

Determinants of salivary cotinine levels among current smokers in Mexico

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The present study describes salivary cotinine levels and their relationship to cigarettes smoked per day in Mexican smokers. Using a sampling strategy based on the number of cigarettes per day, we recruited 1,222 smokers from Mexico City and the state of Morelos in Mexico during 1999. Smoking behaviors and other factors known to affect nicotine intake and cotinine level were identified in an interview using a standardized questionnaire. Salivary cotinine was measured by capillary gas chromatography with nitrogen–phosphorus detection. We used generalized additive models to describe the relationship between salivary cotinine levels and variables of interest. The mean age of the population was 39.7 years ($SD=15.6$ years), with a mean cotinine level of 194.7 ng/ml ($SD=134.8$; range=10.1–767). Participants smoked a mean of 15.5 cigarettes per day ($SD=11.3$). Salivary cotinine and cigarettes smoked per day were positively related, although the association was not linear, flattening above 20 cigarettes per day. After adjusting for cigarettes per day, we found that significant predictors of cotinine levels included age, body mass index, cigarette producer, and smoking behavior variables. These results may have implications for dosing with nicotine medications to aid smoking cessation in Mexican smokers and suggest that whether the cigarette is labeled light or regular has no relationship to nicotine dose from smoking cigarettes.

Introduction

Cigarette smoke contains nicotine, which is present in dry tobacco leaves at a concentration by weight that ranges from 2% to 8% (International Agency for Research on Cancer, 1986; U.S. Department of Health and Human Services [USDHHS], 1988).

Nicotine is the main determinant of tobacco use and addiction, and for the addicted smoker the smoking pattern maintains the needed intake of nicotine (Benowitz, 1996b; USDHHS, 1988). Diverse factors determine the intake of nicotine, including primarily the frequency and number of cigarettes smoked, the pattern of puffing and inhaling, and the characteristics of the cigarettes smoked. Additionally, smoking profiles vary across populations and countries (Corrao, Guindon, Sharma, & Shokoohi, 2000).

Although nicotine levels can be measured in blood and other biological materials, cotinine, the major proximate metabolite of nicotine, has been used most widely to characterize daily nicotine intake or dose (Benowitz, 1996a). For regular smokers, it affords a comparatively stable biomarker related to its half-life of 18–20 hr. Cotinine levels have been measured in a number of populations, thus providing a description of the distribution of levels among smokers and insights into determinants of cotinine levels (Abrams, Follick, Biener, Carey, & Hitti, 1987; Ahijevych & Parsley, 1999; Ahijevych & Wewers, 1994; Benowitz,

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Perez-Stable, Herrera, & Jacob, 2002; Caraballo et al., 1998; Coultas, Stidley, & Samet, 1993; Etter, Vu, & Perneger, 2000; Hill, Haley, & Wynder, 1983; Jaakkola et al., 2003; Jarvis, Boreham, Primatesta, Feyerabend, & Bryant, 2001; Perez-Stable, Herrera, Jacob, & Benowitz, 1998; Perez-Stable, Marin, Marin, Brody, & Benowitz, 1998; Pierce et al., 1987; Quandt, Arcury, Preisser, Bernert, & Norton, 2001; Wagenknecht et al., 1990; Wewers et al., 2000).

The present study was a part of an ongoing multinational survey of cotinine levels in smokers. Findings have been reported for China, where the majority of men smoke cigarettes but less than 5% of women smoke (Jaakkola et al., 2003; Yang et al., 1999). This paper reports findings of a similar survey in Mexico, where 43% of men and 16% of women smoke cigarettes (Tapia-Conyer, Kuri-Morales, & Hoy-Gutierrez, 2001). Additionally, Mexican smokers have been found to smoke fewer cigarettes per day, compared with smokers in many other countries (Tapia-Conyer et al., 2001). The particular goal of this paper is to characterize the relationship between cotinine level and number of cigarettes smoked. The present study provides findings from 1,222 smokers in Mexico City and Cuernavaca in the state of Morelos, representing the first study to our knowledge of cotinine levels in Mexican smokers to date. To account for variables that may have an effect on cotinine levels, we constructed a multivariate model to characterize the relationship between cotinine levels and the number of cigarettes smoked, controlling for other relevant variables that may have a direct or indirect effect on cotinine levels, including age, duration of smoking habit, time since last cigarette was consumed, type of cigarette smoked (filtered light, filtered regular, nonfiltered), gender, and body mass index. The relationship between the Fagerström addiction index (Fagerström & Schneider, 1989) and salivary cotinine concentration also was investigated.

Method

Study population

The study population was recruited using different sampling strategies for Mexico City and Cuernavaca. In Mexico City, eligible smokers were identified among 63,919 participants in a population-based cohort study that was designed to assess risk factors for chronic diseases among adults who were aged 30 years or older and who were residents of a specific district in Mexico City (Coyoacan). This cohort was assembled between 1997 and 1998 by visiting 20,356 houses in the neighborhood of Coyoacan, which was chosen because it is a stable community with low levels of migration, has a wide socioeconomic range within the population, and is a very accessible neighborhood. The eligibility criteria were willingness

to participate and having a family member over age 35 years in the household. Among households with eligible participants, the participation rate for the cohort was 95.4%. At baseline, participants in this cohort completed a general questionnaire that included questions on smoking. From this group, current smokers were identified and a stratified random sample was selected. Because a comparable sampling frame was not available for Morelos, smokers were recruited using a convenience sampling approach. The strategy involved identifying people aged 17 years or older who described themselves as smokers and who agreed to participate in the study. Subjects were identified at schools, health institutions, shopping centers, parks, movie theaters, and other places. Subjects were not provided with any kind of incentive to participate.

To obtain a balanced number of participants in each smoking intensity category, we used a stratified sampling strategy based on the reported number of cigarettes smoked per day. The following categories were used for recruitment: 0–5, 6–10, 11–15, 16–20, 21–30, and 31 or more cigarettes per day. Nondaily smokers were included as long as they identified themselves as smokers. Based on sample size considerations, each stratum was targeted for 100 participants. The research protocol was approved by the Human Subjects Committee of the National Institute of Public Health of Mexico and the Committee on Human Research of the Johns Hopkins Bloomberg School Public Health. All participants received a detailed explanation of the study and procedures used, and all gave their informed consent.

Measures

Participants answered a structured questionnaire that collected information on (a) sociodemographic characteristics, (b) use of nicotine replacement therapy such as nicotine patches or gum to quit smoking, (c) smoking habits, number of cigarettes smoked in the past 24 and 48 hr, intensity of inhalation, time since last cigarette smoked, cigarette smoke inhalation frequency, depth of inhalation, brand of cigarettes, frequency, type of cigarettes smoked (filtered light, filtered regular, nonfiltered), and age at which the person started smoking, and (d) variables used in the Fagerström Tolerance Questionnaire (FTQ; Fagerström & Schneider, 1989). The questionnaire has been used and tested in other international studies (Jaakkola et al., 2003). Sociodemographic items included gender, present occupation, and highest education level. Weight and height measurements also were obtained and were used to estimate body mass index (weight/height² in kg/m). Exclusion criteria were self-identification as a nonsmoker, being ill, or otherwise being unable to answer the questionnaire.

Salivary samples for the cotinine assay were obtained using a stimulant of lemon candy. After answering the questionnaire, participants were asked to first rinse their mouths and then chew the candy. The participants were then asked to spit out a small amount of saliva and then to provide a sample of approximately 3 ml of saliva in a plastic test tube (Schneider et al. 1997). The samples were sent to the Clinical Pharmacology Laboratory at San Francisco General Hospital, where salivary cotinine was measured by capillary gas chromatography with nitrogen-phosphorus detection (Jacob, Wilson, & Benowitz, 1981).

Questionnaire data on brand smoked were used to identify the most popular brands of cigarettes among study participants. Data are not available on tar and nicotine yield according to the Federal Trade Commission (FTC) protocol. For this study, nicotine content was analyzed in the laboratory of Dr. Benowitz. For each of the top 10 brands, at least three cigarettes were analyzed for nicotine content. Each cigarette was cut open and the tobacco within it collected in a vial and weighed. To each vial was added 20 ml of 0.1 M hydrochloric acid, after which the vial was incubated at 90°C for 30 min. The samples were cooled and centrifuged. Supernatant was assayed for nicotine concentration by gas chromatography with nitrogen-phosphorus detection (Jacob et al., 1981).

Statistical analysis

We performed an exploratory analysis of each variable included in the study to produce univariate

statistics and distribution plots and then explored predictors of cotinine levels using bivariate and multivariate regression. The bivariate analysis, after verifying the normality assumption, included a *t*-test (two groups) and an analysis of variance (three or more groups). We modeled the relationship between salivary cotinine and other predictors using generalized additive models (GAMs) with a nonparametric loess smoothing (Hastie & Tibshirani, 1990) to account for a potential nonlinear association between cotinine levels and the number of cigarettes smoked in the past 24 hr, as well as other variables. As an alternative to summarizing the cotinine level and the number of cigarettes smoked using a single parameter (a regression slope) for all levels of smoking as in standard linear regression, GAMs provide an estimate that is a smoothly changing function of the number of cigarettes (Figure 1). We examined a variety of loess spans and found that a span of 0.8 best represented the association and minimized the Akaike's information criterion (Verbeke & Molenberghs, 2000).

Our primary goal was to characterize the relationship between cotinine levels and the number of cigarettes smoked, controlling for other relevant variables that may have a direct or indirect effect on cotinine levels, including age, duration of smoking habit, time since last cigarette was consumed, type of cigarette smoked (filtered light, filtered regular, nonfiltered), gender, and body mass index. The final multivariate model was constructed by examining the correlation between each variable of interest and cotinine levels. The analyses were conducted for the total sample and stratifying by city. In the final model, however, all data were grouped together. To assess brand-specific

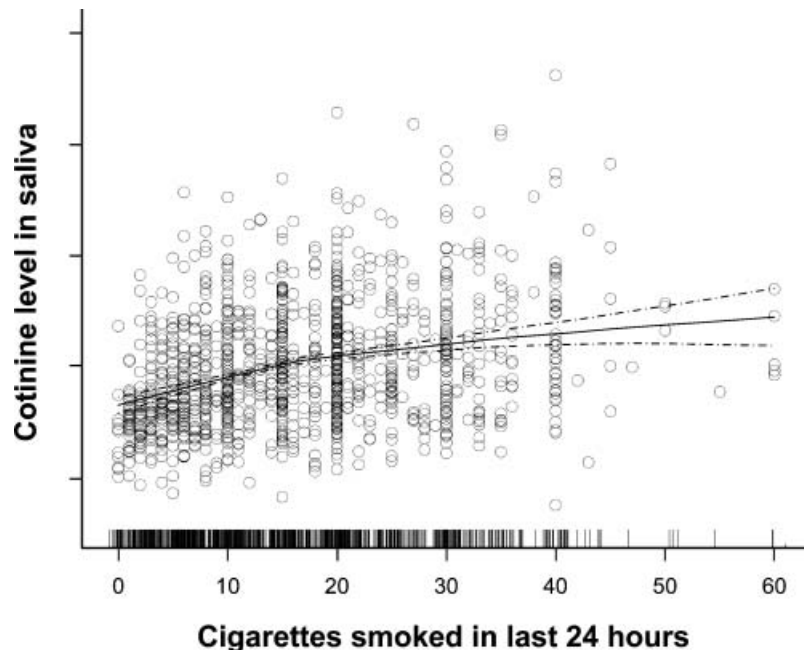


Figure 1. Smooth plot for cotinine and self-reported number of cigarettes smoked in the past 24 h among 1,222 smokers aged 18 years or older. Dashed lines represent confidence bands.

effects, multivariate analyses were conducted that were restricted to participants who reported smoking Marlboro cigarettes, the only brand for which numbers were sufficient.

All statistical analyses were performed using Stata Statistical Software, Release 6.0, and SPLUS v. 4.5.

Results

Population characteristics

The sample included 1,222 smokers aged 17–92 years ($M=39.1$ years; $SD=15.9$) (Table 1). Data from the 23 participants who reported current use of nicotine

Table 1. Sociodemographic characteristics and smoking behavior of self-reported smokers for total sample ($N=1,222$) and by recruitment area in Mexico (all values are percentages)

Variable	Morelos ($n=763$)	Mexico City ($n=459$)	Total ($N=1,222$)
Age (years) ^a			
17–19	24.2	0.0	15.2
20–29	27.3	0.0	17.2
30–39	16.8	17.7	17.1
40–49	17.3	35.3	24.0
50–59	8.5	27.2	15.5
60–92	5.9	19.8	11.1
Gender ^a			
Female	20.0	42.7	28.3
Male	80.0	57.3	71.7
Education (years) ^a			
0–6	15.5	63.0	33.2
7–12	62.3	27.8	49.5
13 or more	22.2	9.2	17.4
Body mass index (kg/m^2) ^a			
18.0–21.9	30.3	13.1	24.0
22.0–24.9	30.9	20.5	27.0
25.0–29.9	29.8	42.6	34.6
30.0–34.9	7.4	17.1	10.9
≥ 35	1.6	6.7	3.5
Age when started smoking (years) ^a			
< 15	31.9	17.2	26.7
15–16	26.0	19.0	23.5
17–20	30.3	29.5	30.0
≥ 21	11.7	34.3	19.8
Cigarettes smoked in the past 24 hr ^a			
< 8	20.7	33.5	25.3
8–15	27.8	29.0	27.9
16–23	22.0	22.3	22.1
≥ 24	30.1	15.2	24.7
Type of cigarettes ^a			
Filtered light	21.8	8.0	16.8
Filtered regular	76.2	64.2	71.8
Nonfiltered	1.9	27.7	11.3
How often do you inhale the cigarette smoke? ^a			
Never	2.6	14.4	6.9
Less than half of the time	8.6	7.8	8.3
Half of the time	15.8	5.6	12.1
More than half of the time	3.5	4.3	3.8
Always	69.3	67.6	68.6
How deeply do you inhale the cigarette smoke? ^a			
Not at all	2.3	13.5	6.4
Slightly	18.8	24.4	20.8
Moderately	45.7	34.9	41.7
Deeply	33.0	27.1	30.8
Time since last cigarette was consumed (hours) ^a			
< 1	76.0	64.7	71.9
1–6	16.2	17.2	16.6
> 6	7.8	18.1	11.6
Cigarette producer ^a			
Cigatam	72.3	51.4	64.6
La moderna	20.8	25.5	22.6
Tabacalera mexicana	6.9	23.1	12.8
Fagerström Tolerance Questionnaire score			
0–2	48.0	49.0	48.5
3–4	19.0	21.0	20.0
5–6	19.0	13.2	15.1
7–10	14.0	16.8	15.4

^aVariables that showed significant differences ($p < .01$) between sites.

replacement therapy to quit smoking were excluded. In the Mexico City cohort, 89.8% ($n=459$) of participants who were invited agreed to participate in the study and provided a sufficient amount of saliva to be included. In Cuernavaca, where we used a convenience recruiting method, 95.7% ($n=763$) of the people who agreed to participate provided a sufficient amount of saliva. Differences existed between the samples recruited in the state of Morelos and in Mexico City (Table 1). Smokers from Morelos were younger, had lower body mass index, were predominantly male, and had higher educational levels than the sample studied in Mexico City. Regarding smoking behavior variables, smokers from Morelos reported an earlier age of smoking initiation and consumed filtered, light cigarettes and Mexican brands more frequently.

The average number of cigarettes consumed in the past 24 hr was 16.5 ($SD=11.3$; range=0–60). The cotinine levels for all participants ranged from 10.1 ng/ml to 767.0 ng/ml, with a mean of 194.7 ng/ml ($SD=134.6$). The number of cigarettes smoked in the past 24 hr was a predictor of cotinine level and explained 21% of the variance (Figure 1). Considerable variation in salivary cotinine levels was observed among those reporting a similar number of cigarettes smoked per day. A simple univariate GAM was used to estimate the relationship between salivary cotinine levels and self-reported number of cigarettes in the past 24 hr. The GAM results indicated that cotinine concentrations increased in a nonlinear fashion across the full range of cigarettes smoked daily. The association between salivary cotinine and cigarettes per day was linear up to 20 cigarettes, but above this number cotinine levels increased less steeply with an attenuated slope associated with a higher number of cigarettes smoked (Figure 1).

Determinants of cotinine levels

Table 2 reports the mean cotinine levels by personal and smoking characteristics, with and without adjustment for number of cigarettes smoked. Significant predictors of cotinine levels in the univariate analyses included gender, place of recruitment, educational level, age, body mass index, type of cigarettes, time since last cigarette was consumed, type of cigarettes, and addiction level as measured by the FTQ. With adjustment for the number of cigarettes smoked, the differences remained significant for all variables except gender. The group who had 10–12 years of education had a substantially lower mean cotinine level (168.0 ng/ml) than groups with 0–9 years (217.2 ng/ml) and 13 or more years (214.2 ng/ml). This group also reported smoking fewer cigarettes per day than the other two groups, and both differences were statistically significant. Participants with body mass index greater than 30 showed substantially lower

cotinine levels. As expected, those who had smoked their last cigarette more than 6 hr before the sample was taken had reduced levels of cotinine (Table 2). The crude mean difference comparing males with females changed from positive to negative after adjusting for differences in the number of cigarettes smoked in the past 24 hr. A small proportion (0.9%) of participants reported smoking zero cigarettes in the past 24 hr, and results remained largely the same when we restricted the analysis to participants who reported they had smoked at least one cigarette in the previous 24 hr.

FTQ score ($M=3.13$; $SD=2.69$) was a significant predictor of cotinine level ($r=0.52$, $p<.01$) and explained 26% of the variance. As expected, FTQ score was significantly associated with the number of cigarettes smoked in the past 24 hr and time elapsed since first cigarette of the day. Both variables accounted for 85% of the variance of the FTQ score.

Multivariate analyses

Age was found to be a significant predictor of cotinine levels. The youngest age group had the lowest mean cotinine level, and cotinine levels increased with increasing age. Compared with participants aged 17–19 years, the older age group (60 years or over) had higher cotinine levels; the mean difference between the groups was 148.3 ng/ml (Table 2). The positive relationship between age and cotinine levels persisted even after adjusting for other variables in the multivariate GAM (Table 3).

Participants in the study reported 32 different commercial brand names; the brands were classified by producer (Cigatam, Cigarrera la Moderna, and Tabacalera Mexicana). Crude mean levels of salivary cotinine for smokers classified according to producer of preferred brand were 167.9, 212.9, and 273.4 ng/ml, respectively. When mean cotinine levels were adjusted by the number of cigarettes smoked in the past 24 hr, participants who consumed Cigatam cigarettes had significantly lower cotinine levels. Mean cotinine differences for La Moderna and Tabacalera Mexicana, compared with Cigatam, were 33.2 and 85.1 ng/ml, respectively. Some 27% of women and 13% of men reported smoking light cigarettes. Levels of cotinine were quite similar in smokers of regular cigarettes and those smoking cigarettes labeled “light.” Self-reported smoking of light cigarettes was not a significant predictor of the cotinine level in the multivariate model (Table 3).

The mean nicotine content for the cigarette brands analyzed was 10.56 mg ($SD=1.56$). Nicotine levels did not vary between light ($M=10.8$ mg; $SD=1.5$) and nonlight cigarettes ($M=10.5$ mg; $SD=1.68$) (Figure 2).

Table 2. Distribution of salivary cotinine levels according to variables of interest and crude and cigarettes/day-adjusted mean differences for 1,222 current smokers aged 17 years or older

Variable	Cotinine level (ng/ml)	Crude mean difference (ng/ml)	Cigarettes/day-adjusted mean difference (ng/ml)
Gender			
Female	177.9		
Male	197.9	21.1*	-3.87
Recruitment area			
Morelos	174.1		
Mexico City	223.5	49.4**	74.8 **
Education level (years)			
0-9	217.2		
10-12	168.0	-49.2**	52.9**
13 or more	214.2	-3.0	24.7*
Age (years)			
17-19	113.3		
20-29	159.1	45.8**	30.3**
30-39	201.1	87.8**	82.0**
40-49	209.5	96.2**	104.4**
50-59	228.4	115.0**	119.2**
60-92	258.7	148.3**	135.7**
Body mass index (kg/m ²)			
18.0-21.9	203.6		
22.0-24.9	206.4	2.51	9.55
25.0-29.9	192.8	-11.9	1.12
30.0-34.9	166.5	-37.2**	-13.61
≥35	155.0	-48.8**	-21.6
Cigarette producer			
Cigatam	167.9		
La moderna	212.9	45.8**	33.2**
Tabacalera mexicana	273.0	106.6**	88.3**
Hours since last cigarette was consumed			
1 hr or less	221.1		
1-6 hr	153.9	-67.1	-26.36
>6 hr	91.9	-129.1**	-60.71**
Type of cigarette			
Filtered light	144.9		
Filtered regular	192.9	47.9**	24.1**
Nonfiltered	259.5	114.5**	87.0**
Fagerström Tolerance Questionnaire score			
0-2	131.1		
3-4	203.1	72.0**	40.1**
5-6	257.6	126.5**	89.9**
7-10	307.9	176.8**	132.0**

Note. Crude mean differences and *p* values were estimated with linear regression models. Adjusted mean differences and associated *p* values were estimated with a bivariate generalized additive model that included the smooth function for number of cigarettes smoked in the past 24 hr and the variable of interest.

p* < .05, *p* < .01.

In the multivariate analyses, significant predictors of cotinine level included cigarettes smoked in the past 24 hr, age, body mass index, cigarette producer, time since last cigarette was smoked, and other behavioral variables (Table 3). A significant effect was found for the variable describing the timing of the first cigarette in the morning on cotinine level. Other variables such as depth of inhalation were not significant predictors. Mean cotinine levels among smokers in Mexico City remained higher than those observed in Morelos (mean difference = 29.5 ng/ml; *p* < .01). Over 50% of smokers reported a preference for Marlboro cigarettes, which are produced in Mexico by Cigatam. Results from the multivariate models were similar when we restricted the analyses to those who reported smoking Marlboro cigarettes (Table 3). As found for all participants, significant differences were not

observed between smokers who reported smoking regular brand cigarettes and those who reported smoking light brand cigarettes, taking other determinants into account.

Discussion

Cigarette smoking is an addiction maintained by the pharmacological action of nicotine delivered by tobacco smoke (USDHHS, 1988). Cotinine is the major proximate metabolite of nicotine. Cotinine levels are determined by the daily intake of nicotine, the extent of metabolism of nicotine to cotinine, and the rate of elimination of cotinine (Benowitz, 1996a). Although considerable individual variability exists in these metabolic parameters, cotinine levels are useful

Table 3. Multivariate model for salivary cotinine level among self-reported smokers aged 17 years or older

Variable	Total sample (N=1,222)		Marlboro smokers (n=590)	
	Coefficient	95% confidence interval	Coefficient	95% confidence interval
Area of recruitment				
Morelos	Ref.			
Mexico City	29.5**	12.2, 46.4	32.7**	16.1, 49.3
Education level (years)				
0-9	Ref.			
10-12	13.7	-2.9, 30.3	10.8	-5.8, 27.4
13 or more	28.7**	8.1, 49.3	24.4**	4.2, 44.6
Age (years)				
17-19	Ref.			
20-29	33.7**	10.9, 56.5	36.9**	15.9, 57.9
30-39	80.8**	56.7, 104.9	81.5**	59.3, 103.7
40-49	102.3**	77.9, 126.5	103.9**	80.7, 127.1
50-59	116.2**	89.6, 142.8	121.8**	197.4, 146.2
≥60	125.8**	97.0, 154.6	125.4**	75.8, 175.0
Body mass index (kg/m ²)				
18.0-21.9	Ref.			
22.0-24.9	-14.4	-31.7, 2.9	-14.4	-36.0, 7.2
25.0-29.9	-35.8**	-52.8, -18.8	-35.5**	14.1, 56.9
30.0-34.9	-57.4**	-80.6, -34.2	-55.0**	-25.8, -84.2
≥35	-72.9**	-108.4, -37.4	-72.2**	-116.4, -28.0
Type of cigarette				
Filter light	Ref.		Ref.	
Filter regular	9.8	-6.8, 26.4	2.9	-10.7, 16.5
No filter	16.3	-9.3, 41.9	NA	
Cigarette producer				
Cigatam	Ref.		NA	
La Moderna	14.7	-0.9, 30.3	NA	
Tabacalera Mexicana	24.2**	-0.8, 49.2	NA	
Hours since last cigarette was consumed				
≤1	Ref.			
1.1-6.0	-15.6	-33.0, 1.8	-17.2*	-34.6, 0.2
>6.0	-67.5**	-88.7, -46.3	-61.3**	-82.3, -40.3
How soon after waking up do you smoke your first cigarette?				
Within the first 5 min	Ref.			
6-30 min	-8.5	-31.3, 14.3	-9.8	-41.2, 21.6
31-60 min	-30.3*	-58.7, -1.9	-32.1**	-61.1, -3.1
More than 60 min	-58.8**	-77.1, -39.9	-61.2**	-85.8, -36.6
Do you smoke more during the first hours of the morning than during the rest of the day? (yes)	20.0**	6.4, 33.6	19.6**	6.2, 33.0
When you are sick, do you smoke? (yes)	15.6*	0.98, 30.2	15.0*	0.6, 29.4

Note. Coefficients were estimated using multivariate generalized additive models including all variables simultaneously and the smooth (loess) function for number of cigarettes smoked in the past 24 hr. NA, not applicable.

* $p < .05$, ** $p < .01$.

in comparing nicotine intake in large populations, as long as the populations do not differ in their profiles of nicotine metabolism. One recent study of several ethnic groups in the United States showed comparable nicotine and cotinine metabolism in Latino and non-Latino White smokers, suggesting that cotinine levels would be a useful comparative indicator of nicotine dose for the two ethnic groups (Benowitz et al., 2002).

In the present study, as expected, the most important predictor of salivary cotinine levels was number of cigarettes smoked per day, which explained 21% of the variation of cotinine levels in the population. In addition, other factors had effects on cotinine levels, including those modifying nicotine disposition, such as age and body composition. We documented that the FTQ score, which combines information about the number of cigarettes smoked

daily, the time to the first cigarette smoked after waking, the amount smoked during the first hours of the morning as compared with the rest of the day, difficulties with not smoking on smoke-free premises, and smoking while being sick, was significantly associated with salivary cotinine ($r = 0.52$, $p < .01$). Our results are in agreement with previous reports that indicate the FTQ correlates with other proposed measures of nicotine dependence (carbon monoxide, nicotine, and cotinine levels; Fagerström & Schneider, 1989). The observed association between salivary cotinine and FTQ in the present study was higher than that observed by Abrams et al. (1987), who reported a correlation of 0.33 between salivary cotinine and FTQ among a group of 96 smokers. No published data for the Mexican population was found to provide a more direct comparison, but our

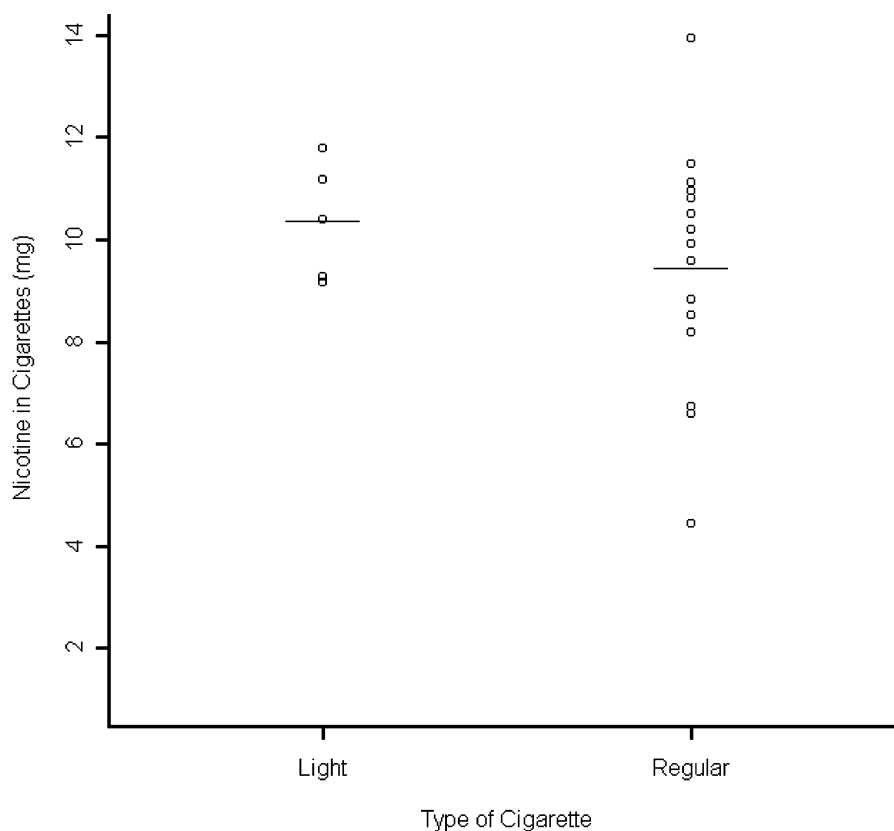


Figure 2. Distribution of nicotine content for most popular brands, by designation as light or nonlight cigarettes.

finding supports the use of the Fagerström addiction index when considering the dose of nicotine replacement therapy in the Mexican population.

Our findings are consistent with other reports that have compared self-reported smoking status with salivary or serum cotinine levels (Abrams et al., 1987; Ahijevych & Parsley, 1999; Ahijevych & Wewers, 1994; Benowitz, 1996a; Benowitz et al., 2002; Caraballo et al., 1998; Coultas et al., 1993; Etter et al., 2000; Hill et al., 1983; Jaakkola et al., 2003; Jarvis et al., 2001; Perez-Stable et al., 1990, 1998; Pierce et al., 1987; Quandt et al., 2001; Wagenknecht et al., 1990; Wewers et al., 2000). A summary of other published studies on the association between cotinine and cigarette consumption is presented in Table 4, which shows that considerable variation exists in cotinine levels across and within populations. The variation in cotinine concentrations among people who report having smoked the same number of cigarettes per day, as well as between populations, is reflected in the variation observed in cigarette consumption or nicotine intake from cigarettes across different countries or different ethnic groups within countries.

Differences in the metabolism of nicotine across populations could explain in part the differing patterns of smoking and possibly of smoking-related diseases across populations. Differing needs for nicotine, reflecting metabolism, also could lead to

differing doses of cigarette smoke toxins. In the United States, the lowest rates of lung cancer are seen in Asians and in Latinos, whereas higher rates are observed in Whites and Blacks (Coultas et al., 1994). Possible explanations include differences in the age at initiation of smoking or differences in smoking behavior. Benowitz et al. (2002) found that Chinese Americans metabolize nicotine more slowly than Latinos and Whites and that they take in less nicotine from each cigarette smoked, compared with Latinos or Whites. This observation is consistent with the hypothesis that slower nicotine metabolism may be partially responsible for the lower intake of nicotine and perhaps lower dose of carcinogens among Chinese-American smokers. However, in the same study, Latinos who consumed fewer cigarettes than Whites had a similar metabolic rate for cotinine. This last observation suggests that cultural factors also are important in determining smoking rates among populations. The present study is part of a large ongoing multinational study to assess smoking and cotinine levels in different populations. When data for all the population studies are available they will contribute to the better understanding of sources of variation in nicotine intake from tobacco.

Women in Mexico, as in many other parts of the world, smoke less frequently and on average smoke fewer cigarettes than men (Aghi, Asma, Yeong, & Vaithinathan, 2001; Tapia-Conyer et al., 2001).

Table 4. Summary of reported studies relating serum or salivary cotinine to cigarette consumption

Author, year, and location of study	Ethnic/racial group, sample size and age (years), and gender composition	Biological sample	Assay method	Cigarettes/day (SD)	Mean cotinine (ng/ml)
Perez-Stable et al., 1982–1983 (Arizona, California, Colorado, New Mexico, and Texas, USA)	Mexican-American males ($n=287$), age=39 Mexican-American males ($n=260$), age=38	Serum	GC	13.4 12.0	163.4 158.3
Hill et al., 1983 (New Jersey, USA)	Volunteers at blood banks ($n=450$), age=36, 52% males	Plasma	RIA	21.8 (9.7)	259 (6.4)
Pierce et al., 1983 (Melbourne and Sydney, Australia)	Random sample ($n=336$), age=42, 45% males	Saliva	GC	19.1	314
Abrams et al., 1987 (USA)	Worksite-based sample ($n=63$), age=41, 34% males	Saliva	RIA	28	349.2 (195.4)
Wagenknecht et al., 1985 (Alabama, Illinois, Minnesota, and California, USA)	Black women ($n=434$), age=25 White women ($n=329$), age=25 Black men ($n=388$), age=24 White men ($n=273$), age=25.4	Serum	RIA	10.6 (7.2) 14.9 (9.8) 10.8 (7.2) 18.1 (10.6)	251.2 (175.6) 176.4 (137.6) 244.8 (156.2) 210.2 (145.1)
Etter et al., 1995 (Geneva, Switzerland)	Students, faculty, and staff of the University of Geneva ($n=207$), age=28, 43% males	Saliva	GC	10.7	166 (170)
Benowitz et al., 1990–1993 (San Francisco Bay Area, USA)	Recruited from local community colleges: Chinese Americans ($n=37$), age=25, 48% males Latinos ($n=40$), age=30, 48% males Whites ($n=54$), age=35, 63% males	Serum	GCMSD	11.2 12.0 20.2	104 126 234
Ahijevich et al., 1994 (USA)	Black women ($n=142$)	Saliva	HPLC	14.5 (9.4)	392 (212)
Perez-Stable, 1998 (California, USA)	Whites ($n=39$), age=32, 51% males Blacks ($n=40$), age=32, 50% males	Plasma	GC	14.7 14.0	177 (135) 234 (131)
Ahijevich et al., 1999 (USA)	Recruited from community and worksite settings: White women ($n=47$), age=35 Black women ($n=48$), age=35	Plasma	HPLC	19 15	182 249
Jarvis, 1998 (England)	Random sample ($n=2,031$), age=40.6, 42.8% males	Saliva	GC	14.6	312
Quandt et al., 1999 (USA)	Latino tobacco workers ($n=182$), age=28, 99% males	Saliva	HP-LC-API-MS-MS	4 (median)	145 (20)
Jaakkola, 1999 (China)	Random sample of males ($n=510$), age=43	Saliva	GC	20	210
Wewers et al., 2000 (USA)	Southeast-Asian males ($n=299$), age=39 Southeast-Asian, females ($n=28$), age=43.1	Saliva	HPLC	11.6 (7.6) 7.3 (4.4)	66 (44) 56 (34)
Present study, 1999 (Mexico)	Men in Mexico City ($n=263$), age=51 Women in Mexico City ($n=196$), age=48 Men in Cuernavaca ($n=634$), age=32 Women in Cuernavaca ($n=158$), age=32	Saliva	GC	15 (11.0) 12 (9.4) 18 (11.4) 15 (10.7)	242.3 (136.4) 198.1 (128.2) 179.4 (130.0) 152.7 (134.3)

Note. GC, gas chromatography; GCMSD, gas chromatography with mass selective detection; HPLC, high-performance liquid chromatography; HP-LC-API-MS-MS, high-performance liquid chromatography-tandem mass spectrometry; RIA, radioimmunoassay.

Nicotine is crucial in establishing tobacco dependence; therefore, variability in nicotine metabolism between genders may explain in part the different smoking behaviors observed in men and women. However, no meaningful difference in salivary cotinine, controlled for cigarette consumption, was observed between males and females in the present study. This finding suggests that nicotine metabolism may be similar in Mexican men and women, as found in other studies (Perez-Stable et al., 1998). However, Benowitz et al. (2002) reported that males had significantly higher salivary cotinine levels (84 ng/ml; $p < .01$), although the difference was attenuated after adjusting for cigarettes per day (56 ng/ml; $p < .01$). More studies are needed to better understand gender differences in cotinine and nicotine metabolism and their implications for nicotine replacement therapy.

The meaning of the cigarette description "light" to the Mexican public has not been studied adequately. Cigarettes that are labeled as "light" in Mexico are frequently purchased using the English word *lights*, which may lead the Mexican population to believe that such cigarettes are less damaging to health. In other parts of the world the tobacco industry has misled consumers into perceiving that these cigarettes pose less risk to health and should be considered as an alternative to quitting (Pollay & Dewhirst, 2001). In the present study we observed that smokers of light and regular cigarettes had similar salivary cotinine levels and that nicotine levels did not vary between light and non-light cigarettes. In fact, the range of variation of nicotine content was quite restricted. This observation supports there being a high degree of compensation for nicotine delivery and indicates that switching to low-yield cigarettes would not be expected to reduce doses of tobacco toxins nor to reduce the risk of disease from smoking. The unsupported belief that individuals who cannot stop smoking could benefit from switching to low-yield cigarettes may mislead smokers to postpone cessation (USDHHS, 2001).

We observed a positive association between age and salivary cotinine levels, after controlling for cigarette consumption. This finding is consistent with reports from other populations (Caraballo et al., 1998; Coultas et al., 1993; Pollay & Dewhirst, 2001; Swan, Habina, Means, Jobe, & Esposito, 1993). Although not all studies have documented this association, Etter et al. (2000) reported a nonsignificant increase of 2.0 ng/ml in salivary cotinine per year, adjusted for the number of cigarettes per day. This estimate is similar to the estimate in the present study (2.6 ng/ml/year). Smith et al. (1996) reported that adolescents (mean age = 15.2 years) who smoked 20 cigarettes or more per day had lower cotinine levels than adults with comparable levels of smoking.

Changes in nicotine metabolism may contribute to the age-related differences in cotinine levels. The clearance of nicotine is slower in elderly smokers compared with younger smokers (Molander, Hansson, & Lunell, 2001). Although there are no reports on the clearance of cotinine in the elderly, cotinine metabolism may be slower in the elderly, given that nicotine and cotinine are metabolized by the same liver enzyme, CYP2A6.

An inverse relationship between body mass index and smoking has been reported (Istvan, Nides, Buist, Greene, & Voelker, 1994; Klesges, Meyers, Klesges, & La Vasque, 1989). In cross-sectional studies, it is difficult to disentangle a possible direct effect of nicotine on the metabolic processes affecting body weight from a possible effect from increased dilution of cotinine in individuals with a higher body mass index. We cannot explain our finding, which is consistent with other reports.

On average, smokers from Mexico City had higher salivary cotinine levels than those from Morelos. We do not have an explanation other than incomplete control for the confounding effect of smoking behaviors and age. When the Morelos sample was restricted to participants who were over age 30 years, the difference between groups decreased markedly (10 ng/ml) and was not statistically significant. Another potential explanation may involve the collection method (Schneider et al., 1997). Salivary flow rate can affect cotinine levels in saliva. Stimulation of saliva with candy results in a slightly lower salivary cotinine concentration (approximately 25% lower), compared with collection of unstimulated saliva (Schneider et al.). Participants from Mexico City were less likely to accept the sugar-stimulated method for salivary collection, but the specific method for collection was not documented for individual participants. We cannot rule out the possibility that participants from Mexico City under-reported their cigarette consumption to a larger extent than did participants from Morelos.

The present study has potential limitations that may affect the inferences derived from these data. The participants were primarily low- and middle-class smokers who voluntarily agreed to participate in the study. Study subjects were not selected at random but were recruited using purposive sampling. Interviewers were trained to prescreen potential participants for their smoking behavior to achieve a proportional sampling quota of all smoking intensities in order to maximize the statistical power of the study. As a result, the mean cotinine concentration and the mean number of cigarettes are biased upward from the true population mean, but associations of cotinine levels with predictors should be unbiased. Therefore, it is unlikely that selection bias could explain our findings. The bias-corrected numbers of cigarettes per day were

10.2 for men and 5.8 for women, and the mean cotinine levels were 160 ($SD=14$) and 117 ng/ml ($SD=8.2$), respectively.

Another limitation of the study is recruitment differences between the participants in Cuernavaca and those in Mexico City, and we cannot accurately determine the effects of these differences on the results. The fact that the Mexico City participants came from a cohort of people previously known to be smokers and the Cuernavaca participants were recruited by convenience may explain differences in cotinine levels among people with corresponding FTQ scores. These differences also may be explained, however, by the fact that the Mexico City participants were older on average compared with Cuernavaca participants and had been smoking longer, suggesting higher degrees of addiction.

We did not assess secondhand smoke exposure, but the additional information provided by this variable would not likely have a major impact in improving our prediction models. Error in reporting the number of cigarettes smoked in the past 24 hr may have occurred. This possibility is suggested by the high degree of rounding error observed in the number of cigarettes smoked. Nonetheless, pure random error in reporting the number of cigarettes may have attenuated the observed association, although it is unlikely that this type of error explains our results regarding the shape of the dose-response curve between cotinine and the number of cigarettes smoked.

Information on the intake of nicotine from smoking is needed to determine optimal doses of nicotine replacement for aiding smoking cessation. In Mexico, as in other parts of Latin America, smokers tend to smoke fewer cigarettes than non-Hispanic Whites in the United States (Aghi et al., 2001). Thus, a dosage system based on U.S. smoking patterns could lead to the provision of either too little or too much nicotine. Following the recommendations of Dale et al. (1995), for smokers who consume 40 or more cigarettes per day, only 5.3% of the present study population would be eligible for the high-dose replacement scheme. However, using cotinine levels as a therapeutic guide, the percentage eligible for high doses would be considerably higher (21.4%). A fixed dose of transdermal nicotine will likely not be effective for all smokers. In the absence of controlled clinical trials based on local patients, individualized therapy might reasonably be guided as much as possible by biological measures, such as cotinine levels. In addition, the present study showed that an addiction index based on the FTQ was significantly related to salivary cotinine concentration, suggesting that this index could be a useful tool for guiding nicotine replacement therapy in the Mexican population.

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