Elevated Levels of Mercapturic Acids of Acrolein and Crotonaldehyde in the Urine of Chinese Women in Singapore Who Regularly Cook at Home

Stephen S. Hecht1 *, Woon-Puay Koh2,3, Renwei Wang4, Menglan Chen1, Steven G. Carmella1, Sharon E. Murphy1, Jian-Min Yuan4,5

1 Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States of America, 2 Duke-NUS Graduate Medical School, Singapore, Singapore, 3 Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore, 4 Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, United States of America, 5 Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America

* hecht002@umn.edu

Abstract

Lung cancer is unusually common among non-smoking women in Southeastern Asia but the causes of this frequently fatal disease are not well understood. Several epidemiology studies indicate that inhalation of fumes from high temperature Chinese style cooking with a wok may be a cause. Only one previous study investigated uptake of potential toxicants and carcinogens by women who cook with a wok. We enrolled three-hundred twenty-eight non-smoking women from Singapore for this study. Each provided a spot urine sample and answered a questionnaire concerning their cooking habits and other factors. The urine samples were analyzed by liquid chromatography-tandem mass spectrometry for mercapturic acid metabolites of acrolein (3-hydroxypropylmercapturic acid), crotonaldehyde (3-hydroxy-1-methylpropylmercapturic acid), and benzene (S-phenylmercapturic acid), accepted biomarkers of uptake of these toxic and carcinogenic compounds. We observed statistically significant effects of wok cooking frequency on levels of 3-hydroxypropylmercapturic acid and 3-hydroxy-1-methylpropylmercapturic acid, but not S-phenylmercapturic acid. Women who cooked greater than 7 times per week had a geometric mean of 2600 (95% CI, 2189-3090) pmol/mg creatinine 3-hydroxypropylmercapturic acid compared to 1901 (95% CI, 1510-2395) pmol/mg creatinine when cooking less than once per week (P for trend 0.018). The corresponding values for 3-hydroxy-1-methylpropylmercapturic acid were 1167 (95% CI, 1022-1332) and 894 (95% CI, 749-1067) pmol/mg creatinine (P for trend 0.008). We conclude that frequent wok cooking leads to elevated exposure to the toxicants acrolein and crotonaldehyde, but not benzene. Kitchens should be properly ventilated to decrease exposure to potentially toxic and carcinogenic fumes produced during Chinese style wok cooking.
Introduction

Lung cancer is the third most common cancer to occur among women in Singapore, and the second leading cause of cancer death [1]. There were 5520 lung cancer deaths among women in Singapore in the period 2008–2012. The age standardized mortality rate for lung cancer in females in Singapore for that period was 12.2 per 100,000, a figure which has declined only slightly in the past 40 years. Considering that most females in Singapore are non-smokers, these figures are remarkably high and reflect similar incidence data among women in China and some other parts of Southeastern Asia [2]. The relatively high incidence of female lung cancer, particularly adenocarcinoma, in Singapore and other parts of Asia, has been consistently observed and documented [2,3]. Neither tobacco smoking which is relatively rare among women in Asia nor use of solid fuels in poorly ventilated spaces which occurs only in specific regions can explain this unusually pervasive occurrence of adenocarcinoma of the lung in Asian women.

One hypothesis which has been investigated in multiple studies is that fumes from high temperature Chinese style wok cooking may be an etiologic factor for lung cancer. Women do most of the cooking in traditional Chinese households, including in Singapore. The heated oil used in wok cooking, including the common techniques of stir frying and deep frying, produces vapors which contain a variety of potentially mutagenic and carcinogenic compounds [4,5]. Multiple epidemiologic studies have examined this hypothesis with generally, although not exclusively, positive results demonstrating an association between extents of wok cooking and lung cancer risk among non-smoking Asian women [6–16]. In one recent prospective study, there was a significant association between fried meat intake, as a surrogate for high temperature wok cooking, and adenocarcinoma of the lung in non-smoking Chinese women and men in Singapore [17]. The International Agency for Research on Cancer concluded that emissions from high-temperature frying are “probably carcinogenic to humans” [18].

While mutagens and carcinogens have been identified in vapors from high temperature wok cooking, there had been no reports on uptake of these substances by the cooks. Therefore, we carried out a study in which we evaluated uptake of a variety of potential toxicants and carcinogens by 54 non-smoking Singapore women of Chinese ethnicity who regularly did wok cooking at home compared to 50 randomly chosen non-smoking women from among participants in the Singapore Chinese Health Study [19]. The results of that study demonstrated the presence of significantly higher levels of urinary mercapturic acid metabolites of benzene, acrolein, and crotonaldehyde in the cooks compared to the controls. Mercapturic acids are formed by reaction of these compounds or their metabolites with cellular glutathione, followed by metabolic processing and excretion, and are widely used biomarkers for exposure to volatile toxicants and carcinogens [20,21]. Benzene is a carcinogen while acrolein and crotonaldehyde are powerful irritants and DNA-reactive compounds which may play some role in lung carcinogenesis [22,23]. The goal of the study reported here was to further evaluate the relationship between wok cooking and urinary levels of mercapturic acids formed from benzene, acrolein, and crotonaldehyde. The analyses were carried out on urine samples from 328 Chinese women from Singapore who did varying amounts of wok cooking.

Materials and Methods

Study Design

We used a cross-sectional study design for the present study. The eligible study participants were healthy Chinese women between the ages of 45 and 74 years residing in Singapore in 2011 who never smoked cigarettes (i.e., less than 100 cigarettes in their lifetime). The estimated
The sample size of the study was 350 including 100 women who never or rarely did home cooking (≤1 time per week), 150 women who did home cooking 2–6 times per week, and 100 women who did home cooking 7 or more times per week. This sample size would provide a statistical power of 80% to detect an approximately 20–25% difference in urinary mercapturic acids of volatile organic toxicants between the two extreme exposure groups. Study subjects were identified using a combination of purposive sampling and ‘snowballing’. Eligible women, after they provided a written consent, were interviewed in person by a trained interviewer using a structured questionnaire eliciting information on study subjects’ demographics, passive smoking, coffee consumption, and home cooking (including frequency, method, and kitchen ventilation use). Each study participant provided a spot urine sample. All subjects provided written consent for participation. The study was approved by the Institutional Review Boards of the National University of Singapore, the University of Pittsburgh, and the University of Minnesota.

After the collected urine samples were brought to the laboratory at the National University of Singapore, the total volume of the urine and the pH value of the urine were measured and recorded on the study questionnaire. Four aliquots per subject, each containing 4.5 ml of urine, were prepared and stored at −80°C until analysis.

Biomarker analyses

One of the four aliquots was taken from the repository at the National University of Singapore and shipped in dry ice to the analytical chemistry laboratory at the University of Minnesota. The mercapturic acids of acrolein, crotonaldehyde, and benzene are 3-hydroxypropylmercapturic acid (3-HPMA, abbreviated HPMA in our previous study [19]), 3-hydroxy-1-methylpropylmercapturic acid (HMPMA, abbreviated HBMA previously [19]), and S-phenylmercapturic acid (SPMA), respectively. (Also note that the previously reported HBMA values must be multiplied by 5 due to an error in that report; see Cancer Epidemiology Biomarkers and Prevention doi: 10.1158/1055-9965.EPI-12-0196). The analyses for 3-HPMA and HMPMA were performed by high throughput liquid chromatography-tandem mass spectrometry using [N-acetyl-D3]3-HPMA and [N-acetyl-D3]HMPMA as internal standards, as described [24], while the following modifications were made for analysis of SPMA: 1. [D5]SPMA (12.5 ng, Toronto Research Chemicals) was also added to the urine samples as internal standard; 2. Following washing of the 96-well Oasis MAX plates with 0.7 ml of 30% methanol in 2% aqueous formic acid to elute 3-HPMA and HMPMA, the plates were washed with 0.7 ml 50% methanol in 2% formic acid to collect the fraction containing SPMA and [D5]SPMA; 3. The MS transitions monitored were m/z 238.05 → m/z 109.05 for SPMA and m/z 243.05 → m/z 114.05 for [D5]SPMA. Accuracy and precision were as follows: compound, accuracy (%), precision (coefficient of variation); 3-HPMA, 92, 9.1; HMPMA, 97, 11.0; SPMA, 99, 10.

Creatinine Analysis

Creatinine was analyzed using a colorimetric microplate assay (CRE34-K01) purchased from Eagle Bioscience (http://stores.eaglebio.com/creatinine-microplate-assay-kit).

Statistical analyses

A total of 350 women were enrolled in the study. Among them, 2 reported having smoked cigarettes based on the in-person interview (ineligible for the study). In addition, the following number of women had missing values on age (n = 1), any one of urinary mercapturic acids (n = 7), or urinary creatinine (n = 12). After excluding ineligible women or those with any missing study variables, the present study included 328 women.
Urinary levels of mercapturic acids 3-HPMA, MHBMA and SPMA were expressed in units of pmol per mg creatinine to correct for varying water contents of individual overnight urine samples. The distributions of these urinary biomarkers were markedly skewed toward high values, which were corrected, to a large extent, by transformation to logarithmic values. Therefore, formal statistical testing was performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented.

One-way analysis of variance (ANOVA) was used to examine the difference in urinary levels of mercapturic acids measured across different categories of demographics (e.g., age, body mass index, level of education, and marital status) and lifestyle factors (e.g., passive smoking exposure and consumption of coffee). Analysis of covariance (ANCOVA) was used to examine the difference in levels of these mercapturic acids by home cooking frequency. The median value of times of home cooking per week within each cooking frequency and the median value of time interval between the last cooking and urine collection were used for linear trend tests with urinary levels of these mercapturic acids. We also examined the difference in levels of these mercapturic acids by other home cooking characteristics only among women with at least 2 or more times of home cooking per week with adjustment for demographic and lifestyle factors as well as cooking frequency.

Statistical analyses were carried out using SAS software version 9.1 (SAS Institute, Cary, NC). All $P$-values reported are two-sided. $P$'s less than 0.05 were considered to be statistically significant.

### Results

The mean age at interview was $59.8 \pm 7.7$ (SD) years. Demographic characteristics and their possible effects on the mercapturic acids are summarized in Table 1. Although we did not obtain information on alcohol consumption, virtually all these nonsmoking Chinese women in Singapore were non-drinkers. There were no significant effects of age, body mass index, level of education, passive smoking exposure, or coffee drinking during the last 3 days on levels of the mercapturic acids. Married women had statistically significantly lower levels of urinary SPMA than women with other marital status. But there was no significant difference in urinary levels of 3-HPMA and HMPMA between the two marital status groups.

The levels of the mercapturic acids were similar to those reported previously[19]. Effects of cooking frequency and the time interval between the last home cooking and urine collection on levels of the mercapturic acids are summarized in Table 2. There was a statistically significant effect of cooking frequency on levels of 3-HPMA and HMPMA. Women who cooked more than 7 times per week had a geometric mean of $2600 \ (95\% \ CI, \ 2189–3090) \ pmol/mg \ creatinine$ 3-HPMA compared to $1901 \ (95\% \ CI, \ 1510–2395) \ pmol/mg \ creatinine$ for those who cooked less than one time per week ($P = 0.046$). The corresponding values for HMPMA were $1167 \ (95\% \ CI, \ 1022–1332) \ and \ 894 \ (95\% \ CI, \ 749–1067) \ pmol/mg \ creatinine$ ($P = 0.027$). In each case there were intermediate levels of the biomarkers in the women who cooked 2–6 times per week and the trends were significant, $P<0.018$ for 3-HPMA and $P<0.008$ for HMPMA. In contrast, there was no effect of cooking frequency on levels of SPMA.

Most (90%) of the study participants collected their spot urine between 9 am and 12 noon. The median time interval between the last home cooking and urine collection was 18.8 hours. There was no statistically significant difference in urinary levels of the mercapturic acids across different time intervals between the last home cooking and urine collection after adjustment for the frequency of home cooking (Table 2).

Most Chinese wok cooking is frying that includes stir-frying, pan-frying, and deep-frying methods. Among 238 women who did home cooking 2 or more times per week, 188 (79%)
reported using any of the frying cooking methods only, 19 (8%) reported using non-frying cooking methods including boiling and steaming only, and the remaining 31 (13%) used both frying and non-frying cooking methods for their home cooking. Different types of home cooking methods did not have a strong impact on levels of the three urinary mercapturic acids (Table 3). Women who did both frying and non-frying cooking had a higher level of 3-HPMA than women who did non-frying cooking only or frying cooking only, and a higher level of HMPMA than women who did frying cooking only. Women who usually used sunflower cooking oil for home cooking had significantly higher levels of urinary SPMA, 3-HPMA and HMPMA than their counterparts using other cooking oils (Table 3). Not using a ventilating hood in the kitchen did not significantly impact levels of the urinary mercapturic acids. Adding

Table 1. Levels of urinary mercapturic acids by demographic variables in non-smoking Chinese women in Singapore.

| Demographic variables          | N (%)       | Geometric means (95% CI) of mercapturic acids (pmol/mg creatinine) |
|-------------------------------|-------------|------------------------------------------------------------------|
|                               |             | SPMA                | 3-HPMA             | HMPMA             |
| Age, years                    |             |                     |                    |                   |
| 45–54                         | 99 (30.2)   | 0.50 (0.42–0.58)    | 1799 (1556–2080)   | 879 (785–984)     |
| 55–64                         | 125 (38.1)  | 0.48 (0.42–0.56)    | 2629 (2311–2991)   | 1134 (1026–1254)  |
| 65–74                         | 104 (31.7)  | 0.56 (0.48–0.64)    | 2107 (1829–2427)   | 1023 (916–1142)   |
| P for trend                   |             | 0.365               | 0.149              | 0.068             |
| Body mass index, Kg/m²        |             |                     |                    |                   |
| <20                           | 45 (13.7)   | 0.52 (0.40–0.66)    | 2096 (1682–2612)   | 1093 (923–1295)   |
| 20–<24                        | 141 (43.0)  | 0.54 (0.46–0.60)    | 2146 (1896–2430)   | 969 (881–1066)    |
| 24–<28                        | 95 (29.0)   | 0.52 (0.44–0.60)    | 2163 (1859–2516)   | 1067 (950–1199)   |
| 28+                           | 47 (14.3)   | 0.44 (0.36–0.56)    | 2455 (1980–3044)   | 991 (840–1170)    |
| P for trend                   |             | 0.339               | 0.334              | 0.913             |
| Level of education            |             |                     |                    |                   |
| No                            | 35 (10.7)   | 0.50 (0.38–0.66)    | 2316 (1806–2969)   | 1044 (861–1265)   |
| Primary                       | 112 (34.1)  | 0.52 (0.44–0.60)    | 2386 (2077–2742)   | 1049 (942–1168)   |
| Secondary                     | 140 (42.7)  | 0.46 (0.40–0.52)    | 2094 (1849–2371)   | 1008 (916–1110)   |
| High school or high           | 41 (12.5)   | 0.72 (0.56–0.92)    | 1896 (1507–2385)   | 937 (784–1119)    |
| P for trend                   |             | 0.290               | 0.085              | 0.321             |
| Marital status                |             |                     |                    |                   |
| Married                       | 237 (72.3)  | 0.48 (0.44–0.54)    | 2183 (1983–2402)   | 1003 (931–1080)   |
| Others*                       | 91 (27.7)   | 0.60 (0.52–0.72)    | 2193 (1879–2560)   | 1051 (933–1184)   |
| P for difference              |             | 0.019               | 0.959              | 0.514             |
| Passive smoking               |             |                     |                    |                   |
| No                            | 275 (83.8)  | 0.52 (0.46–0.56)    | 2152 (1969–2351)   | 1027 (959–1100)   |
| Yes                           | 53 (16.2)   | 0.52 (0.42–0.64)    | 2372 (1938–2903)   | 962 (823–1124)    |
| P for difference              |             | 0.928               | 0.388              | 0.450             |
| Coffee drinking during last 3 days |         |                     |                    |                   |
| Not drink                     | 87 (26.5)   | 0.48 (0.40–0.56)    | 1995 (1703–2336)   | 963 (853–1088)    |
| 1–3 cups                      | 168 (51.2)  | 0.52 (0.46–0.58)    | 2254 (2011–2525)   | 1049 (961–1145)   |
| 4–6 cups                      | 59 (18.0)   | 0.54 (0.44–0.66)    | 2335 (1927–2828)   | 1021 (704–1293)   |
| 7+ cups                       | 14 (4.3)    | 0.62 (0.40–0.94)    | 2026 (1366–3004)   | 954 (704–1293)    |
| P for trend                   |             | 0.228               | 0.363              | 0.703             |

* Includes never married, separated, widowed, and divorced.

doi:10.1371/journal.pone.0120023.t001
cooking wine to food during cooking was associated with a slightly increased urinary level of SPMA (P = 0.083), but did not impact urinary levels of 3-HPMA or HMPMA.

Discussion

The results of this study confirm and significantly extend our previous observation of elevated levels of the mercapturic acids of acrolein (3-HPMA) and crotonaldehyde (HMPMA) in the urine of Chinese women from Singapore who regularly cook using a wok [19]. Acrolein and crotonaldehyde are structurally related reactive α,β-unsaturated aldehydes (Fig. 1), both of which have been identified in emissions from heated cooking oil. These volatile aldehydes have multiple toxic effects which may be involved in carcinogenesis, although they are not themselves strong carcinogens. Thus, exposure to acrolein and crotonaldehyde might contribute to lung cancer etiology in non-smoking Chinese women. Our results did not however confirm our earlier observation of increased levels of SPMA, the mercapturic acid derived from benzene metabolism, in the urine of these women [19].

Many studies have identified acrolein in emissions from various heated cooking oils [4,25–34]. The type of oil and temperature to which the oil is heated are both important in determining the amount of acrolein produced. For example, Fullana and co-workers demonstrated that canola oil was a more significant source of acrolein than olive oil, at both 180°C and 240°C, with the highest amounts emitted at 240°C [27]. The presence of crotonaldehyde in these vapors has been reported less frequently and generally in lower concentrations than acrolein [27,29,31]. Both acrolein and crotonaldehyde persisted for hours in the indoor environments

| Times of cooking/week (median) | SPMA      | 3-HPMA    | HMPMA     |
|-------------------------------|-----------|-----------|-----------|
| ≤ 1 (0)                       | 0.52 (0.40–0.66) | 1901 (1510–2395) | 894 (749–1067) |
| 2–6 (5)                       | 0.46 (0.40–0.54) | 1994 (1733–2295) | 920 (826–1024) |
| 7+ (10)                       | 0.52 (0.44–0.64) | 2600 (2189–3090) | 1167 (1022–1332) |

* All geometric means were adjusted for age, body mass index (kg/m²), level of education (no, primary, secondary, high school or high), passive smoking (no, yes), marital status (married and others), and coffee consumption (none, 1–3 cups, 4–6 cups, and 7+ cups during last 3 days). For times of cooking per week, the time interval between last cooking and urine collection (<12 hours, 12–<24 hours, 24–<48 hours, 48+ hours or no cooking) was additionally adjusted. For hours between last cooking and urine collection, the frequency of cooking (<1, 1–6, and 7+/week) was additionally adjusted.

a,b The different superscript letters denote a statistically significant difference in the geometric mean of a given mercapturic acid between the two exposure levels in a pairwise multi-comparison test (P < 0.05).

† Among women who did two or more times of home cooking per week.
in which they were generated, and the concentrations could be relatively high. For example, deep-frying with different types of cooking oils resulted in indoor concentrations of acrolein ranging from 26.4–64.5 μg/m³, with a half-life of 14–22 h, indicating considerable persistence under conditions of poor ventilation [31]. Crotonaldehyde also persisted under these conditions, with a reported half-life of 20 h [31]. The findings of elevated urinary levels of all three mercapturic acids in women who regularly used sunflower oil for home cooking are intriguing and warrant further investigation.

![Fig 1. Structures of acrolein and crotonaldehyde.](https://doi.org/10.1371/journal.pone.0120023.g001)
Acrolein reacts readily with physiologic nucleophiles such as glutathione, ascorbic acid, DNA, and a variety of proteins, and induces multiple toxic effects including in the respiratory tract [34,35]. In humans, it causes intense eye and respiratory irritation which could limit exposure. Multiple inhalation studies of acrolein in laboratory animals have consistently demonstrated irritation, inflammation, cell proliferation, squamous metaplasia, interference with pulmonary function, immunosuppression, weight loss, and other toxic effects [34,35]. Acrolein is toxic to A549 lung cells in vitro and upregulates several acrolein-responsive protein markers [36]. It also activates transient receptor potential ankyrin 1 (TRPA1) channels and causes relaxation of smooth muscle in mouse isolated tracheal segments [37]. Acrolein forms well-characterized adducts in DNA and has a binding pattern in the p53 tumor suppressor gene similar to the pattern of mutations in this gene found in human lung cancer [38–40]. But carcinogenicity tests of acrolein in mice and rats have yielded negative results [35]. In one study in rats, an increased incidence of urinary bladder papillomas was observed upon intraperitoneal injection of acrolein in combination with dietary uracil [35].

Crotonaldehyde, like acrolein, is a strong irritant to the eye, skin, and respiratory tract in humans, and causes similar effects in laboratory animals [41]. It forms cyclic 1,N2-deoxyguanosine adducts in DNA, structurally similar to those produced from acrolein [38–40]. Crotonaldehyde produced increased levels of altered liver cell foci, liver damage, and neoplastic nodules in rats treated with the compound in their drinking water [42]. The International Agency for Research on Cancer has evaluated both acrolein and crotonaldehyde as “not classifiable as to their carcinogenicity in humans” [35,41].

While direct evidence for the carcinogenicity of acrolein and crotonaldehyde is weak or lacking, there is no question about their multiple toxic effects and DNA reactivity. It is plausible therefore that these properties of acrolein and crotonaldehyde may play an exacerbating role in carcinogenesis, but the specific pathways involved, if any, remain unclear. Considering these data, it would be prudent to take measures such as improved ventilation to reduce human exposure to acrolein and crotonaldehyde.

The simplest explanation for our results is a direct relationship between frequency of wok cooking and exposure to airborne acrolein and crotonaldehyde. However, it is also possible that the observed increases in mercapturic acid excretion result from exposure via the diet. Acrolein and crotonaldehyde may react with proteins or other nucleophiles in food, and these reactions may be reversible upon consumption of the cooked food, resulting in release of acrolein or crotonaldehyde, which are then detoxified by reaction with glutathione and excreted as mercapturic acids.

We did not observe an effect of frequency of wok cooking on levels of SPMA, the mercapturic acid derived from benzene metabolism, in contrast to the results of our previous study. We have no firm explanation for this result. It is possible however that it could be related to differences in mechanisms of mercapturic acid formation from acrolein, crotonaldehyde, and benzene. Acrolein and crotonaldehyde are highly reactive α,β-unsaturated carbonyl compounds which easily react with glutathione in the absence of catalysis. It is not clear whether catalysis by glutathione-S-transferases is necessary for this reaction to occur. Benzene on the other hand is completely unreactive with glutathione. It requires metabolism to benzene oxide, catalyzed mainly by cytochrome P450 2E1, as a prerequisite for formation of SPMA [22]. Most benzene oxide rapidly rearranges to phenol, but some is captured by glutathione with catalysis by glutathione-S-transferases leading ultimately to excretion of SPMA in urine. While SPMA is a well validated biomarker of benzene exposure, its more complex route of formation may detract from its overall sensitivity compared to 3-HPMA and HMPMA.

Most Chinese wok cooking is frying, including stir-frying, pan-frying, and deep-frying. Seventy-nine percent of the women in our study reported using exclusively frying methods. As
described above, deep-frying could generate substantial amounts of both acrolein and crotonaldehyde [31] whereas there are no reported data on the emission of these volatile toxicants from stir-frying or pan-frying, which use relatively lower oil temperatures than deep-frying. In our questionnaire, we did not separate these different frying methods, and were therefore unable to examine the impact of the different frying methods on urinary levels of the biomarkers. Only 6% of the women prepared their food using non-frying methods, which presumably produce lower quantities of volatile toxicants than frying. In the present study, the mean level of 3-HPMA in urine from women who did non-frying cooking was lower than those who did frying cooking, but the difference was statistically borderline significant due to the small sample size of non-frying cooking. We did observe however that the use of both cooking methods resulted in a significant increase in 3-HPMA and HMPMA compared to frying cooking only, but the basis for this observation is unclear.

This study has certain limitations. Our results were based on a single spot urine sample. We do not know if the results of that analysis are generalizable to a given woman’s typical exposure. We also did not perform air monitoring for volatiles in the kitchens where the women cooked. Multiple urine samples and air monitoring could be considered in future studies, but these activities were not compatible with our budget for the study reported here. A more detailed examination of frying methods could also be incorporated in future investigations. The relatively small sample size, especially with respect to specific cooking methods, might have limited the study power to detect a statistically significant effect.

In summary, the present study demonstrates that women who did frequent wok cooking but did not smoke cigarettes or drink alcoholic beverages had significantly increased levels of urinary 3-HPMA and HMPMA, the respective mercapturic acids of acrolein and crotonaldehyde, and the levels of the biomarkers increased with increasing cooking frequency. These findings confirm and significantly extend our previous observation of elevated levels of these biomarkers in a convenience sample of Chinese women in Singapore with a much smaller sample size. The present data, together with multiple epidemiologic studies, demonstrate the need for preventive measures such as improved ventilation to efficiently remove cooking oil fumes.

Acknowledgments

We thank Ms. Siew-Hong Low of the National University of Singapore for supervising the field work of the Singapore Chinese Health Study.

Author Contributions

Conceived and designed the experiments: SSH W-PK J-MY. Performed the experiments: MC SGC SEM. Analyzed the data: SSH RW SGC SEM J-MY. Contributed reagents/materials/analysis tools: RW MC SGC SEM. Wrote the paper: SSH SEM J-MY.

References

1. National Registry of Diseases Office Health Promotion Board. Singapore Cancer Registry interim annual registry report. Trends in cancer incidence in Singapore, 2008–2012. Singapore: National Registry of Diseases Office; 2013. doi: 10.1136/bjophthalmol-2012-302032 PMID: 23462834
2. International Agency for Research on Cancer. The global and regional burden of cancer. In: Stewart BW, Wild CP, editors. World cancer report 2014. Lyon, FR: International Agency for Research on Cancer; 2014. pp. 16–53.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55: 74–108. PMID: 15761078
4. Pellizzari ED, Michael LC, Thomas KW, Shields PG, Harris C. Identification of 1,3-butadiene, benzene, and other volatile organics from wok oil emissions. J Expo Anal Environ Epidemiol. 1995; 5: 77–87. PMID: 7663151
5. Shields PG, Xu GX, Blot WJ, Fraumeni J, Trivers GE, Pellizzari ED, et al. Mutagens from heated Chinese and U.S. cooking oils. J Natl Cancer Inst. 1995; 87: 836–841. PMID: 7791233
6. Yu IT, Chiu YL, Au JS, Wong TW, Tang JL. Dose-response relationship between cooking fumes exposures and lung cancer among Chinese nonsmoking women. Cancer Res. 2006; 66: 4961–4967. PMID: 16651454
7. Gao YT, Zheng W, Ershow AG, Hsu CW, Levin LI, Zhang R, et al. Lung cancer among Chinese women. Int J Cancer. 1987; 40: 604–609. PMID: 2824385
8. Ko YC, Lee CH, Chen MJ, Huang CC, Chang WY, Lin HJ, et al. Risk factors for primary lung cancer among non-smoking women in Taiwan. Int J Epidemiol. 1997; 26: 24–31. PMID: 9126500
9. Zhong L, Goldberg MS, Gao YT, Jin F. Lung cancer and indoor air pollution arising from Chinese-style cooking among nonsmoking women living in Shanghai, China. Epidemiology. 1999; 10: 488–494. PMID: 10468420
10. Seow A, Poh WT, Teh M, Eng P, Wang YT, Tan WC, et al. Fumes from meat cooking and lung cancer risk in Chinese women. Cancer Epidemiol Biomarkers Prev. 2000; 9: 1215–1221. PMID: 11097230
11. Metayer C, Wang Z, Kleinerman RA, Wang L, Brenner AV, Cui H, et al. Cooking oil fumes and risk of lung cancer in women in rural Gansu, China. Lung Cancer. 2002; 35: 111–117. PMID: 11804682
12. Wang XR, Chiu YL, Qiu H, Au JS, Yu IT. The roles of smoking and cooking emissions in lung cancer risk among Chinese women in Hong Kong. Ann Oncol. 2009; 20: 746–751. doi:10.1093/annonc/mdn699 PMID: 19150939
13. Lin Y, Cai L. Environmental and dietary factors and lung cancer risk among Chinese women: a case-control study in southeast China. Nutr Cancer. 2012; 64: 508–514. doi:10.1080/01635581.2012.668743 PMID: 22489989
14. Chiu YL, Wang XR, Qiu H, Yu IT. Risk factors for lung cancer: a case-control study in Hong Kong women. Cancer Causes Control. 2010; 21: 777–785. doi:10.1007/s10552-010-9506-9 PMID: 20084541
15. Mu L, Liu L, Niu R, Zhao B, Shi J, Li Y, et al. Indoor air pollution and risk of lung cancer among Chinese female non-smokers. Cancer Causes Control. 2013; 24: 439–450. doi:10.1007/s10552-012-0130-8 PMID: 23314675
16. Tang L, Lim WY, Eng P, Leong SS, Lim TK, Ng AW, et al. Lung cancer in Chinese women: evidence for an interaction between tobacco smoking and exposure to inhalants in the indoor environment. Environ Health Perspect. 2010; 118: 1257–1260. doi: 10.1289/ehp.0901587 PMID: 20472525
17. Butler LM, Montague JA, Koh WP, Wang R, Yu MC, Yuan JM. Fried meat intake is a risk factor for lung adenocarcinoma in a prospective cohort of Chinese men and women in Singapore. Carcinogenesis. 2013; 34: 1794–1799. doi:10.1093/carcin/bgt113 PMID: 23568952
18. International Agency for Research on Cancer. Household use of solid fuels and high-temperature frying. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, v. 95. Lyon, FR: IARC; 2010. pp. 392–393.
19. 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: 11185–11192. doi: 10.1158/1055-9965.EPI-09-1291 PMID: 20406956
20. Hecht SS, Yuan J-M, Hatsukami DK. Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. Chem Res Toxicol. 2010; 23: 1001–1008. doi:10.1021/tx100056m PMID: 20485654
21. Mathias PI, B’Hymer C. A survey of liquid chromatographic-mass spectrometric analysis of mercapturic acid biomarkers in occupational and environmental exposure monitoring. J Chromatogr B Analyt Technol Biomed Life Sci. 2014; 964C: 136–145.
22. International Agency for Research on Cancer. Chemical agents and related occupations. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, v. 100F. Lyon, France: IARC; 2012. pp. 249–294.
23. International Agency for Research on Cancer. Dry Cleaning, Some chlorinated solvents and other industrial chemicals. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vol. 63. Lyon, France: IARC; 1995. pp. 337–391. PMID: 9097100
24. 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 Chromatogr B. 2013; 935: 36–40. doi: 10.1016/j.jchromb.2013.07.004 PMID: 23934173
25. Umano K, Shibamoto T. Analysis of acrolein from heated cooking oils and beef fat. J Agric Food Chem. 1987; 35: 909–912.
26. Lin JM, Liou SJ. Aliphatic aldehydes produced by heating Chinese cooking oils. Bull Environ Contam Toxicol. 2000; 64: 817–824. PMID: 10858338
27. Fullana A, Carbonell-Barrachina AA, Sidhu S. Comparison of volatile aldehydes present in the cooking fumes of extra virgin olive, olive, and canola oils. J Agric Food Chem. 2004; 52: 5207–5214. PMID: 15291498

28. da Silva TO, Pereira PA. Influence of time, surface-to-volume ratio, and heating process (continuous or intermittent) on the emission rates of selected carbonyl compounds during thermal oxidation of palm and soybean oils. J Agric Food Chem. 2008; 56: 3129–3135. doi: 10.1021/jf0734525 PMID: 18422332

29. Schauer JJ, Kleeman MJ, Cass GR, Simoneit BR. Measurement of emissions from air pollution sources. 4. C1-C27 organic compounds from cooking with seed oils. Environ Sci Technol. 2002; 36: 567–575. PMID: 11883419

30. Bastos LC, Pereira PA. Influence of heating time and metal ions on the amount of free fatty acids and formation rates of selected carbonyl compounds during the thermal oxidation of canola oil. J Agric Food Chem. 2010; 58: 12777–12783. doi: 10.1021/jf1028575 PMID: 21105653

31. Seaman VY, Bennett DH, Cahill TM. Indoor acrolein emission and decay rates resulting from domestic cooking events. Atmospheric Environment. 2009; 43: 6199–6204.

32. Katragadda HR, Fullana A, Sidhu S, Carbonell-Barrachina AA. Emissions of volatile aldehydes from heated cooking oils. Food Chem. 2010; 120: 59–65.

33. Ho SSH, Yu JZ, Chu KW, Yeung LL. Carbonyl emissions from commercial cooking sources in Hong Kong. J Air Waste Manage Assoc. 2006; 56: 1091–1098.

34. Stevens JF, Maier CS. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Mol Nutr Food Res. 2008; 52: 7–25. doi: 10.1002/mnfr.200700412 PMID: 18203133

35. International Agency for Research on Cancer. Acrolein. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vol. 63. Lyon, FR: IARC; 1995. pp. 337–372. PMID: 9097100

36. Burcham PC, Raso A, Thompson CA. Toxicity of smoke extracts towards A549 lung cells: role of acrolein and suppression by carbonyl scavengers. Chem Biol Interact. 2010; 183: 416–424. doi: 10.1016/j.cephb.2009.12.006 PMID: 20015449

37. Cheah EY, Burcham PC, Mann TS, Henry PJ. Acrolein relaxes mouse isolated tracheal smooth muscle via a TRPA1-dependent mechanism. Biochem Pharmacol. 2014; 89: 148–156. doi: 10.1016/j.bcp.2014.02.009 PMID: 24561178

38. Chung FL, Young R, Hecht SS. Formation of cyclic 1,N2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. Cancer Res. 1984; 44: 990–995. PMID: 6318992

39. Minko IG, Kozeckov ID, Harris TM, Rizzo CJ, Lloyd RS, Stone MP. Chemistry and biology of DNA containing 1,N2-deoxyguanosine adducts of the alpha,beta-unsaturated aldehydes acrolein, crotonaldehyde, and 4-hydroxynonenal. Chem Res Toxicol. 2009; 22: 759–778. doi: 10.1021/tr9000489 PMID: 19397281

40. Feng Z, Hu W, Hu Y, Tang M-S. Acrolein is a major cigarette-related lung cancer agent. Preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proc Natl Acad Sci USA. 2006; 103: 15404–15409. PMID: 17030796

41. International Agency for Research on Cancer. Dry cleaning, some chlorinated solvents and other industrial chemicals. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vol. 63. Lyon, France: IARC; 1995. pp. 373–391. PMID: 9097101

42. Chung FL, Tanaka T, Hecht SS. Induction of liver tumors in F344 rats by crotonaldehyde. Cancer Res. 1986; 46: 1285–1289. PMID: 3002613