*Nutrition and Cancer*, 66(1), 128–139

ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635581.2014.852686

Routledge
Taylor & Francis Group

# Cruciferous Vegetables and Risk of Colorectal Neoplasms: A Systematic Review and Meta-Analysis

**Genevieve Tse and Guy D. Eslick**

*The Whiteley-Martin Research Centre, The Discipline of Surgery, The University of Sydney, Sydney Medical School, Nepean, Penrith, New South Wales, Australia*

**Evidence shows cruciferous vegetables exhibit chemoprotective properties, commonly attributed to their rich source of isothiocyanates. However, epidemiological data examining the association between cruciferous vegetable intake and colorectal neoplasms have been inconclusive. This meta-analysis examines the epidemiological evidence to characterize the association between cruciferous vegetable intake and risk of developing colorectal neoplasms. Thirty-three articles were included in the meta-analysis after a literature search of electronic databases. Subgroup analysis for individual cruciferae types ($n = 8$ studies) and GST polymorphism ($n = 8$ studies) were performed. Pooled adjusted odds ratios (ORs) comparing highest and lowest categories of dietary pattern scores were calculated. Results show a statistically significant inverse association between cruciferous vegetable intake and colon cancer [OR = 0.84; 95% confidence interval (CI): 0.72–0.98; *P* value heterogeneity < 0.001]. Broccoli in particular exhibited protective benefits against colorectal (CRC) neoplasms (OR = 0.80; 95% CI: 0.65–0.99; *P* value heterogeneity = 0.02). Stratification by GST genotype reveals that the *GSTT1* null genotype confers a reduction in CRC risk (OR = 0.78; 95% CI: 0.64–0.95; *P* value heterogeneity = 0.32). This study provides support to the hypothesis that cruciferous vegetable intake protects against cancer of the colon. This study also demonstrates the significance of gene–diet interactions and the importance of assessing individual cruciferous vegetables.**

## INTRODUCTION

Epidemiologic studies suggest that diet is an important environmental factor that contributes to the etiology of colorectal neoplasms (1). Moreover, consumption of vegetables of the family *Cruciferae*, rather than vegetables as a group, have drawn a great deal of attention in cancer research because of their potential protective properties (2). The most commonly consumed group of cruciferous vegetables in the Western diet are vegetables of the *Brassica* genus, these include cabbage, brussel sprouts, and broccoli (3).

Like other vegetables, cruciferous vegetables contain a number of phytochemicals with potential cancer chemopreventive properties, including carotenoids, vitamin C, fiber, and flavonoids. However, what makes them unique is that they are rich sources of glucosinolates (4). The breakdown of plant cells triggers the enzymatic myrosinase activity, which hydrolyzes glucosinolates into the biologically active compound isothiocyanate (ITC). ITCs exert their protective effects via multiple mechanisms, including inhibition of carcinogen-activating enzymes, inhibition of angiogenesis, detoxification of carcinogens, induction of apoptosis, and arrest of cell cycle progression (5).

Based on our understanding, we expect to see an inverse association between cruciferous vegetable intake and the risk of developing colorectal neoplasms. However, the data accumulated to date has been weak and inconsistent. Results of large prospective cohort studies and case-control studies taking into account individual genetic variation suggest that the relationship may be complicated by genetics, in particular polymorphism of GST, a phase II conjugating enzyme (6).

GSTs are important in metabolizing ITCs, hence, the protective effect of cruciferous vegetables may predicate on GST genotype (6). *GSTM1* and *GSTT1* are the 2 variants most frequently studied (7). One hypothesis is that individuals with the null genotype of the *GSTM1* or *GSTT1* polymorphism would less readily conjugate and excrete ITCs, and hence would experience a greater protective effect against colorectal neoplasm development (8). Such effect has been observed among individuals who were null for both GSTM1 and/or GSTT1 colorectum, lung and breast. However, a recent meta-analysis showed that null genotypes conferred additional risk for colorectal cancer in Caucasian populations (9). The type of cruciferae consumed may also influence the protective outcome against developing colorectal neoplasms. Certain cruciferae, broccoli for example, contain much greater amounts of glucosinolate, exposing the individual to higher levels of ITC and therefore may provide greater anticarcinogenic benefits (1,7). Cohort studies that have examined vegetable subgroups have found protective benefits

Submitted 9 October 2012; accepted in final form 9 July 2013.

Address for correspondence Guy D. Eslick, The Whiteley-Martin Research Centre, Discipline of Surgery, The University of Sydney, Nepean Hospital, Level 5, South Block, Penrith, NSW 2751, Australia. E-mail: eslickg@med.usyd.edu.au

TABLE 1
Characteristics of studies reporting odd ratios and 95% confidence intervals for the association between total cruciferous vegetable intake and colorectal neoplasm risk

| Reference | Country | Ethnic group | Study design | Study population | Neoplasm type | Case/control size | Cohort size | Type of dietary assessment |
|---|---|---|---|---|---|---|---|---|
| Young 1988 (35) | United States | Caucasian | Case-control | Population | Colon | 353/618 | | FFQ |
| Lee 1989 (36) | Singapore | Asian | Case-control | Hospital | CRC | 101/212 | | Interview, FFQ |
| West 1989 (37) | United States | Caucasian | Case-control | Population-Women | Colon | 112/185 | | Interview |
| Peters 1992 (38) | United States | Caucasian | Case-control | Population | Colon | 746/746 | | Interview, FFQ |
| Bidoli 1992 (39) | Italy | Caucasian | Case-control | Hospital | Colon, rectal | 54/460 | | Interview |
| Steinmetz 1993 (40) | Australia | Caucasian | Case-control | Population-Men | Colon | 55/109 | | FFQ |
| Steinmetz 1994 (41) | United States | Caucasian | Cohort | Population-Women | Colon | 212 | 41837 | Questionnaire |
| Freedman 1996 (33) | United States | Caucasian | Case-control | Hospital | CRC | 41/80 | | FFQ |
| Lin 1998 (24) | United States | Caucasian | Case-control | Hospital | CR adenoma | 114/116 | | FFQ |
| Hsing 1998 (42) | United States | Caucasian | Cohort | Population-Men | CRC | 47 | 17663 | Questionnaire |
| Sellers 1998 (43) | United States | Caucasian | Cohort | Population-Women | Colon | 63 | 35216 | FFQ |
| Franceschi 1998 (39) | Italy | Caucasian | Case-control | Hospital | Colon, rectal | 1225/3763, 728,3763 | | Interview, FFQ |
| Pietinen 1999 (44) | Finland | Caucasian | Cohort | Population-Men smokers | CRC | 42 | 27111 | Questionnaire |
| Voorrips 2000 (45) | Netherlands | Caucasian | Cohort | Population | Colon | 910 | 120816 | Questionnaire |
| Michels 2000 (46) | United States | Caucasian | Cohort | Population-health workers | Colon, rectal | 1181 | 136089 | Questionnaire |
| Seow 2002 (6) | Singapore | Asian | Cohort | Population | CRC | 86/595 | | Interview, FFQ |
| Seow 2002 (47) | Singapore | Asian | Case-control | Hospital | CRC | 90/154 | | Interview, FFQ |
| Hara 2003 (48) | Japan | Asian | Case-control | Hospital | CRC | 29/70 | | FFQ |
| McCollough 2003 (49) | United States | Caucasian | Cohort | Population | Colon | 508 | 133163 | FFQ |
| Chiu 2003 (50) | China | Asian | Case-control | Population | Colon | 232/388 | | Interview, FFQ |
| Turner 2004 (51) | UK | Caucasian | Case-control | Population | CRC | 103/174 | | Interview |
| Tijhuis 2005 (3) | Netherlands | Caucasian | Case-control | Hospital | CR adenoma | 410/349 | | FFQ |
| Michels 2006 (46) | United States | Caucasian | Cohort | Population- Health workers | CR adenoma | 255/5199 | | Questionnaire |
| Hu 2007 (52) | Canada | Caucasian | Case-control | Population | Rectal | 576/1277 | | Questionnaire |
| Hu 2007 (53) | Canada | Caucasian | Case-control | Population | Colon | 196/715 | | Questionnaire |
| Moy 2008 (18) | China | Asian | Cohort | Population | CRC | 54/280 | | Interview |
| Epplein 2009 (19) | United States | Multiethnic | Cohort | Population | CRC | 134/256 | | FFQ |
| Ramadas 2009 (54) | Malaysia | Asian | Case-control | Hospital | CR adenoma | 35/44 | | FFQ |
| Yang 2010 (17) | China | Asian | Case-control | Population | CRC | 209/826 | | FFQ |

CRC = colorectal cancer; CR = colorectal; FFQ = food-frequency questionnaire.

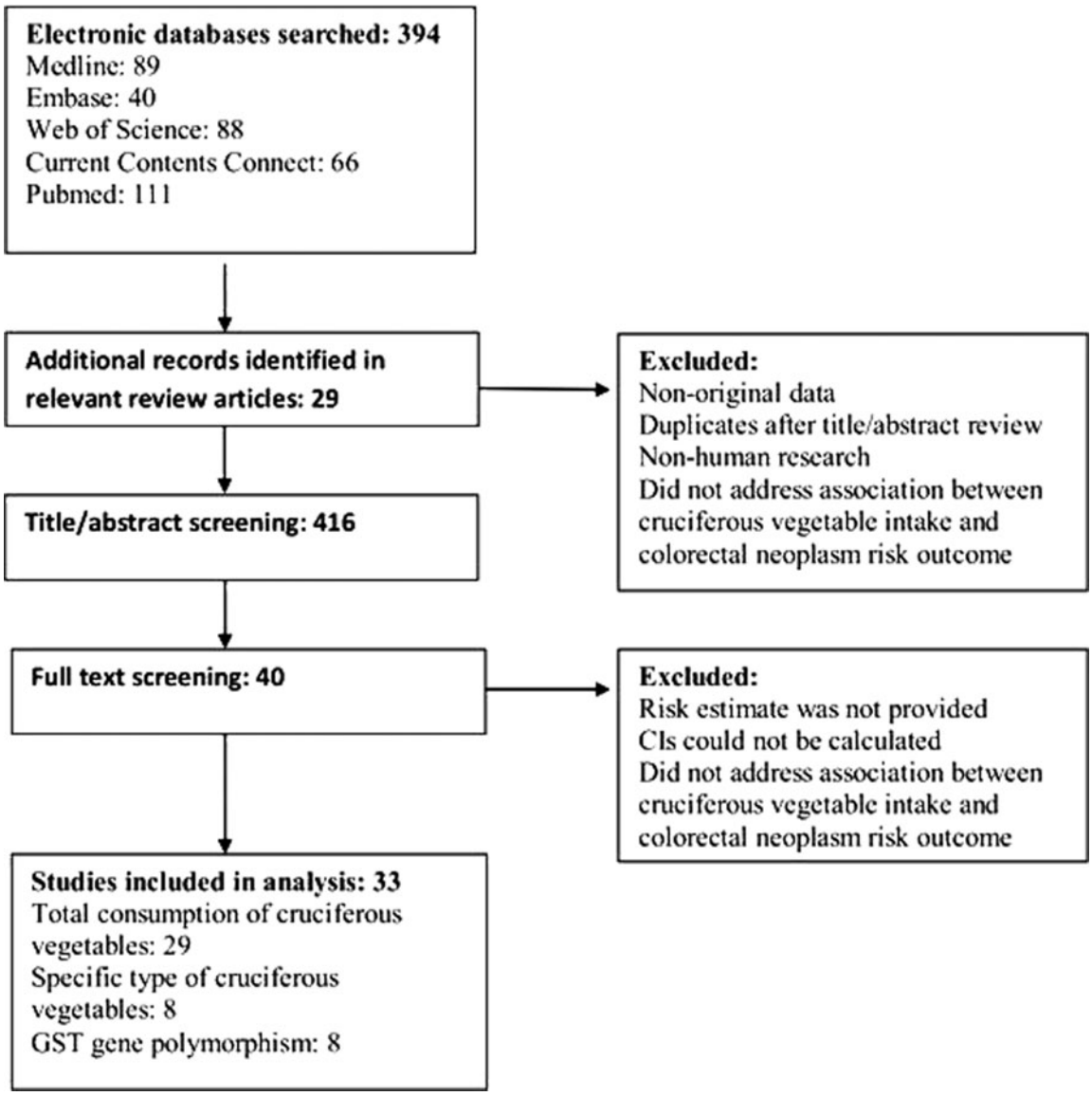


FIG. 1. Flowchart of the study selection process.

with broccoli (10). Site specific differences have also been reported when comparing the different parts of the colorectal tract. No systematic reviews however, have previously been conducted to thoroughly unify this information and assess this evidence.

This systematic review and meta-analysis aims to summarize epidemiological findings to investigate the relationship between cruciferous vegetable intake and colorectal neoplasm risk, including the influence of individual genetic variation of the *GSTM1* and *GSTT1* genes as well as the specific type of cruciferae consumed.

## METHODS

### Study Protocol

Literature searches of epidemiological studies in this systematic review were performed using the Meta-Analysis of Observational Studies in Epidemiology guidelines where possible (11). The following electronic databases were searched: Medline, PubMed, ISI Web of Science, Current Contents Connect, and Embase. The search included all studies published up to May 9, 2013. Key terms including *cruciferous vegetables* OR *Brassica vegetables* AND *colorectal neoplasm* were searched as text words and as exploded medical subject headings where possible. References in the relevant review articles from the bibliographic database search were also checked for appropriate studies. No language restrictions were used in either the search or study selection. A search for unpublished literature was not performed.

### Study Selection

The following inclusion criteria was applied in the screening of articles: 1) original data was provided; 2) the association

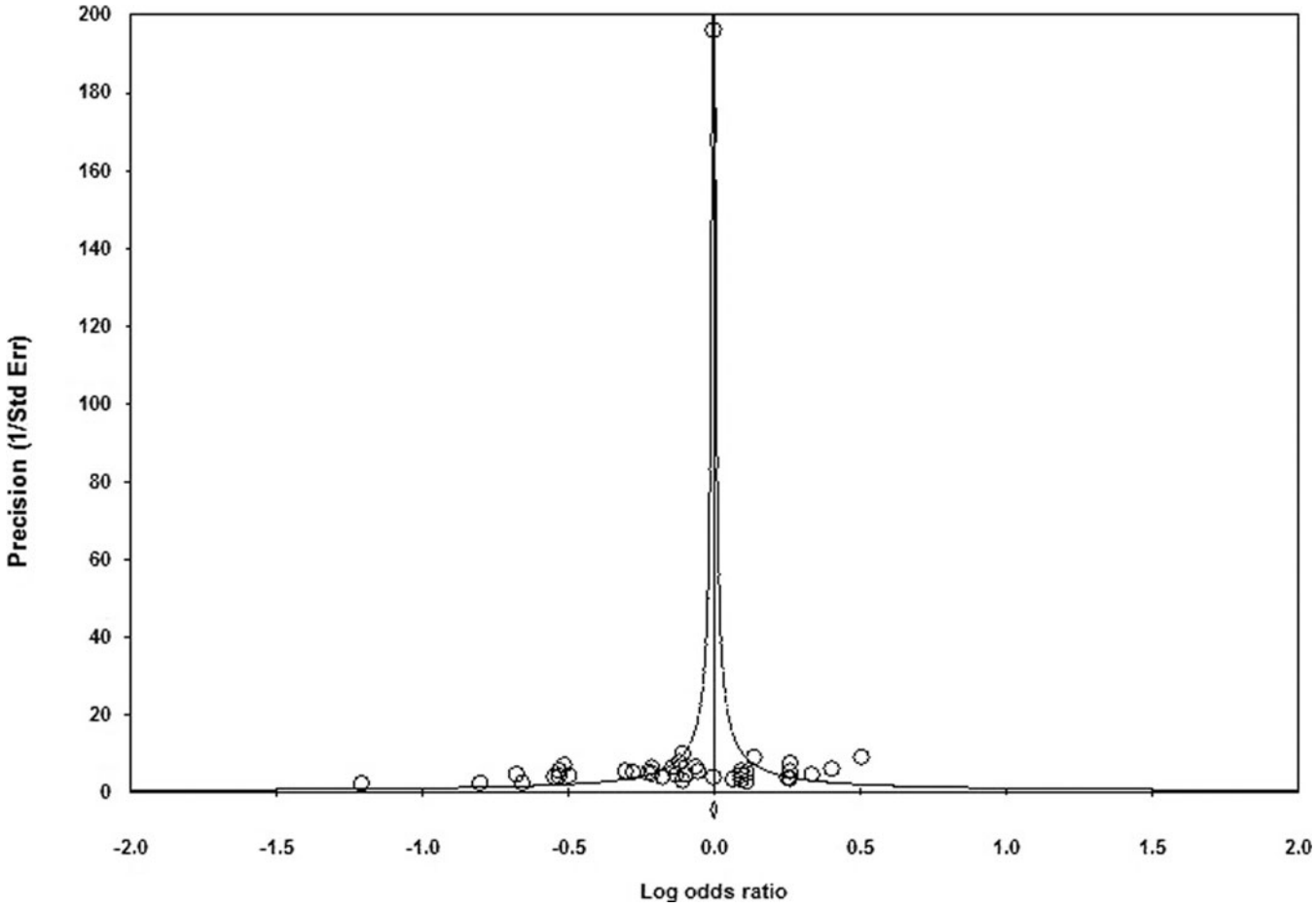


FIG. 2. Funnel plot showing studies assessed for publication bias of total cruciferous vegetable intake. Std Err = standard error.

between cruciferous vegetable intake and colorectal neoplasm risk was addressed; 3) the risk point estimate was reported as an odds ratio (OR) or relative risk (RR), or the data was presented such that an OR could be calculated; and 4) the 95% confidence interval (CI) was reported, or the data was presented such that the CI could be calculated.

## Data Extraction

Data was performed via a standardized data extraction form, collecting information on the publication year, study design, number of cases and controls, total sample size, temporal direction, population type, country, ethnicity of sample group, economic development, case control matching, mean age, response rate, exposure, neoplasm type, number of adjusted variables, the risk estimates or data used to calculate the risk estimates, CIs. Quality of the studies was not assessed and authors were not contacted for missing data. Adjusted ORs were extracted in preference to nonadjusted ORs, however, where ORs were not provided, unadjusted ORs and CIs were calculated. Where more than 1 adjusted ratio was reported, the ratio with the highest number of adjusted variables was chosen. Where multiple risk estimates were available in the same study, for example, due to the use of mutually exclusive comparator groups, they were included as separate risk estimates. Where ORs were provided in tertiles, quantiles, or quintiles, the middle tiles were included.

## Statistical Analysis

Pooled ORs and 95% CIs were calculated for the effect of cruciferous vegetable intake on the risk of colonic neoplasms using a random effects model, model of DerSimmonian and Laird (12). Heterogeneity with Cochran's $Q$ statistic was tested, with $P < 0.10$ indicating heterogeneity, and the degree of heterogeneity was quantified using the $I^2$ statistic, which represents the percentage of the total variability across studies due to heterogeneity. $I^2$ values of 25%, 50%, and 75% corresponded to low, moderate, and high degrees of heterogeneity, respectively (13). Publication bias was quantified using the Egger's regression model (14). All analyses were performed with comprehensive meta-analysis (version 2.0; Biostat, Englwood, NJ [2005]).

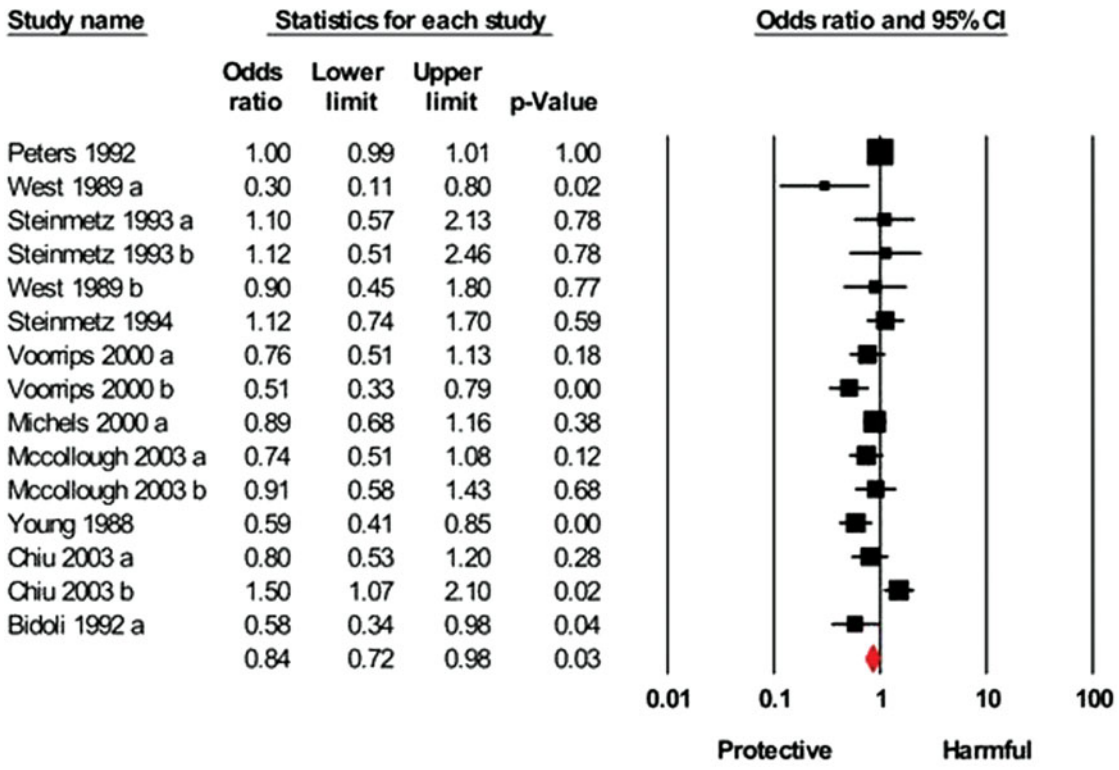


FIG. 3. Forest plot of total cruciferous vegetable consumption and colon cancer risk. CI = confidence interval (color figure available online).

## RESULTS

### Literature Search

The literature searches identified 416 articles for evaluation. Of these, 33 articles were eligible for inclusion. Exclusion reasons for the remainder included original epidemiological data on the association between cruciferous vegetable intake and colorectal neoplasm risk was not provided; risk estimates could not be obtained; CIs were not provided and could not be calculated; only abstract was available. Of the 33 studies included in the final meta-analyses, 29 studies reported on total cruciferous vegetable intake. Two subgroup analyses were developed: 8 studies reported on intake of individual cruciferous vegetables, 8 studies examined the effect of gene variant of the GST gene on cruciferous vegetable intake and colorectal neoplasm risk outcome (Fig. 1). Egger's regression analysis for assessment of publication bias of the studies included in this meta-analysis found no significant bias ($P = 0.13$) (Fig. 2).

### Association Between Total Cruciferous Intake and Colorectal Neoplasm

There were 11 prospective cohort studies and 18 case-control studies, representing a total of 5994 colorectal cancers, 5370 colon cancers, 1900 rectal cancers, and 814 colonic adenoma cases (Table 1). Only colon cancer showed a significant association with total cruciferous vegetable intake (OR = 0.84; 95% CI: 0.72–0.98; $P$ value heterogeneity $< 0.001$; $I^2 = 64.44$) (Fig. 3), with moderate heterogeneity. Additional analysis for colorectal cancer, colonic adenoma and rectal cancer all produced no significant association with total cruciferous vegetable intake (OR = 0.92; 95% CI: 0.83–1.01; $P$ value heterogeneity $< 0.001$; $I^2 = 66.16\%$; OR = 1.09; 95% CI: 0.90–1.33; $P$ value heterogeneity = 0.39; $I^2 = 0.00\%$; and OR = 0.99; 95% CI: 0.67–1.46; $P$ value heterogeneity $< 0.001$; $I^2 = 87.16\%$, respectively).

Analysis of additional variables including country, gender, ethnicity, study population, vegetable assessment method, study design and direction were conducted (Table 2). All other factors were not statistically significant.

### Results Stratified by Individual Cruciferous Vegetables

Two cohort and 6 case-control studies investigated the association between intake of individual cruciferous vegetable types and colonic tumor risk (Table 3). Data for broccoli and cabbage was provided in 7 of the studies, brussel sprouts was reported in 3 studies. Broccoli and cabbage were inversely associated with colonic tumor risk, whereas brussel sprouts showed no such association. In the data generated, the pooled ORs for CRC cancer risk comparing the highest versus lowest categories of intake were 0.80 (95% CI: 0.65–0.99; $P$ value heterogeneity = 0.02; $I^2 = 56.16\%$) for broccoli (Fig. 4), 0.95 (95% CI: 0.80–1.14; $P$ value heterogeneity = 0.34; $I^2 = 11\%$) for cabbage and 1.00

TABLE 2
Odd ratios (ORs) and 95% confidence intervals (CIs) for additional variables examined for the association between total cruciferous vegetable intake and colorectal neoplasm risk

| Factor | OR (95% CI) |
|---|---|
| Ethnicity | |
| Asian | 0.92 (0.76–1.11) |
| Caucasian | 0.93 (0.83–1.04) |
| Study design | |
| Cohort | 0.92 (0.76–1.12) |
| Case control | 0.91 (0.8–1.03) |
| Temporal direction | |
| Prospective | 0.92 (0.76–1.12) |
| Retrospective | 0.91 (0.8–1.03) |
| Population type | |
| Population | 0.87 (0.74–1.02) |
| Hospital | 0.83 (0.65–1.07) |
| Population gender | |
| Male | 0.85 (0.67–1.08) |
| Female | 1.09 (0.75–1.59) |
| Method of assessment for vegetable intake | |
| Interview | 0.66 (0.51–0.86)[a] |
| Self-administered questionnaire | 1.02 (0.79–1.32) |
| Food-frequency questionnaire | 0.95 (0.86–1.06) |
| Questionnaire | 1 (0.59–1.69) |
| Neoplasm type | |
| Colon | 0.84 (0.72–0.98)[a] |
| Rectal | 0.99 (0.67–1.46) |
| Colorectal | 0.89 (0.77–1.03) |
| Adenoma | 1.09 (0.9–1.33) |

[a]Statistically significant.

for brussel sprouts (95% CI: 0.75–1.34, *P* value heterogeneity = 0.83, $I^2 = 0\%$). Only broccoli intake showed statistically significant inverse association with colonic tumor risk.

### Results Stratified by GST Genotypes

Two prospective cohort studies and 6 case-control studies examined GST-diet interaction and thus estimates for the association between dietary ITCs and colonic tumor risk (Table 4). These 8 studies, representing 1777 colorectal cancer and 1546 adenoma cases, reported on the association between cruciferous vegetable consumption and colonic tumor risk stratified by *GSTM1* and *GSTT1* genotypes. Cruciferous vegetable consumption was measured by food-frequency questionnaire (FFQ) or dietary questionnaire in 6 studies, with one study measuring intake using urinary ITC levels. Analysis of variables including country, gender, ethnicity, study population, vegetable assessment method, study design, and direction were conducted (Table 5). The only significant findings were for the *GSTT1* null gene which produced an odds ratio of 0.78 (95% CI: 0.64–0.95; *P* value heterogeneity 0.32; $I^2 = 13.67$).

## DISCUSSION

Epidemiological evidence collated in this systematic review show that there is an inverse association between total cruciferous vegetable intake and risk of developing colorectal neoplasm. However, such association is not statistically significant, except in the case for colon cancers. A comprehensive review of epidemiologic studies published prior to 1996 (15) reported that the majority (67%) of 87 case-control studies reported a negative correlation between consumption of at least 1 or more cruciferous vegetables and cancer risk. According to the review, the inverse association was most consistent for cancers of the lung and gastrointestinal tract.

Much emphasis regarding the anticarcinogenic properties of cruciferous vegetables is placed on its unique source of glucosinolates (1,16). Glucosinolates are converted to ITCs by the action of a myrosinase (5). Several experimental studies and animal models support a potential anticancer role of ITCs against colorectal cancers (17, 18). However, most studies used in this systematic review do not allow inferences to pinpoint ITCs as the key anticarcinogenic constituent of cruciferous vegetables because other nutrients and phytochemicals (e.g., flavonols, folate, and carotenoids) found in cruciferous vegetables may also contribute to the inverse association with colorectal neoplasm (1). Only a number of studies specifically evaluated the ITC levels in servings of cruciferous vegetables as a measure of exposure (6,19–21).

Moreover, although this study attempts to analyze cruciferous vegetables as a separate factor, the consumption of these individual vegetables is likely positively correlated with the total consumption of vegetables in general. In most of the epidemiological studies the effect of cruciferous vegetables was not completely separated from the effect of total vegetables by adjusting for the consumption of these vegetables. Studies that measure the intake of individual cruciferous vegetables in accordance with their ITC levels may better define the benefits of ITC and distinguish protective properties of the cruciferae from other vegetables.

Amongst the studies that looked at the outcome of consuming individual types of cruciferous vegetables, broccoli, cabbage, and brussel sprouts were most examined. Intake of broccoli in particular showed the strongest evidence (statistically significant) against CRC development. A plausible explanation for this is due to broccoli's high glucosinolate content (22). In a series of laboratory studies, broccoli was found to be rich in phytochemicals that induced phase II detoxification enzymes and enhanced antioxidant activities in mammalian cells (16,23,24). One serving can contain up to 60 mg of glucoraphanin, a type of glucosinolate (1). Broccoli sprouts in particular contain about 20–100 times more glucoraphanin than a full-grown broccoli head (16).

TABLE 3
Characteristics of studies reporting odds ratios and 95% confidence intervals for the association between individual cruciferous vegetable intake and colorectal neoplasm risk

| Reference | Country | Ethnic group | Study design | Study population | Neoplasm type | Exposure | Case/control size | Cohort size |
|---|---|---|---|---|---|---|---|---|
| Young 1988 (35) | United States | Caucasian | Case-control | Population | Colon | Cabbage | 353/618 | |
| Steinmetz 1993 (40) | Australia | Caucasian | Case-control | Population-Men | Colon | Broccoli, brussel sprouts cabbage | 110/218 | |
| Steinmetz 1994 (41) | United States | Caucasian | Cohort | Population-Women | Colon | Broccoli, brussel sprouts, cabbage | 159 | 41873 |
| Lin 1998 (24) | United States | Caucasian | Case-control | Hospital | CR adenoma | Broccoli, cabbage | 299/374 | |
| Slattery 2000 (55) | United States | Caucasian | Case-control | Population | Colon | Broccoli, brussel sprouts, cabbage | 301/326 | |
| Flood 2002 (56) | United States | Caucasian | Cohort | Population-Women | CRC | Broccoli | 97/9098 | 45490 |
| Evans 2002 (57) | UK | Caucasian | Case-control | Population | CRC | Broccoli | 512/512 | |
| Hara 2003 (48) | Japan | Asian | Case-control | Hospital | CRC | Broccoli, cabbage | 81/2110 | |

CR = colorectal; CRC = colorectal cancer.

TABLE 4
Characteristics of studies reporting odds ratios and 95% confidence intervals for the association between individual cruciferous vegetable intake and colorectal neoplasm risk with stratification by GST genotype

| Reference | Country | Ethnicity | Study design | Population | Disease type | Exposure | Genes assessed | Case control number | Cohort number | Type of dietary assessment |
|---|---|---|---|---|---|---|---|---|---|---|
| Lin 1998 (24) | United States | Caucasian | Case-control | Hospital | CR adenoma | Cruciferous vegetables | GSTM1 | 134/152 | | FFQ |
| Slattery 2000 (55) | United States | Caucasian | Case-control | Population | Colon | Broccoli | GSTM1 | 326/627 | | Interview |
| Lin 2002 (58) | United States | Caucasian | Case-control | Hospital | CR adenoma | Cruciferous vegetables | GSTM1, GSTT1 | 558/940 | | Interview |
| Seow 2002 (6) | Singapore | Asian | Cohort | Population | CRC | Cruciferous vegetables (Dietary ITC) | GSTM1, GSTT1 | 360/2205 | 63257 | Interview, FFQ |
| Turner 2004 (51) | UK | Caucasian | Case-control | Population | CRC | Cruciferous vegetables | GSTM1, TT1 | 706/932 | | Questionnaire |
| Tijhuis 2005 (3) | Netherlands | Caucasian | Case-control | Hospital | CR adenoma | Cruciferous vegetables | GSTM1, TT1 | 1221/1047 | | FFQ |
| Epplein 2009 (19) | United States | Multiethnic | Cohort | Population | CRC | Urinary ITC levels | GSTM1, GSTT1 | 137/269 | 67594 | FFQ |
| Yang 2010 (17) | China | Asian | Case-control | Population-Women | CRC | Cruciferous vegetables, urinary ITC levels | GSTM1, TT1 | 574/2195 | | FFQ, Urinary ITC |

CR = colorectal; CRC = colorectal cancer; ITC = isothiocyanates; FFQ = food-frequency questionnaire.

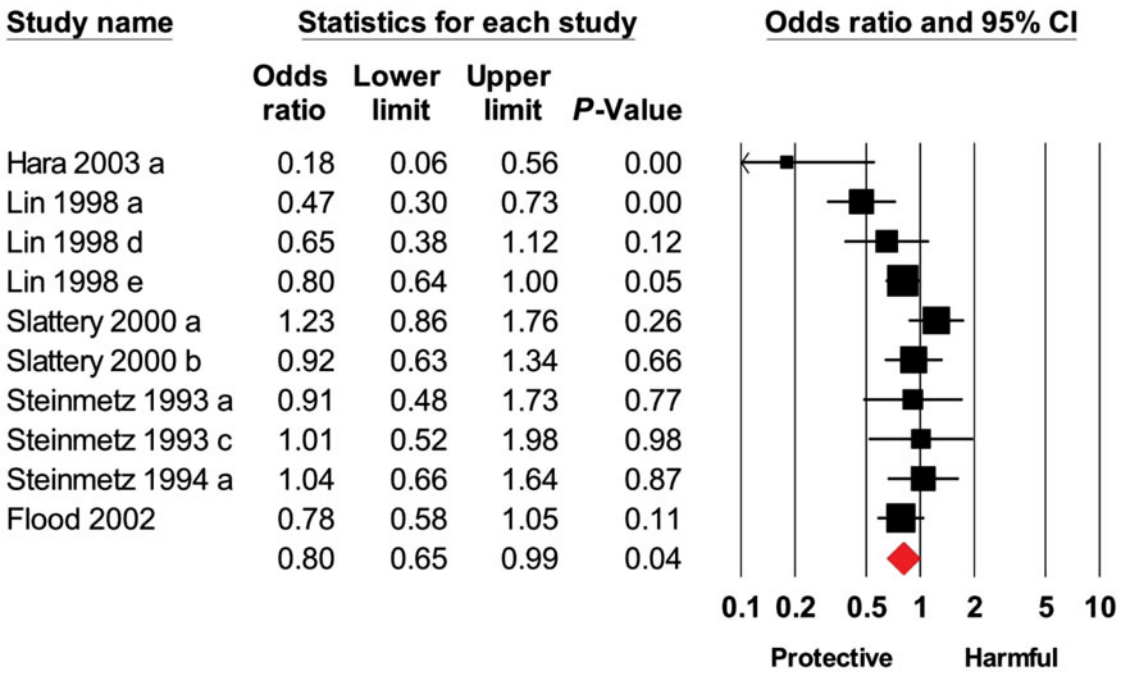


FIG. 4. Forest plot of broccoli consumption and colorectal neoplasm risk (color figure available online).

Data from the studies used in this review did not account for the maturity of vegetables consumed (young sprouts vs. full-grown) nor the preparation methods of these vegetables. Such variability may act to undermine the validity of the results.

Inherited differences in the capacity of individuals to metabolize and eliminate glucosinolate hydrolysis products have been hypothesized to contribute to differences in the degree of protection conferred by cruciferous vegetable intake (25). GSTs conjugate ITCs to glutathione and lead to excretion (1). Variants of several GST enzymes have been described, of which *GSTM1* and *GSTT1* have been most extensively studied. Roughly half the population lack *GSTM1* due to gene deletion (26). The homozygous deletion of *GSTM1* gives rise to the null phenotype in which there is no expression of the GSTM1 protein. The hypothesis is that individuals with the null genotype of the *GSTM1* or *GSTT1* polymorphism would have less conjugation activity, and thus permitting ITC to remain biologically active for a longer period to provide potentially greater protection against cancer (27).

Previous epidemiological data have been inconsistent and there is little evidence to support this theory in the context of colorectal neoplasm risk. A recent comprehensive meta-analysis have in fact demonstrated increased risk with *GSTM1* and *GSTT1* null genotypes in Caucasian populations (9). However, results from this study reveal statistically significant protective effects of cruciferae consumption against colorectal neoplasms among individuals with a single null *GSTT1* genotype. This discrepancy may be due to the fact that the mentioned study did not consider gene–environment interactions that may significantly influence the outcome. In which case cruciferous vegetable intake seems play a significant role in colorectal cancer risk reduction.

A weakness of the evidence that comprises this systematic review is the measurement error that inherently exists when using dietary questionnaires. FFQs used in epidemiological studies query a limited range of food items (thus omitting some sources of ITC), and rely on quantifying phytochemical exposure to constituents based on the weight or servings of cruciferous vegetable consumed (22). This approach has limitations in that the difference in levels of glucosinolates between different cruciferous vegetables is not accounted for. In addition, selection bias and recall bias could have accounted for part of the inverse associations observed in many case-control studies, leading to differential reporting. The observed risk estimate for case-control studies was similar to that for cohort studies which suggests that in this instance recall bias does not play a prominent role.

Although most studies adjusted for age and gender in the calculation of risk estimates, not all parameters were considered. A meta-analysis would not adequately adjust for this. As such, unadjusted confounders may undermine the association between cruciferous vegetables and colorectal neoplasm risk.

Another weakness in the evidence is that the biological potency of cruciferous vegetables may differ, depending on cooking practices, because this influences the bioavailability of ITCs (28). Glucosinolates are water-soluble compounds that leach into cooking water. Some cooking methods, including boiling, steaming, and microwaving at high power can inactivate myrosinase, decreasing the bioavailability of ITCs (29–31). The

TABLE 5
Odd ratios (ORs) and 95% confidence intervals (CIs) for additional variables examined for the association between cruciferous vegetable intake and colorectal neoplasm risk with stratification by GST genotype

| Factor | OR (95% CI) |
|---|---|
| Ethnicity | |
| Asian | 0.91 (0.82–1.01) |
| Caucasian | 0.98 (0.90–1.08) |
| Study design | |
| Cohort | 0.90 (0.74–1.09) |
| Case control | 0.97 (0.90–1.04) |
| Temporal direction | |
| Prospective | 0.90 (0.74–1.09) |
| Retrospective | 0.97 (0.90–1.04) |
| Population type | |
| Population | 0.96 (0.87–1.06) |
| Hospital | 0.97 (0.84–1.11) |
| Population gender | |
| Female | 0.91 (0.80–1.04) |
| Neoplasm type | |
| Colon | 1.06 (0.9–1.26) |
| CRC | 0.92 (0.84–1.01) |
| Adenoma | 0.97 (0.84–1.11) |
| Gene status | |
| *GSTM1* null | 1.05 (0.92–1.19) |
| *GSTM1* nonnull | 1.02 (0.92–1.13) |
| *GSTT1* null | 0.78 (0.64–0.95)[a] |
| *GSTT1* nonnull | 1.02 (0.90–1.13) |
| *GSTM1, GSTT1* double null | 0.86 (0.70–1.06) |
| *GSTM1, GSTT1* double nonnull | 1.11 (0.86–1.43) |

[a]Statistically significant.

lack of information about cooking methods thus introduces a potential source of heterogeneity in the results.

### Recommendation

To address issues relating the specificity of the protective effects attributable to cruciferous vegetables, further epidemiological research should pay specific attention to simultaneous modeling of different vegetables (or vegetable groups) and adjustment for confounding due to other chemoprotective constituents.

Based on assessments of cruciferous vegetable intake and measurements of the maximal amounts of ITC that can be released, researchers have attempted to calculate human ITC exposure (22). A food composition database may be used to provide a more consistent and accurate assessment of dietary ITC intake.

Because other factors may alter the amount of ITC formed and absorbed, assessing dietary intake alone may not accurately measure an individual's exposure to ITC. Urinary ITC measurement was significantly correlated with cruciferae intake derived from 2 dietary assessment approaches (32,33). Urinary ITC levels could supplement traditional food database data by providing an index of recent cruciferous vegetable intake not susceptible to reporting biases.

Although there is evidence to support the idea of gene–diet interaction, more research can be done to explore the influence of gene polymorphisms other than GST genotypes. Genes such as *p53* (34) and *Ki-ras* (35) have been reported to show significant associations with cruciferae intake. The study of alternative genetic factors can provide a better understanding of the different pathways and mechanisms which underpin cruciferae's anti-carcinogenic properties.

## CONCLUSION

In this systematic review and meta-analysis, intake of cruciferous vegetable is inversely associated with colon cancer risk. The consumption of broccoli in particular is demonstrated to be positively correlated with protection against colorectal neoplasm development. There is a positive correlation between GST polymorphism and the capacity of cruciferous vegetables to exhibit chemoprotective properties, in which the *GSTT1* null genotype confers a reduction colorectal neoplasm development risk. Future epidemiological studies should use food databases or urinary ITC levels as methods for more accurate cruciferae intake measurements. Individual cruciferae types and preparation methods should be considered during analysis. More research on alternative gene polymorphisms would also be helpful in studying gene–diet interactions with cruciferous vegetables.

## REFERENCES

1. Herr I and Buchler MW: Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer. *Cancer Treat Rev* **36**, 377–383, 2010.
2. Willett WC: Diet and cancer: One view at the start of the millennium. *Cancer Epidem Biomar* **10**, 3–8, 2001.
3. Tijhuis MJ, Wark PA, Aarts J, Visker M, Nagengast FM, et al.: GSTP1 and GSTA1 polymorphisms interact with cruciferous vegetable intake in colorectal adenoma risk. *Cancer Epidem Biomar* **14**, 2943–2951, 2005.
4. Higdon JV, Delage B, Williams DE, and Dashwood RH: Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol Res* **55**, 224–236, 2007.
5. Zhang Y, Yao S, and Li J: Vegetable-derived isothiocyanates: antiproliferative activity and mechanism of action. *P Nutr Soc* **65**, 68–75, 2006.
6. Seow A, Yuan JM, Sun CL, Van den Berg D, Lee HP, et al.: Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. *Carcinogenesis* **23**, 2055–2061, 2002.
7. Lam TK, Gallicchio L, Lindsley K, Shiels M, Hammond E, et al.: Cruciferous Vegetable Consumption and Lung Cancer Risk: A Systematic Review. *Cancer Epidem Biomar* **18**, 184–195, 2009.
8. Seow A, Vainio H, and Yu MC: Effect of glutathione-S-transferase polymorphisms on the cancer preventive potential of isothiocyanates: An epidemiological perspective. *Mutat Res* **592**, 58–67, 2005.

9. Economopoulos KP and Sergentanis TN: GSTM1, GSTT1, GSTP1, GSTA1 and colorectal cancer risk: A comprehensive meta-analysis. *Eur J Cancer* **31**, 1617–1631, 2010.
10. Flood A, Velie EM, Chaterjee N, Subar AF, Thompson FE, et al.: Fruit and vegetable intakes and the risk of colorectal cancer in the Breast Cancer Detection Demonstration Project follow-up cohort. *Am. J. Clin. Nutrit* **43**, 936–943, 2002.
11. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, et al.: Meta-analysis of observational studies in epidemiology - A proposal for reporting. *JAMA* **283**, 2008–202, 2000.
12. DerSimonian R and Laird N: Meta-analysis in clinical trials. *Control Clin Trials* **7**, 177–188, 1986.
13. Higgins JPT, Thompson SG, Deeks JJ, and Altman DG: Measuring inconsistency in meta-analyses. *Brit Med J* **327**, 557–560, 2003.
14. Egger M, Smith GD, Schneider M, and Minder C: Bias in meta-analysis detected by a simple, graphical test. *Brit Med J* **315**, 629–634, 1997.
15. Verhoeven DTH, Goldbohm RA, vanPoppel G, Verhagen H, and vanden-Brandt PA: Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidem Biomar* **5**, 733–748, 1996.
16. Fahey JW, Zhang YS, and Talalay P: Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *P Natl Acad Sci USA* **94**, 10367–10372, 1997.
17. Chung FL, Conaway CC, Rao CV, and Reddy BS: Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethy l isothiocyanate. *Carcinogenesis* **21**, 2287–2291, 2000.
18. Clapper ML, Szarka CE, Pfeiffer GR, Graham TA, Balshem AM, et al.: Preclinical and clinical evaluation of broccoli supplements as inducers of glutathione S-transferase activity. *Clin Cancer Res* **3**, 25–30, 1997.
19. Yang G, Gao YT, Shu XO, Cai QY, Li GL, et al.: Isothiocyanate exposure, glutathione S-transferase polymorphisms, and colorectal cancer risk. *Am J Clin Nutr* **91**, 704–711, 2010.
20. Moy KA, Yuan JM, Chung FL, Van Den Berg D, Wang RW, et al.: Urinary total isothiocyanates and colorectal cancer: A prospective study of men in Shanghai, China. *Cancer Epidem Biomar* **17**, 1354–1359, 2008.
21. Epplein M, Wilkens LR, Tiirikainen M, Dyba M, Chung FL, et al.: Urinary isothiocyanates; glutathione s-transferase M1, T1, and P1 Polymorphisms; and risk of colorectal cancer: The Multiethnic Cohort Study. *Cancer Epidem Biomar* **18**, 314–320, 2009.
22. McNaughton SA and Marks GC: Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. *Brit J Nutr* **90**, 687–697, 2003.
23. Zhang YS, Talalay P, Cho CG, and Posner GH: A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *P Natl Acad Sci USA* **89**, 2399–2403, 1992.
24. Fahey JW and Talalay P: Antioxidant functions of sulforaphane: a potent inducer of phase II detoxication enzymes. *Food Chem Toxicol* **37**, 973–979, 1999.
25. Lampe JW and Peterson S: Brassica, biotransformation and cancer risk: Genetic polymorphisms alter the preventive effects of cruciferous vegetables. *J Nutr* **132**, 2991–2994, 2002.
26. Lin HJ, Probst-Hensch NM, Louie A, Kau IH, Witte JS, et al.: Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. *Cancer Epidem Biomar* **7**, 647–652, 1998.
27. Cotton SC, Sharp L, Little J, and Brockton N: Glutathione S-transferase polymorphisms and colorectal cancer: A HuGE review. *Am J Epidemiol* **151**, 7–32, 2000.
28. Vermeulen M, Van den Berg R, Freidig AP, Van Bladeren PJ, and Vaes WHJ: Association between consumption of cruciferous vegetables and condiments and excretion in urine of isothiocyanate mercapturic acids. *J Agr Food Chem* **54**, 5350–5358, 2006.
29. Shapiro TA, Fahey JW, Wade KL, Stephenson KK, and Talalay P: Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: Metabolism and excretion in humans. *Cancer Epidem Biomar* **10**, 501–508, 2001.
30. Rouzaud G, Young SA, Duncan AJ. Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. *Cancer Epidem Biomar* **13**, 125–131, 2004.
31. Conaway CC, Getahun SM, Liebes LL, Pusateri DJ, Topham DKW, et al.: Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr Cancer* **38**, 168–178, 2000.
32. Fowke JH, Hebert JR, and Fahey JW: Urinary excretion of dithiocarbamates and self-reported Cruciferous vegetable intake: application of the 'method of triads' to a food-specific biomarker. *Public Health Nutr* **5**, 791–799, 2002.
33. Kristensen M, Krogholm KS, Frederiksen H, Bugel SH, and Rasmussen SE: Urinary excretion of total isothiocyanates from cruciferous vegetables shows high dose-response relationship and may be a useful biomarker for isothiocyanate exposure. E *Eur J Nutr* **46**, 377–382, 2007.
34. Freedman AN, Michalek AM, Marshall JR, Mettlin CJ, Petrelli NJ, et al.: Familial and nutritional risk factors for p53 overexpression in colorectal cancer. *Cancer Epidem Biomar* **5**, 285–291, 1996.
35. Slattery ML, Curtin K, Anderson K, Ma KN, Edwards S, et al.: Associations between dietary intake and Ki-ras mutations in colon tumors: A population-based study. *Cancer Res* **60**, 6935–6941, 2000.
36. Young TB and Wolf DA. Case-control study of proximal and distal colon cancer in Wisconsin. *Int J Cancer* **42**, 167–175, 1988.
37. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, et al.: Colorectal-cancer and diet in an Asian population - a case-control study among Singapore Chinese. *Int J Cancer* **43**, 1007–1016, 1989.
38. West DW, Slattery ML, Robison LM, Schuman KL, Ford MH, et al.: Dietary-intake and colon cancer - sex-specific and anatomic site-specific associations. *Am J Epidemiol* **130**, 883–894, 1989.
39. Peters RK, Pike MC, Garabrant D, and Mack TM: Diet and colon cancer in Los-Angeles county, California. *Cancer Cause Control* **3**, 457–473, 1992.
40. Bidoli E, Franceschi S, Talamini R, Barra S, and Lavecchia C: Food-consumption and cancer of the colon and rectum in North-Eastern Italy. *Int J Cancer* **50**, 223–229, 1992.
41. Steinmetz KA and Potter JD: Food-group consumption and colon cancer in the Adelaide case-control study .1. Vegetables and fruit. *Int J Cancer* **53**, 711–719, 1993.
42. Steinmetz KA, Kushi LH, Bostick RM, Folsom AR, and Potter JD: Vegetables, fruit, and colon-cancer in the Iowa Womens Health Study. *Am J Epidemiol* **139**, 1–15, 1994.
43. Hsing AW, McLaughlin JK, Chow WH, Schuman LM, Chien HTC, et al.: Risk factors for colorectal cancer in a prospective study among US white men. *Int J Cancer* **77**, 549–553, 1998.
44. Sellers TA, Bazyk AE, Bostick RM, Kushi LH, Olson JE, et al.: Diet and risk of colon cancer in a large prospective study of older women : an analysis stratified on family history (Iowa, United States). *Cancer Cause Control* **9**, 357–367, 1998.
45. Franceschi S, Parpinel M, Vecchia CL, and Favero A: Role of different types of vegetables and fruit in the prevention of cancer of the colon, rectum, and breast. *Epidemiology* **9**, 338–341
46. Pietinen P, Malila N, Virtanen M, Hartman TJ, Tangrea JA, et al.: Diet and risk of colorectal cancer in a cohort of Finnish men. *Cancer Cause Control* **10**, 387–396, 1999.
47. Voorrips LE, Goldbohm RA, van Poppel G, Sturmans F, Hermus RJJ, et al.: Vegetable and fruit consumption and risks of colon and rectal cancer in a prospective cohort study - The Netherlands Cohort Study on Diet and Cancer. *Am J Epidemiol* **152**, 1081–1092, 2000.
48. Michels KB, Giovannucci E, Joshipura KJ, Rosner BA, Stampfer MJ, et al.: Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. *J. Natl. Cancer Inst* **92**, 1740–1752, 2000.

49. Seow A, Quah SR, Ph D, Nyam D, Straughan PT, et al.: Food groups and the risk of colorectal carcinoma in an Asian population. *Cancer* **95**, 2390–2396, 2002.
50. Hara M, Hanaoka T, Kobayashi M, Otani T, Adachi HY, et al.: Cruciferous vegetables, mushrooms, and gastrointestinal cancer risks in a multicenter, hospital-based case-control study in Japan. *Nutr Cancer* **46**, 138–147, 2003.
51. McCullough ML, Robertson AS, Chao A, Jacobs EJ, Stampfer MJ, et al.: A prospective study of whole grains, fruits, vegetables and colon cancer risk. *Cancer Cause Control* **14**, 959–970, 2003.
52. Chiu BCH, Ji BT, Dai Q, Gridley G, McLaughlin JK, et al.: Dietary factors and risk of colon cancer in Shanghai, China. *Cancer Epidem Biomar* **12**, 201–208, 2003.
53. Turner F, Smith G, Sachse C, Lightfoot T, Garner RC, et al.: Vegetable, fruit and meat consumption and potential risk modifying genes in relation to colorectal cancer. *Int J Cancer* **112**, 259–264, 2004.
54. Michels KB, Giovannucci E, Chan AT, Singhania R, Fuchs CS, et al.: Fruit and Vegetable Consumption and Colorectal Adenomas in the Nurses' Health Study. *Cancer Res* **66**, 3942–3953, 2006.
55. Hu JF, Mery L, Desmeules M, Macleod M, and Canadian Cancer Registries: Diet and vitamin or mineral supplementation and risk of rectal cancer in Canada. *Acta Oncol* **46**, 342–354, 2007.
56. Hu J, Morrison H, Mery L, DesMeules M, and Macleod M: Diet and vitamin or mineral supplementation and risk of colon cancer by subsite in Canada. *Eur J Cancer Prev* **16**, 275–291, 2007.
57. Ramadas A and Kandiah M: Food intake and colorectal adenomas: a case-control study in Malaysia. *Asian Pac J Cancer P* **10**, 925–932, 2009.
58. Slattery ML, Kampman E, Samowitz W, Caan BJ, and Potter JD: Interplay between dietary inducers of GST and the GSTM-1 genotype in colon cancer. *Int J Cancer* **87**, 728–733, 2000.
59. Evans RC, Fear S, Ashby D, Hackett A, Williams E, et al.: Diet and colorectal cancer: an investigation of the lectin/galactose hypothesis. *Gastroenterology* **122**, 1784–1792, 2002.
60. Lin HJ, Zhou HY, Dai AH, Huang HF, Lin JH, et al.: Glutathione transferase GSTT1, broccoli, and prevalence of colorectal adenomas. *Pharmacogenetics* **12**, 175–179, 2002.