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Garlic

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5 Garlic
Chemistry, Function, and Implications for Health and Disease

Sharon A. Ross and Craig S. Charron

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5.1 INTRODUCTION

Garlic (Allium sativum) has been valued for its medicinal properties for centuries. In recent years, this interest has been reflected by numerous studies investigating the potential of garlic to reduce the risk of cardiovascular disease and cancer.1-4 The ability of garlic and related components to serve as antioxidants5 and influence immunocompetence6 and possibly cognitive function7 suggest that its health implications may be extremely widespread.

A member of the Alliaceae family, garlic is one of the more economically important cultivated spices. Large amounts of garlic are produced annually in China and India. In 2015, 3.83 million cwt. of garlic were harvested from 23,600 acres in the United States.8 About 95% of this amount is produced in California. Although considerable consumption occurs as fresh garlic, it is also found as dehydrates, flakes, and salts in a variety of food preparations. Dozens of garlic supplements are also commercially available as essential oils, garlic-oil macerate, garlic powder, or garlic extract. Garlic has continued to be one of the top-selling herbs in the United States.
Garlic is often referred to as a spice, herb, or vegetable. Along with onions, leeks, shallots, and chives, it is one of the major allium foods consumed by humans. The garlic bulb consists of several individual cloves, each weighing about 3 g. Actual garlic intakes are not known with certainty, especially as garlic is not typically considered in dietary assessment surveys. Intakes are thought to vary from region to region and from individual to individual. Annual per capita retail consumption of fresh garlic has ranged from 2.2 to 2.8 lbs. since 2000. Data used in a meta-analysis of colorectal and stomach cancer suggested that the mean intake (±SD) of raw and cooked garlic intake across all published reports was 18.3 ± 14.2 g per week, or about 6 cloves of garlic per week. Consumption ranged from none to 3.5 g per week (about 1 clove), whereas the highest intake exceeded 28.8 g per week (about 9 to 10 cloves).

Although negative consequences are not always an outcome of high garlic intake, some individuals may be more susceptible to side effects than others. While their incidence is low, a spectrum of adverse allergic reactions can occur following contact with garlic. Even though garlic is recognized as a powerful irritant, a few reports of allergic contact dermatitis appear in the literature. Avoidance of direct contact seems to be the most logical approach for food handlers who are sensitive, but this may be more difficult than anticipated, as diallyl disulfide (DADS), an active irritant, penetrates most commercially available gloves.

Excessive garlic intake has also been reported to lead to hemolytic anemia. The severity of the anemia correlates with a reduction in erythrocyte-reduced glutathione (GSH) and plasma ascorbic acid. Incubations of canine erythrocytes with sodium 2-propanyl thiosulfate from garlic were found to increase methemoglobin concentration and Heinz body occurrences, indicating that this compound may be the cause of oxidative damage in canine erythrocytes. Umar et al. found that ascorbic acid or vitamin E supplements prevented the garlic-precipitated reduction in GSH and plasma ascorbic acid, thereby providing greater protection to the erythrocyte membrane. Other possible adverse effects of garlic include herb-drug interactions, particularly with regard to anticoagulant or antiretroviral therapy, and mild gastrointestinal discomfort, which is the most common adverse effect.

5.2 GARLIC COMPOSITION AND CHEMISTRY

The use of garlic typically centers on its unique flavor and odor characteristics. Unlike other foods, garlic is distinctive in that about 1% of its dry weight is sulfur. Garlic is of somewhat limited nutritional value because its total intake is typically low, although it is more nutritious than onions on a fresh-weight basis. A 3-g serving of garlic provides about 4.5 mg of potassium, 0.6 g of carbohydrate, and trace amounts of calcium, fiber, iron, and vitamin C. Table 5.1 provides some compositional information about garlic. Carbohydrates provide about 33% of garlic’s weight, whereas protein accounts for another 6.4%. Whereas many of garlic’s health benefits have been attributed to its sulfur components, other constituents, including arginine, selenium, oligosaccharides, and flavonoids, may also convey health benefits.

The chemistry of sulfur compounds found in garlic is exceedingly complex and not completely understood. Regardless, it is known that the primary sulfur-containing constituents in garlic bulbs are γ-glutamyl-S-alk(en)yl-L-cysteines and S-alk(en)yl-L-cysteine sulfoxides. The content of S-alk(en)ylcysteine sulfoxide in garlic typically ranges between 0.53% and 1.3% of the fresh weight, with alliin (S-allylcysteine sulfoxide) the largest contributor. This variation likely reflects environmental factors, including climate or cropping conditions. Similarly, the processing method used can markedly influence the amounts and types of individual sulfur compounds. Alliin concentrations can increase during storage as a result of the transformation of γ-glutamylcysteines. In addition to alliin, garlic bulbs contain small amounts of (±)-S-allylcysteine sulfoxide (methyl) and (±)-S-(trans-1-propenyl)-L-cysteine sulfoxide, S-(2-carboxypropyl) glutathione, γ-glutamyl-S-allyl-L-cysteine, γ-glutamyl-S-(trans-1-propenyl)-L-cysteine, and γ-glutamyl-S-allylmercapto-L-cysteine.
Garlic

The characteristic odor of garlic arises from allicin (thio-2-propene-1-sulfinic acid S-allyl ester) and oil-soluble sulfur compounds formed when the bulb is crushed or damaged. This membrane destruction yields organosulfur degradation products as a result of the release of the enzyme alliinase, which rapidly converts alliin to form the odiferous alkyl alkane-thiosulfinates, including allicin. Because allicin is unstable, it further decomposes to sulfides, ajoene, and dithiins. Tamaki and Sonoki reported that strong garlic flavor and scent were linked to a higher content of volatile sulfur. Not surprisingly, heating garlic reduced allyl mercaptan (AM), methyl mercaptan, and allyl methyl sulfide (AMS) concentrations and reduced its odor, possibly because of an inactivation of alliinase. The specific formulation of a garlic product has a clear impact on its sulfur compounds. Aged garlic extract (AGE) is sold in tablet, capsule, and liquid form, and is produced by extraction of chopped garlic in aqueous ethanol, followed by filtration and concentration. S-allylcysteine, S-trans-1-propenylcysteine, and S-allylmercaptocysteine arise as predominant compounds, whereas concentrations of γ-glutamyl-S-alkenylcysteines decrease due to the aging process. Steam-distilled garlic oil has high levels of mono- and polysulfides, particularly diallyl disulfide, diallyl trisulfide, allyl methyl disulfide, and allyl methyl trisulfide. This product is normally diluted 100–200-fold in vegetable oil. Another garlic formulation, oil-extracted garlic macerate, involves the extraction of macerated garlic in soybean or other vegetable oil, which is then sold as capsules. 2-vinyl-4-H-1,3-dithiin constitutes about half of the sulfur compounds on a weight basis, whereas 3-vinyl-4-H-1,2-dithiin constitutes less than a quarter.

The composition of sulfur compounds in commercial preparations is highly variable. Nevertheless, the stability of some of them appears acceptable, according to Lawson and Gardner. They reported that the allyl thiosulfimates of blended fresh garlic were stable for at least 2 years when stored at −80°C. Likewise, they found the dissolution release of thiosulfimates from enteric-coated garlic tablets was near 95%, and the bioavailability, as determined by breath allyl methyl sulfide, was virtually complete and equivalent to that occurring with crushed fresh garlic. The S-allylcysteine (SAC) occurring in deodorized garlic preparations was found to be stable for 12 months when stored at ambient temperature. More compositional information should be provided about garlic preparations available in the marketplace, especially when claims are being made about a specific preparation.

### Table 5.1

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, g</td>
<td>58.6</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>149.0</td>
</tr>
<tr>
<td>Protein, g</td>
<td>6.4</td>
</tr>
<tr>
<td>Total lipid (fat), g</td>
<td>0.5</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>33.1</td>
</tr>
<tr>
<td>Fiber, total dietary, g</td>
<td>2.1</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>181.0</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>25.0</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>153.0</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>401.0</td>
</tr>
<tr>
<td>Selenium, mcg</td>
<td>14.2</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>31.2</td>
</tr>
<tr>
<td>Folate, µg</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Source: Data adapted from the USDA Nutrient Database for Standard Reference, Release 28 (May 2016).
amounts of active compounds in the various products will likely resolve some of the inconsistencies in the literature about the potential health benefits of garlic and commercially prepared extracts, solutions, or tablets. Standardization of the various garlic preparations with respect to one constituent is not a possibility, as the various preparations available in the marketplace have different active components. The development of reference assays that can evaluate the relative bioactivity/potency across preparations may be one of the only solutions for comparing the various preparations available.

The in vivo pharmacokinetics of allyl sulfur compounds have been studied. Lachmann et al. reported the distribution of allicin and vinyldithiines in the form of an oil macerate of the $^{35}$S-labeled substance in rats. Overall, the absorption and the elimination of $^{35}$S-alliin was faster than for other garlic constituents, with maximum blood levels reached within the first 10 min after exposure. Alliin elimination from the blood was almost complete after 6 h. Maximum blood concentrations of $^{35}$S-allicin were not reached until 30 to 60 min after treatment, and for vinyldithiines, the maximum was not achieved until 120 min. Both allicin and vinyldithiines were present in blood at the end of their 72-h study. Urinary excretion suggested an absorption rate approximating 65% for allicin and 73% for vinyldithiines.

Lawson and Wang suggested that allicin absorption in humans is about 95%, although precision was limited because of the rapid metabolism and absence in the blood after consumption. The presence of allicin in blood is transient. Allicin is rapidly transformed in the liver to DADS and allyl mercaptan and, even when incubated in heparinized blood, is converted within 5 min to DADS. DADS can also be further transformed into AM, allyl methyl sulfide, allyl methyl sulfoxide, and allyl methyl sulphone.

Teyssier et al. provided evidence that DADS can be reconverted to diallyl thiosulfinate (allicin) in tissues principally by oxidation arising from cytochrome P450 monooxygenases, and to a limited extent by flavin-containing monooxygenases. Interestingly, their data suggest DADS is preferentially metabolized in human liver to allicin by cytochrome P450 2E1 (CYP2E1). As DADS can also cause autocatalytic CYP2E1 destruction, it is unclear how much allicin might be formed under physiological conditions. Flavin-containing monooxygenases in liver are probably responsible for the oxidization of S-allyl cysteine, among many other sulfur compounds.

Rarely have comparisons of water- and oil-soluble compounds from garlic been examined in the same study. Preclinical studies suggest that garlic extracts of differing composition may inhibit cancer or cardiovascular disease, but it is difficult to compare studies due to the varying doses, times of exposure, animals, cell types, and other experimental procedures. Differences that occur in response to various preparations very likely relate to the content and effectiveness of individual sulfur constituents. The number of sulfur atoms present in the molecule seems to influence the response with diallyl trisulfide (DATS), generally found to be more effective than DADS, which is better than diallyl sulfide (DAS). Likewise, the presence of the allyl group generally enhances the response over that provided by the propyl moiety.

5.3 IMPLICATIONS IN HEALTH

Garlic and a host of its allyl sulfur compounds have been reported to possess a variety of health benefits. Notable among these are the antimicrobial, anticarcinogenic, and protective benefits against cardiovascular disease. Figure 5.1 illustrates some of the most common compounds associated with the health benefits of garlic, and their derivation from the processing of garlic. While there is a need for long-term intervention studies, a variety of preclinical and epidemiological studies suggest that key molecular targets involved in the risk of several diseases can be influenced by these organosulfur compounds arising from garlic.

5.4 ANTIMICROBIAL ACTIVITY

Numerous plants are reported to act as antimicrobial agents. Those rich in tannins, terpenoids, alkaloids, flavonoids, and sulfur compounds have been found to be particularly effective. Historically,
Garlic extracts have been labeled as universal antibiotics. Considerable evidence indicates that garlic extracts can inhibit a range of Gram-negative and Gram-positive bacteria and serve as antifungal agents. Ruddock et al. examined the microbial activity of several garlic products found in the Canadian marketplace and observed a general trend toward increased *in vitro* antibacterial activity among those products containing higher amounts of allicin. Products with marginal antibacterial activity often contained lower concentrations of active constituents than their product labels indicated, which indicates the need to standardize garlic preparations used in research.
*Helicobacter pylori* colonization of the gastric mucosa is responsible for most ulcers of the stomach and upper small intestine\(^5\) and increases risk of non-cardia gastric cancer.\(^5\) Studies by Cellini et al.\(^5\) provide rather convincing evidence that aqueous garlic extracts (2–5 mg/mL) inhibit *H. pylori* proliferation. Reduced effectiveness occurred when the garlic was heated prior to extraction.\(^5\) This depression in activity suggests the need for breakdown products from alliin to achieve a maximum response. As both DAS and DADS are recognized to elicit a dose-dependent depression in *Helicobacter pylori* proliferation in culture,\(^5\) a reduction in their formation may account for the loss of effectiveness caused by heating. Raw garlic extracts and three commercially available garlic tablets were found to vary in their efficacy, as indicated by a minimum inhibitory concentration in the range between 10 and 17.5 \(\mu\)g dry weight/mL.\(^5\)

The ability of garlic to reduce *H. pylori* infection in humans is inconclusive. Although an epidemiological study suggested an association between increased garlic consumption and reduced *H. pylori* infection,\(^5\) three clinical studies testing different garlic preparations in *H. pylori*-infected subjects did not show efficacy.\(^5\) These interventions did not result in the elimination of the organism, change in the severity of gastritis, or a significant change in symptom scores. The studies were not randomized and had small sample sizes. A larger clinical study with 36 outpatients tested the effects of 4 g of garlic powder daily for 8 weeks, and concluded that the result was not different from the placebo.\(^6\) A study with 15 subjects reported that 3 g of garlic cloves consumed twice a day for 3 days suppressed *H. pylori*, but the small sample size and lack of placebo group limits drawing inferences from this result.\(^6\)

Allium plants, including garlic, are effective in suppressing fungal growth.\(^4\) Allicin has been reported to be protective against *Candida albicans* and many other strains. These organisms were extremely sensitive to garlic extracts, some to a greater degree than to nystatin, a known effective antibiotic.\(^4\) Ajoene is also noted for its antimycotic activity both in vitro and in vivo. A fungal infection of the skin known commonly as ringworm and medically as tinea corporis can also be influenced by sulfur compounds found in garlic. Ledeza et al.\(^6\) found that treatment with ajoene (0.6% ajoene or 1% ajoene gel) was as effective as terbinafine (1% cream) in healing tinea corporis and tinea cruris in 70 soldiers with dermatophytosis. As ajoene can be prepared easily from garlic it may be particularly useful as a public health strategy, particularly in developing countries.

The primary antimicrobial effect of garlic may reflect chemical reactions that take place with selected thiol groups of various enzymes and/or a change in the overall redox state of the organism. Specifically, the antimicrobial action of allicin and its breakdown products has been suggested to result from its rapid interaction with thiol or sulfide-containing molecules, including amino acids and cellular proteins within microbial organisms.\(^4\) An example of such an in vivo reaction is that between allicin and glutathione (GSH), which is thought to be the major intracellular mammalian thiol, and investigators have isolated the product of the reaction, established its structure, and examined its interaction with thiol-containing proteins.\(^6\) GSH reacted with allicin in the following fashion:

\[
2\text{GSH} + \text{CH}_2\text{CH-CH}_2\text{(SO)-S-CH}_2\text{-CH} = \text{CH}_2\text{(allicin)} \rightarrow 2\text{GS-S-CH}_2\text{-CH} = \text{CH}_2\text{(S-allylmercaptoglutathione)} (\text{GSSA}) + \text{H}_2\text{O}
\]

As proof of principle, in an in vitro setting, GSSA was found to react with the thiol-containing proteins papain and alcohol dehydrogenase from *Thermoanaerobium brockii* and inhibit their activity, whereas both proteins were reactivated using either reducing agent dithiothreitol or 2-mercaptoethanol. The concomitant release of allylmercaptan in these reactions indicated that the thioallyl moiety binds to inactivated proteins just as allicin has been shown to do. It is interesting to note that one enzyme that may be similarly affected by allicin breakdown products (i.e., DATS, SAC) is squalene monooxygenase.\(^5\) Such activity may explain the antifungal properties of allicin, as squalene monooxygenase is an important enzyme for the formation of the fungal cell wall.\(^6\)
Garlic

Despite promising in vitro results, there is relatively little clinical evidence for the efficacy of garlic as an antibiotic in humans. One research direction of particular importance may be that of determining the effects of garlic intake on the human gastrointestinal microbiome. Gastrointestinal microbiota metabolize dietary components and are modulated by dietary components, hence having a critical role in nutrient metabolism and health status. An in vitro study using the fecal inoculum of a single donor found that garlic powder had a temporal effect on gut commensal bacteria, but specific gut pathogens were not measured. The ample evidence that garlic compounds affect microorganisms in vitro suggests that garlic may have significant effects on the human gastrointestinal microbiome.

5.5 CANCER

There is increasing awareness that several foods and certain dietary patterns may contribute to health, including a reduction in cancer risk. Although limitations exist in defining the precise role that garlic may have in the cancer process, garlic and garlic constituents have been actively studied using both epidemiological and laboratory investigations. There is epidemiological support for the association between increased intake of garlic and/or its active constituents with certain cancers, but the data are limited and show inconsistent results. More than 20 years ago, results from the Iowa Women’s Health Study, a prospective cohort study, found that the strongest association among fruits and vegetables for colon cancer risk reduction was for garlic consumption, with a reduced risk of approximately 50% in distal colon cancer associated with high garlic consumption. This association has since been examined in different populations. In the previous report of the World Cancer Research Fund/American Institute for Cancer Research, the evidence for the association between garlic and colorectal cancer was judged as “probably decreases risk,” whereas in their recent continuous update project on this relationship, it was judged as “limited-no conclusion.” The recent report included studies on garlic and garlic supplement intakes. Other cancer sites have also been studied. Results from a meta-analysis of 14 case control studies, 2 randomized controlled studies, and 1 cohort study with a total of 8621 cases and 14,889 controls found that high, low, and any garlic intake was associated with reduced risk of gastric cancer, with a reduced risk of approximately 50% in distal colon cancer associated with high garlic consumption. This association has since been examined in different populations. In the previous report of the World Cancer Research Fund/American Institute for Cancer Research, the evidence for the association between garlic and colorectal cancer was judged as “probably decreases risk,” whereas in their recent continuous update project on this relationship, it was judged as “limited-no conclusion.” The recent report included studies on garlic and garlic supplement intakes. Other cancer sites have also been studied. Results from a meta-analysis of 14 case control studies, 2 randomized controlled studies, and 1 cohort study with a total of 8621 cases and 14,889 controls found that high, low, and any garlic intake was associated with reduced risk of gastric cancer, with a reduced risk of approximately 50% in distal colon cancer associated with high garlic consumption. However, recent results for two large prospective U.S. cohort studies—women in the Nurses’ Health Study (1984–2014) and men in the Health Professionals Follow-Up Study—found that garlic intake did not reduce gastric cancer risk or modify H. pylori infection. The authors suggested that further studies need to differentiate between cooked and uncooked garlic and consider different gastric cancer types. Interestingly, in a Chinese population, raw garlic intake compared to no garlic intake was associated with lower risk of development of lung cancer with a dose-response pattern. Garlic consumption has also been associated with decreased risk of prostate cancer. A meta-analysis of six case-control and three prospective cohort studies reported a significantly decreased risk of prostate cancer for intake of allium vegetables and that in subgroup analysis stratified by allium vegetable types, significant associations were observed for garlic. Epidemiologic approaches assist in determining associations with cancer risk in large populations, but they have limitations, including multiple testing and potential for false discovery. In addition, studying associations between garlic and cancer risk is also challenging due to the difficulty in assessment of intake levels. The common use of quantiles in epidemiologic studies makes it difficult to compare results across studies, as many cohorts have different ranges of garlic intake. Improved methods for assessment of garlic intake, including the amount, form, and preparation method, may help to further clarify the relationship between garlic and cancer risk.

Few intervention studies have been performed to examine the efficacy of garlic in preventing or treating cancer. In a double-blind, randomized study of Japanese patients with colorectal adenomas, a higher-dose AGE was shown to reduce the risk of new colorectal adenomas compared to a lower-dose garlic extract. Due to observations of a case-control study of gastric cancer in Shandong, China, which indicated that persons in the highest quartile of intake of allium-containing vegetables (including garlic, garlic stalks, scallions, chives, and onions) had only 40% of the risk of those in the
lowest quartile of intake, investigators included a garlic-supplementation arm (800 mg of garlic extract plus 4 mg steam-distilled garlic oil daily) in the Shandong Intervention Trial, a randomized multi-intervention trial to inhibit the progression of precancerous gastric lesion in this same region of China. The interventions included a 2-week course of amoxicillin and omeprazole for subjects who were seropositive for Helicobacter pylori and 7 years of oral supplementation with garlic or with a mixture of vitamin C, vitamin E, and selenium. The outcome of the trial showed that amoxicillin and omeprazole statistically significantly reduced the prevalence and average histological severity of precancerous gastric lesions, whereas the garlic and vitamin treatments did not. Another clinical trial in this same area of China examined the combination of synthetic DATS (200 mg/d of allitridum) plus selenium (100 mg every other day) for a 2-year period on the prevention of gastric cancer. Incidence of total or gastric cancer did not decrease significantly in the overall study population after 5 years, but in males only, the combined intervention resulted in a decreased relative risk for all tumors and gastric cancer.

Preclinical models (Table 5.2) provide some of the most compelling evidence that garlic and its related sulfur components suppress cancer risk and alter the biological behavior of tumors. Overall, garlic and its associated sulfur components have been found to suppress the incidence of mammary, colon, skin, uterine, esophageal, lung, renal, stomach, and liver cancers. Aberrant crypt foci (ACF) are a proposed early preneoplastic lesion of adenoma-carcinoma in humans and chemically induced colon cancer in rodents. In many preclinical studies, both water- and lipid-soluble allyl sulfur compounds administered to animals through their diet have been reported to inhibit ACF, and/or induction of apoptosis. It is possible, and quite probable, that several of these cellular events are modified simultaneously.

### 5.5.1 Nitrosamine and Heterocyclic Amine Formation

Humans are exposed to an array of chemical substances through food sources that may be involved in cancer causation. Nitrosamines, heterocyclic amines (HCAs), and polycyclic aromatic hydrocarbons are potential dietary carcinogens that are not normally present in foods but may arise during preservation or cooking. Human exposure to these suspect carcinogens occurs through the ingestion or inhalation of preformed N-nitroso compounds (NOCs) or by the ingestion of precursors that are combined endogenously. Evidence points to the ability of garlic to suppress the formation of several NOCs. The ability of garlic to reduce NOCs may actually be secondary to an increase in the formation of nitrosothiols. Several sulfur compounds have been proposed to foster the formation of nitrosothiols, thereby reducing the quantity of nitrite available for NOC formation. Studies by Dion et al. revealed that not all allyl sulfur compounds are equally effective in stopping the formation of NOCs. The ability of SAC and its non-allyl analog S-propyl cysteine to retard NOC formation—but not DADS, dipropyl disulfide, and DAS—reveal the critical role that the cysteine residue has in this inhibition. As the content of allyl sulfur can vary among preparations, it is likely that not all garlic sources are equal in the protection they provide against NOC formation. Because of the potential for bacterial-mediated formation of nitrosamines, some of the protection against NOC exposure may also relate to antimicrobial properties associated with garlic.

In a human study, providing 5 g garlic per day completely blocked the enhanced urinary excretion of nitrosoproline that occurred after ingesting supplemental nitrate and proline. The significance of this observation comes from the predictive value that nitrosoproline has for the synthesis of potential carcinogenic nitrosamines. Another human study suggested that 1 g of garlic per day may be sufficient to suppress nitrosoproline formation.

The anticancer benefits attributed to garlic are also associated with the ability of its allyl sulfur compounds to suppress carcinogen bioactivation. Evidence from a variety of sources reveals that garlic is effective in blocking DNA alkylation, a primary step in nitrosamine carcinogenesis.
### TABLE 5.2

**Anticarcinogenic Effects of Garlic and/or Associated Allyl Sulfur Compounds**

<table>
<thead>
<tr>
<th>Site/Carcinogen</th>
<th>Garlic Compound</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Diallyl thioethers</td>
<td>Mouse&lt;sup&gt;239&lt;/sup&gt;</td>
</tr>
<tr>
<td>Buccal pouch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,12-dimethylbenz[a]anthracene</td>
<td>Aqueous garlic extract</td>
<td>Hamster&lt;sup&gt;240&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-dimethylhydrazine</td>
<td>S-allylcysteine</td>
<td>Rat&lt;sup&gt;241&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azoxyomethane</td>
<td>Diallyl sulfide</td>
<td>Mouse&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-methylcholanthrene</td>
<td>Aqueous garlic extract</td>
<td>Mouse&lt;sup&gt;89&lt;/sup&gt;</td>
</tr>
<tr>
<td>Esophagus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosomethylbenzylamine</td>
<td>Diallyl sulfide</td>
<td>Rat&lt;sup&gt;90&lt;/sup&gt;</td>
</tr>
<tr>
<td>Forestomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,12-dimethylbenz[a]anthracene</td>
<td>Diallyl sulfide</td>
<td>Hamster&lt;sup&gt;93&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>Allyl group-containing derivatives</td>
<td>Mouse&lt;sup&gt;243&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-nitrosodiethylamine</td>
<td>Diallyl disulfide</td>
<td>Mouse&lt;sup&gt;244&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gastric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl nitrotrisoguanidine</td>
<td>Diallyl sulfide</td>
<td>Rat&lt;sup&gt;245&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>Fresh garlic, garlic oil</td>
<td>Toad&lt;sup&gt;246&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-nitrosodimethylamine</td>
<td>Diallyl sulfide</td>
<td>Rat&lt;sup&gt;247&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>Diallyl sulfide, allyl methyl disulfide</td>
<td>Mouse&lt;sup&gt;243&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mammary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,12 Dimethylbenz(a)anthracene</td>
<td>Selenium-enriched garlic, garlic powder</td>
<td>Rat&lt;sup&gt;87,190&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-methyl-N-nitrosourea</td>
<td>S-allylcysteine, diallyl disulfide, garlic powder</td>
<td>Rat&lt;sup&gt;16&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP)</td>
<td>Diallyl disulfide</td>
<td>Rat&lt;sup&gt;49&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nasal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodiethylamine</td>
<td>Diallyl sulfide</td>
<td>Rat&lt;sup&gt;280&lt;/sup&gt;</td>
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<tr>
<td>N-nitrosodiethylamine</td>
<td>Diallyl sulfide</td>
<td>Rat&lt;sup&gt;280&lt;/sup&gt;</td>
</tr>
<tr>
<td>4-(methyl nitrosoamino)-1-(3-pyridyl)-1-butanol</td>
<td>Diallyl sulfide</td>
<td>Rat&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>4-(methyl nitrosoamino)-1-(3-pyridyl)-1-butanol</td>
<td>Diallyl sulfide</td>
<td>Mouse&lt;sup&gt;91&lt;/sup&gt;</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
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</tr>
<tr>
<td>N-diethyl nitrosamine</td>
<td>Diallyl disulfide</td>
<td>Rat&lt;sup&gt;62&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,12 Dimethylbenz(a)anthracene</td>
<td>Diallyl sulfide, diallyl disulfide, diallyl trisulfide</td>
<td>Mouse&lt;sup&gt;251,252&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>Diallyl sulfide</td>
<td>Mouse&lt;sup&gt;154&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vinyl carbamate</td>
<td>Diallyl sulfide</td>
<td>Mouse&lt;sup&gt;132&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Consistent with this reduction in bioactivation, Dion et al.\textsuperscript{102} found that both water-soluble SAC and lipid-soluble DADS were effective in retarding the mutagenicity of N-nitrosomorpholine in \textit{Salmonella typhimurium} TA100. A block in mutagenicity following aqueous garlic-extract exposure has also been noted following treatment with ionizing radiation, peroxides, adriamycin, and N-methyl-N-nitro-nitrosoguanidine.\textsuperscript{107}

A block in nitrosamine bioactivation may reflect changes in several enzymes. However, substantial evidence points to the involvement of CYP2E1.\textsuperscript{108,109} An autocatalytic destruction of CYP2E1 may account for some of the chemoprotective effects of DAS, and possibly other allyl sulfur compounds.\textsuperscript{110} Variation in the concentration and overall activity of CYP2E1 may be an important variable in the degree of protection provided by garlic and associated allyl sulfur components.

HCAs are produced by high-temperature cooking of protein-rich foods such as beef and chicken.\textsuperscript{98} Food preparation may be key, as addition of garlic powder to hamburger meat before cooking decreased the HCAs 2-amino-3,8-dimethylimidazo (4,5-f) quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) by 66.2\% and 85.0\%, respectively.\textsuperscript{111} In a study testing the ability of oil marinades containing various levels of onion, garlic, and lemon juice to inhibit HCA formation, increasing garlic and onion, but not lemon juice, in the marinades significantly decreased the formation of MeIQx.\textsuperscript{112} The concentrations of onion, garlic, and lemon that led to the maximal MeIQx reduction were 31.2\%, 28.6\%, and 14.6\%, respectively. Once ingested, the \textit{in vivo} bioactivation of heterocyclic amines to carcinogenic species is known to be initiated by N-oxidation. This reaction occurs primarily in the liver, and in humans is catalyzed by cytochrome P4501A2 (CYP1A2). Davenport and Wargovich\textsuperscript{113} reported the puzzling finding that in rats, the administration of a single bolus of 200 mg/kg DAS and AMS increased hepatic CYP1A2 protein (but not mRNA) by 282\% and 70\%, respectively. Acetylation or sulfation of the N-hydroxy-heterocyclic amine can also occur through the action of acetyltransferases (NAT) and sulfotransferases, which generate N-acetoxy and N-sulfonyloxy esters, electrophiles that are much more reactive with DNA. Several studies provide evidence that organosulfur compounds arising from garlic can effectively reduce NAT activity. Studies by Yu et al.\textsuperscript{114} demonstrated that a suppression in NAT mRNA expression accounts for the majority of the reduction in activity by garlic.

### 5.5.2 CARCINOGEN ACTIVITY MODULATION

Garlic and several of its allyl sulfur compounds can also effectively block the bioactivation and carcinogenicity of non-NOCs and HCAs (Table 5.2). This protection, which involves a diverse array of compounds and several target-tissue sites, suggests either multiple mechanisms of action or a widespread biological effect.

Garlic extract and compounds have also been found to reduce the incidence of tumors resulting from treatment with methylnitrosourea (MNU), a known direct-acting carcinogen.\textsuperscript{115} Providing water-soluble SAC and lipid-soluble DADS at 57 $\mu$mol/kg in the diet has been reported to cause a comparable reduction in MNU-induced O6-methylguanine adducts bound to mammary cell DNA.\textsuperscript{116} However, not all evidence supports SAC for protection against MNU-induced mammary tumors.\textsuperscript{117} The reason for this discrepancy is unknown but may relate to the quantity of lipid in the diet or the quantity of carcinogen provided. If garlic compounds are effective blockers of these carcinogens, the mechanism(s) remain unresolved.

Studies by Ludeke et al.\textsuperscript{118} revealed that DAS diminished the DNA hypermethylation of esophagus, liver, and nasal mucosa that arose from treatment with N-nitrosomethylbenzylamine. This finding suggests that the bioactivation of several carcinogens known to influence DNA methylation patterns\textsuperscript{119} may also be influenced by garlic and many of its sulfur constituents.\textsuperscript{101}

Metabolic activation is required for many carcinogens used in studies aimed at examining the anticarcinogenic properties of garlic. Thus, possible cancer-preventive mechanisms include modulation
of the activity of phase I enzymes, such as cytochrome P450s, which activate carcinogens, or phase II enzymes, such as glutathione S-transferases, that detoxify carcinogens. Recent observations show that the activity of several phase I enzymes, in addition to CYP1A2 and CYP2E1 discussed earlier, are modified following treatment with garlic or related sulfur compounds.\textsuperscript{113,120–122}

The influence of organosulfur compounds (OSCs) on phase I metabolizing enzymes is reportedly quite diverse. For example, previous studies demonstrated that DAS competitively inhibited CYP2E1 activity, but robustly increased the transcriptional levels of CYP1A1, CYP2B1, and CYP3A1 in rat liver.\textsuperscript{121,123} A recent study evaluated organosulfur compounds of aged garlic extract—SAC, S-methyl-L-cysteine, and trans-S-1-propenyl-L-cysteine—for their effects on the activities of five major isoforms of human CYP enzymes: CYP1A2, 2C9, 2C19, 2D6, and 3A4.\textsuperscript{124} The authors found little CYP inhibition or activation after the human intake of these garlic compounds. Therefore, the role of garlic OSCs in carcinogenic biotransformation may be substrate specific.

The significance of any slight induction of certain cytochrome P450 activities is not clear, but some reports suggest the induction of cytochrome P450 metabolic enzymes may increase the rate of clearance of toxic metabolites.\textsuperscript{125} Other enzymes and pathways are involved in the bioactivation or removal of carcinogenic metabolites in the observed protection from garlic constituents. Singh et al.\textsuperscript{126} provided evidence that the efficacy of various organosulfurides to suppress benzo(a)pyrene tumorigenesis was correlated with their ability to induce NAD(P)H:quinone oxidoreductase (NQO), an enzyme involved with the removal of quinones associated with this carcinogen. Investigators have also found that this inductive effect of organosulfur compounds appears to be mediated by the resident antioxidant response element (ARE) enhancer sequence bound by the nuclear factor E2-related factor 2 (Nrf2) in the NQO1 and the heme oxygenase 1 (HO1) gene promoters.\textsuperscript{127} In fact, it was found that the organosulfur compounds DAS, DADS, or DATS differentially mediated the transcriptional levels of NQO1 and HO1. The third sulfur in the structure of OSCs appeared to have a major contribution to this bioactivity, and the allyl-containing OSCs were more potent than the propyl-containing OSCs. The data also suggested that the upregulation of detoxifying enzymes by garlic OSCs through Nrf2 protein accumulation and ARE activation might be partly due to the stress signals originating from the oxidative stress and/or calcium-dependent signaling pathways.\textsuperscript{127} More recently, findings suggest that DATS may directly interact with the Cys288 residue of Kelch-like ECH-associated protein-1 (Keap1), a cytosolic repressor of Nrf2, which partly accounts for DATs ability to induce Nrf2 activation and upregulate defensive gene expression.\textsuperscript{128}

Changes in glutathione concentration and the activity of specific glutathione-S-transferases (GSTs), both factors involved in phase II detoxification, may be important in the protection provided by garlic. Both DADS and DATS have been shown to increase activity of GSTs in a variety of rat tissues.\textsuperscript{129} Moreover, gene expression of various GSTs (e.g., \textit{GSTp1}, \textit{GSTa2}, \textit{GSTm1}) have been shown to be modulated by garlic constituents in several tissues.\textsuperscript{130} The preventive effects of garlic powders containing variable levels of sulfur compounds on the development of preneoplastic foci initiated by aflatoxin B1 (AFB1) in rats has been characterized.\textsuperscript{131} The ultimate metabolite of AFB1, aflatoxin B1-8,9-epoxide, is conjugated with glutathione by GST and more specifically by GSTA5; thus, GST was explored as a mechanism responsible for any chemoprotective properties of garlic against AFB1-induced carcinogenesis. Consumption of garlic was efficient in protecting against AFB1 carcinogenesis, and DADS treatment induced GST protein levels and activity, particularly GSTA5. Thus, not all GST isozymes may be influenced equally. Earlier evidence from Hu et al.\textsuperscript{134} provided support that the induction of glutathione S-transferase pi 1 (GSTP1) may be particularly important in the anticarcinogenic properties associated with garlic and allyl sulfur components. Modulation of both phase I and II enzymes by garlic oil was explored in nitrosodiethylamine (NDEA)-induced hepatocarcinogenesis.\textsuperscript{133} The authors found that changes of the activities, mRNA, and protein levels of phase I enzymes (including CYP2E1, CYP1A2, and CYP1A1) and phase II enzymes (including GSTs and UDP-glucuronosyltransferases) contributed to the protective effects of garlic oil against NDEA-induced hepatocarcinogenesis in rats.
5.5.3 CELL CYCLE ARREST/APOTOPSIS

Evidence indicates that garlic constituents (i.e., DADS, DATS, S-allylmercaptocysteine [SAMC], ajoene) have the ability to suppress proliferation of several different cancer cells by blocking cell-cycle progression and/or causing apoptosis (also known as programmed cell death).\textsuperscript{134–136} Several mechanisms have been cited for the effect of garlic constituents on cell cycle arrest, including reduced Cdk1/cyclin B kinase activity, activation of extracellular signal-regulated kinases (ERK1/2), or induction of phosphorylated checkpoint kinase-1.\textsuperscript{134,137,138} Knowles and Milner\textsuperscript{139} showed that the DADS-mediated suppression of Cdk1 kinase activity during cell-cycle arrest in G2/M was not due to direct interaction with the protein, but was associated with (a) a temporal and dose-dependent increase in cyclin B1 protein level, (b) a reduction in the level of Cdk1–cyclin B1 complex formation, (c) inactivating hyperphosphorylation of Cdk1, and (d) a decrease in Cdc25C protein level. The evidence suggests a complex and coordinated interaction of many factors for the observed DADS-induced cell-cycle arrest. Furthermore, gene expression analysis suggested that alterations in DNA repair and cellular adhesion factors may also be involved in the G2/M block following DADS exposure.\textsuperscript{140}

Current knowledge of the mechanisms by which garlic compounds cause apoptosis indicates that they target various apoptosis-signaling molecules from initiation to execution, including mitogen-activated protein kinases (Jun N-terminal kinase (JNK), ERK1/2, and p38), PS3, nuclear factor kappa-light-chain enhancer of activated B cells (NF–kB), B-cell lymphoma 2 (bcl-2) family, and caspases,\textsuperscript{135} but not all of the signaling molecules are affected by each of the garlic constituents. In many studies, however, the apoptotic effects of garlic constituents were triggered by increased intracellular production of reactive oxygen species (ROS), suggesting the importance of the intracellular redox environment for apoptosis induction.\textsuperscript{141} An example is shown by the ability of DADS to induce apoptosis, as well as cell-cycle arrest at the G2/M phase, in human A549 lung cancer cells in a time- and dose-dependent manner.\textsuperscript{136} In this study, DADS caused not only a dose-dependent increase, but also a time-dependent change of ROS production, and an oxidative burst was found to be an early event, occurring less than 0.5 h after DADS treatment. These investigators hypothesized that the increased ROS may also act on the important signaling molecule in the observed DADS-induced cell cycle arrest. In a recent review of the activity of DATS in cancer prevention,\textsuperscript{142} several studies reported that DATS induces G2/M phase cell cycle arrest and that this may occur through the generation of ROS. Additionally, increased pro-apoptotic capacity as a result of regulating intrinsic and extrinsic apoptotic pathway components was widely reported following DATS treatment.\textsuperscript{142} The importance of the redox environment for apoptosis is also suggested by a study in which DATS increased hydrogen peroxide formation, lowered thiol levels, and induced caspase-3 activity in HepG2 cells.\textsuperscript{143}

5.5.4 DNA REPAIR

Exposing cells to mutagens, including intracellular by-products of cellular metabolism (ROS, endogenous alkylating agents) or extracellular influences (carcinogens, UV, or ionizing radiation), can cause DNA damage that is manifested as genomic instability, cellular senescence, and/or cell death. Initially the cell attempts to repair the damage, but if too extensive, a cascade of alternative cellular responses, including cell-cycle arrest or the induction of apoptosis, may occur.

There are three major DNA repairing mechanisms: base excision, nucleotide excision, and mismatch repair. Very little information exists about garlic or its organosulfur constituents as a modifier of DNA repair, although evidence exists that pretreatment with garlic extracts has been reported to stimulate DNA repair in human fibroblasts following cadmium chloride, gamma-radiation, and 4-nitroquinoline-1-oxide treatment.\textsuperscript{144} Interestingly, investigators found that gene expression of DNA repair genes did not correlate with growth inhibition by DADS.\textsuperscript{145} Regardless, several studies have demonstrated that histone/chromatin modifications such as acetylation, methylation,
Garlic and phosphorylation have a crucial role in DNA-repair processes, and some evidence suggests that garlic could influence one or more of these determinants of repair.

5.5.5 Epigenetic Modulation

Cancer progression is probably also highly dependent on epigenetic changes. Several regulatory proteins, including DNA methyltransferases, methyl-cytosine guanine dinucleotide binding proteins, histone-modifying enzymes, chromatin-remodeling factors, and their multimolecular complexes, are involved in controlling the epigenetic process to influence the regulation of gene expression.\textsuperscript{146} Because epigenetic events can be influenced by several dietary components, they represent another plausible site for intervention with bioactive food components.\textsuperscript{146} Garlic and its constituents have been shown to influence gene expression both \textit{in vitro} and \textit{in vivo},\textsuperscript{130} but few studies have examined whether epigenetic processes influence gene expression following garlic treatment. In one recent study, SAC treatment of human ovarian cancer A2780 cells was shown to decrease global DNA methylation levels, DNA methyltransferase (DNMT) activity, mRNA and protein levels of DNMT1. Additionally, SAC treatment resulted in re-expression of the mRNA and proteins of the silenced tumor suppressor gene \textit{CDKN1A} accompanied by reduced cell proliferation and induced cell cycle arrest in the G1/S phase.\textsuperscript{147}

Evidence suggests that some garlic constituents can influence histone homeostasis, which may influence gene expression and cellular phenotype. Lea et al.\textsuperscript{148} first reported that at least part of the ability of DADS to induce differentiation in DS19 mouse erythroleukemic cells might relate to its ability to increase histone acetylation. DADS caused a marked increase in the acetylation of H4 and H3 histones in DS19 and K562 human leukemic cells. Consistent with other studies, disulfide was found more effective than mono-sulfide. Moreover, these investigators found that the inhibition of cell proliferation by SAC and SAMC of DS19, Caco-2 human colon cancer, and T47D human breast cancer cells was associated with increased histone acetylation.\textsuperscript{149} Additionally, Druesne et al.\textsuperscript{150} reported DADS and allyl mercaptan, a metabolite of DADS, effectively increased histone H3 acetylation in cultured Caco-2 and HT-29 cells. The histone H4 hyperacetylation was found to occur preferentially at the lysine residues 12 and 16. The reason for this hyperacetylation was thought to be due to the observed reduction in histone deacetylase activity. This change in hyperacetylation was also accompanied by an increase in p21(waf1/cip1) expression, at mRNA and protein levels, again demonstrating that epigenetic events may influence subsequent gene expression patterns and lead to the accumulation of cells in the G2 phase of the cell cycle.\textsuperscript{150} Using an \textit{in vivo} model, DADS was found to inhibit the growth of tumors in SCID mice bearing HL-60 peritoneal neoplasms, which was accompanied by increased expression of acetylated histone H3 and H4, as well as increased protein expression of p21WAF1.\textsuperscript{151} There is now ample evidence from preclinical studies that DADS, AM, and other garlic compounds increase histone acetylation.\textsuperscript{152} These observations are thought to be due to inhibition of histone deacetylase activity (HDAC).\textsuperscript{152} Nian et al.\textsuperscript{153} screened several garlic compounds for their ability to inhibit HDAC activity \textit{in vitro}. AM was found to be the most potent HDAC inhibitor, acting as a competitive HDAC inhibitor \textit{in vitro}, with a \textit{K}_i on the order of 24 \textmu M for human HDAC8. Using HT29 cells, Nian et al.\textsuperscript{153} found that inhibition of HDAC activity by AM was associated with increased global histone acetylation, as well as localized hyperacetylation of histone H3 on the \textit{P21WAF1} promoter. The garlic constituents DADS and AM, for which there is the most evidence for this activity, join the list of other food components with demonstrated weak histone deacetylase inhibitor activity.\textsuperscript{155}

MicroRNAs are a class of small non-coding RNAs (approximately 23 nucleotides in length) that regulate post-transcriptional gene expression by binding to the 3'-untranslated region (3'-UTR) of target mRNAs, leading to mRNA cleavage or translational repression.\textsuperscript{156} MicroRNAs regulate the expression of a wide variety of target genes and are therefore involved in a broad range of biological processes, and their expression is dysregulated in cancer.\textsuperscript{157} Garlic compounds have been found to influence microRNA or miR expression in cancer cells. For example, DADS suppressed
proliferation and invasion in human breast cancer cells by upregulating miR-34a to target repression of the cytoplasmic tyrosine kinase SRC mRNA, consequently reducing SRC/Ras/ERK oncogene signaling. These results add to the variety of gene regulatory mechanisms that may be modified by consumption of garlic and its constituents.

5.5.6 Redox and Antioxidant Capacity

It is well documented that dysregulation of ROS is involved in the etiology of a variety of diseases, including cancer. As a result, attention has been given to the identification of antioxidants in human foods. How the classification of antioxidant activity in a food or food component translates to human health continues to be a topic of discussion. However, both water- and lipid-soluble organosulfur compounds from garlic have been studied for their antioxidant capacity. A variety of methods have been used to evaluate the total antioxidant activity of garlic preparations available in the marketplace. Any single method is insufficient, as the response depends not only on its ability to reduce oxidation radicals, but on its metal-chelating capabilities. Both alliin and allicin are have been shown to possess antioxidant properties in a Fenton oxygen-radical generating system. Additionally, the antioxidant actions of garlic and its constituents have been documented through their ability to scavenge ROS, inhibit lipid peroxide formation, retard low-density lipoprotein (LDL) oxidation, and enhance endogenous antioxidant systems.

It should be noted that not all organosulfur compounds have been found to exhibit antioxidant properties. DADS, but not DAS, dipropyl sulfide, or dipropyl disulfide, has been found to inhibit liver microsomal-lipid peroxidation induced by NADPH, ascorbate, and doxorubicin. The presence of both the allyl and sulfur groups appears to magnify the antioxidant capabilities of the molecule. Both the number of sulfur atoms and the oxidation state of sulfur atoms can influence the overall antioxidant potential. Whereas allicin is effective in retarding methyl linoleate oxidation, it is less than that caused by \( \alpha \)-tocopherol. Organosulfur compounds such as SAC are recognized to be powerful antioxidants and radical scavengers with the strong capacity to minimize oxidation. Antioxidant activity of garlic shows great variation depending on the genotype or species evaluated for activity. Moreover, processing of garlic may affect antioxidant efficacy; the heating of garlic can not only denature proteins, but also its antioxidant properties.

5.5.7 Immunocompetence/Immunonutrition

Diet is increasingly recognized to influence the development and functionality of immunocompetent cells. Several dietary components, including garlic extracts and allyl sulfur compounds, may have physiologically important immunomodulatory effects. Both an aqueous and an ethanolic extract of garlic powder significantly stimulated proliferation of rat spleen lymphocytes in culture, which was correlated with the upregulation of the Interleukin 2 receptor alpha expression and an increase in interleukin (IL)-2 production. This data also suggested that the potentiating effect of the garlic extract on lymphocyte proliferation in vitro differed, depending on specific stimulators of cell proliferation, speculating that the in vivo response would depend on the type of responding cells. These investigators also demonstrated that aqueous and ethanolic extracts from two garlic powders significantly modulated proliferation of rat thymocytes and splenocytes in vitro to concanavalin A. Both garlic extracts significantly modulated lymphocyte proliferation, triggered by this potent T-cell mitogen, but the response was dependent on the type and dilutions of extracts, and concentrations of concanavalin A. Interestingly, at higher concentrations of the extracts, an inhibitory effect on T-cell proliferation was observed, whereas at lower concentrations, a significant increase in T-cell proliferation occurred. In an in vivo study, DAS treatment of BALB/c mice has been reported to block the suppression of the antibody response caused by N-nitrosodimethylamine to T-cell-dependent antigens, and the lymphoproliferative response to T-cell and the B-cell mitogens. These results support the concept that garlic may be a modulator of T cell-mediated immune functions in vivo.
However, most studies have been carried out in vitro, and there is a need to design studies using well-defined systems and chemically pure active garlic compounds at defined concentrations, and to develop animal models to test the immunomodulatory effect of garlic in cancer prevention. It is important to note that very little research to determine the immunomodulatory effects of garlic on cancer or cancer prevention in humans has been performed. In one randomized double-blind clinical trial, the effects of AGE were evaluated on the quality of life (QOL) and immune functions of patients with advanced cancer of the digestive system. Although the authors reported no differences in QOL, they did find that the number of natural killer (NK) cells and NK cell activity increased significantly in the patients that consumed AGE. The immunomodulatory effects of garlic are not limited to sulfur compounds, as a protein fraction isolated from aged-garlic extract was also found to enhance cytotoxicity of human peripheral blood lymphocytes (PBL) against both natural killer-sensitive K562 and NK-resistant M14 cell lines. The mechanism(s) by which sulfur or non-sulfur components of garlic influence immunocompetence remains to be determined. Recent insights about their mechanism of action are suggested from the results of a human randomized crossover trial. In this study, a single meal containing raw, crushed garlic resulted in increased expression of immune function and cancer pathway–related genes in whole blood 3 h after consumption. The seven genes that were upregulated have a variety of functions, including roles in xenobiotic metabolism, inflammation, B cell and T cell development, apoptosis, and tumorigenesis, and five of these genes were also upregulated in the monocytic cell line Mono Mac 6 when treated with garlic extract.

Garlic compounds may also be modulators of inflammatory molecules, including cytokines that exhibit a vast array of regulatory functions in both adaptive and innate immunity. DADS and AMS, in addition to DAS, demonstrated different effects on the production of cytokines in lipopolysaccharide (LPS)-activated macrophages. DAS inhibited both pro- and anti-inflammatory cytokines, including tumor necrosis factor (TNF)α, IL-β, IL-6, and IL-10, in stimulated macrophages. DADS enhanced pro-inflammatory cytokines IL-β and IL-6, but suppressed anti-inflammatory cytokine IL-10, indicating the effect of DADS may be more toward pro-inflammation. On the other hand, AMS, to a lesser extent, decreased production of nitric oxide (NO) and TNF-α in activated macrophages, but significantly enhanced IL-10 production, suggesting that AMS may be a potential anti-inflammatory compound.

Allicin and ajoene have been reported to cause a dose-dependent inhibition of the inducible nitric oxide synthase (iNOS) system in LPS-stimulated RAW 264.7 murine macrophages. Such inhibition has been correlated with a reduction in iNOS protein, as well as in its mRNA. Thus, changes in the amount or ratio of NO and peroxynitrite concentrations may be significant in the observed lowering of inflammation by garlic and associated sulfur components. DAS, DADS, and AMS have also been shown to display unique regulatory properties in suppressing NO in stimulated macrophages. DAS was found to decrease stimulated NO and prostaglandin E2 (PGE2) production by inhibiting inducible NO synthase and cyclooxygenase-2 expressions, and to indirectly enhance NO clearance. DADS inhibited activated NO production by decreasing inducible NO synthase expression and by directly clearing NO, whereas AMS suppressed NO mainly through its direct NO clearance activity.

It has been hypothesized that consumption of garlic OSC may assist in shifting the balance from a pro-inflammatory to an anti-tumor response by dampening the pro-inflammatory response and/or strengthening the anti-tumor immunity toward tumor eradication. Using a colitis-induced colorectal cancer AOM/DSS mouse model, investigators recently examined whether DADS exerts its protective effects against colorectal tumors by suppressing inflammation. Supplementation with DADS resulted in a reduction in tumor incidence, tumor number, and tumor burden. Furthermore, the DADS-supplemented diet resolved the initial DSS-induced inflammation faster than those on the control diet, preventing prolonged inflammation and cellular transformation. Mechanistic studies using the human CRC SW480 cell line suggested that DADS interferes with nuclear translocation of pro-inflammatory transcription factor NF-κB and the expression of tumorigenic enzyme COX2 via the serine/threonine kinase GSK-3 (α/β) inactivation. These findings suggest that the antitumor effect of allyl sulfurs may be related to their anti-inflammatory as well as immune-stimulatory properties.
5.5.8 COX/LOX Pathways

Cyclooxygenase (COX), lipoxygenase (LOX), and pathway-associated enzymes have been found to activate carcinogens. Smith et al.\textsuperscript{180} reported that prostaglandin H synthase could metabolize the bay region diol of benzo(a)pyrene to electrophilic diol epoxides that were capable of binding to DNA. It has also been reported that both cyclooxygenase and lipoxygenase are involved with 7,12-dimethylbenz(a)anthracene (DMBA) bioactivation.\textsuperscript{181,182} Garlic may influence this bioactivation. For example, Song and Milner\textsuperscript{182} found that feeding DADS or SAC markedly reduced DMBA-induced DNA adducts in rat mammary tissue. Ali\textsuperscript{183} provided evidence that garlic could block cyclooxygenase activity. Moreover, garlic and associated sulfur components may also inhibit lipoxygenase activity.\textsuperscript{184}

With regard to the influence of allyl sulfur compounds on the lipoxygenase and cyclooxygenase signaling pathways, DAS, DADS, and to a lesser extent AMS, were found to differentially regulate NO and PGE\textsubscript{2} production in mouse RAW 264.7 macrophages stimulated by LPS.\textsuperscript{178} In another study, ajene was found to act similarly to several non-steroidal anti-inflammatory drugs in that this garlic compound inhibited, in a dose-dependent fashion, the release of PGE\textsubscript{2} from LPS-activated RAW 264.7 cells, which was associated with a dose-dependent inhibition of COX-2 enzyme activity.\textsuperscript{185} Collectively, these studies pose interesting questions about the role of both cyclooxygenase and lipoxygenase in not only forming prostaglandins, and therefore modulating tumor cell proliferation and immunocompetence, but also their involvement in the bioactivation of carcinogens. Clearly, additional attention is needed to clarify what role, if any, these enzymes have in determining the biological response to dietary garlic or its allyl sulfur components.

5.5.9 Diet as a Modifier

Garlic’s influence on cancer processes cannot be considered in isolation, as certain dietary patterns and foods may influence the overall response. For example, the effects of combining tomato and garlic were examined using several carcinogenesis models.\textsuperscript{186–188} The combination suppressed the incidence and mean tumor burden of hamster buccal-pouch carcinomas more than either alone, and appeared to relate to a decrease in phase I enzymes and an increase in phase II enzyme activities.

A variety of individual food components may also influence the response to garlic. Notable are the modifications made by the quantity of fat, selenium, methionine, and vitamin A in the diet.\textsuperscript{189–191} Amagase et al.\textsuperscript{190} and Ip et al.\textsuperscript{191} reported that selenium supplied either as a component of the diet or as a constituent of the garlic supplement enhanced the protection against DMBA mammary carcinogenesis beyond that provided by garlic alone. Suppression in carcinogen bioactivation, as indicated by a reduction in DNA adducts, may partially account for this combined benefit of garlic and selenium.\textsuperscript{189} Because both selenium and allyl sulfur compounds are recognized to suppress tumor cell proliferation and to induce apoptosis,\textsuperscript{192–194} the synergistic response to allyl sulfur and selenium may relate to changes in cancer-related processes other than those associated with carcinogen metabolism.

Dietary fatty acid supply can influence the bioactivation of DMBA and ultimately the metabolites of this carcinogen, which binds to rat mammary cell DNA. A significant portion of the enhancement in mammary DNA adducts caused by increasing dietary corn oil consumption can be attributed to linoleic acid intake.\textsuperscript{195} Whereas exaggerated oleic acid consumption also increases DMBA-induced DNA adducts, it was found to be far less effective in promoting adduct formation than was linoleic acid. The influence of garlic in modifying the effect of corn oil on rat mammary cell DNA adducts resulting from DMBA treatment has been studied.\textsuperscript{190} Garlic supplementation was found to prevent the increase in DNA adducts caused by increasing dietary corn oil.

The diversity of molecular targets that can be influenced by various food components demonstrates the complexity in dealing with nutrient–nutrient interactions. Although the effect of combining bioactive food components on garlic’s ability to influence cellular proliferation has not
been adequately examined, there are potentially several combinations that would produce more dramatic effects. For example, and similar to information with chemical carcinogenesis mentioned above, there is evidence of a greater effect of allyl sulfur when combined with selenium than when provided alone.196 Likewise, a combination of garlic and onion oils was more effective in blocking the proliferation of HL60 cells in culture than when used singly.197 Furthermore, the combination of garlic and lemon aqueous extracts was more effective in inhibiting breast cancer in vitro and in vivo, using Balb/C mice inoculated with EMT6/P breast cancer cells, than either aqueous extract alone.198 The rationale for this study was that lemon extract may reduce the strong smell and flavor of garlic extract and thus enhance consumption. In addition, it was thought that the phytochemicals in lemon extract may act synergistically to enhance the anticancer activity of garlic constituents. Although the molecular basis for these enhanced effects needs to be investigated in more detail, they serve as proof-of-principle that interactions among food components must be considered when developing strategies for using diet for cancer prevention.

5.6 CARDIOVASCULAR DISEASE

Garlic may have a role in the genesis and progression of cardiovascular disease. These effects may be mediated through a variety of biological responses, including modulation of serum lipids and fibrinogen concentrations, lowering arterial blood pressure, and/or an inhibition of platelet aggregation.

5.6.1 CHOLESTEROL AND LIPOPROTEINS

Several studies have attempted to clarify the effects that garlic has on serum total cholesterol, LDL, high-density lipoprotein (HDL), and triglycerides.1,199,200 While some studies have reported that garlic reduces LDL concentrations, others have not. Evaluating cardioprotective responses is made complicated by the use of various quantities of garlic, different preparations, variations in the duration of treatment, and qualities of study design. One randomized controlled clinical study compared the lipid-lowering effects of 4.0 g of raw garlic, an equivalent (based on allicin) dose of dried garlic, AGE, and a placebo in a moderately hypercholesterolemic population (LDL cholesterol, 130–190 mg/dL, mean 151 ± 15 mg/dL).201 Total cholesterol, LDL, HDL, total/HDL ratio, and triglycerides did not change in response to 6 months of any of the interventions (consumed 6 days/week).201 In contrast, in a study of 51 participants with mean baseline serum LDL = 186 ± 9 mg/dL, LDL decreased in men taking garlic powder tablets (350 mg/day) for 12 months compared to placebo, but not in women.202 This difference in response may have been associated with differences in the intervention period, the garlic dose, and/or the baseline LDL levels of the populations studied. A recent meta-analysis including 39 studies using various garlic preparations reported that total serum cholesterol and LDL cholesterol were reduced by 17 ± 6 mg/dL and 9 ± 6 mg/dL, respectively, in individuals with total cholesterol >200 mg/dL and who had taken the garlic preparation for more than 2 months.203 Triglycerides were not affected. Lipoprotein(a) [Lp(a)], which is related to LDL but is covalently bound to the protein apo(a), is an independent risk factor for cardiovascular disease. A recent meta-analysis did not indicate a significant effect of garlic supplementation on Lp(a) levels.204

As demonstrated for cancer models, lipid changes in response to intake of garlic preparations may depend on the formation of bioactive sulfur compounds. Jabbari et al.205 found that swallowing undamaged garlic had no lowering effect on serum lipids, but consuming crushed garlic reduced cholesterol, triglycerides, malondialdehyde, and blood pressure. Similarly, heating garlic modifies the ability of garlic to inhibit in vivo binding of mammary carcinogen [7,12-dimethylbenzene(a)anthracene, DMBA] metabolites to rat mammary epithelial cell DNA.206 Differential responses to garlic that is crushed or heated may be related to effects on the activity of alliinase in converting alliin to allicin.

LDL oxidation has been investigated as a contributor to the initiation and progression of atherosclerosis.207 Some preclinical and clinical studies suggest that AGE may protect against...
oxidation. Munday et al. found a modest reduced susceptibility of LDL particles to Cu-mediated oxidation from subjects given 2.4 g of AGE daily for 7 d. Interestingly, a similar response was not observed when subjects were given 6 g of raw garlic as a daily supplement for 7 d. This result was repeated in subjects taking 1.2 g of AGE for 2 weeks. In contrast, Byrne et al. did not find that 900 mg of garlic powder for 6 months had an impact on LDL susceptibility to oxidation. It is unclear if the discrepancies in the literature about garlic and LDL oxidation relate to the subjects examined or the preparations used. DADS has been reported to protect human LDL, erythrocyte membranes, and platelets from oxidation and/or glycation. The protective effects of six organosulfur compounds (DAS, DADS, SAC, S-ethylcysteine, S-methylcysteine, and S-propylcysteine) were tested for their ability to reduce further oxidation and glycation in already partially oxidized and glycated samples from patients with non-insulin-dependent diabetes. These studies revealed that DAS and DADS were superior in delaying LDL oxidation compared to the four cysteine-containing compounds tested. However, the cysteine-containing agents were superior to DAS and DADS in delaying glycative deterioration in already partially glycated LDL. Both responses were highly concentration dependent. Thus, the content or potential for forming bioactive compounds likely explains much of the variability that has been observed in the published literature.

### 5.6.2 Blood Pressure

Blood pressure is an important risk factor in cardiovascular disease. A large meta-analysis showed that lowering systolic blood pressure by 10 mm Hg or diastolic blood pressure by 5 mm Hg by any of the main classes of blood pressure-lowering drugs reduced cardiovascular disease events (fatal and non-fatal) by about 25% and stroke by about 33%. Diet, as well as age, sex, hormonal state, and genetic factors, probably influences blood pressure. Increasing evidence suggests garlic may be a dietary component with the ability to reduce blood pressure and cause relaxation in arterial walls. A recent meta-analysis which included 20 studies reported an average 5.1 mm Hg reduction in systolic blood pressure and an average 2.5 mm Hg reduction in diastolic blood pressure. The response to garlic was higher in hypertensive subjects, whose systolic blood pressure decreased by 8.7 mm Hg and diastolic blood pressure decreased by 6.1 mm Hg. Other studies suggest that garlic reduces blood pressure in hypertensive subjects but not in normotensive subjects. Formulations of AGE and garlic powder seemed to be similarly efficacious.

Garlic treatment has been found to lead to a dose-dependent vasorelaxation in both endothelium-intact and mechanically endothelium-disrupted pulmonary arterial rings. This vasorelaxation was diminished by the administration of NG-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor. The inducible nitric oxide synthase is recognized to occur in human atherosclerotic lesions. Studies have demonstrated that garlic exerts its therapeutic effect by increasing NO production and by suppressing the reduction of cellular nitric oxide synthase by oxidized LDL. The relaxant effect on vascular smooth muscle appears to be mediated through a decrease in cGMP and the subsequent release in endothelium-derived relaxing factors, as well as a depression in prostaglandins via a suppression in cyclooxygenase activity. It is known that ROS counteract the vasodilating and antiproliferative actions of nitric oxide by rapidly degrading it to peroxynitrites. It is possible that part of the blood pressure changes caused by garlic may relate to its ability to reduce radical formation. Another mechanism by which blood pressure may be moderated by garlic involves hydrogen sulfide (H₂S), which has been shown to be produced by metabolism of garlic polysulfides. H₂S promotes sulfhydration of ATP-sensitive potassium channels, thereby opening voltage-sensitive channels and triggering subsequent relaxation of vascular smooth muscle cells.

### 5.6.3 Plaque and Platelet Aggregation

Acute coronary syndromes can occur when an unstable atherosclerotic plaque erodes or ruptures, thereby exposing the highly thrombogenic material inside the plaque to the circulating blood.
This exposure triggers a rapid formation of a thrombus that occludes the artery. Budoff et al.\textsuperscript{225} found in a pilot study that providing AGE for a year inhibited the rate of progression of coronary calcification compared to a placebo. A follow-up clinical trial with 65 patients reported that ingesting AGE (250 mg) supplemented with B vitamins, folic acid, and L-arginine daily for 1 year slowed the progression of subclinical atherosclerosis as assessed by coronary artery calcium scanning.\textsuperscript{226} In a study that measured low attenuation plaque (non-calcified plaque) in 55 subjects with metabolic syndrome, daily intake (2400 mg) of AGE for 1 year significantly decreased low attenuation plaque compared to placebo.\textsuperscript{227} Low attenuation plaque was an independent predictor of adverse cardiac events in a population with stable angina, and therefore the moderating effects of AGE on low attenuation plaque may be clinically important.\textsuperscript{228}

Other garlic preparations have also been reported to inhibit plaque formation in humans. Providing 900 mg of garlic powder daily for 48 weeks in a randomized trial reduced arteriosclerotic plaque volumes in both the carotid and femoral arteries by 5\%–18\%.\textsuperscript{229} Another randomized trial determined that garlic powder (standardized to 2400 \(\mu\)g/day) ingested daily for 3 months was superior to placebo in retarding the progression of carotid intima-media thickness (CIMT), a measurement of the innermost two layers of the artery walls, and which is strongly associated with atherosclerosis.\textsuperscript{230} Time-released garlic powder tablets were effective in decreasing CIMT when taken daily for 1 year, whereas there was a moderate progression of CIMT in the placebo group.\textsuperscript{231} These investigators proposed that this result was a consequence of serum atherogenicity inhibition, a measurement of the ability of serum to induce cholesterol accumulation. Similarly, Zahid et al.\textsuperscript{232} suggested that garlic may exert its beneficial effect on plaque formation by reducing cholesterol as well as maintaining NO-mediated endothelial function, possibly secondary to an inhibition of LDL oxidation and an increase in HDL.

Aggregates of activated platelets are also likely have a pivotal role in coronary syndromes. Garlic and some of its organosulfur components have been found to be potent inhibitors of platelet aggregation \textit{in vitro}.\textsuperscript{220,233} Some of the platelet-inhibitory compounds arising from allium plants include ajoene, allicin, SAC, methylallyl trisulfide, and alk(en)nyl thiosulfates such as sodium 2-propenyl thiosulfate and sodium n-propyl thiosulfate. Heating garlic by boiling decreases its ability to inhibit platelet aggregation.\textsuperscript{233} Recently, Rahman et al.\textsuperscript{234} studied platelets from 14 subjects and found that AGE significantly decreased cGMP and cAMP, inhibited the binding of activated platelets to fibrinogen, and thereby prevented changes in platelet shape that favor aggregation.

A few studies have documented that garlic can inhibit platelet aggregation \textit{in vivo}. Steiner and Lin\textsuperscript{235} provided evidence that consumption of AGE reduced epinephrine and collagen-induced platelet aggregation, although it failed to influence adenosine diphosphate-induced aggregation. Their studies also provided evidence that platelet adhesion to fibrinogen could be suppressed by consumption of this garlic supplement. In a study with 23 subjects who had consumed 5 mL of AGE daily for 13 weeks, AGE did inhibit adenosine diphosphate-induced aggregation.\textsuperscript{236} Finally, DAS, a constituent of garlic oil, induced a reduction in adenosine-induced platelet aggregation in women with type 2 diabetes mellitus.\textsuperscript{237}

Overall, the potential of garlic to reduce sterol synthesis, hyperlipidemia, hypertension, and thrombus formation make it a strong candidate for lowering the risk of heart disease and stroke. Nevertheless, the literature provides evidence for considerable variability in response. Additional studies are needed to help clarify who might benefit most from added garlic, and the most efficacious garlic formulations and dose levels.

5.7 SUMMARY AND CONCLUSIONS

Garlic has significant physiological attributes that may promote health. Although it is possible that other allium foods possess similar health attributes, few comparative studies have been undertaken. As garlic causes relatively few side effects, except for possibly its lingering odor, there is little reason to avoid its use. Odor does not appear to be a necessary prerequisite for many of the benefits, as
water-soluble SAC generally gives comparable benefits to those compounds associated with smell. Although garlic and its bioactive components may influence several key molecular events that are involved with health, to do so it must achieve an effective concentration within the target site, be in the correct metabolic form, and lead to changes in small molecular weight signals in the cellular milieu (metabolomic effects). Whereas most can savor the culinary experiences identified with garlic, some individuals, because of their gene profile and/or environmental exposure, may be particularly responsive to more exaggerated intakes.

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