Title
Different profiles of carcinogen exposure in Chinese compared with US cigarette smokers

Journal
Tobacco Control, 24(e4)

ISSN
0964-4563

Authors
Benowitz, Neal L
Gan, Quan
Goniewicz, Maciej L
et al.

Publication Date
2015-12-01

DOI
10.1136/tobaccocontrol-2014-051945

Peer reviewed

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

Neal L. Benowitz,[1-2] Quan Gan,[3] Maciej L. Goniewicz,[4] Wei Lu,[3] Jiying Xu[3], Xinjian Li,[3] Peyton Jacob III,[1-2] and Stanton Glantz[2,5]

[1]Division of Clinical Pharmacology and Experimental Therapeutics,
Medical Service, Departments of Medicine, and Biopharmaceutical Sciences,
University of California, San Francisco, California, USA

[2]Center for Tobacco Control Research and Education, University of California, San Francisco, California, USA

[3] Shanghai Center for Disease Control and Prevention, Shanghai, China

[4] Department of Health Behavior, Division of Cancer Prevention and Population Sciences, Roswell Park Cancer Institute, Buffalo, New York, USA

[5]Division of Cardiology, Department of Medicine,
University of California, San Francisco, California, USA

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

Keywords: Cigarette smoking, carcinogens, cancer, China, biomarkers, nitrosamines, polycyclic aromatic hydrocarbons, nicotine

Corresponding Author:

Neal L. Benowitz, M.D.

Chief, Division of Clinical Pharmacology and

Experimental Therapeutics

University of California, San Francisco, Box 1220

San Francisco, California 94143-1220

Tel. (415) 206-8324

Fax (415) 206-4956

Words: 3377

Figures: 2

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 [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 [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 [18] and the other participants in a clinical trial of reduced nicotine content cigarettes (baseline evaluation) [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.

Analytical Chemistry

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 [20]. Urine concentrations of total NNAL were measured by LC-MS/MS as described previously [21]. 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 [22]. 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 [23] [24]. 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 [25]. 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.[26]

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 1. Demographics and Urine Biomarkers in Chinese and U.S. Smokers (values are medians and IQR, unless otherwise indicated)

	Chinese (n=238)	U.S. (n=203)	p-value[a]	U.S. Men (n=118)	p-value[a]
Age	44.3 (43.0-45.5)[b]	38.3 (36.7-39.8)[b]	< 0.0001[c]	39-8 (3.77-41.8)[b]	0.0001[c]

Male (%)	238 (100%)	118 (58%)	< 0.0001	118 (100%)	
Tar	12.2 (11.9-12.6)[b]	13.6 (13.0-14.2)[b]	< 0.0001[c]	14.2 (13.4-14.9)[b]	<0.0001[c]
CPD	18.0 (16.7-19.3)[b]	19.5 (18.2-20.7)[b]	NS[c]	20.6 (18.9-22.4)[b]	0.02[c]
Nicotine Eq (nmol/mg creat)	53.4 (32.8-78.9)	54.8 (42.7-81.6)	NS	54.6 (38.2-78.6)	NS
NNAL (pmol/mg creat)	0.29 (0.18-0.47)	1.19 (0.63-2.06)	< 0.0001	1.12 (0.62-1.81)	<0.0001
1HP (pmol/mg creat)	3.64 (2.64-5.48)	1.17 (0.75-1.79)	< 0.0001	1.10 (0.70-1.52)	<0.0001
2Nap (pmol/mg creat)	103 (65.3-149)	98.3 (59.7-149)	NS	88.7 (52.0-130.3)	0.03
Sum of Fluorenes (pmol/mg creat)	23.5 (15.8-32.9)	16.6 (10.8-24.5)	< 0.0001	16.0 (10.6-22.4)	<0.0001
Sum of Phenantrenes (pmol/mg creat)	9.52 (6.56-13.5)	3.54 (2.44-5.07)	< 0.0001	3.53 (2.52-4.90)	<0.0001
Sum of PAHs (pmol/mg creat)	145 (95.8-201)	123 (78.5-184)	0.0082	110 (79-158)	0.0001
Nicotine Eq/CPD (nmol/mg creat)	3.16 (1.76-5.23)	3.13 (2.07-5.06)	NS	2.87 (1.88-4.02)	NS
NNAL/CPD (fmol/mg creat)	18.9 (11.3-32.7)	73.4 (37.4-112)	< 0.0001	62.1 (28.8-99.3)	<0.0001
1HP/CPD (pmol/mg creat)	0.24 (0.15-0.40)	0.06 (0.04-0.10)	< 0.0001	0.05 (0.04-0.08)	<0.0001
2Nap/CPD (pmol/mg creat)	6.04 (3.69-9.75)	5.53 (3.54-8.37)	NS	4.68 (3.10-6.52)	0.0001
Sum of Fluorenes/CPD (pmol/mg creat)	1.47 (0.93-2.21)	0.98 (0.56-1.42)	< 0.0001	0.82 (0.53-1.25)	<0.0001
Sum of Phenantrenes/CPD (pmol/mg creat)	0.60 (0.39-0.93)	0.19 (0.13-0.29)	< 0.0001	018 (0.11-0.29)	<0.0001
Sum PAHs/CPD (pmol/mg creat)	8.41 (5.56-12.9)	7.00 (4.55-10.3)	0.0002	5.66 (4.02-8.25)	<0.0001
NNAL/Nicotine Eq x 10^6	5.55	22.0	< 0.0001	21.5	<0.0001

	(3.74-11.8)	(14.2-31.8)		(12.9-31.4)	
1HP/ Nicotine Eq x 10^3	0.07	0.02	< 0.0001	0.02	<0.0001
	(0.05-0.13)	(0.01-0.03)		(0.01-0.03)	
2Nap/Nicotine Eq x10^3	1.91	1.54	< 0.0001	1.46	<0.0001
	(1.50-2.61)	(1.13-2.42)		(1.05-2.36)	
Sum of Fluorene/Nicotine	0.43	0.29	< 0.0001	0.30	<0.0001
Eq x 10^3	(0.33-0.61)	(0.21-0.38)		(0.23-0.37)	<0.0001
Sum of Phenantrene/Nicotine	0.16	0.06	< 0.0001	0.06	<0.0001
Eq x 10^3	(0.11-0.33)	(0.04-0.08)		(0.04-0.08)	
Sum PAHs/Nicotine Eq x 10^3	2.65	1.99	< 0.0001	1.81	<0.0001
	(2.08-3.68)	(1.44-3.00)		(1.40-2.73)	

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.

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

* - 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[10 27]. Other authors have reported higher levels of a metabolite of the PAH phenanthrene (r-1,t-2,3,c-4-Tertrahydroxy-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)[28].

Tobacco specific nitrosamines and PAHs are two major classes of tobacco carcinogens [29]. The nitrosamine NNK has been implicated causing in oral cancer, lung cancer, and pancreatic cancer [30]. Tobacco specific nitrosamine levels in cigarette tobacco vary widely among cigarettes and across countries [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 [12]. Tobacco specific nitrosamines are formed during the curing, processing and fermenting of cigarette tobacco, and may also form during cigarette combustion [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 [14,15]. 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 [31]. PAHs form adducts with DNA are found in p53 mutations and have been associated with increased risk of human lung cancer [32,33]. 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 [34].

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 expousure comparing smokers of cigarettes vs water pipers.[35] We observed that country differences were greater for some PAHs (pyrene and phenanthrene) than for others (fluorene and napthylene). 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 [15,36]. 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.[15]. 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.[37] Because smokers smoke to obtain desired levels of nicotine, it is important to consider carcinogen exposure in relation to nicotine intake [38]. 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[28 39]. 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[40 41]. 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 [8 10 42 43]. 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.[44] 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 [45]. 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.

Acknowledgements:

We thank Yao Haihong, Miao Sun, Wang Yuheng and Chen Yisheng for their help in conducting the survey. We are grateful to Lisa Yu and Trisha Mao for performing the nicotine metabolite analyses, to Olivia Yturralde for the PAH metabolite analyses and to Christopher

Havel for analytical chemistry advice and for performing the NNAL analyses. Funding for laboratory infrastructure in the Division of Clinical Pharmacology at UCSF was provided by the National Institutes of Health, P30 DA012393.

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.

Funding:

National Cancer Institute Training Grant CA-113710, the William Cahan Endowment and the UCSF Bland Lane Center of Excellence on Secondhand Smoke, both funded by the Flight Attendants Medical Research Institute, USPHS Grant DA01293 from National Institute on Drug Abuse and the UCSF Helen Diller Family Comprehensive Cancer Center, and the China CDC. The external funding agencies played no role in the design of the project, collection and analysis of the data or preparation of the manuscript.

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.

Ethics approval:

This study was conducted with the approval of the UCSF and Shanghai CDC.

Provenance and peer review:

Not commissioned; not externally peer reviewed.

REFERENCES

1. Gu D, Kelly TN, Wu X, et al. Mortality attributable to smoking in China. N Engl J Med 2009;**360**(2):150-9.
2. Zhang J, Ou JX, Bai CX. Tobacco smoking in China: prevalence, disease burden, challenges and future strategies. Respirology 2011;**16**(8):1165-72.
3. Thun MJ, Carter BD, Feskanich D, et al. 50-year trends in smoking-related mortality in the United States. N Engl J Med 2013;**368**(4):351-64.
4. Fujieda M, Yamazaki H, Saito T, et al. Evaluation of CYP2A6 genetic polymorphisms as determinants of smoking behavior and tobacco-related lung cancer risk in male Japanese smokers. Carcinogenesis 2004;**25**(12):2451-8.
5. Liu T, Xie CB, Ma WJ, et al. Association between CYP2A6 genetic polymorphisms and lung cancer: A meta-analysis of case-control studies. Environ Mol Mutagen 2012.
6. Hecht SS. Lung carcinogenesis by tobacco smoke. Int J Cancer 2012;**131**(12):2724-32.
7. Yuan JM, Butler LM, Stepanov I, et al. Urinary tobacco smoke-constituent biomarkers for assessing risk of lung cancer. Cancer Res 2014;**74**(2):401-11.
8. Church TR, Anderson KE, Caporaso NE, et al. A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. Cancer Epidemiol Biomarkers Prev 2009;**18**(1):260-6.
9. Hecht SS, Murphy SE, Stepanov I, et al. Tobacco smoke biomarkers and cancer risk among male smokers in the Shanghai Cohort Study. Cancer Letters 2013;**334**(1):34-38.
10. Yuan JM, Gao YT, Murphy SE, et al. Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. Cancer Res 2011;**71**(21):6749-57.
11. Ashley DL, Beeson MD, Johnson DR, et al. Tobacco-specific nitrosamines in tobacco from U.S. brand and non-U.S. brand cigarettes. Nicotine Tob Res 2003;**5**(3):323-31.
12. Wu W, Zhang L, Jain RB, et al. Determination of carcinogenic tobacco-specific nitrosamines in mainstream smoke from U.S.-brand and non-U.S.-brand cigarettes from 14 countries. Nicotine Tob Res 2005;**7**(3):443-51.
13. Gray N, Zaridze D, Robertson C, et al. Variation within global cigarette brands in tar, nicotine, and certain nitrosamines: analytic study. Tob Control 2000;**9**(3):351.
14. Fischer S, Spiegelhalder B, Preussmann R. Preformed tobacco-specific nitrosamines in tobacco--role of nitrate and influence of tobacco type. Carcinogenesis 1989;**10**(8):1511-7.
15. Hoffmann D, Hoffmann I. The changing cigarette, 1950-1995. J Toxicol Environ Health 1997;**50**(4):307-64.
16. Adams JD, Lee SJ, Hoffmann D. Carcinogenic agents in cigarette smoke and the influence of nitrate on their formation. Carcinogenesis 1984;**5**(2):221-3.
17. Gan Q, Lu W, Xu J, et al. Chinese 'low-tar' cigarettes do not deliver lower levels of nicotine and carcinogens. Tob Control 2010;**19**(5):374-9.
18. Benowitz NL, Dains KM, Dempsey D, et al. Racial differences in the relationship between number of cigarettes smoked and nicotine and carcinogen exposure. Nicotine Tob Res 2011;**13**(9):772-83.

19. Benowitz NL, Dains KM, Hall SM, et al. Smoking behavior and exposure to tobacco toxicants during 6 months of smoking progressively reduced nicotine content cigarettes. Cancer Epidemiol Biomarkers Prev 2012;**21**(5):761-9.
20. Dempsey D, Tutka P, Jacob P, 3rd, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. Clin Pharmacol Ther 2004;**76**(1):64-72.
21. Jacob P, 3rd, Havel C, Lee DH, et al. Subpicogram per milliliter determination of the tobacco-specific carcinogen metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in human urine using liquid chromatography-tandem mass spectrometry. Anal Chem 2008;**80**(21):8115-21.
22. Jacob P, 3rd, Wilson M, Benowitz NL. Determination of Phenolic Metabolites of Polycyclic Aromatic Hydrocarbons in Human Urine as Their Pentafluorobenzyl Ether Derivatives Using Liquid Chromatography-Tandem Mass Spectrometry. Anal Chem 2007;**79**(2):587-98.
23. Benowitz NL, Jacob P, 3rd, Fong I, et al. Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine. The Journal of pharmacology and experimental therapeutics 1994;**268**(1):296-303.
24. Feng S, Kapur S, Sarkar M, et al. Respiratory retention of nicotine and urinary excretion of nicotine and its five major metabolites in adult male smokers. Toxicol Lett 2007;**173**(2):101-6.
25. Benowitz NL, Dains KM, Dempsey D, et al. Estimation of nicotine dose after low-level exposure using plasma and urine nicotine metabolites. Cancer Epidemiol Biomarkers Prev 2010;**19**(5):1160-6.
26. Benowitz NL, Hukkanen J, Jacob P, 3rd. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb Exp Pharmacol 2009;(192):29-60.
27. Xia Y, Bernert JT, Jain RB, et al. Tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers in the United States: NHANES 2007-2008. Biomarkers 2011;**16**(2):112-9.
28. Yuan JM, Butler LM, Gao YT, et al. Urinary metabolites of a polycyclic aromatic hydrocarbon and volatile organic compounds in relation to lung cancer development in lifelong never smokers in the Shanghai Cohort Study. Carcinogenesis 2014;**35**(2):339-45.
29. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer 2003;**3**(10):733-44.
30. Gray N, Boyle P. The case of the disappearing nitrosamines: a potentially global phenomenon. Tob Control 2004;**13**(1):13-6.
31. Humans IWGotEoCRt. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum 2004;**83**:1-1438.
32. Smith LE, Denissenko MF, Bennett WP, et al. Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. J Natl Cancer Inst 2000;**92**(10):803-11.
33. HHS. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2010.
34. Akpan V, Huang S, Lodovici M, et al. High levels of carcinogenic polycyclic aromatic hydrocarbons (PAH) in 20 brands of Chinese cigarettes. J Appl Toxicol 2006;**26**(6):480-3.

35. Jacob P, 3rd, Abu Raddaha AH, Dempsey D, et al. Comparison of nicotine and carcinogen exposure with water pipe and cigarette smoking. Cancer Epidemiol Biomarkers Prev 2013;**22**(5):765-72.
36. Ding YS, Zhang L, Jain RB, et al. Levels of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. Cancer Epidemiol Biomarkers Prev 2008;**17**(12):3366-71.
37. Benowitz NL. Compensatory smoking of low-yield cigarettes. National Cancer Institute Risks associated with smoking cigarettes with low machine yields of tar and nicotine Smoking and Tobacco Control Monograph No 2001;**13**:39-63.
38. Burns DM, Dybing E, Gray N, et al. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. Tob Control 2008;**17**(2):132-41.
39. Wu MT, Simpson CD, Christiani DC, et al. Relationship of exposure to coke-oven emissions and urinary metabolites of benzo(a)pyrene and pyrene in coke-oven workers. Cancer Epidemiol Biomarkers Prev 2002;**11**(3):311-4.
40. Bernert JT, Pirkle JL, Xia Y, et al. Urine concentrations of a tobacco-specific nitrosamine carcinogen in the U.S. population from secondhand smoke exposure. Cancer Epidemiol Biomarkers Prev 2010;**19**(11):2969-77.
41. Suwan-ampai P, Navas-Acien A, Strickland PT, et al. Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons in the United States, 1999 to 2002. Cancer Epidemiol Biomarkers Prev 2009;**18**(3):884-93.
42. Yuan JM, Gao YT, Wang R, et al. Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers. Carcinogenesis 2012;**33**(4):804-9.
43. Hecht SS, Murphy SE, Stepanov I, et al. Tobacco smoke biomarkers and cancer risk among male smokers in the Shanghai Cohort Study. Cancer Lett 2012.
44. HHS. The Health Consequences of Smoking-50 Years of Progress. A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.
45. Alshaarawy O, Zhu M, Ducatman A, et al. Polycyclic aromatic hydrocarbon biomarkers and serum markers of inflammation. A positive association that is more evident in men. Environ Res 2013;**126**:98-104.

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.

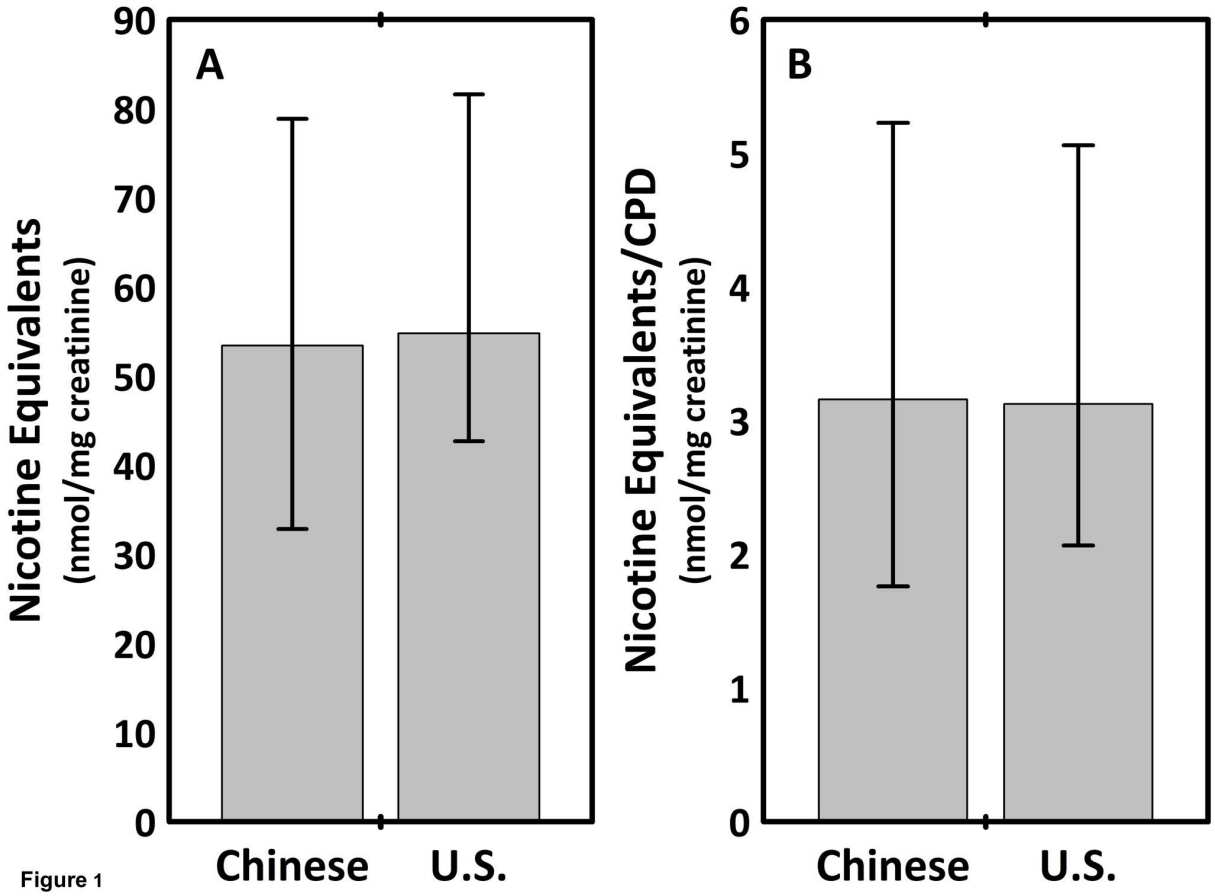
A
Nicotine Equivalents
(nmol/mg creatinine)
90
80
70
60
50
40
30
20
10
0
Chinese
U.S.
B
Nicotine Equivalents/CPD
(nmol/mg creatinine)
6
5
4
3
2
1
0
Chinese
U.S.

Figure 1

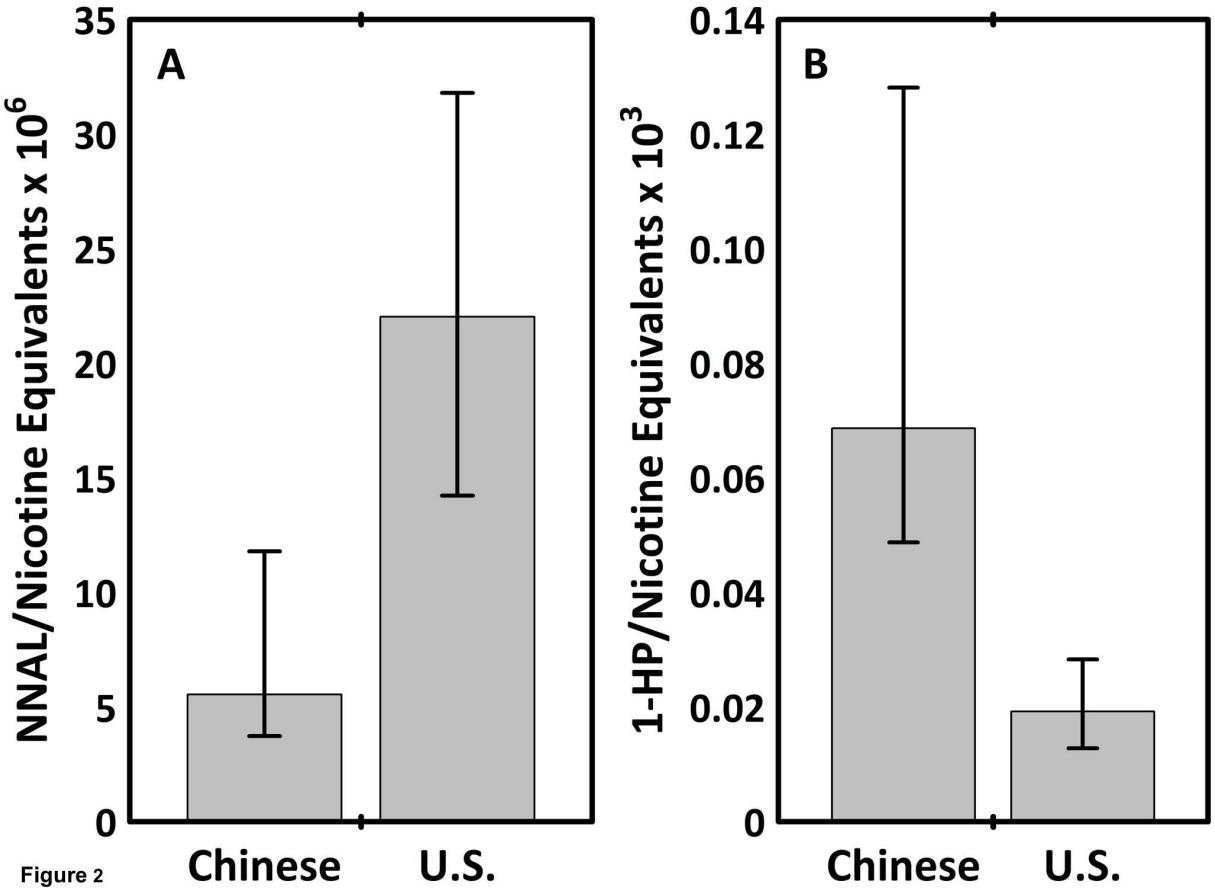
A
NNAL/Nicotine Equivalents x 10^6
35
30
25
20
15
10
5
0
Chinese
U.S.
B
1-HP/Nicotine Equivalents x 10^3
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
Chinese
U.S.

Figure 2