

Cruciferous vegetables and colo-rectal cancer

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Cruciferous vegetables have been studied extensively for their chemoprotective effects. Although they contain many bioactive compounds, the anti-carcinogenic actions of cruciferous vegetables are commonly attributed to their content of glucosinolates. Glucosinolates are relatively biologically inert but can be hydrolysed to a range of bioactive compounds such as isothiocyanates (ITC) and indoles by the plant-based enzyme myrosinase, or less efficiently by the colonic microflora. A number of mechanisms whereby ITC and indoles may protect against colo-rectal cancer have been identified. In experimental animals cruciferous vegetables have been shown to inhibit chemically-induced colon cancer. However, the results of recent epidemiological cohort studies have been inconsistent and this disparity may reflect a lack of sensitivity of such studies. Possible explanations for the failure of epidemiological studies to detect an effect include: assessment of cruciferous vegetable intake by methods that are subject to large measurement errors; the interaction between diet and genotype has not been considered: the effect that post-harvest treatments may have on biological effects of cruciferous vegetables has not been taken into account.

Cruciferous vegetables: Broccoli: Colo-rectal cancer

Colo-rectal cancer (CRC) is a major public health burden in Westernised societies. In the UK >33 000 new cases are diagnosed each year (Quinn *et al.* 2001). Inherited cancer syndromes, such as familial adenomatous polyposis, account for 5–10% CRC, but most appear to arise sporadically (Lynch & de la Chapelle, 2003). A number of environmental factors appear to influence risk, including alcohol intake, smoking, physical activity, non-steroidal anti-inflammatory drugs and diet (Giovannucci *et al.* 1994; Colditz *et al.* 1997; World Cancer Research Fund, 1997; Giovannucci, 2001).

Estimates vary, but ≤80% of the sporadic CRC may be attributable to diet (Bingham, 2000). Epidemiological and experimental studies have identified a number of putative risk factors and protective factors in the human diet. For example, some studies indicate that diets high in fat and meat may increase risk, whereas diets high in dietary fibre and vegetables may be protective (World Cancer Research Fund, 1997; Bingham *et al.* 2003; International Agency for Research on Cancer, 2003).

Cruciferous vegetables, which include the genus *Brassica*, have received much interest as possible cancer-protective components of the human diet (Verhoeven *et al.* 1996, 1997). In the mid 1990s a number of epidemiological reviews

concluded that the consumption of cruciferous or brassica vegetables is associated with a reduced risk of CRC (Steinmetz & Potter, 1996; Verhoeven *et al.* 1996; Kohlmeier & Su, 1997). However, at that time few results from cohort studies had been published, but more recent cohort studies have cast doubt on the strength of the inverse association between cruciferous vegetable intake and CRC (Steinmetz *et al.* 1994; Hsing *et al.* 1998; Pietinen *et al.* 1999; Michels *et al.* 2000; Voorrips *et al.* 2000; Flood *et al.* 2002; McCullough *et al.* 2003). However, in animal models of colon cancer cruciferous vegetables have consistently been shown to exhibit anti-carcinogenic effects (International Agency for Research on Cancer, 2004).

Mechanisms through which cruciferous vegetables may protect against colo-rectal cancer

Cruciferous vegetables contain a number of bioactive components such as folate, vitamin C, tocopherols, carotenoids and polyphenols (DeSouza & Eitenmiller, 1986; Price *et al.* 1998; Kurilich *et al.* 1999). However, the anti-carcinogenic actions of cruciferous vegetables are most frequently attributed to their content of glucosinolates

Abbreviations: ACF, aberrant crypt foci; CRC, colo-rectal cancer; CYP, cytochrome P; DMH, 1,2-dimethylhydrazine; GLS, glucosinolates; GST, glutathione S-transferases; I3C, indole-3-carbinol; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; ITC, isothiocyanates.

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(GLS; van Poppel *et al.* 1999; Lampe & Peterson, 2002). GLS are a group of compounds that have a common basic structure of a β -D-thioglucose and a sulfonated oxime moiety, but differ in the structure of their side chains, which may be aliphatic, aromatic or heterocyclic (Mithen *et al.* 2000; Holst & Williamson, 2004). GLS are historically regarded as biologically inert, but can be hydrolysed to a range of more bioactive products (Johnson, 2002a). The hydrolysis of GLS is largely dependent on the activity of a plant-based β -D-thioglucosidase enzyme, myrosinase (Bones & Rossiter, 1996). GLS and myrosinase appear to be separated within plant cells but they come into contact with each other on disruption of the cells. Myrosinase then catalyses the hydrolysis of the thioglucose bond leading to the production of an unstable aglucone that undergoes spontaneous rearrangement to form a range of breakdown products such as isothiocyanates (ITC), nitriles and S, oxazolidine-2-thiones and indolyl compounds (Holst & Williamson, 2004). The product formed depends on the side-chain structure of the parent GLS, pH, the presence of Fe^{2+} and, in the case of some GLS, the presence of a cofactor, epithiospecifier protein (Bones & Rossiter, 1996; Matusheski *et al.* 2004).

The mechanisms through which cruciferous vegetables and their GLS breakdown products may protect against CRC include the modulation of xenobiotic-metabolising enzymes, antioxidant effects, the induction of apoptosis and cell cycle arrest (Plumb *et al.* 1997; van Poppel *et al.* 1999; Smith *et al.* 2003, 2005).

Modulation of xenobiotic-metabolising enzymes

Chemical carcinogens can be classified as either direct acting or indirect acting, with the latter being the most common (Hodgson & Akunda, 2001). The activation and detoxification of carcinogens is largely catalysed by the phase 1 and phase 2 enzyme families that comprise the xenobiotic-metabolising system (Williams, 1967). Generally, phase 1 enzymes from the cytochrome P (CYP) 450 family catalyse the activation of indirect-acting carcinogens, whereas phase 2 enzymes such as the glutathione S-transferases (GST) catalyse the detoxification of both direct-acting and indirect-acting carcinogens (Talalay & Fahey, 2001). Thus, it has been suggested that shifting the balance of phase 1 and phase 2 enzymes in favour of the latter might be an effective chemoprevention strategy (Talalay, 2000). However, this hypothesis has been challenged for a number of reasons, including the fact that individual phase 2 enzymes often activate a specific class of chemicals (Paolini *et al.* 1999).

The induction of phase 2 enzymes has, however, been proposed as the major mechanism through which cruciferous vegetables protect against chemically induced tumours (Steinkellner *et al.* 2001; Jeffery & Stewart, 2004). An extra-colonic increase in phase 2 enzyme activity could protect the colon from carcinogens by reducing the delivery of partially-activated compounds from the systemic circulation (Pool-Zobel, 1999). Kassie *et al.* (2002, 2003a) have shown that in rodents exposed to the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) the ability of juices prepared from cruciferous vegetables to protect against the induction of aberrant crypt foci (ACF) is correlated with their ability to increase the activity of the phase 2 enzyme

UDP-glucuronosyl transferase form 2 in the liver. Cruciferous vegetables may also exert their protective effects through a local induction of phase 2 enzymes in the large intestine. In human subjects the consumption of 300 g Brussels sprouts/d for 1 week results in the elevation of rectal GST- α and - π isoenzymes (Nijhoff *et al.* 1995). Similarly, a diet containing 200 g freeze-dried broccoli (*Brassica oleracea* var. Majestic)/kg potently induces quinone reductase activity in the colonic mucosa of rodents (Keck *et al.* 2003).

Although cruciferous vegetables induce phase 2 enzymes, they also tend to induce phase 1 enzymes, particularly CYP1A1 and CYP1A2 (Vang *et al.* 1991, 2001; Wortelboer *et al.* 1992). The induction of CYP1A1 and CYP1A2 may sometimes be detrimental because they activate many carcinogens and may also increase the production of reactive oxygen species (Guengerich & Shimada, 1998; Paolini *et al.* 2004). GLS breakdown products are probably the primary compounds responsible for the induction of xenobiotic-metabolising enzymes after the consumption of cruciferous vegetables (Vang *et al.* 2001). Certain ITC, such as sulforaphane, appear to be potent monofunctional inducers of phase 2 enzymes (Fig. 1; Zhang *et al.* 1992). However, most cruciferous vegetables contain a range of GLS with variable modulatory effects on enzymes. For example, broccoli, which is the main dietary source of sulforaphane, also typically contains sizeable quantities of glucobrassicin and neo-glucobrassicin, which hydrolyse to form indole-3-carbinol (I3C; Kushad *et al.* 1999; Mithen *et al.* 2000). On exposure to the acid environment of the stomach, I3C can undergo several condensation reactions to form a range of dimers, trimers, tetramers and oligomers (McDanell *et al.* 1988). In contrast to sulforaphane, I3C and its acid condensation products induce both phase 1 and phase 2 enzymes (Fig. 1; Shertzer & Sainsbury, 1991; Nho & Jeffery, 2004). The effect of cruciferous-vegetable consumption on phase 1 and phase 2 enzyme activities is likely to be unpredictable, varying according to the GLS composition of the vegetable (which is governed by genotype, growing conditions and post-harvest treatment) and possibly the content of other bioactive compounds present at the time of consumption (Vang *et al.* 2001; International Agency for Research on Cancer, 2004; Jeffery & Stewart, 2004).

Evidence from animal models generally indicates that the joint induction of phase 1 and phase 2 enzymes by a variety of cruciferous vegetables results in a favourable metabolic profile for the elimination of certain chemical carcinogens (for a comprehensive review, see International Agency for Research on Cancer, 2004). However, it is unclear whether a similar chemoprotective effect would occur in free-living human subjects, who, in contrast to experimental animals, are chronically exposed to low doses of a wide variety of chemical carcinogens. The outcome of any shift in the balance of xenobiotic-metabolising enzymes is likely to be unpredictable, depending on the range of chemical carcinogens to which an individual is exposed (Paolini *et al.* 1999).

Antioxidant effects

The colonic epithelium may be subject to oxidative damage mediated by free radicals generated near its surface by the

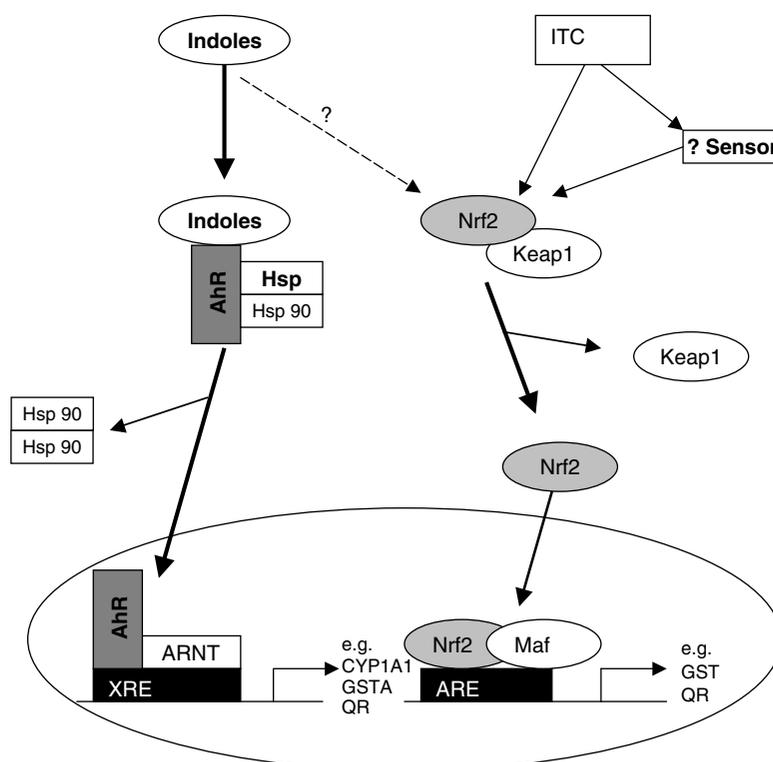


Fig. 1. Regulation of xenobiotic-metabolising enzymes by glucosinolates breakdown products (adapted from Hayes & McMahon, 2001; Lampe & Peterson, 2002). Isothiocyanates (ITC) are monofunctional inducers that interact with an antioxidant responsive element (ARE) that is present in the upstream enhancer region of the genes encoding a number of phase 2 enzymes such as quinone reductase (QR) and several glutathione S-transferases (GST) isoenzymes. Indoles have been classified as bifunctional inducers. Indoles enhance the transcription of a number of genes with xenobiotic-responsive elements (XRE) in their upstream enhancer regions, such as cytochrome P (CYP)1A1, CYP1A2, QR and GSTA. Indoles may also interact with the ARE possibly after metabolism by CYP450 or through some other as yet undefined mechanism (Hayes & McMahon, 2001; Nho & Jeffery, 2004). Hsp, heat-shock protein; AhR, aromatic hydrocarbon receptor; Nrf2, NF-E2-related factor-2; Keap1, Kelch-like ECH-associated protein 1; Maf, musculoaponeurotic fibrosarcoma; ARNT, aryl hydrocarbon receptor nuclear translator.

faecal matrix (Babbs, 1990; Stone *et al.* 2002). Cruciferous vegetables may contribute to the antioxidant defence of the colonic mucosa through non-enzymic and enzymic mechanisms. Various GLS breakdown products can interact with a common antioxidant-responsive element present in the upstream region of the genes encoding several phase 2 enzymes with known antioxidant actions, such as GST and quinone reductase (Fig. 1). Some cruciferous vegetables are also rich sources of carotenoids and polyphenolic compounds (Kurilich *et al.* 1999), which are antioxidants that are poorly absorbed in the proximal gastrointestinal tract and are therefore likely to reach the colon in substantial quantities (Halliwell *et al.* 2000). Within the colonic lumen, carotenoids and polyphenolic compounds may protect the colonic epithelium from free radical attack. Polyphenolic compounds may chelate Fe, thereby helping to reduce the pro-oxidant effect of colonic Fe, and both carotenoids and polyphenols may directly scavenge free radicals (Halliwell *et al.* 2000). Little work appears to have been conducted on the ability of cruciferous vegetables to alter the antioxidant

activity of the large bowel. However, preliminary work has indicated that pigs consuming raw broccoli have an increased faecal antioxidant level, as measured by electron spin resonance (Ratcliffe *et al.* 2001).

Induction of apoptosis

The induction of apoptosis may confer protection against CRC at every stage of the adenoma–carcinoma sequence. Cells with mutational DNA damage may be removed before the mutation becomes fixed. Clonal expansion may be prevented by the removal of initiated cells, and even established lesions may regress (Takayama *et al.* 1998; Johnson, 2002b). Some evidence indicates that cruciferous vegetables and their GLS breakdown products may exert some of their anti-carcinogenic effects through the induction of apoptosis. In highly-proliferative HT29 human colon cancer cells sulforaphane induces apoptosis in a dose-dependent manner (Gamet-Payraastre *et al.* 2000). Furthermore, sinigrin and Brussels sprouts induce

apoptosis in rodents when consumed after the administration of 1,2-dimethylhydrazine (DMH; Smith *et al.* 1998, 2003).

Cell cycle arrest

GLS breakdown products may be able to suppress cancer development in initiated cells by inhibiting cellular proliferation (Gamet-Payraastre *et al.* 2000; Smith *et al.* 2004). For example, allyl ITC causes human HT29 colo-rectal carcinoma cells to become blocked in the G₂M phase (Smith *et al.* 2004).

Cruciferous vegetables and experimental colon cancer

In animal models of colon cancer, cruciferous vegetables have generally been shown to inhibit chemical carcinogenesis. Brussels sprouts in particular have been shown to be protective (Rijken *et al.* 1999; Kassie *et al.* 2003a; Smith *et al.* 2003; Uhl *et al.* 2004). Kassie and associates (Kassie *et al.* 2003a; Uhl *et al.* 2004) have investigated whether juices prepared from two varieties of Brussels sprouts ('Maximus' and 'Cyrus') could provide protection during both cancer initiation and promotion. For rats administered IQ by gavage on ten alternate days supplementation of their drinking water with Brussels sprout juices was found to inhibit the development of ACF. In a further study using the same carcinogen-dosing schedule, the consumption of juice prepared from 'Maximus' Brussels sprouts was found to inhibit the formation of ACF when consumed in the period after IQ exposure (Uhl *et al.* 2004). In contrast, juices prepared from two varieties of red cabbage ('Reliant' and 'Roxy') were found to be much less effective, exerting only a small non-significant inhibiting effect during the initiation period and no effect during the promotion period (Kassie *et al.* 2003a). The authors have hypothesised that the lack of effect of red-cabbage juice might be explained by its 2–3-fold lower content of GLS. However, in another study (Hagiwara *et al.* 2002) an extract of the colour from red cabbage (incorporated into the diet at a level of 50 g/kg) that is devoid of GLS was shown to inhibit adenoma and carcinoma formation in rats initiated with DMH and subsequently fed a diet containing the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine at a concentration of 0.2 g/kg. The red-cabbage-colour extract was also found to protect against the formation of ACF in non-DMH-treated rats fed a diet containing 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

Other cruciferous vegetables have received relatively little attention. Single studies have found that garden cress (*Lepidium sativum*) juice and cruciferous seed meals and hulls are protective, whereas broccoli has been shown to have no effect (Barrett *et al.* 1998; Rijken *et al.* 1999; Kassie *et al.* 2002). The latter finding of no protective effect of broccoli against azoxymethane-induced ACF is somewhat surprising because the breakdown products of glucobrassicin and glucoraphanin, the major GLS found in broccoli, have been shown to inhibit azoxymethane-induced ACF (Chung *et al.* 2000; Kim *et al.* 2003).

There are only two reports of a possible colon-cancer-promoting effect of cruciferous vegetables (Temple & Basu, 1987; Temple & El-Khatib, 1987). In these studies female Swiss mice fed a diet supplemented with cabbage during initiation with DMH or throughout the experimental period were reported to exhibit a modest increase in tumour formation, although in both cases the effect was not significant.

Indoles and isothiocyanates

A variety of GLS hydrolysis products, including I3C, sulforaphane and phenethyl ITC, have been investigated for their ability to inhibit chemically-induced colon cancer (Pence *et al.* 1986; Pereira & Khoury 1991; Guo *et al.* 1995; Wargovich *et al.* 1996; Xu *et al.* 1996, 2001; Chung *et al.* 2000). There is consistent evidence that when heterocyclic amines are used to initiate colon cancer I3C is an effective inhibiting agent (Guo *et al.* 1995; Xu *et al.* 1996). For example, I3C fed at a level of 1 g/kg diet during the initiation phase, post initiation or during the whole experimental period inhibits ACF in male F344 rats exposed to 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (Guo *et al.* 1995; Xu *et al.* 1996). Similarly, I3C fed at a level of 1 g/kg diet inhibits the formation of IQ-induced ACF when fed to male F344 rats throughout the experimental period or during the post-initiation phase (Xu *et al.* 1996, 2001). However, there is some concern that when DMH is used to initiate colon cancer I3C may promote tumour formation. In an early study (Pence *et al.* 1986) an increased incidence of tumours (combined tally of small intestinal and colonic tumours) was found in rats fed I3C in their diet at 1 g/kg before, during and after DMH administration. In contrast, in a more recent study I3C was not found to promote tumour formation when administered after DMH exposure (Xu *et al.* 2001). A striking difference between the studies of Xu *et al.* (2001) and Pence *et al.* (1986) is the number of colon tumours found in the positive control group injected with DMH (87 and 0% respectively). Possible explanations include the higher DMH dose (20 mg/kg *v.* 10 mg/kg) and a greater time lapse from the last dose to the rats being killed (45 weeks *v.* 16 weeks) in the study of Xu *et al.* (2001). No firm conclusion can be drawn about the promoting effect of I3C from the study of Xu *et al.* (2001) because the very high level of tumours in the positive control group would make it difficult to identify any tumour-promoting effect of I3C. However, it is evident that I3C fails to exert a protective effect. Interestingly, I3C fed during the post-initiation phase inhibits ACF formation in male mice administered azoxymethane (a metabolite of DMH), perhaps indicating a species-specific effect (Kim *et al.* 2003).

Chung *et al.* (2000) have compared the ability of sulforaphane, phenethyl ITC and their respective N-acetylcysteine conjugates to prevent azoxymethane-induced ACF in male F344 rats. Sulforaphane and phenethyl ITC were both found to be effective at reducing total ACF and crypt multiplicity when administered during the initiation and promotion periods. In contrast, their respective N-acetylcysteine conjugates were only found to be effective when fed during the post-initiation period. Surprisingly, phenethyl ITC–N-acetylcysteine was found to increase the

formation of ACF when administered during the initiation period. In contrast to the study of Chung *et al.* (2000), two earlier studies have found no protective effect of phenethyl ITC against azoxymethane-induced ACF (Pereira & Khoury, 1991; Wargovich *et al.* 1996).

Epidemiological studies

At least seven cohort studies have investigated the association between the intake of cruciferous or brassica vegetables and colon, rectal or combined CRC risk (summarised in Fig. 2). Overall, the cohort studies provide little evidence for a protective effect of cruciferous vegetable intake against cancer of the large intestine. In fact, the most striking observation is that almost without exception the relative risks cluster around 1 and the 95% CI encompass 1. In only one study, in a Dutch population, is there a significant negative association between brassica vegetable consumption and colon cancer, and even then the effect is only observed in women (relative risk 0.51 (95% CI 0.50, 1.13), $P = 0.05$ for trend; Voorrips *et al.* 2000). Furthermore, for rectal cancer brassica vegetable consumption is associated with an increased risk in women (relative risk 1.66 (95% CI 0.94, 2.94), $P = 0.05$ for trend). Of further concern, a study of male smokers in Finland has found a significant increase in colon-cancer risk in the highest quintile of intake (relative risk 1.6 (95% CI 1.0, 2.4), $P = 0.05$ for trend; Pietinen *et al.* 1999). However, the study population exhibited an atypical consumption pattern with almost no intake of green cruciferous vegetables and the highest overall consumption levels were very low, the median level in the highest category of intake being 38 g/d.

So what factors may explain why large-scale prospective epidemiological studies generally fail to support the hypothesis that cruciferous vegetables protect against colon cancer, whereas animal studies have generally observed chemoprotective effects and experimental studies have identified a number of plausible mechanisms of action? One factor that may need to be considered is that relatively large doses of cruciferous vegetables or GLS exhibit chemoprotection, but normal habitual levels of intake of cruciferous vegetables have no effect. However, a number of limitations in the published cohort studies may have reduced their sensitivity to detect a modest protective effect of cruciferous vegetable consumption. The cohort studies all used food-frequency questionnaires to measure cruciferous vegetable intake. When validated against repeated weighed intakes food-frequency questionnaires tend to perform badly in comparison with other methods of dietary assessment, overestimating vegetable consumption in particular (Bingham *et al.* 1994). The failure of food-frequency questionnaires to accurately assess vegetable intake could lead to an overestimation of vegetable intake and/or misclassification of subjects into the wrong category of consumption. Consistent overestimation of intake among all subjects would not preclude studies from finding an association between cruciferous vegetable intake and CRC, but would create a false impression of the range of intake that does or does not have an effect on cancer risk. Despite the fact that cohort studies may have overestimated the intake

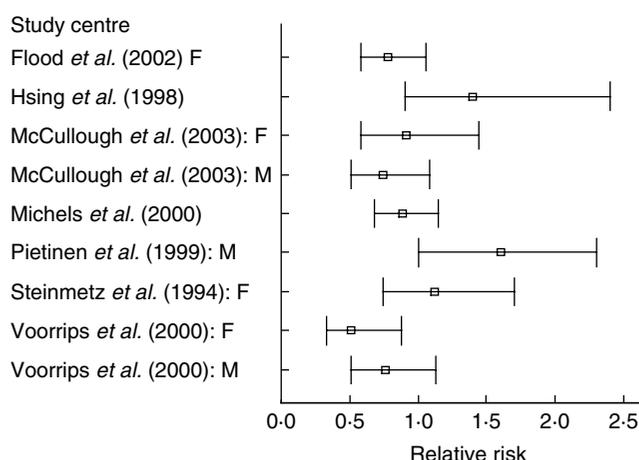


Fig. 2. Cohort studies of colon cancer and cruciferous vegetable consumption. Values are relative risks and 95% CI represented by horizontal bars. F, females; M, males.

of cruciferous vegetables, reported values are still low. For example, in the Cancer Prevention II study cohort, the highest quintile of cruciferous vegetable intake was reported to be half a portion or more per d in women and even lower in men (McCullough *et al.* 2003).

Misclassification of subjects is a potentially more serious problem than overestimation. Comparison of the ability of different methods of dietary assessment to classify individuals into the same quartile of consumption as 16 d weighed records indicates that food-frequency questionnaires correctly classify 30–50% of individuals for most nutrients (Bingham *et al.* 1994). Such an extent of misclassification would attenuate relative-risk estimates and make modest protective effects very difficult to detect.

The ability of cruciferous vegetables to inhibit CRC may be influenced by the GST genotype of the individual (Lin *et al.* 1998; Slattery *et al.* 2000; Seow *et al.* 2002). Thus, the failure of the published cohort studies to consider the modulatory effect of GST genotype may have weakened their sensitivity to detect a protective effect of cruciferous vegetable consumption. The GST are involved in the detoxification of a range of chemical carcinogens and are responsible for the metabolism and excretion of ITC after cruciferous vegetable consumption (Hayes & Pulford, 1995; Kolm *et al.* 1995). A recent nested case-control study has explored the relationship between cruciferous vegetable consumption (measured as ITC intake, using a semi-quantitative food-frequency questionnaire), genotype and CRC risk. In an initial comparison of 213 cases of CRC with 1194 controls selected from the prospective Singapore Chinese Health Study (Seow *et al.* 2002), only a small non-significant reduction in risk was found in subjects with the highest intake of ITC (odds ratio 0.81 (95% CI 0.59, 1.12)). However, further stratification of subjects according to GST genotype has revealed a stronger significant protective effect of ITC consumption for CRC (odds ratio 0.43 (95% CI 0.20, 0.96)) and particularly for colon cancer (odds ratio 0.31 (95% CI 0.12, 0.84)) among individuals with both GSTM1 and GSTT1 null genotypes, but no effect for individuals with a single

null genotype. A protective effect in individuals with null genotypes and therefore reduced GST activity may seem counterintuitive because the up-regulation of GST activity is a mechanism through which cruciferous vegetables appear to inhibit chemical carcinogenesis (Steinkellner *et al.* 2001). However, individuals with GSTM1 and GSTT1 null genotypes may excrete ITC more slowly, leading to an accumulation of ITC in target tissues (Johnson, 2002a; Seow *et al.* 2002). ITC may then induce other chemoprotective phase 2 enzymes or exert other anti-carcinogenic effects, such as blocking the cell cycle or inducing apoptosis in cells carrying DNA damage (Johnson, 2002a; Seow *et al.* 2002).

Before consumption cruciferous vegetables are often stored or subjected to a range of treatments such as washing, chopping, blanching, freezing and cooking that may alter their biological effects. Variations in processing and storage may mean that individuals with similar levels of cruciferous vegetable intake are exposed to different levels of bioactive compounds. This variation could mask any association between cruciferous vegetable intake and CRC risk. Most types of post-harvest treatment result in a loss of bioactive compounds (Price *et al.* 1998; Conaway *et al.* 2000; Vallejo *et al.* 2002, 2003). Processing may also beneficially or negatively alter the bioavailability of such compounds. For example, the location and extent of GLS breakdown in the gastrointestinal tract and the profile of the breakdown products formed is influenced by the extent of thermal processing to which a vegetable is subjected before consumption (Johnson, 2002a; Matusheski *et al.* 2004). When raw or lightly-cooked cruciferous vegetables are consumed, most GLS hydrolysis occurs in the proximal gastrointestinal tract, catalysed by the presence of active plant myrosinase (Johnson, 2002a). However, the profile of the breakdown products formed after consumption of raw and lightly-cooked cruciferous vegetables may vary. A heat-labile cofactor epithiospecifier protein may favour the formation of nitriles over ITC after the consumption of raw cruciferous vegetables, whereas mild cooking may shift the balance in favour of ITC by inactivating epithiospecifier protein whilst conserving some myrosinase activity (Mithen *et al.* 2003; Matusheski *et al.* 2004). If myrosinase is inactivated by prolonged cooking, intact GLS will be delivered to the colon and degraded by the colonic microflora (some of which have myrosinase activity), resulting in the production of ITC and possibly other, as yet undetermined, breakdown products (Johnson, 2002a).

Only a few studies appear to have investigated the effect of processing on the actions of cruciferous vegetables *in vivo*. In a rodent study, juice or tissue prepared from raw Brussels sprouts has been shown to induce apoptosis and inhibit DMH-induced ACF, whereas blanched Brussels sprouts (with inactive myrosinase) have no effect (Smith *et al.* 2003). In contrast, Kassie *et al.* (2003a) have found that juices prepared from raw Brussels sprouts or Brussels sprouts that have been cooked for 10 min at 100°C are similarly as effective at inhibiting IQ-induced ACF. Juice prepared from raw Brussels sprouts would be expected to contain GLS breakdown products, whereas juice prepared from cooked Brussels sprouts would be expected to contain intact GLS and no active myrosinase. Kassie *et al.* (2003a) have suggested that the colonic microflora or the acidic

conditions of the stomach facilitate the hydrolysis of the GLS in the cooked Brussels sprout juice. However, neither of these routes would be expected to be as effective at supplying anti-carcinogenic GLS breakdown products as consuming juice containing hydrolysed GLS breakdown products, so it is surprising that both juices have similar effects.

The effect of common industrial food-processing techniques on the effects of cruciferous vegetables *in vivo* has not been reported. An animal experiment has been conducted to investigate the effect of the commercial freezing process on the ability of broccoli to affect a variety of markers of possible relevance to CRC risk. The markers that were measured included xenobiotic-metabolising enzyme activities in the liver and colon, SCFA concentrations in colonic contents, alterations in the faecal microflora and DNA strand breakage in colonocytes. Pigs were fed diets supplemented with 600 g raw or blanched-frozen broccoli (*var.* Marathon)/d for 12 d. Neither of the broccoli treatments was found to alter the activity of any of the enzymes measured (Table 1; Lynn *et al.* 2005) or the total SCFA content and the ratio of individual SCFA in the colonic contents (A Lynn, Z Fuller, K Hillman and B Ratcliffe, unpublished results). Both raw and blanched-frozen broccoli were found to exert similar effects on the colonic microflora, reducing total coliform and *Escherichia coli* numbers (A Lynn, Z Fuller, K Hillman and B Ratcliffe, unpublished results). Surprisingly, it was found that raw broccoli consumption causes a significant 21% increase in DNA strand breaks in the colon ($P < 0.05$), whereas blanched-frozen broccoli has no significant effect (Fig. 3). The lack of effect of the blanched-frozen broccoli indicates that the blanching-freezing process causes a loss of the bioactive component responsible for causing DNA damage and/or alters its supply to the colonocytes.

The damaging effect of raw broccoli consumption on colonocyte DNA has been confirmed in a second experiment. Pigs fed diets supplemented with a different variety of raw broccoli (*var.* Monaco) were found to exhibit a 50% increase in DNA strand breaks ($P < 0.05$; A Lynn, A Collins, Z Fuller, K Hillman and B Ratcliffe, unpublished results). To investigate whether a raw vegetable devoid of GLS would cause DNA damage, a second group of pigs were fed supplemental raw carrots. Raw carrots had no significant effect (A Lynn, A Collins, Z Fuller, K Hillman and B Ratcliffe, unpublished results). These studies appear to be the first to find an increase in DNA damage in the colon of animals fed raw cruciferous vegetables. In contrast to these studies, Kassie *et al.* (2003b) have found that juice prepared from raw garden cress decreases DNA damage in the colon of rats. The different effect of garden cress juice and broccoli may be explained by variations in their content of bioactive compounds such as GLS. For example, garden cress is rich in glucotropaeolin, whereas broccoli typically contains sizeable quantities of glucobrassicin, glucoraphanin and gluconapin (Kushad *et al.* 1999; Kassie *et al.* 2003b). Whilst no other studies have reported an increase in DNA damage in the colon after the consumption of raw broccoli, one study has reported an increase in oxidative DNA damage (measured as 8-oxodeoxyguanosine) in the liver of rats fed an extract of cooked Brussels sprouts (Sorensen *et al.* 2001).

Table 1. Activities of glutathione S-transferase (GST), quinone reductase (QR), ethoxyresorufin *O*-deethylation (EROD) and methoxyresorufin *O*-demethylation (MROD) in pigs following consumption of raw or blanched–frozen broccoli (from Lynn *et al.* 2005)*
(Values are means and standard deviations for five pigs)

	Raw broccoli		Blanched–frozen broccoli		Control	
	Mean	SD	Mean	SD	Mean	SD
GST activity (nmol/min per mg protein):						
Liver	2108.7	549.2	2177.6	546.9	2213.2	254.7
Colon	120.3	15.7	137.6	23.2	134.9	24.5
QR activity (nmol/min per mg protein):						
Liver	160.0	89.2	171.3	56.5	149.1	94.3
Colon	133.8	25.9	130.8	27.1	106.7	30.8
EROD activity (pmol/min per mg protein):						
Liver	106.1	31.9	111.6	33.8	103.2	20.8
MROD activity (pmol/min per mg protein):						
Liver	38.3	9.7	39.1	9.2	37.2	5.0

*The animals (five per treatment group) were fed diets unsupplemented (control) or supplemented with 600 g raw or blanched–frozen broccoli (var. Marathon)/d for 12 d. All enzyme activities were determined in triplicate. Two-way ANOVA was used to assess statistical significance of differences.

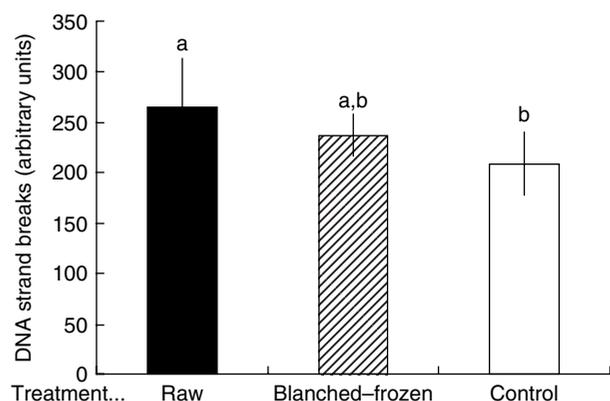


Fig. 3. The effect of raw and processed broccoli on the level of DNA strand breaks in the colonocytes of pigs. The animals (five per treatment group) were fed diets unsupplemented (control) or supplemented with 600 g raw or blanched–frozen broccoli (var. Marathon)/d for 12 d. DNA damage was measured in cryopreserved colonocytes by single-cell gel electrophoresis (comet assay) using a modification of the method of Collins *et al.* (1993). Results are expressed as pooled mean values (in arbitrary units, scored 0–400) for each treatment group and standard deviations represented by vertical bars. Two-way ANOVA and the post-hoc Tukey test were used to assess statistical difference. ^{a,b}Treatment groups with unlike superscript letters were significantly different ($P < 0.05$).

The mechanism through which raw broccoli may cause DNA damage in the colon of pigs is unclear. Evidence from studies *in vitro* and *in vivo* suggests that the induction of CYP450 enzymes, including CYP1A1 and CYP1A2, by GLS breakdown products may in some circumstances increase the production of reactive oxygen species and cause oxidative DNA damage (Park *et al.* 1996; Paolini *et al.* 2004). In the authors' studies, no increase in ethoxyresorufin *O*-deethylation or methoxyresorufin *O*-demethylation activity in the liver (possible measures of CYP1A1/2) has been observed; however, because CYP450 activities in the colon were not measured a local induction of

CYP450 cannot be ruled out, although a major induction seems unlikely.

There is some evidence that various GLS hydrolysis products can generate reactive oxygen species and cause DNA damage under certain circumstances (Murata *et al.* 2000). The $-N=C=S$ group of ITC appears to be able to undergo spontaneous hydrolysis leading to the production of superoxide and H_2O_2 (Murata *et al.* 2000) and the presence of these pro-oxidants in colonocytes could lead to oxidative DNA damage. The hydrolysis of certain GLS present in raw broccoli may result in the generation of nitriles (Mithen *et al.* 2003; Matusheski *et al.* 2004). It has been speculated that nitriles have toxic effects and they may have contributed to the genotoxic effect observed with raw broccoli. It is also possible that other, unidentified, compounds present in raw broccoli may have been responsible.

Conclusion

Experimental studies have identified a number of plausible mechanisms through which cruciferous vegetables may protect against CRC. Also, high intakes of cruciferous vegetables, GLS and their breakdown products have consistently been shown to protect against colon cancer in animal models. However, the results from epidemiological cohort studies have been unconvincing, which may reflect a lack of sensitivity of these studies to detect an effect. Stratifying populations according to GST genotype appears to strengthen the negative association between cruciferous vegetable consumption and CRC risk. Considering how a vegetable has been processed before consumption may also increase the sensitivity of epidemiological studies. Conversely, the lack of a negative association between cruciferous vegetable intake and CRC in epidemiological studies may indicate that normal intakes of cruciferous vegetables do not exert chemoprotective effects.

In animal experiments 600 g raw broccoli (equivalent to five large servings)/d has been found to exhibit a genotoxic effect, increasing DNA strand breakage in colonocytes. The

relevance of this finding to cancer risk is unclear. However, further investigations are warranted to identify the compounds responsible and their mechanism(s) of action.

Acknowledgements

A. L. was supported by a stipend from The Robert Gordon University, Aberdeen. The studies were funded by the World Cancer Research Fund. Animal facilities were provided by the Rowett Research Institute, Aberdeen.

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