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Determinants of salivary cotinine concentrations in Chinese male smokers

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Abstract

Background. Identifying factors that affect cotinine levels in smokers may be useful for smoking cessation programs. Our aims were to characterize the distribution of salivary cotinine levels in Chinese smokers and to investigate factors that influence cotinine concentrations.

Methods. In a cross-sectional study, 600 Chinese adult smokers answered a questionnaire on smoking habits and provided a saliva sample for cotinine analysis. Modification of the relation between number of cigarettes smoked and cotinine concentration by individual characteristics, smoking behavior, and type of tobacco was evaluated.

Results. Quadratic model provided the best fit for the relation between number of cigarettes smoked in the previous 24 hours and salivary cotinine concentration. Among those smoking up to 20 cigarettes, the median cotinine concentration was higher among younger subjects, those smoking cigarettes without filter and regular rather than light cigarettes, and those inhaling frequently and deeply. Such trends were not observed among heavier smokers. The increase in cotinine per cigarette tended to be larger in those with lower median cotinine level.

Conclusions. Our findings show that smoking behavior-related factors modify the relation between number of cigarettes smoked and salivary cotinine concentration. This suggests that smokers may regulate their smoking behavior to achieve a certain optimum nicotine level.

Keywords: Tobacco smoking; Cotinine; Smoking behavior; Modification

Introduction

There are now about 1.1 billion smokers in the world, using diverse tobacco products. Nicotine addiction is central in the maintenance of sustained smoking, but little is known about nicotine delivery and dose in smokers around the world. Measurement of cotinine, the major proximate metabolite of nicotine, in smokers can provide a characterization of nicotine dose received by a population.

Cotinine can be measured in body fluids, such as plasma, saliva, and urine, as a biomarker of inhaled or ingested nicotine [1,2]. Cotinine is readily detectable in smokers with a distribution of levels quite distinct from that of nonsmokers. Earlier studies have shown different mean salivary and serum concentrations of cotinine among U.S. smokers of different ethnic backgrounds including Hispanics, blacks, and whites [3–5]. These differences may reflect differences in smoking behavior, types of cigarettes smoked, and/or nicotine metabolism between smokers of different ethnic groups [5–9]. The roles of smoking behavior and product characteristics in modifying cotinine concentrations are not yet well understood.

Since nicotine is the major addictive substance in cigarette smoke, cotinine levels of body fluids, as an indicator of nicotine dose, may prove to be important predictors of smoking cessation rates. Identification of factors affecting

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cotinine levels in body fluids may provide useful information for planning of smoking cessation programs, and for determining the appropriate dosage for nicotine replacement therapy. In addition, the intake of nicotine can be taken as a surrogate for exposure to tar and other toxic substances in tobacco smoke and may thus be predictive of the adverse health effects of smoking [9].

In China, the proportion of active smokers is high among men and low among women. In the National Smoking Prevalence Survey conducted in China in 1996, 63% of men and 4% of women were current smokers [10]. The distribution of cotinine levels in the Chinese smoking population has not been studied previously.

The aims of the present study were to characterize the distribution of salivary cotinine levels in Chinese smokers from Beijing and Shanghai, and to investigate factors that influence salivary cotinine levels in smokers. In addition, this study forms the first part of a multicountry study that will compare the relation of the number of cigarettes smoked with salivary cotinine concentration across different national groups, including China, Mexico, and Brazil.

Methods

Study design

A cross-sectional study was conducted among adults in Beijing and Shanghai, China, with interviews and saliva specimen collection taking place between January and March 1999. The Human Research Committees of the Johns Hopkins School of Public Health and the Chinese Academy of Preventive Medicine approved the study.

Study population

The study sample included 600 Chinese adult smokers 15 years or older 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 performed by the Chinese Academy of Preventive Medicine. It was confirmed that the person was a regular smoker (i.e., smoked at least one cigarette per day) and had not used any form of nicotine replacement therapy within the last 3 days. Subjects fulfilling these requirements were asked to participate in the study. They received information on the study and were asked to sign a consent form. They were first requested to answer a 52-item interviewer-administered survey questionnaire on smoking habits and attitudes toward smoking used in a previous study of adult smokers, and they then answered a short questionnaire including additional questions on smoking habits and provided a saliva specimen.

Of the 600 self-reported smokers participating in the study, only 20 participants (3.3%) were women, while two (0.3%) had missing gender information. Only four participants (0.7%) were cigar or pipe smokers. In addition, one smoker reported use of nicotine gum (0.2%). These subjects were excluded from further analyses. Altogether 63 subjects (10.5%) had salivary cotinine levels that were nondetectable ($n = 22$) or below the quantification limit of 10 ng/ml ($n = 41$). Since these subjects had cotinine levels below the cut-off point for regular active smoking [11], they were excluded from the analysis. Finally, seven persons with outlying values (1.2%) mentioned in the statistical analyses section were excluded. Some of these groups overlapped, so altogether 90 subjects were excluded. Thus, the final study population consisted of 510 male cigarette smokers.

Measurement methods

Questionnaire

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 hours and 48 hours, the duration of smoking, the brand of the cigarettes smoked most often, the brands of the 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. Standardized questions based on the American Thoracic Society's (ATS) adult respiratory questionnaire were used when appropriate [12].

Salivary cotinine

The subjects were asked to rinse their mouth and chew a lemon candy. They were asked to first spit out a small amount of saliva, and then to spit approximately 6 ml in a test tube [13]. The specimen was frozen to −20°C and then shipped for cotinine analysis to the University of California, San Francisco. The cotinine concentration was determined by using gas chromatography with a nitrogen-phosphorus detection technique, as described earlier [14].

Height and weight

Height was measured in centimeters by using a tape mounted on the wall. Participants were asked to remove their shoes. Weight was measured in kilograms with a portable scale. The portable scales were calibrated every day with a standard scale before recording the weight of the participants.

Data analysis

Outcome

The outcome of interest was salivary cotinine concentration.

Exposure

Nicotine is very specific for tobacco and thus tobacco was considered to be the only relevant source of salivary

cotinine in active smokers. The half-life of cotinine is on average 17 hours [2], so the number of cigarettes smoked during the previous 24 hours was chosen as the determinant of interest.

Modifiers

The relation between cigarette smoking and salivary cotinine may be modified by factors related to uptake and metabolism of nicotine. The following factors were considered as potential modifiers of the relation of interest: smoking behavior, i.e., frequency and depth of inhaling smoke, and butt length to which the cigarette is smoked; type of tobacco, i.e., smoking of light or regular cigarettes and use of filter tips; and individual characteristics, i.e., age and body mass index (BMI; weight/height^2 in kg/m^2).

Statistical analyses

Salivary cotinine concentration was plotted in relation to the number of cigarettes smoked in the previous 24 hours. The SPSS statistical package (SPSS 10.0 for windows, SPSS Inc., Chicago, IL, USA) was used for fitting curves of linear and quadratic models. A spline model with a knot at 20 cigarettes/24 hours was fit to the data as well. Five subjects had exceptionally high salivary cotinine concentration in relation to a low reported number of cigarettes smoked and two subjects had exceptionally low cotinine in relation to a high reported number of cigarettes smoked. Their data were checked and there were no data entry mistakes and the cotinine concentrations were of similar magnitude in duplicate analyses, so these subjects were excluded from the final data analysis as outliers. Log transformation of cotinine concentration or log transformation of the number of cigarettes did not improve the fit of the data, so untransformed cotinine was used as the outcome in the final analyses to allow conceptually easier understanding of the results.

The median and 25th and 75th percentiles of salivary cotinine concentrations were computed for different categories of potential modifiers to explore trends. This was done stratified by the number of cigarettes smoked, since the smoking rate and other dimensions of smoking habits are likely to be correlated. To assess potential modifying effect by the factors mentioned earlier, the relation between the number of cigarettes smoked and salivary cotinine concentration was analyzed in linear regression analysis stratifying by the categories of the modifier of interest. Finally, statistical significance of potential modification was tested in linear regression analysis by including in the model smoking in the previous 24 hours, the modifier of interest, and an interaction term between the modifier of interest and the number of cigarettes smoked in the previous 24 hours. In these analyses, the modifiers were categorized as follows: age, 15–54 years or 55–89 years; type of cigarettes, light or regular; use of filter tip, cigarettes with or without filter tip; frequency of inhaling, never or at least sometimes; depth of inhaling, never or at least lightly; cigarette butt length, $\leq$1 cm or $>$1 cm; BMI, $\leq$21.15 or $>$21.15. Since duration of smoking did not show any meaningful trend, it was not tested statistically. Data analyses were carried out by using the SAS System Version Eight for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics and salivary cotinine concentrations of the study population

The distribution of individual characteristics, smoking behavior, and tobacco product characteristics are presented in Table 1 for the total population of 600 subjects and for the final study population of 510 subjects, all cigarette-smoking men. The distributions of most of the characteristics were essentially similar in the study population and in the total population. The number of cigarettes smoked daily was slightly higher in the study population. The median number of cigarettes smoked in the previous 24 hours was 20 cigarettes. The cumulative distribution of salivary cotinine concentration is shown in Fig. 1.

The relation between the number of cigarettes smoked and salivary cotinine concentration

In a simple linear regression model, cotinine concentration was estimated to increase on average by 5.5 ng/ml for each cigarette smoked in the previous 24 hours (95% confidence interval 4.0, 6.9 ng/ml, $R^2 = 0.09$) (Table 2). The spline model showed that for those who smoked 20 cigarettes or less/24 hours, salivary cotinine increased on average by 7.3 ng/ml for each cigarette (95% CI 5.2, 9.5 ng/ml). This relationship weakened dramatically among those smoking more than 20 cigarettes. Quadratic model provided the best fit to the data ($R^2 = 0.11$). Fig. 2a and b show salivary cotinine concentration in relation to the number of cigarettes smoked during the previous 24 hours. Fig. 2a presents fitting of a linear model, showing that the relation between the number of cigarettes smoked and cotinine fits well a linear assumption. Fig. 2b presents fitting of a quadratic model, showing that a convex model seems to fit the data best.

Modification of the relation between the number of cigarettes smoked and cotinine concentration

Table 3 presents the median salivary cotinine concentration in categories of modifiers of interest stratified by the number of cigarettes smoked in the previous 24 hours. The median cotinine concentration tended to be higher among the younger age groups. The highest cotinine concentrations were observed among subjects with the lowest BMI, although no clear trends were seen from the heaviest to the lightest subjects. Among those who had smoked up to 20 cigarettes, the cotinine concentration increased with in-

Table 1
Characteristics of the total population participating and the study population of the present analyses

Characteristic	All participants (n = 600)		Study population[1] (n = 510)	
	N[2]	%	N[3]	%
Individual characteristics				
Gender				
Male	578	96.7	510	100.0
Female	20	3.3	—	—
Age (in years)				
15–34	131	21.9	110	21.6
35–54	400	66.9	344	67.6
55–89	67	11.2	55	10.8
BMI[4]				
≤21.15	149	25.0	126	24.9
21.16–23.14	140	23.5	126	24.9
23.15–25.34	156	26.2	127	25.0
25.35	151	25.3	128	25.2
Type of tobacco and smoking rate				
Type of tobacco				
Cigarettes w/o filter tip	21	3.5	20	3.9
Cigarettes with filter tip	566	95.0	483	95.1
Cigars	3	0.5	—	—
Pipe	1	0.2	—	—
Other	5	0.8	5	1.0
Type of cigarettes				
Light	154	25.9	119	23.5
Regular	437	73.4	383	75.7
Hand-rolled	4	0.7	4	0.8
No. of cigarettes smoked in the previous 24 hours				
1–10	193	32.3	133	26.1
11–20	315	52.8	297	58.2
21–30	68	11.4	62	12.2
31+	21	3.5	18	3.5
No. of cigarettes smoked daily on average				
1–10	213	35.8	150	29.5
11–20	316	53.1	299	58.9
21–30	52	8.7	48	9.4
31+	14	2.4	11	2.2
Smoking habits				
Frequency of inhaling				
Never	152	25.4	114	22.4
Less than 1/2 of the time	149	24.9	134	26.4
About 1/2 of the time	168	28.1	144	28.4
More than 1/2 of the time	51	8.5	47	9.2
Always	78	13.1	69	13.6
Depth of inhaling				
Never	135	22.6	103	20.3
Lightly	200	33.5	169	33.3
Moderately	208	34.8	188	37.1
Deeply	54	9.1	47	9.3
Butt length (cm)				
≤0.5	180	30.0	159	31.2
0.5–1	233	38.8	200	39.2
1.1–1.5	66	11.0	56	11.0
1.6+	121	20.2	95	18.6
Duration of smoking (years)				
1–10	115	21.1	91	19.4
11–20	152	27.9	142	30.2
21+	278	51.0	237	50.4

[1] The following subjects were excluded: those using nicotine replacement therapy (n = 1), women (n = 20), and those with missing gender information (n = 2), those smoking cigars or pipe (n = 4), those with cotinine concentration under 10 ng/ml (n = 63), and seven outliers. Some of these groups were overlapping, so altogether 90 subjects were excluded from the final analyses.

[2] Information was missing: gender in 2 subjects, age in 2 subjects, BMI in 4 subjects, type of tobacco in 4 subjects, type of cigarettes in 5 subjects, no. of cigarettes smoked in the previous 24 hours in 3 subjects, no. of cigarettes smoked daily on average in 5 subjects, frequency of inhaling in 2 subjects, depth of inhaling in 3 subjects, duration of smoking in 55 subjects.

[3] Information was missing: age in 1 subject, BMI in 3 subjects, type of tobacco in 2 subjects, type of cigarettes in 4 subjects, no. of cigarettes smoked daily on average in 2 subjects, frequency of inhaling in 2 subjects, depth of inhaling in 3 subjects, duration of smoking in 40 subjects.

[4] BMI = body mass index (= weight/height^2 in kg/m^2).

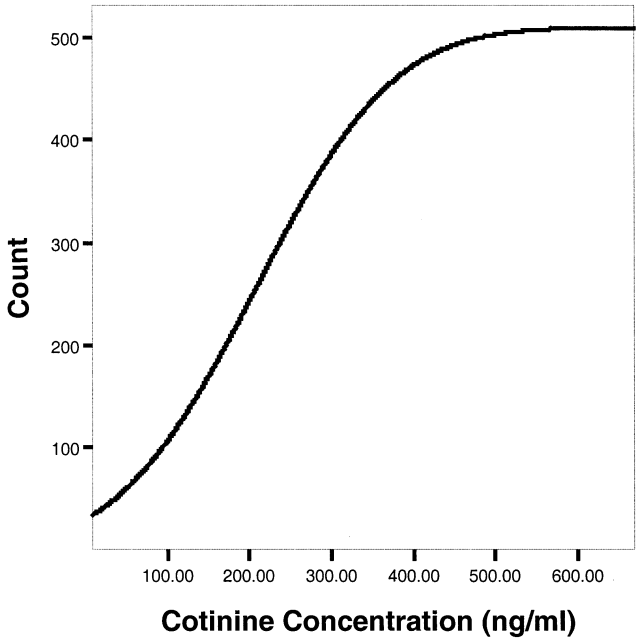

Fig. 1. The cumulative distribution of salivary cotinine concentration among 510 Chinese male smokers. X-axis shows the cotinine concentration in ng/ml and Y-axis shows the cumulative count.

creasing frequency of inhaling as well as with deeper inhaling of smoke, while no clear trend could be observed among those who had smoked more than 20 cigarettes. An increasing cotinine concentration with a decreasing cigarette butt length was observed only in the lightest smoking category. Consistent trends were not observed for duration of smoking. In regard to the type of tobacco, the median cotinine concentration increased with smoking of regular cigarettes compared to light cigarettes and with smoking cigarettes without filter tip compared to cigarettes with filter tip. These trends were seen clearly among those who had smoked up to 20 cigarettes, while the picture was less clear among heavier smokers.

The results of linear regression analysis of the relation between the number of cigarettes smoked and salivary cotinine concentration stratified by the levels of modifiers of interest are shown in Table 4. The average increase in cotinine concentration per one cigarette smoked was larger in young smokers than in old smokers, among those with the lowest BMI, in those leaving a long cigarette butt than in those leaving a small butt, in those never inhaling than in those inhaling frequently and deeply, in those smoking light cigarettes than in those smoking regular cigarettes, and in those smoking cigarettes with filter tip than in those smoking cigarettes without filter tip. Statistical significance ($P < 0.05$) was reached by modification by the frequency of inhaling and by cigarette butt length, and borderline significant ($P = 0.06$) modification was observed by age.

Discussion

This study evaluated the relation between the number of cigarettes smoked in the previous 24 hours and salivary cotinine concentration in a sample of Chinese adults from Beijing and Shanghai. Cotinine concentration as a function of the number of cigarettes smoked fitted well a linear model for the purposes of our analyses. However, the best fit to the data was a convex relation with flattening of the change in cotinine at about 20–30 cigarettes. This form of the curve suggests that there is an upper limit of desired nicotine intake, which is achieved on average by smoking about 20–30 cigarettes per day. Above this consumption level, smokers may modify their smoking pattern to take in less nicotine per cigarette. The form of the curve would also be consistent with saturation of nicotine metabolism so that less cotinine is formed, and with induction of cotinine metabolism at higher levels of cigarette consumption. However, the authors are unaware of any evidence of dose dependence of nicotine or cotinine metabolism [15].

Our results are consistent with earlier studies from United States. A convex type of curve was shown for serum cotinine in the U.S. population of the Third National Health and Nutrition Examination Survey (NHANES III), including black, white, and Mexican-American smokers (5), as well as in a sample of smokers recruited from shopping malls [16]. To our knowledge, the relation between the number of cigarettes smoked and salivary cotinine concentration has not been studied earlier in a population of Chinese origin. The proportion of variation in salivary cotinine explained by the number of cigarettes smoked in the previous 24 hours was 9% in the linear model and 11% in the quadratic model. This finding emphasizes the need to understand the factors that influence the relation between smoking rate and cotinine level.

Several studies have reported differences in mean salivary or serum cotinine concentrations among different ethnic groups [3–5,9]. Differences in the number of cigarettes smoked, in the smoking behavior, in the type of tobacco smoked, in reporting of smoking habits, and in metabolism of nicotine have been suggested as potential explanations for these variable cotinine concentrations [5–7,9,17,18]. Re-

Table 2
Results of fitting different type of regression models for the relation between the number of cigarettes smoked in the previous 24 hours and salivary cotinine concentration (in ng/ml)

Model	Estimate	95% CI[1]	R^2
Simple linear	5.46	3.99, 6.94	0.09
Spline with knot at 20 cigarettes/24 h			0.10
≤20 cigarettes	7.33	5.15, 9.50	
>20 cigarettes	−4.69	−8.74, −0.62	
Quadratic			0.11
Per cigarette	11.05	6.16, 15.9	
Per cigarette^2	−0.14	−0.27, −0.02	

[1] Confidence interval.

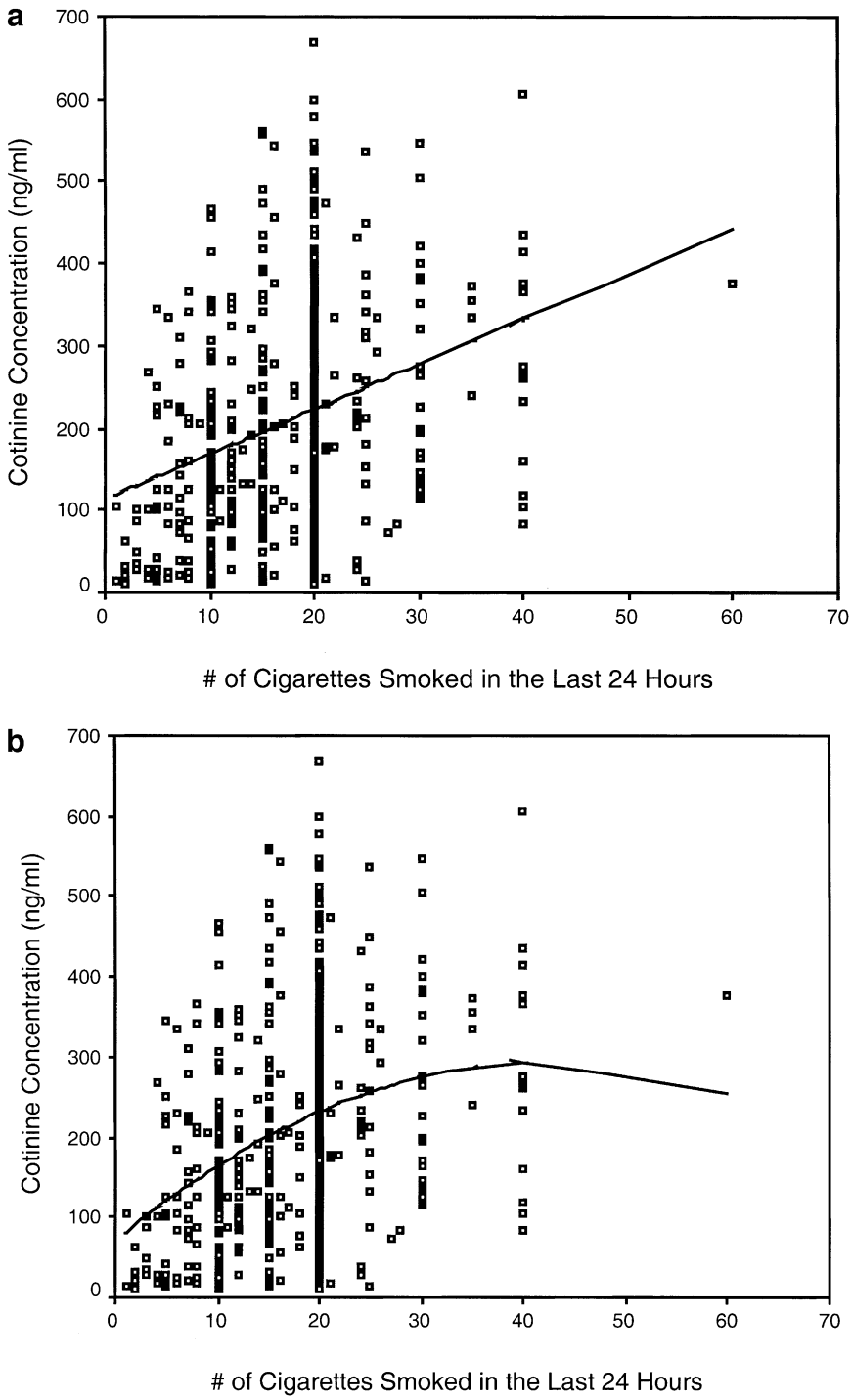

Fig. 2. (a) Salivary cotinine concentration (on Y-axis) in relation to the number of cigarettes smoked during the previous 24 hours (on X-axis) among 510 Chinese male smokers. The line shows fitting of a linear model. (b) Salivary cotinine concentration (on Y-axis) in relation to the number of cigarettes smoked during the previous 24 hours (on X-axis) among 510 Chinese male smokers. The curve shows fitting of a quadratic model.

cent data suggest that Chinese Americans take in, on average, 35% less nicotine per cigarette and metabolize nicotine more slowly than do Hispanics and whites [19]. Another recent study of pharmacokinetics of nicotine in smokers indicated that the higher cotinine levels observed in blacks compared with whites might be explained by lower metabolic clearance of cotinine and by greater nicotine intake per cigarette in blacks than in whites [9]. The determinants of the higher intake were not investigated.

Since identification of sources of variability in cotinine concentrations may be important for planning preventive programs and for determining the dose for nicotine replace-

Table 3
Median and 25th and 75th percentiles of cotinine concentration (in ng/ml) at different levels of potential modifiers stratified by the number of cigarettes smoked in the previous 24 hours

Modifier	1–10 cigarettes				11–20 cigarettes				21–30 cigarettes				31+ cigarettes			
	n[1]	25th	Median	75th	n	25th	Median	75th	n	25th	Median	75th	n	25th	Median	75th
Individual characteristics																
Age (years)																
15–34	39	71.9	128.0	224.0	62	141.0	232.5	344.0	5	114.0	154.0	214.0	4	298.0	344.5	396.0
35–54	79	41.5	130.0	213.0	206	111.0	196.0	289.0	51	163.0	221.0	333.0	8	246.5	374.0	396.5
55–89	15	58.5	126.0	207.0	28	128.0	198.5	257.0	6	194.0	220.0	232.0	6	117.0	200.5	276.0
BMI[2]																
≤21.15	40	92.0	177.0	226.5	65	117.0	251.0	340.0	17	214.0	316.0	450.0	4	360.0	367.5	403.5
21.16–23.14	37	40.0	103.0	223.0	80	114.5	198.5	294.0	7	165.0	265.0	275.0	2	105.0	169.0	233.0
23.15–25.34	29	38.6	119.0	170.0	72	123.5	179.0	284.5	22	146.0	206.0	320.0	4	161.6	251.5	320.0
25.35+	27	58.6	126.0	206.0	78	124.0	201.0	289.0	16	129.0	192.5	226.5	7	161.0	276.0	416.0
Type of tobacco																
Type of cigarettes																
Light	36	39.3	121.5	215.0	66	109.0	168.5	269.0	14	132.0	216.0	259.0	3	356.0	377.0	436.0
Regular	95	58.6	134.0	214.0	226	119.0	205.5	311.0	47	163.0	220.0	333.0	15	161.0	263.0	371.0
Use of filter tip																
Cigarettes w/o filter	6	140.0	159.0	220.0	5	219.0	257.0	330.0	6	200.0	231.5	420.0	3	233.0	276.0	333.0
Cigarettes with filter	126	58.2	125.5	214.0	286	117.0	198.0	289.0	56	150.0	217.5	326.5	15	161.0	356.0	377.0
Smoking habits																
Freq. of inhaling																
Never	38	27.1	82.8	151.0	62	119.0	194.5	273.0	11	154.0	211.0	265.0	3	240.0	364.0	436.0
<1/2 of the time	48	63.2	134.0	215.0	75	95.8	159.0	330.0	8	129.0	191.5	229.0	3	83.2	117.0	377.0
≥1/2 of the time	47	96.3	160.0	232.0	159	131.0	209.0	317.0	42	165.0	225.0	351.0	12	246.5	304.5	374.0
Depth of inhaling																
Never	38	24.2	83.8	194.0	55	124.0	176.0	276.0	8	119.7	193.0	319.5	2	240.0	302.0	364.0
Lightly	57	63.7	138.0	216.0	91	113.0	198.0	297.0	16	129.0	186.5	224.5	5	161.0	276.0	377.0
Moderately or deeply	38	85.9	158.5	225.0	148	127.0	204.5	334.0	38	169.0	262.5	340.0	11	233.0	333.0	377.0
Cigarette butt (cm)																
≤ 0.5	38	58.5	136.0	207.0	87	126.0	219.0	328.0	29	132.0	203.0	275.0	5	117.0	333.0	371.0
0.5–1.0	52	44.9	131.5	215.0	122	103.0	184.5	259.0	18	165.0	223.5	401.0	8	197.0	250.0	269.5
1.1+	43	62.6	106.0	216.0	88	140.5	225.0	361.0	15	200.0	265.0	380.0	5	364.0	377.0	416.0
Duration of smoking (years)																
1–10	32	76.4	129.0	214.5	50	141.0	207.5	344.0	6	71.8	134.0	211.0	3	263.0	356.0	436.0
11–20	32	38.5	118.0	213.5	88	90.8	188.5	287.0	19	146.0	220.0	351.0	3	260.0	333.0	416.0
21+	52	58.1	153.5	210.0	137	125.0	202.0	278.0	36	170.0	227.5	326.5	12	139.0	258.0	374.0

[1] n = number of subjects in each category.
[2] BMI = body mass index.

ment therapy, we evaluated potential modification of the relation between the amount of smoking and cotinine concentration by some personal and smoking behavior-related factors. We found that many smoking behavior features are correlated with the amount of smoking, for example, the proportion of deep and frequent inhalers increases from light to heavy smokers. Thus, we computed the median cotinine concentrations for different levels of potential modifiers, stratified by the amount of cigarette smoking. We found that among those who had smoked up to 20 cigarettes during the previous 24 hours, the median cotinine concentration was higher in younger subjects, in those with the lowest BMI, in frequent and moderate or deep inhalers, in those leaving a small cigarette butt, in those smoking cigarettes without filter tip, and in those smoking regular rather than light cigarettes. Among those who had smoked more than 20 cigarettes, such trends were not consistently observed. This may be explained by the convex form of the relation, i.e., after a certain nicotine (and cotinine) level is attained, the intake of nicotine per cigarette becomes smaller. However, the number of subjects in the highest smoking category of 31 or more cigarettes was small (n = 18), so the estimates in this category are less precise.

Regression analyses of relation between the number of cigarettes smoked and salivary cotinine concentration stratified by the levels of modifiers revealed a tendency of a higher cotinine concentration increase per cigarette among those groups that had a lower median level of cotinine. The mechanisms underlying our findings should be evaluated further. A potential pathway leading to such results is the following: For example, frequent inhalers have achieved a higher serum nicotine (and salivary cotinine) level and have already reached the level needed to satisfy their desire. Consequently, they can adjust the puff volume and other

Table 4
Average increase in cotinine concentration (in ng/ml) per one cigarette smoked during the previous 24 hours in linear regression analyses stratified by the levels of potential modifiers

Modifier	n[1]	Estimate	95% CI[2]
Individual characteristics			
Age (years)			
15–34	110	6.1	3.0, 9.3
35–54	344	6.3	4.5, 8.1
55–89	55	2.3	−0.9, 5.5
BMI[3]			
≤21.15	126	7.3	4.3, 10.4
21.16–23.14	126	5.5	2.6, 8.4
23.15–25.34	127	4.7	2.0, 7.4
25.35+	128	5.1	2.1, 8.1
Type of tobacco			
Type of cigarettes			
Light	119	6.4	3.4, 9.4
Regular	383	5.2	3.6, 6.8
Use of filter tip			
Cigarettes w/o filter	20	4.2	−1.5, 9.9
Cigarettes with filter	483	5.4	4.0, 6.9
Smoking habits			
Freq. of inhaling			
Never	114	7.9	5.6, 10.2
<1/2 of the time	134	4.2	1.2, 7.2
≥1/2 of the time	260	4.0	1.9, 6.1
Depth of inhaling			
Never	103	7.1	4.3, 9.9
Lightly	169	4.6	2.2, 7.0
Moderately or deeply	235	4.4	2.1, 6.7
Cigarette butt (cm)			
≤0.5	159	4.6	1.9, 7.3
0.5–1.0	200	4.6	2.6, 6.6
1.1+	151	7.9	5.2, 10.6
Duration of smoking (years)			
1–10	91	5.9	2.3, 9.5
11–20	142	7.0	4.0, 10.0
21+	237	4.7	2.7, 6.7

[1] n = number of subjects in each category.
[2] 95% CI = 95% confidence interval for the estimate.
[3] BMI = body mass index.

aspects of their smoking behavior to take less nicotine in per cigarette. In contrast, those not inhaling have achieved a low serum nicotine level and must take more nicotine in per cigarette to approach the desired level. Thus, our findings suggest that smokers may regulate (unconsciously) their smoking behavior according to their nicotine concentration to achieve a certain serum nicotine level and consequently salivary cotinine level. An alternative pathway explaining our results would be that nicotine and/or cotinine metabolism is induced at high concentrations, so that the increase in cotinine concentration per cigarette becomes smaller. Our findings may have relevance when thinking about potential benefits of low-nicotine cigarettes.

Validity issues

Our study population was recruited among volunteers, so the information on smoking rates and habits cannot be generalized to the general Chinese population. However, it is unlikely that the volunteers would have been selected according to their ability to metabolize nicotine, and thus it is likely that our results on the relation between smoking and salivary cotinine levels are valid.

Information on smoking rate and habits was collected mainly by using a short questionnaire that was developed for this study. The questions of it were based on the ATS adult respiratory questionnaire [12] where appropriate. The larger survey questionnaire was modified from the World Health Organization questionnaire [20]. The questionnaires were developed in English, translated into Chinese, and then back-translated into English to assure comparability of the questionnaires in both languages. Any differential misclassification of smoking habits in relation to cotinine level is unlikely, but some nondifferential misclassification is possible, so our estimates may slightly underestimate the true relation between smoking and cotinine concentration.

Cotinine is the major proximate metabolite of nicotine and it is commonly used to estimate the absorbed dose of nicotine [1,2,11]. The absorbed dose is best reflected in serum cotinine, but levels of cotinine in serum are highly correlated with levels in saliva, the typical concentration ratio for saliva to blood being 1.1 to 1.4 [2,21,22]. Those with a salivary cotinine concentration below 10 ng/ml were excluded to ensure that our study population consisted of regular smokers who had provided a proper saliva sample.

Nicotine, and consequently cotinine, is practically specific for tobacco. Small amounts of nicotine can be absorbed from some dietary sources, such as tea and vegetables from the Solanaceae family, but these amounts are negligible compared to nicotine absorbed due to active smoking [2,23,24]. Absorption of nicotine due to passive smoking is also negligible compared to that due to active smoking [11] and the most important source of environmental tobacco smoke exposure of a smoker is his/her own smoking. Thus, we did not control for dietary or environmental sources of nicotine in our analyses. Other potential confounders (i.e., direct determinants of serum nicotine and cotinine) are not known.

Conclusions

In a population of Chinese male smokers, salivary cotinine concentration increased significantly with the number of cigarettes smoked in the previous 24 hours, a quadratic model providing the best fit to the data. Flattening of this relation was observed at 20–30 cigarettes. Among those smoking up to 20 cigarettes, the median cotinine concentration was higher among younger subjects, among those smoking cigarettes without filter and regular cigarettes rather than light cigarettes, among those leaving a small cigarette butt, and among those inhaling frequently and deeply. Such trends were not consistently observed among

those smoking more than 20 cigarettes. The increase in cotinine per cigarette tended to be larger in those groups with a lower median salivary cotinine level. Our results suggest that smokers regulate their smoking behavior to achieve a certain optimum nicotine (and, hence, cotinine) level.

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