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Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China

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Epidemiological evidence has suggested that consumption of a diet rich in cruciferous vegetables reduces the risk of several types of cancers and chronic degenerative diseases. In particular, broccoli sprouts are a convenient and rich source of the glucosinolate, glucoraphanin, which can release the chemopreventive agent, sulforaphane, an inducer of glutathione *S*-transferases. Two broccoli sprout-derived beverages, one sulforaphane-rich (SFR) and the other glucoraphanin-rich (GRR), were evaluated for pharmacodynamic action in a crossover clinical trial design. Study participants were recruited from the farming community of He Zuo Township, Qidong, China, previously documented to have a high incidence of hepatocellular carcinoma with concomitant exposures to aflatoxin and more recently characterized with exposures to substantive levels of airborne pollutants. Fifty healthy participants were randomized into two treatment arms. The study protocol was as follows: a 5 days run-in period, a 7 days administration of beverage, a 5 days washout period and a 7 days administration of the opposite beverage. Urinary excretion of the mercapturic acids of acrolein, crotonaldehyde, ethylene oxide and benzene were measured both pre- and postinterventions using liquid chromatography tandem mass spectrometry. Statistically significant increases of 20–50% in the levels of excretion of glutathione-derived conjugates of acrolein, crotonaldehyde and benzene were seen in individuals receiving SFR, GRR or both compared with their preintervention baseline values. No significant differences were seen between the effects of SFR versus GRR. Intervention with broccoli sprouts may enhance detoxication of airborne pollutants and attenuate their associated health risks.

Introduction

Human populations are continuously exposed to varying amounts of chemicals or manufacturing by-products that are carcinogenic in animal models; over 100 such compounds have been designated as human carcinogens by the International Agency for Research on Cancer and the National Toxicology Program (1,2). Exposures to these exogenous agents occur through the environmental vectors of food, water and air. In many cases, the pathway to reducing cancer burden from these exposures is obvious—eliminate exposures. However, in some instances, exposures are largely unavoidable, such as exposures to aflatoxins and other mycotoxins in food or require substantial behavioral changes (e.g. smoking cessation) or economic investments (e.g. clean air in developing megacities) that are exceedingly difficult to implement in individuals or populations.

Chemoprevention offers reasonable prospects for disease risk reduction in these settings. Elucidation of the mechanisms of chemical carcinogenesis, in turn, provides insights into targets for chemoprevention. Environmental carcinogens typically undergo metabolic activation in target cells to form reactive electrophiles that damage DNA. Several completed clinical trials have attempted to reduce the burden of DNA damage imparted by environmental exposures to heterocyclic amines (3), tobacco smoke (4) and aflatoxins (5–7). The end points for these trials were short-term modulations of biomarkers of carcinogen metabolism and/or DNA adducts and other forms of damage. Modulation of these biomarkers is presumptive evidence for a cancer risk reduction, a concept well validated in animal models (8). Multiple strategies for modifying the bioactivation and/or detoxication of environmental carcinogens have been developed (9).

One means to alter the balance between bioactivation and detoxication is to induce the expression of glutathione *S*-transferases (GSTs), a multigene family of enzymes that facilitate the nucleophilic addition of glutathione to electrophilic centers in carcinogens, among many substrates (10). The importance of this detoxification pathway is highlighted by studies in cell culture in which overexpression of GSTs renders them resistant to the DNA-damaging actions of carcinogens, whereas disruption of GST expression in cells or mice renders them more sensitive (11,12). Pharmacological induction of GST expression leads to reduced DNA damage and substantive reduction in tumor incidence in animal models, as best exemplified by the effects of the chemopreventive agent oltipraz on aflatoxin metabolism, adduction and carcinogenesis (13). We have also shown in a randomized, placebo-controlled double-blind clinical trial in Qidong China, where risk for development of hepatocellular carcinoma is high in part from dietary exposures to aflatoxins, that treatment with oltipraz substantively elevated the rate of excretion of the mercapturic acid of aflatoxin (5). Mercapturic acids (*N*-acetylcysteine conjugates) are the water soluble, terminal products of glutathione conjugation typically excreted via urine.

Early studies by Sparnins *et al.* and Wattenberg (14,15) demonstrated that minor dietary consitutents were effective inhibitors of carcinogenesis. Moreover, Pantuck *et al.* (16) observed that feeding a diet enriched in Brussels sprouts- and cabbage-enhanced acetaminophen conjugation in healthy subjects relative to control diet. Therefore, in this study, we have examined the impact of a food-derived inducer of GSTs that exhibits strong chemopreventive action in animal models of chemical carcinogenesis: sulforaphane (17,18). Sulforaphane is a bioactive phytochemical originally isolated from broccoli (19). Earlier, this year, we reported a simple crossover design trial conducted with 50 participants to evaluate and compare the bioavailability of sulforaphane from broccoli sprouts in two forms: enterically generated from glucoraphanin by gut microflora or prereleased by treatment of the former preparation with myrosinase from the plant *Raphanus sativus* (20). Although the study was designed and powered to evaluate the pharmacokinetics of sulforaphane uptake and elimination, the crossover design in which each individual can serve as their own control allows us to probe for signals of pharmacodynamic action. The results reported here indicate that detoxication of toxic and carcinogenic airborne pollutants through increased elimination of mercapturic

Abbreviations: GRR, glucoraphanin-rich; GST, glutathione *S*-transferase; HBMA, crotonaldehyde [4-hydroxybut-2-yl mercapturic acid; HEMA, ethylene oxide [N-acetylcysteinyl]ethanol, also called 2-hydroxyethyl mercapturic acid; HPMA, acrolein [3-hydroxypropyl mercapturic acid; PAH, polycyclic aromatic hydrocarbon; PheT, r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; SFR, sulforaphane-rich; SPMA, *S*-phenylmercapturic acid.

acids is enhanced by consumption of broccoli sprout beverages rich in sulforaphane or its plant-derived precursor glucoraphanin. Selection of the pollutant biomarkers for this analysis was predicated on (i) their known metabolism to mercapturic acids via GSTs (21–23), (ii) availability of sensitive and selective analytical methods for quantitation under conditions of ambient environmental exposures (24), (iii) the omnipresent 'Asian Brown Cloud' that covers this region for substantial portions of the year (25) and (iv) earlier detection of biomarkers of the air pollutant phenanthrene in biospecimens obtained from nonsmoking residents of rural areas of Qidong that indicated exposure levels several fold higher than those found in urban smokers in the USA (7).

Materials and methods

Study design and participants

Adults in good general health without a history of major chronic illnesses were randomized into two intervention arms in a crossover trial design for comparing two forms of broccoli sprout extracts: glucoraphanin-rich (GRR) and sulforaphane-rich (SFR). Full details of the study design and pharmacokinetic measures have been published recently (20). Study participants were recruited from the villages of Yuan He and Wu Xing in the rural farming community of He Zuo Township, Qidong, Jiangsu Province, China in the fall of 2009. There were 17 men (34%) and 33 women (66%) with a median age of 46 (range 29–62) years enrolled in the study. Written informed consent was obtained from all participants. The protocol was approved by the Institutional Review Boards of the Johns Hopkins Medical Institutions, the University of Minnesota, and the Qidong Liver Cancer Institute and registered with ClinicalTrials.gov (NCT01008826).

As shown in Figure 1, participants consumed a 'placebo' beverage during a 5 days run-in before randomization to either a GRR or an SFR beverage for the next 7 days. After an additional 5 days washout period, participants received the alternate broccoli-derived beverage from their first allocation for a final 7 days. The study was conducted using lyophilized broccoli sprout powders rich in either glucoraphanin or sulforaphane that were produced by the Cullman Chemoprotection Center at Johns Hopkins University, School of Medicine, Department of Pharmacology and Molecular Sciences and were rehydrated in dilute mango juice just prior to dosing. Briefly, specially selected broccoli seeds were grown for 3 days, at a BroccoSprouts™ commercial green-sprouting facility, under license from Johns Hopkins University using controlled light and moisture conditions. All further processing was performed at a food processing facility (Oregon Freeze Dry, Albany, OR) by plunging them into boiling deionized water in a steam-jacketed kettle and boiling for 30 min. The resulting GRR aqueous extract was then lyophilized and the glucoraphanin titer of the resulting powder was determined by high-performance liquid chromatography (26) to be 329 μmol/g powder (14.3%) when assayed just prior to use in the clinical study. To prepare an SFR powder, the GRR aqueous extract was filtered, cooled to 37°C and treated with myrosinase, an enzyme released from a small amount of daikon (*Raphanus sativus*) sprouts, for 4 h in order to hydrolyze the glucosinolates to isothiocyanates and then lyophilized. Isothiocyanate and sulforaphane levels were quantified by cyclocondensation analysis (27) and by direct high-performance liquid chromatography (26,28), respectively. Sulforaphane content at time of use was 202 μmol/g powder (3.6%), which represented 91% of the total isothiocyanate content in the powder. The bulk powders were tested for microbial contaminants prior to release by Oregon Freeze Dry and again upon receipt in Baltimore. Both powder preparations were stored in sealed bags in a locked, dedicated −80°C freezer until reconstitution of the study beverages. Daily allotments of each powder were dissolved in sterile water such that resultant doses were 2.43 g of GRR powder or 0.743 g of SFR powder. An equal volume of mango juice (Del Monte; Lotte Chilsung Beverage Co., Seoul, Korea) was added with vigorous mixing prior to transfer of 100 ml individual doses into sterile 330 ml commercial bottled water bottles for daily distribution to study participants. The juice served to mask flavor and taste but had no effect on the stability of the phytochemicals; nor did the juice contribute any enzyme inducer activity to the beverage (data not shown). The daily doses were 800 μmol of glucoraphanin in GRR and 150 μmol of sulforaphane in SFR beverages. The mango-water beverage without addition of any powder was administered to participants as a placebo during the run-in and washout phases of the study. Participants were requested to refrain from eating cruciferous vegetables during the 24 days period of the study. Overnight (roughly 12 h), urine samples were collected by participants and delivered to study supervisors each morning in urine collection bottles containing ascorbic acid. Volumes were measured, and aliquots prepared and transported to the Qidong Liver Cancer Institute for initial storage at −20°C.

Biomarker analyses

Urinary mercapturic acid metabolites of the following airborne pollutants were quantified: for acrolein [3-hydroxypropyl mercapturic acid (HPMA)], for crotonaldehyde [4-hydroxybut-2-yl mercapturic acid (HBMA)], for benzene [*S*-phenyl mercapturic acid (SPMA)] and for ethylene oxide [*N*-acetylcysteinyl]ethanol, also called 2-hydroxyethyl mercapturic acid (HEMA)]. All mercapturic acids were quantified by isotope-dilution mass spectrometry as described previously (29). *r*-1,*t*-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) was measured by gas chromatography–mass spectrometry as described by Hecht *et al.* (30). Creatinine was assayed by the Hagerstown Medical Laboratory,

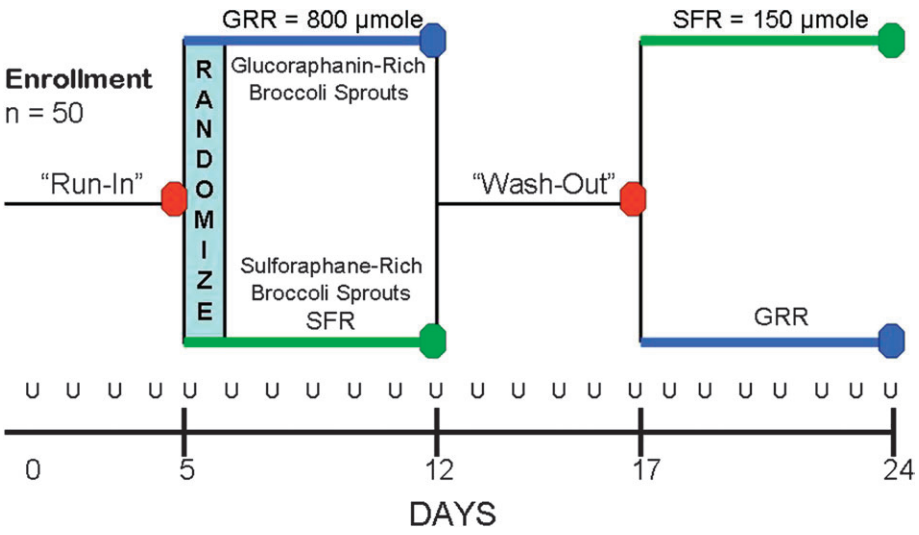

Fig. 1. Outline of the intervention protocol, schedule and timeline. Placebo beverages (dilute mango juice) were administered daily, shortly before dinner, for five consecutive days (run-in). Participants were randomized to receive either the GRR beverage containing 800 μmol glucoraphanin or the SFR beverage containing 150 μmol sulforaphane for seven consecutive days. The placebo beverage was then administered for five consecutive days (washout), at which time crossover assignments of the study beverages were consumed nightly for an additional 7 days. Overnight voids (0–12 h) were collected each morning on days 1 through 24. In addition, follow-up daytime voids (12–24 h) were collected on days 6, 12, 18 and 24. Symbols indicate the samples selected for determinations of the mercapturic acid biomarkers of airborne pollutants (days 5, 12, 17 and 24).

Hagerstown, MD. Limits of quantification were as follows (pmol/mg creatinine): HPMA 2, HBMA 0.2, SPMA 0.01, HEMA 0.2 and PheT 0.0001.

Statistical analysis

The primary outcomes of the analyses were levels of four urinary biomarkers: mercapturic acid conjugates of crotonaldehyde, acrolein, ethylene oxide and benzene, collected at Day 5 (end of run-in), Day 12 (end of first treatment phase), Day 17 (end of wash-out) and Day 24 (end of second treatment phase). The analysis consisted of describing and comparing: (i) the pretreatment biomarker levels (Day 5 and Day 17) and (ii) the posttreatment levels relative to the pretreatment levels. Medians and interquartile ranges were the primary summary statistics for biomarker levels. To determine the effectiveness of the run-in and wash-out phases, the within-individual biomarker levels at Day 5 and Day 17 were compared (i.e. H_o: pre-SFR level at Day 5/pre-GRR level at Day 17 = 1 and vice versa for the other treatment arm) as well as the pretreatment levels between treatment arms (i.e. H_o: pre-SFR level at Day 5 = pre-SFR level at Day 17 and the same for pre-GRR levels). The putative effect of treatment on biomarker levels was described by the within-individual ratio of posttreatment level to pretreatment level (i.e. posttreatment at Day 12/pretreatment at Day 5 and posttreatment at Day 24/pretreatment at Day 17). For each treatment (SFR or GRR), the null hypothesis was that there was no difference between posttreatment level and pretreatment level (i.e. H_o: the median ratio is equal to 1).

Non-parametric tests were used to detect significant differences in biomarker levels by run-in/washout and treatment phase at the $P < 0.05$ level. The Wilcoxon signed-rank test was used for within-individual comparisons (i.e. paired data), in which individuals serve as his or her own control. The rank-sum test was used for two sample comparisons (e.g. comparing pre-SFR biomarker levels on Day 5 versus Day 17).

Results

Levels of mercapturic acid biomarkers at baseline

Urinary levels of the mercapturic acids of acrolein, crotonaldehyde, ethylene oxide and benzene were measured in all study participants on Day 5 (the last day of the run-in phase) and on Day 17 (the last day of the washout phase). As noted in Figure 1, these samples were collected during the overnight period just prior to the initiation of the first and second waves of the crossover interventions with SFR and GRR.

Table I presents the medians, interquartile ranges and geometric means for pretreatment levels, where each individual contributes two urine samples while not receiving treatment (i.e. at Day 5 and Day 17). The geometric means combining smokers and non-smokers tended to slightly overestimate the medians indicating some extreme values in a positively skewed distribution. These values are largely due to 1.5- to 5-fold higher levels among smokers. The geometric means among non-smokers are presented for a comparison with previously reported values in a different non-smoking population [i.e. Singaporean women (29)]. Mercapturic acid levels of crotonaldehyde and acrolein in the Qidong non-smoking population were slightly higher than those in the Singaporean population while that of ethylene oxide was about the same. Levels of the benzene biomarker SPMA were 5-fold higher in the Qidong population.

Effects of GRR and SFR on excretion of mercapturic acid biomarkers

Biomarker levels prior to each treatment were compared at the within-individual level to determine whether the levels after the washout phase were different from levels at run-in (i.e. prior to treatment). The ratio of biomarker levels of Day 17 to Day 5 were calculated for each individual by treatment arm. There was no significant difference between biomarker levels at Day 17 compared with Day 5 in either treatment arm (signed rank $P > 0.10$ for each comparison; results available in Supplementary Table 1, *Carcinogenesis* Online). Since these levels were not different within individuals, the pre-SFR and pre-GRR biomarker levels between treatment arms were compared (e.g. Day 5 for the SFR → GRR treatment group versus Day 17 for the GRR → SFR treatment group). The pretreatment levels were not different between treatment arms (rank sum $P > 0.35$; results available in Supplementary Table 1, *Carcinogenesis* Online). This provided statistical justification to collapse the different arms by treatment since both arms began each treatment at about the same biomarker level.

The pre- and posttreatment biomarker levels are summarized in Table II. The distributions of posttreatment levels were higher than pretreatment levels, with the exception of ethylene oxide levels while on GRR. Figure 2 summarizes the distributions of within-individual change in biomarker levels as the ratio of posttreatment to pretreatment levels as percentile boxplots. In most instances, 20–50% increases in the levels of mercapturide excretion were observed comparing the post- to pretreatment measures. SFR treatment was significantly associated with elevated levels of HBMA and HPMA, the mercapturic acids of crotonaldehyde and acrolein and was marginally significantly associated with elevated levels of SPMA, the mercapturic acid of benzene ($P = 0.079$). GRR treatment was associated with elevated levels of HPMA and SPMA but not HBMA or HEMA. Although the median ratio of HEMA was not statistically different from 1, it was still elevated for both treatments. A comparison of treatments (i.e. SFR versus GRR) at the within-individual level showed no differences between treatments (results not shown).

As a secondary analysis of this dataset, we also compared the change in biomarker levels among smokers ($n = 9$) and non-smokers ($n = 40$). Prior to receiving treatment, smokers had significantly higher levels of HBMA, HPMA and HEMA, but not SPMA. Smokers had higher biomarker levels after SFR treatment, and the ratio of post- to pretreatment levels were higher than non-smokers, although this result was not statistically significant. Interestingly, the effect of GRR treatment was roughly similar between smokers and non-smokers. It is important to note that the study and analysis were not designed to determine how smoking modifies the effect of treatment on biomarker levels, and these results are presented as a glimpse toward potential future research.

Polycyclic aromatic hydrocarbon Biomarker PheT

Many polycyclic aromatic hydrocarbons (PAHs) are metabolically activated by the formation of diol epoxides that bind to DNA leading to mutations (31). Detoxication pathways including glutathione conjugation, glucuronidation, sulfation and phenol formation compete with metabolic activation (32). PheT levels have been reported to be higher in smokers than non-smokers indicating the urinary levels of the biomarker reflect exposure and metabolic processing.

Table I. Medians and interquartile ranges and geometric means for pretreatment biomarker levels of samples collected on Days 5 and 17 from 48 participants

Biomarker	Day 5 and 17 combined, median (IQR), $n = 96$	Day 5 and 17 combined, geometric mean, $n = 96$	Day 5 and 17 combined, geometric mean among non-smokers, $n = 78$ or (smokers $n = 18$)	Singapore population[a] (non-smoking), geometric mean, $N = 50$
Crotonaldehyde, nmol/mg creatinine	0.413, (0.300, 0.660)	0.512	0.410, (1.342)	0.142
Acrolein, nmol/mg	1.711, (1.362, 2.792)	2.221	1.713, (6.869)	1.370
Ethylene oxide, pmol/mg	25.08, (17.13, 49.67)	28.39	25.43, (45.60)	28.30
Benzene, pmol/mg	0.911, (0.657, 1.483)	1.034	0.975, (1.328)	0.180

Geometric means for urban, non-smoking Chinese women in Singapore (29) are provided for reference.
[a]Hecht *et al.* (29).

Table II. Median (IQR) for pretreatment and posttreatment biomarker levels, combined treatment arms ($n = 48$)

Biomarker	SFR treatment		GRR treatment	
	Pretreatment levels	Posttreatment levels	Pretreatment levels	Posttreatment levels
Crotonaldehyde, nmol/mg	0.413 (0.291, 0.642)	0.481 (0.319, 0.721)	0.408 (0.302, 0.730)	0.486 (0.312, 0.904)
Acrolein, nmol/mg	1.751 (1.363, 3.210)	2.317 (1.674, 3.651)	1.630 (1.334, 2.662)	2.288 (1.472, 3.956)
Ethylene oxide, pmol/mg	23.47 (15.96, 37.50)[a]	26.21 (16.01, 37.32)	29.90 (19.19, 56.71)	29.86 (23.05, 56.13)
Benzene, pmol/mg	0.884 (0.654, 1.284)	1.346 (0.730, 1.943)	1.003 (0.660, 1.881)	1.719 (1.035, 2.330)

IQR, interquartile range.
[a]For the pre-SFR level of ethylene oxide, the total $n = 49$.

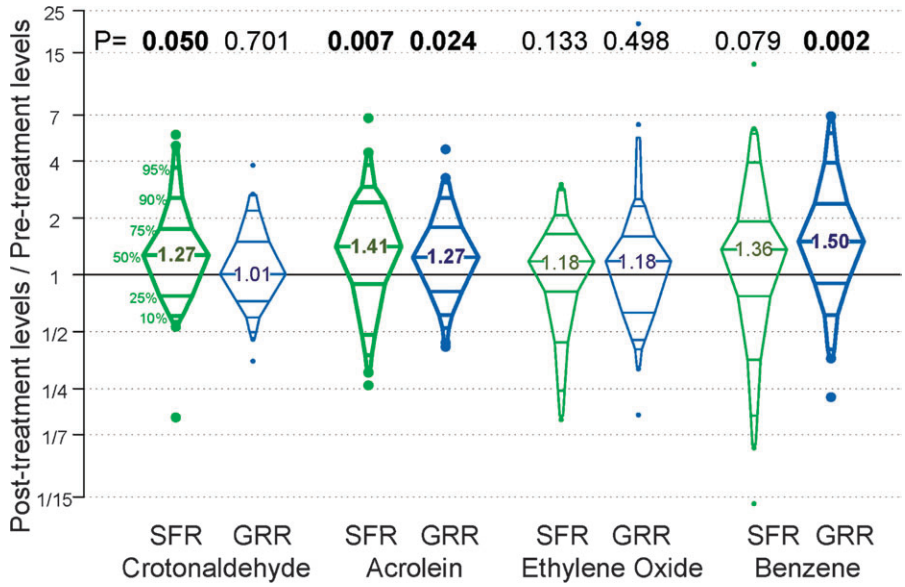

Fig. 2. Distributions of ratios of posttreatment/pretreatment biomarker levels by SFR and GRR treatment levels. Bold percentile boxplots indicate the median ratio was significantly different from 1 (signed-rank test, $P < 0.05$). One extreme observation was omitted from the graph (ethylene oxide, SFR treatment, ratio = 0.02) but was included in the analysis.

Phenanthrene is also a very abundant component of indoor air pollution resulting from biomass combustion during cooking and heating (33). In the present study, we find geometric mean levels of urinary PheT in non-smokers to be 6.5 pmol/mg creatinine during the washout and run-in periods. As shown in Table III, these levels are substantially higher than measured in non-smokers in the USA, marginally higher than measured in Singaporean non-smokers and considerably lower than those measured in non-smoking Shanghai residents, albeit in samples collected more than two decades earlier under conditions when industrial pollution was lower, but coal combustion for heating was commonplace.

The predicted impact of sulforaphane on PheT formation and disposition is uncertain, as this phytochemical could influence both oxidation and conjugation pathways (35,36). Levels of this biomarker were analyzed in an earlier clinical trial in which a broccoli sprout beverage containing 400 μmol of glucoraphanin per daily dose had no overall effect when compared with placebo (7). However, a secondary analysis indicated an inverse association between PheT levels and urinary metabolites of sulforaphane ($r = -0.39$; $P < 0.001$), suggesting that sulforaphane might enhance the conjugation elimination of PheT or its metabolic precursors. In contrast, in this study, a 16% ($P < 0.007$) increase in PheT was detected after a 7 days treatment with GRR (800 μmol), whereas a 30% ($P < 0.001$) increase resulted from treatment with SFR (150 μmol). Nonetheless, a non-significant inverse association between total urinary sulforaphane metabolites from GRR and PheT ($r = -0.169$; $P = 0.259$) was also observed here (data not shown). No association with PheT was seen in individuals receiving SFR ($r = -0.014$; $P = 0.929$), where sulforaphane metabolite excretion was considerably higher and interindividual variation lower. Given the lack of clarity of potentially altered metabolic pathways of phenanthrene metabolism, the utility of PheT as a marker of

Table III. PheT levels (geometric means) in urine from different study cohorts

Study (year)	PheT (pmol/mg creatinine)	Reference
Qidong (2009)		Current study
Non-smokers ($n = 40$)	6.5	
Smokers ($n = 9$)	10.1	
Qidong (2003)		Kensler *et al.* (7) [a]
Non-smokers ($n = 87$)	8.0	
Smokers ($n = 12$)	11.6	
Singapore (2009)		Hecht *et al.* (29)
Non-smokers ($n = 50$)	2.4	
Shanghai (1986–1989)		unpublished results[b]
Non-smokers ($n = 84$)	19.3	
Twin cities, USA (2000–2003)		Hecht *et al.* (30) [a]
Non-smokers ($n = 30$)	1.2	
Smokers ($n = 31$)	3.8	
Twin cities, USA (2003–2004)		Hecht *et al.* (34)
Non-smokers ($n = 10$)	1.4	
Smokers ($n = 12$)	3.7	

[a]Data presented in original publications are means.
[b]Yuan, J.M. and Hecht S.S., unpublished results.

pharmacodynamic action of chemopreventive agents such as broccoli sprouts should be considered cautiously.

Discussion

Levels of outdoor air pollution in China are among the highest in the world (37). Increases in fossil fuel use in China's industry, transport and residential sectors have resulted in a steep rise in emissions. The Yangtze River Delta region, which includes Qidong in eastern Jiangsu Province, is the fastest growing economic development area in China (38). This region, which constitutes only 2% of the area of China, contributes upwards of 15% of countrywide emissions of greenhouse gases (38). In rural areas, such as He Zuo Township where this study was conducted, biomass fuel and coal are burned for cooking and heating, resulting in elevated indoor air pollution as well. Visible impact of air pollution is manifest as the Asian Brown Cloud, a haze composed of pollutants and particulates that resides over the sub-Asian continent as well as China for months on end, especially in the winter. Outdoor air pollution has been found to be associated with a wide range of adverse health outcomes, including increased mortality, increased rates of hospital admissions and exacerbation of chronic respiratory conditions along with decreased lung function (39).

There is a relative scarcity of air monitoring data in this region and precious little human biomonitoring for exposures. Biomonitoring in China has centered on PAHs and benzene, the latter an environmental carcinogen with especially high exposures in some workplace settings. PAHs, such as phenanthrene, are ubiquitous in the general environment and are released into the ambient air by tobacco smoke, vehicle exhausts and other incomplete combustion sources such as cooking stoves. Analysis of the organic extract of indoor air particles from homes in Yunnan Province, China, indicated phenanthrene to be the most abundant PAH (36). Hecht *et al.* (30) previously reported mean PheT levels of 1.2 pmol/mg creatinine (Table III) in non-smokers in the USA, whereas smokers had levels of 3.8 pmol/mg. In a study conducted in He Zuo Township in the winter of 2003, we observed a modest increase in PheT levels in the 12 smokers compared with the 87 non-smokers who were in the placebo arm (11.6 versus 8.0). Although the magnitude of the difference between smokers and non-smokers is lower in Qidong than in the US study, the baseline values of the PAH biomarker are substantially higher in the non-smoking residents of Qidong compared with the US non-smokers. Thus, non-tobacco-derived sources, such as industrial, automotive and cooking emissions, seem to account for the bulk of PAH airborne exposures in this rural area adjacent to and downwind of Shanghai. Consumption of vegetables contaminated with PAHs in the fields as well as those produced by broiling meat may provide additional vectors for exposure. Lower levels of PheT (6.5 pmol/mg creatinine) were detected in the urine of non-smokers in this study conducted in the fall of 2009. Seasonal differences in levels of air pollution—highest in winter, previously observed in Shanghai (40), may drive the lower levels seen in the current study. We also find in this study that the levels of mercapturic acids of acrolein, crotonaldehyde and benzene were 25, 290 and 540% higher in non-smoking, rural Qidong residents than found in non-smoking residents of Singapore (29). The levels of the benzene biomarker, SPMA, were in accord with earlier findings by Kim *et al.* (41) in a control population in Tianjin, China. No differences were seen in levels of the urinary ethylene oxide mercapturic acid. Reference values for these biomarkers in USA or European populations are limited (42,43). In addition, health effects of these ambient levels of exposure, if any, are unknown.

Effective low cost means (i.e. functional foods) to empower individuals to reduce body burden of genotoxic air pollutants may exert benefits for public health but are not to be considered as substitutes for regional and countrywide efforts to control pollution. In this study, consumption of broccoli sprout beverage led to reasonably consistent increases (~20 to 50%) in excretion of mercapturides of acrolein, crotonaldehyde, ethylene oxide and benzene. Interestingly, increases were seen in both smokers and non-smokers indicating that level of exposure upon entry into the intervention did not have much influence on the inductive response. However, broccoli sprout related increases in mercapturic acid excretion of the four air pollutants did not track among individual participants. That is, elevated excretion of one mercapturic acid was not linked to elevations in excretion rates for the others. This heterogeneous outcome may relate to the involvement of distinct isoforms of GSTs in the conjugation of these different substrates. Overlaid on that effect is the knowledge that 34% of the participants were null for *GSTM1* alleles, whereas 51% were null for *GSTT1*; 29% were null for both (20). The induction of different isoforms of human GSTs in response to sulforaphane and their proportional contributions to the conjugations of the four substrates in this study are not well characterized. Considerable evidence suggests that polymorphisms in *GSTM1* and *GSTT1* profoundly affect the formation of SPMA from benzene (44), and to a lesser extent ethylene oxide (45). In contrast, no effects of these isoforms on acrolein conjugation have been observed (Hecht S., unpublished results).

The central question addressed in the clinical trial was to determine the bioavailability and tolerability of two preparations of beverages prepared from 3-day-old broccoli sprouts in order to administer the chemopreventive agent sulforaphane: (i) enterically generated from its cognate glucosinolate (glucoraphanin) by thioglucosidases found in the gut microflora (GRR) and (ii) prereleased when the GRR beverage was treated with myrosinase from daikon to catalyze hydrolysis of glucoraphanin to sulforaphane (SFR). The bioavailability of sulforaphane administered as SFR was far superior to GRR (20). Estimates of the area under the curves derived from the excretion patterns suggest that 70% of the administered sulforaphane in the SFR beverage was eliminated in 24 h, whereas only 5% of the administered glucoraphanin in GRR beverage was recovered as sulforaphane metabolites (20). Somewhat surprisingly, despite the higher bioavailability of sulforaphane from the SFR, there was no indication of differential efficacy of urinary mercapturic acid excretion between the two treatment arms (Figure 2). This result suggests that the 800 μmol dose of glucoraphanin yielded sufficient sulforaphane to provoke a maximal inductive response. Whether an adequate peak concentration (C_{max}) or extended half-live relative to the administration of sulforaphane itself explain the equieffectiveness is not clear at this time. What has clearly been demonstrated, both in *in vitro* and animal models, is that the potential of broccoli sprout extract to upregulate cytoprotective enzymes can be completely attributed to the sulforaphane in those extracts and that no other phytochemical components contributed significantly to the induction of these enzymes (7,17). Whether other pathways affecting the metabolism of these pollutants are also modulated by the broccoli sprout beverages was not addressed in this study.

The mercapturic acids are measures of internal dose following exposures to air pollutants. As such, it is challenging and perhaps inappropriate to use them as estimates of risk. Excretion rates reflect multiple processes. These rates increase with exposure, such as seen with smoking, but are influenced (up and down) by polymorphisms in metabolizing enzymes. To control for variable exposures, reverse phase 0 studies in which microdoses of heavy isotope-labeled compounds, as has been done with deuterated phenanthrene (46), could separate effects of exposure from metabolism (47). Nonetheless, an intriguing question lies in the health impact of 20–50% increases in rates of detoxication of these air pollutants as seen with the broccoli sprout intervention. A few insights can be gleaned from animal experiments where dose–response and time series studies explore the relationships between modulation of expression of conjugating enzymes, metabolic flux of carcinogens, DNA adduct levels and cancer chemopreventive efficacy. In rats treated with the chemopreventive agent ethoxyquin, a 4-fold increase in the biliary excretion of aflatoxin–glutathione conjugate was associated with 75% reductions in DNA adducts and >95% reductions in tumor burden following challenge with the hepatocarcinogen aflatoxin (48). In rats fed 0.02% oltipraz, hepatic GST activity increased by 50%, DNA adduct burden dropped by 40% and volume percent of preneoplastic lesions were reduced by >95% (49). Perhaps, one can also extrapolate from the experience in Beijing in 2008 when stringent air quality control measures were implemented before and during the Olympic games. Reductions of 27–66% in atmospheric content of hydrocarbons were

reported (50); comparable reductions were measured for PAHs. Jia *et al.* (51) have estimated that these PAH reductions, if sustained, could contribute to a 46% reduction in cancer risk. Thus, it is reasonable to expect that seemingly modest increases in detoxication capacity, as afforded by broccoli sprouts, can lead to risk reduction in individuals and populations unavoidably exposed to environmental carcinogens.

Supplementary material

Supplementary Table 1 can be found at http://carcin.oxfordjournals.org/

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