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Urinary Isothiocyanate Levels and Lung Cancer Risk Among Non-Smoking Women: a Prospective Investigation

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Abstract

Background—Aside from tobacco carcinogen metabolism, isothiocyanates (ITC) from cruciferous vegetables may induce apoptosis or steroid metabolism to reduce lung cancer risk. To separate the effect of these divergent mechanisms of action, we investigated the association between urinary ITC levels and lung cancer risk among non-smoking women.

Methods—We conducted a nested case-control within the Shanghai Women's Health Study. Subjects included 209 incident lung cancer cases who never used tobacco, and 787 individually matched non-smoking controls. Conditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) summarizing the association between urinary ITC levels and lung cancer. Secondary analyses stratified the ITC-lung cancer analyses by menopausal status, exposure to environmental tobacco smoke, and GSTM1 and GSTT1 genotypes.

Results—Urinary ITC levels were not significantly associated with lower lung cancer risk among non-smoking women, regardless of exposure to environmental tobacco smoke or menopausal status. Furthermore, this association was not modified by GSTT1 genotype. However, an inverse association was suggested among women with a GSTM1-positive genotype (Q1: OR=1.0 (reference); Q2: OR=0.35 (0.14, 0.89); Q3: OR=0.47 (0.20, 1.10); Q4: OR=0.63 (0.35, 1.54), p-trend = 0.38)). In contrast, lung cancer risk was positively associated with urinary ITC levels among women with the GSTM1-null genotype (Q1: OR=1.0 (reference); Q2: OR=1.67 (0.80, 3.50); Q3: OR=1.54 (0.71, 3.33); Q4: OR=2.22 (1.05, 4.67), p-trend = 0.06)).

Conclusion—Urinary ITC levels were not associated overall with lower lung cancer risk among non-smoking women, but secondary analyses suggested an interaction between urinary ITC levels, GSTM1 genotype, and lung cancer risk.

Keywords

isothiocyanate; lung neoplasm; diet; genetic susceptibility; women

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Introduction

Tobacco is clearly the dominant risk factor for lung cancer. However, approximately 25% of all lung cancer world-wide are diagnosed among persons that have never smoked (1), and lung cancer among non-smokers has a distinct histology and epidemiology. Unlike squamous cell carcinomas common among tobacco–users, the majority of lung tumors among non-smokers are adenocarcinomas located in the more distal and peripheral airways and alveoli. Furthermore, gender differences become reversed in that non-smoking women are at greater risk for lung cancer than non-smoking men (1).

Exposure to environmental tobacco smoke (ETS) or other environmental carcinogens may contribute to lung carcinogenesis among non-smokers (2). Interestingly, several recent case-control studies report cruciferous vegetable intake, such as cabbage and bok choy, is associated with lower lung cancer risk among non-smoking men and women (3–5). These plants are the primary dietary source for isothiocyanates (ITCs) and indoles known to induce Phase I (i.e. cytochrome P-450) and Phase II (e.g, glutathione S-transferases) systems responsible for metabolizing tobacco procarcinogens. Indeed, metabolism of the tobacco carcinogen nitrosamine 4-(methylnitrosamino)-1-3-pyridyl)-1-butanone (NNK) increases when smokers are exposured to ITCs (6–8), indole-3-carbinol (I3C) (9–12) or cruciferous vegetables (13). Cruciferous vegetable intake is associated with reduced risk of lung cancer among tobacco users, often jointly with genetic variation in GST enzymes (3;14;15). One study also reported that genetic variation in GSTM1 modified the protective association between cruciferous vegetable intake and lung cancer among non-smokers (5).

Other factors perhaps involved in lung carcinogenesis among non-smokers may include exposure to reactive oxygen species (ROS) from radiation or other sources, bacterial or viral infection, or increased exposure to estrogens (2). ITCs or indoles derived from crucifers reduce systemic oxidative stress levels (16–18), alter cytokine activity associated with the inflammatory response (19), induce apoptosis (20–22), and inhibit angiogenesis (23). ITCs also have shown anti-bacterial (24) and anti-viral (25) properties. Furthermore, Phase I induction in response to indoles from cruciferous vegetables may be sufficient to induce estrogen metabolism (26;27) or reduce estrogen receptor signal transduction (28;29), thus reducing ER activation on lung epithelial cells.

This study will prospectively investigate the association between cruciferous consumption and lung cancer among women who have never smoked. Non-smoking Asian women are among the non-smoking populations with the highest risk of lung cancer (30–32), and our investigation will utilize data from women living in Shanghai, China. To avoid reporting biases associated with food frequency questionnaires, we measured urinary ITC levels as an estimate of the internalized exposure to the biologically active agents derived from cruciferous vegetable consumption (33;34).

Materials and Methods

Study Subjects

The detailed methods for the SWHS has been published elsewhere (35). The study was approved by the institutional review boards of all collaborating institutions. From 1997 to 2000, 74,942 Chinese women between the ages of 40 and 70 years and residing in seven urban communities of Shanghai were recruited into the cohort study. Each woman signed a consent form at the time of enrollment and completed an in-person survey that included information on medical history and medication use, demographic characteristics, anthropometrics, and other lifestyle factors. Each subject was also asked questions to ascertain their exposure to environmental tobacco smoke from their husband or from

colleagues in the workplace. Usual dietary habits were ascertained using a validated food frequency questionnaire (FFQ), and included queries on bok choy, cabbage, Chinese greens, and turnip consumption. The participation rate for the baseline survey was 92.7%.

Urine Collection

Of the study participants, 65,754 (87.7%) provided a spot urine sample. Urine samples were collected into a sterilized cup containing 125 mg ascorbic acid to prevent oxidation of labile metabolites. After collection, the samples were kept in a portable Styrofoam box with ice packs (at approximately 0 to 4°C) and processed within 6 hours for long-term storage at −70°C. At the time of sample procurement, a biospecimen collection form was completed for each woman, which included the date and time of sample collection, time of last meal, and day of last menstruation (for premenopausal women), as well as intake of selected foods, smoking, and use of any medications over the previous 24 hours and during the previous week.

Follow-Up

The cohort was followed through biennial home visits as well as annual record linkage to cancer incidence and mortality data collected by the Shanghai Cancer Registry and death certificate data collected by the Shanghai Vital Statistics Unit. Follow-up rates for the first, second and the third follow-up surveys were 99.8%, 98.7%, and 96.7% respectively. For cohort members who were diagnosed with cancer, medical charts were reviewed to verify the diagnosis, and detailed information on the pathologic characteristics of the cancer was obtained.

Nested Case-Control Study

The nested case-control study includes 228 incident lung cancer cases diagnosed before February 2003. We included only participants who donated a urine sample before any cancer diagnosis. The incidence-density method was used for case-control matching. For each case, a set of controls was selected from women who donated a urine sample at baseline and were free of any cancer at the time of cancer diagnosis for the index case. In addition, cases and controls were individually matched on age at baseline (± 2 years), date (≤30 days) and time (morning or afternoon) of urine collection, interval since last meal (≤2 hours), menopausal status (pre- or post-), and antibiotic use (yes/no) in the past week. Controls for assay were randomly selected from among the pool of eligible controls within each stratum.

Urinary ITC Assay

The method for analysis of urinary total isothiocyanates (ITCs) and their thiol metabolites described in detail previously (33) was slightly revised. Briefly, frozen urine samples were thawed and vortexed; 1 ml samples were placed in 2 ml glass vials and centrifuged (2800 rpm) for 15 min to sediment suspended matter, then placed on ice. Triplicate aliquots of 100 μl clarified urine were pipetted into 2 ml HPLC vials (Chromacol, Inc., Trumbull, CT)) containing 600 μl degassed 2-propanol solution of 10 mM 1,2-benzenedithiol (Lancaster Synthesis, Inc., Waldham NM) and 500 μl degassed 0.1 M potassium phosphate. The reaction mixtures in capped vials were vortexed, and incubated for 2 h at 65° C in a shaking water bath. The reaction mixtures were cooled and centrifuged (2800 rpm, 20 min) prior to analysis of the reaction product 1,3-benzendithiol-2-thione by HPLC.

Following incubation, samples were analyzed by reverse-phase HPLC using a Waters μBondapak C_{18} (150 × 3.9 mm) with a Waters C_{18} guard column and a detection wavelength of 365 nm. The mobile phase consisted of a mixture of methanol and H_2O (7:3

v/v) with a flow rate of 1.75 ml/min. A Shimadzu model SCL-10A controller, dual LEC-10AS pumps, and SIL-10A autosampler (Shimadzu Scientific Instruments, Inc., Columbia, MD) were utilized; Axxiom 727 software (Axxiom Chomatography, Inc., Moorpark, CA) was utilized to collect and integrate HPLC data. Concurrent triplicate standards of the *N*-acetyl conjugate of phenethyl ITC (PEITC-*N*AC) were prepared in 20 mM phosphate buffer at pH 5 and concentrations of 0 μM (H_2O), 5 μM, 15μM and 25 μM; concurrent standards were analyzed with each batch of urine samples. PEITC-*N*AC was prepared, and purity and structure were verified by NMR and HPLC in the Organic Synthesis Laboratory (36). A standard curve (1–100 μM PEITC-*N*AC in 20 mM phosphate buffer, pH 5, in triplicate), prepared and analyzed weekly, was used for quantification of urinary total ITC concentrations. All lab assays were performed in 2007–2008.

GSTM1 and GSTT1 genotyping

DNA was extracted from blood (86.4%) or exfoliated buccal cells (13.6%). Copy number for the *GSTM1* and *GSTT1* genes was determined by a duplex real-time quantitative PCR-based assay, following the method described in the NCI SNP500 project with modifications(37). The assay was designed to detect whether an individual has zero, one, or two or more copies of the *GSTM1* and *GSTT1* genes. The primers used are: GSTM1_F (5′-GGA CAT TTT GGA GAA CCA GAC C-3′), GSTM1_R (5′-CAC TCA CAA ATT CTG GAT TG-3′), GSTT1_F (5′-TGT GTC CTT TAA TCA CTG CAT TTC-3′), GSTT1_R (5′-GGG ACA GAG GAA AGT CAA ATA AAT C-3′), BRCA1_F (5′-AAA CAT GTT CCT CCT AAG GTG CTT T-3′), and BRCA1_R (5′-ATG AAA CCA GAA GTA AGT CCA CCA GT-3′). Each 5 μL volume contained 5 ng of DNA, 300 nM of each primer, 2.5 μL of TaqMan Master Mix (2X), and 250 nM of each probe. The thermal cycling conditions are: 10 minutes at 95°C follow by 40 cycles at 95°C for 30 seconds and 60°C for 1 minute. The real-time PCR were performed in a 384-well plate in ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA).

Laboratory staff was blind to the case-control status of the samples. Coriell DNA samples containing the zero, one, or two copies of the *GSTM1* and *GSTT1* genes were included to serve as internal quality controls. The concordance rate for quality control samples, including water, Coriell DNA, and blinded DNA samples, was 100%. GSTT1 genotypes were within Hardy-Weinburg (H-W) equilibrium among the cases (p=0.73) and the controls (p=0.15). Similarly, GSTM1 genotypes were within H-W equilibrium among the cases (p=1.00) and the controls (p=0.10).

Statistical Analyses

We excluded 17 cases and 23 controls currently using tobacco, and 2 cases and 8 controls who were former tobacco users. We also excluded one control with total energy intake greater than 3,500 kcal/day because of uncertain data quality. The final analytic dataset included 209 lung cancer cases and 787 individually matched controls. The frequency of each case:control matched set was: 1:1 (n=3), 1:2 (n=7), 1:3 (n=26), and 1:4 (n=173).

Urinary ITC levels were standardized to urinary creatinine levels (ng ITC/mg creatinine). The distribution was right-skewed and ITC values were natural log transformed prior to analysis. Median and percentages of selected baseline characteristics for cases and controls were calculated. The Wilcoxon signed rank test for paired data was used to compare ITC values and other continuous variables between cases and matched controls. The Wald test from a conditional logistic regression model was used to compare the frequency of categorical variables between cases and controls. Multivariable conditional logistic regression was used to calculate odds ratios and 95% confidence intervals summarizing the association between urinary ITC levels and lung cancer. Urinary ITC levels were analyzed

as a continuous variable, or categorized as two (non-detected, detected), three (non-detected, below median, median or above), or four (quartiles of control group distribution) categories. A *P* value for linear trend was determined by modeling the categorical variable for urinary ITC levels as a continuous variable in the logistic model. Analyses were stratified by menopausal status, exposure to ETS, and GST genotypes using unconditional logistic regression with the matching variables included in the model. Tests for interaction involved the significance of cross-product terms between ITC and GST variables while controlling for each main effect and other covariates.

Results

Cases and controls reported a similar intake of fat, soy food, and cruciferous vegetable, and were generally similar in overall health-related parameters (Table 1). Cases were marginally more likely to have a past diagnosis of asthma.

Lung cancer was not significantly associated with total ETS exposure (OR=0.83 (0.53, 1.29), p=0.40) or having a husband who smoked (OR=1.08 (0.79, 1.48), p=0.61)). Similarly, GSTM1-positive (OR=1.25 (0.91, 1.71), p=0.17) and GSTT1-positive (OR=0.88 (0.65, 1.19, p=0.40) genotypes were not significantly associated with lung cancer.

There was a weak but significant correlation between urinary ITC levels and reported cruciferous vegetable intake across all subjects ($r_{spearman}$ = 0.12, p<0.01), regardless of GSTM1 (r_s (GSTM1-null) = 0.14, p<0.01; r_s (GSTM1-present) = 0.10, p=0.04) or GSTT1 (r_s (GSTT1-null) = 0.15, p<0.01; r_s (GSTT1-present) =0.09, p=0.04) genotypes. However, urinary ITC levels were not significantly correlated with cruciferous vegetable intake among lung cancer cases ((r_s (cases) = −0.01, p=0.86; r_s (controls) = 0.16, p<0.01). Average urinary ITC levels did not significantly differ with GSTM1 genotype (p=0.32), GSTT1 genotype (p=0.72), exposure to ETS (p=0.19), smoking status of husband (p=0.20), or menopause status (p=0.07).

Median urinary ITC did not significantly differ between cases and controls (Table 1). Table 2 summarizes the association between lung cancer and urinary ITC. To support interpretation against other studies, we developed a priori four scales of ITC exposure, including ITC levels as a continuous variable (log transformed) and three categorization methods. Lung cancer risk was not significantly associated with urinary ITC levels. Adjusting for ETS exposure, GST genotypes, or other potential risk factors did not substantively change these results.

We stratified analyses by menopausal status and exposure to ETS. Urinary ITC levels were not significantly associated with lung cancer within either premenopausal or postmenopausal women (Table 3). Similarly, the association between urinary ITC levels and lung cancer was not modified by exposure to ETS.

We investigated the association between urinary ITC levels and lung cancer stratified by GSTM1 or GSTT1 genotypes (Table 4). Urinary ITC levels appeared to be protective for lung cancer with the GSTM1-present genotype. The test for interaction between GSTM1 and ITC levels (continuous) was significant (p-int=0.03), but not for GSTT1 (p-int = 0.62), suggesting the association between urinary ITC levels and lung cancer was modified by GSTM1 genotype. In contrast, greater urinary ITC levels were associated with an increase in lung cancer risk with the GSTM1-null genotype. GSTT1 genotypes did not substantially modify associations between urinary ITC and lung cancer.

Discussion

The incidence of lung cancer is increasing among non-smoking women. Glucosinolates found in cruciferous vegetables are enzymatically degraded following consumption to yield multiple ITCs and indoles, and these compounds are believed to affect not only tobacco carcinogen metabolism but also infection and steroid hormone activity potentially relevant to lung carcinogenesis. However, contrary to initial hypothesis, this prospective analysis within the SWHS found greater urinary ITC levels were not consistently associated with lower lung cancer risk among non-smoking women.

Most prior studies report that the association between cruciferous vegetable intake and lung cancer is modified by smoking status in a manner consistent with a mechanism involving tobacco carcinogen metabolism (3–5;15). For example, London and colleagues measured urinary ITC levels among men in Shanghai with a high prevalence of smoking (cases: 81.5%; controls 47.5%). Despite a high level of non-detectable ITC levels (15%-24% of participants), a detectable ITC level was associated with lower lung cancer risk among smokers (RR=0.63 (0.41, 0.95)). The inverse association between urinary ITC levels and lung cancer was stronger among subjects with the GSTM1-null (RR=0.63 (0.44, 0.91)) or GSTT1-null (RR=0.82 (0.57, 1.19)) genotypes compared to the respective GST-positive genotype (14). Perhaps unexpectedly, a similar relationship also was reported among non-smokers. Zhao and colleagues investigated Chinese women who had never smoked and found a reduced risk associated with ITC level only among women with the GSTM1-null genotype (GSTM1-null: OR=0.54 (0.30, 0.95); GSTM1-positive: OR=1.07 (0.50, 2.29)) (5). Two other studies also reported reduced lung cancer risk with cruciferous intake in non-smokers, although they found no evidence of modification by GSTM1 or GSTT1 genotypes (3;4).

Results from our prospective investigation of non-smoking women living in Shanghai found no overall association between urinary ITC levels and lung cancer risk. We did not find that lung cancer risk was associated with cruciferous vegetable intake within any GSTT1 or M1 genotypes. Furthermore, urinary ITC levels were not associated with lung cancer among women exposed to ETS. We also explored menopausal status as a proxy for potential biologic interaction between cruciferous intake and estrogens, but did not find any substantive differences in urinary ITC and lung cancer associations between premenopausal and postmenopausal women.

We also note that lung cancer risk was positively associated with greater urinary ITC levels among GSTM1-null subjects. This was unexpected, but may suggest indole exposure and the resulting effects on Phase I and Phase II enzyme induction could have an overall unintended effect to activate procarcinogenic species leading to an increased lung cancer risk (13). For example, perhaps subjects with a GSTM1-null genotype may not be able to invoke a sufficient or balanced Phase II enzyme response to rapidly conjugate and excrete these carcinogens(38;39). We also cannot rule out residual confounding effects or chance as possible explanations. Further investigation of the association between cruciferous intake and lung cancer among non-smoking women is clearly needed.

There are several strengths of this study. This study utilized a prospective cohort design, and all biospecimens and data were collected prior to the diagnosis of lung cancer. Furthermore, lung cancer cases and controls were individually matched on the timing of urine collection and other factors, and we controlled for exposure to ETS. Furthermore, we estimated cruciferous vegetable intake using a well characterized biomarker. Glucosinolate levels may vary substantially across plant cultivars (40–42), or differences associated with plant age, weather, or soil conditions. Glucosinolates have little biologic activity (43), but the plant

enzyme myrosinase responsible for glucosinolate hydrolysis is inactivated when the plant is cooked. Urinary ITC levels provide a cumulative index of glucosinolate exposure across plants and myrosinase activity from the plant and the gut microflora necessary for estimating internalized exposure to ITCs and indoles derived from cruciferae intake. We also scaled urinary ITC levels using several approaches to allow comparisons with other investigations.

One concern is that urinary ITC levels directly represent cruciferous consumed within the past 8 to 72 hours (43), and inference from a single urinary ITC level may limit our ability to estimate exposure to cruciferous phytochemicals over an extended period of time. However, dietary patterns are less varied in Shanghai than what is found in Western regions (44), consistent with so few non-detected urinary ITC levels. Most persons living in Shanghai eat cruciferous vegetables on a routine basis, and there was a significant, albeit modest, correlation between urinary ITC levels and cruciferous vegetable intake estimated by FFQ. Most cruciferous vegetables express indole glucosinolates at high levels, although indoles do not form stable ITCs when hydrolyzed by myrosinase and would not contribute to the urinary ITC content. However, urinary ITC levels might be expected to provide an index of myrosinase activity and glucosinolate hydrolysis necessary to produce indoles as well as ITCs. Median duration of follow-up was just 3.2 years, and additional genes of interest, such as GSTP1 or CYP1A2, will be investigated in future studies.

In summary, we prospectively investigated the hypothesis that urinary ITC levels would be associated with lower lung cancer risk among never-smoking women. Overall, we found no association between urinary ITC and lung cancer risk among female non-smokers. However, there was also indication that GSTM1 genotypes may determine the direction of the association between urinary ITC levels and lung cancer among non-smoking women.

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Reference List

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. CA: A Cancer Journal for Clinicians. 2005 Mar 1; 55(2):74–108. [PubMed: 15761078]
2. Subramanian J, Govindan R. Lung cancer in never smokers: a review. J Clin Oncol. 2007 Feb 10; 25(5):561–70. [PubMed: 17290066]
3. Brennan P, Hsu CC, Moullan N, Szeszenia-Dabrowska N, Lissowska J, Zaridze D, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, et al. Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a mendelian randomisation approach. Lancet. 2005 Oct 29; 366(9496):1558–60. [PubMed: 16257343]
4. Wang LI, Giovannucci EL, Hunter D, Neuberg D, Su L, Christiani DC. Dietary intake of Cruciferous vegetables, Glutathione S-transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. Cancer Causes Control. 2004 Dec; 15(10):977–85. [PubMed: 15801482]
5. Zhao B, Seow A, Lee EJD, Poh W-T, Teh M, Eng P, Wang T-T, Tan W, Yu MC, Lee H-P. Dietary isothiocyanates, glutathione S-transferase -M1, T1 polymorphisms and lung cancer risk among Chinese women in Singapore. Cancer Epidemiol Biomarkers Prev. 2001; 10:1063–7. [PubMed: 11588132]
6. Brooks JD, Paton VG, Vidanes G. Potent induction of phase 2 enzymes in human prostate cells by sulforaphane. Cancer Epidemiol Biomarkers Prev. 2001 Sep; 10(9):949–54. [PubMed: 11535546]
7. Hecht SS, Chung FL, Richie JP Jr, Akerkar SA, Borukhova A, Skowronski L, Carmella SG. Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers. Cancer Epidemiol Biomarkers Prev. 1995 Dec; 4(8):877–84. [PubMed: 8634661]
8. Hecht SS, Carmella SG, Murphy SE. Effects of watercress consumption of urinary metabolites of nicotine in smokers. Cancer Epidemiol Biomarkers Prev. 1999; 8:907–13. [PubMed: 10548320]

9. Morse MA, LaGreca SD, Amin SG, Chung F-L. Effects of Indole-3-carbinol on lung tumorigenesis and DNA methylation induced by 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butone (NNK) and on the metabolism and disposition of NNK in A/J mice. Cancer Res. 1990; 50:2613–7. [PubMed: 2328487]
10. Morse MA, Wang C-X, Amin SG, Hecht SS, Chung F-L. Effects of dietary sinigrin or indole-3-carbinol on O6-methylguanine-DNA-transmethylase activity and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA methylation and tumorigenicity in F344 rats. Carcinogenesis. 1988; 9(10):1891–5. [PubMed: 3168166]
11. Chung FL, Morse MA, Eklind KI, Xu Y. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis by compounds derived from cruciferous vegetables and green tea. Ann N Y Acad Sci. 1993; 686:186–201. [PubMed: 8512247]
12. Hecht SS, Carmella SG, Kenney P, Low S-H, Arakawa K, Yu MC. Effects of Cruciferous vegetable consumption on urinary metabolites of the tobacco-specific lung carcinogen NNAL in Singapore. Cancer Epidemiol Biomarkers Prev. 2004; 13(6):997–1004. [PubMed: 15184256]
13. Hecht SS, Carmella SG, Kenney PMJ, Low SH, Arakawa K, Yu MC. Effects of Cruciferous Vegetable Consumption on Urinary Metabolites of the Tobacco-Specific Lung Carcinogen 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone in Singapore Chinese. Cancer Epidemiol Biomarkers Prev. 2004 Jun 1; 13(6):997–1004. [PubMed: 15184256]
14. London SJ, Yuan J-M, Chung F-L, Gao Y-T, Coetzee GA, Ross RK, Yu MC. Isothiocyanates, glutathioneS-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet. 2000; 356:724–9. [PubMed: 11085692]
15. Spitz MR, Duphorne CM, Detry MA, Pillow PC, Amos CI, Lei L, de Andrade M, Gu X, Hong WK, Wu X. Dietary intake of isothiocyanates; evidence of a joint effect with glutathione S-transferasen polymorphisms in lung cancer risk. Cancer Epidemiol Biomarkers Prev. 2000; 9:1017–20. [PubMed: 11045782]
16. Verhagen H, Poulsen HE, Loft S, van Poppel GA, Willems M, vanBladeren PJ. Reduction of oxidative DNA-damage in humans by Brussels sprouts. Carcinogenesis. 1995; 16(4):969–70. [PubMed: 7728983]
17. Verhagen H, de Bries A, Nijhoff WA, Schouten A, van Poppel GA, Peters WH, vandenBerg H. Effect of Brussels sprouts on oxidative DNA-damage in man. Cancer Lett. 1997; 114:127–30. [PubMed: 9103270]
18. Fowke JH, Morrow J, Motley S, Bostick R, Ness R. Brassica Vegetable Consumption Reduces Urinary F2-isoprostane Levels, a Biomarker of Systemic Oxidative Stress. Cancer Epidemiol Biomarkers Prev. 2005; 14(2):546.
19. Xue L, Firestone GL, Bjeldanes LF. DIM stimulates IFNgamma gene expression in human breast cancer cells via the specific activation of JNK and p38 pathways. Oncogene. 2005 Mar 31; 24(14): 2343–53. [PubMed: 15735741]
20. Ge X, Fares FA, Yannai S. Induction of apoptosis in MCF-7 cells by indole-3-carbinol is independent of p53 and bax. Anticancer Res. 1999; 19(4B):199–203.
21. Tang L, Zhang Y. Mitochondria are the primary target in isothiocyanate-induced apoptosis in human bladder cancer cells. Mol Cancer Ther. 2005 Aug; 4(8):1250–9. [PubMed: 16093441]
22. Chinni SR, Sarkar FH. Akt inactivation is a key event in indole-3-carbinol-induced apoptosis in PC-3 cells. Clin Cancer Res. 2002 Apr; 8(4):1228–36. [PubMed: 11948137]
23. Chang X, Firestone GL, Bjeldanes LF. Inhibition of growth factor-induced Ras signaling in vascular endothelial cells and angiogenesis by 3,3′-diindolylmethane. Carcinogenesis. 2006 Mar; 27(3):541–50. [PubMed: 16199440]
24. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, Lozniewski A. 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 May 28; 99(11):7610–5. [PubMed: 12032331]
25. Jin L, Qi M, Chen D-Z, Yang AAG-Y, Arbeit JM, Auborn KJ. Indole-3-carbinol prevents cervical cancer in human papilloma virus type 16 (HPV16) transgenic mice. Cancer Res. 1999; 59:3991–7. [PubMed: 10463597]

26. Bradlow HL, Michnovicz JJ, Halper M, Miller DG, Wong GYC, Osborne M. Long-term responses of women to indole-3-carbinol or a high fiber diet. Cancer Epidemiol Biomarkers Prev. 1994; 3:591–5. [PubMed: 7827590]
27. Fowke JH, Longcope C, Hebert JR. *Brassica* vegetable consumption shifts estrogen metabolism in healthy postmenopausal women. Cancer Epidemiol Biomarkers Prev. 2000; 9:773–9. [PubMed: 10952093]
28. Fan S, Meng Q, Auborn K, Carter T, Rosen EM. BRCA1 and BRCA2 as molecular targets for phytochemicals indole-3-carbinol and genistein in breast and prostate cancer cells. Br J Cancer. 2006 Feb 13; 94(3):407–26. [PubMed: 16434996]
29. Meng Q, Yuan F, Boldberg ID, Rosen EM, Auborn K, Fan S. Indole-3-carbinol is a negative regulator of estrogen receptor-alpha signaling in huma tumor cells. J Nutr. 2000; 130:2927–31. [PubMed: 11110848]
30. Chen JG, Zhu J, Parkin DM, Zhang YH, Lu JH, Zhu YR, Chen TY. Trends in the incidence of cancer in Qidong, China, 1978–2002. Int J Cancer. 2006 Sep 15; 119(6):1447–54. [PubMed: 16596645]
31. Devesa SS, Bray F, Vizcaino AP, Parkin DM. International lung cancer trends by histologic type: male:female differences diminishing and adenocarcinoma rates rising. Int J Cancer. 2005 Nov 1; 117(2):294–9. [PubMed: 15900604]
32. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers--a different disease. Nat Rev Cancer. 2007 Oct; 7(10):778–90. [PubMed: 17882278]
33. Chung F-L, Jiao D, Getahun SM, Yu MC. A urinary biomarker for uptake of dietary isothiocyanates in humans. Cancer Epidemiol Biomarkers Prev. 1998; 7:103–8. [PubMed: 9488583]
34. Zhang Y, Wade KL, Prestera T, Talalay P. Quantitative determination of isothiocyanates, dithiocarbamates, carbon disulfide, and related thiocarbonyl compounds by cyclocondensation of 1,2-benzenedithiol. Anal Biochem. 1996; 239:160–7. [PubMed: 8811900]
35. Zheng W, Chow WH, Yang G, Jin F, Rothman N, Blair A, Li HL, Wen W, Ji BT, Li Q, Shu XO, Gao YT. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. Am J Epidemiol. 2005 Dec 1; 162(11):1123–31. [PubMed: 16236996]
36. Jiao D, Conaway CC, Wang M-H, Yang CS, Koehl W, Chung F-L. Inhibition of *N*-nitrosodimethylamine demethylase in rat and human liver micosomes by isothiocyanates and their glutathione, L-cysteine, and *N*-acetyl-L-cysteine conjugates. Chem Res Toxicol. 1996; 9:932–8. [PubMed: 8870979]
37. Moore LE, Huang WY, Chatterjee N, Gunter M, Chanock S, Yeager M, Welch B, Pinsky P, Weissfeld J, Hayes RB. GSTM1, GSTT1, and GSTP1 polymorphisms and risk of advanced colorectal adenoma. Cancer Epidemiol Biomarkers Prev. 2005 Jul; 14(7):1823–7. [PubMed: 16030123]
38. Canistro D, Croce CD, Iori R, Barillari J, Bronzetti G, Poi G, Cini M, Caltavuturo L, Perocco P, Paolini M. Genetic and metabolic effects of gluconasturtiin, a glucosinolate derived from cruciferae. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2004 Jan 12; 545(1–2):23–35.
39. Paolini M, Perocco P, Canistro D, Valgimigli L, Pedulli GF, Iori R, Croce CD, Cantelli-Forti G, Legator MS, Abdel-Rahman SZ. Induction of cytochrome P450, generation of oxidative stress and in vitro cell-transforming and DNA-damaging activities by glucoraphanin, the bioprecursor of the chemopreventive agent sulforaphane found in broccoli. Carcinogenesis. 2004 Jan 1; 25(1):61–7. [PubMed: 14514654]
40. McNaughton SA, Marks GC. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. Br J Nutr. 2003; 90(3):687–97. [PubMed: 13129476]
41. Mullin WJ, Sahasrabudhe MR. Glucosinolate content of cruciferous vegetable crops. Canadian Journal of Plant Science. 1977; 57:1227–30.
42. Mullin WJ, Sahasrabudhe MR. An estimate of the average daily intake of glucosinolates via cruciferous vegetables. Nutrition Reports International. 1978; 18:273–9.

43. Shapiro TA, Fahey JW, Wade KL, Stephenson KK, Talalay P. Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of *Cruciferous* vegetables. Cancer Epidemiol Biomarkers Prev. 1998; 7:1091–100. [PubMed: 9865427]

44. Fowke JH, Schlundt DG, Gong Y, Jin F, Shu X-O, Wen W, Liu D, Gao Y, Zheng W. Season of food frequency questionnaire administration affects reporting of habitual dietary intake. Ann Epidemiol. 2004; 14(10):778–85. [PubMed: 15519900]

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Table 1

Population Description

		Cases (n=209)		Controls (n=787)		
		Median	**25th, 75th**	**Median**	**25th, 75th**	***P****
Age (years)		61.0	52.0, 66.0	61.0	51.0, 66.0	0.13
BMI		24.3	22.4, 26.7	24.3	22.0, 26.7	0.78
WHR		0.83	0.79, 0.86	0.82	0.79, 0.86	0.27
Energy (kcal/d)		1623	1378, 1891	1609	1381, 1881	0.84
Fat (g/d)		24.4	18.2, 32.3	26.2	18.8, 34.5	0.18
Soy (g/d)		90.0	43.6, 232.8	107.3	55.8, 225.5	0.25
Isoflavone (nmol/mg cre)		25.5	14.0, 39.9	25.9	16.6, 41.9	0.49
Cruciferous (g/d)		82.9	52.5, 113.4	81.1	48.5, 128.4	0.33
Urinary ITC (ng/mg cre)		1.70	0.70, 4.52	1.67	0.67, 4.19	0.66
		n	%	n	%	p
Currently Married		175	83.7	656	83.4	0.84
Any Alcohol Use		2	1.0	19	2.4	0.18
Current HRT Use		3	1.4	16	2.0	0.74
Postmenopausal		162	77.5	608	77.3	0.62
TB (ever)		16	7.7	54	6.9	0.71
Bronchitis (ever)		12	5.7	64	8.1	0.20
Asthma (ever)		8	3.8	13	1.7	0.06
Age at menarche	13 yrs or less	142	67.9	491	62.4	0.31
	14 yrs	32	15.3	150	19.1	
	15 yrs or more	35	16.8	146	18.6	
Education	Elementary	87	41.6	315	40.0	0.70
	Middle	58	27.8	220	28.0	
	High School	37	17.7	165	21.0	
	College	27	12.9	87	11.1	
Number of live births	0	10	4.8	19	2.4	0.02
	1	63	30.1	227	28.8	
	2	45	21.5	215	27.3	

		Cases (n=209)		Controls (n=787)		
		Median	25th, 75th	Median	25th, 75th	p*
	3 or more	91	43.5	326	41.4	
ETS	None	61	29.2	191	14.3	0.30
	One Source	100	47.9	413	52.5	
	Multiple Sources	48	22.9	183	23.3	
Family Income	Low	47	22.5	161	20.5	0.36
	Moderate-low	75	35.9	294	37.4	
	Moderate-high	47	22.5	216	27.5	
	High	39	18.7	115	14.6	
GSTM1	null/null	110	52.8	456	58.1	0.35
	null/positive	83	39.9	273	34.8	
	positive/positive	15	7.2	56	7.1	
GSTT1	null/null	108	51.9	381	48.6	0.60
	null/positive	82	39.4	344	43.9	
	positive/positive	18	8.7	59	7.5	

* p-values from wilcoxon signed rank test or wald test from conditional logistic regression.

Table 2

Association between urinary ITC levels and lung cancer

	Case/Ctls	OR*	95% CI	OR**	95% CI
ITC (ng/mg creat)***	209 / 787	1.01	0.92, 1.10	1.01	0.93, 1.11
Non-detected	18 / 71	1.0	Ref	1.0	Ref
Detected	191 / 716	1.04	0.58, 1.89	1.11	0.60, 2.04
Non-detected	18 / 71	1.0	Ref	1.0	ref
<1.91 ng ITC/mg cre	94 / 359	1.03	0.59, 2.01	1.09	0.58, 2.04
>1.91 ng ITC/mg cre	97 / 357	1.06	0.60, 2.09	1.13	0.60, 2.13
Quartile 1	52 / 196	1.0	Ref	1.0	Ref
Quartile 2	49 / 197	0.90	0.57, 1.43	1.01	0.62, 1.62
Quartile 3	49 / 197	0.93	0.58, 1.47	1.00	0.62, 1.62
Quartile 4	59 / 197	1.08	0.69, 1.69	1.10	0.69, 1.76
Cruciferous (g/day)	209 / 787	1.00	0.99, 1.00	1.00	1.00, 1.00
Quartile 1	50 / 196	1.00	Ref	1.0	Ref
Quartile 2	52 / 197	1.11	0.72, 1.72	1.12	0.71, 1.77
Quartile 3	63 / 197	1.26	0.83, 1.93	1.39	0.89, 2.18
Quartile 4	44 / 197	0.94	0.60, 1.47	0.94	0.58, 1.53

* adjusted for age;

** adjusted for age, a prior asthma diagnosis, fat intake, soy intake, ETS, education, alcohol intake, WHR, number of births, and gstm1 and gstt1 genotypes.

*** ORs reflect association for log transformed ITC

Table 3

Association between ITC and lung cancer, by menopause or ETS exposure

	Menopause status				Exposure to ETS			
	Premenopausal		Postmenopausal		Yes		No	
ITC category	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Quartile 1	1.0	Ref	1.0	Ref	1.0	Ref	1.0	Ref
Quartile 2	0.81	0.33, 1.97	1.00	0.60, 1.67	0.85	0.51, 1.43	1.25	0.52, 3.02
Quartile 3	0.83	0.32, 2.20	1.02	0.62, 1.69	0.95	0.56, 1.61	0.97	0.42, 2.24
Quartile 4	0.72	0.30, 1.72	1.26	0.76, 2.06	1.19	0.73, 1.96	0.88	0.37, 2.13
p-trend	0.48		0.37		0.41		0.67	

Odds ratios adjusted for age, (menopause or ETS), morning or evening urine collection, antibiotic use, date of sample collection, and time since last meal. Further adjustment for fat intake, soy intake, asthma, education, alcohol intake, WHR, and gstm1 and gstt1 genotypes (i vs. d) did not affect results.

Table 4

Association between any urinary ITC levels and lung cancer; by GST genotypes and menopausal status

	GSTM1					
	null*			positive		
	Cases/ctls	**OR**	**95% CI**	**Cases-ctls**	**OR**	**95% CI**
ITC**	110/510	1.01	0.99, 1.04	98/329	0.97	0.92, 1.04
Non-detected	9/44	1.0	Ref	9/27	1.0	Ref
Detected	101/412	1.12	0.44, 2.88	89/302	0.97	0.29, 3.23
Non-detected	9/44	1.0	Ref	9/27	1.0	Ref
<1.91 ng ITC/mg cre	50/209	1.02	0.39, 2.69	44/150	0.92	0.27, 3.20
>1.91 ng ITC/mg cre	51/203	1.25	0.47, 3.32	45/152	1.03	0.30, 3.55
Quartile 1	19/114	1.0	Ref	35/82	1.0	Ref
Quartile 2	32/115	1.67	0.80, 3.50	17/83	0.35	0.14, 0.89
Quartile 3	25/113	1.54	0.71, 3.33	23/81	0.47	0.20, 1.10
Quartile 4	34/114	2.22	1.05, 4.67	23/83	0.63	0.26, 1.54
p-trend		0.06			0.38	
	GSTT1					
	Null			positive		
ITC	108/381	1.02	0.98, 1.06	100/403	1.00	0.99, 1.01
Non-detected	9/37	1.0	Ref	9/91	1.0	Ref
Detected	99/344	1.88	0.72, 4.94	91/369	0.82	0.30, 2.25
Non-detected	9/37	1.0	Ref	9/34	1.0	Ref
<1.91 ng ITC/mg cre	47/171	1.75	0.65, 4.71	47/186	1.02	0.36, 2.90
>1.91 ng ITC/mg cre	52/173	2.09	0.76, 5.74	44/183	0.66	0.23, 1.87
Quartile 1	26/85	1.0	Ref	28/115	1.0	Ref
Quartile 2	26/99	0.96	0.48, 1.93	26/94	0.86	0.43, 1.73
Quartile 3	24/97	1.29	0.61, 2.72	24/109	0.53	0.24, 1.15
Quartile 4	32/100	1.23	0.59, 2.57	22/85	0.72	0.34, 1.52
p-trend		0.41			0.24	

[*] Null-homozygous deleted, positive: heterozygous or homozygous inserted. Odds ratios adjusted for age, menopause, morning or evening urine collection, antibiotic use, date of sample collection, and time since last meal. Further adjustment for fat, soy intake, ETS exposure at home or in workplace, education, any alcohol use, WHR, and number of births did not substantially alter odds ratios but substantially widened confidence intervals.

[***] ORs reflect association for log transformed ITC