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Different Profiles of Carcinogen Exposure in Chinese compared to U.S. Cigarette Smokers

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Running Title: Carcinogen exposure in U.S. and Chinese smokers

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Tables: 2
Abstract

Background

Differences in carcinogen exposure from different cigarette products could contribute to differences in smoking-associated cancer incidence among Chinese compared to U.S. smokers.

Methods

Urine concentrations of metabolites of nicotine, the tobacco-specific nitrosamine (TSNA) 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and polycyclic aromatic hydrocarbon metabolites (PAHs) were compared in 238 Chinese and 203 U.S. daily smokers.

Results

Comparing Chinese vs U.S. smokers, daily nicotine intake and nicotine intake per cigarette smoked was similar. When normalized for cigarettes per day urine NNAL excretion was 4-fold higher in U.S. smokers, while the excretion of urine metabolites of the PAHs fluorene, phenanthrene and pyrene metabolites were 50% to 4-fold higher in Chinese smokers (all, p<0.0001). Similar results were seen when NNAL and PAHs excretion was normalized for daily nicotine intake.

Conclusions

Patterns of carcinogen exposure differ, with lower exposure to TSNA and higher exposure to PAHs in Chinese compared to U.S. smokers. These results likely reflect country differences in cigarette tobacco blends and manufacturing processes, as well different environmental exposures.
**Introduction**

China is the world’s largest producer and consumer of tobacco products\(^1\). In 2010 the adult smoking prevalence was 28%, including 53% of men age 15 or older\(^2\). Cancer accounts for a large fraction of tobacco-caused deaths, with lung cancer the most common cancer. While lung cancer is a major health problem in Chinese smokers, the relative risk of lung cancer is lower for Chinese compared to U.S. smokers (RR 2.5 and 25, respectively)\(^1,3\). Three widely cited explanations for this difference are 1) that Chinese smokers smoke fewer cigarettes per day, 2) Chinese smokers start smoking at a later age, and 3) genetic differences make Chinese smokers less susceptible to smoking-induced lung cancer than U.S. smokers\(^1,4,5\). Another explanation that should be considered is a difference in cigarette products that results in different profiles of exposure to tobacco smoke carcinogens.

Cigarettes expose smokers to more than 70 carcinogens. Of particular concern with respect to lung cancer are: tobacco-specific nitrosamines (TSNAs, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK] and N’-nitrosonornicotine [NNN]) and polycyclic aromatic hydrocarbons (PAHs).\(^6,7\) Biomarker levels of TSNAs and PAHs independently correlate with the risk of lung cancer\(^8-10\), and are the focus of our analysis.

Cigarettes sold in China contain and generate by machine testing lower levels of TSNAs compared to U.S. cigarettes, even within the same global brands such as Marlboro or Camel\(^11-13\). Consistent with this observation, biomarkers of NNK exposure are on average lower in Chinese compared to U.S. smokers\(^10\). An important factor in cigarette design that determines TSNA levels is nitrate content; high nitrate content results in greater nitrosation of nicotine and more generation of TSNAs during curing and smoking\(^14\). High nitrate content of tobacco is also inversely correlated with the pyrosynthesis of PAHs when cigarettes are smoked\(^15\). Different
types of tobacco have different nitrate content and generate different levels of TSNAs and PAHs in mainstream smoke \(^\text{16}\).

To better understand possible reasons for differences in cancer risk among Chinese and U.S. smokers, we measured biomarkers of TSNA and PAH exposure in smokers from the two countries. Since smokers smoke to obtain desired levels of nicotine, we also assessed a biomarker of daily nicotine intake so that we could normalize carcinogen exposure for nicotine intake.

**Methods**

**Subjects**

As described previously \(^\text{17}\), the Chinese subjects for the present analysis were taxi drivers smoking Chinese brand cigarettes who were recruited in driver physical examination centers in Shanghai, China from January to April 2006. 238 subjects were selected randomly from a total of 543 smokers in the original study group for analysis in the present study. The subjects were healthy men between the ages of 18 and 65 who reported smoking 5 or more cigarettes per day. Each subject provided a smoking history and a single void spot urine sample. The United States smokers came from two studies conducted in San Francisco in which 203 subjects provided a detailed smoking history and urine sample: one of smokers who attended a research clinic \(^\text{18}\) and the other participants in a clinical trial of reduced nicotine content cigarettes (baseline evaluation) \(^\text{19}\).

The studies were approved by the University of California San Francisco Committee on Human Research and the Shanghai Center for Disease Control and Prevention Committee on Human Subjects. Subjects provided written consent.
The urine samples from China were frozen and shipped to San Francisco General Hospital for analysis. Urine total (free + conjugated) concentrations of nicotine, cotinine and trans-3’-hydroxycotinine were measured by LC-MS/MS as described previously. Urine concentrations of total NNAL were measured by LC-MS/MS as described previously. Several PAH metabolites: 2-napthol (2-Nap), 1-hydroxyfluorene, 2-hydroxyfluorene, 3-hydroxyflourene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3+4-hydroxyphenanthrene, and 1 hydroxypyrene (1-HP) were determined by LC-MS/MS as described previously. Details on limits of quantitation and quality control measures for various assays are provided in the assay methodology papers cited above. Urine creatinine was measured in the San Francisco General Hospital clinical laboratory using a colorimetric assay.

Urine total nicotine equivalents (TNE) was determined as the molar sum of nicotine, cotinine, trans 3’-hydroxycotinine and their respective glucuronides, normalized for creatinine concentration. When measured at steady state, the sum of these metabolites accounts for 80 to 90% of a daily dose of nicotine. Nicotine equivalents measured in this way are highly correlated with daily intake of nicotine validated by the administration of labeled nicotine in steady state conditions. Because it represents the sum of nicotine metabolites generated by various pathways at steady state, urine total nicotine equivalents are expected to be unaffected by racial differences in rates and pathways of nicotine metabolism, as are known to occur in people of Chinese, African and Caucasian ancestry. We assessed fluorene and phenanthrene exposure as the molar sum of their several metabolites measured in urine.
Statistical Analysis

Comparisons of demographic characteristics and urine biomarker levels of Chinese vs U.S. smoker groups were performed using t test to compare means (age, tar level and cigarettes per day, which were normally distributed) and the Mann-Whitney rank sum test to compare medians (urine biomarkers, which were not normally distributed). Because all Chinese smokers were men but U.S. smokers were both men and women, we performed analyses including and excluding women. Spearman rank correlations between cigarettes per day and various biomarkers among Chinese and among U.S. smokers were computed.

Results

Demographic Data

Demographic data for our subjects are provided in Table 1. The U.S. population included 134 white and 69 black smokers. The average age was significantly higher for Chinese compared to U.S. smokers. Chinese smokers were all men, whereas about 60% of U.S. smokers were men. The nominal tar delivery was significantly lower for cigarettes used by Chinese compared to U.S. smokers (12.2 vs 13.6 mg, p < 0.0001). Chinese smokers smoked an average of 18.0 cigarettes per day compared to 19.5 for U.S. smokers, but the difference was not statistically significant.

<table>
<thead>
<tr>
<th></th>
<th>Chinese (n=238)</th>
<th>U.S. (n=203)</th>
<th>p-valuea</th>
<th>U.S. Men (n=118)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>44.3 (43.0-45.5)b</td>
<td>38.3 (36.7-39.8)b</td>
<td>&lt; 0.0001c</td>
<td>39-8 (3.77-41.8)b</td>
<td>0.0001c</td>
</tr>
</tbody>
</table>

Table 1. Demographics and Urine Biomarkers in Chinese and U.S. Smokers (values are medians and IQR, unless otherwise indicated)
<table>
<thead>
<tr>
<th></th>
<th>Male (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>238 (100%)</td>
<td>118 (58%)</td>
<td>&lt; 0.0001</td>
<td>118 (100%)</td>
</tr>
<tr>
<td>Tar</td>
<td>12.2 (11.9-12.6)</td>
<td>13.6 (13.0-14.2)</td>
<td>&lt; 0.0001</td>
<td>14.2 (13.4-14.9)</td>
</tr>
<tr>
<td>CPD</td>
<td>18.0 (16.7-19.3)</td>
<td>19.5 (18.2-20.7)</td>
<td>NS</td>
<td>20.6 (18.9-22.4)</td>
</tr>
<tr>
<td>Nicotine Eq (nmol/mg creat)</td>
<td>53.4</td>
<td>54.8</td>
<td>NS</td>
<td>54.6</td>
</tr>
<tr>
<td>NNAL (pmol/mg creat)</td>
<td>0.29 (32.8-78.9)</td>
<td>1.19 (42.7-81.6)</td>
<td>&lt; 0.0001</td>
<td>1.12 (38.2-78.6)</td>
</tr>
<tr>
<td>1HP (pmol/mg creat)</td>
<td>3.64 (0.18-0.47)</td>
<td>1.17 (0.63-2.06)</td>
<td>&lt; 0.0001</td>
<td>1.10 (0.62-1.81)</td>
</tr>
<tr>
<td>2Nap (pmol/mg creat)</td>
<td>103 (65.3-149)</td>
<td>98.3 (59.7-149)</td>
<td>NS</td>
<td>88.7 (52.0-130.3)</td>
</tr>
<tr>
<td>Sum of Fluorenes (pmol/mg creat)</td>
<td>23.5 (15.8-32.9)</td>
<td>16.6 (10.8-24.5)</td>
<td>&lt; 0.0001</td>
<td>16.0 (10.6-22.4)</td>
</tr>
<tr>
<td>Sum of Phenantrenes (pmol/mg creat)</td>
<td>9.52 (6.56-13.5)</td>
<td>3.54 (2.44-5.07)</td>
<td>&lt; 0.0001</td>
<td>3.53 (2.52-4.90)</td>
</tr>
<tr>
<td>Sum of PAHs (pmol/mg creat)</td>
<td>145 (6.56-13.5)</td>
<td>123 (2.44-5.07)</td>
<td>0.082</td>
<td>110 (2.52-4.90)</td>
</tr>
<tr>
<td>Nicotine Eq/CPD (nmol/mg creat)</td>
<td>3.16 (1.76-5.23)</td>
<td>3.13 (2.07-5.06)</td>
<td>NS</td>
<td>2.87 (1.88-4.02)</td>
</tr>
<tr>
<td>NNAL/CPD (fmol/mg creat)</td>
<td>18.9</td>
<td>73.4</td>
<td>&lt; 0.0001</td>
<td>62.1 (11.3-32.7)</td>
</tr>
<tr>
<td>1HP/CPD (pmol/mg creat)</td>
<td>0.24 (11.3-32.7)</td>
<td>0.06 (37.4-112)</td>
<td>&lt; 0.0001</td>
<td>0.05 (28.8-99.3)</td>
</tr>
<tr>
<td>2Nap/CPD (pmol/mg creat)</td>
<td>6.04 (0.15-0.40)</td>
<td>5.53 (0.04-0.10)</td>
<td>NS</td>
<td>4.68 (0.04-0.08)</td>
</tr>
<tr>
<td>Sum of Fluorenes/CPD (pmol/mg creat)</td>
<td>1.47 (0.93-2.21)</td>
<td>0.98 (0.56-1.42)</td>
<td>&lt; 0.0001</td>
<td>0.82 (0.53-1.25)</td>
</tr>
<tr>
<td>Sum of Phenantrenes/CPD (pmol/mg creat)</td>
<td>0.60 (0.39-0.93)</td>
<td>0.19 (0.13-0.29)</td>
<td>&lt; 0.0001</td>
<td>0.18 (0.11-0.29)</td>
</tr>
<tr>
<td>Sum PAHs/CPD (pmol/mg creat)</td>
<td>8.41 (5.56-12.9)</td>
<td>7.00 (4.55-10.3)</td>
<td>0.0002</td>
<td>5.66 (4.02-8.25)</td>
</tr>
<tr>
<td>NNAL/Nicotine Eq x 10^6</td>
<td>5.55</td>
<td>22.0</td>
<td>&lt; 0.0001</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>1HP/ Nicotine Eq x 10^3</td>
<td>2Nap/Nicotine Eq x10^3</td>
<td>Sum of Fluorene/Nicotine Eq x 10^3</td>
<td>Sum of Phenantrene/Nicotine Eq x 10^3</td>
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<td></td>
<td>(3.74-11.8)</td>
<td>(14.2-31.8)</td>
<td>(12.9-31.4)</td>
<td>(0.05-0.13)</td>
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<tr>
<td></td>
<td>0.07</td>
<td>0.02</td>
<td>&lt; 0.0001</td>
<td>0.02</td>
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<td></td>
<td>(0.01-0.03)</td>
<td>&lt; 0.0001</td>
<td>(1.13-2.42)</td>
<td>1.54</td>
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<td>(0.01-0.03)</td>
<td>0.43</td>
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<td>&lt; 0.0001</td>
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<td>1.99</td>
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<td>(0.06-0.08)</td>
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<td>(0.04-0.08)</td>
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<td></td>
<td></td>
<td>(1.99-2.65)</td>
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</table>

Among U.S. smokers, men were significantly older (39.7 vs 36.1 years, p = 0.02), tar yield was higher (14.2 vs 12.8, p = 0.03) and cigarettes per day were higher (20.6 vs 17.8, p = 0.02) compared to women. Among U.S. smokers, comparing black vs white smokers, there were significant differences in age (42.0 vs 36.3 years), tar yield (16.2 vs 12.3 mg), and cigarettes per day (17.5 vs 20.5). There were no significant black vs white differences in TNE, NNAL or metabolites of 1-HP, fluorene or phenanthrenes per cigarette smoked. 2-napthol excretion per cigarette was significantly lower in blacks vs whites (medians 4.49 vs 5.79, p = 0.008).

Nicotine and Carcinogen Exposure (Table 1, Figure 1)

Daily nicotine intake, assessed as urinary TNE per mg creatinine or TNE per cigarette smoked per day were not significantly different in Chinese compared to U.S. smokers. Urine NNAL levels, expressed either as absolute values, normalized for cigarettes per day or normalized for urine nicotine equivalents were approximately 4-fold higher in U.S. compared to Chinese smokers. Urine concentrations of three PAH metabolites (1-HP, sum of fluorene and sum of phenanthrene metabolites) as well as the sum of all PAH metabolites were significantly higher in Chinese compared to U.S. smokers, both for absolute values or when normalized for
cigarettes per day or for nicotine equivalents. The excretion of 1-HP and phenanthrene metabolites was approximately 3-fold higher in Chinese compared to U.S. smokers. The excretion of the PAH metabolite 2-napthol, expressed as absolute values or normalized for cigarettes per day, was not significantly different in Chinese vs U.S. smokers, but when normalized for urine nicotine equivalents 2-naphthol excretion was significantly higher in Chinese smokers. Differences between Chinese and U.S. smokers were similar when women were included or excluded from the analysis.

**Table 2.** Correlation between biomarkers within groups by country (Chinese vs. U.S.). Spearman rank coefficients.

<table>
<thead>
<tr>
<th></th>
<th>CPD</th>
<th>Nicotine Eq</th>
<th>NNAL</th>
<th>1HP</th>
<th>2NP</th>
<th>Sum of Fluor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine Eq</td>
<td>0.23* vs. 0.25*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNAL</td>
<td>0.28* vs. 0.27*</td>
<td>0.49* vs. 0.65*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1HP</td>
<td>0.20* vs. 0.19</td>
<td>0.39* vs. 0.39*</td>
<td>0.32* vs. 0.39*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2NP</td>
<td>0.19* vs. 0.37*</td>
<td>0.76* vs. 0.62*</td>
<td>0.48* vs. 0.47*</td>
<td>0.48* vs. 0.44*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of Fluor</td>
<td>0.26* vs. 0.23*</td>
<td>0.73* vs. 0.66*</td>
<td>0.53* vs. 0.54*</td>
<td>0.54* vs. 0.72*</td>
<td>0.75* vs. 0.65*</td>
<td></td>
</tr>
<tr>
<td>Sum Phen</td>
<td>0.18* vs. 0.13</td>
<td>0.22* vs. 0.41*</td>
<td>0.29* vs. 0.35*</td>
<td>0.70* vs. 0.79*</td>
<td>0.32* vs. 0.43*</td>
<td>0.56* vs. 0.78*</td>
</tr>
</tbody>
</table>

* - significant correlation, p<0.05

**Discussion**

We present novel data on urine biomarkers of nicotine and carcinogen exposure among Chinese compared to U.S. smokers. We found that the daily intake of nicotine, assessed by urinary nicotine equivalents, as well as nicotine intake per cigarette, was similar in Chinese compared to U.S. smokers. Measured either as absolute concentrations or as concentrations normalized for cigarettes smoked per day, exposure to the tobacco specific nitrosamine NNK,
assessed by urinary total NNAL, was 4-fold higher in U.S. compared to Chinese smokers. On the other hand, exposure to three PAHs was significantly higher among Chinese compared to U.S. smokers. On average pyrene and phenanthrene exposures were 3 to 4-fold higher and fluorene exposure 50% higher in Chinese compared to U.S. smokers. Differences in NNAL and PAH exposure were similar after normalization by daily nicotine intake, as assessed by urine nicotine equivalents. These findings may have implications in understanding differences in tobacco-related disease risks among smokers in the two countries.

Although not directly compared to U.S. smokers and not normalized for nicotine intake, urine NNAL levels in Chinese smokers as reported by Yuan were lower than those typically found in U.S. smokers\textsuperscript{10,27}. Other authors have reported higher levels of a metabolite of the PAH phenanthrene (\textit{r-1,t-2,3,c-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; Phe-T}) in non-smokers from Shanghai compared to non-smokers in the U.S., but we unaware of a direct comparison of multiple PAH metabolites in Chinese vs U.S. smokers, and no prior studies in which PAH exposure was normalized for nicotine intake (a marker of total smoke exposure)\textsuperscript{28}.

Tobacco specific nitrosamines and PAHs are two major classes of tobacco carcinogens\textsuperscript{29}. The nitrosamine NNK has been implicated causing in oral cancer, lung cancer, and pancreatic cancer\textsuperscript{30}. Tobacco specific nitrosamine levels in cigarette tobacco vary widely among cigarettes and across countries\textsuperscript{11,12}. Tobacco specific nitrosamine levels in mainstream smoke of one popular brand Chinese cigarettes were found to be almost 40-fold lower than those of two popular U.S. cigarette brands\textsuperscript{12}. Tobacco specific nitrosamines are formed during the curing, processing and fermenting of cigarette tobacco, and may also form during cigarette combustion\textsuperscript{29,31}. The level of TSNAs depends on the type of tobacco used, the nitrate content of the tobacco and the curing process. Chinese cigarettes tend to be made of bright tobacco which is flue-cured
and is relatively low in TSNA content. In contrast, U.S. cigarettes are typically made of blends of tobacco that contain considerable amounts of burley and reconstituted tobacco that have much higher TSNA levels. Thus, country difference in the type of tobacco used and the way tobacco is processed likely underlie our observed country differences in NNK exposure.

PAHs are a diverse group of carcinogens formed during the combustion of tobacco and other organic materials. PAHs are found in tobacco smoke, broiled foods and polluted environments. Several of the higher molecular weight PAHs such as benzo(a)pyrene are highly carcinogenic in animals, including induction of lung tumors. PAHs form adducts with DNA are found in p53 mutations and have been associated with increased risk of human lung cancer. Akplan reported that the PAH levels in mainstream smoke from Chinese cigarettes were higher than those of European cigarettes, although in that study the nicotine and tar levels were also higher in Chinese cigarettes.

To the best of our knowledge, our study is the first to describe the excretion of multiple tobacco smoke-derived PAH metabolites in Chinese smokers. We measured several PAH metabolites because different sources of combustion may result in different patterns of PAH generation and exposure. For example, we recently reported different patterns of PAH exposure comparing smokers of cigarettes vs water pipers. We observed that country differences were greater for some PAHs (pyrene and phenanthrene) than for others (fluorene and naphthylene). As mentioned previously phe-T, a metabolite of phenanthrene, has been found to be higher in Shanghai non-smokers compared to U.S. non-smokers. PAH generation from cigarettes appears to be related to nitrate content and the type of tobacco. PAH generation is inversely related to nitrate content, believed to be a result of nitrogen oxides formed during tobacco combustion scavenging carbon and hydrogen radicals that are major precursors for the pyrosynthesis of
PAHs. PAH yields are higher from bright tobacco, the primary type of tobacco in Chinese cigarettes; and PAH yields are lower in reconstituted and burley tobacco, as found in U.S. blends. Thus, our findings of country differences in urinary PAH excretion are consistent with expectations from differences in the type of tobacco used in Chinese vs. U.S. cigarettes. In general factors that increase TSNA levels decrease PAHs generation and vice versa. In the present study, machine-determined tar yields were lower on average for cigarettes smoked by Chinese compared to U.S. smokers. Such differences are not likely to explain our results because tar emissions by machine testing are not meaningful measures of smoke exposure.

Because smokers smoke to obtain desired levels of nicotine, it is important to consider carcinogen exposure in relation to nicotine intake. We found that large country differences in nitrosamine and PAHs exposure remained after normalizing for each individual’s daily intake of nicotine.

PAHs are important environmental pollutants, formed by incomplete combustion of organic materials. Major sources are motor vehicle exhaust, coal and oil fed power plants and cooking. Since industrial pollution may be higher in Shanghai than in San Francisco, one must consider the contribution of environmental sources for the higher PAHs observed in Chinese smokers, which has been observed for phenanthrene and pyrene in Chinese nonsmokers exposed to industrial pollution. Also there may be greater exposure to fried foods, another source of PAHs, among Chinese smokers. An argument against environmental sources as the sole explanation is that in Chinese as well as U.S. smokers there were similarly strong correlations between various urine PAH metabolite levels and level of cigarette smoke exposure, evidenced either by cigarettes per day or urine TNE. We reasoned that if the PAHs metabolites in Chinese smokers were derived primarily from environmental pollution rather than tobacco smoke, there
would be weaker correlations between PAH metabolites and tobacco smoke exposure among Chinese compared to U.S. smokers. That the correlations were similarly strong suggests that differences in environmental exposures do not fully explain country differences in PAH exposure. We cannot however exclude the possibility that among Chinese smokers there is a correlation between cigarettes smoking and exposure to environmental pollutants which could contribute to the positive correlations between PAH exposure and cigarettes per day or TNE.

A limitation of our study is that we do not have a non-smoker control group for PAH exposure in China. In addition, our Chinese smokers came primarily from one city in China, Shanghai, and U.S. smokers came from one city in the U.S., San Francisco. This raises questions about generalizability. In support of generalizability among the San Francisco smokers is that biomarker data of this population are similar to that reported in NHANES, which is a representative U.S. population. We know of no similar biomarker data collected from the general Chinese population. However, since all Chinese cigarette brands are sold nationally, one would expect that similar products are used in Shanghai compared to other parts of China, and we can expect biomarkers of carcinogen exposure to be similar to national values. The Chinese smokers worked as taxi drivers, which may have exposed them to higher levels of air pollution than the U.S. smokers who were volunteer research subjects with various occupations. Another generalizability concern arises from the heterogeneity of sex and race in U.S. smokers. Sex does not appear to be an explanation in that differences in biomarkers of exposure between Chinese and U.S. smokers were similar with or without inclusion of women in the analysis. Comparison of differences in biomarkers in U.S. African American vs Caucasian smokers indicated few differences, so we do not believe that racial heterogeneity explains the observed country differences.
Both TSNAs and PAHs are tumorigenic and urine metabolite levels have been independently associated with the risk of lung cancer among smokers. Our data demonstrate that the ratio of NNK/PAH exposure is much higher in U.S. compared to Chinese smokers. The ratio of NNK/PAH exposure among U.S. smokers has changed from the 1950’s to the present, during which time PAH deliveries have declined while NNK levels have increased. Among U.S. smokers the incidence of lung cancer has increased during this period of time, with a substantial increase in the proportion lung cancers that are adenocarcinoma compared to squamous cell carcinoma. In that Chinese smokers have markedly lower NNK exposure, lung cancer risk would be expected to decrease, but as they have higher PAH exposure, their lung cancer risk would be expected to be increased compared to U.S. smokers. The net effect of this oppositional change in carcinogen exposure is unknown, but based on historical trends in exposure and lung cancer risk in U.S. smokers, one must consider the possibility that differences in toxicant exposure contribute to country differences in lung cancer risk. Since the Chinese smoker toxicant profile resembles the U.S. smoker profile of many years ago, a lower overall risk of lung cancer and a greater proportion of squamous cell carcinoma might be expected. We are unaware of any data on the histologic types of lung cancer among Chinese smokers. It would be of interest to determine is the proportion of squamous cell vs adenocarcinoma is higher in Chinese smokers, resembling that of U.S. smokers in the 1950s.

Higher PAH and lower NNK exposure among Chinese smokers could be associated with different risks of tobacco-associated cancers other than lung cancer. In addition, urinary PAH metabolite excretion, independent of cigarette smoking, has been associated with inflammatory biomarkers that are predictive of cardiovascular disease risk. In any case, country differences
in cigarette composition and related differences in exposure to tobacco smoke toxicants should be considered in comparative disease epidemiology studies.

In summary, Chinese and U.S. smokers have strikingly different profiles of carcinogen exposure, with or without normalization for cigarettes smoked or intake of nicotine per day. Lower nitrosamine exposure among Chinese smokers could explain at least in part lower lung cancer rates in Chinese compared to U.S. smokers. Higher PAH exposure among Chinese smokers from cigarette smoking and environmental pollution could result in increases in other types of cancer, and this possibility warrants further exploration. Country-specific differences in tobacco carcinogen exposure should be considered in assessing international differences in smoking-related disease epidemiology.

What this study adds:

Patterns of carcinogen exposure differ, with lower exposure to tobacco specific nitrosamines and higher exposure to polycyclic aromatic hydrocarbons in Chinese compared to U.S. smokers. Most likely this reflects country differences in cigarette tobacco blends and manufacturing processes, and possibly environmental exposures. Country differences in cigarette composition and exposure to tobacco smoke toxicants should be considered in comparative smoking and health epidemiology studies.

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Contributors:
Drs. Benowitz, Gan and Glantz contributed to planning, conduct and reporting of the study; Drs. Goniewicz, Lu, Xu, Li and Jacob contributed to the conduct and reporting of the study. Dr. Benowitz is responsible for the overall content as guarantor.

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Competing Interests:
NLB serves as a paid consultant to pharmaceutical companies that are developing or that market smoking cessation medications. He also has been a paid expert witness in litigation against tobacco companies, including on issues related to light cigarettes. None of the other authors have any competing interests to declare.
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This study was conducted with the approval of the UCSF and Shanghai CDC.

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Figure Legends

Figure 1. Panel A - urine nicotine equivalents (TNE); panel B urine equivalents / cigarettes per day ratio (TNE/CPD) in Chinese compared to U.S. smokers. Data shown as medians and interquartile intervals.

Figure 2. Panel A – urine NNAL / urine nicotine equivalents ratio (NNAL/TNE); panel B – urine1-hydroxypyrene / urine nicotine equivalents ratio (1-HP/TNE) in Chinese compared to U.S. smokers. Data shown as medians and interquartile intervals.
Figure 2