Allium Vegetables and Organosulfur Compounds: Do They Help Prevent Cancer?

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Allium vegetables have been shown to have beneficial effects against several diseases, including cancer. Garlic, onions, leeks, and chives have been reported to protect against stomach and colorectal cancers, although evidence for a protective effect against cancer at other sites, including the breast, is still insufficient. The protective effect appears to be related to the presence of organosulfur compounds and mainly allyl derivatives, which inhibit carcinogenesis in the forestomach, esophagus, colon, mammary gland, and lung of experimental animals. The exact mechanisms of the cancer-preventive effects are not clear, although several hypotheses have been proposed. Organosulfur compounds modulate the activity of several metabolizing enzymes that activate (cytochrome P450s) or detoxify (glutathione *S*-transferases) carcinogens and inhibit the formation of DNA adducts in several target tissues. Antiproliferative activity has been described in several tumor cell lines, which is possibly mediated by induction of apoptosis and alterations of the cell cycle. *Allium* vegetables and organosulfur compounds are thus possible cancer-preventive agents. Clinical trials will be required to define the effective dose that has no toxicity in humans.
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The *Allium* genus includes approximately 500 species, the most widely used of which are onions (*Allium cepa*), garlic (*Allium sativum*), leeks (*Allium porrum*), chives (*Allium schoenoprasum*), and shallots (*Allium ascalonicum*). Garlic is widely cultivated and consumed worldwide, and its beneficial effects have been known for thousands of years. It has been considered to increase longevity and to confer stamina and physical strength, and it has been used empirically as a vermifuge, antiseptic, antimicrobic, antipyretic, and analgesic. The scientific community has now become interested in the pharmacologic properties of *Allium* vegetables and their chemical constituents, particularly with regard to their effects on the cardiovascular system and in the prevention of cancer. Garlic has antihypertensive and antiarrythmic properties and exerts an antithrombotic effect through fibrinolytic activity and the reduction of platelet aggregation. Ingestion of garlic has also been reported to lower the concentration of triglycerides, cholesterol, and low-density lipoproteins and to increase the concentration of high-density lipoproteins in blood. These findings suggest that garlic has a preventive effect against atherosclerosis and its complications, including stroke, myocardial infarction, and thrombotic disorders. The findings in relation to cancer prevention that have been accumulated over the last 20 years are summarized here.

We performed a systematic Medline search of the published literature on epidemiologic studies involving the relationship between consumption of *Allium* vegetables—garlic, onions, and leeks—and neoplasms/tumors. For the animal studies and for studies on mechanisms of action, we conducted searches for review articles. These lists of references were then updated using references from review papers and original research articles.

Active Compounds in *Allium* Vegetables

Fresh garlic contains water, carbohydrates, proteins, fiber, and fat, as well as essential amino acids, vitamins, and minerals. When garlic is cut, chopped, or crushed, the clove's membrane is disrupted and *S*-allylcysteine sulfoxide (an odorless compound called alliine) is transformed enzymatically into allicin by alliinase (*1*). Allicin is responsible for the typical odor of garlic, but it is unstable and converts readily into mono-, di-, and trisulfides and other compounds such as ajoene. Onions mainly contain *S*-propenylcysteine sulfoxide (*1*), but also other sulfoxides, including *S*-propylcysteine sulfoxide and *S*-methylcysteine sulfoxide (*2*). *S*-Propenylcysteine sulfoxide, positional isomer of alliin, is called lacrimatory precursor, because it is tranformed by alliinase into the lacrimatory factor propanethial *S*-oxide. The lacrimatory factor is highly reactive and hydrolyzes to propionaldehyde, sulfuric acid, and hydrogen sulfide; it is also the precursor of several sulfur derivatives. Organosulfur compounds present in *Allium* vegetables, which are either lipid or water soluble (Table 1), are considered responsible for the beneficial effects of these herbs. Garlic derivatives generally have a thioallyl moiety, whereas onion extracts contain a thiopropyl group with somewhat different chemical properties. The amounts of volatile compounds present in garlic bulbs are reported in Table 2.

Further transformation of organosulfur compounds can occur after interaction with free sulfhydryl groups, including those present in cysteine, glutathione, or proteins (*4,5*). Incubation of cystein with allyldisulfide or diallylsulfide groups produces *S*-allylmercaptocysteine and allylmercaptane, respectively (*6*). *S*-Allylmercaptocysteine is further transformed to allylmercaptane following incubation with fresh blood. One study (*7*) in the isolated, perfused rat liver showed that allicin is first metabolized to diallyldisulfide, which is further reduced to allylmercaptane. Another study identified allylmercaptane and allylmethylsulfide as metabolites of diallyldisulfide and diallylsulfide in primary rat hepatocytes (*8*). Urine from healthy individuals consuming garlic or onions contains *N*-acetyl-*S*-allylcysteine and *N*-acetyl-*S*-(2-carboxylpropyl)-cysteine (*9–11*). Allylmercaptane and diallyldisulfide have been found in human breath after ingestion of garlic (*12,13*).

Epidemiologic Studies

The association between consumption of *Allium* vegetables and risk for cancer has been assessed in several epidemiologic, mainly case–control, studies. One of the first indications of a role of *Allium* vegetables in the prevention of stomach cancer came from China. The 10-fold difference in the death rate from stomach cancer in two Chinese provinces was suggested to be attributable to garlic consumption, which was approximately 20 g/day in the low-risk area and < 1 g/day in the high-risk area (*14*). This hypothesis was further supported by the results of an ecologic study in China (*15*), which reported that 82.3% of men and 74.7% of women in a low-risk area consumed garlic more than

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three times per week, whereas only 0.5% of the population in a high-risk area did so. Similar differences were found for the consumption of Welsh onions, onions, and Chinese chives.

Several case–control studies in Asia and Europe have been conducted to assess the effect of *Allium* vegetables on the risk of stomach cancer [Table 3 (*16–27*)]. Most reported a decreased risk with increasing consumption. In one study (*17*) a higher risk for stomach cancer was found with increasing frequency of onion consumption, but the controls had severe gastric diseases, including chronic or acute gastritis, gastric ulcer, and polyps. No association with onion consumption was found in two other studies (*19*,*23*). In a multicenter case–control study in areas of high and low risk for stomach cancer in Italy (*20*), use of garlic and onions as condiments decreased the risk of stomach cancer. When questions on the frequency of garlic consumption were added to a questionnaire given to a subgroup of subjects, an inverse association was observed between consumption of raw garlic and cancer risk, persons in the highest tertile of intake having only 40% of the risk of those in the lowest. The effects of different *Allium* vegetables have been evaluated separately in a few studies. You et al. (*21*) showed that patients with gastric cancer consumed approximately 3.5 kg less *Allium* vegetables per year than controls (15.5 vs. 19). Each *Allium* vegetable was associated with a decreased risk, which was most marked for scallions, garlic, and Chinese chives. Similar results were obtained when the usual diet 25–30 years before diagnosis (before the Cultural Revolution) was considered. In a population-based study, the age- and sex-adjusted odds ratio for stomach cancer decreased inversely with the frequency of consumption of garlic, onions, Welsh onions, and Chinese chives (*26*). This study also showed a decreased risk for esophageal cancer with a higher frequency of consumption of individual *Allium* vegetables [Table 4 (*26*,*28*,*29*)].

A cohort study in the Netherlands addressed the effect of daily use of onions, leeks, and garlic supplements for at least 1 year in the 5 years before baseline on the risk for stomach cancer (*27*) and for colorectal, breast, and lung cancers. Overall, cases were associated with lower consumption of onions and leeks, but slightly greater use of garlic supplements (9.2% vs. 8.8%). Onion consumption was significantly inversely correlated with the risk of stomach cancer, with a statistically significant test for trend (Table 3). Slightly different results were obtained in stratified and multivariate analyses, but the trends remained statistically significant. Leek consumption was associated with a decreased risk only for persons in the highest category of consumption, but there was no clear decreasing trend. The relative risk associated with use of garlic supplements was slightly greater than 1.0. Similar results were obtained when cases occurring in the first year of follow-up were excluded from the analysis and when only subjects without a history of gastric disorders were considered. This study has the advantages of good completeness of follow-up and adjustment for several dietary and nondietary confounding factors, although no distinction was made between raw and cooked onions and leeks.

The chemopreventive effects of *Allium* vegetables against stomach and esophageal cancers may be related to their antibacterial properties. Inhibition of bacterial growth in the gastric cavity may result in less conversion of nitrate to nitrite in the stomach (*14*), a decreased probability of endogenous formation of carcinogenic *N*-nitroso compounds, and reduction in *Helicobacter pylori* infection specifically. *In vitro*, garlic oil and extracts of individual organosulfur compounds strongly inhibit the bacterial growth of *H. pylori* (*30–33*). In contrast, some studies in humans showed no correlation between seropositivity to *H. pylori* and the consumption of garlic (*34*) or use of garlic to treat *H. pylori* infection (*35*).

An intervention trial is being conducted in China in which capsules containing steam-distilled garlic oil and aged garlic extract (in addition to amoxicillin and omeprazole or antioxidants) are given to determine whether they reduce the prevalence of dysplasia and other preneoplastic lesions in the stomach (*36*). No results have yet been published.

Studies of the effects of *Allium* vegetables against colorectal cancer are summarized in Table 5 (*17*,*37–46*). Apart from the study of Tajima and Tominaga (*17*) discussed above, the case–control studies generally found decreased risks for colorectal cancer. The effect was particularly significant for consumption of cooked onions and leeks in Belgium (*38*), for a combination of garlic, onions, and pepper in Argentina (*40*), and for garlic in Switzerland (*43*). In a case–control study in Australia, Steinmetz and Potter (*41*) reported a lower risk for both sexes, with a more pronounced decrease for women and for cancer of the proximal compared with the distal colon. Nevertheless, two of the three cohort studies reported decreased risks for cancer of the distal colon with higher consumption of garlic (*44*,*45*). The third cohort study showed no significant effect of consumption of onions, leeks, and garlic supplements (*46*).

Table 1. Main organosulfur compounds present in *Allium* vegetables.

Chemical structure	Compound	Abbreviation
Lipid-soluble compounds		
CH_2=CH-CH_2-S(O)-CH_2-CH(NH_2)-COOH	*S*-Allylcysteine sulfoxide (Alliin)	
CH_3-CH=CH-S(O)-CH_2-CH(NH_2)-COOH	*S*-Propenylcysteine sulfoxide (lacrimatory precursor)	
CH_3-CH_2-CH_2-S(O)-CH_2-CH(NH_2)-COOH	*S*-Propylcysteine sulfoxide	
CH_3-S(O)-CH_2-CH(NH_2)-COOH	*S*-Methylcysteine sulfoxide	
CH_2=CH-CH_2-S(O)-S-CH_2-CH=CH_2	Allicin	
CH_2=CH-CH_2-S(O)-CH_2-CH=CH-S-S-CH_2-CH=CH_2	Ajoene	
CH_3-CH_2-CH=SO	Propanethial *S*-oxide (lacrimatory factor)	
CH_2=CH-CH_2-S-CH_2-CH=CH_2	Diallylsulfide	DAS
CH_2=CH-CH_2-S-S-CH_2-CH=CH_2	Diallyldisulfide	DADS
CH_2=CH-CH_2-S-S-S-CH_2-CH=CH_2	Diallyltrisulfide	DATS
CH_2=CH-CH_2-S-CH_3	Allylmethylsulfide	AMS
CH_2=CH-CH_2-S-S-CH_3	Allylmethyldisulfide	AMDS
CH_2=CH-CH_2-S-S-S-CH_3	Allylmethyltrisulfide	AMTS
CH_3-CH_2-CH_2-S-CH_2-CH_2-CH_3	Dipropylsulfide	DPS
CH_3-CH_2-CH_2-S-S-CH_2-CH_2-CH_3	Dipropyldisulfide	DPDS
CH_3-CH_2-CH_2-S-S-S-CH_2-CH_2-CH_3	Dipropyltrisulfide	DPTS
CH_3-CH_2-CH_2-S-CH_3	Propylmethylsulfide	PMS
CH_3-CH_2-CH_2-S-S-CH_3	Propylmethyldisulfide	PMDS
CH_3-CH_2-CH_2-S-S-S-CH_3	Propylmethyltrisulfide	PMTS
Water-soluble compounds		
CH_2=CH-CH_2-S-CH_2-CH(NH_2)-COOH	*S*-Allylcysteine	SAC
CH_2=CH-CH_2-S-S-CH_2-CH(NH_2)-COOH	*S*-Allylmercaptocysteine	SAMC
CH_2=CH-CH_2-S-H	Allylmercaptan	AM

Table 2. Amounts of some volatile organosulfur compounds in garlic.

Compound	µg/g Garlic bulb
DAS	30–100
DADS	530–610
DATS	900–1,100
AMS	3.8–4.6
AMDS	~ 100
AMTS	250–270
DMDS	2.4–2.5
DMTS	15–19
PMDS	0.7–0.8

The compounds were isolated by water or steam distillation with solvent extraction (*3*).

An increased frequency of consumption of garlic and onions was associated with a decreased risk for breast cancer [Table 6 (*47–50*)] in case–control studies in Switzerland and France (*48,49*), but a case–control study in Greece (*47*) and the cohort study in the Netherlands showed no effect of *Allium* vegetables (*50*).

Studies of the association between consumption of *Allium* vegetables and risk for cancers at other sites are summarized in Table 7 (*51–57*). In case–control studies, significantly decreased risks with onion consumption for cancer of the lung in India (*51*) and of the brain in China (*56*) were reported. A significantly decreased risk for cancer of the larynx with garlic consumption was reported in China (*53*). A case–control study in England (*55*) reported a significant protective effect against prostate cancer when garlic was eaten as a food and as a food and supplement, but no effect was seen with onions. However, adjustment for social class reduced the association with garlic to nonsignificance. A moderate protection of endometrial cancer was also reported with *Allium* vegetable consumption (*57*).

Studies summarized above can suffer from some limitations, including use of hospital-based controls, low response rates among cases or controls, low rates of histologic confirmation of the tumors, use of recent diet as a reference, use of frequency instead of amount consumed, bias due to differential recall of dietary intake associated with awareness of disease, and absence of information on vegetable preparation (i.e., raw or cooked). These factors could explain some inconsistencies in

Table 3. Epidemiologic studies of consumption of *Allium* vegetables and risk for stomach cancer.

Country	Cases/controls	Time of reference	*Allium* vegetable	OR[a] (*p*-value for trend)	Adjusted for confounding	Reference
Case–control studies						
Hawaii (Japanese)	220/440 (hospital)	Current diet	Onions (frequency)	0.48 Hawaiian Japanese 0.67 Japanese migrants 0.31 Migrant offspring	Sex	(*16*)
Japan	93/186 (hospital)	1–2 Years before onset	Onions (frequency)	2.13	Age, sex	(*17*)
Greece	110/100 (hospital)	Before onset	Onions (frequency)	0.68 (< 0.001)	Age, sex, socio-economic status	(*18*)
China	241/241 (hospital)	Before onset	Onions, chives (kg/year)	No significant effect	—	(*19*)
Italy	1,016/1,159 (population)	2 Years before interview	Onions, garlic as condiments (frequency)	0.8 (0.04)	Age, sex, study area, social class, residence, migration, Quetelet index, family history of gastric cancer	(*20*)
			Raw onions (frequency)	0.8 (< 0.01)		
			Cooked onions (frequency)	1.1		
			Cooked garlic (frequency)	0.4 (< 0.001)		
China	564/1,131 (population)	6–8 Years before onset	Total *Allium* vegetables (kg/year)	0.4 (< 0.01)	Age, sex, family income, intake of other *Allium* vegetables	(*21*)
			Scallions (kg/year)	0.8 (0.02)		
			Garlic (kg/year)	0.7 (0.03)		
			Chinese chives (kg/year)	0.6 (< 0.01)		
			Onions (kg/year)	1.0 (0.46)		
Poland	741/741 (hospital)	Before onset	Onions (frequency)	0.72 (0.01)	Age, sex, occupation, education, residence	(*22*)
Spain	354/354 (hospital)	1 Year before onset	Onions (high vs low)	0.9	Total calories, intake of salad, tomatoes, chard, spinach, borage, green beans, cruciferous vegetables	(*23*)
Belgium	449/3,524 (population)	Before onset	Cooked onions (g/week)	0.3 (< 0.0001)	Age, sex, province	(*24*)
			Cooked leeks (g/week)	0.29 (< 0.001)		
Sweden	338/669 (population)	20 Years before interview	Onions (frequency)	0.84 (0.44)	Age, sex, socio-economic status	(*25*)
			Leeks (frequency)	0.63 (0.001)		
			Garlic (frequency)	0.89		
China	153/234 (population)	Not specified	Garlic (frequency)	0.31	Age, sex, income, smoking, drinking, tea consumption, intake of leftover gruel, pickled vegetables, meat, fruit, tomatoes, eggs, snap beans	(*26*)
			Onions (frequency)	0.17		
			Welsh onions (frequency)	0.22		
			Chinese chives (frequency)	0.4		
Cohort study						
The Netherlands	139/3,123	Last year before interview	Onions (*n*/day)	0.50 (0.03)	Age, sex, education, smoking status, intake of alcohol, vitamin C, β-carotene, history of stomach disorders, family history of stomach cancer	(*27*)
			Leeks (frequency)	0.69 (1.23)		
			Garlic supplements	1.27		

[a]Odds ratio: highest versus lowest consumption.

the results. Publication bias, with limited number of published data with no apparent protective effect, could also be operating and detracting from the evidence of an effect. However, studies assessing the role of *Allium* vegetables in cancer prevention have generally not been designed for this purpose, and it is therefore possible that finding no effect for *Allium* vegetables did not really affect the publication.

For calculation of odds ratios, the data were generally adjusted for age and sex and other variables, including socioeconomic status, anthropometry, and family history of gastric disorders and cancer. Alcohol, smoking, and energy intake were also taken into account in some studies. However, consumption of other food items, including vegetables, was adjusted for in few studies (*23,26,27,43,46,50,52*). Such adjustment seems essential in studies of gastric cancer, because low consumption of green vegetables and fruits has been described as an important risk factor for this cancer. Epidemiologic studies summarized here are generally very consistent in reporting a protective effect of *Allium* vegetables in gastric cancer, and the adjustment for fruits and vegetables was made in studies showing either protection (*26,27*) or lack of effect (*23*). It seems, therefore, that such adjustment is not the only factor explaining differences in results.

Experimental Carcinogenesis

The effects of *Allium* vegetables have been studied experimentally by testing individual organosulfur compounds or extracts and oils from garlic and onions [Table 8 (*58–74*)]. The sulfur compounds were generally administered intragastrically before the carcinogen, at doses ranging between 0.01 and 0.2 mmol/mouse or 50–200 mg/kg body weight (bw)/rat or mouse, diluted in the diet (200–2,000 ppm) or percutaneously. Garlic was administered as a powder at 1–4% in the diet or as an extract at 400 mg/kg bw intragastrically.

Garlic contains compounds that inhibit the initiation step of carcinogenesis at a variety of sites in different animal species and with different initiators. Diallylsulfide (DAS) generally inhibited cancers of the forestomach, colon, esophagus, mammary gland, and lung (*58,60,64,65*). Contradictory results were obtained with regard to the prevention of liver cancer (*62,69*). Other allyl derivatives, including diallyldisulfide, allylmethyldisulfide, and allylmercaptan (DADS, AMDS, and AM, respectively), decreased tumor formation in the forestomach and lung, but the related saturated propyl derivatives propylmethyldisulfide, propylmethyltrisulfide, dipropylsulfide, and dipropyltrisulfide (PMDS, PMTS, DPS, and DPTS, respectively) did not (*58,59*). Diallyltrisulfide (DATS) and allylmethylsulfide (AMTS) inhibited forestomach cancer, DATS, which contains two allyl groups, being more potent than AMTS, the analogue derivative with only one allyl group (*58*). This finding underlines the importance of the allyl group for cancer-preventive activity. These trisulfide derivatives, AMTS and DATS, were not effective against pulmonary adenoma formation, suggesting that the number of sulfur atoms in the molecule is also important, possibly determining the organ sites at which protection is achieved against carcinogenesis (*58*). DADS was also protective against cancers of the colon, mammary gland, and kidney (*62,63,65,67*). *S*-Allylcysteine (SAC) inhibited the incidence and frequency of 1,2-dimethylhydrazine (DMH)-induced colon tumors (*61*). Contradictory results were reported with SAC for *N*-methylnitrosourea (MNU)-induced mammary carcinogenesis (*67,68*). These inconsistencies could be explained by differencies in the doses of MNU and SAC used and the type of diet administered. Garlic powder in the diet inhibited mammary tumors (*65–67*), and a garlic extract decreased the incidence of cervical carcinoma (*70*). DAS and garlic oil applied topically during the initiation phase of skin cancer reduced the number of tumors per mouse (*72,74*). In addition, garlic extract and garlic and onion oil decreased the promotion step of skin carcinogenesis induced by 12-*O*-tetradecanoylphorbol 13-acetate (*71,73*). The protection provided by garlic against carcinogenesis may be modulated by various dietary components, including selenium, vitamin A, and lipids (*65,75,76*).

The finding of lack of cancer-preventive effect of organosulfur compounds could have affected the publication of the study; however, the protective effect seems to be very consistent, and publication bias appears unlikely.

Mechanisms of Cancer Prevention

Several mechanisms have been proposed to explain the cancer-preventive effects of *Allium* vegetables and related organosulfur compounds. These include inhibition of mutagenesis, modulation of enzyme activities, inhibition of DNA adduct formation, free-radical scavenging, and effects on cell proliferation and tumor growth. Although there is evidence supporting these mechanisms for organosulfur compounds, they are still speculative, and further research is needed to support a causality between such properties and the cancer-preventive activity in experimental animals.

Inhibition of mutagenesis. Aqueous and methanolic garlic extracts inhibited the mutagenic activity of aflatoxin B_1 in *Salmonella typhimurium* (*77*). Aqueous garlic extract also decreased the mutagenicity of 4-nitroquinoline-1-oxide in *Escherichia coli* (*78*) and the mutagenicity of γ-radiation, hydrogen peroxide, cumene, and *t*-butyl hydroperoxides in *S. typhimurium* (*79*). Onion juice protected *S. typhimurium* against cooked mutagens present in hamburgers (*80*).

Modulation of enzyme activities. Organosulfur compounds have been shown to modulate the activity of glutathione *S*-transferases (GST), a family of enzymes important in detoxification of carcinogens, and cytochromes P450 (CYP), a family of enzymes that activate many chemical carcinogens in experimental animals.

Sparnins and co-workers (*81*) first showed that AMTS increased the activity of

Table 4. Case–control studies of consumption of *Allium* vegetables and risk for esophageal cancer in China.

Cases/controls	Time of reference	*Allium* vegetable	OR[a] (*p*-value for trend)	Adjusted for confounding	Reference
196/392 (hospital)	Before onset	Onions (kg/year) Garlic (kg/year)	0.7 (0.33) 1.0 (0.24)	Alcohol, income, occupation, smoking	(*28*)
902/1,552	5 Years before interview	*Allium* vegetables (frequency)	Men 1.1 (0.69) Women 1.1 (0.34)	Age, education, birthplace, cigarette smoking, tea and alcohol drinking	(*29*)
153/234 (population)	Not specified	Garlic (frequency) Onions (frequency) Welsh onions (frequency) Chinese chives (frequency)	0.30 0.25 0.15 0.57	Age, sex, income, smoking, drinking, tea consumption, intake of leftover gruel, pickled vegetables, meat, fruits, tomatoes, eggs, snap beans	(*26*)

[a]Odds ratio: highest versus lowest consumption.

GST in the forestomach, small-bowel mucosa, liver, and lung of mice. Other allyl derivatives also increased GST activity in these tissues (*58*). Derivatives with a propyl instead of an allyl group were less active or inactive. The induction of GST paralleled the inhibition of benzo[*a*]pyrene-induced carcinogenesis in the forestomach, but not in the lung, suggesting that increased carcinogen detoxification is only one of the factors responsible for the cancer-preventive effects of organosulfur compounds. These results were partially confirmed by Sumiyoshi and Wargovich (*61*), who found a greater effect of thioallyl than thiopropyl derivatives in inducing hepatic and colonic GST in mice. In contrast, DAS did not increase GST activity in mouse liver (*60*) or in a culture of rat hepatocytes (*69*).

The activity of mammary and liver GST was increased by the addition of garlic powder to the diet of rats (*66*). The maximum activity of GST did not coincide with maximum inhibition of carcinogenesis, however, further indicating that increased GST activity does not account fully for the protection provided by garlic powder against carcinogenesis. Thus, the effects on enzymes that activate chemical carcinogens are not sufficient to explain the cancer-preventive activity. For example, an oral dose of DAS suppressed

Table 5. Epidemiologic studies of consumption of *Allium* vegetables and risk for colorectal cancer.

Country	Cases/controls	Time of reference	*Allium* vegetable	OR[a] (*p*-value for trend)	Adjusted for confounding	Reference
Case–control studies						
Japan	588/1,176 (hospital)	Not specified	Japanese leeks (frequency)	Colorectum 0.78 Low rectum 0.65	Age, sex, prefecture	(*37*)
Japan	93/186 (hospital)	1–2 Years before onset	Onions (frequency)	Colon 3.84 Rectum (2.99)	Age, sex	(*17*)
Belgium	Colon 453/2,851 Rectum 365/2,851 (population)	1 Week before onset	Cooked onions (g/week) Cooked leeks (g/week)	Colon 0.16 Rectum 0.17 Colon 0.34 Rectum 0.23	Age, sex, province	(*38*)
China	336/336 (hospital)	Before onset	Garlic (yes/no)	Rectum, women 0.21		(*39*)
Argentina	110/220 (population)	5 Years before interview	Garlic, onions, pepper (frequency)	Colon 0.22 (0.004)		(*40*)
Australia	220/438 (population)	1 Year before interview	Onions (frequency)	Colon Men 0.86 Women 0.42 Proximal colon Men 0.23 Women 0.38 Distal colon Men 1.01 Women 0.57	Occupation (men), age at first live birth (women), Quetelet index, protein and alcohol intake	(*41*)
Hawaii	1,192/1,192 (population)	3 Years before onset	Onions (g/day)	Colorectum Men 0.9 (0.34) Women 0.9 (0.37)	Age, Quetelet index, cigarette smoking, total calories, egg and calcium intake, alcohol consumption, lifetime recreational activity	(*42*)
			Garlic (g/day)	Colorectum Men 0.7 (0.11) Women 0.7 (0.23)		
Switzerland	223/491 (hospital)	2 Years before diagnosis	Garlic (frequency)	Colorectum 0.39 (< 0.01)	Age, sex, education, body mass index, physical activity, smoking, alcohol, meat and vegetable consumption, total energy intake	(*43*)
Cohort studies						
United States	212/35,004 Postmenopausal women	Last year before interview	Garlic (frequency)	Colon 0.68 Proximal colon 1 Distal colon 0.52 (< 0.05)	Age, energy intake	(*44*)
United States	205/47,949	Last year before interview	Garlic (frequency)	Colon 0.77 (0.14) Distal colon 0.63 (0.07)	Age, energy intake	(*45*)
The Netherlands	443/3,123	Last year before interview	Onions (*n*/day)	Colon Men 0.87 Women 1.49 Rectum Men 0.66 Women 1.34	Age, education, Quetelet index, smoking status, intake of alcohol, vitamin C, β-carotene, history of intestinal diseases, family history of cancer	(*46*)
			Leeks (frequency)	Colon Men 1.1 Women 1.18 Rectum Men 0.72 Women 1.31		
			Garlic supplements	Colon 1.36 Rectum 1.28		

[a]Odds ratio: highest versus lowest consumption.

esophageal carcinogenesis induced by *N*-nitrosomethylbenzylamine in rats and significantly reduced the microsomal conversion of this nitrosamine in liver but not in esophagus (*64*). In addition, the prevention of benzo[*a*]pyrene-induced forestomach cancer in mice by organosulfur compounds is not attributable to a reduction in the activity of CYP1A1 (*82*).

DADS in the diet increased not only the activity of GST but also that of other detoxifying enzymes, including reduced nicotinamide adenine dinucleotide phosphate [NAD(P)H]-dependent quinone oxidoreductase, which is involved in detoxification of activated quinone metabolites of benzo[*a*]pyrene, and of uridine diphosphate (UDP)-glucuronosyl transferase in rat tissues (*63,83*).

DAS acted as a competitive inhibitor of *N*-dimethylnitrosamine demethylase activity (*84*). It also decreased the activity of CYP2E1 in a time- and dose-dependent manner and induced the activities of CYP2B1 and pentoxy- and ethoxyresorufin dealkylases in hepatic microsomes (*85*). An increase in CYP2B1 mRNA was also observed. Treatment with the DAS metabolites diallyl sulfoxide (DASO) and diallylsulfone ($DASO_2$) had similar effects on rat hepatic monooxygenase activities (*85,86*). Reicks and Crankshaw (*87*) reported that DAS, DADS, and AMS decreased *p*-nitrophenol hydroxylase activity and CYP2E1 protein concentration in rat liver. When the diet of rats was supplemented with DAS or DADS (*88*), DADS increased the activities of several monooxygenases and transferases in intestine and liver; the protein levels of epoxide hydrolase and CYP2B1/2 were also increased. DADS also decreased CYP2E1 level in liver. The effects of DAS were similar to those of DADS in liver, but only epoxide hydrolase activity and CYP2B1/2 protein levels were increased in the intestine.

In a study of the effect of garlic oil, DAS, and DADS on the activities of several metabolizing enzymes in the liver of rats fed high-fat diets (*89*), GST activity was increased by all treatments. Garlic oil induced the expression of the placental form of GST and CYP2B1 and decreased the expression of CYP2E1. DAS and DADS also modulated these enzymes, but DAS increased mainly CYP2B1, whereas DADS increased mainly GST activity; similar effects were observed on CYP2E1 expression.

DAS and its oxidation derivatives DASO and $DASO_2$ are conjugated with glutathione, in rats (*90*). To our knowledge, no study has investigated the effects of possible GST polymorphisms in the deactivation of these *Allium* vegetable-derived compounds, although this could provide some explanations of differential effects in humans.

Modulation of the activity of arylamine *N*-acetyltransferase, a polymorphic enzyme that deactivates arylamines and activates some heterocyclic dietary amines, was addressed in a few studies. The slow and fast acetylator phenotypes have been associated with increased risk for cancers of the bladder and colon, respectively. DAS and DADS decreased the activity of this enzyme in strains of *H. pylori* from peptic ulcer patients (*31*) and inhibited its activity in a human colon tumor cell line (*91*) and in human bladder tumor cells (*92*) in a dose-dependent manner.

Inhibition of DNA adduct formation. DNA adducts are believed to be an initial step in carcinogenesis by chemicals. In rat mammary gland, garlic powder decreased the occurrence of 7,12-dimethylbenz[*a*]anthracene (DMBA)–DNA adducts *in vivo* and the amounts of total and individual adducts correlated positively with mammary tumor incidence (*66*). Garlic powder, garlic water extract, a deodorized garlic powder, a garlic powder with a high sulfur content, and SAC were also effective against mammary DMBA–DNA binding (*93*).

DNA adducts induced by incubation of human bladder tumor cells with 2-aminofluorene were inhibited by DAS and DADS (*92*). In contrast, a water extract of raw garlic and SAC, but not DAS, significantly inhibited benzo[*a*]pyrene–DNA adduct formation in simulated human peripheral blood lymphocytes *in vitro* (*94*).

N-Nitroso compounds, a class of potential human carcinogens that can be synthesized in humans from precursors present in the diet, are metabolized to alkylating agents that can bind to DNA. Shenoy and Choughuley (*95*) showed that onion and garlic juices inhibit the nitrosation reactions *in vitro* in a dose-dependent manner. The occurrence of 7-methyldeoxyguanosine (7-MedG) and O^6-methyldeoxyguanosine (O^6-MedG) was decreased in rat liver when garlic powder was added to a diet containing aminopyrine and sodium nitrite (*96*). Garlic powder also decreased DNA methylation in the livers of rats treated with *N*-nitrosodimethylamine and in mammary tissue of rats treated with *N*-methylnitrosourea. Garlic, SAC, and DADS also decreased the formation of 7-MedG and O^6-MedG induced by *N*-methylnitrosourea in mammary DNA; this decrease correlated with the inhibition of mammary tumors by these compounds (*67*).

Table 6. Epidemiologic studies of consumption of *Allium* vegetables and risk for breast cancer.

Country	Cases/controls	Time of reference	*Allium* vegetable	OR[a] (*p*-value for trend)	Adjusted for confounding	Reference
Case–control studies						
Greece	120/120 (hospital)	Before onset	Onions, leeks (frequency)	No significant effect	Age, education	(*47*)
Switzerland	107/318 (hospital)	Before onset	Garlic (frequency) Onions (frequency)	0.6 0.5 (< 0.01)	Age	(*48*)
France	345/345 (selected population)	Before interview	Garlic, onions (frequency)	0.3 (< 0.0001)	Total caloric intake, parity, weight, corporeal surface	(*49*)
Cohort study						
The Netherlands	469/1,713	Last year before interview	Onions (*n*/day) Leeks (frequency) Garlic supplements	0.95 (0.65) 1.08 (0.32) 0.75	Age, parity, age at menarche, age at first birth, age at menopause, artificially induced menopause, oral contraceptive use, alcohol consumption, education, Quetelet index, smoking status, dietary intake of vitamin C and β-carotene, history of benign breast diseases, breast cancer in mother and sister	(*50*)

[a]Odds ratio: highest versus lowest consumption.

Free-radical scavenging. Free radicals have been related to several age-related diseases, including cancer (*97*). Reduced glutathione (GSH) is not only a cofactor for GST but also serves as a reductant for glutathione peroxidase (GPX), an enzyme involved in natural protection by free radicals, in addition to superoxide dismutase and catalase. Garlic and onion oils stimulated the activity of GPX and inhibited the decreased ratio of reduced to oxidized glutathione produced by 12-*O*-tetradecanoylphorbol-13-acetate in epidermal cells (*98*). GPX activity was also increased in animal tissues with DAS (*99*), DADS and garlic oil (*89*). DAS and DADS also increased the activity of glutathione reductase, and garlic oil increased the activity of superoxide dismutase (*89*). In contrast, DAS and garlic homogenates decreased catalase in the livers of rats and mice (*100*). *S*-Allylmercaptosysteine (SAMC) and SAC increased the synthesis of GSH in human prostate cancer cells (*101*).

Aged garlic extract, SAC, and SAMC exhibited radical scavenging activity (*102*). DAS, DADS, and AMS showed selective actions on different markers in tests for their ability to react with carbon tetrachloride-derived free radicals (*103*). DADS also inhibited carbon tetrachloride-induced lipid peroxidation. The antioxidant properties of *Allium* vegetables might therefore result from the contributions of various sulfur components at different steps of the process.

Effects on cell proliferation and tumor growth. Inhibition of tumor cell proliferation by organosulfur compounds has been reported in several studies using different cell cultures, including canine mammary tumor cells (*104*), human colon, lung, and skin tumor cell lines (*105,106*), human neuroblastoma cells (*107*), human and murine melanoma cells (*108*), and human prostatic carcinoma cells (*101*).

Contradictory results have been obtained with regard to modulation of the proliferative activity of non-neoplastic cell lines by organosulfur compounds, with some studies showing inhibition (*108,109*) and others no effect (*106*).

Garlic and onion oils caused a marked suppression of proliferation of human promyelocytic leukemia cells (*110*). Garlic powder and an alliin-enriched garlic extract inhibited the growth of a human lymphatic leukemia cell line in a dose-dependent manner, but inhibited the growth of human hepatoma and human colorectal carcinoma cells only when applied as a mixture. This finding indicates that the antiproliferative effect of garlic is due to breakdown products of alliin catalyzed by the alliinase enzyme system present in garlic powder (*111*).

Polyamines, mainly spermine, play an important role in cell division and differentiation. SAMC, but not SAC, has been shown to alter polyamine concentrations in human prostate carcinoma cells, increasing that of spermidine and decreasing those of putrescine and spermine (*101*). Ornithine decarboxylase,

Table 7. Epidemiologic studies of consumption of *Allium* vegetables and risk for cancers at other sites.

Study	Country	Cases/controls	Time of reference	*Allium* vegetable	OR[a] (*p*-value for trend)	Adjusted for confounding	Reference
Lung							
Case–control	India	281/1,281 (selected population)	Before onset	Onions (frequency)	0.03 (< 0.001)	Age, education, religion, smoking	(*51*)
Cohort	The Netherlands	484/3,123	Last year before interview	Onions (*n*/day) Leeks (frequency) Garlic supplements	0.80 (0.7) 1.08 (0.58) 1.78	Age, sex, education, past and current smoking, dietary intake of vitamin C and β-carotene, history of chronic obstructive pulmonary disease	(*52*)
Laryngeal							
Case–control	China	201/414 (population)	10 Years before interview	Garlic (g/month)	Men 0.5 (0.02) Women 0.7	Age, education, smoking	(*53*)
Nasal							
Case–control	China	60/414 (population)	10 Years before interview	*Allium* vegetables (g/month)	0.6 (NS)	Age	(*54*)
Prostate							
Case–control	England	328/328 (population)	5 Years before interview	Garlic (frequency) Onions (frequency)	0.64 (0.13) 0.85 (0.65)	Social class	(*55*)
Brain							
Case–control	China	129/258 (hospital)	1 Year before onset	Garlic (g/month) Onions (g/month)	1.34 (0.13) 0.1 (< 0.01)	Income, education, selected occupational exposures, cigarette smoking, alcohol intake, total energy intake	(*56*)
Endometrium							
Case–control	China	268/268 (population)	10 Years before interview	*Allium* vegetables (g/week)	0.7 (0.12)	Age, number of pregnancies, body mass index, total calories	(*57*)

NS, not significant.
[a]Odds ratio: highest versus lowest consumption.

a rate-limiting enzyme involved in the synthesis of polyamines, is also reduced by DAS (*98,112,113*), although there is evidence of an increase in the livers of rat not treated with initiators (*114*).

The antiproliferative effect of organosulfur compounds appears to be related to the induction of apoptosis. Exposure to DADS and DATS caused cells to undergo apoptosis, as determined by morphologic changes and/or DNA fragmentation (*105,106*). A positive correlation was found between DADS-induced DNA fragmentation and increased intracellular free-calcium concentration, which may activate calcium-dependent endonucleases leading to apoptosis. A recent study (*115*) showed that DAS, DADS, and garlic extract increase the number of non–small-cell lung cancer cells in the apoptotic state. This increase followed the induction of p53 protein by DADS or the increase of the expression of Bax and decrease of the expression of Bcl-2 by DAS and garlic extract. Ajoene induced apoptosis in human leukemic cells but not in peripheral mononuclear blood cells from healthy donors (*116*). The cell cycle was also affected by DADS, which decreased the percentage of human colon tumor cells in the G_1 and S phases and concomitantly increased the percentage of those in the G_2/M phase (*117*). These effects depended on the dose of DADS and the length of incubation. The ability of DADS to inhibit cell proliferation was related to induction of G_2/M phase arrest and to inhibition of $p34^{cdc2}$ kinase activity, which modulates the progression of cells from G_2 into the M phase of the cell cycle. The suppression of the $p34^{cdc2}$ kinase activity by DADS resulted not from a direct interaction with the protein but from modulation of the factors involved in the formation and conversion of the enzyme to its active form (*118*). DADS also significantly inhibited the growth of H-*ras* oncogene-transformed tumors implanted in nude mice by suppressing the association of $p21^{H\text{-}ras}$ with the cell membrane (*119*).

Conclusions

Overall, evidence shows that *Allium* vegetables, mainly garlic and related organic allyl sulfur components, have cancer-preventive effects. In the 37 case–control or cohort studies for cancer at any site reported here, 28 showed some protective effect of *Allium* vegetables. The evidence is particularly strong for stomach cancer (9 out of 12 studies) and colon cancer (9 out of 11 studies), while there is still insufficient evidence for an effect on breast, lung, and other cancers. A recent meta-analysis also showed that a high intake of garlic may be associated with decreased risks for stomach and colorectal cancer (*120*).

However, the epidemiologic data do have some limitations, which decrease the strength of the evidence currently available. For example, consumption is often reported as frequency instead of amount consumed, and the consumption categories differ widely among studies. In addition, the meta-analysis (*120*) indicated that the apparent protective effect found between *Allium* vegetable consumption and cancers of the stomach and colon may have been overestimated, suggesting a possible publication bias. Lack of adjustment in the statistical analyses also limits the strength of the conclusion. For example, the data for stomach cancer were generally not adjusted for fruit and vegetable consumption, which are known to be protective and could therefore be a confounder in the analysis. Clinical trials are not currently available, but they could be useful for assessing the ability of garlic and other *Allium* vegetables to prevent cancer or preneoplastic lesions. There are difficulties in standardizing the real intake of *Allium* vegetables, and in estimating the composition in organosulfur compounds and other chemicals. In addition, these trials cannot use cancer as the end point and should therefore rely on surrogate intermediate biomarkers (*121*). Such end points are not fully validated, and further research is needed to develop and validate suitable biomarkers.

Studies in experimental animals indicate that the benefits of *Allium* vegetables are not limited to one species, tissue, or carcinogen. Organosulfur compounds can hinder activation of a carcinogen from its precursor, increase its metabolic detoxification, or

Table 8. Effects of *Allium* vegetable components on carcinogenesis in experimental animals.

Organ	Chemical component	Animal species, doses	Carcinogen	Effect on carcinogenesis	Reference
Forestomach	AMDS, AMTS, DAS, DATS	Mouse, 0.02 mmol, po	BaP	Inhibition	(*58*)
	PMDS, PMTS, DPS, DPTS			No effect	
	AMDS, DADS, AM	Mouse, 0.01–0.02 mmol, po	NDEA	Inhibition (mainly DADS, AM)	(*59*)
Colon	DAS	Mouse, 200 mg/kg bw, po	DMH	Inhibition	(*60*)
	SAC	Mouse, 50–100 mg/kg bw, po	DMH	Inhibition	(*61*)
	DAS, DADS	Rat, 50–200 mg/kg bw, po	Combination	Inhibition (DADS)	(*62*)
	DADS	Rat, 200 ppm in diet	AOM	Inhibition	(*63*)
Esophagus	DAS	Rat, 200 mg/kg bw, po	NMBA	Inhibition	(*64*)
Mammary gland	DAS, DADS, AMS	Rat, 1.8 mmol/kg bw, po	DMBA	Inhibition	(*65*)
	Garlic powder	20 g/kg diet			
	Garlic powder	Rat, 1–4% in diet	DMBA	Inhibition	(*66*)
	SAC, DADS	Rat, 57 µmol/kg diet	MNU	Inhibition	(*67*)
	Garlic powder	20 g/kg diet			
	SAC	Rat, 666 and 2,000 ppm in diet	MNU	No effect	(*68*)
Lung	AMDS, DAS,	Mouse, 0.02 mmol, po	BaP	Inhibition	(*58*)
	AMTS, DATS, PMDS, PMTS, DPS, DPTS			No effect	
	AMDS, DADS, AM	Mouse, 0.01–0.02 mmol, po	NDEA	Inhibition	(*59*)
Liver	DAS	Rat, 50–100 mg/kg bw, po	DMH	Inhibition	(*69*)
	DAS, DADS	Rat, 50–200 mg/kg bw, po	Combination	Increase (DAS)	(*62*)
Kidney	DAS, DADS	Rat, 50–200 mg/kg b.w., po	Combination	Inhibition (DADS)	(*62*)
Uterine cervix	Garlic extract	Mouse, 400 mg/kg bw, po	MCA	Inhibition	(*70*)
Skin	Garlic oil, onion oil	Mouse, 10 µg–10 mg, pc	DMBA-TPA	Inhibition (mainly onion oil)	(*71*)
	Garlic oil	Mouse, 10% solution, pc	BaP-croton oil	Inhibition	(*72*)
	Garlic extract	Mouse, 5 mg dry weight, pc	DMBA-TPA	Inhibition	(*73*)
	DAS	Mouse, 250 µg, pc	DMBA, BaP	Inhibition	(*74*)

Abbreviations: AOM, azoxymethane; BaP, benzo[*a*]pyrene; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMH, 1,2-dimethylhydrazine; MCA, 3-methylcholanthrene; MNU, *N*-methylnitrosourea; NDEA, *N*-nitrosodiethylamine; NMBA, *N*-nitrosomethylbenzylamine; pc, percutaneously; po, per os; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

prevent its reaction with vulnerable target cells. Additional mechanisms include a delay or a reversion of the expression of malignancy by antiproliferative activity in tumor cells and modification of signal transduction mechanisms. These speculative mechanisms in animal models should be verified in human studies to establish a causative link between some molecular properties and the cancer-preventive activity.

Extrapolation of the doses of pure chemicals that are effective in animals to their equivalents in terms of *Allium* vegetables leads to unrealistic estimates of the amounts that would have to be consumed by humans to benefit from the antitumor effects of these substances. For example, because the dose of DAS that is effective in animals ranges from 50 to 400 mg/kg bw, a person weighing 70 kg would have to consume 3.5–28 g of DAS per day. The concentration of DAS in garlic ranges from 0.03 to 0.1 mg/g; thus, 40–100 g of garlic would have to be consumed per day, corresponding to 10–100 garlic cloves per day. Epidemiologic studies have found a protective effect of consumption of much lower doses, the mean intake being 18.3 g/week (*120*).

Little is known about the stability of organosulfur compounds during cooking or about the metabolism, pharmacokinetics, and toxicity of the active compounds in humans. All these aspects require further attention if the use of garlic and other *Allium* vegetables are to be recommended for cancer prevention.

References and Notes

1. Block E. The chemistry of garlic and onions. Sci Am 252:94–99 (1985).
2. Virtanen AL, Matikkala EJ. The isolation of *S*-methyl-*L*-cysteine sulphoxide and *S*-*n*-propyl-*L*-cysteine sulphoxide from onion (*Allium cepa*) and the antibiotic activity of crushed onion. Acta Chem Scand 13:1898–1900 (1976).
3. Yu TH, Wu CM, Liou YC. Volatile compounds from garlic. J Agric Food Chem 37:725–730 (1989).
4. Weisberger AS, Pensky J. Tumor inhibition by a sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). Cancer Res 18:1301–1308 (1958).
5. Gilbert HF. Molecular and cellular aspects of thiol-disulfide exchange. Adv Enzymol Relat Areas Mol Biol 63:69–172 (1990).
6. Lawson LD, Wang ZJ. Pre-hepatic fate of the organosulfur compounds derived from garlic (*Allium sativum*). Planta Med 59:A688–A689 (1993).
7. Egen-Schwind C, Eckard R, Kemper FH. Metabolism of garlic constituents in the isolated perfused rat liver. Planta Med 58:301–305 (1992).
8. Sheen LY, Wu CC, Lii CK, Tsai SJ. Metabolites of diallyl disulfide and diallyl sulfide in primary rat hepatocytes. Food Chem Toxicol 37:1139–1146 (1999).
9. Jandke J, Spiteller G. Unusual conjugates in biological profiles originating from consumption of onions and garlic. J Chromatogr 421:1–8 (1987).
10. de Rooij BM, Boogaard PJ, Rijksen DA, Commandeur JN, Vermeulen NP. Urinary excretion of *N*-acetyl-*S*-allyl-*L*-cysteine upon garlic consumption by human volunteers. Arch Toxicol 70:635–639 (1996).
11. de Rooij BM, Boogaard PJ, Commandeur JN, van Sittert NJ, Vermeulen NP. Allylmercapturic acid as urinary biomarker of human exposure to allyl chloride. Occup Environ Med 54:653–661 (1997).
12. Laakso I, Seppanen-Laakso T, Hiltunen R, Muller B, Jansen H, Knobloch K. Volatile garlic odor components: gas phases and adsorbed exhaled air analysed by headspace gas chromatography-mass spectrometry. Planta Med 55:257–261 (1989).
13. Minami T, Boku T, Inada K, Morita M, Okazaki Y. Odor components of human breath after the ingestion of grated raw garlic. J Food Sci 54:763–765 (1989).
14. Mei X, Wang MC, Xu HX, Pan XY, Gao CY, Han N, Fu MY. Garlic and gastric cancer—the effect of garlic on nitrite and nitrate in gastric juice. Acta Nutr Sin 4:53–56 (1982).
15. Takezaki T, Gao CM, Ding JH, Liu TK, Li MS, Tajima K. Comparative study of lifestyles of residents in high and low risk areas for gastric cancer in Jiangsu Province, China; with special reference to *Allium* vegetables. J Epidemiol 9:297–305 (1999).
16. Haenszel W, Kurihara M, Segi M, Lee RK. Stomach cancer among Japanese in Hawaii. J Natl Cancer Inst 49:969–988 (1972).
17. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. Jpn J Cancer Res 76:705–716 (1985).
18. Trichopoulos D, Ouranos G, Day NE, Tzonou A, Manousos O, Papadimitriou C, Trichopoulos A. Diet and cancer of the stomach: a case-control study in Greece. Int J Cancer 36:291–297 (1985).
19. Hu JF, Zhang SF, Jia EM, Wang QQ, Liu SD, Liu YY, Wu YP, Cheng YT. Diet and cancer of the stomach: a case-control study in China. Int J Cancer 41:331–335 (1988).
20. Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, Biserni R, Cipriani F, Cocco P, Giacosa A. A case-control study of gastric cancer and diet in Italy. Int J Cancer 44:611–616 (1989).
21. You WC, Blot WJ, Chang YS, Ershow A, Yang ZT, An Q, Henderson BE, Fraumeni JF Jr, Wang TG. *Allium* vegetables and reduced risk of stomach cancer. J Natl Cancer Inst 81:162–164 (1989).
22. Boeing H, Jedrychowski W, Wahrendorf J, Popiela T, Tobiasz-Adamczyk B, Kulig A. Dietary risk factors in intestinal and diffuse types of stomach cancer: a multicenter case-control study in Poland. Cancer Causes Control 2:227–233 (1991).
23. Gonzalez CA, Sanz JM, Marcos G, Pita S, Brullet E, Saigi E, Badia A, Riboli E. Dietary factors and stomach cancer in Spain: a multi-centre case-control study. Int J Cancer 49:513–519 (1991).
24. Tuyns AJ, Kaaks R, Haelterman M, Riboli E. Diet and gastric cancer. A case-control study in Belgium. Int J Cancer 51:1–6 (1992).
25. Hansson LE, Nyren O, Bergstrom R, Wolk A, Lindgren A, Baron J, Adami HO. Diet and risk of gastric cancer. A population-based case-control study in Sweden. Int J Cancer 55:181–189 (1993).
26. Gao CM, Takezaki T, Ding JH, Li MS, Tajima K. Protective effect of *Allium* vegetables against both esophageal and stomach cancer: a simultaneous case-referent study of a high-epidemic area in Jiangsu Province, China. Jpn J Cancer Res 90:614–621 (1999).
27. Dorant E, van den Brandt PA, Goldbohm RA, Sturmans F. Consumption of onions and a reduced risk of stomach carcinoma. Gastroenterology 110:12–20 (1996).
28. Hu J, Nyren O, Wolk A, Bergstrom R, Yuen J, Adami HO, Guo L, Li H, Huang G, Xu X. Risk factors for oesophageal cancer in northeast China. Int J Cancer 57:38–46 (1994).
29. Gao YT, McLaughlin JK, Gridley G, Blot WJ, Ji BT, Dai Q, Fraumeni JF Jr. Risk factors for esophageal cancer in Shanghai, China. II: Role of diet and nutrients. Int J Cancer 58:197–202 (1994).
30. Cellini L, Di Campli E, Masulli M, Di Bartolomeo S, Allocati N. Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). FEMS Immunol Med Microbiol 13:273–277 (1996).
31. Chung JG, Chen GW, Wu LT, Chang HL, Lin JG, Yeh CC, Wang TF. Effects of garlic compounds diallyl sulfide and diallyl disulfide on arylamine *N*-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. Am J Chin Med 26:353–364 (1998).
32. Jonkers D, van den Broek E, van Dooren I, Thijs C, Dorant E, Hageman G, Stobberingh E. Antibacterial effect of garlic and omeprazole on *Helicobacter pylori*. J Antimicrob Chemother 43:837– 839 (1999).
33. O'Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. Appl Environ Microbiol 66:2269–2273 (2000).
34. Shinchi K, Ishii H, Imanishi K, Kono S. Relationship of cigarette smoking, alcohol use, and dietary habits with *Helicobacter pylori* infection in Japanese men. Scand J Gastroenterol 32:651–655 (1997).
35. Graham DY, Anderson SY, Lang T. Garlic or jalapeno peppers for treatment of *Helicobacter pylori* infection. Am J Gastroenterol 94:1200–1202 (1999).
36. Gail MH, You WC, Chang YS, Zhang L, Blot WJ, Brown LM, Groves FD, Heinrich JP, Hu J, Jin ML, et al. Factorial trial of three interventions to reduce the progression of precancerous gastric lesions in Shandong, China: design issues and initial data. Control Clin Trials 19:352–369 (1998).
37. Haenszel W, Locke FB, Segi M. A case-control study of large bowel cancer in Japan. J Natl Cancer Inst 64:17–22 (1980).
38. Tuyns AJ, Kaaks R, Haelterman M. Colorectal cancer and the consumption of foods: a case-control study in Belgium. Nutr Cancer 11:189–204 (1988).
39. Hu JF, Liu YY, Yu YK, Zhao TZ, Liu SD, Wang QQ. Diet and cancer of the colon and rectum: a case-control study in China. Int J Epidemiol 20:362–367 (1991).
40. Iscovich JM, L'Abbe KA, Castelleto R, Calzona A, Bernedo A, Chopita NA, Jmelnitzsky AC, Kaldor J. Colon cancer in Argentina. I: Risk from intake of dietary items. Int J Cancer 51:851–857 (1992).
41. Steinmetz KA, Potter JD. Food-group consumption and colon cancer in the Adelaide Case-Control Study. I: Vegetables and fruit. Int J Cancer 53:711–719 (1993).
42. Le Marchand L, Hankin JH, Wilkens LR, Kolonel LN, Englyst HN, Lyu LC. Dietary fiber and colorectal cancer risk. Epidemiology 8:658–665 (1997).
43. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S. Food groups and colorectal cancer risk. Br J Cancer 79:1283–1287 (1999).
44. Steinmetz KA, Kushi LH, Bostick RM, Folsom AR, Potter JD. Vegetables, fruit, and colon cancer in the Iowa Women's Health Study. Am J Epidemiol 139:1–15 (1994).
45. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 54:2390–2397 (1994).
46. Dorant E, van den Brandt PA, Goldbohm RA. A prospective cohort study on the relationship between onion and leek consumption, garlic supplement use and the risk of colorectal carcinoma in The Netherlands. Carcinogenesis 17:477–484 (1996).
47. Katsouyanni K, Trichopoulos D, Boyle P, Xirouchaki E, Trichopoulou A, Lisseos B, Vasilaros S, MacMahon B. Diet and breast cancer: a case-control study in Greece. Int J Cancer 38:815–820 (1986).
48. Levi F, La Vecchia C, Gulie C, Negri E. Dietary factors and breast cancer risk in Vaud, Switzerland. Nutr Cancer 19:327–335 (1993).
49. Challier B, Perarnau JM, Viel JF. Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. Eur J Epidemiol 14:737–747 (1998).
50. Dorant E, van den Brandt PA, Goldbohm RA. *Allium* vegetable consumption, garlic supplement intake, and female breast carcinoma incidence. Breast Cancer Res Treat 33:163–170 (1995).
51. Sankaranarayanan R, Varghese C, Duffy SW, Padmakumary G, Day NE, Nair MK. A case-control study of diet and lung cancer in Kerala, South India. Int J Cancer 58:644–649 (1994).
52. Dorant E, van den Brandt PA, Goldbohm RA. A prospective cohort study on *Allium* vegetable consumption, garlic supplement use, and the risk of lung carcinoma in The Netherlands. Cancer Res 54:6148–6153 (1994).
53. Zheng W, Blot WJ, Shu XO, Gao YT, Ji BT, Ziegler RG, Fraumeni JF Jr. Diet and other risk factors for laryngeal cancer in Shanghai, China. Am J Epidemiol 136:178–191 (1992).
54. Zheng W, Blot WJ, Shu XO, Diamond EL, Gao YT, Ji BT, Fraumeni JF Jr. A population-based case-control study of cancers of the nasal cavity and paranasal sinuses in Shanghai. Int J Cancer 52:557–561 (1992).
55. Key TJ, Silcocks PB, Davey GK, Appleby PN, Bishop DT. A case-control study of diet and prostate cancer. Br J Cancer 76:678–687 (1997).
56. Hu J, La Vecchia C, Negri E, Chatenoud L, Bosetti C, Jia X, Liu R, Huang G, Bi D, Wang C. Diet and brain cancer in adults: a case-control study in northeast China. Int J Cancer 81:20–23 (1999).
57. Shu XO, Zheng W, Potischman N, Brinton LA, Hatch MC, Gao Y-T, Fraumeni JF Jr. A population-based case-con-

trol study of dietary factors and endometrial cancer in Shanghai, People's Republic of China. Am J Epidemiol 137:155–165 (1993).
58. Sparnins VL, Barany G, Wattenberg LW. Effects of organosulfur compounds from garlic and onions on benzo[*a*]pyrene-induced neoplasia and glutathione *S*-transferase activity in the mouse. Carcinogenesis 9:131–134 (1988).
59. Wattenberg LW, Sparnins VL, Barany G. Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. Cancer Res 49:2689–2692 (1989).
60. Wargovich MJ. Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. Carcinogenesis 8:487–489 (1987).
61. Sumiyoshi H, Wargovich MJ. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. Cancer Res 50:5084–5087 (1990).
62. Takahashi S, Hakoi K, Yada H, Hirose M, Ito N, Fukushima S. Enhancing effects of diallyl sulfide on hepatocarcinogenesis and inhibitory actions of the related diallyl disulfide on colon and renal carcinogenesis in rats. Carcinogenesis 13:1513–1518 (1992).
63. Reddy BS, Rao CV, Rivenson A, Kelloff G. Chemoprevention of colon carcinogenesis by organosulfur compounds. Cancer Res 53:3493–3498 (1993).
64. Wargovich MJ, Woods C, Eng VW, Stephens LC, Gray K. Chemoprevention of *N*-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. Cancer Res 48:6872–6875 (1988).
65. Ip C, Lisk DJ, Stoewsand GS. Mammary cancer prevention by regular garlic and selenium-enriched garlic. Nutr Cancer 17:279–286 (1992).
66. Liu J, Lin RI, Milner JA. Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors and DNA adducts by garlic powder. Carcinogenesis 13:1847–1851 (1992).
67. Schaffer EM, Liu JZ, Green J, Dangler CA, Milner JA. Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea induced rat mammary carcinogenesis. Cancer Lett 102:199–204 (1996).
68. Cohen LA, Zhao Z, Pittman B, Lubet R. *S*-allylcysteine, a garlic constituent, fails to inhibit *N*-methylnitrosourea-induced rat mammary tumorigenesis. Nutr Cancer 35:58–63 (1999).
69. Hayes MA, Rushmore TH, Goldberg MT. Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. Carcinogenesis 8:1155–1157 (1987).
70. Hussain SP, Jannu LN, Rao AR. Chemopreventive action of garlic on methylcholanthrene-induced carcinogenesis in the uterine cervix of mice. Cancer Lett 49:175–180 (1990).
71. Belman S. Onion and garlic oils inhibit tumor promotion. Carcinogenesis 4:1063–1065 (1983).
72. Sadhana AS, Rao AR, Kucheria K, Bijani V. Inhibitory action of garlic oil on the initiation of benzo[*a*]pyrene-induced skin carcinogenesis in mice. Cancer Lett 40:193–197 (1988).
73. Nishino H, Iwashima A, Itakura Y, Matsuura H, Fuwa T. Antitumor-promoting activity of garlic extracts. Oncology 46:277–280 (1989).
74. Singh A, Shukla Y. Antitumour activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. Cancer Lett 131:209–214 (1998).
75. Zoumas C, Amagase H, Milner JA. Impact of dietary lipid, protein and methionine on ability of garlic to decrease binding of 7,12-dimethylbenz[*a*]anthracene to mammary DNA. FASEB J 6:A13–A13 (1992).
76. Amagase H, Schaffer EM, Milner JA. Dietary components modify the ability of garlic to suppress 7,12-dimethylbenz[*a*]anthracene-induced mammary DNA adducts. J Nutr 126:817–824 (1996).
77. Soni KB, Lahiri M, Chackradeo P, Bhide SV, Kuttan R. Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Cancer Lett 115:129–133 (1997).
78. Zhang YS, Chen XR, Yu YN. Antimutagenic effect of garlic (*Allium sativum* L.) on 4NQO-induced mutagenesis in *Escherichia coli* WP20. Mutat Res 227:215–219 (1989).
79. Knasmuller S, de Martin R, Domjan G, Szakmary A. Studies on the antimutagenic activities of garlic extract. Environ Mol Mutagen 13:357–365 (1989).
80. Kato T, Michikoshi K, Minowa Y, Maeda Y, Kikugawa K. Mutagenicity of cooked hamburger is reduced by addition of onion to ground beef. Mutat Res 420:109–114 (1998).
81. Sparnins VL, Mott AW, Barany G, Wattenberg LW. Effects of allyl methyl trisulfide on glutathione *S*-transferase activity and BP-induced neoplasia in the mouse. Nutr Cancer 8:211–215 (1986).
82. Srivastava SK, Hu X, Xia H, Zaren HA, Chatterjee ML, Agarwal R, Singh SV. Mechanism of differential efficacy of garlic organosulfides in preventing benzo[*a*]pyrene-induced cancer in mice. Cancer Lett 118:61–67 (1997).
83. Munday R, Munday CM. Low doses of diallyl disulfide, a compound derived from garlic, increase tissue activities of quinone reductase and glutathione transferase in the gastrointestinal tract of the rat. Nutr Cancer 34:42–48 (1999).
84. Brady JF, Li DC, Ishizaki H, Yang CS. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. Cancer Res 48:5937–5940 (1988).
85. Brady JF, Wang MH, Hong JY, Xiao F, Li Y, Yoo JS, Ning SM, Lee MJ, Fukuto JM, Gapac JM. Modulation of rat hepatic microsomal monooxygenase enzymes and cytotoxicity by diallyl sulfide. Toxicol Appl Pharmacol 108:342–354 (1991).
86. Pan J, Hong JY, Li D, Schuetz EG, Guzelian PS, Huang W, Yang CS. Regulation of cytochrome P450 2B1/2 genes by diallyl sulfone, disulfiram, and other organosulfur compounds in primary cultures of rat hepatocytes. Biochem Pharmacol 45:2323–2329 (1993).
87. Reicks MM, Crankshaw DL. Modulation of rat hepatic cytochrome P-450 activity by garlic organosulfur compounds. Nutr Cancer 25:241–248 (1996).
88. Haber D, Siess MH, Canivenc-Lavier MC, Le Bon AM, Suschetet M. Differential effects of dietary diallyl sulfide and diallyl disulfide on rat intestinal and hepatic drug-metabolizing enzymes. J Toxicol Environ Health 44:423–434 (1995).
89. Sheen LY, Chen HW, Kung YL, Liu CT, Lii CK. Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets. Nutr Cancer 35:160–166 (1999).
90. Jin L, Baillie TA. Metabolism of the chemoprotective agent diallyl sulfide to glutathione conjugates in rats. Chem Res Toxicol 10:318–327 (1997).
91. Chen GW, Chung JG, Hsieh CL, Lin JG. Effects of the garlic components diallyl sulfide and diallyl disulfide on arylamine *N*-acetyltransferase activity in human colon tumour cells. Food Chem Toxicol 36:761–770 (1998).
92. Chung JG. Effects of garlic components diallyl sulfide and diallyl disulfide on arylamine *N*-acetyltransferase activity in human bladder tumor cells. Drug Chem Toxicol 22:343–358 (1999).
93. Amagase H, Milner JA. Impact of various sources of garlic and their constituents on 7,12-dimethylbenz[*a*]anthracene binding to mammary cell DNA. Carcinogenesis 14:1627–1631 (1993).
94. Hageman GJ, van Herwijnen MH, Schilderman PA, Rhijnsburger EH, Moonen EJ, Kleinjans JC. Reducing effects of garlic constituents on DNA adduct formation in human lymphocytes *in vitro*. Nutr Cancer 27:177–185 (1997).
95. Shenoy NR, Choughuley AS. Inhibitory effect of diet related sulphydryl compounds on the formation of carcinogenic nitrosamines. Cancer Lett 65:227–232 (1992).
96. Lin XY, Liu JZ, Milner JA. Dietary garlic suppresses DNA adducts caused by *N*-nitroso compounds. Carcinogenesis 15:349–352 (1994).
97. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915–7922 (1993).
98. Perchellet JP, Perchellet EM, Abney NL, Zirnstein JA, Belman S. Effects of garlic and onion oils on glutathione peroxidase activity, the ratio of reduced/oxidized glutathione and ornithine decarboxylase induction in isolated mouse epidermal cells treated with tumor promoters. Cancer Biochem Biophys 8:299–312 (1986).
99. Gudi VA, Singh SV. Effect of diallyl sulfide, a naturally occurring anti-carcinogen, on glutathione-dependent detoxification enzymes of female CD-1 mouse tissues. Biochem Pharmacol 42:1261–1265 (1991).
100. Chen L, Hong JY, So E, Hussin AH, Cheng WF, Yang CS. Decrease of hepatic catalase level by treatment with diallyl sulfide and garlic homogenates in rats and mice. J Biochem Mol Toxicol 13:127–134 (1999).
101. Pinto JT, Qiao C, Xing J, Rivlin RS, Protomastro ML, Weissler ML, Tao Y, Thaler H, Heston WD. Effects of garlic thioallyl derivatives on growth, glutathione concentration, and polyamine formation of human prostate carcinoma cells in culture. Am J Clin Nutr 66:398–405 (1997).
102. Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H, Itakura Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. Planta Med 60:417–420 (1994).
103. Fanelli SL, Castro GD, de Toranzo EG, Castro JA. Mechanisms of the preventive properties of some garlic components in the carbon tetrachloride-promoted oxidative stress. Diallyl sulfide, diallyl disulfide, allyl mercaptan and allyl methyl sulfide. Res Commun Mol Pathol Pharmacol 102:163–174 (1998).
104. Sundaram SG, Milner JA. Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture. Cancer Lett 74:85–90 (1993).
105. Sundaram SG, Milner JA. Diallyl disulfide induces apoptosis of human colon tumor cells. Carcinogenesis 17:669–673 (1996).
106. Sakamoto K, Lawson LD, Milner JA. Allyl sulfides from garlic suppress the *in vitro* proliferation of human A549 lung tumor cells. Nutr Cancer 29:152–156 (1997).
107. Welch C, Wuarin L, Sidell N. Antiproliferative effect of the garlic compound *S*-allyl cysteine on human neuroblastoma cells *in vitro*. Cancer Lett 63:211–219 (1992).
108. Takeyama H, Hoon DS, Saxton RE, Morton DL, Irie RF. Growth inhibition and modulation of cell markers of melanoma by *S*-allyl cysteine. Oncology 50:63–69 (1993).
109. Lee ES, Steiner M, Lin R. Thioallyl compounds: potent inhibitors of cell proliferation. Biochim Biophys Acta 1221:73–77 (1994).
110. Seki T, Tsuji K, Hayato Y, Moritomo T, Ariga T. Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. Cancer Lett 160:29–35 (2000).
111. Siegers CP, Steffen B, Robke A, Pentz R. The effects of garlic preparations against human tumor cell proliferation. Phytomedicine 6:7–11 (1999).
112. Baer AR, Wargovich MJ. Role of ornithine decarboxylase in diallyl sulfide inhibition of colonic radiation injury in the mouse. Cancer Res 49:5073–5076 (1989).
113. Hu PJ, Wargovich MJ. Effect of diallyl sulfide on MNNG-induced nuclear aberrations and ornithine decarboxylase activity in the glandular stomach mucosa of the Wistar rat. Cancer Lett 47:153–158 (1989).
114. Takada N, Matsuda T, Otoshi T, Yano Y, Otani S, Hasegawa T, Nakae D, Konishi Y, Fukushima S. Enhancement by organosulfur compounds from garlic and onions of diethylnitrosamine-induced glutathione *S*-transferase positive foci in the rat liver. Cancer Res 54:2895–2899 (1994).
115. Hong YS, Ham YA, Choi JH, Kim J. Effects of allyl sulfur compounds and garlic extract on the expression of Bcl-2, Bax, and p53 in non small cell lung cancer cell lines. Exp Mol Med 32:127–134 (2000).
116. Dirsch VM, Gerbes AL, Vollmar AM. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kB. Mol Pharmacol 53:402–407 (1998).
117. Knowles LM, Milner JA. Depressed $p34^{cdc2}$ kinase activity and G2/M phase arrest induced by diallyl disulfide in HCT-15 cells. Nutr Cancer 30:169–174 (1998).
118. Knowles LM, Milner JA. Diallyl disulfide inhibits $p34^{(cdc2)}$ kinase activity through changes in complex formation and phosphorylation. Carcinogenesis 21:1129–1134 (2000).
119. Singh SV, Mohan RR, Agarwal R, Benson PJ, Hu X, Rudy MA, Xia H, Katoh A, Srivastava SK, Mukhtar H, et al. Novel anti-carcinogenic activity of an organosulfide from garlic: inhibition of H-RAS oncogene transformed tumor growth *in vivo* by diallyl disulfide is associated with inhibition of $p21^{H\text{-}ras}$ processing. Biochem Biophys Res Commun 225:660–665 (1996).
120. Fleischauer AT, Poole C, Arab L. Garlic consumption and cancer prevention: meta-analyses of colorectal and stomach cancers. Am J Clin Nutr 72:1047–1052 (2000).
121. Miller AB, Bartsch H, Boffetta P, Dragsted L, Vainio H, eds. Biomarkers in Cancer Prevention. IARC Sci Publ 154:1–214 (2001).