


## Research Article

**Rapid and Sustainable Detoxication of Airborne Pollutants by Broccoli Sprout Beverage: Results of a Randomized Clinical Trial in China** 

Patricia A. Egner<sup>1</sup>, Jian-Guo Chen<sup>6</sup>, Adam T. Zarth<sup>4</sup>, Derek K. Ng<sup>2</sup>, Jin-Bing Wang<sup>6</sup>, Kevin H. Kensler<sup>1</sup>, Lisa P. Jacobson<sup>2</sup>, Alvaro Muñoz<sup>2</sup>, Jamie L. Johnson<sup>1</sup>, John D. Groopman<sup>1</sup>, Jed W. Fahey<sup>3</sup>, Paul Talalay<sup>3</sup>, Jian Zhu<sup>6</sup>, Tao-Yang Chen<sup>6</sup>, Geng-Sun Qian<sup>6</sup>, Steven G. Carmella<sup>4</sup>, Stephen S. Hecht<sup>4</sup>, and Thomas W. Kensler<sup>1,3,5</sup>

**Abstract**

Broccoli sprouts are a convenient and rich source of the glucosinolate, glucoraphanin, which can generate the chemopreventive agent, sulforaphane, an inducer of glutathione S-transferases (GST) and other cytoprotective enzymes. A broccoli sprout-derived beverage providing daily doses of 600  $\mu\text{mol}$  glucoraphanin and 40  $\mu\text{mol}$  sulforaphane was evaluated for magnitude and duration of pharmacodynamic action in a 12-week randomized clinical trial. Two hundred and ninety-one study participants were recruited from the rural He-He Township, Qidong, in the Yangtze River delta region of China, an area characterized by exposures to substantial levels of airborne pollutants. Exposure to air pollution has been associated with lung cancer and cardiopulmonary diseases. Urinary excretion of the mercapturic acids of the pollutants, benzene, acrolein, and crotonaldehyde, were measured before and during the intervention using liquid chromatography tandem mass spectrometry. Rapid and sustained, statistically significant ( $P \leq 0.01$ ) increases in the levels of excretion of the glutathione-derived conjugates of benzene (61%), acrolein (23%), but not crotonaldehyde, were found in those receiving broccoli sprout beverage compared with placebo. Excretion of the benzene-derived mercapturic acid was higher in participants who were *GSTT1*-positive than in the null genotype, irrespective of study arm assignment. Measures of sulforaphane metabolites in urine indicated that bioavailability did not decline over the 12-week daily dosing period. Thus, intervention with broccoli sprouts enhances the detoxication of some airborne pollutants and may provide a frugal means to attenuate their associated long-term health risks. *Cancer Prev Res*; 7(8); 813–23. ©2014 AACR.

**Introduction**

The International Agency for Research on Cancer (IARC) has recently classified air pollution and particulate matter from air pollution as carcinogenic to humans (1). China is now the world's largest emitter of anthropogenic air pollution, and levels of outdoor air pollution in China are among the highest in the world (2, 3). The Yangtze River

delta region of China, which includes our study site of Qidong, is the fastest growing economic development area in China. Air pollution from expanding industrialization in this region masks the horizon on many days, especially during the winter months. Increases in fossil fuel use in China's industry, transport, and residential sectors have resulted in a steep increase in emissions. The Yangtze River delta region, which constitutes only 2% of the area of China, contributes upward of 15% of countrywide emissions of greenhouse gases (4). These emissions include particulate matter. There is substantial evidence that the most harmful components of particulate matter are in the fine fraction of particulate matter (particles with an aerodynamic diameter  $< 2.5 \mu\text{m}$ ;  $\text{PM}_{2.5}$ ) which can be inhaled into the deep lungs (5, 6). In Chinese cities, until recently, only the larger particulate matter,  $\text{PM}_{10}$ , were routinely monitored and reported. A large, recent study in Europe indicated that particulate matter, irrespective of particle size, contributes to lung cancer incidence (7). Adsorbed onto these inhaled particles are heavy metals, as well as carcinogenic polycyclic aromatic hydrocarbons and volatile organic chemicals such as benzene and aldehydes, which, following desorption

**Authors' Affiliations:** Departments of <sup>1</sup>Environmental Health Sciences and <sup>2</sup>Epidemiology, Johns Hopkins Bloomberg School of Public Health; <sup>3</sup>Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland; <sup>4</sup>Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota; <sup>5</sup>Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania; and <sup>6</sup>Qidong Liver Cancer Institute, Qidong, Jiangsu, China

P.A. Egner, J.-G. Chen, A.T. Zarth, and D.K. Ng contributed equally to this work.

**Corresponding Author:** Thomas W. Kensler, Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261. Phone: 412-648-1942; Fax: 412-628-1945; E-mail: tkensler@pitt.edu

doi: 10.1158/1940-6207.CAPR-14-0103

©2014 American Association for Cancer Research.

from particulate matter, may contribute to lung cancer risk (8, 9).

We have previously reported 5-fold higher levels of the polycyclic aromatic hydrocarbon biomarker, phenanthrene tetraol, in the urines of nonsmoking Qidongese than in nonsmoking residents of the Twin Cities region of Minnesota (10), perhaps reflective of different ambient air quality in these 2 regions. Qidong is located on the northeastern tip of the mouth of the Yangtze River delta and is undergoing a rapid transition from isolated rural farm communities to an industrialized manufacturing center. Levels of mercapturic acids formed in the metabolism of benzene [*S*-phenylmercapturic acid (SPMA)], acrolein [3-hydroxypropylmercapturic acid (3-HPMA)], and crotonaldehyde [3-hydroxy-1-methylpropylmercapturic acid (HMPMA)] are also substantially higher in these Qidongese than in nonsmoker residents of Singapore (11). In the United States, the predominant exposure to benzene arises from on-road mobile source emission, although other sources, including emissions from coal and oil combustion, evaporation from industrial sites, and gasoline service stations, are noted (12). Smoking is also an important source of exposure to benzene, acrolein, and crotonaldehyde, based on analyses of their mercapturic acids before and after cessation, as well as other data (13). We have also observed 2- to 3-fold higher rates of excretion of crotonaldehyde and acrolein mercapturic acids in the urine of Qidong smokers than in nonsmokers (10).

Mercapturic acids are detoxication products resulting from glutathione conjugation of the parent aldehydes; or in the case of benzene, a primary metabolite, benzene oxide, is conjugated with glutathione followed by dehydration giving SPMA. They can be formed nonenzymatically or by glutathione *S*-transferase (GST)-catalyzed reactions (14–16). These biomarkers can play multiple, seemingly paradoxical roles in studies on human health. Commonly, they are used as indices of internal dose and as such are physiologically integrated measures of either ambient or occupational exposures that have been applied across study populations amid a range of exposures linked to adverse health effects. Dose–response relationships between workplace air measures of benzene and urinary excretion of SPMA have been reported (17). These biomarkers may also serve as measures of pharmacodynamic action in randomized clinical trials to assess the impact of interventions to enhance carcinogen detoxication (10).

To determine possible enhancement of detoxication of airborne pollutants by a broccoli sprout beverage, we conducted a placebo-controlled, randomized intervention trial in China. A bioactive component derived from broccoli, sulforaphane (18), is an effective anticarcinogen in animal models (19) and acts in part through inducing detoxication enzymes, including GSTs. The safety of broccoli sprout beverage has been well established in several phase I clinical trials (10, 20, 21). Unlike the previous clinical studies, this trial used a beverage with a blended, well-defined content of sulforaphane (40  $\mu$ mol) and its biogenic precursor glucoraphanin (600  $\mu$ mol). Therefore, the primary goals of this

study were to determine (i) to what extent daily consumption of a broccoli sprout beverage could elevate the initial rate of detoxication of pervasive toxic air pollutants among individuals exposed to excessive ambient levels and (ii) whether such a protective response would be sustainable with daily doses across a 12-week time frame.

## Materials and Methods

### Study design and participants

Adults in good general health without a history of major chronic illnesses were randomized into a placebo-controlled trial for assessing the pharmacokinetics and pharmacodynamics of a beverage enriched with glucoraphanin and sulforaphane from broccoli sprouts. Study participants were recruited from the villages of Qing Jia, Ji Zi, and Jiang Lou in the rural farming community of He-He Township, Qidong, Jiangsu Province, China. A total of 1,205 individuals were screened at local clinics over 6 days in September 2011. Written informed consents were obtained from all participants. The protocol was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health (Baltimore, MD), the University of Pittsburgh (Pittsburgh, PA), the University of Minnesota (Minneapolis, MN), and the Qidong Liver Cancer Institute and registered with ClinicalTrials.gov (NCT 01437501). A medical history, physical examination, and routine hepatic and renal function tests were used to screen the individuals, aged 21 to 65 years, by methods identical to those described for our previous interventions in this region (10, 22). Nearly half (539) of the individuals from the screened group were eligible, of which the initial 300 were randomized using a fixed randomization scheme with a block size of 10. A total of 291 of these selected participants returned to the clinics on the first day of the study where they provided informed consents for the intervention study and were given their identification code. Overall, there were 62 men (21%) and 229 women (79%) with a median age of 53 (range, 21–65) years. Although this was a tightly controlled dietary intervention, participants were under no dietary restrictions throughout the trial.

The trial was conducted from mid-October 2011 to early January 2012. Participants consumed a placebo beverage or a broccoli sprout beverage for 84 consecutive days (12 weeks). Participants met local doctors and study investigators at 1 of 10 designated local sites between 16:30 and 18:00 each evening for distribution of the intervention beverages. Compliance was determined by visual observation of consumption and measures of urinary excretion of sulforaphane metabolites (see below). Placebo and broccoli sprout beverages were prepared fresh each afternoon from bulk powders and brought to He-He daily for distribution. To control pH, ascorbic acid was added to urine collection containers shortly before distribution to participants, and complete overnight and daytime (about 12 hour each) urine samples were collected following consumption of the beverage on days 1, 7, 14, 28, 42, 56, 70, and 84. In addition, a 12-hour overnight urine was collected on the day before

consuming the first beverage (day 0). Once collected, urine volumes were measured, and aliquots prepared and transported to the Qidong Liver Cancer Institute for immediate storage at  $-20^{\circ}\text{C}$ . Blood samples were collected on days 0, 28, 56, and 84 of the study. Serum alanine aminotransferase activities were determined on all collected samples. Aliquots of urine and serum from each sample were shipped frozen to Baltimore at the end of the study, and serum samples were transferred immediately to a clinical laboratory (Hagerstown Medical Laboratory, Hagerstown, MD) for comprehensive blood chemistry analyses.

### Preparation of the broccoli sprouts beverages

The study was conducted using rehydrated, previously lyophilized broccoli sprout powders rich in either glucoraphanin or sulforaphane that were produced by the Cullman Chemoprotection Center at Department of Pharmacology, Johns Hopkins University School of Medicine, for clinical study use as an Investigational New Drug. Broccoli sprouts were grown from specially selected BroccoSprouts seeds (cv. DM1999B) with technology licensed from Johns Hopkins University. Briefly, seeds were surface-disinfected and grown in a commercial sprouting facility under controlled light and moisture conditions. After 3 days of sprout growth, an aqueous extract was prepared in a steam-jacketed kettle at a GMP food processing facility (Oregon Freeze Dry). Sprouts were plunged into boiling deionized water and allowed to boil for 30 minutes. The resulting aqueous extract contained about 5 mmol/L glucoraphanin, the biogenic precursor of sulforaphane.

A glucoraphanin-rich powder was prepared by filtering and lyophilizing this aqueous extract at Oregon Freeze Dry. Total glucoraphanin titer was determined in the resulting powder by high-performance liquid chromatography (HPLC; ref. 23) to be  $329\ \mu\text{mol/g}$  powder when assayed just before use in the clinical study. To prepare our sulforaphane-rich powder, the aqueous extract was filtered, cooled to  $37^{\circ}\text{C}$ , and treated with myrosinase, an enzyme released from a small amount of daikon (*Raphanus sativus*) sprouts, for 4 hours to hydrolyze the glucosinolates to isothiocyanates. Total isothiocyanate and sulforaphane levels were then quantified by cyclocondensation analysis (24) and by direct HPLC (25), respectively. This hydrolyzed aqueous extract was also lyophilized at Oregon Freeze Dry. Sulforaphane content at time of use was  $202\ \mu\text{mol/g}$  powder and represented 91% of the total isothiocyanate content in the powder.

The bulk powders were tested for microbial contaminants before release by Oregon Freeze Dry and again upon receipt in Baltimore (IEH-JL Analytical Services and Eurofins Strasburger and Siegel), heavy metals (Elemental Analysis, Inc.), and benzene (TestAmerica). Following air shipment to China, both powder preparations were stored in sealed bags in a locked, dedicated  $-20^{\circ}\text{C}$  freezer until reconstitution of the study beverages.

To prepare 150 daily doses, allotments of each powder (360 g glucoraphanin-rich and 24.8 g sulforaphane-rich

powders) were dissolved in sterile water. An equal volume of pineapple juice (Dole) was added along with lime juice (Safeway) in a final ratio of 47:47:6 water:pineapple juice:lime juice (by volume) with vigorous mixing before transfer of 100-mL individual doses into sterile 330-mL commercial bottled water bottles for daily distribution to study participants. The individual daily dose was 600  $\mu\text{mol}$  of glucoraphanin and 40  $\mu\text{mol}$  of sulforaphane. The placebo beverage contained the same liquid components, to which 1% molasses v/v was added to provide color masking.

### Quality control of beverages

The juices served to mask odor and taste but had no effect on the stability of the phytochemicals and contributed minimal enzyme inducer activity to the beverage. Extra beverages prepared at early, middle, and late time points during the trial were stored at  $-20^{\circ}\text{C}$  and returned to Baltimore for analyses of glucoraphanin and sulforaphane content as well as enzyme inducer activity. NAD(P)H:quinone acceptor oxidoreductase inducer activity in the beverages, measured by the Prochaska assay (26), confirmed  $40 \pm 1.4\ \mu\text{mol}$  of sulforaphane equivalents (mean  $\pm$  SD) in the broccoli beverage in the absence of treatment with myrosinase and  $635 \pm 100\ \mu\text{mol}$  of equivalents following incubation with myrosinase, per 100 mL. Direct analyses of glucoraphanin and sulforaphane content in the broccoli beverage (23, 25) indicated striking concordance with the bioassay results:  $614 \pm 15\ \mu\text{mol}$  of glucoraphanin and  $40.5 \pm 0.8\ \mu\text{mol}$  of sulforaphane. Equivalent measures were seen across the frozen samples saved from the early, middle, and late time point preparations. No glucoraphanin or sulforaphane was detected in the placebo beverage. Negligible basal inducer activity was detected ( $0.87 \pm 0.24\ \mu\text{mol}$  of sulforaphane equivalents) in the placebo beverage.

### Air pollution biomarkers

Data for the  $\text{PM}_{10}$  levels in Qidong during the study period were provided by the Qidong Environmental Monitoring Station. Values for  $\text{PM}_{10}$  in Shanghai were obtained from the Shanghai Environmental Monitoring Center, Shanghai Environmental Protection Bureau. All mercapturic acids were quantified by isotope dilution mass spectrometry as described previously (13, 27). Urinary creatinine was assayed by the Hagerstown Medical Laboratory.

### Glucoraphanin and sulforaphane in urine

Measurement of glucoraphanin and sulforaphane metabolites in urine was performed by isotope dilution mass spectrometric assay as previously reported by Egner and colleagues (28). Positive electrospray ionization tandem mass spectrometry (ESI-MS/MS) was carried out using a Thermo-Finnigan TSQ Advantage triple quadrupole mass spectrometer coupled to a Thermo-Finnigan Accela UPLC and HTC Pal autoinjector (ThermoElectron Corporation). Chromatographic separation of analytes was achieved using a  $1.9\text{-}\mu\text{m}$   $100 \times 1\ \text{mm}^2$  Thermo Hypersil Gold column maintained at  $40^{\circ}\text{C}$ .

### Genotyping and SNP analyses

gDNA was isolated from serum with a Qiagen QIAamp Mini Blood isolation kit. *GSTM1* and *GSTT1* genotypes were identified by real-time PCR as described previously (21). The primer and probe sequences for the NRF2 rSNP-617 were as follows: forward primer sequence CAGTG-GCCCTGCCTAG; reverse primer sequence TCAGGT-GACTGCGAACAC; reporter 1 dye\_VIC TGGACAGCGC-CGGCAG; reporter 2 dye\_FAM TGTGGACAGCTCCGG-CAG (Applied Biosystems).

### Statistical analyses

The analyses comprised 4 components: (i) a comparison of levels of air pollutant biomarkers by treatment arm at baseline, before the administration of the broccoli sprout beverage (i.e., day 0), (ii) a comparison of the persistent effects (days 1 through 84) of the beverage on air pollutant excretion in urine, (iii) a comparison of air pollutant excretion by genotype, and (iv) a description of sulforaphane metabolites excreted in urine at the individual level.

For the baseline comparison of the treatment and placebo arms, a 2-sample *t* test of geometric means for each biomarker was conducted. To describe the acute and persistent effects of treatment, separate log linear mixed-effects (random intercepts and slopes) models for each biomarker were fit. In this setting, the independent variables were treatment assignment (placebo as reference), time in weeks (from day 1 to day 84), and the interaction between treatment assignment and time. Specifically, the model was of the form:

$$\begin{aligned} \log(\text{biomarker}) = & (\alpha_0 + \alpha_1 \times \text{treatment} + a) \\ & + (\beta_0 + \beta_1 \times \text{treatment} + b) \\ & \times \text{time in weeks} + e \end{aligned}$$

where *a* and *b* follow a bivariate normal distribution with means equal to 0, variance components corresponding to the between-individuals differences in intercept (level at day 1) and slopes (change per week) and are statistically independent of the residuals (*e*) which also follow a normal distribution, whose variance corresponds to the within-individual variability of the biomarkers across visits. The parameter  $\alpha_0$  is interpreted as the average biomarker level for the placebo group at day 1 and  $\alpha_1$  describes the difference in biomarker level due to treatment at day 1 (acute effect). The parameter  $\beta_0$  is the average change in biomarker level per each week for the placebo group and  $\beta_1$  is the effect of treatment on this slope (persistent effect).

For benzene, a portion (14%) of urine samples was below the limit of detection (i.e., <0.125 pmol/mL). Because these observations were standardized to heterogeneous urine creatinine concentrations (mg/mL), the resulting left-censored values were also heterogeneous. To appropriately incorporate the left-censored observations to the mixed-effects models, we programmed the maximum likelihood method using the flexible procedure NLMIXED in SAS. The contribution of left-censored values to the maximum likelihood function was determined by the cumulative-distribution function, whereas the contribution of non-

left-censored values was determined by the probability-density function.

For comparing excretion by genotype, the geometric means and interquartile ranges (IQR) of each air pollutant were calculated by day for each genotype-treatment strata and displayed graphically. As a summary of overall levels, the geometric means were calculated within each individual from days 1 through 84. The Wilcoxon rank-sum test compared these individual levels by genotype class.

Sulforaphane pharmacokinetics was described by fitting individual linear regressions of the excreted sulforaphane conjugates in the log scale. The slopes from these regressions describe the average change per week. The sign rank test compared whether the average change per week was significantly different than 0% (28). Statistical significance was assessed at the  $\alpha = 0.05$  level. All analyses were conducted in SAS 9.2 (SAS Institute).

## Results

### Compliance, data collection completeness, and tolerability

As indicated in Fig. 1, 300 individuals were randomized into the 2 intervention arms; 9 declined to participate shortly thereafter. The intervention groups did not differ significantly ( $P > 0.05$ ) by gender, age, or body mass index (Table 1). Of the 24 dropouts, 13 were assigned to the placebo arm whereas 11 were assigned to the broccoli sprout beverage arm. Of 291 participants, 267 (92%) completed the trial: 53% drank every beverage whereas the rest consumed at least 80 of 84 assigned beverages. Ten grade 1

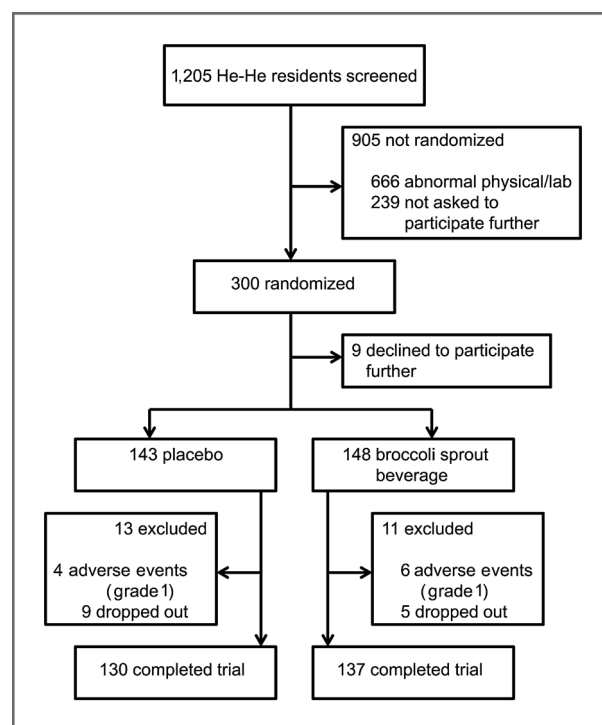


Figure 1. Intervention trial profile.



**Table 1.** Demographic distribution [% (*n*) or median (IQR)] of screened population and enrolled participants by treatment

Variable	Screened population ( <i>n</i> = 1,205)	Treatment group	
		Placebo ( <i>n</i> = 143)	Broccoli sprout ( <i>n</i> = 148)
Female	74% (889)	78% (111)	80% (118)
Age, y	54 (48–59)	53 (48–59)	52 (46–58)
Body mass index	23.9 (21.8–26.2)	23.8 (21.9–25.8)	23.4 (21.3–25.1)
Among women	23.9 (22.0–26.1)	23.8 (22.1–25.4)	23.6 (21.8–25.1)
Among men	23.8 (21.6–26.5)	23.6 (21.5–26.7)	22.5 (20.3–25.3)
Current smoker	12% (146)	13% (18)	9% (14)
Among women	0% (0)	0% (0)	0% (0)
Among men	46% (146)	56% (18)	47% (14)

adverse events were reported; all occurred in the first week of the trial and were distributed as 4 (2.8%) participants drinking the placebo and 6 (4.1%) the broccoli sprout beverage. Unacceptable taste and mild stomach discomfort were the common complaints. One individual, assigned to the broccoli beverage, reported mild vomiting. Most of the remaining 14 dropouts left the study because of the inconvenience of meeting on a daily basis for supervised beverage consumption. The tolerability of the broccoli beverage was vastly improved because of the water:pineapple juice:lime juice formulation recommended by Sensory Spectrum (29) compared with earlier trials in which broccoli sprout extracts were delivered in water (21) or a 50:50 water:mango juice mixture (10). Furthermore, of the 267 participants who completed the trial, only 18 of 4,539 possible urine samples were not collected (0.3%); 99.8% of the blood samples were collected. There were no abnormal clinical chemistry values for blood samples collected on the last day of the intervention.

#### Levels of air pollutant biomarkers at baseline

Levels of SPMA, 3-HPMA, and HMPMA were measured in all study participants in the 12-hour overnight urine samples collected on the morning before consumption of the first beverage. These analytes serve as biomarkers of internal dose from ambient exposures to these pollutants. Because

the 3 biomarkers exhibited strong skewness (7.2, 6.9, and 6.4 for SPMA, 3-HPMA, and HMPMA, respectively), all analyses were performed on the log-transformed scale, which reduced the skewness to  $-0.2$ ,  $0.7$ , and  $1.4$ , respectively. Table 2 presents the geometric means and IQR for these day 0 values segregated by treatment arm assignment. There were no significant differences in biomarker levels in the participants upon entry into the placebo and broccoli sprout beverage arms of the trial.

#### Effects of broccoli sprout beverage on air pollutant biomarkers

No routine monitoring of airborne concentrations of volatile organic chemicals is conducted in China and was not undertaken as part of this study. However, daily tracking of the concentration of PM<sub>10</sub> was recorded for many Chinese cities, including Shanghai and Qidong, at the time of the study. Presented in Fig. 2A are the daily, 24-hour averaged concentrations of PM<sub>10</sub> recorded in central Shanghai and in Qidong during the study period. The means of 2 Qidong monitoring sites, each within 0.5 km of the Qidong Liver Cancer Institute, are presented. The excellent concordance in the daily fluctuations over the 84-day period between the Shanghai and Qidong sites highlights the regional nature of the pollution in the Yangtze River delta area. Exposures were consistently but moderately higher in

**Table 2.** Geometric mean levels (IQR) of benzene, acrolein, and crotonaldehyde mercapturic acids at day 0 by treatment assignment

Mercapturic acids <sup>a</sup>	Placebo ( <i>n</i> = 143)	Broccoli sprout ( <i>n</i> = 148)	<i>P</i>
Carcinogen			
Benzene (SPMA)	0.709 (0.326–1.542)	0.745 (0.395–1.407)	0.885
Irritants			
Acrolein (3-HPMA)	3,361 (1,686–5,486)	3,569 (1,703–6,186)	0.779
Crotonaldehyde (HMPMA)	1,510 (880–1,959)	1,312 (829–1,790)	0.112

<sup>a</sup>pmol/mg creatinine.

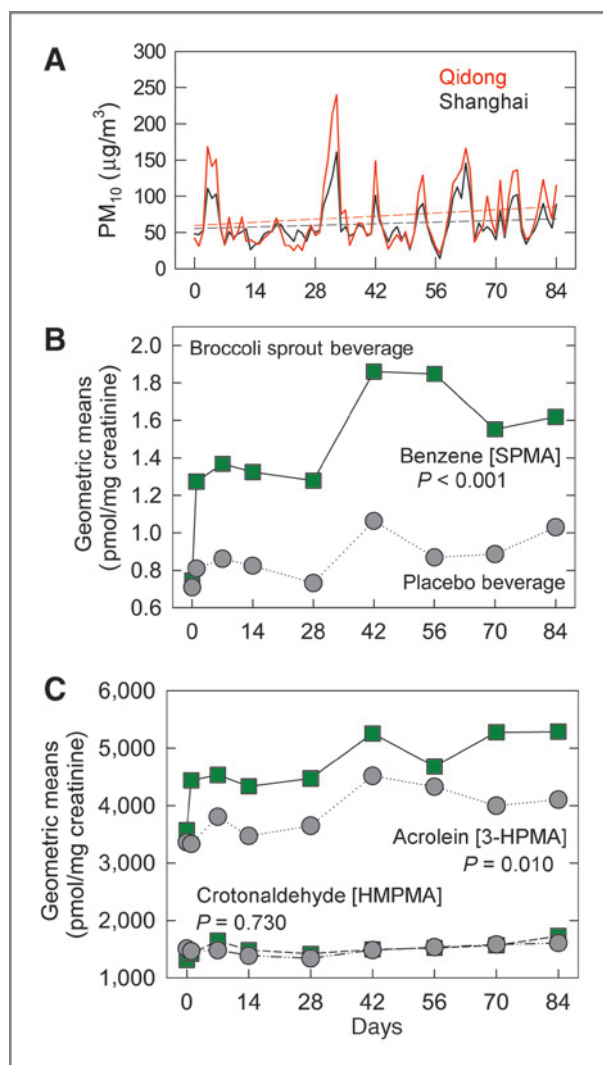


Figure 2. Geometric means for biomarker levels on days 0, 1, 7, 14, 28, 42, 56, 70, and 84 of the intervention. A, daily average levels for PM<sub>10</sub> in Shanghai (black) and Qidong (red) during the study period. B, urinary benzene mercapturic acid levels. C, urinary acrolein and crotonaldehyde mercapturic acid levels. (■) Broccoli sprout beverage arm; (●), placebo beverage arm. The geometric means for benzene appropriately accounted for left-censoring using the flexible PROC NL MIXED command in SAS 9.2.

Qidong than in Shanghai. Moreover, the rates of seasonal increase in PM<sub>10</sub> levels were consistent (+2.0%/week) between the monitoring sites.

Isotope dilution mass spectrometry was used to quantify the urinary excretion of the mercapturic acids of benzene, acrolein, and crotonaldehyde from the 8 overnight 12-hour urine samples collected from each participant over the 12-week intervention period. On the basis of the mixed model, the estimated SPMA excretion for the placebo group was 0.818 pmol/mg creatinine [95% confidence interval (CI), 0.725–0.922] at day 1. As shown in Fig. 2B, those receiving broccoli sprout beverage had 60.6% higher excretion (95% CI, +35.8% to +89.8%;  $P < 0.001$ ) at day 1, and this effect

persisted over time. The average change per week for the placebo arm was +1.7% (95% CI, +0.7% to +2.6%) and it was similar ( $P = 0.204$ ) to the average change per week for the broccoli sprout beverage (+2.5%; 95% CI, +1.6% to +3.5%). Similarly, as shown in Fig. 2C, the broccoli sprout beverage group had a +22.7% higher urinary excretion of 3-HPMA (95% CI, +5.0% to +43.4%;  $P = 0.010$ ) at day 1 than the placebo group, whose average level was 3548 pmol/mg creatinine (95% CI, 3173–3968). This significantly higher level persisted over time, whereas each group had modest increase of 1.7% per week ( $P$  value for difference in change per week between arms = 0.877). Finally, the urinary excretion of HMPMA for the placebo arm at day 1 was 1,412 pmol/mg creatinine and the broccoli sprout beverage was practically identical ( $P = 0.531$ ).

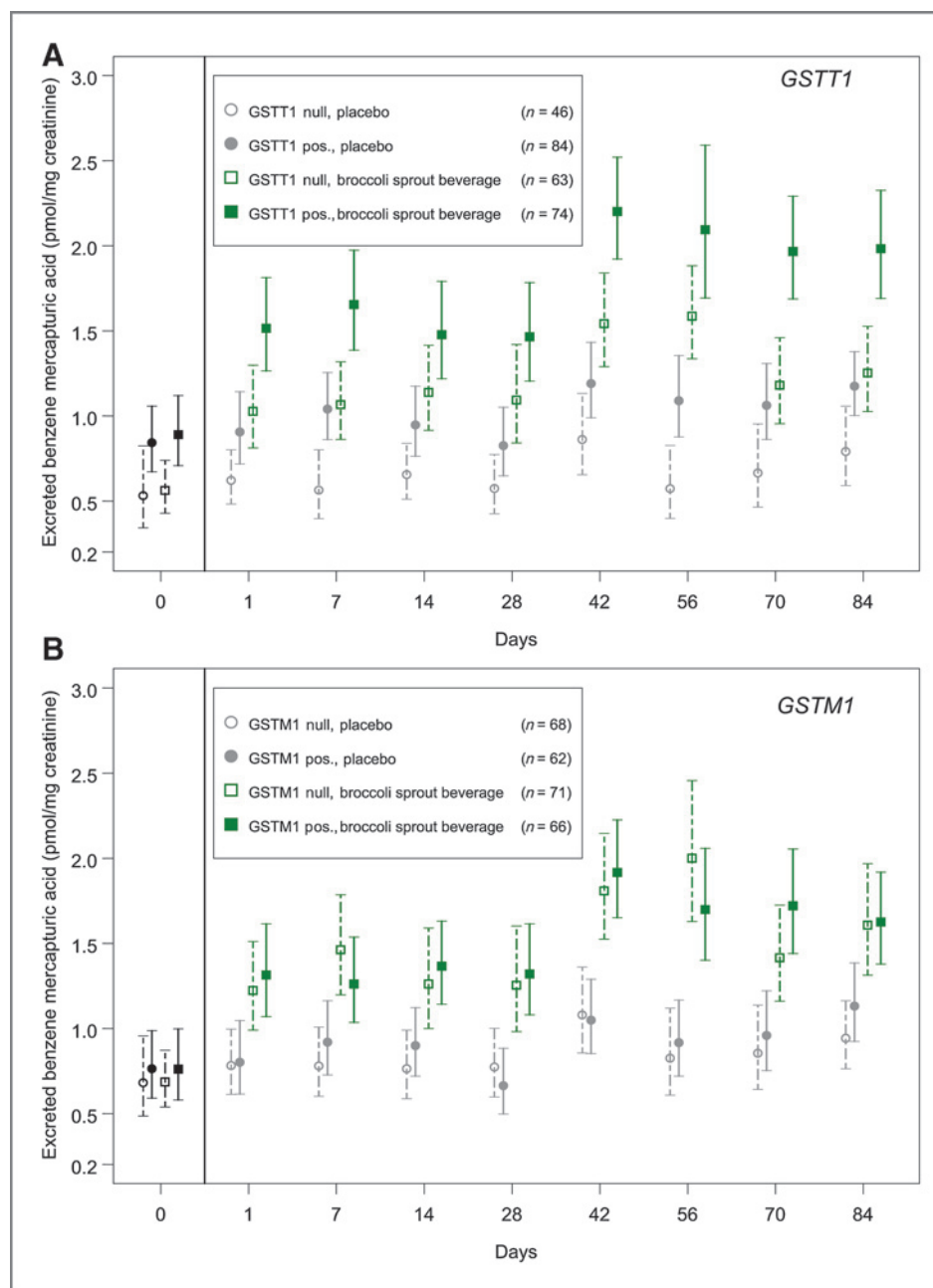
Restriction of the analyses to the 211 women who completed the trial, all of whom were nonsmokers, yielded the same results as seen with all participants. In this subgroup analysis, the increases in the excretion of the mercapturic acids of benzene, acrolein, and crotonaldehyde for the broccoli sprout versus placebo group were +54.7% (+27.2% to +88.1%), +21.7% (+1.8% to 45.5%), and +2.0% (–13.7% to +20.4%), respectively.

#### Effect of GST genotypes and Nrf2 rSNP-617 on biomarker levels

The absence of the *GSTT1* allele is known to diminish SPMA excretion in settings of occupational exposures (17); an effect of *GSTM1* is far less certain (16). The 267 participants who completed this study were genotyped for presence of these 2 GST alleles. The distributions of the null genotype for *GSTT1* (41.9%) and *GSTM1* (52.1%) were in accord with our earlier determinations in this population (10, 21). As shown in Fig. 3A, on day 0, there was a significant 59% elevation of SPMA excretion in those individuals positive for the *GSTT1* allele compared with those who were null ( $P < 0.01$ ). Within the placebo arm, there was a consistent >50% increase in SPMA excretion at each time point evaluated based on a positive *GSTT1* genotype. A similar differential effect of null and positive *GSTT1* genotype was seen in the treatment arm, but starting from a higher baseline value reflecting the intervention effect. Thus, although *GSTT1* genotype is an important modifier of benzene metabolism, the broccoli beverage-induced effects on increased excretion of SPMA appear to be independent of *GSTT1* status. In contrast, shown in Fig. 3B, on day 0 and throughout the trial, the presence or absence of the *GSTM1* alleles had no effect on rates of excretion of SPMA in the overnight voids (day 0;  $P = 0.501$ ). Moreover, the effect of treatment was evident at all time points, irrespective of *GSTM1* genotype.

There is a functional polymorphism in an antioxidant response element-like sequence at –617 of the promoter of the transcription factor *NRF2*, in which A is substituted for C (30). Nrf2 is known to regulate the expression of genes, including GSTs, involved in the detoxication of toxicants and carcinogens (31). In an exploratory analysis, among the 267 participants completing the trial, 123 were

**Figure 3.** Effect of *GSTM1* and *GSTT1* genotypes on urinary excretion of benzene-mercapturic acid. Distributions of benzene-mercapturic acid levels (geometric means and 95% CIs) in participants either null or positive for the *GSTT1* gene (A) or the *GSTM1* gene (B) by assignment group (placebo or treated and day of study). Bars highlighted in green indicate those receiving the broccoli beverage. Open symbols represent geometric means for the participants null for the genotype and solid symbols indicate the geometric means of those who were positive.

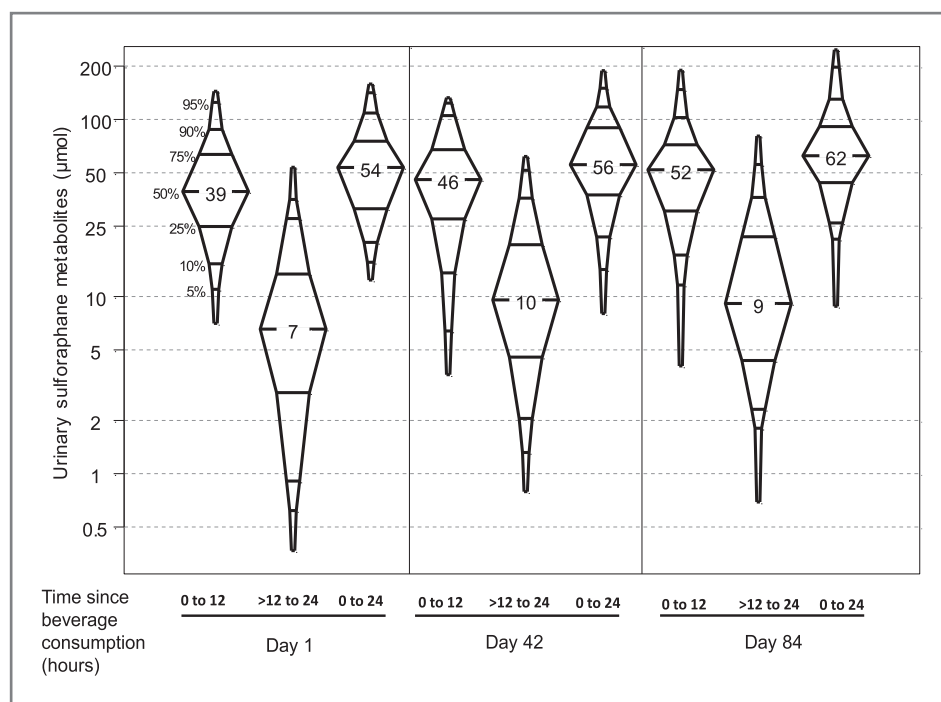


homozygous for the C allele (C/C), 115 heterozygous (C/A), and 29 A/A. There was no significant effect of this SNP (A/A or A/C vs. C/C) on the excretion of SPMA at baseline (day 0;  $P = 0.203$ ). Moreover, no change was seen in the placebo arm throughout the intervention period. The median (IQR) of individual geometric mean levels in pmol/mg creatinine for days 1 through 84 of the trial were 0.750 (0.461–1.140) for C/C and 0.860 (0.443–1.119) for C/A or A/A ( $P = 0.896$ ). However, a significant effect on benzene metabolism and excretion was seen following treatment with the broccoli beverage, indicating a potential, partial role for NRF2 in the actions of sulforaphane in this setting.

The median (IQR) levels in pmol/mg creatinine were 1.104 (0.797–1.519) for C/C and 1.352 (0.938–1.833) for C/A or A/A ( $P = 0.029$ ).

#### Sulforaphane pharmacokinetics

Isotope dilution mass spectrometry was used to measure the levels of glutathione-derived conjugates of sulforaphane excreted in the urine during consecutive 12-hour collections on days 1, 42, and 84, which are shown in Fig. 4. SF-*N*-acetylcysteine (80%–81%), SF-cysteine (12%–14%), and free sulforaphane (5–7%) are the major urinary metabolites; the other glutathione-derived



**Figure 4.** Urinary excretion of sulforaphane and its metabolites (SF-cysteine and SF-mercapturic acid) on days 1, 42, and 84 as measured by isotope dilution mass spectrometry in participants randomized to the broccoli sprout beverage arm.

conjugates SF-glutathione and SF-cysteinyl-glycine account for <1%, as seen previously (28). The distributions of individual urinary metabolites did not change over the course of the intervention. Even with the blended glucoraphanin and sulforaphane formulation designed to extend the biological half-life of each dose, the majority of the sulforaphane metabolites were excreted in the first 12 hours following administration of each dose, although the percentage of total 24-hour excretion increased from 72% to 82% to 84% on days 1 to 42 to 84. Median levels of the 24-hour excretion of sulforaphane metabolites increased from 54 to 56 to 62  $\mu\text{mol}$ . These amounts represent 8.4%, 8.8%, and 9.7% of the administered daily dose of sulforaphane (600  $\mu\text{mol}$  glucoraphanin + 40  $\mu\text{mol}$  sulforaphane). To summarize the within-subject changes in sulforaphane metabolites over time, regression lines were fit to each individual's data. The median of the 136 subject-specific slopes, expressed as percent change, was +10.1% (IQR: -13.7% to +43.9%). This average change was significantly greater than 0 (sign-rank:  $P = 0.01$ ). The intraclass correlation coefficient for the repeated measures of sulforaphane urinary metabolites was 0.35.

All individuals assigned to the placebo arm had urinary sulforaphane metabolite levels below 1  $\mu\text{mol}/\text{mg}$  creatinine with the exception of 6 individuals at day 42 and 4 individuals at day 84. The highest value amongst these individuals was 14  $\mu\text{mol}/\text{mg}$  creatinine per 24 hours. These excursions into the detectable range likely reflected the consumption of broccoli, which was being harvested from the local fields during the second half of the study, as participants were under no dietary restrictions throughout the trial.

## Discussion

The key finding from this clinical trial was the observed rapid and highly durable elevation of the detoxication of benzene, a known human carcinogen, and acrolein metabolites in the participants randomized to the blended glucoraphanin- and sulforaphane-rich broccoli beverage. In this regard, the study demonstrated the persistent effects over the course of 12 weeks, extending the findings first described in our a small, short-term crossover trial in which daily consumption of either a glucoraphanin- or sulforaphane-rich broccoli beverage enhanced the excretion of these analytes at a 7-day endpoint (10, 28). Selection of a proper dose is especially difficult with a food-based intervention. In the crossover trial, we reported that 104  $\mu\text{mol}$  of sulforaphane equivalents were excreted in the urine following the initial dose of sulforaphane (150  $\mu\text{mol}$ ) and 32  $\mu\text{mol}$  following the initial dose of glucoraphanin (800  $\mu\text{mol}$ ). Either intervention resulted in comparable increases in SPMA or acrolein-mercapturic acid excretion, suggesting that the dose-response effects were saturated. In the current trial, using a blend of 600  $\mu\text{mol}$  of glucoraphanin and 40  $\mu\text{mol}$  of sulforaphane, we observed a median excretion of 54  $\mu\text{mol}$  of sulforaphane equivalents over the 24 hours following the first dose (day 1). The magnitude of increased SPMA and acrolein-mercapturic acid excretion were nearly identical at the common time point of day 7 in the 2 studies. The dose levels used in these 2 studies effectively defined the maximum tolerated doses of glucoraphanin, sulforaphane, or the 2 combined. They reflect levels of intake beyond that typically associated with broccoli consumers. Thus, future efforts should evaluate the efficacy of lower doses. Formulations with more consistent bioavailability need to be



considered as well. In our initial 7-day crossover study, the conversion of the glucoraphanin following hydrolysis, absorption and conjugation with glutathione varied from 2% to 50% among study participants. Yield of sulforaphane equivalents in the current study ranged from 6.4% to 48.9%, buttressed at the lower end by the inclusion of some sulforaphane in the formulation. It is well established that sulforaphane-rich beverages provide higher and more consistent levels of sulforaphane than do glucoraphanin-rich beverages (28, 32). Conversion efficiency is likely determined by the composition of the gut microflora of individual participants (32). Interestingly, the bioavailability of the glucoraphanin contribution to systemic sulforaphane appeared to increase over the 84-day period, perhaps due to changes in the composition of the microflora.

The mechanisms underlying the actions of sulforaphane on benzene metabolism or its myriad of other protective effects are unclear. Nonetheless, activation of the NRF2 cytoprotective signaling pathway is a hallmark of sulforaphane mode of action (33, 34). In mice, disruption of *Nrf2* signaling obviates the cancer chemopreventive actions of sulforaphane (35). In this study, *GSTT1* genotype had a dramatic effect on rates of SPMA excretion, but this effect appeared to be independent of the broccoli beverage intervention. *GSTT1* is not known to be a transcriptional target of NRF2 in humans. The nature of the inducible factors contributing to the broccoli-enhanced detoxication of benzene is not resolved. Acrolein is principally conjugated with glutathione through the catalytic actions of GSTP1 (14). Allelic variants in human *GSTP1* are known to influence rates of conjugation of acrolein (15). In contrast, the specific activity of crotonaldehyde with human GSTs is minimal (14), perhaps explaining the absence of modulating effect by the broccoli sprout beverage on excretion of HMPMA.

Sulforaphane-rich broccoli sprout preparations can induce NRF2-regulated gene expression in the upper airway of human subjects (36) and attenuate nasal allergic response to diesel exhaust particles (37). In mice, disruption of *Nrf2* enhances susceptibility to airway inflammatory responses and DNA damage induced by diesel exhaust particles (38, 39). Moreover, the acute toxicity or carcinogenicity of many of the metals and organic molecules adsorbed onto air pollution particles has been shown individually to be exacerbated in *Nrf2*-disrupted mice (35, 40, 41). That NRF2 signaling may be the responsible target for sulforaphane is further buttressed by our finding that a functional polymorphism in the proximal promoter of the *NRF2* gene (−617) affects rates of SPMA excretion following treatment with the broccoli beverage.

Outdoor air pollution is associated with a wide range of adverse health outcomes, including cardiorespiratory mortality, chronic obstructive pulmonary disease, lung cancer along with increased rates of hospital admissions, and exacerbation of chronic respiratory conditions together with decreased lung function (1, 42, 43). The Global Burden of Disease Study of 2010 lists chronic obstructive pulmonary disease as the third leading cause of death in China (44). Two sources of particulate matter, ambient air and

indoor air, were listed only below dietary risk levels, high blood pressure, and tobacco smoke as the prime risk factors for disability-adjusted life years in China. Clearly, control of ambient and, as possible, indoor air pollution (45) must become public policy priorities. Indeed, significant improvements in air quality in the United States, in part, from public policy efforts to control air pollution, have been associated with improvements in life expectancy (46).

A population-based cancer registry has been in place in Qidong since 1972 and documents that the China age-standardized incidence rate for lung cancer has tripled in Qidongese men over the last 40 years, an increase consonant with a 5-fold increase in per capita tobacco sales in Qidong over that time (47). In Qidong, and indeed in many rural areas of China, a majority of men are smokers (~60%), whereas women are largely nonsmokers (<1%; ref. 47). Although smoking was not an exclusion criterion, none of the 211 women enrolled in our study were smokers. Given that there is a several decade lag between cigarette use and development of lung cancer, this increase in smoking from the 1950s into the 1980s and beyond likely drives much of the lung cancer in Qidongese men seen after 1972. There has been a doubling of the China age-standardized incidence rate of lung cancer in women in Qidong, beginning in 2000 (47). Unlikely to be associated with even second-hand smoking, the inflection in lung cancer in women begins a decade or more later than the emergence of economic development in the region. Whether this increase reflects increased exposures to outdoor or indoor air pollutants is not clear at this time, but it is certainly an escalating public health concern and a potential opportunity for evaluation of population-based approaches for chemoprevention.

#### Disclosure of Potential Conflicts of Interest

A. Muñoz is a consultant/advisory board member for Pfizer. No potential conflicts of interest were disclosed by the other authors

#### Authors' Contributions

**Concept and design:** P.A. Egner, J.-G. Chen, J.L. Johnson, J.D. Groopman, J.W. Fahey, P. Talalay, T.W. Kensler

**Development of methodology:** P.A. Egner, J.D. Groopman, J.W. Fahey

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** P.A. Egner, J.-G. Chen, A.T. Zarth, J.-B. Wang, K.H. Kensler, L.P. Jacobson, J.L. Johnson, J.W. Fahey, J. Zhu, T.-Y. Chen, G.-S. Qian, S.G. Carmella, S.S. Hecht, T.W. Kensler

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** J.-G. Chen, D.K. Ng, L.P. Jacobson, A. Muñoz, J.L. Johnson, J.D. Groopman, P. Talalay, J. Zhu, G.-S. Qian

**Writing, review, and/or revision of the manuscript:** P.A. Egner, J.-G. Chen, A.T. Zarth, D.K. Ng, K.H. Kensler, L.P. Jacobson, A. Muñoz, J.D. Groopman, J.W. Fahey, P. Talalay, T.W. Kensler

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** P.A. Egner, J.-G. Chen, D.K. Ng, J.-B. Wang, K.H. Kensler, L.P. Jacobson, T.-Y. Chen

**Study supervision:** P.A. Egner, J.-G. Chen, K.H. Kensler, T.-Y. Chen, T.W. Kensler

#### Acknowledgments

The authors thank the staff of the He-He Public Health Station and the He-He Medical Clinic, the village doctors, and the residents of He-He for their participation; Kristina Wade (Johns Hopkins University) for the quality control analyses; and the laboratory and clinical staff of the Qidong Liver Cancer Institute for their logistical support throughout the study. They also thank Safeway, Inc., for donating the lime juice used in this study.

## Grant Support

This work was supported by the NIH (P01 ES006052 and P30 ES003819 to J.D. Groopman).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked

advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 28, 2014; revised May 13, 2014; accepted May 27, 2014; published OnlineFirst June 9, 2014.

## References

- Loomis D, Groose Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa, et al. The carcinogenicity of outdoor air pollution. *Lancet Oncol* 2013;14:1262–3.
- Chen B, Hong C, Kan H. Exposures and health outcomes from outdoor air pollutants in China. *Toxicology* 2004;198:291–300.
- Lin J, Pan D, Davis SJ, Zhang Q, He K, Wang C, et al. China's international trade and air pollution in the United States. *Proc Natl Acad Sci U S A* 2014;111:1736–41.
- Zhou Y, Fu JS, Zhuang G, Levy JI. Risk-based prioritization among air pollution control strategies in the Yangtze River delta, China. *Environ Health Perspect* 2010;118:1204–10.
- Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002;287:1132–41.
- Laden F, Schwartz J, Speizer FE, Dockery DW. Reduction in fine particulate air pollution and mortality: extended follow-up of the Harvard Six Cities study. *Am J Respir Crit Care Med* 2006;176:667–72.
- Raaschou-Nielsen O, Andersen ZJ, Beeten R, Samoli E, Stafoggia M, Weinmayr G, et al. Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE). *Lancet Oncol* 2013;14:813–22.
- Yuan JM, Gao YT, Wang R, Chen M, Carmella SG, Hecht SS. Urinary levels of volatile organic and toxicant biomarkers in relation to lung cancer development in smokers. *Carcinogenesis* 2012;33:804–9.
- Yuan JM, Butler LM, Gao YT, Murphy SE, Carmella SG, Wang R, et al. Urinary metabolites of a polycyclic aromatic hydrocarbon and volatile organic compounds in relation to lung cancer development in lifelong never smokers in the Shanghai Cohort Study. *Carcinogenesis* 2014;35:339–45.
- Kensler TW, Ng D, Carmella SG, Chen M, Jacobson LP, Muñoz A, et al. Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. *Carcinogenesis* 2012;33:101–7.
- Hecht SS, Seow A, Wang M, Wang R, Meng L, Koh WP, et al. Elevated levels of volatile organic carcinogen and toxicant biomarkers in Chinese women who regularly cook at home. *Cancer Epidemiol Biomarkers Prev* 2010;19:1185–92.
- HEI Air Toxics Review Panel. Mobile-source air toxics: a critical review of the literature on exposure and health effects. HEI Special Report 16. Boston, MA: Health Effects Institute; 2007.
- Carmella SG, Chen M, Han S, Briggs A, Jensen J, Hatsukami DK, et al. Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. *Chem Res Toxicol* 2009;22:734–41.
- Berhane K, Widersten M, Engström A, Kozarich JH, Mannervik B. Detoxication of base propanals and other  $\alpha,\beta$ -unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. *Proc Natl Acad Sci U S A* 1994;91:1480–4.
- Pal A, Hu X, Zimniak P, Singh SV. Catalytic efficiencies of allelic variants of human glutathione S-transferase Pi in the glutathione conjugation of  $\alpha,\beta$ -unsaturated aldehydes. *Cancer Lett* 2000;154:39–43.
- Kim S, Lan Q, Waideyanatha S, Chanock S, Johnson BA, Vermeulen R, et al. Genetic polymorphisms and benzene metabolism in humans exposed to a wide range of air concentrations. *Pharmacogenet Genomics* 2007;17:789–801.
- Qu Q, Shore R, Li G, Su L, Jin X, Melikian AA, et al. Biomarkers of benzene: urinary metabolites in relation to individual genotype and personal exposure. *Chem Biol Interact* 2005;153–154:85–95.
- Zhang Y, Talalay P, Cho CG, Posner GH. A major inducer of anticarcinogenic enzymes from broccoli: isolation and elucidation of structure. *Proc Natl Acad Sci U S A* 1992;89:2399–403.
- Zhang Y, Kensler TW, Cho C-G, Posner GH, Talalay P. Anticarcinogenic activity of sulforaphane and structurally related synthetic norbornanyl isothiocyanates. *Proc Natl Acad Sci U S A* 1994;91:3147–50.
- Shapiro TA, Fahey JW, Dinkova-Kostova AT, Holtzclaw WD, Stephenson KK, Wade KL, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. *Nutr Cancer* 2006;55:53–62.
- Kensler TW, Chen J-G, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo Township, Qidong, PRC. *Cancer Epidemiol Biomarkers Prev* 2005;14:2605–13.
- Jacobson LP, Zhang B-C, Zhu Y-R, Wang J-B, Wu Y, Zhang Q-N, et al. Oltipraz chemoprevention trial in Qidong, People's Republic of China: study design and clinical outcomes. *Cancer Epidemiol Biomarkers Prev* 1997;6:257–65.
- Wade KL, Garrard IJ, Fahey JW. Improved hydrophilic interaction chromatography method for the identification and quantification of glucosinolates. *J Chrom A* 2007;1154:469–72.
- Ye L, Dinkova-Kostova AT, Wade KL, Zhang Y, Shapiro TA, Talalay P. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. *Clin Chim Acta* 2002;316:43–53.
- Tang L, Zhang Y, Jobson HE, Li J, Stephenson KK, Wade KL, et al. Potent activation of mitochondria-mediated apoptosis and arrest in S and M phases of cancer cells by a broccoli sprout extract. *Mol Cancer Ther* 2006;5:935–44.
- Fahey JW, Dinkova-Kostova AT, Talalay P. The "Prochaska" microtiter plate bioassay for inducers of NQO1. *Methods Enzymol* 2004;382:243–58.
- Carmella SG, Chen M, Zarth A, Hecht SS. High throughput liquid chromatography-tandem mass spectrometry assay for mercapturic acids of acrolein and crotonaldehyde in cigarette smokers' urine. *J Chromatog B* 2013;936:36–40.
- Egner PA, Chen J-G, Wang JB, Wu Y, Sun Y, Lu JH, et al. Bioavailability of sulforaphane from two broccoli sprout beverages: results of a short-term cross-over clinical trial in Qidong, China. *Cancer Prev Res* 2011;4:384–95.
- Civille GV, Oftedal KN. Sensory evaluation techniques – make "good for you" taste "good". *Physiol Behav* 2012;107:598–605.
- Marzec JM, Christie JD, Reddy SP, Jedlicka AE, Vuong H, Lanken PN, et al. Functional polymorphisms in the transcription factor NRF2 in humans increase the risk of acute lung injury. *FASEB J* 2007;21:2237–46.
- Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Ann Rev Pharmacol Toxicol* 2007;47:89–116.
- Fahey JW, Wehage SL, Holtzclaw WD, Kensler TW, Egner PA, Shapiro TA, et al. Protection of humans by plant glucosinolates: efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora. *Cancer Prev Res* 2012;5:603–11.
- Zhang Y. The molecular basis that unifies the metabolism, cellular uptake and chemopreventive activities of dietary isothiocyanates. *Carcinogenesis* 2012;33:2–9.
- Kensler TW, Egner PA, Agyeman AS, Visvanathan K, Groopman JD, Chen JG, et al. Keap1-Nrf2 signaling: a target for cancer prevention by sulforaphane. *Top Curr Chem* 2013;329:163–78.
- Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. *Proc Natl Acad Sci U S A* 2002;99:7610–5.

36. Riedl MA, Saxon A, Diaz-Sanchez D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. *Clin Immunol* 2009;130:244–51.
37. Heber D, Li Z, Garcia-Lloret M, Wong AM, Lee TY, Thames G, et al. Sulforaphane-rich broccoli sprout extract attenuates nasal allergic response to diesel exhaust particles. *Food Funct* 2014;5:35–41.
38. Li YJ, Takizawa H, Azuma A, Kohyama T, Yamauchi Y, Takahashi S, et al. Disruption of Nrf2 enhances susceptibility to airway inflammatory responses induced by low-dose diesel exhaust particles in mice. *Clin Immunol* 2008;128:366–73.
39. Aoki Y, Sato H, Nishimura N, Takahashi S, Itoh K, Yamamoto M. Accelerated DNA adduct formation in the lung of the Nrf2 knockout mouse exposed to diesel exhaust. *Toxicol Appl Pharmacol* 2001;173:154–60.
40. Toyama T, Shinkai Y, Yasutake A, Uchida K, Yamamoto M, Kumagai Y. Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury. *Environ Health Perspect* 2011;119:1117–22.
41. Wu KC, Liu JJ, Klaassen CD. Nrf2 activation prevents cadmium-induced acute liver injury. *Toxicol Appl Pharmacol* 2012;263:14–20.
42. Chen Y, Ebenstein A, Greenstone M, Li H. Evidence on the impact of sustained exposure to air pollution on life expectancy from China's Huai River policy. *Proc Natl Acad Sci U S A* 2013;110:12936–41.
43. Samet J, Krewski D. Health effects associated with exposure to ambient air pollution. *J Toxicol Environ Health* 2007;198:291–300.
44. Yang G, Wang Y, Zeng Y, Gao GF, Liang X, Zhou M, et al. Rapid health transition in China, 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet* 2013;381:1987–2009.
45. Zhou Y, Zou Y, Li X, Chen S, Zhao Z, He F, et al. Lung function and incidence of chronic obstructive pulmonary disease after improved cooking fuels and kitchen ventilation: a 9-year prospective cohort study. *PLoS Med* 2014;11:e1001621.
46. Pope CA III, Ezzati M, Dockery DW. Fine-particulate air pollution and life expectancy in the United States. *N Engl J Med* 2009;360:376–86.
47. Chen JG, Kensler TW. Changing rates for liver and lung cancers in Qidong, China. *Chem Res Toxicol* 2014;27:3–6.

# Cancer Prevention Research

## Rapid and Sustainable Detoxication of Airborne Pollutants by Broccoli Sprout Beverage: Results of a Randomized Clinical Trial in China

Patricia A. Egner, Jian-Guo Chen, Adam T. Zarth, et al.

*Cancer Prev Res* 2014;7:813-823. Published OnlineFirst June 9, 2014.

**Updated version** Access the most recent version of this article at:  
doi:[10.1158/1940-6207.CAPR-14-0103](https://doi.org/10.1158/1940-6207.CAPR-14-0103)

**Cited articles** This article cites 46 articles, 12 of which you can access for free at:  
<http://cancerpreventionresearch.aacrjournals.org/content/7/8/813.full#ref-list-1>

**Citing articles** This article has been cited by 5 HighWire-hosted articles. Access the articles at:  
<http://cancerpreventionresearch.aacrjournals.org/content/7/8/813.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cancerpreventionresearch.aacrjournals.org/content/7/8/813>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.