Cotinine Concentration in Smokers from Different Countries: Relationship with Amount Smoked and Cigarette Type

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Abstract

This four-country study examined salivary cotinine as a marker for nicotine intake and addiction among smokers in relation to numbers and types of cigarettes smoked. Smoking characteristics of cigarette smokers in Brazil, China, Mexico, and Poland were identified using a standard questionnaire. Cotinine concentration was measured using a saliva sample from each participant; its relationship with numbers and types of cigarettes smoked was quantified by applying regression techniques. The main outcome measure was salivary cotinine level measured by gas chromatography. In all four countries, cotinine concentration increased linearly with cigarettes smoked up to 20 per day [11.3 ng/mL (95% confidence interval, 10.5-12.2)] and then stabilized as the number of cigarettes exceeded 20 [6.8 ng/mL per cigarette (95% confidence interval, 6.3-7.4) for up to 40 cigarettes]. On average, smokers of regular cigarettes consumed more cigarettes and had higher cotinine levels than light cigarette smokers. Cotinine concentration per cigarette smoked did not differ between regular and light cigarette smokers. Results suggest a saturation point for daily nicotine intake and minimal or no reduction in nicotine intake by smoking light cigarettes. (Cancer Epidemiol Biomarkers Prev 2006;15(10):1799–804)

Introduction

Worldwide, >1 billion adults are regular tobacco smokers, most using manufactured cigarettes (1). These cigarettes are diverse in their design, in the tobacco additives used, and in their yields of tar and nicotine as measured by smoking machine-based protocols. They are made by many companies, and a substantial number of the brands include the words ''light,'' ''ultralight,'' or ''mild'' as a part of the name. Regardless of brand name, manufacturer, or other characteristics, all commercially successful cigarettes deliver nicotine, the addicting component of tobacco smoke, to the smoker (2, 3).

Several studies have now been carried out to assess the relationship of smoking pattern and products smoked with indices of addiction and with levels of nicotine and its metabolite cotinine in body fluids (3, 4). These studies have shown that levels of cotinine increase with the numbers of cigarettes smoked but vary little with self-reported depth of inhalation or cigarette design characteristics. Severity of addiction scores is also associated with biomarker levels.

To date, this evidence largely comes from studies carried out in developed Western countries. We have conducted a multicountry study of smoking and salivary cotinine levels in regular smokers. The protocol has a standard smoking questionnaire, which includes the Fagerström Test for Nicotine Dependence (5), along with collection of a saliva specimen for measurement of cotinine by gas chromatography. Populations have been studied in China (6), Mexico (7), Brazil, and Poland; data collection is under way in India. Although 30% to 35% of adults smoke in each of these countries, the smoking patterns differ markedly. In China, the majority of smokers are men (8). In Poland, consumption is high among 40% of men and ~20% of women who smoke (9). The number of cigarettes smoked in Mexico and Brazil tends to be low, particularly among women. The use of a standardized protocol makes possible the comparison of findings across these populations. In this article, we provide findings from the four groups studied to date, comparing smoking behaviors and saliva cotinine levels across the countries.

Materials and Methods

Overview. The data were collected within each country according to a common protocol, but the approach to population selection differed from country to country (Table 1). All measurements of salivary cotinine were made in the Clinical Pharmacology laboratory at the University of California at San Francisco (San Francisco, CA; N.L.B.). Details of the methods used in the four countries are provided in separate

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publications for China (6), Mexico (7), Brazil (8), and Poland (9). Selected participants were smoking at least one cigarette per day and had not used any form of nicotine replacement therapy within the past 3 days. Informed consent was obtained from all participants.

Study Populations

Brazil. The participants from Brazil were a subsample of the National Household Survey in Rio de Janeiro, carried out annually by the Brazilian Institute of Geography and Statistics. Approximately 360 smokers, ages ≥15 years, were selected using a multistage random sampling. First, a sample of ~200 enumeration districts in Rio de Janeiro was obtained from the National Household Sample by Brazilian Institute of Geography and Statistics. A probability sample of these enumeration districts was obtained and weighted by the number of residences in each district. In the second stage, a weighted probability sample of 38 enumeration districts among these 200 enumeration districts was selected. In the third stage, a random sample of households was selected from each enumeration district, and in each selected household, all residents ages ≥15 years were interviewed. The study sample included both nonsmokers and smokers. After applying the exclusion criteria, ~350 smokers were eligible for the study.

China. The study sample had a target of 600 Chinese adult smokers, ages ≥15 years, who were residents in the cities of Beijing and Shanghai (300 subjects in each city). Trained interviewers from the Chinese Academy of Preventive Medicine contacted adult subjects who had participated in previous studies done by the Chinese Academy of Preventive Medicine. Current smokers who had not used any form of nicotine replacement therapy within the last 3 days were asked to participate in the study.

Mexico. The study population was recruited from two Mexican cities, Mexico City and Cuernavaca, Morelos, using different sampling strategies. In Mexico City, eligible smokers were identified among participants in a population-based cohort study that was designed to assess risk factors for chronic diseases among adults who were ≥30 years of age and who were residents of a specific district in Mexico City. From this cohort, current smokers were identified and a stratified random sample was selected. As a comparable sampling frame was not available for Cuernavaca, smokers were recruited using a convenience sampling approach. The strategy involved identifying smokers ages ≥17 years at schools, health institutions, shopping centers, parks, movie theaters, and other places. To obtain a balanced number of participants in each smoking intensity category, a stratified sampling strategy was used that was based on the number of cigarettes per day that potential participants reported. The following categories were used for recruitment: ≤5, 6 to 10, 11 to 15, 16 to 20, 21 to 30, and ≥31 cigarettes per day. Each stratum was targeted for 100 participants. Current smokers who had not used any form of nicotine replacement therapy within the last 2 days were asked to participate in the study.

Poland. The study aimed to sample 600 Polish adult smokers at ages of 20 to 69 who were residents of Warsaw, smoked at least one cigarette per day during the last 30 days, and have never used any form of nicotine replacement therapy. The study sample was planned to consist of 300 men and 300 women, including 200 people who smoked <20 cigarettes per day, 200 who smoked ~20 cigarettes per day, and 200 who smoked >20 cigarettes per day. Using the random-route method, 125 start points were selected in all Warsaw districts. Each start point had been described by street address, and the number of start points was proportional to number of residents living in each district. Trained interviewers from the collaborating public opinion research center began to enroll participants at the particular start point, obtaining a maximum of five interviews at that point, and moved from residence to residence. Only one interview per residence was given. In total, 636 were recruited for the study and 601 had complete data.

Measurement Methods

Questionnaire. A standard set of questions without modification was used in each country, but additional questions were also permitted. Information on the following items was obtained by the questionnaires: demographic data, the number of cigarettes smoked daily on average, the number of cigarettes smoked during the previous 24 and 48 hours, the duration of smoking, the brand of cigarettes smoked most often, the brands of cigarettes smoked during the survey day and the previous day, frequency of smoking cigarettes with filter tips, the depth and frequency of inhaling, smoking of cigars or pipe, and use of nicotine gum or patches in the previous 3 days. The questions of the Fagerström Test for Nicotine Dependence (5) were also included to separate substance dependent and behavioral smokers. Standardized questions based on the American Thoracic Society's adult respiratory questionnaire were used when appropriate. The questions were translated, and instruments were pilot tested before use.

Saliva Specimen. The subjects were asked to rinse their mouths and chew lemon candy. They were asked to first spit out a small amount of saliva and then to spit ~6 mL in a test tube. The specimen was frozen to −20°C and then shipped for cotinine analysis at the University of California at San Francisco. The cotinine concentration was determined using gas chromatography with a nitrogen-phosphorus detection technique (10).

Height and Weight. Height was measured in centimeters after participants removed their shoes. Weight was measured in kilograms with a portable scale. The body mass index was calculated as weight divided by the square of height in meters. The portable scales were calibrated every day with a standard scale before recording the weight of the participants.

Statistical Methods. A series of exclusion criteria was applied to the sampled populations before pooling the individual country data. Any participants with missing information for cotinine concentration (ng/mL), number of cigarettes smoked in the previous 24 hours, age, gender, weight (kg), height (m), saliva collection time, or type of cigarette most frequently smoked (light versus regular) were excluded. Participants must have reported smoking at least 1 but no more than 60 cigarettes in the previous 24 hours, indicated he/she was a regular smoker, did not smoke cigars, did not use any nicotine replacement therapy (patch, gum, etc.) in the past 3 days, had a saliva collection time of later than 6 a.m., and did not smoke hand-rolled cigarettes. Any participants with a ratio of cotinine concentration (ng/mL) to number of cigarettes smoked >35 ng/mL per cigarette were excluded. A cotinine concentration of >35 ng/mL per cigarette smoked in the previous 24 hours is regarded as an unlikely value from the biological viewpoint, possibly resulting from information or measurement bias. The rationale for excluding values of >35 ng/mL per cigarette is based on studies of how much nicotine a person can take in from a cigarette and rates of nicotine and cotinine metabolism. Commercial cigarettes typically contain 10 to 15 mg nicotine per tobacco rod. The usual systemic absorption of nicotine from a cigarette is 1 to 1.5 mg but can be as high as 3 mg per cigarette with very intense smoking. In experimental studies in which cigarette smoking is measured and in which nicotine and cotinine pharmacokinetic variables are characterized, the typical cotinine concentration per cigarette is 12 ng/mL. Assuming

Table 1. Study populations in a multicountry study of smoking and salivary cotinine

Country	City	Year of study	*n*	*n* (after exclusion criteria)	Sampling approach
Brazil	Rio de Janeiro	2000	2,393	360	Multistage random sampling
China	Beijing and Shanghai	1999	600	490	Convenient sampling
Mexico	Mexico City and Cuernavaca	1999	1,252	1,006	Convenient sampling
Poland	Warsaw	2000	601	517	Random-route method

this reflects an intake of 1 mg nicotine, one can estimate a cotinine level of 36 ng/mL for a person taking in 3 mg nicotine from each cigarette. We set a limit of 35 ng/mL per cigarette as a boundary above which a level would be considered not biologically plausible. Thus, in supplementary sensitivity analyses, we excluded individuals with such values. A total of 161 subjects were excluded from the analysis: 53 subjects in Brazil, 19 subjects in China, 75 subjects in Mexico, and 14 subjects in Poland.

Statistical analyses were run using Statistical Analysis System version 9.1 (SAS Institute, Cary, NC) and freeware R.[8] Cotinine concentrations (ng/mL) were adjusted within country to remove any relationship with sampling time, which had not been standardized across countries. Linear regressions were used to describe the change in cotinine concentration per cigarette smoked, adjusting for age, gender, body mass index, country, and country by cigarette interactions. Quadratic regressions were also used to test for nonlinearity in the cotinine/cigarette relationship. There were few participants who indicated smoking >40 cigarettes per day ($n = 47$), and among them, the range of number of cigarettes was large (42-98 cigarettes). Because the data were sparse and variable for >40 cigarettes, we restricted the analyses to those who smoked ≤40 cigarettes.

Results

The age and gender distributions of the participants varied across the countries, reflecting their demographic and smoking profiles (Table 2). Only a small percentage of women were included in China, and the population in Mexico was the youngest. The patterns of current smoking and type of cigarette smoked also differed across countries. The median body mass index values were 24.0 kg/m^2 for Brazil, 23.1 kg/m^2 for China, 24.7 kg/m^2 for Mexico, and 24.0 kg/m^2 for Poland.

The cotinine distributions for the four countries are provided as box plots in Fig. 1. Mexico had the lowest median and Poland had the highest. We initially explored the distribution of cotinine levels in relation to number of cigarettes smoked using scatter plots and smoothed curves (Fig. 2). The plots showed similar features of the relationship between cotinine level and numbers of cigarettes smoked across the four countries: a linear increase in cotinine level with numbers of cigarettes smoked up to ~20 per day and then a more gradual increase or a plateau with further increase in the number of cigarettes smoked. The curves were similar for smokers of light and regular cigarettes up to 20 per day.

We further explored these relationships using linear regression no-intercept models, which show that the number of cigarettes smoked in the previous 24 hours is significantly associated with cotinine concentration (ng/mL), adjusting for age, body mass index, gender, and country for those who smoked up to 20 cigarettes [$\beta = 11.3$ ng/mL per cigarette; 95% confidence interval (95% CI), 10.5-12.2; $P < 0.001$] and up to 40 cigarettes ($\beta = 6.8$; 95% CI, 6.3-7.4; $P < 0.001$). The change in cotinine per cigarette was not significantly different between the countries (all interaction Ps > 0.10). Quadratic no-intercept regression models indicated that the relationship between cotinine and cigarettes for those who smoked up to 40 cigarettes is not linear, as both the main effect and squared term for cigarettes were significant ($P < 0.001$ and $P < 0.001$, respectively). There were no significant interactions between country and cigarettes in the quadratic model.

We addressed the effect of cigarette type using two different analytic approaches for smokers of ≤40 and ≤20 cigarettes per day. We first explored the effect of cigarette type by examining the distribution of cotinine concentration and number of cigarettes by country for light and regular cigarette smokers (Table 3). Among those who smoked up to 40 cigarettes per day, those who smoked regular cigarettes had significantly higher cotinine levels than light cigarette smokers in Poland and Mexico. This difference was marginally significant in Brazil ($P = 0.08$) and China ($P = 0.06$). After pooling the data and adjusting for country, cotinine levels were higher on average in regular cigarettes smokers than in light cigarette smokers [increase of 43.8 ng/mL (95% CI, 31.4-56.3); $P < 0.0001$]. There was also a significant increase among those who smoked up to 20 cigarettes only [38.4 ng/mL (95% CI, 25.0-51.8)].

Regular cigarette smokers also smoked significantly more cigarettes in Poland, Mexico, and Brazil than light cigarette smokers. There was no difference in China ($P = 0.13$), explained partly by the more homogeneous sample in that country. Pooling the data and adjusting for country showed that regular smokers consumed 3.2 cigarettes per day (95% CI, 2.3-4.0) more than light cigarette smokers. There was also a smaller difference in cigarettes per day among those who smoked up to 20 cigarettes [1.5 cigarettes (95% CI, 0.9-2.2); $P < 0.0001$].

The second approach used two no-intercept models, stratifying by type of cigarette, which included terms for number of cigarettes smoked per day and adjusted for age, body mass index, gender, and country. For those who smoked up to 40 cigarettes, the change in cotinine per cigarette was higher for smokers of light cigarettes [increased by 7.4 ng/mL per cigarette (95% CI, 6.5-8.2)] than smokers of regular cigarettes [6.6 ng/mL per cigarette (95% CI, 6.0-7.2)]. The interaction between type of cigarette and number of cigarettes smoked was found to be significant ($P = 0.0003$) when formally tested in a full model with smokers of both types of cigarettes. This relationship reversed when the analysis was restricted to those who smoked up to 20 cigarettes; the change in cotinine per cigarette was higher for smokers of regular cigarettes [$\beta = 11.6$ ng/mL per cigarette (95% CI, 10.6-12.7)] than light cigarette smokers [$\beta = 10.9$ ng/mL per cigarette (95% CI, 9.6-12.2)]. The interaction between type of cigarette and number of cigarettes was not significant ($P = 0.91$) when formally tested in a combined model.

Discussion

Our study compared smoking behavior and resultant salivary cotinine levels across several countries with different ethnic, racial, and cultural characteristics. We found differences among the countries in age and sex distributions of the

[8] http://www.r-project.org.

Table 2. Characteristics of participants by country

	Brazil (n = 360)	China (n = 490)	Mexico (n = 1,006)	Poland (n = 517)
Gender (%)				
Male	46.9	97.8	72.5	49.1
Female	53.1	2.2	27.5	50.9
Age (%)				
15-34	27.2	21.4	43.8	30.4
35-54	53.6	68.2	43.0	49.9
>54	19.2	10.4	13.1	19.7
No. cigarettes smoked in the previous 24 hours (%)				
1-10	28.0	24.3	34.5	14.1
11-20	52.5	58.9	34.4	54.5
21-30	9.1	12.9	19.5	22.8
>30	10.2	3.9	11.6	8.5
Cigarette type (%)				
Light	71.9	23.9	17.6	56.7
Regular	28.1	76.1	82.4	43.3
Smoking duration, y* (%)				
1-10	14.7	20.2	36.8	19.9
11-20	22.2	28.3	19.3	19.5
>20	62.2	45.9	43.9	59.8

*Participants in Brazil (n = 3), China (n = 27), and Poland (n = 4) had missing values for age began smoking. For participants who reported smoking at an age greater than the current age, this item was recorded as missing.

smokers, numbers of cigarettes smoked per day, the prevalence of light cigarette consumption, and the distributions of cotinine levels among samples across countries (Tables 2 and 3; Fig. 1). The differences in numbers of women in the four samples were particularly striking and were reflective of well-described differences in the smoking patterns by sex in the four countries (6, 9). We also confirmed earlier observations about the significant relationship between level of salivary cotinine and the level of cigarette consumption (6, 7).

In interpreting these descriptive findings, the limitations posed by the different sampling methods need to be considered. In Mexico and in China, convenience sampling was used for feasibility and with the assumption that sampling method should not affect the relationship between cigarette consumption pattern and salivary cotinine level (Table 1). The sampling methods were population-based for Brazil and Poland. In Mexico, smokers of greater numbers of cigarettes were oversampled to characterize the relationship between number of cigarettes smoked and salivary cotinine level; in that country, survey data show that ~62% of smokers, both men and women, smoke one to five cigarettes per day (11). Because of the differing sampling methods, comparisons of smoking patterns across the four countries should be interpreted with great caution; fortunately, national survey data are available for each of the countries (12). In spite of these differing sampling frames, however, comparisons of the dose-response relationships for cotinine level with numbers of cigarettes smoked should be valid.

A principal focus of the study was on the quantitative relationship between number of cigarettes consumed and level of salivary cotinine, a biomarker of nicotine dose. Overall, saliva cotinine concentrations averaged ~200 ng/mL across countries, with Poland having the highest and Mexico the lowest median concentration. This median cotinine value is similar to that seen in smokers in the United States and the United Kingdom (13, 14). In all countries, the level of salivary cotinine increased with increasing numbers of cigarettes smoked, up to ~20 cigarettes per day, and from that number, either the smoothed curve flattened or the slope dropped. The most likely explanation for this observation is that smokers titrate to their intake of nicotine, with an average preferred intake for heavy smokers corresponding to ~200 ng/mL cotinine. At lower levels of cigarette consumption (up to 20 per day), the slope of the saliva cotinine per cigarette smoked curves averaged ~11.3 ng/mL per cigarette. Using pharmacokinetically based estimates as described previously (15), 12 ng/mL cotinine in saliva corresponds to a daily systemic intake of 1 mg nicotine. Therefore, the 11.3 ng/mL per cigarette slope in subjects in the present study corresponds to an average intake of 0.94 mg per cigarette. This nicotine intake per cigarette is similar to the machine-tested nicotine yield of the most popular U.S. cigarettes (0.7-1.1 mg). The slope of saliva cotinine per cigarette up to 40 cigarettes per day is 6.8 ng/mL per cigarette. Thus, smokers seem to take in less nicotine per cigarette at higher cigarette consumption levels, implying that they are puffing less intensively or smoking less of the cigarette than those smoking ≤20 cigarettes per day. The shape of the relationship between cotinine level and number of cigarettes smoked implies that smokers seek some particular dose of nicotine overall regardless of the number of cigarettes smoked.

Light cigarette use varied among the smokers from the different countries; 72% of smokers in Brazil and 57% in Poland but only 24% and 18% consumed light cigarettes in China and Mexico, respectively (Table 2). Presumably, differences in smoking of light cigarettes reflect how cigarettes have been marketed in different countries. Because of these differing patterns of light cigarette use, we were able to assess cigarette type as a determinant of cotinine level. We found that the form of the relationship between cotinine level and numbers of cigarettes smoked was similar by cigarette type across the four countries (Fig. 2). We found that the average cotinine level was lower in smokers of light compared with regular cigarettes (Table 3); however, smokers of light cigarettes and of regular cigarettes had similar quantitative relationships between cotinine level and numbers of cigarettes smoked, for those smoking ≤20 cigarettes per day.

Thus, the finding of higher cotinine levels in smokers of regular compared with light cigarettes is a consequence of regular cigarette smokers smoking a greater number of cigarettes per day. The finding of a similar cotinine level per cigarette for light compared with regular cigarette smokers indicates that smokers are inhaling these cigarettes in a manner that delivers much more nicotine that is predicted by smoking machine testing. A similar conclusion was reached in the National Cancer Institute's Monograph 13 (16), which reviewed the literature and found evidence indicating a higher level of compensation for nicotine in smokers of light cigarettes than predicted by the Federal Trade Commission machine

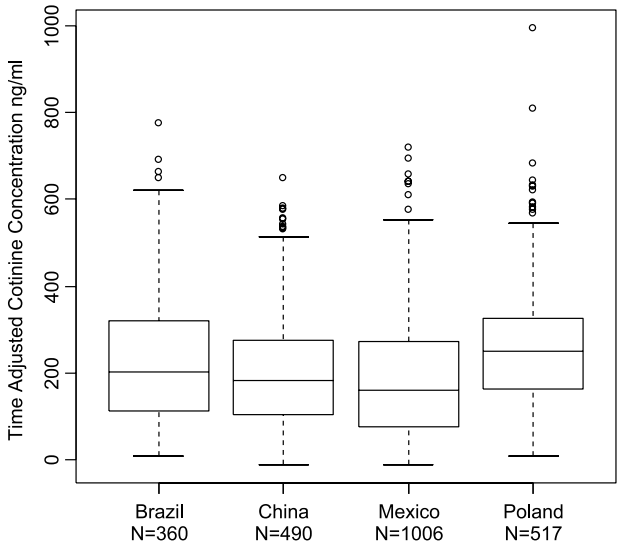

Figure 1. Box plots of time-adjusted cotinine concentrations (ng/mL) by country.

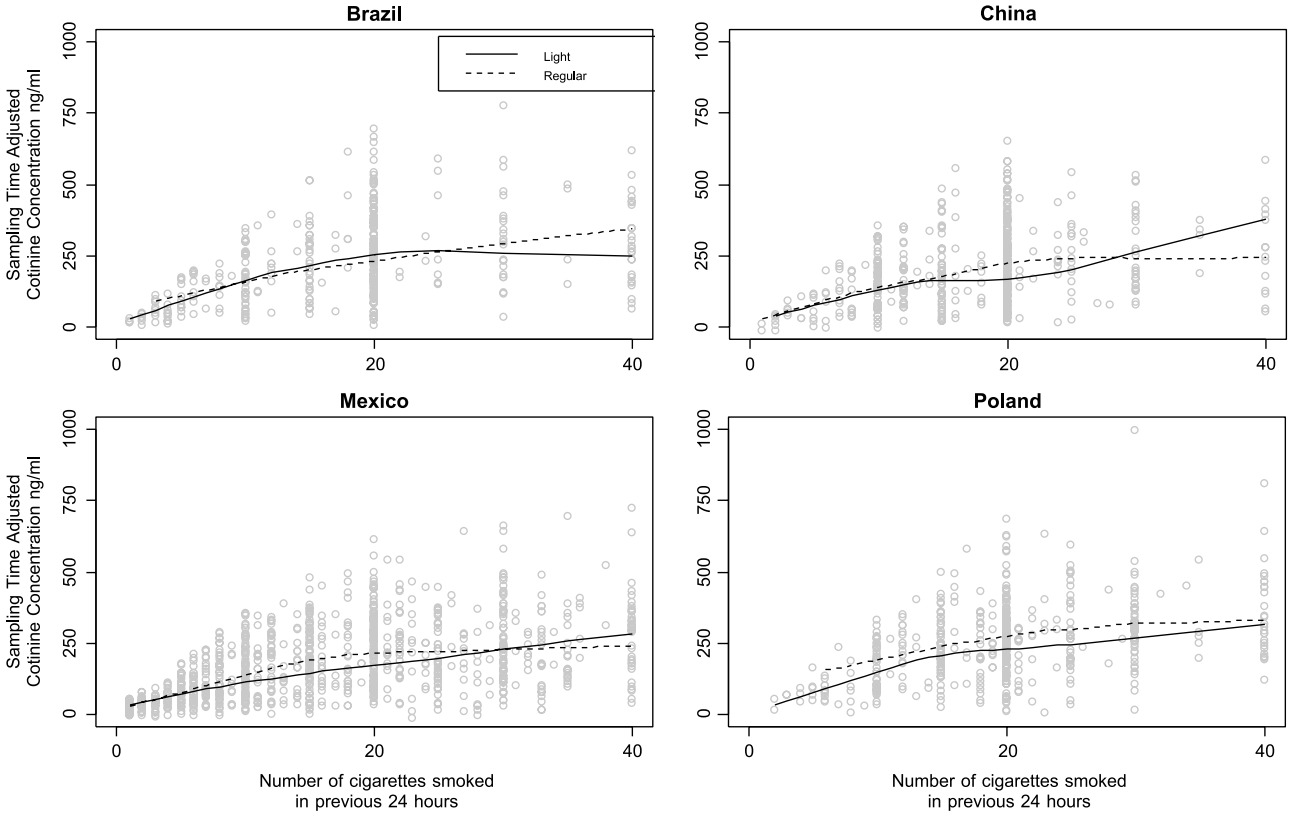

Figure 2. Scatter plots of time-adjusted cotinine concentration (ng/mL) and number of cigarettes smoked in the previous 24 hours by country with LOESS smoothers by type of cigarette.

method. The observation that smokers of light cigarettes smoke fewer cigarettes per day than regular cigarette smokers differs from that observed in the United States. The reason for this discrepancy is unclear.

Assuming that nicotine intake is an indicator of doses of toxins and carcinogens in tobacco smoke, our findings imply that little reduction in disease risk would be anticipated for smokers of light cigarettes compared with those smoking a similar number of regular cigarettes per day. Although studies reported in the 1960s and 1970s showed lower risks for lung cancer in smokers who switched to filtered cigarettes, more recent epidemiologic evidence has shown little indication of reduced risk for smokers of lower delivery cigarettes versus higher delivery cigarettes, with delivery assessed by machine (3, 16, 17). Ongoing surveillance is needed to track risks of cancer and other diseases as the tobacco industry continues to modify its products (18).

Tobacco industry documents show that industry research studies documented the phenomenon of decompensation and the decoupling of actual from machine-measured yields (19). Our findings confirm that compensation is likely to be universal among smokers and provide a strong rationale for prohibiting misleading labeling of cigarettes as ''light'' or similar designations as proposed in Article 11 of the Framework Convention on Tobacco Control; such restrictions have already been introduced in some countries.

What This Article Adds

What is already known on this subject: cotinine concentration, a biomarker of nicotine consumption, has been used to study nicotine intake and addiction among smokers.

What this study adds: (*a*) intake of nicotine per cigarette is comparable across countries; (*b*) smokers of >20 cigarettes per

Table 3. Distribution of cotinine concentration (ng/mL) and cigarettes per day by country, type of cigarette, and number of cigarettes among smokers of up to 40 cigarettes per day

	Light cigarette smokers		Regular cigarette smokers		*P**
	Mean (SD)	*n*	Mean (SD)	*n*	
Cotinine concentration (ng/mL)					
Brazil	217.7 (150.6)	253	249.1 (146.3)	99	0.08
China	180.3 (121.1)	117	206.6 (132.0)	372	0.06
Mexico	140.6 (112.8)	176	188.4 (130.6)	816	<0.0001
Poland	225.3 (128.6)	289	284.2 (131.0)	219	<0.0001
No. cigarettes per day					
Brazil	16.6 (9.2)	253	20.6 (9.8)	99	0.0004
China	16.5 (7.1)	117	17.8 (7.7)	372	0.13
Mexico	13.8 (10.5)	176	17.5 (10.2)	816	<0.0001
Poland	18.5 (7.8)	289	22.0 (8.1)	219	<0.0001

*Ps for t tests for mean differences between light and regular cigarette smokers.

day tend to have a lower nicotine intake per cigarette; and (*c*) the type of cigarette smoked has little effect on nicotine intake.

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