Cruciferous vegetables and risk of cancers of the gastrointestinal tract

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Abbreviations: CRC: colorectal cancer; OSCC: oesophageal squamous cell carcinoma; OAC: oesophageal adenocarcinoma; GST: glutathione S-transferase; IST: isothiocyanate

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Abstract

Cancers of the oropharyngeal tissues, oesophagus, stomach and colorectum are amongst the most common causes of death from cancer throughout the world. Higher consumption of fruits and vegetables is thought to be protective, and cruciferous vegetables are of particular interest because of their unique role as a source of biologically active glucosinolate breakdown products. A literature review of primary studies and meta-analyses indicates that higher consumption of cruciferous vegetables probably reduces the risk of colorectal and gastric cancers by approximately 8% and 19% respectively. Some studies support the hypothesis that the protective effect against colorectal cancer is modified by genetic polymorphisms of genes regulating the expression of enzymes of the glutathione *S*-transferase family, but due to contradictory findings the evidence is currently inconclusive.

Despite these promising findings, future epidemiological research on the protective effects of cruciferous plants will depend critically upon accurate measurement of dietary exposure, both to the vegetables themselves, and to their active constituents. The development of sensitive chemical assays has facilitated the measurement of urinary excretion of isothiocyanate metabolites as an objective biomarker of intake, but sampling strategies need to be optimised in order to assess long-term exposures at the population level.

1.0 Introduction

Globally, tumours of the upper digestive tract, stomach, colon and rectum are amongst the most common causes of death from cancer. However the incidence rates for each of these sites vary greatly, both between countries, and within populations undergoing environmental change. For example, in western industrialised countries, colorectal carcinoma (CRC) is usually the most frequently diagnosed cancer after cancers of the lung

and prostate in men, and breast in women, whereas in many countries of southern Asia and sub-Saharan Africa CRC remains relatively uncommon [1]. Japan provides a striking example of an Asian country where CRC was formerly rare, but where incidence has risen in parallel with increasing prosperity since the middle of the of the twentieth century. Of note is that the increasing lifetime risk of CRC in Japan has occurred alongside a decline in cases of gastric carcinoma [2]. Such changes are clearly related to environmental exposures including diet, tobacco use, alcohol consumption and physical activity, but in most cases the precise biological mechanisms remain poorly understood [3].

Amongst the many environmental exposures thought to influence the aetiology of cancers of the alimentary tract, the protective effect of a high dietary intake of fruits and vegetables has received much attention over the last few decades, both from researchers and from public health bodies. Two comprehensive literature reviews published in the early 1990s were particularly influential. Both Block *et a l* [4] and Steinmetz and Potter [5] concluded that the evidence that higher consumption of plant foods protected against most solid tumours was overwhelming, and this view was endorsed by the World Cancer Research Fund in its wide-ranging report on Food, Nutrition and the Prevention of Cancer, published in 1997 [6]. The strength of the epidemiological evidence focused the attention of laboratory scientists on the mechanisms responsible for the protective effects, and research has since revealed numerous biologically active constituents of fruits and vegetables that may modulate the progress of carcinogenesis in human beings [7].

Brassica vegetables and other cruciferous food plants are of special interest because they contain glucosinolates, a group of sulphur-containing glycosides which are decomposed

by the endogenous plant enzyme myrosinase to yield several biologically active products, including the isothiocyanates and indoles [8]. The presence of these compounds in the human food chain is of particular interest because of their potentially anticarcinogenic effects, including both modulation of the phase I and II enzymes which metabolise environmental carcinogens, and the suppression of proliferation and induction of apoptosis in tumour cells [9]. These topics are dealt with in detail elsewhere in this special volume. This article explores the epidemiological evidence for protective effects of cruciferous vegetables against cancers at the most commonly affected anatomical sites in the alimentary tract. In preparing this article emphasis has been placed on systematic reviews and meta-analyses which, together with the most recent primary studies, have been sourced via *Pubmed*, using appropriate search terms. The first section reviews the general evidence for associations between cruciferous vegetable consumption and cancers of the oropharynx, oesophagus, stomach and colorectum. The second section considers the evidence for interactions between the anticarcinogenic effects of cruciferous vegetables and the common genetic polymorphisms affecting the expression of the enzymes that metabolise isothiocyanates and indoles.

2.0 Cruciferous vegetable consumption as a risk-factor for cancers of the gastrointestinal tract

2.1 Oropharyngeal cancers

Carcinomas of the lip, oral cavity and pharynx are collectively termed oropharyngeal cancers, and they account for about 4% of all cancer cases worldwide [10]. They vary markedly in incidence across populations, and they are thought to have a range of causes, including infectious agents, nutritional deficiencies exposure to environmental and self-administered toxins. Thus, in high-income countries, infection with high-risk human Papillomavirus (HR-HPV) is thought to account for more than twenty

percent of oropharyngeal cancers [11]. Many of the remainder are thought to be linked to the carcinogenic effects of alcohol and tobacco use [12], and to the absence of protective factors associated with fruits and vegetables [13]. In 1996, Verhoeven *et al* [14] published the first comprehensive narrative review of epidemiological studies focussed specifically on Brassica vegetables and risk of cancer. Their findings were based on a total of 87 case-control studies and seven cohort studies available at the time. None of the cohort studies contained relevant data, but five of the case-control studies provided information on cancers of the oropharyngeal tissues, and two of these reported statistically significant lower risks of cancer associated with the highest compared to the lowest quartile for intake of brassica vegetables [15, 16].

In 2002 Chainani-Wu [17] published a comprehensive narrative review exploring the available evidence on the relationship between cancers of the upper digestive tract and consumption of fruits and vegetables, including brassica vegetables as a separate category. The author concluded that consumption of green vegetables, yellow vegetables and cruciferous vegetables, as well as total fruit and citrus fruits, was associated with a significant reduction in risk, but cruciferous vegetables did not emerge as more strongly protective than the other categories.

The World Cancer Research Fund (WCRF) published two comprehensive and influential reports on diet and cancer in 1997 and 2007 respectively. Both reports included detailed appraisals of the evidence for protective effects of vegetables in general, and wherever possible of specific varieties, against all the major cancers, including those of the alimentary tract. The first report [18] assessed the evidence that diets high in vegetables decreased the risk of cancers of the mouth and pharynx as "convincing". The second report [13] addressed the issue in more detail, and included evidence from one cohort study, fourteen case-control studies and a single ecological study. The cohort study [19] provided evidence of a non-significantly increased risk of oropharyngeal cancer associated with a high consumption of cauliflower, and a non-significantly decreased risk for consumption of cabbage. The latter observation was consistent with the ecological study, which also showed a

statistically significant protective effect of cabbage consumption [20]. Four case-control studies provided evidence of statistically significant protective effects of brassica vegetables in general [15, 16, 21, 22], while nine others showed associations which were not statistically significant. No meta-analysis of studies pertaining to cruciferous vegetables and cancer at any site was provided. The overall conclusion was that a protective effect of non-starchy vegetables against cancers of the mouth and pharynx was "probable", but no particular reference was made to cruciferous vegetables.

In 2004 the International Agency for Research on Cancer (IARC) published a detailed systematic review focused specifically on the relationship between risk of cancer and consumption of cruciferous vegetables, isothiocyanates and indoles [23]. The agency's systematic analysis of epidemiological data identified the single cohort study previously mentioned [19], which reported no statistically significant effects on oropharyngeal cancers, and seven case-control studies, of which only two, also previously mentioned, reported statistically significant protective effects [15, 16]. More recently, analysis of a large network of case-control studies conducted by Bosetti *et al* [24] provided evidence for a statistically significant reduction 17% reduction in risk of oropharyngeal cancer in subjects consuming cruciferous vegetables at least once a week, compared to non-consumers.

2.2 Oesophageal cancer

Oesophageal carcinoma is an important cause of death from cancer in both high-income countries and in the developing world. In any discussion of risk-factors it is important to distinguish between two histological sub-types of the disease, which differ markedly in frequency and aetiology. Until the last decades of the twentieth century, oesophageal squamous cell carcinoma (OSCC) was the most common form of oesophageal cancer in all populations, and it remains so in the developing world [25]. Alcohol consumption and tobacco have long been understood to be major risk factors [26] but the disease also shows striking variations in incidence in China and other parts of Asia [27]

which, although genetic variance may play some role [28], seem to be largely attributable to environmental factors. The other major histological sub-type, oesophageal adenocarcinoma (OAC), was relatively uncommon until the 1970s, but the incidence has since risen steeply in many high-income countries for reasons that remain largely unexplained [25]. OAC develops from a specific precursor lesion, Barrett's oesophagus [29], for which the strongest known risk-factors gastro-oesophageal reflux disease and smoking [30]. Although tobacco use is also a risk-factor for EAC, alcohol consumption does not appear to be important [31]. Despite these significant differences between the two forms of oesophageal cancer, the distinction is often not made, particularly in the earlier epidemiological literature.

Verhoeven et al [14] identified four case-control studies that reported on the relationship between brassica vegetable consumption and oesophageal cancers. Three of these were conducted in Chinese populations but, though some reductions in relative risk associated with higher consumption were observed, they were generally statistically non-significant. However a fourth study conducted in America and dealing specifically with OAC, observed a relative risk of 0.3 (p for trend <0.001) in men and women in the highest quartile of brassica vegetable consumption [32].

The two reports on diet and cancer from WCRF concluded that the evidence for protective effects of fruits and vegetables in general against oesophageal cancer was "convincing" [6] and "probable"[13] respectively, but neither came to any specific conclusions with respect to Brassica vegetables. The IARC report of 2004 [23] considered four case-control studies, but only that of Brown et al [32] on EAC showed a statistically significant protective effect of cruciferous vegetables. A later study by the same group on squamous cell carcinoma of the oesophagus showed relative risks less than 1.0 for the highest consumers of brassica vegetables but the relationship was not statistically significant [33]. More recently there have been individual studies providing evidence of protective effects of cruciferous vegetables against unspecified oesophageal cancer [24], against OAC in particular [34, 35], and against Barrett's oesophagus [36]. The most recent systematic review on modifiable risk-

factors for OAC confirmed the protective effect of fruits and vegetables in general but made no specific reference to cruciferous vegetables [31].

2.3 Gastric cancer

Although the incidence of gastric cancer has tended to decline in most countries in recent years, it still ranked fifth for incidence and third as a cause of global cancer mortality in 2015 [37]. Chronic infection with *Helicobacter pylori* is a major risk factor for gastric cancer [38]. Declining rates of infection in high income countries are thought to account for much of the reduction in mortality for this disease during the twentieth century, and this effect has probably complicated attempts to explore the influence of diet in these populations. Nevertheless, case-control studies have provided fairly consistent evidence for a protective effect of total fruit and vegetable consumption against gastric cancer [39].

Verhoeven *et al* [14] recorded eleven case-control studies reporting on associations between brassica vegetable consumption and risk of gastric cancer, out of which eight showed at least one inverse association and three reported a positive association. Amongst the studies observing a negative association, five reported a statistically significant effect [14]. The WCRF reports of 1997 and 2007 both concluded that higher consumption of vegetables probably protected against gastric cancer, but they reached no specific conclusions with regard to cruciferous vegetables. The IARC report of 2004 derived a statistically non-significant negative odds-ratio (OR) of 0.91 (95% confidence interval (CI), 0.67-1.23) for brassica vegetables from an analysis of the small number of cohort studies available [23]. A more consistent protective effect was obtained from a systematic review of case-control studies (OR 0.81; 95% CI 0.73-0.90) but unmeasured confounding with other environmental factors could not be excluded, and the protective effect was somewhat lower than that attributed to total vegetable consumption in an earlier IARC review [39] on fruit and vegetable consumption and cancer (OR 0.66; 95% CI 0.61-0.71).

The most recent available systematic review of cruciferous vegetable consumption and risk of gastric cancer is that of Wu et al from 2013 [40]. From the pooled results of sixteen case-control and six prospective studies, the authors obtained evidence for a protective effect of cruciferous vegetables, giving a relative risk of 0.81 (95% CI 0.75-0.88) when comparing the highest with the lowest intake. As previously observed, the statistical significance was stronger for case-control compared to prospective studies. In a separate analysis of two cohort and five case-control studies, a protective effect specifically for cabbage consumption against gastric cancer was obtained (RR 0.68; 95% CI 0.58 – 0.80) [41].

2.4 Colorectal cancer

Colorectal carcinoma is amongst the most commonly diagnosed forms of cancer in the world; incidence rates are particularly high in prosperous countries, including those of Western Europe, North America, Australia and Japan [1]. It has been suggested that the vulnerability of the colorectal mucosa to cancerous changes is largely a function of the high rates of stem cell division associated with mucosal crypt cell proliferation in these epithelial tissues [42], but the importance of environmental factors is clear from the variations in age-adjusted incidence of up to ten-fold that occur, both across populations and within populations undergoing dietary and other changes associated with increasing prosperity [2]. These epidemiological features have long focused attention on the possible role of diet in the prevention of CRC. Early indications from case-control studies that fruits and vegetables might play a protective role against CRC [4], coupled with the fact that biologically active glucosinolate breakdown products are released by bacterial metabolism in the colon [43], have encouraged the hypothesis that cruciferous vegetables play a particularly important protective role against CRC.

Verhoeven et al [14] recorded a single prospective study on the consumption of brassica vegetables and risk of colon cancer, conducted amongst post-menopausal women in the USA, and subdividing

the exposure into varietal categories including broccoli, cabbage, cauliflower and Brussels sprouts [44]. The relative risk associated with brassica consumption was 0.7 (95% CI 0.4 – 1.3); however, for each variety the relative risk was close to unity, and none of these associations were statistically significant. Overall, in fifteen case-control studies on exposure to brassica vegetables of various types and colon cancer, and ten on rectal cancer, at least one inverse association was reported by 73% and 80% of studies respectively. Amongst these studies, 55% and 50% of the associations were statistically significant [14].

Neither of the WCRF reports on diet and cancer contained meta-analyses of studies on brassica vegetables and CRC, nor came to any specific conclusions on the protective effects of brassica vegetables against CRC. The WCRF report of 1997 [6] found convincing evidence of a protective effect of vegetables in general, but that of 2007 [13] was more cautious and merely concluded that there was limited evidence suggesting a protective effect of non-starchy vegetables. The IARC report on cruciferous vegetables and cancer [23] included a meta-analysis of eleven comparisons drawn from six individual cohort studies on CRC, but no statistically significant association was observed. A similar meta-analysis of six case-control studies did however provide evidence for a statistically significant inverse association (OR of 0.73; 95% CI 0.63 – 0.84).

The literature pertaining to cruciferous vegetables and risk of CRC has continued to expand, and recently two comprehensive systematic reviews have been published. Wu et al [41] conducted meta-analyses on twenty four case-control studies and eleven prospective cohort studies. The combined results indicated a significant inverse association for consumption of all cruciferous vegetables (RR: 0.82; 95% CI 0.75 – 0.90). In sub-group analyses, the associations between cabbage consumption and CRC (eight case-control and one cohort study) and broccoli consumption and CRC (three case-control and three cohort studies) were investigated, yielding RRs of 0.76 (95% CI 0.60 – 0.97) and 0.82 (95% CI 0.65 – 1.02) respectively. As in previous reviews, the association obtained

from analysis of case-control studies (RR 0.74; 95% CI 0.65 – 0.84) was larger and more statistically significant than that derived from cohort studies (RR 0.93; 95% CI 0.87 – 1.00).

Tse and Eslick [45] conducted a meta-analysis of thirty three articles and obtained a statistically significant inverse relationship between intake of all cruciferous vegetables and colon cancer (RR 0.84; 95% CI 0.72 – 0.98). Sub-group analysis suggested an effect of broccoli (RR 0.80; 95% CI 0.65 – 0.99) but no statistically significant effects of cabbage or Brussels sprouts were detected.

3.0 Cruciferous vegetable consumption, GST polymorphisms and gastrointestinal neoplasia

Like many other potentially toxic xenobiotics, isothiocyanates and indoles are rapidly metabolised during and after intestinal absorption by an array of Phase 2 detoxification enzymes, amongst which some of the various sub-classes of the glutathione *S*-transferases (GST) family are particularly important [46]. Sulforaphane and other ITCs are converted to glutathione conjugates by hepatic microsomal GSTs, including GSTM-1, GSTT-1 and GSTP-1, and then further metabolised to water-soluble mercapturic acids, which are readily excreted in urine [47]. This detoxification pathway forms part of a complex metabolic network which, as is discussed at length elsewhere in the present volume, is thought to regulate a variety of anti-carcinogenic mechanisms, including the induction of Phase 2 enzymes that metabolise and help to detoxify environmental carcinogens such as the constituents of tobacco smoke [48]. Another consequence of the rapid metabolism of glucosinolate breakdown products is the appearance in human urine of their metabolites, which can be detected and quantified and used as an objective biomarker of cruciferous vegetable consumption [49] [50].

Human populations display significant inter-individual variations in GST activity due to genetic polymorphisms. For example, the prevalence of homozygous null mutations of *GSTM1* and *GSTT1,* which lead to an absence of any functional enzyme activity for these sub-types, varies between 10% and more than 60% across different population groups [51]. In the case of GSTP1, which is also important for the metabolism of isothiocyanates, a single base pair (A-G) substitution in the *GSTP1*

gene, causes an amino acid substitution in the gene product, and leads to a significantly reduced level of enzyme activity [52]. These natural variations in gene expression, both between and within populations, have been proposed as heritable risk-factors for a variety of cancer including carcinomas of the alimentary tract [53].

Because of the important role that GSTs play in the metabolism of ISTs, variations in their activity may modify the duration and level of exposure of target tissues to the unmetabolised and therefore biologically active compound. Human intervention trials provide some support for this hypothesis in that changes in the level of GST activity in human serum in response to consumption of cruciferous vegetables under laboratory conditions have been shown to be influenced to a measurable extent by GST genotype [54]. Genetic polymorphisms may therefore lead to variations in the chemopreventive effects of cruciferous vegetables detectable in epidemiological studies [47], but this has proved difficult to confirm. In the case of lung cancer for example, two case-control studies appeared to show that individuals homozygous for *GSTM-1* expression gained greater protective benefit from consumption of cruciferous vegetables than individuals null for *GSTM-1* [55, 56]. In contrast, London et al [57], using urinary ITC metabolites as a biomarker of glucosinolate intakes, reported that the protective effect against lung cancer in a cohort of men in Shanghai was significantly greater in subjects null for *GSTT1* and *GSTM1* compared to those positive for each gene, and strongest in subjects who were null for both. The inconsistency between these studies remains unexplained. The current state of evidence for any effects of *GST* polymorphisms on the anticarcinogenic effects of brassica vegetable consumption for sites in the gastrointestinal tract is discussed below.

3.1 Oropharyngeal cancer

There has been little work done on the interrelationship between diet, *GST* polymorphisms and risk of oropharyngeal cancer. However, Gaudet *et al* [58] conducted a relatively small case-control study (149 cases and 180 controls) on head and neck cancer (which includes both oropharyngeal and

laryngeal squamous cell carcinomas) to test the hypothesis that individuals with a null genotype for *GSTM1* and *GSTT1* would benefit from greater exposure of tissues to chemopreventive compounds in fruits and vegetables through mechanisms analogous to those postulated by London *et al* [57] in relation to lung cancer. Although there was a statistically non-significant negative relationship between higher consumption of raw vegetables and risk of cancer (OR 0.66; 95% confidence interval 0.3 – 1.33) there was no evidence for protective effects of consumption of cruciferous vegetables, and no effect of *GST* polymorphisms. More recently Karen-Ng *et al* [59] reported a similarly sized case-control study (115 cases and 116 controls) in which they searched for effects of estimated IST intake on risk of squamous cell carcinoma of the oral cavity among subjects with differing *GSTM1, GSTT1* and *GSTP1* polymorphisms. Overall there were no statistically significant interactions between IST intake and *GST* polymorphisms but a statistically significant later age of onset was observed in subjects who were non-null for *GSTP1* and consuming low levels of IST. This observation would perhaps imply and adverse effect of cruciferous vegetable consumption but given the known problems of bias in case-control studies, coupled with the difficulty of estimating the consumption of phytochemicals from dietary records, the significance of these observations is difficult to assess.

3.2 Oesophageal cancer

The relationship between *GST* polymorphisms and risk of oesophageal cancer has been studied in some depth, and there is evidence that *GSTM1*-null individuals are at higher risk, at least in Asian populations[60]. However, a search for publications concerned with interactions between GST polymorphisms, cruciferous vegetable consumption and risk of oesophageal cancer produced no results.

3.3 Gastric cancer

In the same male cohort used by London *et al* [57] to study the effects of cruciferous vegetable consumption on risk of lung cancer in Shanghai, China, Moy *et al* [61] investigated the inter-

relationships between urinary isothiocyanate excretion, *GSTM1* and *GSTT1* genotype, and risk of gastric cancer in 18,244 middle-aged and older men. Overall, a higher level of urinary ITCs was associated with a lower risk of gastric cancer in this cohort, regardless of genotype (OR for highest tertile versus lowest tertile: 0.66, 95% CI 0.47 – 0.94). However, the association was larger in magnitude in those homozygous for deletion of *GSTM1* (OR for first tertile versus third tertile 0.5; 95% CI 0.27 – 0.93) or *GSTT1* (OR for first tertile versus third tertile 0.47; 95% CI 0.25 – 0.88) and largest for those with both deletions (OR for first tertile versus second and third tertiles 0.44; 95% CI 0.21 – 0.93). The authors concluded that, at least in their study population, the protective effect of cruciferous vegetable consumption was greatest in those with lower functional activity of the GSTM1 and GSTT1 subtypes.

3.4 Colorectal cancer

The first study to address the possible influence of *GST* polymorphisms on the relationship between cruciferous vegetable consumption and colorectal carcinogenesis was that of Lin et al [62], who explored the effects on the occurrence of adenomatous polyps, the precursor lesion for the majority of colorectal cancers, in a case-control study. The cases were 459 men and women in California USA, with adenomas in the distal colon, confirmed by flexible sigmoidoscopy, matched with 507 controls from the same population but shown to be free of adenomas. Intakes of cruciferous vegetables were assessed by questionnaire, and all subjects were genotyped for *GSTM1* polymorphisms. Subjects in the highest quartile for intake of broccoli had a lower risk of polyps compared to those who reported never consuming broccoli (OR 0.47; 95% Confidence interval 0.3 – 0.73) but the effect was only statistically significant in subjects who were null for *GSTM1* ($P<0.01$). There was weaker evidence for protective effects of cruciferous vegetables in general, but further analysis of the data for effects of brassica varieties other than broccoli was prevented by low levels of consumption. Another case-control study conducted in California USA by Slattery et al [63] looked for an interplay between colorectal cancer and consumption of cruciferous vegetables and coffee, both of which act

as inducers of GST, in subjects with and without the *GSTM1* deletion polymorphism. There were no associations for the group of 1579 cases and 1898 controls as a whole, but among *GSTM1*-null individuals less than 55 years of age, a protective effect of high consumption of cruciferous vegetables compared to no consumption was observed (OR 0.23 95% confidence interval 0.1 – 0.54), with the strongest effect evident for broccoli. Among younger individuals non-null for *GSTM1*, there was a lower risk of colorectal cancer, independently of any differences in consumption of cruciferous vegetables.

Seow *et al* [64] investigated the effects of ITC intake and GST polymorphisms on risk of colorectal cancer in a nested case-control study conducted within a large prospective trial, the Singapore Chinese Health Study. IST intakes were estimated from a food frequency questionnaire, and 213 cases of colorectal cancer were compared with 1194 controls, all of whom were genotyped for *GSTM1, GSTT1* and *GSTP1* polymorphisms. The results were consistent with those of Lin et al [62] in that amongst individuals null for both *GSTT1* and *GSTM1*, high consumers of ITCs were at significantly lower risk of colorectal cancer compared to low consumers (OR 0.43 95% confidence interval 0.20 – 0.96). A more comprehensive case-control study conducted by Turner *et al* [65] in the UK looked at the effects of meat, fruit and vegetable consumption, including cruciferous vegetables as a separate category, on risk of colorectal cancer in subjects genotyped for polymorphisms affecting six genes coding for metabolic enzymes (*GSTT1, GSTM1, GSTP1, CYP1A1, EPHX1, NQO1*). A protective effect of vegetables in general, and of cruciferous vegetables independently, was only detected in those with reduced expression of *GSTT1*. Some other interactions were detectable, but the authors commented that the multiple comparisons inherent in their study meant that their results needed independent corroboration.

Tijhuis *et al* [66] obtained results inconsistent with those of Turner *et al* in a case-control study of the effects of cruciferous vegetable consumption on risk of adenomatous polyps in a Dutch population. A group of 746 polyp cases were compared to 698 controls, for all of whom genetic

polymorphisms in *GSTM1, GSTT1, GSTP1* and *GSTA1* were determined. Across the whole population, higher consumption of cruciferous vegetables was associated with increased risk of polyps, though this relationship did not reach statistical significance (OR 1.15; 95% confidence interval 0.92 – 1.44). There was no detectable effect of *GSTM1* and/or *GSTT1* deletion polymorphisms in this study but the positive relationship between cruciferous vegetable intake and risk of polyps was only detectable in individuals with the *GSTP1* low-activity (GG) genotype (OR 1.94; 95% confidence interval 1.02 – 3.69). A similar effect was noted for the low expression (TT) polymorphism of *GSTA1*, although this relationship was weaker and not statistically significant (OR 1.57; 95% confidence interval 0.93 – 2.65). No attempt was made to analyse any effects of cruciferous vegetable consumption by variety. The authors concluded that variations in GST activity might modulate the relationship between consumption of cruciferous vegetables and colorectal adenomas, but not in a way consistent with their initial hypothesis or with the work of others.

Epplein *et al* [67] carried out a study of the relationship between urinary IST metabolite levels, GST polymorphisms and risk of CRC in a case-control study nested within a multi-ethnic cohort study based in Hawaii and California USA. Detection of IST metabolites in urine was associated with a statistically significant reduction in risk of CRC (OR 0.59; 95% confidence interval 0.36 – 0.98) relative to subjects with undetectable levels. There was no detectable effect of *GSTM1* or *GSTT1* deletions on this relationship and only a weak suggestion of an interaction such that those with *GSTP1* AG or GG genotypes and detectable urinary IST had greater protection against CRC compare to those with the *GSTP1* AA genotype. Yang *et al* [68] also explored the association between exposure to food-borne isothiocyanates and risk of colorectal cancer using urinary IST excretion, in a nested case-control study derived from a cohort of 74,942 women recruited between 1996 and 2000 in Shanghai, China. Urinary IST concentrations were again inversely related to risk of CRC, and the relationship was only statistically significant in women null for *GSTM1* ($p<0.04$) or for both *GSTM1* and *GSTT1* ($p< 0.03$), amongst whom the OR was 0.51 (95% CI 0.27 – 0.95). In contrast however,

Vogtmann et al [69] explored the same question, using a nested case-control study within the Shanghai Men's Heath cohort, but found no association between urinary IST and risk of CRC in the group as a whole, and no effect of *GST* polymorphisms.

The most recent systematic review on cruciferous vegetable consumption and colorectal cancer was published in 2014 by Tse and Eslick , and included a meta-analysis of six case-control [62, 63, 65, 66, 68, 70] and two nested case-control studies [64, 67] from which the association between cruciferous vegetable intake and risk of colorectal cancer could be quantified after stratification by *GSTM1* and *GSTT1* genotype. According to their analysis the only statistically significant result was a reduction in risk in high consumers of cruciferous vegetables who were homozygous for the *GSTT1* deletion genotype (OR 0.78; 95% CI 0.64 – 0.95). It should be noted that this meta-analysis did not include the study of Vogtmann et al [69], also published in 2014, which showed no significant effect of *GST* polymorphisms.

4.0 Discussion

All epidemiological investigations on diet and cancer are attempts to extract meaningful signals from a sea of necessarily noisy data; even the largest individual studies are rarely definitive. The European prospective investigation into cancer and nutrition (EPIC) for example is one of the largest studies of its type ever undertaken, with a final cohort of 477,312 individuals. No statistically significant protective effects of cabbage intake, or indeed vegetable intake in general, against gastric or other cancers of the gastrointestinal tract have been observed [71]. The strongest sources of evidence upon which to judge the protective effects of cruciferous vegetables are systematic reviews; fortunately those by Wu et al [41] and Tse and Eslick [45] on CRC are reasonably consistent in their findings. From a meta-analysis of 11 prospective studies Wu et al derived an RR of 0.93 (95% CI 0.87 – 1.0) for the highest versus lowest consumers of cruciferous vegetables, whereas Tse and Eslick obtained an OR of 0.92 (95% CI 0.83 – 1.01 for the same comparison). Tse and Eslick also reported a

slightly larger and more statistically significant protective effect for cancer of the colon only (OR 0.84 95% CI 0.72 – 0.98) [45]. Thus, both studies reported modest protective effects against CRC, with low or marginal statistical significance. It is worth noting that the prospective studies included in their analysis by Wu et al have appeared only since 2007, which perhaps explains why the WCRF reviews of 1997 [6] and 2007 [13], and the IARC report on cruciferous vegetables and cancer of 2004 [23] all acknowledged evidence in favour of protective effects against CRC, but lacked sufficient data to reach a definitive conclusion (See Table 1). It seems reasonable to conclude therefore that the two most recent systematic reviews provide sufficient evidence of a protective effect of cruciferous vegetable against CRC. To put the effect-size in context however, it should be noted that the reduction in risk is similar in magnitude to the protective effects reported for vegetables in general [72], and smaller than that reported for dietary fibre, another protective component of plant foods [73].

For cancers at sites other than the large bowel, there is less evidence available with which to form a definitive conclusion. The impact of diet on the risk of oropharyngeal and oesophageal cancers has received much less attention than for CRC, possibly because these cancers are less common than CRC in high income countries. More data are available for gastric cancer, and the systematic review of Wu et al from 2013 [40] indicates a reduction in risk of 19% in the highest consumers of cruciferous vegetables compared to the lowest (Table 1). This finding was based on six prospective studies, in contrast to the eleven included in the meta-analysis on cruciferous vegetables and CRC by the same authors [41], but it seems reasonable to conclude that the gastric epithelia are also sensitive to protective effects from the components of cruciferous vegetables, and these benefits may be larger in magnitude. This possibility should receive more attention from both experimental and epidemiological researchers.

Despite the balance of evidence in favour of effects of cruciferous vegetable consumption against both CRC and gastric cancer, it is not yet possible to conclude that the mechanisms are influenced

consistently by genetic polymorphisms of the genes coding for the GST enzyme family. The mechanistic hypothesis that lower expression of the principal GSTs involved in the metabolism and excretion of absorbed ITCs leads to higher and/or more prolonged exposure of target tissues to active compunds has received support from some, but by no means all, of the epidemiological studies designed to test it. That the issue remains unresolved is evident from the meta-analysis of Tse & Eslick [45], which identified only one statistically significant protective association between cruciferous vegetable consumption and the presence of the *GSTT1* null genotype. The possibility remains that this is a chance finding; the authors themselves concluded that there was little evidence to support the hypothesis of protective null *GST* genotypes, at least in the context of colorectal neoplasms.

The difficulty of testing hypotheses relating to GST polymorphisms and the consumption of cruciferous vegetables at the population level is probably largely a reflection of the uncertainties associated with the assessment of dietary exposure to glucosinolate breakdown products. Dietary assessment by means of food frequency questionnaires is prone to error, and the glucosinolate content of the cruciferous vegetables consumed varies with variety, season, storage and cooking methods. The development of reliable and accurate analytical techniques, including the 1,2-benzenedithiol-based cyclocondensation assay [74] and improved HPLC-mass spectrometry methods for isothiocyanate [75] and indole derivatives [76], has greatly facilitated the detection and quantification of isothiocyanates and indoles in foods, plasma and urine. The use of urinary markers to measure dietary exposure to cruciferous vegetables is a potentially valuable advance. Although statistically significant correlations between urinary ITC levels and self-reported intake of cruciferous vegetables can be detected in human populations [50], the clearance of glucosinolate breakdown products from plasma is relatively rapid [77]. Spot urine samples are therefore an indicator of recent intake rather than long-term exposure. Improved sampling strategies, including 24h urine collections repeated at regular intervals, are needed to optimise the estimation of long-term

exposure to isothiocyanates and their metabolites, but this will be difficult to achieve in large population samples.

Apart from the problem of accurately measuring exposure, there are other variables that potentially hamper the detection of biological effects of cruciferous vegetable consumption in culturally diverse populations. For example, the quantities and varieties of cruciferous vegetables eaten by American, European and Asian populations differ markedly. Chinese populations have been reported to consume as much as 80 -100 g of cruciferous vegetables per day [78], whereas intakes as low as 19 g per day have been reported for Dutch adults [79]. Moreover oriental populations tend to consume a higher proportion of their cruciferous vegetables as pak choi and similar varieties [78]. In laboratory studies, glucosinolate breakdown products from broccoli and pak choi exert markedly different effects on both gene expression and inflammation induced carcinogenesis in mouse colon [80]. Together with the differing frequencies of *GSTT1* and *GSTM1* null genotypes in Caucasian and Asian populations, variations in consumption pattern need to be carefully considered when interpreting epidemiological findings from different geographical regions.

5.0 References

1 Arnold M., Sierra M. S., Laversanne M., Soerjomataram I., Jemal A., Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017, *66*, 683-691.

2 Katanoda K., Hori M., Matsuda T., Shibata A., Nishino Y., Hattori M., Soda M., Ioka A., Sobue T., Nishimoto H. An updated report on the trends in cancer incidence and mortality in Japan, 1958-2013. *Jpn J Clin Oncol* 2015, *45*, 390-401.

3 Key T. J., Allen N. E., Spencer E. A., Travis R. C. The effect of diet on risk of cancer. *Lancet* 2002, *360*, 861-8.

4 Block G., Patterson B., Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer* 1992, *18*, 1-29.

5 Steinmetz K. A., Potter J. D. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* 1991, *2*, 325-57.

6 WCRF/AICR. Food, Nutrition and the Prevention of Cancer: a Global Perspective. Washington DC: American Institute for Cancer Research, 1997; 216-251

7 Johnson I. T. Mechanisms and possible anticarcinogenic effects of diet related apoptosis in colorectal mucosa. *Nutrition Research Reviews* 2001, *14*, 229-256.

8 Mithen R. F., Dekker M., Verkerk R., Rabot S., Johnson I. T. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agriculture* 2000, *80*, 967-984.

9 Higdon J. V., Delage B., Williams D. E., Dashwood R. H. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol Res* 2007, *55*, 224-36.

10 Shield K. D., Ferlay J., Jemal A., Sankaranarayanan R., Chaturvedi A. K., Bray F., Soerjomataram I. The global incidence of lip, oral cavity, and pharyngeal cancers by subsite in 2012. *CA Cancer J Clin* 2017, *67*, 51-64.

11 Walvik L., Svensson A. B., Friborg J., Lajer C. B. The association between human papillomavirus and oropharyngeal squamous cell Carcinoma: Reviewed according to the Bradford Hill criteria for causality. *Oral Oncol* 2016, *63*, 61-65.

12 Franceschi S., Levi F., La Vecchia C., Conti E., Dal Maso L., Barzan L., Talamini R. Comparison of the effect of smoking and alcohol drinking between oral and pharyngeal cancer. *Int J Cancer* 1999, *83*, 1-4.

13 WCRF/AICR. Food, Nutrition, Physical Activity and the Prevention of Cancer: A global perspective. Washington, 2007

14 Verhoeven D. T., Goldbohm R. A., van Poppel G., Verhagen H., van den Brandt P. A. Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiol Biomarkers Prev* 1996, *5*, 733-48.

15 Gridley G., McLaughlin J. K., Block G., Blot W. J., Winn D. M., Greenberg R. S., Schoenberg J. B., Preston-Martin S., Austin D. F., Fraumeni J. F., Jr. Diet and oral and pharyngeal cancer among blacks. *Nutr Cancer* 1990, *14*, 219-25.

16 McLaughlin J. K., Gridley G., Block G., Winn D. M., Preston-Martin S., Schoenberg J. B., Greenberg R. S., Stemhagen A., Austin D. F., Ershow A. G., et al. Dietary factors in oral and pharyngeal cancer. *J Natl Cancer Inst* 1988, *80*, 1237-43.

17 Chainani-Wu N. Diet and oral, pharyngeal, and esophageal cancer. *Nutr Cancer* 2002, *44*, 104-26.

18 WCRF/AICR. Food, nutrition and the prevention of Cancer: a global perspective. Washington DC, USA.: American Institute for Cancer Research, 1997

19 Kjaerheim K., Gaard M., Andersen A. The role of alcohol, tobacco, and dietary factors in upper aerogastric tract cancers: a prospective study of 10,900 Norwegian men. *Cancer Causes Control* 1998, *9*, 99-108.

20 Hebert J. R., Landon J., Miller D. R. Consumption of meat and fruit in relation to oral and esophageal cancer: a cross-national study. *Nutr Cancer* 1993, *19*, 169-79.

21 Rajkumar T., Sridhar H., Balaram P., Vaccarella S., Gajalakshmi V., Nandakumar A., Ramdas K., Jayshree R., Munoz N., Herrero R., Franceschi S., Weiderpass E. Oral cancer in Southern India: the influence of body size, diet, infections and sexual practices. *Eur J Cancer Prev* 2003, *12*, 135-43.

22 Zheng T., Boyle P., Willett W. C., Hu H., Dan J., Evstifeeva T. V., Niu S., MacMahon B. A case-control study of oral cancer in Beijing, People's Republic of China. Associations with nutrient intakes, foods and food groups. *Eur J Cancer B Oral Oncol* 1993, *29B*, 45-55.

23 IARC. Cruciferous vegetables, isothiocyanates and indoles. IARC Handbooks of Cancer Prevention volume 9. Lyon: IARC Press, 2004

24 Bosetti C., Filomeno M., Riso P., Polesel J., Levi F., Talamini R., Montella M., Negri E., Franceschi S., La Vecchia C. Cruciferous vegetables and cancer risk in a network of case-control studies. *Ann Oncol* 2012, *23*, 2198-203.

25 Jemal A., Center M. M., DeSantis C., Ward E. M. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010, *19*, 1893-907.

26 Freedman N. D., Murray L. J., Kamangar F., Abnet C. C., Cook M. B., Nyren O., Ye W., Wu A. H., Bernstein L., Brown L. M., Ward M. H., Pandeya N., Green A. C., Casson A. G., Giffen C., Risch H. A., Gammon M. D., Chow W. H., Vaughan T. L., Corley D. A., Whiteman D. C. Alcohol intake and risk of oesophageal adenocarcinoma: a pooled analysis from the BEACON Consortium. *Gut* 2011, *60*, 1029-37.

27 Kmet J., Mahboubi E. Esophageal cancer in the Caspian littoral of Iran: initial studies. *Science* 1972, *175*, 846-53.

28 Li W. Q., Hu N., Hyland P. L., Gao Y., Wang Z. M., Yu K., Su H., Wang C. Y., Wang L. M., Chanock S. J., Burdett L., Ding T., Qiao Y. L., Fan J. H., Wang Y., Xu Y., Shi J. X., Gu F., Wheeler W., Xiong X. Q., Giffen C., Tucker M. A., Dawsey S. M., Freedman N. D., Abnet C. C., Goldstein A. M., Taylor P. R. Genetic variants in DNA repair pathway genes and risk of esophageal squamous cell carcinoma and gastric adenocarcinoma in a Chinese population. *Carcinogenesis* 2013, *34*, 1536-42.

29 Kim R., Weissfeld J. L., Reynolds J. C., Kuller L. H. Etiology of Barrett's metaplasia and esophageal adenocarcinoma [see comments]. *Cancer Epidemiol Biomarkers Prev* 1997, *6*, 369-77.

30 Ronkainen J., Aro P., Storskrubb T., Johansson S. E., Lind T., Bolling-Sternevald E., Vieth M., Stolte M., Talley N. J., Agreus L. Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology* 2005, *129*, 1825-31.

31 Castro C., Peleteiro B., Lunet N. Modifiable factors and esophageal cancer: a systematic review of published meta-analyses. *J Gastroenterol* 2017

32 Brown L. M., Swanson C. A., Gridley G., Swanson G. M., Schoenberg J. B., Greenberg R. S., Silverman D. T., Pottern L. M., Hayes R. B., Schwartz A. G. Adenocarcinoma of the esophagus: role of obesity and diet. *J Natl Cancer Inst* 1995, *87*, 104-9.

33 Brown L. M., Swanson C. A., Gridley G., Swanson G. M., Silverman D. T., Greenberg R. S., Hayes R. B., Schoenberg J. B., Pottern L. M., Schwartz A. G., Liff J. M., Hoover R., Fraumeni J. F., Jr. Dietary factors and the risk of squamous cell esophageal cancer among black and white men in the United States. *Cancer Causes Control* 1998, *9*, 467-74.

34 Freedman N. D., Park Y., Subar A. F., Hollenbeck A. R., Leitzmann M. F., Schatzkin A., Abnet C. C. Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. *Int J Cancer* 2007, *121*, 2753-60.

35 Gonzalez C. A., Pera G., Agudo A., Bueno-de-Mesquita H. B., Ceroti M., Boeing H., Schulz M., Del Giudice G., Plebani M., Carneiro F., Berrino F., Sacerdote C., Tumino R., Panico S., Berglund G., Siman H., Hallmans G., Stenling R., Martinez C., Dorronsoro M., Barricarte A., Navarro C., Quiros J. R., Allen N., Key T. J., Bingham S., Day N. E., Linseisen J., Nagel G., Overvad K., Jensen M. K., Olsen A., Tjonneland A., Buchner F. L., Peeters P. H., Numans M. E., Clavel-Chapelon F., Boutron-Ruault M. C., Roukos D., Trichopoulou A., Psaltopoulou T., Lund E., Casagrande C., Slimani N., Jenab M., Riboli E. Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). *Int J Cancer* 2006, *118*, 2559-66.

36 Keszei A. P., Schouten L. J., Driessen A. L., Huysentruyt C. J., Keulemans Y. C., Goldbohm R. A., van den Brandt P. A. Vegetable, fruit and nitrate intake in relation to the risk of Barrett's oesophagus in a large Dutch cohort. *Br J Nutr* 2014, *111*, 1452-62.

37 Global Burden of Disease Cancer C., Fitzmaurice C., Allen C., Barber R. M., Barregard L., Bhutta Z. A., Brenner H., Dicker D. J., Chimed-Orchir O., Dandona R., Dandona L., Fleming T., Forouzanfar M. H., Hancock J., Hay R. J., Hunter-Merrill R., Huynh C., Hosgood H. D., Johnson C. O., Jonas J. B., Khubchandani J., Kumar G. A., Kutz M., Lan Q., Larson H. J., Liang X., Lim S. S., Lopez A. D., MacIntyre M. F., Marczak L., Marquez N., Mokdad A. H., Pinho C., Pourmalek F., Salomon J. A., Sanabria J. R., Sandar L., Sartorius B., Schwartz S. M., Shackelford K. A., Shibuya K., Stanaway J., Steiner C., Sun J., Takahashi K., Vollset S. E., Vos T., Wagner J. A., Wang H., Westerman R., Zeeb H., Zoeckler L., Abd-Allah F., Ahmed M. B., Alabed S., Alam N. K., Aldhahri S. F., Alem G., Alemayohu M. A., Ali R., Al-Raddadi R., Amare A., Amoako Y., Artaman A., Asayesh H., Atnafu N., Awasthi A., Saleem H. B., Barac A., Bedi N., Bensenor I., Berhane A., Bernabe E., Betsu B., Binagwaho A., Boneya D., Campos-Nonato I., Castaneda-Orjuela C., Catala-Lopez F., Chiang P., Chibueze C., Chitheer A., Choi J. Y., Cowie B., Damtew S., das Neves J., Dey S., Dharmaratne S., Dhillon P., Ding E., Driscoll T., Ekwueme D., Endries A. Y., Farvid M., Farzadfar F., Fernandes J., Fischer F., TT G. H., Gebru A., Gopalani S., Hailu A., Horino M., Horita N., Husseini A., Huybrechts I., Inoue M., Islami F., Jakovljevic M., James S., Javanbakht M., Jee S. H., Kasaeian A., Kedir M. S., Khader Y. S., Khang Y. H., Kim D., Leigh J., Linn S., Lunevicius R., El Razek H. M. A., Malekzadeh R., Malta D. C., Marcenes W., Markos D., Melaku Y. A., Meles K. G., Mendoza W., Mengiste D. T., Meretoja T. J., Miller T. R., Mohammad K. A., Mohammadi A., Mohammed S., Moradi-Lakeh M., Nagel G., Nand D., Le

Nguyen Q., Nolte S., Ogbo F. A., Oladimeji K. E., Oren E., Pa M., Park E. K., Pereira D. M., Plass D., Qorbani M., Radfar A., Rafay A., Rahman M., Rana S. M., Soreide K., Satpathy M., Sawhney M., Sepanlou S. G., Shaikh M. A., She J., Shiue I., Shore H. R., Shrime M. G., So S., Soneji S., Stathopoulou V., Stroumpoulis K., Sufiyan M. B., Sykes B. L., Tabares-Seisdedos R., Tadese F., Tedla B. A., Tessema G. A., Thakur J. S., Tran B. X., Ukwaja K. N., Uzochukwu B. S. C., Vlassov V. V., Weiderpass E., Wubshet Terefe M., Yebyo H. G., Yimam H. H., Yonemoto N., Younis M. Z., Yu C., Zaidi Z., Zaki M. E. S., Zenebe Z. M., Murray C. J. L., Naghavi M. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol* 2017, *3*, 524-548.

38 Tsukamoto T., Nakagawa M., Kiriyama Y., Toyoda T., Cao X. Prevention of Gastric Cancer: Eradication of Helicobacter Pylori and Beyond. *Int J Mol Sci* 2017, *18*

39 IARC. Fruit and Vegetables. IARC Handbooks of Cancer Prevention volume 8. Lyon: IARC Press, 2003

40 Wu Q. J., Yang Y., Wang J., Han L. H., Xiang Y. B. Cruciferous vegetable consumption and gastric cancer risk: a meta-analysis of epidemiological studies. *Cancer Sci* 2013, *104*, 1067-73.

41 Wu Q. J., Yang Y., Vogtmann E., Wang J., Han L. H., Li H. L., Xiang Y. B. Cruciferous vegetables intake and the risk of colorectal cancer: a meta-analysis of observational studies. *Ann Oncol* 2013, *24*, 1079-87.

42 Tomasetti C., Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* 2015, *347*, 78-81.

43 Krul C., Humblot C., Philippe C., Vermeulen M., Van Nuenen M., Havenaar R., Rabot S. Metabolism of sinigrin (2-propenyl glucosinolate) by the human colonic microflora in a dynamic in vitro large-intestinal model. *Carcinogenesis* 2002, *23*, 1009-16.

44 Steinmetz K. A., Kushi L. H., Bostick R. M., Folsom A. R., Potter J. D. Vegetables, fruit, and colon cancer in the Iowa Women's Health Study. *Am J Epidemiol* 1994, *139*, 1-15.

45 Tse G., Eslick G. D. Cruciferous vegetables and risk of colorectal neoplasms: a systematic review and meta-analysis. *Nutr Cancer* 2014, *66*, 128-39.

46 Higgins L. G., Hayes J. D. Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents. *Drug Metab Rev* 2011, *43*, 92-137.

47 Lampe J. W., Peterson S. Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. *J Nutr* 2002, *132*, 2991-4.

48 Hecht S. S. Chemoprevention by isothiocyanates. *J Cell Biochem Suppl* 1995, *22*, 195-209.

49 Chung F. L., Jiao D., Getahun S. M., Yu M. C. A urinary biomarker for uptake of dietary isothiocyanates in humans. *Cancer Epidemiol Biomarkers Prev* 1998, *7*, 103-8.

50 Vogtmann E., Yang G., Li H. L., Wang J., Han L. H., Wu Q. J., Xie L., Cai Q., Li G. L., Waterbor J. W., Levitan E. B., Zhang B., Gao Y. T., Zheng W., Xiang Y. B., Shu X. O. Correlates of self-reported dietary cruciferous vegetable intake and urinary isothiocyanate from two cohorts in China. *Public Health Nutr* 2015, *18*, 1237-44.

51 Cotton S. C., Sharp L., Little J., Brockton N. Glutathione S-transferase polymorphisms and colorectal cancer: a HuGE review. *Am J Epidemiol* 2000, *151*, 7-32.

52 Harries L. W., Stubbins M. J., Forman D., Howard G. C., Wolf C. R. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* 1997, *18*, 641-4.

53 McIlwain C. C., Townsend D. M., Tew K. D. Glutathione S-transferase polymorphisms: cancer incidence and therapy. *Oncogene* 2006, *25*, 1639-48.

54 Navarro S. L., Chang J. L., Peterson S., Chen C., King I. B., Schwarz Y., Li S. S., Li L., Potter J. D., Lampe J. W. Modulation of human serum glutathione S-transferase A1/2 concentration by cruciferous vegetables in a controlled feeding study is influenced by GSTM1 and GSTT1 genotypes. *Cancer Epidemiol Biomarkers Prev* 2009, *18*, 2974-8.

55 Spitz M. R., Duphorne C. M., Detry M. A., Pillow P. C., Amos C. I., Lei L., de Andrade M., Gu X., Hong W. K., Wu X. Dietary intake of isothiocyanates: evidence of a joint effect with glutathione S-transferase polymorphisms in lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2000, *9*, 1017-20.

56 Wang L. I., Giovannucci E. L., Hunter D., Neuberg D., Su L., Christiani D. C. Dietary intake of Cruciferous vegetables, Glutathione S-transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. *Cancer Causes Control* 2004, *15*, 977-85.

57 London S. J., Yuan J. M., Chung F. L., Gao Y. T., Coetzee G. A., Ross R. K., Yu M. C. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. *Lancet* 2000, *356*, 724-9.

58 Gaudet M. M., Olshan A. F., Poole C., Weissler M. C., Watson M., Bell D. A. Diet, GSTM1 and GSTT1 and head and neck cancer. *Carcinogenesis* 2004, *25*, 735-40.

59 Karen-Ng L. P., Marhazlinda J., Rahman Z. A., Yang Y. H., Jalil N., Cheong S. C., Zain R. B. Combined effects of isothiocyanate intake, glutathione S-transferase polymorphisms and risk habits for age of oral squamous cell carcinoma development. *Asian Pac J Cancer Prev* 2011, *12*, 1161-6.

60 Lu Q. J., Bo Y. C., Zhao Y., Zhao E. J., Sapa W. B., Yao M. J., Duan D. D., Zhu Y. W., Lu W. Q., Yuan L. Glutathione S-transferase M1 polymorphism and esophageal cancer risk: An updated meta-analysis based on 37 studies. *World J Gastroenterol* 2016, *22*, 1911-8.

61 Moy K. A., Yuan J. M., Chung F. L., Wang X. L., Van Den Berg D., Wang R., Gao Y. T., Yu M. C. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms and gastric cancer risk: a prospective study of men in Shanghai, China. *Int J Cancer* 2009, *125*, 2652-9.

62 Lin H. J., Probst-Hensch N. M., Louie A. D., Kau I. H., Witte J. S., Ingles S. A., Frankl H. D., Lee E. R., Haile R. W. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 1998, *7*, 647-52.

63 Slattery M. L., Kampman E., Samowitz W., Caan B. J., Potter J. D. Interplay between dietary inducers of GST and the GSTM-1 genotype in colon cancer. *Int J Cancer* 2000, *87*, 728-33.

64 Seow A., Yuan J. M., Sun C. L., Van Den Berg D., Lee H. P., Yu M. C. Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. *Carcinogenesis* 2002, *23*, 2055-61.

65 Turner F., Smith G., Sachse C., Lightfoot T., Garner R. C., Wolf C. R., Forman D., Bishop D. T., Barrett J. H. Vegetable, fruit and meat consumption and potential risk modifying genes in relation to colorectal cancer. *Int J Cancer* 2004, *112*, 259-64.

66 Tijhuis M. J., Wark P. A., Aarts J. M., Visker M. H., Nagengast F. M., Kok F. J., Kampman E. GSTP1 and GSTA1 polymorphisms interact with cruciferous vegetable intake in colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev* 2005, *14*, 2943-51.

67 Epplein M., Wilkens L. R., Tiirikainen M., Dyba M., Chung F. L., Goodman M. T., Murphy S. P., Henderson B. E., Kolonel L. N., Le Marchand L. Urinary isothiocyanates; glutathione S-transferase M1, T1, and P1 polymorphisms; and risk of colorectal cancer: the Multiethnic Cohort Study. *Cancer Epidemiol Biomarkers Prev* 2009, *18*, 314-20.

68 Yang G., Gao Y. T., Shu X. O., Cai Q., Li G. L., Li H. L., Ji B. T., Rothman N., Dyba M., Xiang Y. B., Chung F. L., Chow W. H., Zheng W. Isothiocyanate exposure, glutathione S-transferase polymorphisms, and colorectal cancer risk. *Am J Clin Nutr* 2010, *91*, 704-11.

69 Vogtmann E., Xiang Y. B., Li H. L., Cai Q., Wu Q. J., Xie L., Li G. L., Yang G., Waterbor J. W., Levitan E. B., Zhang B., Zheng W., Shu X. O. Cruciferous vegetables, glutathione S-transferase polymorphisms, and the risk of colorectal cancer among Chinese men. *Ann Epidemiol* 2014, *24*, 44-9.

70 Lin H. J., Zhou H., Dai A., Huang H. F., Lin J. H., Frankl H. D., Lee E. R., Haile R. W. Glutathione transferase GSTT1, broccoli, and prevalence of colorectal adenomas. *Pharmacogenetics* 2002, *12*, 175-9.

71 Gonzalez C. A., Lujan-Barroso L., Bueno-de-Mesquita H. B., Jenab M., Duell E. J., Agudo A., Tjonneland A., Boutron-Ruault M. C., Clavel-Chapelon F., Touillaud M., Teucher B., Kaaks R., Boeing H., Steffen A., Trichopoulou A., Roukos D., Karapetyan T., Palli D., Tagliabue G., Mattiello A., Tumino R., Ricceri F., Siersema P. D., Numans M. E., Peeters P. P., Parr C. L., Skeie G., Lund E., Quiros J. R., Sanchez-Cantalejo E., Navarro C., Barricarte A., Dorronsoro M., Ehrnstrom R., Regner S., Khaw K. T., Wareham N., Key T. J., Crowe F. L., Blaker H., Romieu I.,

Riboli E. Fruit and vegetable intake and the risk of gastric adenocarcinoma: a reanalysis of the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study after a longer follow-up. *Int J Cancer* 2012, *131*, 2910-9.

72 Vieira A. R., Abar L., Chan D. S. M., Vingeliene S., Polemiti E., Stevens C., Greenwood D., Norat T. Foods and beverages and colorectal cancer risk: a systematic review and meta-analysis of cohort studies, an update of the evidence of the WCRF-AICR Continuous Update Project. *Ann Oncol* 2017, *28*, 1788-1802.

73 Aune D., Chan D. S., Lau R., Vieira R., Greenwood D. C., Kampman E., Norat T. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *Bmj* 2011, *343*, d6617.

74 Zhang Y. The 1,2-benzenedithiole-based cyclocondensation assay: a valuable tool for the measurement of chemopreventive isothiocyanates. *Crit Rev Food Sci Nutr* 2012, *52*, 525-32.

75 Egner P. A., Kensler T. W., Chen J. G., Gange S. J., Groopman J. D., Friesen M. D. Quantification of sulforaphane mercapturic acid pathway conjugates in human urine by high-performance liquid chromatography and isotope-dilution tandem mass spectrometry. *Chem Res Toxicol* 2008, *21*, 1991-6.

76 Fujioka N., Ransom B. W., Carmella S. G., Upadhyaya P., Lindgren B. R., Roper-Batker A., Hatsukami D. K., Fritz V. A., Rohwer C., Hecht S. S. Harnessing the Power of Cruciferous Vegetables: Developing a Biomarker for Brassica Vegetable Consumption Using Urinary 3,3'-Diindolylmethane. *Cancer Prev Res (Phila)* 2016, *9*, 788-793.

77 Gasper A. V., Al-Janobi A., Smith J. A., Bacon J. R., Fortun P., Atherton C., Taylor M. A., Hawkey C. J., Barrett D. A., Mithen R. F. Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. *Am J Clin Nutr* 2005, *82*, 1283-91.

78 Fowke J. H., Shu X. O., Dai Q., Shintani A., Conaway C. C., Chung F. L., Cai Q., Gao Y. T., Zheng W. Urinary isothiocyanate excretion, brassica consumption, and gene polymorphisms among women living in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2003, *12*, 1536-9.

79 Wark P. A., Grubben M. J., Peters W. H., Nagengast F. M., Kampman E., Kok F. J., van 't Veer P. Habitual consumption of fruits and vegetables: associations with human rectal glutathione S-transferase. *Carcinogenesis* 2004, *25*, 2135-42.

80 Lippmann D., Lehmann C., Florian S., Barknowitz G., Haack M., Mewis I., Wiesner M., Schreiner M., Glatt H., Brigelius-Flohe R., Kipp A. P. Glucosinolates from pak choi and broccoli induce enzymes and inhibit inflammation and colon cancer differently. *Food Funct* 2014, *5*, 1073-81.

Table 1 Results of published meta-analyses of effects of higher cruciferous vegetable consumption on risk of cancer at six anatomical sites in the human alimentary tract.

Reference	**Effects of higher consumption of cruciferous vegetables on relative risk.**					
	Oropharynx	**Oesophagus**	**Stomach**	**Colorectum**	**Colon**	**Rectum**
IARC, 2004 [23]	Insufficient data for meta-analysis	Insufficient data for meta-analysis	Cohort studies All CV: OR 0.91 (0.67-1.23) Case-control studies All CV: OR 0.81 (0.73-0.90)	Cohort studies All CV: OR 0.96 (0.85-1.09) Case-control studies All CV: OR 0.73 (0.63-0.84)	N/A	N/A
Wu et al, 2013a [40]	N/A	N/A	Cohort studies All CV: RR 0.89 (0.77-1.02) Case-control studies All CV: RR 0.78 (0.71-0.86) Cohort studies (Cabbage) RR 0.77 (95% CI 0.56-1.06) Case-control studies (Cabbage) RR 0.66 (0.55-0.79)	N/A	N/A	N/A

Wu et al, 2013b [41]	N/A	N/A	N/A	Cohort studies All CV: RR 0.93 (0.87-1.00) Case-control studies All CV: RR 0.74 (0.65-0.84) Cohort studies (Cabbage) RR 0.96 (0.54-1.72) Case-control studies (Cabbage) RR 0.74 (0.57-0.97) Cohort studies (Broccoli) RR 0.91 (0.8-1.03) Case-control studies (Broccoli) RR 0.60 (0.32-1.13)	N/A	N/A
Tse & Eslick, 2014 [45]	N/A	N/A	N/A	All CV: RR 0.89 (0.77-1.03) Cabbage: RR 0.95 (0.80-1.14) Broccoli:	All CV: RR 0.84 (0.72-0.98)	All CV): RR 0.99 (0.67-1.46)

				RR 0.80 (0.65-0.99) Brussels sprouts: RR 1.00 (0.75-1.34)		

Footnotes. OR: odd ratio; RR: relative risk; All CV: All cruciferous vegetables; N/A: The study did not address risk of cancer at this site.

Graphical Abstract

IT Johnson

Higher consumption of cruciferous vegetables may protect against cancers of the gastrointestinal tract. A literature review of primary studies and meta-analyses indicates protective effects of 8% and 19% for colorectal and gastric cancers respectively. Some studies suggest that these protective effects are modified by common genetic polymorphisms of genes for the glutathione *S*-transferase enzyme family but the evidence remains inconclusive.