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Dietary Phytoestrogen Intake Is Associated with Reduced Colorectal Cancer Risk[1]

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Abstract

Evidence suggests dietary phytoestrogens may reduce the risk of certain hormonal cancers (e.g. breast and prostate). There is a paucity of data regarding phytoestrogens and colorectal cancer risk. Phytoestrogens are plant compounds with estrogen-like activities. Main classes include isoflavones (found in legumes such as soy) and lignans (found in grains, seeds, nuts, fruits, and vegetables). Although isoflavones have dominated phytoestrogen cancer research, lignans may be more relevant to North American diets. Food questionnaires and analytic databases have recently been modified to incorporate some lignan information. We conducted a case-control study to evaluate the association between phytoestrogen intake and colorectal cancer risk. Colorectal cancer cases were diagnosed in 1997–2000, aged 20–74 y, identified through the population-based Ontario Cancer Registry, and recruited by the Ontario Familial Colorectal Cancer Registry. Controls were a sex and age-group matched random sample of the population of Ontario. Epidemiologic and food frequency questionnaires were completed by 1095 cases and 1890 control subjects. Multivariate logistic regression analysis was used to obtain adjusted odds ratio (OR) estimates. Dietary lignan intake was associated with a significant reduction in colorectal cancer risk [OR (T3 vs. T1) = 0.73; 95% CI: 0.56, 0.94], as was isoflavone intake [OR (T3 vs. T1) = 0.71; 95% CI: 0.58, 0.86]. We evaluated interactions between polymorphic genes that encode enzymes possibly involved in metabolism of phytoestrogens (CYPs, catechol *O*-methyl transferase, GSTs, and UGTs) and found no significant effect modification with respect to phytoestrogen intake. This finding that phytoestrogen intake may reduce colorectal cancer risk is important, because dietary intake is potentially modifiable.

Introduction

Colorectal cancer is the third most common cancer and the second leading cause of cancer death in Canada (1,2). Despite improvements in surgical and chemotherapeutic treatments, colorectal cancer has a poor 5-y survival rate of 60% (2). Identifying modifiable factors associated with colorectal cancer is of importance, the ultimate goal being primary prevention.

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Phytoestrogens, contained in plant foods, have nonsteroidal estrogen-like activities (3,4). The most important dietary sources include isoflavones (e.g. soy products) and lignans (e.g. flaxseed, grains, nuts, vegetables, fruits) (5). Isoflavones have dominated phytoestrogen research, because there is convincing in vitro evidence of their cancer inhibitory effects and soy foods are a major dietary component in Asia where hormone-related cancers are less prevalent. However, lignans may be more relevant in North America as they are plentiful in Western diets (6-8); lignan-containing foods include legumes, seeds, cereals/grains, berries, dried fruit, and vegetables (9). There is epidemiologic evidence that phytoestrogens may protect against the development of hormone-dependent cancers (e.g. breast and prostate) (10-13), and it has been suggested that this may extend to colorectal cancer (13-19). The development of colorectal cancer is thought to be influenced by estrogen exposure; for example, hormone-replacement therapy (HRT)[7] halves the risk of colorectal cancer among women (20,21). Phytoestrogens may act through hormonal mechanisms to reduce cancer risk by binding to estrogen receptors (ER) (22) or interacting with enzymes involved in sex steroid biosynthesis and metabolism (23).

Although epidemiologic findings are inconsistent, several studies have reported lowered colorectal cancer risk associated with the consumption of soy foods, although findings vary by type of soy food, colorectal cancer sub-site, and sex (13,15,17-19,24-28). Most studies were conducted in Asia, were small, and did not assess total phytoestrogen intake but only individual soy food consumption (proxy for isoflavone intake). The only population-based study conducted in North America (Hawaii) reported no association between tofu and colorectal cancer risk and found “legumes and soy products” intake was associated with a reduction in colorectal cancer risk (only significant for women) (18). Recent development of comprehensive phytoestrogen databases (6,7,9) now permits a more thorough evaluation of intake (including lignans) among North Americans.

Both genetic and environmental factors are involved in the development of colorectal cancer. Some 20% of colorectal cancers display a familial component whereby relatives exhibit a doubling of risk (29). Less than 5% of colorectal cancer cases are explained by known genetic syndromes (30,31); thus, common inherited polymorphisms may be of greater public health importance. Genetic variation exists within genes that code enzymes possibly involved in phytoestrogen metabolism [e.g. CYP1A1/1A2/1B1,2E1, catechol O-methyl transferase (COMT), GSTs, UGTs] (32-38) and these genetic variants may alter phytoestrogen metabolism and consequently modify any phytoestrogen and cancer risk association. Although the metabolism of phytoestrogens is not well understood, both intestinal bacteria and host metabolic enzymes are known to be involved; in particular, enzymes important in the metabolism of estrogen as their substrates are structurally similar (32-38).

We evaluated the association between dietary phytoestrogen intake (isoflavones, lignans, and total phytoestrogens) and colorectal cancer risk among subjects and control subjects participating in the population-based Ontario Familial Colorectal Cancer Registry (OFCCR). In addition, possible effect modification of this association by genetic polymorphisms in enzymes possibly involved in the metabolism of phytoestrogens was assessed.

Study Design and Methods

The OFCCR is 1 of 6 international sites participating in the Cooperative Familial Registry for Colorectal Studies established by the U.S. National Cancer Institute. The methods of the

[7]Abbreviations used: COMT, catechol *O*-methyl transferase; ER, estrogen receptors; HNPCC, hereditary nonpolyposis colorectal cancer; HRT, hormone-replacement therapy; NSAID, nonsteroidal antiinflammatory drug; OCR, Ontario Cancer Registry; OFCCR, Ontario Familial Colorectal Cancer Registry; OR, odds ratio.

OFCCR have been described previously (39,40) and are outlined below. Colorectal cancer cases and population control subjects participating in the OFCCR were used to conduct this study.

Case and control recruitment (subjects)

The population-based Ontario Cancer Registry (OCR) was used to identify and recruit into the OFCCR, living, incident colorectal cancer cases (pathology confirmed; International Classification of Diseases 9th revision codes 153.0–153.9, 154.1–154.3, 154.8) (41) aged 20–74 y and diagnosed between July 1, 1997 and June 30, 2000. The OCR registers all cases of invasive cancer diagnosed among residents of Ontario using computerized probabilistic record linkage to resolve the 4 main sources of cancer information (i.e. pathology reports with any mention of cancer, hospital discharge summaries that include a diagnosis of cancer, reports from Ontario's regional cancer centers, and death certificates).

Control subjects recruited by the OFCCR were comprised of a random sample of Ontario residents identified using 2 methods with the same eligibility criteria. Population-based controls were randomly selected and frequency-matched, within sex and 5-y age groups, to the colorectal cancer cases. In 1999–2000, persons were identified using a list of residential telephone numbers in Ontario provided by Infodirect (Bell Canada). 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 population-based control subjects were recruited in 2001. An age- and sex-stratified random sample of persons was selected from a listing of all Ontario residents (homeowners and occupants) based on population-based assessment rolls maintained and made available by the provincial government (database fields included name, age, sex, and address). A reabstraction study was able to link >95% of persons in the OCR to this population database, suggesting that its accuracy and completeness are high (42).

Data collection

Physicians identified from pathology reports were asked to permit contact with their patient(s) and to provide the patient address, telephone number, and vital status. Once a physician provided consent, his/her patient was mailed a package containing a letter, a brochure describing the various phases of the OFCCR, a family history questionnaire, and a return postage paid envelope. A reminder postcard was sent several weeks after this mailing and nonresponders were then followed up with a telephone call approximately 8 wk after the initial mailing. Over 90% of physicians consented, and the response rate for phase 1 of the OFCCR (completion of the family history questionnaire and agreement to complete epidemiologic questionnaires, provide blood sample, and enroll kin) was ~61% for colorectal cancer cases (39).

Following the completion and return of the family history questionnaire, pedigrees were constructed based on the family information provided. Each colorectal cancer case was then classified as *1*) high familial risk [satisfying hereditary nonpolyposis colorectal cancer (HNPCC) Amsterdam criteria] (43), *2*) intermediate familial risk, or *3*) low (sporadic) risk. Intermediate familial risk has a very broad definition and consists of cases satisfying at least 1 of the following: *1*) 2 relatives with HNPCC cancers (this includes 14 cancer sites), and 2 (of 3) are first degree kin, *2*) case and relative both with colorectal cancer <50 y of age, and *3*) any relative with colorectal cancer <35 y of age. All other cases not classified as high or intermediate familial risk were classified as sporadic (with the exception of a few cases categorized as intermediate due to selected “pathology criteria” such as multiple polyps). All high and intermediate risk cases and a 25% random sample of the low risk cases were selected

to participate in phase 2 of the OFCCR. Subjects were asked to complete a self-administered mailed epidemiologic risk factor questionnaire and FFQ, provide a blood sample, and grant permission to contact their relatives.

Control subjects were mailed a cover letter along with the family history, epidemiologic, and FFQs. They were also asked to provide a blood sample.

Epidemiologic and dietary information

Daily phytoestrogen intake was determined based on the FFQ adopted by the CFR and used by the OFCCR. It was important that all CFR sites use the same FFQ; thus, although the chosen FFQ may not be ideal for use in Canada, it was the one selected by the international CFR consortium. This 19-page FFQ, which asked about foods "eaten about two years ago," was developed by the Epidemiology Program, Cancer Research Center of Hawaii and has been previously described and validated against 24-h recalls among a multi-ethnic Hawaiian/Southern Californian population (44). FFQs were analyzed using food composition databases that include values for macro- and micronutrients, as well as isoflavones (44). In addition, food composition data for lignans (secoisolariciresinol and matairesinol) was recently added based on wet weight values reported or calculated from direct food analyses (6,7,45-51). When multiple values were reported in the literature, averages were calculated; when values were given in ranges, midpoint values were used. Values for foods that could not be directly matched to those in the literature were imputed based on those for similar foods defined by their fiber content and botanical family. The 3 daily phytoestrogen intake variables (isoflavone, lignan, and total) were defined based on the tertile distribution in the controls.

The 32-page epidemiologic questionnaire included many close-ended questions about colorectal screening, medical conditions, medication use, diet, reproductive factors, physical activity, sociodemographics, and anthropometric measures.

Response rates/numbers

The 1536 incident colorectal cancer cases participating in phase 2 of the OFCCR were mailed questionnaires and asked to provide blood. A total of 1138 (74%) completed the FFQ and 1124 (73%) completed the epidemiologic questionnaire. The 1095 cases completed both the epidemiologic questionnaire and FFQ and are included in this data analysis. The OFCCR classified cases based on their familial cancer history. Of the 1095 cases in this dietary analysis, 42 (4%) were high (HNPCC) risk, 483 (44%) were intermediate familial risk (defined above), and 570 (52%) were low risk.

Of the 4876 eligible controls identified and invited to participate, 2131 refused (43%), and of the 2745 mailed the questionnaire package, 1928 (70%) completed the FFQ, and 1944 (71%) completed the epidemiology questionnaire. The 1890 control subjects completed both the epidemiologic questionnaire and FFQ and are included in this data analysis. Reasons for nonparticipation included language barrier, illness, too busy, and questionnaire too long; however, the majority of cases and controls did not provide a reason. Ninety-five percent of participants in the OFCCR are Caucasian.

Persons (n = 83) who reported extreme energy intakes (females: <700 or >4200 kcal and males: <800 or >4900 kcal)[8] were considered outliers and removed from data analysis. Of the 1095 cases and 1890 controls that completed both questionnaires, 842 cases and 1251 controls provided a blood (DNA) sample, and these persons comprised the dataset used to evaluate gene-environment interactions.

[8] 1 kcal = 4.184 kJ.

DNA preparation and genotyping

The OFCCR obtained 40 mL of blood from participating cases and controls. DNA was extracted from lymphocytes using organic solvents or spin columns (Qiagen) and banked at 4°C.

Genetic variants were chosen for investigation based on a minor allele frequency of ≥5% with a preference given to polymorphisms with a potential impact on function. Genotyping assays are standard assays from the literature. Assays to query the single nucleotide polymorphism of interest were performed using the TaqMan 5′ nuclease allele discrimination assay (Applied Biosystems). In general, an allele-specific oligonucleotide probe, labeled with a fluorescent reporter and quencher dye, is cleaved during the amplification process, generating an increased intensity of fluorescence directly related to the accumulation of PCR product. The reaction mix consisted of 5 μL Taqman Universal Master mix-no UNG (Applied Biosystems), combined primer, and probe mix (per manufacturer's instructions), 20–50 ng of DNA template, and water for a total reaction volume of 10 μL. Cycling conditions for the reaction were 95°C for 10 min, followed by 40–45 cycles of 94°C for 15 s and 60°C for 1 min. Following PCR amplification, end-point fluorescence was read using an ABI 7900HT Sequence Detection system and genotypes assigned using Allelic Discrimination software (Applied Biosystems SDS Software v2.1). Appropriate controls representative of each genotype and multiple template controls were included in each analysis. We used microsatellite fragment analysis to genotype UGT1A1*28. Briefly, PCR was performed on 50 ng of DNA in buffer [100 mmol/L Tris-HCl (pH 8.0), 500 mmol/L KCl, 1.5 mmol/L MgCl2, 0.2 mmol/L dNTP, 0.2 umol/L of each primer, and 1 unit of Taq Polymerase (Applied Biosystems)]. Cycling conditions were: initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, with a 15-min final extension at 72°C. Microsatellite fragment analysis was performed using the ABI 3730 DNA analyzer and Genemapper 3.5 software (Applied Biosystems).

Approximately 5% of the samples were selected for duplicate analysis (verification), with an estimated genotyping error rate of 0.25%.

Statistical data analysis

Associations between each phytoestrogen variable (lignan, isoflavone, and total) and colorectal cancer risk were examined by computing age-adjusted odds ratio (OR) estimates and approximate 95% confidence intervals (52). Multivariate logistic regression analyses were performed to obtain OR estimates while simultaneously controlling for potential confounders (53). For each analysis, potential confounding variables were evaluated based on the 10% change in OR estimate methods (54). Potential confounders were evaluated and only true confounders, plus age and sex, remained in the final multivariate model(s). Potential confounders included: family history of colorectal cancer, prior diagnosis of inflammatory bowel disease, BMI, colonic screening, intake of nonsteroidal antiinflammatory drugs (NSAIDs), folate, calcium, vegetables, fruits, red meat, dietary fiber, saturated fat, total energy (calories), alcohol consumption, smoking history, and lifetime physical activity. Only dietary fiber and energy intake were identified as true confounders in our dataset, and these variables remained in the relevant final multivariate model(s). All statistical analysis was done using SAS v8.2 (SAS Institute).

The possibility of interactions between selected genetic variants (CYP1A2–163C > A, rs762551; CYP2E1 1293G > C, rs3813867; CYP2E1 7632T > A, rs6413432; COMT 472G > A, rs4680; CYP1A1 2455A > G, rs1048943; CYP1A1 3801T > C, rs4646903; CYP1B1 142C > G, rs10012; CYP1B1 4326C > G, rs1056836; CYP1B1 4390A > G, rs1800440; GSTM1-locus deletion; GSTT1-locus deletion; GSTM1 K173N, rs1065411; GSTM3delAGG,

rs1799735; UGT1A7 W208R, rs11692021; UGT1A7 N129K, rs17868323; UGT1A1*28 A (TA)6TAA > A(TA)7TAA) and phytoestrogen intake was initially evaluated by stratified analyses, where summary OR estimates were computed as appropriate (54). Interaction was formally assessed by the significance of the Likelihood Ratio Statistic ($P < 0.05$) after the addition of the product term to the model (55).

A test for trend consisted of entering the ordinal version of the exposure variable into a logistic regression model as a continuous variable. The chi-square statistic from the Wald test for estimated coefficient was used to test the hypothesis that the slope of the effect was equal to 0.

Ethics approval

Ethics approval was granted from the Office of Research Services, University of Toronto, the Mount Sinai Hospital Research Ethics Board, and the Hospital for Sick Children Research Ethics Board.

Results

The distribution of colorectal cancer cases and controls by selected subject characteristics was recently published in greater detail (Table 1) (40). As expected, family history of colorectal cancer, increased BMI, greater red meat intake, and higher energy intake were considerably more common among the colorectal cases than the control subjects. NSAID use was associated with a decreased risk of colorectal cancer and no association was observed regarding dietary fiber intake and colorectal cancer risk.

We found that higher dietary lignan intake was associated with a considerable reduction in colorectal cancer risk [Table 2; MVOR (T3 vs. T1) = 0.73; 95% CI: 0.56, 0.94]. Dietary isoflavone intake was also associated with a reduction in colorectal cancer risk [MVOR (T3 vs. T1) = 0.71; 95% CI: 0.59, 0.86]. It follows that higher total phytoestrogen intake (lignans and isoflavones combined) was associated with a reduced risk of colorectal cancer [MVOR (T3 vs. T1) = 0.71; 95% CI: 0.58, 0.86].

The genotypes ($n = 16$) assessed for interaction with each of the 3 phytoestrogen variables are listed in the Methods section. None were found to be important effect modifiers of the association between phytoestrogen intake and colorectal cancer risk (data not shown). However, it should be noted that 2 variants, CYP1B1 142C > G and CYP2E1 1293G > C, were borderline significant effect modifiers (Likelihood Ratio Statistic interaction, $P = 0.06$) with the reduced colorectal cancer risk observed among persons with high lignan intake limited to those carrying the CC and GG genotypes, respectively.

The association between phytoestrogen intake and cancer risk was evaluated separately for microsatellite instability-high and microsatellite instability-low/stable colorectal cancer cases. We did not observe a significant difference; phytoestrogen intake was associated with a considerable decreased risk of colorectal cancer for both these types of tumors (as compared with controls; data not shown). Based on previous isoflavone (soy) and colorectal cancer literature, effect modification by sex and cancer sub-site (colon/rectal) was also explored. OR estimates did not differ across sex or sub-site strata (data not shown).

As a disproportionate number of cases in the OFCCR were from families classified as high/intermediate risk (vs. low risk), we assessed effect modification by familial risk. No differences across familial risk strata were identified with respect to the association between phytoestrogen intake and colorectal cancer risk (data not shown).

Discussion

Our finding that dietary phytoestrogens, both lignans and isoflavones, are associated with a reduction in colorectal cancer risk is important, because diet is potentially modifiable. Dietary intake resulting in classification within our top tertile of daily isoflavone or lignan intake is quite achievable. For example, daily isoflavone intake (>1.0 mg) could come from 1 small glass of soy milk or a small bowl of miso soup, and lignan intake (>0.26 mg) could come from 2 peaches, 1/2 slice of multigrain bread, or a pinch of flaxseed (9). To our knowledge, no previous epidemiologic study has evaluated lignan intake, the phytoestrogen most prevalent in North American diets, in relation to colorectal cancer risk (6,13,28,56,57). Epidemiologic studies have assessed isoflavone intake and cancer risk, with some reporting that increased soy product intake is associated with reduced colorectal cancer risk (17-19,24-27).

Several epidemiologic studies conducted in Asia (19,24,58,59) and 1 conducted in Hawaii (18) evaluated the association between individual soy foods (main source of isoflavones) and colorectal cancer risk. Although some of these studies reported reduced colorectal cancer risk, particularly for nonfermented soy foods (e.g. tofu) (19,24), lack of an association between tofu intake and colorectal cancer risk was also reported (26,27), and occasionally findings differed by colorectal cancer sub-site, type of soy food, and sex (25). The only case-control study in North America found that legumes and soy products (analyzed as 1 group) were associated with a reduction in colorectal cancer risk; however, tofu alone showed no association (18).

The main limitations of previous studies were small sample size and the assessment of specific soy food intake rather than total phytoestrogen intake. None of these studies were designed to evaluate phytoestrogen intake; thus, evidence of an association is indirect. Findings from animal studies suggest phytoestrogens are associated with a reduction in colorectal cancer (13,60-62). To our knowledge, our study is the first epidemiologic study to examine the association between colorectal cancer and lignans common in Western diets; recently available food composition data for lignans (secoisolariciresinol and matairesinol) were added to the analytic database applied to the FFQ.

Only recently have researchers modified FFQs and analytic databases such that total phytoestrogen (both isoflavone and lignan) intake can be measured in epidemiologic studies (6,7,9,50,63). Historically, the lignan content of foods was not included in standard nutrient databases. Biomarkers of phytoestrogen intake have also been examined, and although promising (64-67), have limited usefulness in a case-control studies where biologic specimens are obtained postdiagnosis.

It is thought that phytoestrogens may act via: *1*) hormonal effects mediated by ER binding; *2*) nonhormonal actions by altering processes involved in carcinogenesis such as apoptosis and antioxidant activity; or *3*) interaction with enzymes involved in sex steroid biosynthesis and metabolism (23,33,68). Isoflavones may alter CYP(1A1,1A2,1B1)-mediated estradiol metabolism by reducing formation of carcinogenic hydroxylated metabolites (33) while increasing less reactive 2-OH estrone and 16α-OH estrone metabolites (69). Also, phytoestrogens may inhibit CYP-dependent estrogen metabolism by acting as competitive substrates, or they may reduce circulating levels of estradiol by induction of CYP enzymes (70,71).

Much is known about the protective association between estrogens and colorectal cancer risk. Epidemiologic studies and trials consistently report a significant reduction in colorectal cancer risk among women who used HRT (20,21,72-74). It has been suggested that estrogens may influence colorectal cancer risk by modification of lipids and bile acids thought to be involved in carcinogenesis (74,75) or by reducing the likelihood of estrogen-receptor methylation (76), because the ER gene is thought to play a tumor suppressor role (76-78). English et al. (79)

report that estrone decreases colonic cell proliferation, whereas estradiol does not, suggesting the HRT-protective effect may be due to estrone and the metabolism of estrogens may also be important (74,80).

Phytoestrogen metabolism is poorly understood (32,33), although its large interindividual variation (13,81,82) likely involves both mammalian enzymes and intestinal bacteria (33,36, 82). Phase I and II enzymes, important in metabolism of endogenous estrogens, may be important in phytoestrogen metabolism because of the structural similarities of these substrates and because they are abundant in the liver and small intestine where phytoestrogen metabolism occurs (33). Isoflavones and lignans are metabolized by CYP(1A1/1A2/1B1,2E1), COMT, GST, and UGT enzymes and distinct variants acting on the same phytoestrogens produce different metabolites with varying bioactivities (32-38,83). The role of intestinal microbes in phytoestrogen metabolism has also been demonstrated and variant bacterial species or strains may explain interindividual variation (82-86).

Far more is known about the metabolism of endogenous estrogens. It is reasonable to assume that, given the structural similarity, phytoestrogens may be metabolized similarly to estrogens. Variation in estrogen metabolism affects the level of circulating estrogen metabolites. Most pathways of estradiol and estrone metabolism involve CYP enzymes (CYP1A1, CYP1A2, CYP1B1, and CYP3A4), which carry out irreversible hydroxylation (87-89). COMT also plays an important role by converting hydroxylated estrone/estradiol into methoxy derivatives that are inactive estrogens (90). These enzymes are polymorphic, with genetic variants exhibiting varying levels of enzyme activity/inducibility (91-99).

Our evaluation of gene-environment interactions was of a hypothesis-generating nature. An improved understanding of interactions between phytoestrogen intake and genetic factors may provide insight into the mechanisms of carcinogenesis, as well as help in the development of colorectal cancer prevention strategies. Our data suggest the reduction in colorectal cancer risk associated with phytoestrogen intake is not markedly modified by polymorphisms in genes suspected of involvement in phytoestrogen metabolism (e.g. CYPs, COMT, GST, and UGTs genes).

Possible limitations of our study should be noted. Because fatal cases were excluded, survival bias may be a concern; if phytoestrogen intake affects survival, then our findings may be biased as cases with better survival are overrepresented. However, most colon cancer risk factors do not differ by stage of disease (100). As both our cases and control subjects were selected from population-based sampling frames, selection bias is unlikely. Furthermore, many known risk factors (101) were found to be associated with colorectal cancer risk in our dataset, suggesting the cases and controls are representative (40). However, response bias is always a concern if high response rates are not achieved. Although unlikely, bias could have been introduced if participation was differential regarding both case and exposure status.

Possible confounding by known colorectal cancer risk factors was evaluated, and adjusted for, in our analyses. Because dietary fiber may be a confounder of the lignan and colorectal cancer risk association, dietary fiber was controlled for in the lignan analysis. Fruit and vegetable consumption was also evaluated as potential confounders but were not identified as confounders. It is possible that phytoestrogen-rich food intake may be a marker for other factors associated with colorectal cancer risk or may be associated with the intake of other protective food components. Although potential confounders were evaluated, residual or unknown confounding remains a possibility. It should be noted that whereas the Hawaii FFQ has been validated among a multi-ethnic American population (44), it has never been validated among a Canadian population. Recall bias is always a concern in case-control studies, because cases may report exposures differently than controls; however, because information on a wealth of

factors was collected in the epidemiologic questionnaire, it would be unlikely that participants would focus on our particular study hypothesis. Furthermore, the usual concern is that cases overreport the exposure of interest, but in this study, cases had a lower intake of phytoestrogens. It is unlikely that overreporting of phytoestrogen intake (misclassification) by the control subjects is responsible for the protective effect we observed.

Phytoestrogen intake is likely underestimated for some subjects, because the FFQ did not include all phytoestrogen-containing foods. Flaxseed, which contains a high concentration of lignans, is omitted, although this is not a frequently consumed food item. Soy milk, alfalfa sprouts, and mung bean sprouts, all of which contain isoflavones, were not captured by the FFQ. Future cancer studies should expand upon the standard FFQs available such that they include all important food sources of phytoestrogens. Many of these food items (e.g. flaxseed, flax bread, soy milk, sprouts) are not typically included in the common FFQs available for epidemiologic studies.

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TABLE 1

Distribution of colorectal cancer cases, controls, and age-adjusted OR (AOR) estimates for subject characteristics and selected colorectal cancer risk factors

Variable	Cases, *n* = 1095	Controls, *n* = 1890	AOR (95% CI)
	n[1] (%)		
Age group,[2] *y*			
20–44	71 (7)	126 (7)	N/A
45–49	76 (7)	105 (6)	N/A
50–54	135 (12)	282 (15)	N/A
55–59	172 (16)	356 (19)	N/A
60–64	245 (22)	400 (21)	N/A
65–69	250 (23)	384 (20)	N/A
70–74	145 (13)	231 (12)	N/A
Sex			
Female	516 (47)	881 (47)	1.00
Male	579 (53)	1009 (53)	0.98 (0.84, 1.14)
Education			
High school	638 (59)	960 (51)	1.00
College/Bachelor	331 (31)	695 (37)	0.72 (0.61, 0.86)
Graduate	112 (10)	222 (12)	0.76 (0.60, 0.98)
NSAID use[3]			
No	668 (62)	1062 (57)	1.00
Yes	400 (38)	804 (43)	0.78 (0.67, 0.91)
BMI 2 y ago (kg/m^2)			
<25	393 (37)	769 (41)	1.00
25–29.9 (overweight)	479 (45)	771 (41)	1.23 (1.04, 1.45)
≥30 (obese)	197 (18)	323 (17)	1.21 (0.98, 1.50)
Family history of CRC in 1st degree relative[6]			
No	781 (71)	1660 (89)	1.00
Yes	312 (29)	212 (11)	3.11 (2.56, 3.78)
Red meat,[4] *servings/wk*			
0–2	307 (29)	697 (37)	1.00
2.1–3	224 (21)	370 (20)	1.37 (1.10, 1.70)
3.1–5	265 (25)	417 (22)	1.45 (1.18, 1.78)
>5	276 (26)	378 (20)	1.67 (1.36, 2.05)
Energy intake,[5] *kcal/d*			
≤1514.0 (Q1)	201 (19)	467 (25)	1.0
1514.1–1937.0 (Q2)	259 (25)	466 (25)	1.29 (1.03, 1.62)
1937.1–2435.0 (Q3)	266 (26)	466 (25)	1.35 (1.08, 1.69)
>2435.0 (Q4)	310 (30)	467 (25)	1.56 (1.25, 1.94)
Dietary fiber intake,[5] *g/d*			
≤17.0 (Q1)	240 (23)	466 (25)	1.0
17.1–23.3 (Q2)	258 (25)	468 (25)	1.09 (0.87, 1.35)
23.4–30.8 (Q3)	263 (25)	466 (25)	1.09 (0.88, 1.36)
>30.8 (Q4)	275 (27)	466 (25)	1.15 (0.93, 1.43)

[1] Numbers may not add to total due to missing values.

[2] Age at colorectal cancer diagnosis date for cases and referent date (June 30, 1999) for controls.

[3] Took aspirin or ibuprofen-based medications at least twice a week for more than a month.

[4] Intake 2 y ago (epidemiology questionnaire); quartile distribution based on controls.

[5] Intake 2 y ago (based on Hawaii FFQ); quartile distribution based on controls; 1 kcal = 4.184 kJ.

[6] CRC, colorectal cancer.

TABLE 2

Distribution of colorectal cancer cases and controls, multivariate-adjusted OR (MVOR) estimates and 95% CI for dietary phytoestrogen intake

Phytoestrogen intake	**Cases, *n* = 1095**	**Controls, *n* = 1890**	**MVOR (95% CI)**
mg/d	*n*[1] *(%)*		
Lignans[2]			
T1: 0–0.158	353 (33)	612 (33)	1.00
T2: 0.159–0.255	375 (35)	647 (35)	0.86 (0.69, 1.06)
T3: >0.255	330 (31)	598 (32)	0.73 (0.56, 0.94)
			P trend[4] *= 0.01*
Isoflavones[3]			
T1: 0–0.289	399 (39)	619 (33)	1.00
T2: 0.290–1.097	337 (33)	640 (34)	0.80 (0.67, 0.96)
T3: >1.097	300 (29)	607 (33)	0.71 (0.58, 0.86)
			P trend[4] *< 0.01*
Total phytoestrogen (lignans + isoflavones)[3]			
T1: 0–0.531	387 (38)	611 (33)	1.00
T2: 0.532–1.338	337 (33)	640 (35)	0.79 (0.65, 0.95)
T3: >1.338	301 (29)	602 (33)	0.71 (0.59, 0.86)
			P trend[4] < 0.01

[1] Numbers may not add to total due to missing values, and tertile distribution is based on the controls.

[2] MVOR adjusted for age, sex, dietary fiber, and total energy intake.

[3] MVOR adjusted for age, sex, and total energy intake.

[4] Chi-square test for trend.