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Allicin and Allicin-Derived Garlic Compounds Increase Breath Acetone through Allyl Methyl Sulfide: Use in Measuring Allicin Bioavailability

LARRY D. LAWSON*,† AND Z. JONATHAN WANG‡

Plant Bioactives Research Institute, Inc., Orem, Utah 84058, and Nature's Way Products, Inc., Springville, Utah 84663

Progress in establishing systemic pharmacological effects for fresh, crushed garlic (*Allium sativum* L) in humans has been hindered by (1) the inability to measure allicin bioavailability, (2) lack of direct evidence that allicin has significant systemic activity at doses of garlic normally consumed, and (3) lack of a model for an acute effect. We have addressed these problems by quantifying the increases in breath acetone and breath allyl methyl sulfide (AMS). The area under the 48 h curve was measured in humans after consumption of standardized garlic preparations, allicin, and allicin-derived compounds, at the equivalent of 7 g of crushed garlic. It was shown that the allyl thiosulfinates (mainly allicin) are solely responsible for breath AMS and increased breath acetone. Diallyl trisulfide, diallyl disulfide, ajoene, and *S*-allylmercaptocysteine, at isomolar dithioallyl, showed the same quantitative effects as allicin. Consumption of AMS at isomolar allyl also gave the same effects as allicin, indicating that AMS is the main metabolite of allicin and is an active metabolite. In conclusion, allicin and allicin-derived compounds are rapidly metabolized to AMS, a compound which stimulates the production of acetone and which can be used to measure the bioavailability of allicin and, hence, the ability of garlic supplements to represent fresh garlic.

KEYWORDS: Garlic; allicin; serum lipids; allyl methyl sulfide; acetone; bioavailability; metabolism; diallyl disulfide; diallyl trisulfide; diallyl sulfide; ajoene

INTRODUCTION

The numerous clinical trials with garlic supplements have proceeded at a much greater rate than would be justified by the paucity of research on the pharmacology of its possible active compounds, resulting in a series of conflicting results that have precluded any clearly established systemic pharmacological effect for garlic (*1*−*3*). The conflicts have been particularly prominent among the 52 controlled trials on serum lipids, the most studied effect of garlic (*2*). Although differences in trial designs may account for some of the conflict (*2*, *3*), recent studies have shown that most garlic powder supplements, including those used in the clinical trials, release far less allicin (diallyl thiosulfinate), the main suspected active compound of crushed fresh garlic, under simulated gastrointestinal conditions than is found in an equivalent amount of crushed garlic (*4*, *5*). These recent results reveal a large quality (bioequivalence) difference between most garlic supplements and fresh garlic and establish the absolute need of a method for determining the bioavailability of allicin from garlic supplements before the results of clinical trials with such supplements can be considered valid for fresh garlic.

The difficulty of releasing allicin from garlic powder supplements is related to the fact that allicin is not present in uncrushed garlic or in garlic powder but is produced by the action of the garlic enzyme, alliinase, upon its most abundant sulfur compound, alliin, L-(+)-*S*-allylcysteine sulfoxide (*6*, *7*). When one consumes raw garlic, the acts of chopping, crushing, chewing, or blending activate alliinase, causing maximum allicin production in less than 6 s, well before reaching the intestinal tract (*8*). With supplements, however, alliinase does not have the opportunity to convert alliin to allicin until they disintegrate in the body, requiring the preservation of alliinase activity throughout the manufacturing procedures as well as from the acidic and hydrolytic conditions of the gastrointestinal tract.

The term "allicin bioavailability" is being used in this study to represent the sum of two processes: (1) the formation of allicin from alliin in the intestinal tract and (2) the absorption and metabolism of allicin to a quantifiable metabolite or its effect upon a metabolic process. Determining the bioavailability of allicin directly has failed in all attempts, being undetectable in the blood, urine, or stool, even after consuming large amounts of crushed fresh garlic (25 g) or pure allicin (60 mg) (*9*, *10*). *In vitro* studies have shown that when allicin is added to fresh blood that it is rapidly metabolized (half-life, <1 min) to allyl

* To whom correspondence should be addressed: Plant Bioactives Research Institute, 95 S. Mountain Way Drive, Orem, UT 84058. Telephone: 801-226-3041. Fax: 801-226-3083. E-mail: lawson@plant-bioactives.com.
† Plant Bioactives Research Institute, Inc.
‡ Nature's Way Products, Inc.

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mercaptan (allyl-SH) (*11*), but attempts to find allyl mercaptan in the blood, urine, or stool after consuming garlic or allyl mercaptan have also failed, indicating that it is also rapidly metabolized (*10*). Although finding allicin or its metabolites in the blood or urine after garlic consumption appeared elusive, it has been known for some time that allyl mercaptan and allyl methyl sulfide (AMS) are components of the breath (*12*−*14*) soon after garlic consumption and that AMS is also found in urine vapor (*14*). The presence of allyl mercaptan is transient, reaching maximum levels in 1−2 min and disappearing by 1 h, indicating that it is formed in the epithelial cells of the mouth and throat, making it unacceptable as an indicator of allicin bioavailability (*12*, *13*). However, AMS has been found to reach maximum levels in 4 h and to persist for over 30 h, indicating that it is a product of systemic metabolism (*15*). Although identity of the garlic compound that was metabolized to AMS was not suggested by the authors (*15*), it seemed possible that it might be related to allicin and we decided to pursue this possibility.

Another possible indicator of allicin bioavailability, through effects on general metabolism (bioassay), was found in 1996 when Taucher et al. showed that consumption of a single dose of crushed garlic doubled the breath acetone output over a 32-h period, an effect that may indicate increased triglyceride metabolism (*15*). This study was limited in that it involved only one person and examined only one very large dose (38 g), with no indication of the active compound. However, because of its potential as a marker of allicin bioavailability, we chose to further examine this effect at lower doses and to determine if allicin is responsible for it.

The purpose of the present studies was to determine if breath AMS and increased breath acetone could be useful measures of allicin bioavailability. This was accomplished by comparing the effects of standardized garlic preparations representing crushed fresh garlic (allicin present), microwave-cooked garlic (allicin absent because of inhibition of alliinase), unheated allicin-free garlic (removed by vacuum-drying), and a brand of garlic powder tablets previously shown to give high allicin release under simulated gastrointestinal conditions (*4*). Afterward, studies with pure allicin as well as with allicin metabolites and allicin transformation compounds found in commercial garlic oils were conducted.

MATERIALS AND METHODS

Allicin. Allicin (98%) was synthesized by hydrogen peroxide oxidation of diallyl disulfide, as previously described (*4*) and stored at 1−2 mg/mL in water at −80 °C.

***S*-Allylmercaptocysteine.** *S*-allylmercaptocysteine was synthesized by modification of the method of Cavallito et al. (*16*). To 8 mmol of L-cysteine in water was added 6 mmol (a 50% excess) of allicin, and the solution was stirred for 30 min. The pH was adjusted to about 6.5 with NaOH, and the solution was stirred 10 min. The crystals were filtered and washed with dichloromethane to give a yield of 5.7 mmol of >98% purity with a mp of 178−180 °C and extinction coefficients of 1950 and 590 M^{-1} at 220 and 240 nm, respectively, measured at 0.26 mM in 0.025 N HCl. Purity was determined by two C18 HPLC methods (90% 0.05 M phosphate buffer at pH 4.5 and 10% methanol at 220 nm and 55% methanol in water at 240 nm) and TLC (*17*).

Ajoene. Ajoene was synthesized by incubation of allicin in hot methanol, by the method of Block et al. (*18*). Purity was determined C18 HPLC, with resolution of the isomers requiring silica-HPLC (*19*). An overall yield of 20% of 89% pure ajoene was obtained, with an E/Z ratio of 11.7 and a density of 1.18. It was stored without solvent at −80 °C.

Vinyldithiins. Vinyldithiin oil (97% vinyldithiins at 9.7 mg/g in soybean oil) was synthesized by incubation of neat allicin in soybean oil. Allicin (2 mg/mL in water) was extracted once with 1 volume of dichloromethane, and the solvent was removed by rotary evaporation to obtain neat allicin. The allicin was then added to soybean oil at 10 mg/mL and allowed to sit for 4 days at ambient temperature before centrifugation and analysis (*19*). Commercial oil-macerate garlic oils are prepared by incubation of crushed garlic with a vegetable oil, resulting in a similar composition.

Other Compounds. Diallyl disulfide, diallyl trisulfide, L-(+)-*S*-allylcysteine sulfoxide (alliin), and *S*-allylcysteine (each ≥98%) were purchased from LKT Labs (St. Paul, MN). Diallyl sulfide (99%), AMS (99%), and allyl mercaptan (80%) were purchased from Aldrich Chemical (Milwaukee, WI). Allyl mercaptan was further purified to 95.5% by fractional distillation. Steam-distilled garlic oil in soybean oil was consumed as commercial softgel capsules (KAL Garlic-Oil 1500) (Makers of KAL, Inc., Park City, UT).

Standardized Garlic Preparations. Products representing crushed fresh (uncooked) garlic, microwave-cooked garlic (containing alliin and other cysteine sulfoxides), and alliin-free uncooked garlic were prepared as powders. The product representing crushed garlic was prepared by freeze-drying peeled whole cloves that had been cut into 3−4 mm thick slices, followed by pulverization in a coffee-bean grinder. Garlic cloves were also microwave-heated to inhibit alliinase. The powder of microwaved garlic was prepared by microwaving 20 g of peeled cloves for 45 s (650 W, 2450 mHz), followed by freeze drying and pulverization. Addition of water to the powder revealed no detectable amount of allicin, indicating the absence of active alliinase. Alliin-free garlic powder was prepared without heat by homogenizing peeled cloves in water at 2 mL/g followed by freeze drying. Because freeze drying only removes about 50% of the allicin (the least volatile of the garlic thiosulfinates), water was added to the dry material at 2 mL/g and freeze-dried again. This was repeated 6 times until only a trace amount of allicin remained. The final powder possessed a high amount of alliinase activity, indicating little protein damage. A single set of garlic bulbs was used to prepare all three products. Enteric-coated garlic powder tablets, Garlicin (Nature's Way Products, Inc., Springville, UT), weighed 600 mg each and were label-standardized to contain 300 mg of garlic powder and to yield at least 2.5 mg of allicin per tablet when crushed and added to water.

Consumption. A total of seven persons (four women and three men), with an age range of 23−51, participated in various sections of this study. The garlic powders were consumed at a dose of 3.0 g (representing 7.0 g of whole garlic), after adding water at 2.5 mL/g to make a slurry. Thiosulfinate formation in the slurry was complete in 5 min. Immediately prior to consumption, the slurry was transferred by a positive displacement pipet to 14 size 0 gelatin capsules. Allicin consumption required special care, because of stability concerns. Immediately prior to consumption, frozen allicin solution was thawed and extracted 2 times with one volume of cold dichloromethane. Dichloromethane was removed by rotary evaporation in ambient water to near constant weight. The solvent-free allicin was kept in an ice−water bath until used (stable for at least 5 h at 2 °C and for 1 h at 23 °C). Upon use, specific weights were transferred by micropipet to gelatin capsules. The vinyldithiins were consumed in soybean oil (5.4 g) in gelatin capsules, their usual supplement form. All other compounds were consumed in pure, undiluted form in gelatin capsules. The products were usually consumed between 9:00 am and 10:30 am. Immediately before consumption, 280 g of sweetened yogurt was eaten; this was done to provide a somewhat similar gastric environment for all of the tests. If any gastrointestinal disturbances occurred after consuming the products, the disturbance was quickly relieved by eating additional food. For some products, gastric irritation was greatly reduced or eliminated by consuming them in several smaller portions over a 30−60 min period, as was done for the crushed garlic product, allicin (30−59 mg), and ajoene.

Analysis of the Garlic Preparations. Aqueous extracts of the dehydrated garlic preparations (25−100 mL water/g) were used for analysis of all compounds, except for the allyl sulfides, which required extraction with acetonitrile/water (1:1). Allicin and other thiosulfinates were analyzed using C18-HPLC upon elution with methanol/water (1:1) at 240 nm (*20*). Alliin and methiin (*S*-methylcysteine sulfoxide) were analyzed by C18-HPLC after derivatization with *o*-phthaldialdehyde/

Table 1. Sulfur Compounds in Rehydrated Garlic Preparations (mg/g Dry Weight)[a]

	crushed garlic (powder)	microwaved garlic (powder)	alliin-free garlic (powder)	enteric-coated garlic tablets
alliin	<0.1	32.8	<0.1	<0.1
other cysteine sulfoxides[b]	<0.1	6.9	<0.1	<0.1
allicin	13.5	0.1	<0.1	4.9
allyl thiosulfinates[c]	19.1	<0.1	<0.1	6.6
total thiosulfinates[d]	19.6	<0.1	<0.1	6.7
allyl sulfides[e]	0.15	0.02	0.07	0.11
γ-glutamyl-*S*-allylcysteine	5.9	6.7	6.6	7.2
γ-glutamyl-*S*-*trans*-1-propenylcysteine	5.8	6.9	6.9	6.1
S-allylcysteine	0.28	0.30	0.37	0.20
S-allylmercaptocysteine	<0.02	<0.02	<0.02	0.08

[a] Values are expressed as mg/g dry weight (powder or pulverized tablet) after adding water. [b] Other cysteine sulfoxides (microwaved garlic): methiin (4.7 mg/g *S*-methylcysteine sulfoxide) and isoalliin (2.2 mg/g *S*-*trans*-1-propenylcysteine sulfoxide). [c] Allyl thiosulfinates: allicin, allyl methyl thiosulfinates (CH_2═CH–CH_2–SS(═O)–CH_3; 3.6 mg/g for crushed garlic, 1.5 mg/g for tablets) and allyl *trans*-1-propenyl thiosulfinates (CH_2═CH–CH_2–SS(═O)–CH═CH–CH_3; 1.9 mg/g for crushed garlic, 0.2 mg/g for tablets). [d] Total thiosulfinates: allyl thiosulfinates (96–98%), dimethyl thiosulfinate (1–2%), and both methyl *trans*-1-propenyl thiosulfinate regioisomers (0.5–2.5%). [e] Allyl sulfides: diallyl trisulfide (56–87%), diallyl disulfide (9–31%), diallyl tetrasulfide (3–8%), and AMS (4–13%). Diallyl sulfide, AMS, and allyl methyl disulfide were undetectable (<0.01 mg/g).

tert-butylthiol and elution with acetonitrile/dioxane/tetrahydrofuran/0.05 M sodium phosphate buffer pH 7.15 (21:4:2:73) at 337 nm (*21*). This method does not separate alliin from isoalliin (*S*-*trans*-1-propenylcysteine sulfoxide). Hence, isoalliin was calculated on the basis of the amount of 1-propenyl thiosulfinates formed (*20*). Allyl sulfides were analyzed by C18-HPLC upon elution with acetonitrile/water (7:3) at 240 nm (*19*). The γ-glutamylcysteines, *S*-allylcysteine, and *S*-allylmercaptocysteine were analyzed by C18-HPLC upon elution with 0.05 M KH_2PO_4 (pH 4.5)/methanol (97.5:2.5) at 220 nm (*22*).

Breath Analysis. Whole breath samples were collected in 1.2 L Teflon bags (Alltech, Deerfield, IL) containing a stainless steel on−off valve, to which a 4-cm piece of Tygon tubing was added, and a septum port for the syringe. Subjects were instructed to flatten the bag and then exhale a normal breath (avoiding the natural tendency to take a deep breath first) until the bag was mostly full. After the test material was consumed, breaths were taken every 1−2 h for 48 h, except during sleep. The average rate of disappearance of compounds from the bags was 0.3%/h (range 0.1−0.9%/h) for acetone and 0.4%/h (range 0.1−0.6%/h) for AMS. The disappearance rate for each bag was determined, and only the better bags were used for the breaths that could not be analyzed within 4 h. Moisture accumulation in the bags was found to have no effect on the concentrations, in agreement with another report (*23*). Occasionally the bags were dried overnight at 37 °C.

Duplicate 5-mL samples were injected with a gastight syringe directly into a gas chromatograph (Hewlett−Packard 5880A) fitted with an FID detector and a 30 m × 0.32 mm × 0.25 μm Supelcowax 10 capillary column (Supelco, Bellefonte PA), using helium at 3.2 mL/min and a split ratio of 20:1. The column was operated isothermally at 50 °C, while the injection port and FID detectors were operated at 225 and 250 °C, respectively. The AMS and acetone vapor standards were prepared by quickly transferring 4.00 μL to a 4.3 L nitrogen-filled glass bottle. After waiting at least 20 min, we transferred 43 mL of vapor via a gastight syringe to a second 4.3 L bottle, giving final concentrations of 6.27 μg/L for acetone and 7.44 μg/L for AMS. Typical retention times were 1.5 min for acetone, 2.2 min for AMS, 1.8 min for allyl mercaptan, and 5.2 min for diallyl sulfide, which remained stable for at least 1 year. All compounds of interest were baseline-separated from adjacent compounds found in the breath. Consumption of raw carrots and apples was avoided within 30 min of taking a breath sample because of a large ethanol peak (2.03 min) that interfered with AMS. Normal levels of breath acetone can sometimes show considerable fluctuation, such as after poor sleep. Therefore, an experiment was not begun if the breath acetone levels were not moderately stable during the previous 24 h period.

After most of the experiments were conducted, it was found that alveolar breath (lung air only), determined using an alveolar bag (Quintron, Milwaukee, WI) attached to the Teflon bag, gave 7 and 18% higher concentrations of acetone and AMS, respectively, than whole breath. However, because all experiments were conducted in the same manner and because only relative amounts were critical, this was not considered an important deficiency. It was also difficult to use alveolar bags for breaths taken at home.

Identity of acetone and AMS in breath samples was confirmed using an HP Model 5989A GCMS by electron impact and selected ion monitoring of ion 58 for acetone and ion 88 for AMS (conducted by San Rafael Chemical Services, Salt Lake City, UT).

Quantitation of the Breath Effects. The effect of consuming a product or compound was determined by plotting the breath concentrations of acetone and AMS versus time over 48 h to obtain the area under the curve (AUC_{48}) or, for the increase in breath acetone, the AUC_{48} to the basal value. The basal value for acetone was determined by drawing a line from the value at 0 h to the value at 48 h. One GC area unit was found to represent acetone at 1.3 μg/L or AMS at 1.2 μg/L.

Statistical Analysis. Statistical analyses were conducted using Microsoft Excel software. Data were examined for homogeneity among variances. Differences between groups were analyzed by Student's *t* test (2 tail). p values < 0.05 were considered to be significant. Data are presented as means ± standard deviation (SD).

RESULTS

Composition of the Garlic Preparations. The composition of sulfur compounds present in the rehydrated standardized garlic powders and tablets used in the study are given in **Table 1**. Because rehydration activated alliinase, alliin was not found in the products representing crushed or alliin-free garlic. However, microwave heating of the garlic was found to effectively inhibit alliinase because a large amount of alliin and no detectable amount of thiosulfinates were found. The allyl thiosulfinates constituted 97−98% of the total thiosulfinates, while allicin accounted for 69−73% of the total. All products were similar in their amounts of γ-glutamylcysteines. The low levels of allyl sulfides, *S*-allylcysteine, and *S*-allylmercaptocysteine indicate that little degradation had taken place during preparation of the powders. In agreement with another report (*24*), neither acetone, AMS, or allyl mercaptan were detectable (<0.08 μg/L) in the 2-h vapor above the wetted crushed garlic preparation.

Basal Breath Values and Experimental Variation. For the seven persons included in the study, the breath acetone level prior to consuming the garlic products was 0.68 ± 0.21 μg/L, with a range of 0.4 to 1.7. No AMS was detected (<0.08 μg/L) in the breath prior to the experiments, which is in agreement with others who reported 0.04 μg/L (about 12 ppb) with a more sensitive detector (*15*). A fair amount of variation is to be expected in breath experiments. **Table 2** reveals the amount of

Table 2. Experimental Variation

source of variation	degree of variation (% cv) acetone	AMS
between duplicate GC injections for the same breath ($n = 24$)	3.7	3.0
between breaths about 20 min apart ($n = 12$)	7.7	7.4
between weekly baseline acetone levels[a]	18.3	

[a] Breath acetone level before start of an experiment. The value represents the average percent coefficient of variation (cv) for four individuals and a total of 130 experiments over an average time period of 22 months per person.

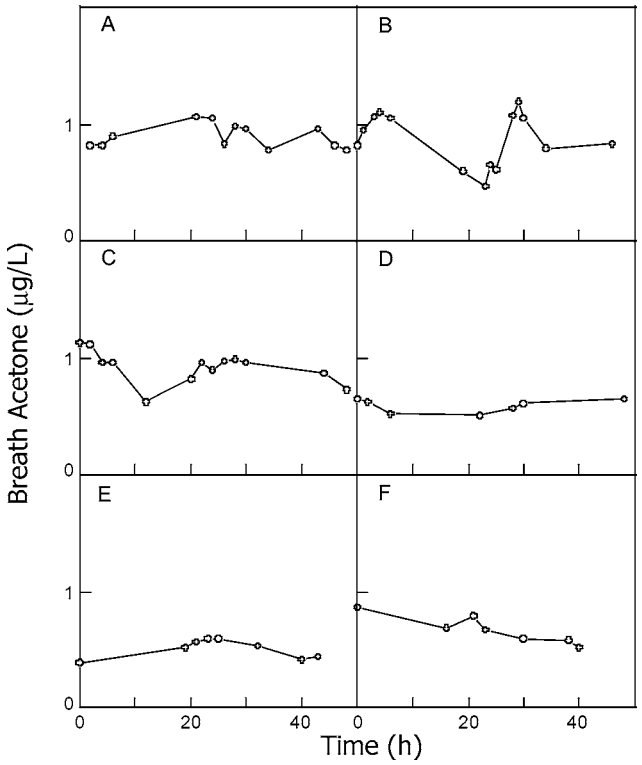

Figure 1. Examples of normal breath acetone fluctuations in five persons who did not consume garlic but did consume the standard yogurt meal at time zero with (A–D) or without (E and F) empty gelatin capsules. A and B are from the same person.

variation found between duplicate analyses for the same breath and between two breaths about 20 min apart. The variations found for acetone were similar to those found for AMS. A larger amount of variation was found in the week to week basal acetone concentrations. This variation was consistent among the four individuals tested at least 10 times over a time span of at least 9 months each (20, 19, 14, and 20%). Hence, larger variation is expected in measuring acetone in the breath than in measuring AMS, because AMS is normally absent from the breath. However, by measuring the increase in breath acetone over the course of an experiment, rather than the absolute amount of acetone, the amount of variation found is minimized. **Figure 1** depicts examples of the variation found in breath acetone levels found in control tests when neither garlic nor garlic-related compounds were consumed.

Breath Composition after Consuming Crushed Fresh Garlic Cloves. Initial qualitative experiments were designed to confirm the original report of Taucher et al. (*15*), who used an almost intolerably large amount (38 g) of finely chopped fresh garlic, and to determine a dose to use with the standardized

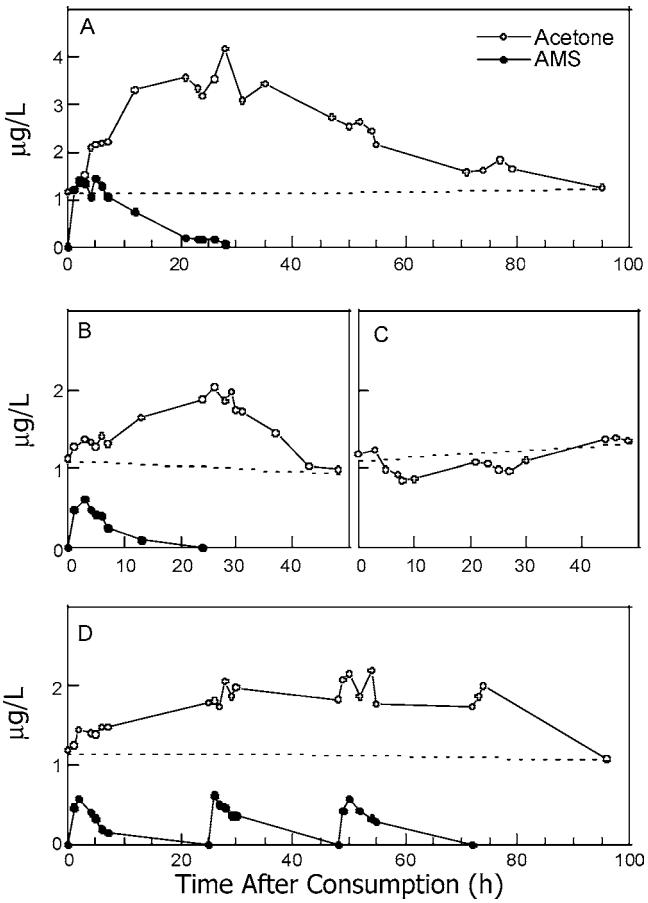

Figure 2. Effect of consuming crushed fresh garlic cloves on breath levels of acetone and AMS. Breath levels were measured after consuming (A) 20 g, (B) 7 g, (D) 7 g on three consecutive days, and (C) 7 g of microwave-heated garlic. The dashed line indicates the basal acetone level.

preparations that would be more tolerable to consume while still permitting reliable measurements. As shown in **Figure 2A**, consumption of 20 g of crushed California Early garlic (on bread), with an allicin yield of 108 mg, resulted in a large increase in acetone, which reached a maximum level at 28 h and required 96 h to return to normal. AMS reached maximum concentration in 2 h and required about 30 h to become undetectable. Consuming this much fresh garlic caused substantial irritation to the mouth; therefore, the dose was decreased to a very tolerable 7 g (**Figure 2B**), which still gave a substantial increase above the basal acetone fluctuation. This is not an uncommon size for a garlic clove and is only moderately above the average U.S. per capita consumption of 4.2 g/day in 1999 (*25*). At this dose, breath acetone became maximal at about 20−26 h and returned to normal levels in about 48 h. A total of 7 g of this crushed garlic yielded 38 mg of allicin and 56 mg of total allyl thiosulfinates. Hence, in subsequent experiments, standardized garlic powder that represented crushed garlic was consumed at similar thiosulfinate levels (**Table 3**).

Crushed fresh garlic was also consumed on 3 consecutive days to determine if the effect on breath acetone would change after the first dose. As shown in **Figure 2D**, consumption of 7 g of crushed garlic at 0, 24, and 48 h resulted in a constant increase in acetone that returned to normal levels 48 h after consumption of the third dose. Hence, the effect on breath acetone appears to be sustained as long as garlic is consumed. Inactivation of alliinase by microwave heating of the garlic before crushing (**Figure 2C**), eliminated the increase in breath acetone and prevented formation of detectable amounts of AMS,

Table 3. Effect of Various Dried Garlic Preparations and Allicin on Breath Acetone and Breath AMS for One Person[a]

	AUC_{48} [(μg h)/L]		
product	acetone (total)	acetone increase[b]	AMS
control (4)	40.1 ± 5.2[c]	1.2 ± 3.7[c]	nd[c,d]
crushed garlic[e] (5)	65.8 ± 10.6[f]	21.2 ± 4.1[f]	6.0 ± 1.1
microwaved garlic[e] (2)	47.6 ± 2.9[g]	0.2 ± 0.3[c]	nd
alliin-free garlic[e] (4)	45.5 ± 6.8[c]	3.5 ± 1.7[c]	nd
alliin-free garlic[e] + added allicin (59 mg) (2)	64.6 ± 2.1[f]	22.1 ± 7.0[f]	5.1 ± 0.6
allicin (59 mg, 730 μmol of *S*-allyl) (8)	67.9 ± 8.2[f]	23.6 ± 6.7[f]	8.2 ± 1.6[g]
enteric-coated tablets[h] (3)	76.1 ± 12.9[f]	25.5 ± 11.9[f]	6.1 ± 1.9

[a] Data are means ± SD for (*n*) experiments. [b] Increase above the basal line, drawn from 0 to 48 h. [c] Different from crushed garlic, $p < 0.01$. [d] nd = not detectable. limit of detection = 0.3 (μg h)/L. [e] Water was added to the powders (3.0 g) immediately prior to consumption. The crushed garlic product liberated 59 mg of total thiosulfinates, including 41 mg of allicin plus 16.5 mg of allyl methyl and allyl 1-propenyl thiosulfinates (620 μmol of *S*-allyl). [f] Different from the control, $p < 0.02$. [g] Different from crushed garlic, $p < 0.05$. [h] The enteric-coated tablets had the potential of producing 60 mg of total thiosulfinates, including 44 mg of allicin, 13.4 mg of allyl methyl, and 1.8 mg of allyl 1-propenyl thiosulfinates (652 μmol of *S*-allyl).

Table 4. Effect of Various Dried Garlic Preparations and Allicin on Breath Acetone and Breath AMS for Several Persons[a]

	AUC_{48} [(μg h)/L]	
product	acetone increase	AMS
control (7)	1.4 ± 2.4[b]	nd
crushed garlic (4)	13.7 ± 7.7[c]	3.5 ± 1.9
microwaved garlic (3)	1.1 ± 0.8[b]	nd
alliin-free garlic (4)	2.9 ± 1.6[d]	nd
allicin (59 mg, 730 μmol of *S*-allyl)[e] (3)	16.0 ± 7.1[c]	5.2 ± 3.7
enteric-coated tablets[f] (7)	14.7 ± 6.7[c]	3.3 ± 1.8

[a] Data are means ± SD for (*n*) persons. [b] Different from crushed garlic, $p < 0.02$. [c] Different from the control, $p < 0.02$. [d] Different from crushed garlic, $p < 0.05$. [e] Total acetone = 51.8 ± 6.6 (μg h)/L. [f] Total acetone = 49.0 ± 17.7 (μg h)/L.

indicating that the increase in acetone was associated with enzyme activity, such as the alliinase-generated thiosulfinates, and that AMS was a metabolite of enzyme-generated compounds containing allyl groups, such as the allyl thiosulfinates.

Breath Composition after Consuming Standardized Garlic Preparations and Allicin. The effects of these preparations on breath composition are given in **Table 3** for one person and in **Table 4** for several persons. Example plots of their effects on breath acetone have been previously published (*10*). The increase in breath acetone caused by consumption of *crushed garlic* was eliminated upon microwave inactivation of alliinase (alliin present) and by removal of alliin. However, addition of allicin to the alliin-free powder, at the same weight of THSs originally present, completely restored the ability to stimulate acetone production. Hence, virtually all of the effect of crushed garlic on acetone production is due to the thiosulfinates.

Breath levels of AMS, caused by consuming crushed garlic, were also eliminated by inactivation of garlic alliinase or by removal of alliin and restored by addition of allicin, demonstrating that AMS is strictly an allyl thiosulfinate metabolite. Ingestion of pure *allicin* also gave high amounts of breath AMS. Because allicin contains no methyl group, the source of the methyl group must come from metabolism, probably from *S*-adenosylmethionine in the liver. Pure allicin at a dose of 59 mg gave significantly greater amounts of breath AMS than crushed garlic containing 59 mg of allyl thiosulfinates because only 85 mol % of the garlic thiosulfinate alkyl groups are allyl groups and hence capable of forming AMS. When corrected for equimolar amounts of allyl, the difference between crushed garlic and pure allicin is insignificant. The smaller amount of AMS found when 59 mg of allicin was added to alliin-free garlic powder (**Table 3**) is probably due to the variability associated with the small number of experiments (2 versus 8) conducted with this product.

Table 5. Time to Reach Maximum Breath Levels[a]

	n	acetone (h)	AMS (h)
crushed garlic	4	21.1 ± 3.9	3.4 ± 0.5[b]
allicin	3	22.2 ± 1.2	3.5 ± 0.5[b]
enteric-coated tablets	7	25.1 ± 2.8	6.3 ± 1.8
tablet delay[c]		3.5	2.9

[a] Data represent mean ± SD for *n* persons. All were consumed at a content or potential (tablets) of 59–60 mg of total thiosulfinates. [b] Different from enteric-coated tablets, $p < 0.02$. [c] Tablet delay, average time for crushed garlic and allicin minus time for the tablets.

The effects of the products on breath acetone and AMS observed with one person remained significant when expanded to several persons (**Table 4**); however, the variation was greater. The person−person variation (cv) in the increase in exhaled acetone for crushed garlic and allicin was 44−56%, while the variation between experiments for one person was only 19−32% (**Table 3**). For AMS, the person−person variation ranged from 54 to 71%, while the variation for one person ranged from 12 to 20%.

The effects of *enteric-coated tablets* on breath acetone and AMS (**Tables 3** and **4**) were not significantly different from crushed garlic or an equivalent amount of pure allicin, demonstrating that allicin formation *in vivo* from such tablets can be complete. However, these results cannot be generalized to other brands, because most brands, even most enteric-coated brands, have been found to release much less allicin under USP/NF-defined simulated gastrointestinal dissolution conditions, because of low or inhibited tablet alliinase activity (*4*, *5*). Furthermore, the amount of AMS exhaled has been found to correlate well ($R = 0.985$) over a 20-fold range with the dissolution allicin release (USP/NF) for four sets of tablets of widely varying alliinase activity, demonstrating that the amount of allicin produced in the body from tablets depends highly upon the quality of the tablet (*4*).

The amount of time needed to reach maximum breath levels of acetone and AMS is depicted in **Table 5**. No significant difference was found between consuming pure allicin and consuming the crushed garlic product. However, consumption of enteric-coated tablets caused an average delay of about 3 h (range of 1−5 h) in maximum concentrations, which indicates the amount of time required for the tablets to disintegrate and form allicin in the gastrointestinal tract. This time delay is similar to that of the conditions required in the USP/NF dissolution monograph for enteric-coated garlic powder tablets (2 h in acid and 1 h in buffer) (*26*). However, the range of delays that was

Table 6. Effect of Orally Consumed Allicin, Allicin-Derived Compounds, and *S*-Allylcysteine on Breath Acetone and Breath AMS[a]

compound consumed (dose in μmol of allyl, dose in μmol of compound, if different)	*n*	acetone increase[b] AUC_{48} [(μg h)/L]	acetone increase[b] T_{max} (h)	AMS AUC_{48} [(μg h)/L]	AMS C_{max} (μg/L)	AMS T_{max} (h)	AMS $T_{1/2}$ (h)
allicin (730, 365)	4	24.1 ± 7.5	22.8 ± 3.4	9.4 ± 1.6	0.97 ± 0.21	3.5 ± 0.7	10.0 ± 0.9
allicin-derived compounds in garlic oils							
diallyl trisulfide (730, 365)	3	26.5 ± 14.6	20.7 ± 2.3	9.3 ± 2.0	0.86 ± 0.21	5.0 ± 0.9[c]	13.2 ± 1.8
diallyl disulfide (730, 365)	3	19.8 ± 2.2	19.5 ± 2.1	9.1 ± 1.2	0.84 ± 0.17	9.2 ± 1.0[d]	13.7 ± 2.3
diallyl sulfide (730, 365)	4	8.7 ± 1.0[d]	18.8 ± 3.6	nd[e]			
diallyl sulfide (1460, 730)	2	22.5 ± 0.8	21 ± 2.8	nd			
ajoene (730, 365)	2	12.9 ± 2.2[f]	20.3 ± 2.1	3.9 ± 0.6[g]	0.42 ± 0.02	2.5 ± 0.3	10.8 ± 0.4
vinyldithiin oil (725, 370)[h]	2	1.5 ± 2.0[d]		0.3 ± 0.1[d]			
distilled garlic oil (730, 380)[i]	2	22.2 ± 7.1	22.5 ± 3.5	10.4 ± 0.4	1.04 ± 0.23	8.5 ± 0.7[d]	14.4 ± 0.9
allicin metabolites							
allyl mercaptan (730)	2	20.9 ± 1.0	18 ± 1.4	7.7 ± 0.8	0.87 ± 0.01	1.8 ± 0.4[g]	6.8 ± 1.1[g]
AMS (730)	2	23.5 ± 8.5	21 ± 1.4	10.5 ± 1.0	1.21 ± 0.09	3.0 ± 0.3	8.5 ± 0.7
other							
S-allylmercaptocysteine (730)	2	21.6 ± 5.7	19 ± 0	10.0 ± 0.6	1.11 ± 0.06	3.3 ± 1.1	8.8 ± 0.7
S-allylmercaptocysteine (1460)	1	49.1	24	21.1	1.62	5	10.0
S-allylcysteine (730)	2	1.1 ± 0.8[d]		nd			

[a] Data are means ± SD for *n* experiments with the same person. C_{max} = maximum concentration reached; T_{max} = time after consumption to reach maximum concentration; $T_{1/2}$ = time after consumption for the concentration to decrease to one-half of the maximum. [b] Increase above basal line, drawn from 0 to 48 h. [c] Different from allicin, $p = 0.052$. [d] Different from allicin, $p < 0.01$. [e] nd = not detectable; AMS limit of detection = 0.3 (μg h)/L. [f] Different from allicin, $p = 0.12$. [g] Different from allicin, $p < 0.05$. [h] The vinyldithiin oil contains 7.05 mg of 1,3-vinyldithiin, 2.4 mg of 1,2-vinyldithiin, 0.23 mg of ajoene, 0.05 mg of diallyl disulfide per gram of soybean oil, and no allicin. The value used for micromoles of allyl is based on inclusion of both the external and internal allyl groups present in these cyclic compounds. [i] The commercial steam-distilled garlic oil contains 0.14 mg of diallyl sulfide, 2.9 mg of diallyl disulfide, 4.1 mg of diallyl trisulfide, 0.70 mg of diallyl tetrasulfide, 0.10 mg of diallyl pentasulfide, 0.14 mg of allyl methyl disulfide, and 0.50 mg of allyl methyl trisulfide per gram of soybean oil.

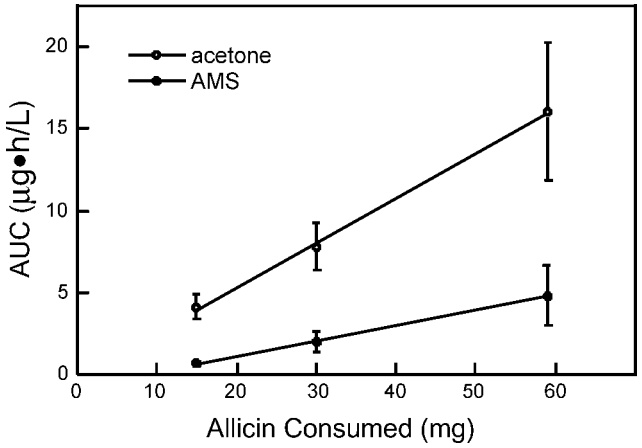

Figure 3. Effect of allicin dose on the acetone increase and AMS content of human breath. Values represent mean ± SEM for three persons.

found indicates that there is considerable person−person variability. The production of AMS at a substantially earlier time than production of acetone indicates that AMS or a further metabolite of AMS may be responsible for the effect of allicin on acetone formation.

Dose−response curves for acetone and AMS production after consumption of pure allicin are shown in **Figure 3**. The amount of allicin consumed (59, 30, and 15 mg) represents the total allyl thiosulfinates produced after consumption of 7, 3.5, and 1.8 g of thoroughly crushed fresh garlic. The response was linear for both acetone and AMS. The *y* intercept for acetone production was near 0, but it was negative [−0.9 (μg h)/L] for AMS. Upon consuming 7.5 mg of allicin (not shown), the effect on breath acetone could not be clearly distinguished from normal breath acetone fluctuation.

Breath Composition and Pharmacokinetics after Consuming Allicin, Allicin-Derived Compounds, Allicin Metabolites, and *S*-Allylcysteine. Allicin is a moderately unstable compound that can be readily transformed through self-reactions into a variety of oil-soluble thioallyl (mainly dithioallyl or SS-allyl) compounds when crushed garlic is commercially processed (**Figure 5**) (*18*, *19*, *27*). The types of compounds formed depends strongly on the temperature and polarity of the medium. Diallyl trisulfide and diallyl disulfide are the main products formed when crushed garlic is steam-distilled, while ring-structured vinyldithiins and oxygenated ajoene are the main products formed when it is incubated in a vegetable oil (oil macerate) at ambient temperature. Furthermore, both allicin and its dithioallyl transformation compounds (diallyl trisulfide, diallyl disulfide, and ajoene) are known to react rapidly (slower for diallyl disulfide) with the amino acid cysteine to form *S*-allylmercaptocysteine (allyl-SS-Cys) at a mole ratio of one cysteine per *S*-allyl, a reaction which certainly takes place in the digestive tract, where both protein-bound cysteine and free cysteine, formed by protein hydrolysis, are present (*11*, *16*). When these compounds are incubated in fresh human blood, they, as well as *S*-allylmercaptocysteine, are metabolized almost quantitatively to stable amounts of allyl mercaptan, while the vinyldithiins and diallyl sulfide (monosulfide) do not form allyl mercaptan (*11*). These *in vitro* results indicate that allicin and its noncyclic dithioallyl transformation products have a similar metabolic fate. To determine if this common metabolism might also be true *in vivo*, the effects of consuming these compounds on breath acetone and breath AMS levels were determined. The results of these experiments, all conducted on the same person to provide better comparisons, are given in **Table 6**, with example plots given in **Figure 4**.

In agreement with *in vitro* results, consumption of equimolar amounts of allicin, *diallyl disulfide*, *diallyl trisulfide*, and steam-distilled garlic oil was found to result in the same amount of AMS exhaled and to have the same stimulating effect on breath acetone (**Table 6**). *Ajoene*, on the other hand, produced only about half as much AMS and had only about half of the effect on breath acetone because only one of its two allyl groups is adjacent to a dithio group. Consumption of the *vinyldithiins* did not result in production of AMS, nor did they stimulate acetone production. Consumption of *diallyl sulfide* also did not result in the production of AMS, but it did increase acetone production in a dose-dependent manner by about half as much as allicin. After diallyl sulfide was consumed, only trace amounts of diallyl

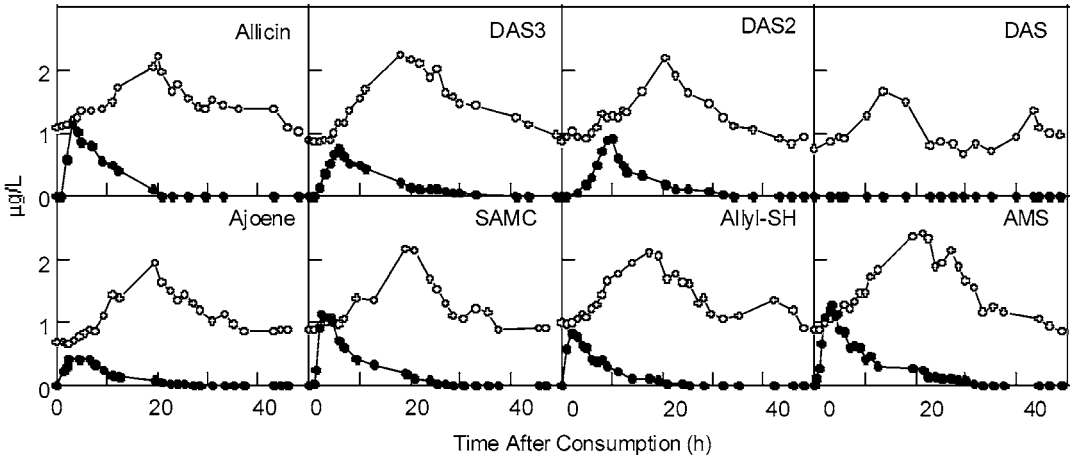

Figure 4. Example AUC profiles of the breath levels of acetone (○) and AMS (●) after consuming 730 allyl-μmol of allicin and allicin-derived compounds. DAS3, diallyl trisulfide; DAS2, diallyl disulfide; DAS, diallyl sulfide; SAMC, *S*-allylmercaptocysteine; Allyl-SH, allyl mercaptan; and AMS, allyl methyl sulfide.

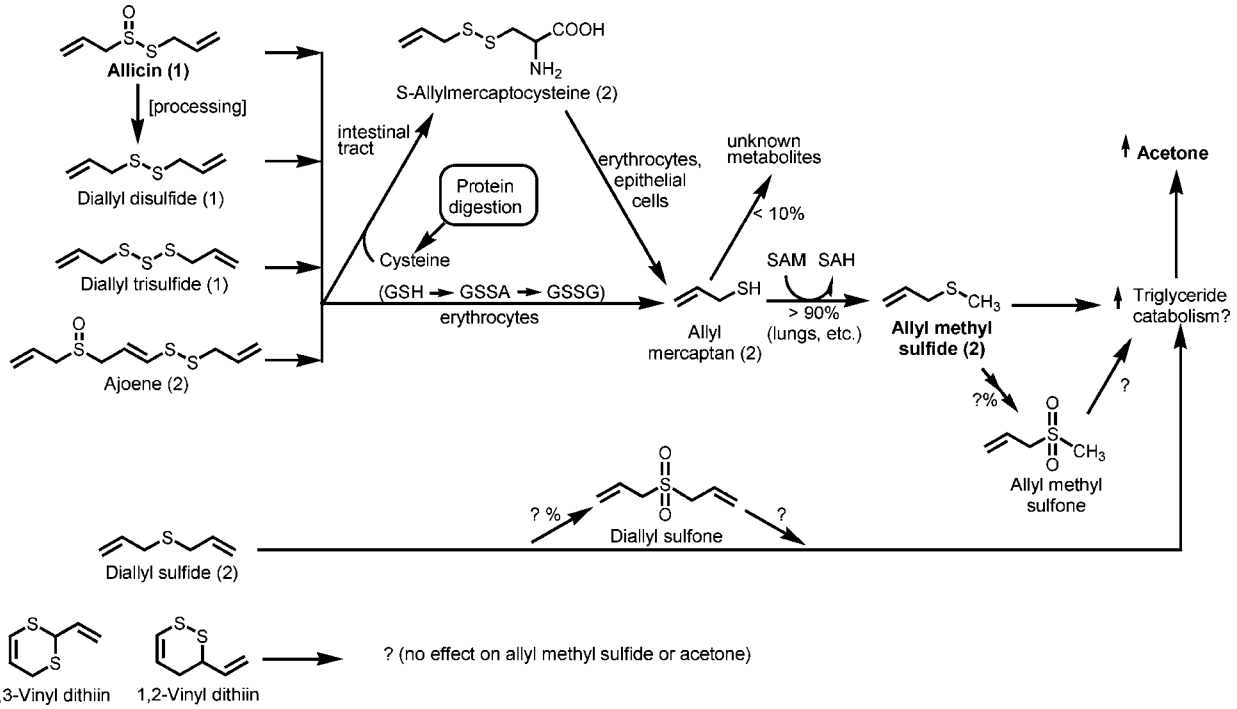

Figure 5. Current understanding of the metabolic fate and metabolic effect of allicin and allicin transformation compounds. Numbers in parentheses indicate number of moles required to produce 2 mol of AMS or, for diallyl sulfide, an equivalent amount of acetone. GSH, glutathione (γ-Glu-Cys-Gly); GSSA, *S*-allylmercaptoglutathione (γ-Glu-Cys-(*S*-allyl)-Gly); GSSG, oxidized glutathione; SAM, *S*-adenosylmethionine; and SAH, *S*-adenosylhomocysteine.

sulfide [AUC = 0.1 (μg h)/L] were found in the breath (not shown), with maximum concentrations at 5−10 min and complete disappearance by 30 min, indicating rapid metabolism to a compound that may be responsible for its effect on acetone production. Indeed, rats have been shown to metabolize diallyl sulfide to diallyl sulfone (allyl-SO_2-allyl), a compound that has pharmacological activity, as shown by its ability to inhibit the metabolism of cytochrome P450 2E1 (*28*, *29*). Consumption of *S-allylmercaptocysteine* produced as much breath AMS and acetone as allicin for the same amount of *S*-allyl consumed and in a dose-dependent manner. However, consumption of *S-allylcysteine* (allyl-S-cys), the hydrolysis product of γ-glutamyl-*S*-allylcysteine, which lacks a dithio group, did not result in AMS production or stimulation of breath acetone. The lack of production of AMS after consuming diallyl sulfide and *S*-allylcysteine demonstrate the requirement of a dithioallyl group for AMS production.

A unique exception to this requirement is *allyl mercaptan.* Consumption of allyl mercaptan also resulted in production of about the same amount of AMS and acetone as consumption of allicin at the same moles of allyl. In agreement with *in vitro* studies (*11*), allyl mercaptan appears to be an intermediate in the metabolism of dithioallyl compounds to AMS, as the recipient of the methyl group from *S*-adenosylmethionine (**Figure 5**). Further evidence for the intermediate role of allyl mercaptan is found in the fact that maximum AMS production by allyl mercaptan was found to occur in half the time as AMS production from allicin. However, because allyl mercaptan is so rapidly metabolized to AMS, it is only a transient metabolite. It was not found in the breath after consuming allicin or other dithioallyl compounds in gelatin capsules. After allyl mercaptan in capsules was consumed, only small amounts of allyl mercaptan [AUC = 0.4 (μg h)/L, about 5% that of AMS] were found in the breath, with maximum concentrations at 0.5−1 h

and complete disappearance by 3−4 h, indicating that it was entering the breath directly from the stomach rather than from the lungs.

Consumption of *AMS* itself, at the same allyl moles as allicin, resulted in the same increase in breath acetone and only about 10% more (not significant) breath AMS, demonstrating that (1) AMS is a physiologically active metabolite of allicin and other related dithioallyl compounds and (2) allicin is essentially completely (≥90%) metabolized to AMS. The fact that consumption of allicin and AMS resulted in the same time maxima for breath AMS production indicates that metabolism of allicin to AMS is extremely rapid and that accumulation of the intermediate, allyl mercaptan, would not be expected. The delayed maximum AMS times for diallyl trisulfide and especially for diallyl disulfide, relative to allicin, parallel results found *in vitro* with isolated blood where the half-lives for disappearance of allicin, diallyl trisulfide, and diallyl disulfide were <1, 4, and 60 min, respectively (*11*). These enhanced delays appear to be related to the strength of the S−S bond, being weaker for thiosulfinates and trisulfides than for disulfides (*30*).

Side Effects. The only side effect noticed in these acute studies, other than breath odor, was stomach irritation, which occurred briefly and only for the crushed garlic product and larger amounts of allicin (30−59 mg) or ajoene (94 mg). Breath odor was noticeable for all products and compounds that produced AMS. Stomach irritation was usually only significant (a clearly noticeable discomfort or pain) if the person's stomach was mostly empty before consuming the yogurt and product, such as when the person had eaten only a small breakfast (e.g., a piece of toast) or had not consumed the product until shortly before lunch.

DISCUSSION

In addition to providing two methods for the evaluation of allicin bioavailability from garlic and garlic supplements, this study has provided important information on the *metabolic pathway* and a *metabolic effect* of allicin and allicin-derived compounds that contain a dithioallyl group, as is summarized in **Figure 5**. Because these compounds react readily with cysteine, which is present in the digestive tract when co-ingested with protein, to form *S*-allylmercaptocysteine, the pathway to allyl mercaptan formation takes place by two routes, the proportioning of which depends on the abundance of cysteine present: (1) by metabolism of S-allylmercaptocysteine to allyl mercaptan in erythrocytes (possibly also in epithelial cells) and (2) by a glutathione-mediated pathway in erythrocytes (*30*, *31*), where the cysteine end of glutathione reacts with allyl groups to form S-allylmercaptoglutathione (GSSA) as an intermediate. Miron et al. have shown this intermediate to abundantly exist when allicin is incubated with erythrocytes (*32*, *33*). *S*-Adenosylmethionine is the postulated and probable methyl donor for AMS formation; however, initial attempts to prove this *in vitro* with liver microsomes have failed (*34*). Although AMS does stimulate acetone production and appears to be the effector metabolite of allicin and other dithioallyl compounds, it may be that a metabolite of AMS is directly responsible for the effect. Indeed, Germain et al. have shown that rats rapidly metabolize diallyl disulfide to allyl methyl sulfone (allyl-SO_2-methyl) and much smaller amounts of AMS and allyl methyl sulfoxide (allyl-SO-methyl), as found in liver and plasma (*35*). The presence of these compounds in human plasma after consumption of garlic or allicin has not yet been reported. The similar metabolic pathways and metabolic effects for all dithioallyl compounds has an important implication. Although pure allicin has been used in few animal studies and no prior human studies, because of its instability, steam-distilled garlic oils containing predominantly diallyl disulfide and diallyl trisulfide have been used in numerous animal and clinical studies (*10*). Hence, the results of these garlic oil studies can be considered as valid for allicin for effects that occur after its metabolism, which would exclude, for example, intestinal antimicrobial effects.

The extent to which *allicin is absorbed* has been difficult to determine because of its rapid metabolism and absence in the blood after consumption. The well-known persistent garlic odor from the breath and skin after fresh garlic consumption has qualitatively indicated good absorption. Animal studies have shown that ^{35}S-labeled allicin is at least 79% absorbed within 30−60 min (*36*). *In vitro* studies have shown that allicin crosses phospholipid bilayers as rapidly as if no membrane were present (at the rate of diffusion) and that it rapidly enters erythrocytes, reacting with intracellular glutathione to produce maximum concentrations of *S*-allylmercaptoglutathione in 1 min without causing leakage or damage to the membrane (*32*). The present human study indicates that allicin absorption (as allicin itself or bound to cysteine) must be very high, because consumption of allicin resulted in the same amount of breath AMS (and at similar rates) as consuming equivalent amounts of smaller molecules (AMS and allyl mercaptan) as well as after consuming diallyl trisulfide. Diallyl trisulfide has been shown to be 99% absorbed in rabbits (*37*). Hence, it can be concluded that allicin absorption in humans is at least 95%.

An important question is the amount of *acetone production* that is stimulated by allicin. The average breath output of acetone for a normal person is 12 mg (0.207 mmol) per day (*38*), but because acetone has a blood/breath partition coefficient of 330 (*39*), the amount of acetone produced and found in the blood over 48 h would be 137 mmol. The average increase in breath acetone over 48 h caused by 0.365 mmol of allicin for the three persons in **Table 4** was 45% [16/(51.8−16)] or 62 mmol, which corresponds to 170 mmol of acetone produced per millimole of allicin consumed. The true acetone/allicin ratio is expected to be even higher, after including the amount of acetone present in the tissues and the amount lost to metabolism.

The *mechanism* by which allicin stimulates acetone production is unclear. Rat studies have shown that garlic powder, alliin, diallyl trisulfide, and diallyl disulfide but not diallyl sulfide increase triglyceride catabolism (hence, acetone production) by increasing secretion of norepinephrine by a β-adrenergic mechanism (*40*). On the other hand, blood acetone levels in rats have been shown to increase upon administration of diallyl sulfide by inhibition of cytochrome P450 2E1, causing a decrease in acetone catabolism (*41*). The mechanism could be resolved by measuring blood levels of the ketone bodies, acetoacetate, and β-hydroxybutyrate, which increase upon triglyceride catabolism but not upon inhibition of P450 2E1.

Because a known amount of AMS was consumed and the AUC for AMS was measured (**Table 6**), the *body/breath partition coefficient* can be calculated ("body" includes all tissues, blood, urine, and losses through the skin). Upon consuming 64.4 mg (730 μmol) of AMS, the average breath AMS concentration over 48 h was 0.22 μg/L = [10.5 (μg h)/L]/(48 h). The person who consumed the AMS had an average exhale volume of 0.7 L and a breath rate of 18 breaths/min. Hence, the total amount of AMS exhaled was 8.0 mg, which gives a body/breath partition coefficient of 8.1. This is much lower than the blood/breath partition coefficient of 330 that has been reported for acetone (*39*) and reflects both the volatility

of AMS (although less volatile than acetone) and especially the low water solubility of AMS (3.3 mg/mL).

The absence of AMS in the breath after consumption of microwave-cooked garlic, in which alliin is abundant, demonstrates the *absence of alliinase* activity in the body. Early *in vitro* work demonstrated that several intestinal bacteria possess alliinase-like activity and led these and other workers to conclude that the alliin present in cooked garlic can be converted to allicin in the body by bacteria (*7*, *42*, *43*). The contrary results found in the present *in vivo* study may indicate that the alliinase-like activity of bacteria is suppressed in the intestinal tract or that alliin is absorbed too rapidly by the intestinal epithelial cells to be available to the bacteria. However, it may also be possible that a small percent of garlic alliin is converted to allicin by intestinal bacteria, enough to produce a noticeable garlic breath odor (there are anecdotal reports of this) but insufficient to be detected using an FID detector. The finding of insignificant alliinase activity in the body demonstrates that cooked garlic will not have the same pharmacological effects as fresh garlic, at least as far as thiosulfinate-related effects are concerned. It also underscores the need to prove the allicin bioavailability of alliin-containing garlic supplements that are intended to provide the same health benefits as fresh garlic.

Breath AMS is generated not only from allicin but also from the other allyl thiosulfinates (mainly allyl methyl thiosulfinates) present in crushed garlic, because both halves of the thiosulfinate molecules undergo the same reactions (*44*). Hence, it follows that breath should contain dimethyl sulfide after consuming crushed garlic, because of metabolism of allyl methyl thiosulfinates. Indeed, upon using a more sensitive detector, dimethyl sulfide has been found in the breath after garlic consumption but at about one order of magnitude less than AMS, because of the lower abundance of allyl methyl thiosulfinates (*15*).

Because fasting is well-known to increase breath acetone levels, the effects of a short fast on acetone output were compared to that of consuming allicin or crushed garlic. Three 20-h fasts were conducted on the person tested in **Tables 3** and **6** (not shown). The average increase in breath acetone caused by the fasts was 1.79 times greater than that of consuming 59 mg of allicin (**Table 3**). Upon extrapolation of the AUC plots to correspond to a 17 h fast, the breath acetone increase was nearly equal to that of 59 mg of allicin. The allicin yield of crushed garlic varies from 2.5 to 6.5 mg/g (*20*). Hence, consumption of 7−19 g (corrected by 20% for other allyl thiosulfinates) of crushed garlic results in the same amount of acetone production as fasting for 17 h (skipping breakfast). Therefore, consumption of normal amounts of fresh garlic will not produce harmful levels of acetone.

In summary, this study has demonstrated that consumption of a single dose of allicin-containing garlic increases breath acetone in a dose-dependent manner, that allyl thiosulfinates account for all of the effects of crushed garlic, and that AMS, the main metabolite of allicin, is essential to the effects. For evaluating a pharmacological effect of garlic that may be caused by allicin, either breath AMS or breath acetone (as AUC) can be used in determining the bioequivalence (allicin bioavailability) of garlic supplements relative to crushed fresh garlic. Measurement of breath acetone is limited to consumption of 15 mg of allicin (30 mg practical limit) because of fluctuating basal breath acetone levels. Measurement of breath AMS is more reliable than acetone because AMS is normally absent when garlic has not been consumed. We have recently found the limit of allicin consumption for measuring breath AMS to be as low as 0.5−1 mg when using a sulfur-selective pulsed flame photometric detector (OI Analytical, College Station, TX), rather than FID. Because garlic supplements vary greatly in their ability to produce allicin in the body and because allicin is involved in many of the pharmacological effects of garlic, the results of clinical trials with garlic supplements can only be extrapolated to garlic itself when the supplement has been shown to have similar allicin bioavailability to crushed fresh garlic. Breath AMS and breath acetone provide new tools to assess allicin bioavailability.

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