1 bar/35 g	O	O					
151 CANDY (without chocolate)	1 caramel	O	O					
Miscellaneous								
152 TOTAL TEMPEH	½ cup 2" x 2" x 1" slice	O	O					
153 KETCHUP	1 tbs	O	O					
154 MAYONNAISE/ MIRACLE WHIP Regular fat (or used salad, fresh, etc.)	1 tbs	O	O					
155 MAYONNAISE/ MIRACLE WHIP Light (or bread, salad dressing, fresh, etc.)	1 tbs	O	O					
156 SALAD DRESSING Regular fat (French, Italian etc.)	1 tbs	O	O					
157 OIL (in cooking)	1 tsp	O	O					

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	Section A			Section B				Section
		Your Portion Size, if NOT Average		**HOW OFTEN?** (Complete one column only)				**If Ate Foo In Season Only**
FOOD	Average Portion Size							
		Smaller	Larger	per DAY (enter a number)	per WEEK (enter a number)	per MONTH (enter a number)	NEVER or RARELY (check)	number Months per Year
159	MARGARINE (on vegetables or bread, exclude use in baked or mixed dishes)	1 pat/1 tsp	O	O					
161	PEANUTS	30g/1 oz	O	O					
163	JAM, JELLY, HONEY, SYRUP (1)	1 tbs	O	O					
165	CHOCOLATE or STRAWBERRY SYRUP	1 tbs	O	O					
167	SAUCES (white, cream, Mornay)	30 ml/1oz/2 tbs	O	O					
169	WHEAT BRAN	1 tbs	O	O					
193	WHEAT GERM	1 tbs	O	O					

Continue on next page ⟶

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Now we would like to ask you a few questions about how you prepared certain foods ABOUT ONE YEAR BEFORE DIAGNOSIS and whether you followed any special diets. For the following questions, please check the circle or fill in the appropriate answer:

1. About 1 year before diagnosis, how much of the visible fat on your meat did you eat?
- O Most of it
- O Some of it
- O As little as possible
- O Do not eat meat

6. About 1 year before diagnosis, what type of oil did you use in other preparations (for example, in salad dressings)?

2. About 1 year before diagnosis, how often did you eat the skin on chicken?
- O Most of it
- O Some of it
- O As little as possible
- O Do not eat chicken

7. About 1 year before diagnosis, what type of the following items did you usually use? Please check one box per line.

	Mayonnaise/Miracle Whip	O Light		
O regular	O Light	O Both	O None
Cream cheese			
O regular	O Light	O Both	O None

3. About 1 year before diagnosis, what kind of fat did you usually use for stir-frying?
- O Vegetable oil
- O Vegetable shortening
- O Lard pork fat
- O Butter
- O Margarine
- O Do not add fat or oil
- O Other, please specify

8. About 1 year before diagnosis, were you on a (please check one box only):
- O Non-vegetarian (eats all meat, chicken, fowl)
- O Partly non-vegetarian (eats chicken, fish, no meat)
- O Vegan (eats no dairy, no eggs, no meat)
- O Lacto-vegetarian (eats dairy, no eggs, no meat)
- O Lacto-ovo-vegetarian (eats dairy & eggs, no meat)

4. About 1 year before diagnosis, what kind of fat did you usually use for deep frying?
- O Vegetable oil
- O Vegetable shortening
- O Lard pork fat
- O Butter
- O Margarine
- O Do not fry
- O Other, please specify

9. About 1 year before diagnosis, were you on a special diet?
- O No
- O Yes

If yes, what type of diet?
- O To lose Weight
- O To lower cholesterol
- O Diabetes
- O Heart disease
- O Hypertension
- O Gastric ulcer
- O Bowel disease
- O Low fat
- O High fibre
- O Other type _____

5. About 1 year before diagnosis, what kind of fat did you usually use for baking?
- O Butter
- O Margarine
- O Vegetable Oil
- O Vegetable shortening
- O Lard pork fat
- O Do not bake

If yes, how long were you on the special diet?

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PART 2 - USE OF VITAMINS AND DIETARY SUPPLEMENTS

Now we would like to know about your use of vitamins and dietary supplements.
ABOUT ONE YEAR BEFORE DIAGNOSIS, did you take any of the following? If Yes, then specify usual brand and amount and how long you took them.

	Vitamin and Amount	— if used, ▶		How many pills did you take per week?	How long had you taken them?
Vitamin C					
O None	O Below 500	O 500-1000	O above 1000 mg	`0 5` per week	`2 4` months
Multivitamins that include minerals					
O No	O Yes:	If yes, usual brand _____		per week	months
Multivitamins, no minerals					
O No	O Yes	If yes, usual brand _____		per week	months
B Complex vitamins					
O No	O Yes	If yes, usual brand _____		per week	months

In the following items, DO NOT INCLUDE use of the above MULTIVITAMINS

Vitamin A					
O None	O Below 10000	O 10000-15000	O above 15000 IU	per week	month
Vitamin C					
O None	O Below 500	O 500-1000	O above 1000 mg	per week	months
Vitamin E					
O None	O Below 400	O 400-800	O above 800 IU	per week	months
Beta-carotene					
O None	O Below 10000	O 10000-15000	O above 15000 IU	per week	months
Folic acid					
O None	O Below 1.0	O 1.0 mg	O above 1.0 mg*	per week	months
Calcium					
O None	O Below 250	O 250-500	O above 500 mg	per week	months
Iron					
O None	O Below 100	O 100-200	O above 200 mg	per week	months
Other dietary supplements (e.g., yeast, cod liver oil, etc)					
O No	O Yes, specify type _____			per week	months
				per week	months

* 1 mg = 1000 micrograms

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FOOD PHOTOGRAPH PAGE

Please use these photos for foods in the questionnaire that refer you to pictures A, B, or C to help estimate your usual portion size. The ruler on the left will help you to visualize the size of the 9" dish shown.

A

SMALL (S)   MEDIUM (M)   LARGE (L)

Can be used for Vegetables, Rice, Spaghetti, Salads, Potatoes etc.

B

SMALL (S)   MEDIUM (M)   LARGE (L)

Can be used for Meats, Steaks, Chops, Fish etc.

C

SMALL (S)   MEDIUM (M)   LARGE (L)

Can be used for Chicken, Turkey etc.
Size of Dinner Plates shown above is 9 inches

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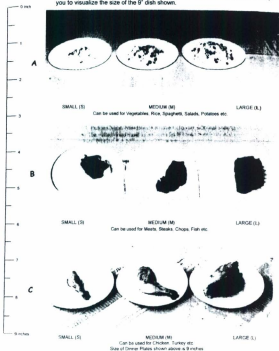
We welcome any other information or comments that you would like to give us:

_____

_____

_____

THANK YOU VERY MUCH for your assistance in this research!

Appendix C. Food frequency questionnaire used in Ontario

Diet Questionnaire

IDENTIFICATION NO:

NAME _____

ADDRESS _____

MARKING INSTRUCTIONS

- Use No. 2 pencil only.
  One is provided for your use.

- Do NOT use ink or ballpoint pens.

- Fill in the circle completely,
  staying within the circle line.   CORRECT MARK   INCORRECT MARKS

- Erase cleanly any answer you wish to change.

- Do NOT make any stray marks in this booklet.

PLEASE BEGIN THIS SURVEY ON PAGE 1.

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These questions are about your usual eating habits *ABOUT 2 YEARS AGO*. For each food group, please fill in the circle that best describes HOW OFTEN you ate those items about 2 years ago and then fill in the circle that best describes your USUAL SERVING SIZE.

Most categories include examples. They are only suggestions, and you may not eat all of the listed items. Some ethnic foods are also listed. If you don't recognize the name, you probably don't eat that item.

For each item, please include any fresh, frozen, canned, and packaged foods you ate, such as TV dinners, frozen entrees, vegetables, or side dishes.

If you did not eat an item, or if you *ate an item less than once a month*, fill in the circle in the first column. *DO NOT LEAVE BLANK.*

For some categories, portions of food on a dinner plate are included to help you estimate your usual serving size. Please note that "1 cup" refers to an 8-ounce (240 mL) measuring cup.

For EACH FOOD GROUP, fill in the circle that best describes HOW OFTEN you ate those items *ABOUT 2 YEARS AGO*. Then fill in the circle that best describes your USUAL SERVING SIZE.

SOUPS, RAMEN, AND JOOK	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Cream Soup or Chowder	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Dried Bean or Pea (Legume) Soup (such as Portuguese bean, split pea)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Tomato or Vegetable Soup (may include meat, poultry or fish)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Miso Soup	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Broth with Noodles or Rice (such as beef noodle or chicken rice	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Mexican Meat Soup or Stew (such as menudo, albondigas, cocido, posole)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Oriental Noodles with Broth (such as saimin, ramen, wún tun mein or long mein)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)
Jook (rice gruel, may include meat, poultry, fish, or vegetables)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (about 1 cup) OR ○ Large bowl (2 cups or more)

1

For EACH FOOD GROUP fill in the circle that best describes HOW OFTEN you ate these items **ABOUT 2 YEARS AGO**. Then fill in the circle that best describes your **USUAL SERVING SIZE**.

A   B   C

NOODLES, SPAGHETTI, AND MIXED DISHES:	AVERAGE USE ABOUT 2 YEARS AGO								YOUR VISUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 4 times a week	5 to 6 times a week	Once a day	2 or more times a day	
Chow Mein, Chow Fun, or Yakisoba (Oriental fried noodles)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Spaghetti, Ravioli, Lasagna, or Other Pasta with Tomato Sauce	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Macaroni and Cheese or Other Pasta and Cheese Casseroles	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Macaroni or Potato Salad (with mayonnaise)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Pasta or Ramen Salad	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Noodle Casseroles (with tuna, chicken or turkey)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Pasta with Cream Sauce (such as linguine with clam sauce, beef stroganoff)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Arroz Con Pollo (rice with chicken)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) **OR** ○ Photo B (about 1 cup) **OR** ○ Photo C (2 cups or more)
Stew, Curry, Pot Pie or Empanada (with beef or lamb)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Part A (1/2 cup or 1 Empanada) **OR** ○ Part B (about 1 cup or 1 pie) **OR** ○ Part C (2 cups or more)
Stew, Curry, Pot Pie or Empanada (with chicken or turkey)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Part A (1/2 cup or 1 Empanada) **OR** ○ Part B (about 1 cup or 1 pie) **OR** ○ Part C (2 cups or more)

For EACH FOOD GROUP, fill in the circle ( ) that best describes HOW OFTEN you ate these items *ABOUT 2 YEARS AGO*. Then fill in the circle ( ) that best describes your USUAL SERVING SIZE.

A B C

MIXED DISHES	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Stir-Fried Beef or Pork and Vegetables, or Fajitas (beef and broccoli, pork fajitas, chop suey, subgum)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (2 cups or more)
Stir-Fried Chicken and Vegetables, or Fajitas (such as cashew chicken, chicken fajitas, chicken lo mein, moo goo gai pan)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (2 cups or more)
Stir-Fried Shrimp or Fish and Vegetables	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (2 cups or more)
Stir-Fried Vegetables (no meat)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (2 cups or more)
Pork and Greens or Laulaus	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (2 cups or more)
Chili	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ Small bowl (1 cup) OR ○ Large bowl (2 cups or more)
Hamburgers (on a bun)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 regular size burger OR ○ 1 quarter pound burger OR ○ 1 large double burger
Cheeseburgers (on a bun)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 regular size burger OR ○ 1 quarter pound burger OR ○ 1 large double burger
Meat Loaf, Meatballs, or Patties (not fast food hamburgers)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 3 meatballs OR ○ 1 patty or slice or 3 meatballs OR ○ 2 large patty or 6 meatballs
Pizza	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 piece or slice or less OR ○ 2 to 3 pieces OR ○ 4 pieces or more

For EACH FOOD GROUP, fill in the circle ○ that best describes HOW OFTEN you ate these items *ABOUT 2 YEARS AGO*. Then fill in the circle ○ that best describes your USUAL SERVING SIZE.

A

B

C

MEATS (NOT PART OF MIXED DISHES)	AVERAGE USE ABOUT 2 YEARS AGO							YOUR USUAL SERVING SIZE
	Never or less than once/month	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	2 or more times a day	
Beef Steak or Roast, Veal or Lamb (includes beef tongue, chop, cold cuts and corned variety)	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B (3 oz. or 1 sirloin chop) OR ○ Photo C (5 ounces or more)
Short Ribs			○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B (or 2 short ribs) OR ○ Photo C (or 3 ribs or more)
Corned Beef (fresh or canned)	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B (or 1-4 oz. box) OR ○ Photo C (or 16 fl.oz. or more)
Corned Beef Hash			○	○	○	○	○	CHOOSE ONE ○ Photo A (or 1 patty) OR ○ Photo B (or 2 patties) OR ○ Photo C (or 3 patties or more)
Pork Chops or Roasts, Kolba Pig, or Carnitas (includes chile verde)	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B (3 ounces) OR ○ Photo C (5 ounces or more)
Ham (includes ham-al, fresh, or sandwich)			○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B (3 ounces) OR ○ Photo C (5 ounces or more)
Ham Hocks or Pig's Feet	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B (3 ounces) OR ○ Photo C (5 ounces or more)
Spareribs			○	○	○	○	○	CHOOSE ONE ○ 1 small or 1 slog rib or less OR ○ 2 to 3 slog ribs OR 1 medium OR ○ 4 ribs or more
Liver	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1 ounce or less) OR ○ Photo B or 3 chicken livers OR ○ Photo C (5 ounces or more)
Chicken or Turkey Wings			○	○	○	○	○	CHOOSE ONE ○ 1 chicken wings or less OR ○ 2 chicken wings OR ○ 3 pieces or 4 chicken wings or more

A   B   C

POULTRY AND FISH (NOT PART OF MIXED DISHES)	AVERAGE USE ABOUT 2 YEARS AGO								FOUR USUAL SERVING SIZE
	Never or less than once/mon	Once a month	2-3 a month	Once a week	2 to 4 a week	5 to 6 a week	Once a day	2 or more day	
Fried Chicken (includes fried chicken sandwich, nuggets)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A or 1 drumstick, OR ○ Photo B or 1 breast, 2 thighs, 2 wings, or 1 sandwich OR ○ Photo C or 5 breasts or 4 thighs
Roasted, Baked, Grilled or Stewed Chicken (includes grilled chicken sandwich)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A or 1 drumstick, OR ○ Photo B or 1 breast, 2 thighs, 2 wings, or 1 sandwich OR ○ Photo C or 5 breasts or 4 thighs
Turkey (includes roast, ground, deli-style, or sandwich)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A ½ ounce or less OR ○ Photo B (4 ounces) OR ○ Photo C (6 ounces or more)
Fried Shrimp or Other Shellfish (includes tempura, fried calamari or squid)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 3 items OR ○ 4 to 5 items or 1 ½ cup OR ○ 6 items or more
Cooked, Canned, or Raw Shellfish (such as crab, squid, shrimp)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ ¼ cup or less OR ○ ½ cup or 1 cup OR ○ 1 lobster tail or 1 cup or more
Fried Fish (includes pan-fried too, frozen fish sticks, fried fish sandwiches)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A about 1 ounce OR ○ Photo B (4 ounces) OR ○ Photo C (7 ounces or more)
Baked, Broiled, Boiled or Raw Fish (fresh ao not snapper, salmon, sashimi)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A about 1 ounce OR ○ Photo B (3 ounces) OR ○ Photo C (6 ounces or more)
Canned Tuna fish (plain, salad, or sandwich)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ ¼ cup or 1 sandwich OR ○ 1 cup or 2 sandwiches OR ○ 1 cup or 2 sandwiches
Other Canned Fish (such as salmon, mackerel, sardines)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small sardines or ¼ cup OR ○ ½ cup with OR ○ 1 cup fish or more
Salted and Dried Fish (such as ika, codfish, raw)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 slice or strip or piece OR ○ 2 slices OR ○ 4 slices or more

PROCESSED MEATS AND MEXICAN DISHES	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Bacon (includes Canadian bacon)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 slice or strip or piece OR ○ 2 slices OR ○ 3 slices or more
Regular Hot Dogs (beef or pork)				○	○	○			CHOOSE ONE ○ 1/2 hot dog OR ○ 1 hot dog OR ○ 2 hot dogs or more
Chicken or Turkey Hot Dogs or Luncheon Meats	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 hot dog or 1 slice OR ○ 1 hot dog or 2 slices OR ○ 2 hot dogs or 3 slices or more
Spam, Bologna, Salami, Pastrami or Other Luncheon Meats	○	○	○	○					CHOOSE ONE ○ 1 slice (1 ounce or less) OR ○ 2 slices OR ○ 3 slices or more
Sausage (such as pork, beef, chorizo, Polish, Vienna, Portuguese, hot links)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 piece or link OR ○ 2 pieces or links or 1 patty OR ○ 3 pieces or links or more
Tacos, Tostadas, Sopes, or Taco Salad (with beef or pork)	○	○	○	○	○				CHOOSE ONE ○ 1 item or taco OR ○ 2 items OR ○ 3 items or more
Tacos, Tostadas, Sopes, or Taco Salad (with chicken)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 item or taco OR ○ 2 items OR ○ 3 items or more
Meat Burritos (includes beef and bean and other combinations)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 fast food burrito OR ○ 1 medium burrito OR ○ 1 large or 2 fast food burritos
Vegetable or Bean Burritos, Tacos, or Tostadas (no meat)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 item or taco OR ○ 2 items OR ○ 3 items or more
Enchiladas with Chicken	○	○	○	○	○				CHOOSE ONE ○ 1 enchilada or less OR ○ 2 enchiladas OR ○ 3 enchiladas or more
Enchiladas with Beef	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 enchilada or less OR ○ 2 enchiladas OR ○ 3 enchiladas or more
Enchiladas with Cheese, Quesadillas, or Nachos with Cheese	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 enchilada or quesadilla OR ○ 2 enchiladas or 1 serving nachos OR ○ 3 enchiladas
Tamales	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 tamale or less OR ○ 1 tamale OR ○ 2 tamales or more
Chili Rellenos	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 chili relleno or less OR ○ 1 chili relleno OR ○ 2 chili rellenos or more

RICE, POTATOES, TARO, AND POI	AVERAGE USE: ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 4 times a week	5 to 6 times a week	Once a day	2 or more times a day	
White Rice (includes instant)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or 1 scoop or less OR ○ 1 rice bowl (1 cup) or 1 mound OR ○ 2 rice bowls or 2 mounds or more
Sushi or Barazushi	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1-2 pieces or small scoop OR ○ 3-4 pieces or 1 large scoop or 1/2 cup OR ○ 5 pieces or 1 cup or more
Brown or Wild Rice	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or 1 scoop or less OR ○ 1 cup or 2 scoops OR ○ 2 cups or more
Mexican or Spanish Rice	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup OR ○ 2 cups or more
Fried Rice	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup OR ○ 2 cups or more
French-Fried, Hash-Browned or other Fried Potatoes	○	○	○	○	○	○	○	○	CHOOSE ONE ○ fast food small order or 1 cup OR ○ fast food medium order OR ○ fast food large order or more
Mashed, Scalloped or Au Gratin Potatoes	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or 1 scoop or less OR ○ 1 cup or 2 scoops OR ○ 2 cups or more
Baked or Boiled White Potatoes	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small or 1/2 medium or less OR ○ 1 medium (about 5 inches) OR ○ 1 large potato or more
Yellow-Orange Sweet Potatoes or Yams	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small or 1/2 medium or less OR ○ 1 medium (about 5 inches) OR ○ 1 large potato or more
White or Purple Sweet Potatoes	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small or 1/2 medium or less OR ○ 1 medium (about 5 inches) OR ○ 1 large potato or more
Taro	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/4 taro or less OR ○ 1/2 taro OR ○ 1 whole taro or more
Poi	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1/2 cup OR ○ 1 cup or more

For EACH FOOD GROUP, fill in the circle ⬤ that best describes HOW OFTEN you ate these items *ABOUT 2 YEARS AGO.* Then fill in the circle ⬤ that best describes your USUAL SERVING SIZE.

A   B   C

SALAD ITEMS, EGGS, AND OTHER NON-MEAT ITEMS	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	3 to 4 times a week	5 to 6 times a week	Once a day	2 or more times a day	
Light Green Lettuce or Tossed Salad (such as iceberg or head lettuce)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (1-1/2 cups or more)
Dark Green Lettuce (such as romaine, red, butter, mesclun, endive)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/2 cup or less) OR ○ Photo B (about 1 cup) OR ○ Photo C (1-1/2 cups or more)
Tomatoes	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 2 slices or wedges or 7 cherry tomatoes or less OR ○ 4 slices or 1/2 medium tomato OR ○ 1 medium tomato or more
Coleslaw	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/4 cup or less OR ○ 1/2 cup OR ○ 1 cup or more
Low-Calorie or Diet Dressings Added to Salads	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 2 teaspoons or less OR ○ 1 Tablespoon OR ○ 2 Tablespoons or more
Regular Salad Dressings or Mayonnaise Added to Salads	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 2 teaspoons or less OR ○ 1 Tablespoon OR ○ 2 Tablespoons or more
Eggs, Cooked or Raw (including egg nolik)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 egg OR ○ 1 egg or 1 sandwich OR ○ 2 eggs or more
Tofu, Plain or in Salads (soybean curd)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 2 cubes or 1/4 cup OR ○ 1/4 block or 1/2 cup OR ○ 1/2 block or more
Fried Tofu (such tau foo pok, plain or stuffed)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup OR ○ 1 cup OR ○ 1-1/2 cups
Vegetarian Meat Loaf, Meatballs or Patties	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 inch/1 meatball OR ○ 1 patty or slice or 3 meatballs OR ○ 1 thin patty, 1 meatball or less

For EACH FOOD GROUP, fill in the circle ___ that best describes HOW OFTEN you ate these items *ABOUT 2 YEARS AGO*. Then fill in the circle ___ that best describes your USUAL SERVING SIZE.

| A | B | C |

RAW OR COOKED VEGETABLES (NOT IN SOUPS OR MIXED DISHES):	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Broccoli (raw or cooked)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Cabbage (such as head, Chinese or Napa cabbage, Brussels sprouts)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Dark Leafy Greens (such as spinach, collard, mustard or turnip greens, bok choy, escarole, chard)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Green Beans or Peas	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Other Green Vegetables (such as zucchini, celery, asparagus, green pepper, okra)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Cauliflower	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Carrots (raw or cooked)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (4 sticks or less) **OR** ○ Photo B (3 cup or 1 raw) **OR** ○ Photo C (1 cup or more)
Corn (fresh, frozen, or canned)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (1/2 cup or 1 cob) **OR** ○ Photo C (1 cup or more)
Pumpkin or Yellow-Orange Winter Squash	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)
Other Vegetables (such as okra or summer squash, beets, eggplant)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) **OR** ○ Photo B (about 1/2 cup) **OR** ○ Photo C (1 cup or more)

For EACH FOOD GROUP, fill in the circle ⬤ that best describes HOW OFTEN you ate those items *ABOUT 2 YEARS AGO*. Then fill in the circle ⬤ that best describes your *USUAL SERVING SIZE*.

AVERAGE USE ABOUT 2 YEARS AGO

DRIED BEANS (NOT IN SOUPS OR MIXED DISHES)	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	YOUR USUAL SERVING SIZE
Refried Beans (not in burritos or tostadas)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) OR ○ Photo B (about 1/2 cup) OR ○ Photo C (1 cup or more)
Baked Beans or Pork and Beans	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) OR ○ Photo B (about 1/2 cup) OR ○ Photo C (1 cup or more)
Boiled Dried Beans or Peas (such as red, lima, pinto or soy beans, black-eyed peas, frijoles de la olla)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Photo A (1/4 cup or less) OR ○ Photo B (about 1/2 cup) OR ○ Photo C (1 cup or more)

AVERAGE USE ABOUT 2 YEARS AGO

FRUITS AND JUICES	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	YOUR USUAL SERVING SIZE
Oranges	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 orange or 1/2 cup or less OR ○ 1 orange or 1 cup OR ○ 2 oranges or more
Tangerines or Mandarin Oranges	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 tangerine or 1/2 cup or less OR ○ 2 tangerines or 1 cup OR ○ 3 tangerines or more
Grapefruit or Pomelo	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/4 cup or less OR ○ 1/2 grapefruit or 1/2 cup OR ○ 1 cup or more
Papaya	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/4 papaya or less OR ○ 1/2 papaya OR ○ 1 papaya or more
Pineapple (fresh or canned)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 slice or wedge or less OR ○ 1/2 cup or 2 slices or wedges OR ○ 1 cup or more
Peaches (fresh, canned, or dried)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 peach or less OR ○ 1 peach or 2 halves or 1/2 cup OR ○ 2 peaches or 1 cup or more
Apricots (fresh, canned, or dried)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 apricot or less OR ○ 3 apricots or 1/2 cup OR ○ 5 apricots or more
Pears (fresh, canned, or dried)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 pear or 1/2 cup OR ○ 1 pear or 1 cup OR ○ 2 pears or more

For EACH FOOD GROUP, fill in the circle ● that best describes HOW OFTEN you ate these items *ABOUT 2 YEARS AGO*. Then fill in the circle ● that best describes your USUAL SERVING SIZE.

FRUITS AND JUICES (continued)	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	3 to 4 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Apples and Applesauce	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 apple or 1/2 cup OR ○ 1 apple or 1 cup OR ○ 2 apples or more
Bananas	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 banana OR ○ 1 banana OR ○ 2 bananas or more
Cantaloupe (in season)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/4 cantaloupe or less OR ○ 1/2 cantaloupe OR ○ 1 cantaloupe or more
Watermelon (in season)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 quarter slice or 1/2 cup OR ○ 1 half slice or 1 cup OR ○ 1 whole slice or more
Mangoes (in season)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup slices OR ○ 1 medium or Pint or 1 cup OR ○ 1 large or Regular or more
Avocados and Guacamole	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 2 slices or 2 Tablespoons OR ○ 1/4 avocado or 1/4 cup OR ○ 1/2 avocado or 1/2 cup or more
Any Other Fruit (fresh, canned, or dried)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 fruit or 1 cup OR ○ 2 fruits or more
Orange or Grapefruit Juice (not orange drink) or orange soda	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Small juice glass (1/2 cup) OR ○ Large glass (8 ounces) OR ○ 12 ounce can or more
Tomato or V-8 Juice	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Small juice glass (1/2 cup) OR ○ Large glass (8 ounces) OR ○ 12 ounce can or more
Other Fruit Juices or Fruit Drinks	○	○	○	○	○	○	○	○	CHOOSE ONE ○ Small juice glass (1/2 cup) OR ○ Large glass (8 ounces) OR ○ 12 ounce can or more

BREAD ITEMS	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	3 to 4 times a week	4 to 6 times a week	Once a day	2 or more times a day	
White Bread (includes sandwich, French, sourdough, pan dulce, Portuguese sweet bread)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 slice or less OR ○ 2 slices OR ○ 3 slices or more

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For EACH FOOD GROUP, fill in the circle ● that best describes HOW OFTEN you ate these items ABOUT 2 YEARS AGO. Then fill in the circle ○ that best describes your USUAL SERVING SIZE.

AVERAGE USE ABOUT 2 YEARS AGO

BREAD ITEMS (continued)	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	YOUR USUAL SERVING SIZE
Whole Wheat or Rye Bread (includes pumpernickel, whole wheat pita bread)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 slice or less OR ○ 2 slices OR ○ 3 slices or more
Other Bread (such as mixed grain, oat bran, raisin bread)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 slice or less OR ○ 2 slices OR ○ 3 slices or more
Rolls, Buns, Biscuits, or Flour Tortillas (includes bagels, English muffins)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 item or less OR ○ 2 items or 1 bagel or English muffin OR ○ 3 items or more
Corn Tortillas	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 tortilla OR ○ 2 tortillas OR ○ 3 tortillas or more
Corn Muffins, Cornbread, or Cornbread Stuffing	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 piece cornbread or 1/2 cup stuffing OR ○ 1 muffin or 1 cup stuffing OR ○ 2 muffins or 2 pieces cornbread or more
Bran, Blueberry or Other Muffins, Banana or Mango Bread	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 regular muffin or 1 slice OR ○ 1 large muffin or 2 slices OR ○ 3 muffins or 3 slices or more
Sweet Rolls, Croissants, Doughnuts, Danish Pastry, or Coffee Cake	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 item or less OR ○ 2 items OR ○ 3 items or more
Pancakes, Waffles, or French Toast	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 item or less OR ○ 2 items OR ○ 3 items or more
Margarine Added to Bread Items	○	○	○	○	○	○	○	○	CHOOSE ONE ○ spread thin OR ○ spread thick
Butter Added to Bread Items	○	○	○	○	○	○	○	○	CHOOSE ONE ○ spread thin OR ○ spread thick
Peanut Butter Added to Bread Items	○	○	○	○	○	○	○	○	CHOOSE ONE ○ spread thin OR ○ spread thick
Jam or Jelly Added to Bread Items	○	○	○	○	○	○	○	○	CHOOSE ONE ○ spread thin OR ○ spread thick
Mayonnaise in Sandwiches	○	○	○	○	○	○	○	○	CHOOSE ONE ○ spread thin OR ○ spread thick

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BREAKFAST CEREALS, MILK, AND CHEESE

	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or less than once per month	Once a month	2 to 3 times a month	Once a week	2 to 4 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Highly Fortified Cereals (such as Product 19, Total, Most)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or individual box OR ○ 1-1/2 cups or more
Bran or High Fiber Cereals	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or individual box OR ○ 1-1/2 cups or more
Other Cold Cereals (such as corn flakes, Cheerios, granola)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or individual box OR ○ 1-1/2 cups or more
Cooked Cereals (such as oatmeal, cream of wheat, corn grits)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or individual packet OR ○ 1-1/2 cups or more
Nonfat or Skim Milk or Buttermilk (as beverage or added to cereal)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or half pint carton OR ○ 2 cups or more
Lowfat Milk (1% or 2%) (as beverage or added to cereal - includes lactaid and acidophilus milk)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or half pint carton OR ○ 2 cups or more
Whole Milk (as beverage or added to cereal)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup or half pint carton OR ○ 2 cups or more
Yogurt (includes lowfat and nonfat)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or 4-6 oz. carton OR ○ 1 cup or 8 oz. carton OR ○ 2 cups or more
Chocolate Milk, Cocoa, or Ovaltine	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1 cup OR ○ 2 cups or more
Milkshakes or Malts	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 milkshake or malt OR ○ 1 milkshake or malt (12 oz.) OR ○ 2 milkshakes or malts
Cottage Cheese (includes farmer's and ricotta cheese)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 cup or less OR ○ 1/2 cup or 1 scoop OR ○ 1 cup or more
Lowfat Cheese (such as lowfat American, lowfat Swiss, mozzarella)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 slice OR ○ 1 slice (1 ounce) OR ○ 2 slices (2 ounces) or more
Other Cheese (such as American, cheddar, cream cheese)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 slice or 1 Tablespoon OR ○ 1 slice (1 ounce) OR ○ 2 slices (2 ounces) or more

DESSERTS AND SNACKS	Never or rarely ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	YOUR USUAL SERVING SIZE
				AVERAGE USE ABOUT 2 YEARS AGO					
Ice Cream	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 scoop (1/2 cup) or less OR ○ 2 scoops (1 cup) or 1 bar OR ○ 3 to 4 scoops (1 pint) or more
Ice Milk, Frozen Yogurt, or Sherbet	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 scoop (1/2 cup) or less OR ○ 2 scoops (1 cup) or 1 bar OR ○ 3 to 4 scoops (1 pint) or more
Cookies, Brownies, or Fruit Bars	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 2 average size cookies OR ○ 3 to 4 average or 1 extra large cookie or 1 brownie or fruit bar OR ○ 2 large cookies or 2 brownies or more
Cake	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small piece or cupcake OR ○ 1 average piece (1/12 of cake) OR ○ 2 pieces or more
Apple or Other Fruit Pies, Tarts, Cobblers, or Turnovers	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small piece OR ○ 1 piece (1/8 pie) or 1 item OR ○ 1/4 pie or more
Pumpkin, Sweet Potato, or Carrot Pies	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small piece OR ○ 1 average piece (1/8 pie) OR ○ 1/4 pie or more
Cream or Custard Pies, Eclairs, or Cream Puffs	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small piece OR ○ 1 average piece or 1 item OR ○ 1/4 pie or more
Puddings or Custards (includes flan)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 small size or 1/2 cup OR ○ 2 small size or 1 cup OR ○ 3 small size or 1 1/2 cups
Chocolate Candy	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 3 pieces OR ○ 1 regular size bar OR ○ 1 giant size bar or more
Dim Sum, such as Bao or Momplus (Chinese bun with meat and vegetables)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 bao or less OR ○ 1 bao OR ○ 2 bao or more
Other Dim Sum (such as pork balls, gau gee, fried won ton, eggroll)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 2 pieces OR ○ 3 to 4 pieces OR ○ 5 pieces or more
Crackers and Pretzels (such as soda, graham, saltines, rice crackers, wheat thins)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 4 to 5 small or 1 large cracker OR ○ 6 to 10 small or 2 large crackers OR ○ 1 large crackers or more
Peanuts or Other Nuts	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 buds or less OR ○ 1/4 cup OR ○ 1/2 cup or more

For EACH FOOD GROUP, fill in the circle ○ that best describes HOW OFTEN you ate those items *ABOUT 2 YEARS AGO*. Then fill in the circle ○ that best describes your USUAL SERVING SIZE.

SNACKS (continued)	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Potato, Corn, Tortilla or Other Chips, or Crackers (pork rinds)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 snack bag or 1/2 cup OR ○ 1 package or 1 cup OR ○ 1/2 twin pack or more
Popcorn	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 to 3 cups or less OR ○ 1 microwave bag OR ○ 1 medium theater size or more

ALCOHOLIC AND OTHER BEVERAGES	AVERAGE USE ABOUT 2 YEARS AGO								YOUR USUAL SERVING SIZE
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Light Beer	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 can or bottle or less OR ○ 2 cans or bottles OR ○ 3 cans or bottles OR ○ 4 cans or bottles or more
Regular or Draft Beer	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 can or bottle or less OR ○ 2 cans or bottles OR ○ 3 cans or bottles OR ○ 4 cans or bottles or more
White or Pink Wine (includes champagne and wine)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 glass or less OR ○ 2 glasses OR ○ 3 glasses OR ○ 4 glasses or more
Red Wine	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 glass or less OR ○ 2 glasses OR ○ 3 glasses OR ○ 4 glasses or more
Hard Liquor (such as bourbon, scotch, gin, vodka, tequila, rum, cocktails)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1 drink or less OR ○ 2 drinks OR ○ 3 drinks OR ○ 4 drinks or more
Diet Sodas (such as Diet Coke, Diet Pepsi, Diet 7-Up)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 can or small glass OR ○ 1 can or large glass OR ○ 2 cans or glasses OR ○ 3 cans or glasses or more
Regular Sodas (such as Coca-Cola, Pepsi, 7-Up)	○	○	○	○	○	○	○	○	CHOOSE ONE ○ 1/2 can or small glass OR ○ 1 can or large glass OR ○ 2 cans or glasses OR ○ 3 cans or glasses or more

OTHER BEVERAGES	AVERAGE USE ABOUT 2 YEARS AGO									WHAT DID YOU USUALLY ADD?
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 4 times a week	4 to 6 times a week	Once a day	2 to 3 times a day	4 or more times a day	
Cappuccino - 1 cup or mug (includes café au lait, caffe latte, café con leche)	○	○	○	○	○	○	○	○	○	MARK ALL THAT APPLY ○ Sugar or honey ○ Sugar substitute
Regular Coffee - 1 cup or mug (brewed or instant)	○	○	○	○	○	○	○	○	○	MARK ALL THAT APPLY ○ Cream or half & half ○ Milk ○ Non-dairy cream ○ Sugar or honey ○ Sugar substitute
Decaffeinated ("Decaf") Coffee - 1 cup or mug (brewed or instant)	○	○	○	○	○	○	○	○	○	MARK ALL THAT APPLY ○ Cream or half & half ○ Milk ○ Non-dairy cream ○ Sugar or honey ○ Sugar substitute
Black Tea - 1 cup or glass (such as Lipton's, oolong, iced tea)	○	○	○	○	○	○	○	○	○	MARK ALL THAT APPLY ○ Cream or half & half ○ Milk ○ Non-dairy cream ○ Sugar or honey ○ Sugar substitute
Green Tea - 1 cup	○	○	○	○	○	○	○	○	○	
Fortified Diet Beverages 1 glass or can (such as Slimfast)	○	○	○	○	○	○	○			

HOW OFTEN DID YOU EAT THE FOLLOWING ITEMS?	AVERAGE USE ABOUT 2 YEARS AGO								
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
Western Pickles or Relish (such as dill or sweet pickles)	○	○	○	○	○	○	○	○	
Olives	○	○	○	○	○	○	○	○	
Salsa or Hot Chili Peppers (red or green)	○	○	○	○	○	○	○	○	
Garlic	○	○	○	○	○	○	○	○	
Onions	○	○	○	○	○	○	○	○	
Oriental Salted or Pickled Vegetables (such as salted cabbage or leafy greens, takuwan, kim chee)	○	○	○	○	○	○	○	○	
Seaweed (fresh or dried) (such as ogo, nori, furikake)	○	○	○	○	○	○	○	○	
Gravy on meat, potatoes, rice	○	○	○	○	○	○	○	○	

HOW OFTEN DID YOU ADD THE FOLLOWING ITEMS TO YOUR FOODS AT THE TABLE...	AVERAGE USE ABOUT 2 YEARS AGO							
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day
Salt	○	○	○	○	○	○	○	○
Shoyu (Soy Sauce) or Teriyaki Sauce	○	○	○	○	○	○	○	○
Mustard	○	○	○	○	○	○	○	○
Catsup	○	○	○	○	○	○	○	○
Sour Cream	○	○	○	○	○	○	○	○

HOW OFTEN DID YOU EAT RED MEAT THAT HAD BEEN MARINATED IN...	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day
Barbecue Sauce	○	○	○	○	○	○	○	○
Teriyaki Sauce or Shoyu (Soy Sauce)	○	○	○	○	○	○	○	○

HOW OFTEN DID YOU EAT CHICKEN THAT HAD BEEN MARINATED IN...	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day
Barbecue Sauce	○	○	○	○	○	○	○	○
Teriyaki Sauce or Shoyu (Soy Sauce)	○	○	○	○	○	○	○	○

HOW OFTEN DID YOU EAT MEAT, CHICKEN, OR FISH COOKED WITH...	AVERAGE USE ABOUT 2 YEARS AGO							
	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day
Vegetable Oil	○	○	○	○	○	○	○	○
Salt Pork, Lard, or Bacon Fat	○	○	○	○	○	○	○	○
Vegetable Shortening (such as Crisco)	○	○	○	○	○	○	○	○
Margarine	○	○	○	○	○	○	○	○
Butter	○	○	○	○	○	○	○	○

ANSWER THE FOLLOWING FOR THE TIME PERIOD ABOUT 2 YEARS AGO:

WHEN YOU ATE RED MEAT, HOW WAS IT USUALLY PREPARED? (mark only one)
- ○ Rare
- ○ Medium
- ○ Well-done
- ○ Don't eat meat

WHEN YOU ATE RED MEAT, DID YOU EAT THE FAT? (mark only one)
- ○ Most of the time
- ○ Some of the time
- ○ Never or hardly ever
- ○ Don't eat meat

WHEN YOU ATE CHICKEN, DID YOU EAT THE SKIN? (mark only one)
- ○ Most of the time
- ○ Some of the time
- ○ Never or hardly ever
- ○ Don't eat chicken

WHAT KIND OF MARGARINE DID YOU USUALLY USE? (mark only one)
- ○ Regular Stick **OR**
- ○ Regular Tub **OR**
- ○ Diet or Spread **OR**
- ○ Don't use margarine
- ○ Don't know

WHAT KIND OF BUTTER DID YOU USUALLY USE? (mark only one)
- ○ Regular **OR**
- ○ Whipped **OR**
- ○ Don't use butter
- ○ Don't know

WHAT KIND OF VEGETABLE OIL DID YOU USUALLY USE? (mark only one)
- ○ Soybean or corn oil **OR**
- ○ Olive oil **OR**
- ○ Canola oil **OR**
- ○ Any other oil
- ○ Don't use oil   ○ Don't know

For EACH FOOD, fill in the circle ⬤ that describes HOW OFTEN you ate ABOUT 2 YEARS AGO meats and fish that were pan-fried, oven-broiled, and grilled or barbecued, and fill in the circle ⬤ that describes HOW BROWN it usually was on the outside.

COOKING METHOD	FOOD ITEM	AVERAGE USE ABOUT 2 YEARS AGO								HOW BROWN WAS THE OUTSIDE?
		Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	
PAN-FRIED (cooked in a pre-heated frying pan or griddle)	Beef Steak	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Hamburger (Not Fast Food)	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Chicken	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Sausage	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Spam or Ham	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Bacon	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Fish	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
OVEN-BROILED (cooked at the "broil" setting)	Beef Steak	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Hamburger (Not Fast Food)	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Shortribs or Spareribs	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Chicken	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Fish	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
GRILLED OR BARBECUED (cooked over charcoal or on an electric or gas grill)	Beef Steak	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Hamburger (Not Fast Food)	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Shortribs or Spareribs	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Chicken	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Sausage	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown
	Fish	○	○	○	○	○	○	○	○	○ light brown / ○ medium brown / ○ dark brown

VITAMINS AND MINERALS

DID YOU TAKE ANY OF THE FOLLOWING VITAMINS OR MINERALS WITH MINERALS ABOUT 2 YEARS AGO (at least once a week)?

		IF YES, HOW MANY TABLETS DID YOU TAKE?	IF YES, HOW MANY YEARS DID YOU TAKE THEM?
STRESS-TABS TYPE	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more
THERAPEUTIC, THERAGRAN TYPE	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more
ONE-A-DAY TYPE	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more

DID YOU TAKE ANY OF THE FOLLOWING VITAMINS OR MINERALS BY ITSELF ABOUT 2 YEARS AGO (at least once a week)?

		IF YES, HOW MANY TABLETS DID YOU TAKE?	IF YES, HOW MANY YEARS DID YOU TAKE THEM?	IF YES, WHAT WAS THE DOSE PER TABLET?
VITAMIN A (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 5,000 I.U. International units or less ○ 8,000 to 15,000 I.U. ○ 11,000 to 24,000 I.U. ○ 25,000 I.U. or more ○ Don't know
VITAMIN C (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 250 mg (milligrams) or less ○ 300 to 500 mg ○ 500 to 4,000 mg ○ 5,000 to 9,500 mg ○ 10,000 mg. or more ○ Don't know
VITAMIN E (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 200 I.U. International units or less ○ 250 to 600 I.U. ○ 405 to 1,000 I.U. ○ 1,250 I.U. or more ○ Don't know
FOLATE OR FOLIC ACID (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 250 mcg (micrograms) or less ○ 400 to 600 mcg ○ 700 to 850 mcg ○ 1,000 mcg or more ○ Don't know
CALCIUM (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 250 mg (milligrams) or less ○ 300 to 600 mg ○ 625 to 1,000 mg ○ 1,250 mg. or more ○ Don't know
SELENIUM (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 75 mcg (micrograms) or less ○ 100 to 150 mcg ○ 200 to 225 mcg ○ 250 mcg. or more ○ Don't know
IRON (BY ITSELF)	○ No ○ Yes	○ 1 to 3 a week ○ 4 to 6 a week ○ 1 a day ○ 2 a day ○ 3 or more a day	○ 1 year or less ○ 2 to 4 years ○ 5 years or more	○ 50 mg (milligrams) or less ○ 51 to 150 mg ○ 151 mg. or more ○ Don't know