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Determinants of salivary cotinine level: a population-based study in Brazil

Determinantes dos níveis de cotinina salivar: um estudo de base populacional no Brasil

ABSTRACT

OBJECTIVE: A cross-sectional population-based study was conducted to assess, in active smokers, the relationship of number of cigarettes smoked and other characteristics to salivary cotinine concentrations.

METHODS: A random sample of active smokers aged 15 years or older was selected using a stepwise cluster sample strategy, in the year 2000 in Rio de Janeiro, Brazil. The study included 401 subjects. Salivary cotinine concentration was determined using gas chromatography with nitrogen-phosphorus detection. A standard questionnaire was used to collect demographic and smoking behavioral data. The relation between the number of cigarettes smoked in the last 24h and cotinine level was examined by means of a nonparametric fitting technique of robust locally weighted regression.

RESULTS: Significantly ($p<0.05$) higher adjusted mean cotinine levels were found in subjects smoking their first cigarette within five minutes after waking up, and in those smoking 1–20 cigarettes in the last 24h who reported inhaling more than ½ the time. In those smoking 1–20 cigarettes, the slope was significantly higher for those subjects waiting for more than five minutes before smoking their first cigarette after waking up, and those smoking "light" cigarettes when compared with their counterparts. These heterogeneities became negligible and non-significant when subjects with cotinine >40 ng/mL per cigarette were excluded.

CONCLUSIONS: There was found a positive association between self-reporting smoking five minutes after waking up, and inhaling more than ½ the time are consistent and higher cotinine levels. These can be markers of dependence and higher nicotine intake. Salivary cotinine proved to be a useful biomarker of recent smoking and can be used in epidemiological studies and smoking cessation programs.

KEY WORDS: Smoking. Cotinine, pharmacokinetics. Cross-sectional studies.

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RESUMO

OBJETIVO: Realizou-se um estudo transversal para analisar a relação entre número de cigarros fumados e outras características com a concentração de cotinina salivar entre fumantes.

MÉTODOS: Fumantes ativos de 15 anos ou mais foram selecionados por meio de amostra probabilística em múltiplos estágios no ano 2000, município do Rio de Janeiro, Brasil. O estudo incluiu 401 fumantes. A concentração de cotinina salivar foi determinada utilizando-se cromatografia gasosa com detector de nitrogênio/fósforo. Coletaram-se informações demográficas e sobre o comportamento tabágico utilizando-se questionário padronizado. A relação entre o número de cigarros fumados nas últimas 24h e o nível de cotinina foi analisada utilizando técnica não paramétrica baseada em regressões robustas locais ponderadas.

RESULTADOS: O nível médio ajustado de cotinina foi significativamente ($p<0,05$) mais elevado entre indivíduos que fumaram o primeiro cigarro até cinco minutos depois de acordar e entre os que fumaram de um a 20 cigarros nas últimas 24h e tragavam mais de metade das vezes. Considerando-se fumantes de um a 20 cigarros, a inclinação da curva de regressão foi significativamente maior entre os que, após acordar, esperam mais de cinco minutos para fumar e para os que consumiam cigarros "suaves", quando comparados a seus opostos. Essas heterogeneidades desaparecem ao se excluir indivíduos com cotinina inferior a 40 ng/ml/cigarro.

CONCLUSÕES: Houve associação positiva entre referir fumar até cinco minutos depois de acordar e tragar mais da metade das vezes e níveis de cotinina salivar. Essas informações podem ser marcadores de dependência e maior absorção de nicotina entre fumantes. A cotinina salivar mostrou-se útil como biomarcador do uso recente de tabaco a ser usado em estudos epidemiológicos e programas de cessação de fumar.

DESCRITORES: Tabagismo. Cotinina, farmacocinética. Estudos transversais.

INTRODUCTION

Nicotine has a short half-life and its major metabolite is cotinine. Cotinine has a half-life of 16–20 hours and is a useful indicator of nicotine intake from recent smoking. About 70% to 80% of nicotine absorbed into the blood stream is converted into cotinine.[5] Thus, measurement of cotinine in biological samples provides is an objective approach to identify recent smoking exposure.[5]

In smokers, the level of cotinine is determined by many factors which vary widely for the same number of cigarettes smoked per day. Factors potentially influencing cotinine levels relate to the product smoked (filter or non-filter and nicotine yield), the way the product is smoked (depth of inhalation and butt length), and smoker characteristics (age, gender, and phenotype of nicotine metabolism).[15] Cotinine levels also vary across racial groups, perhaps reflecting differences in some of these factors.[3,10] These racial differences are of interest and may reflect intrinsic metabolic differences, differences related to the circumstances of exposure, such as frequency and depth of smoking,[3,22] or differential accuracy in self-reported number of cigarettes smoked recently.[1] The heterogeneity of effects by ethnic background also suggests that other variables may modify the observed relationship between number of cigarettes smoked and cotinine concentration. If this is the case, these variables would have to be taken into consideration when assessing the value of cotinine as a marker of recent smoking exposure.

Although the relationship between salivary cotinine and number of cigarettes smoked has been studied for specific ethnic groups, the replication of prior findings in a previously unstudied country with different genetic and cultural characteristics is novel and important. The present study is part of a multi-country study, which also included China, Mexico and Poland, aiming at the assessment of the association between cigarette characteristics and smoking behavior and salivary cotinine. The objective of the present study was to assess

in active smokers the relationship between number of cigarettes and other characteristics and salivary cotinine concentrations in an admixed population, and to examine whether certain variables modify the relationship of number of cigarettes to cotinine.

METHODS

In 2000, a cross-sectional survey was conducted based on a random sample of the metropolitan population of the city of Rio de Janeiro, Brazil. A stepwise cluster strategy was used. Eligible household members consisted of individuals aged 15 years or older living in Rio. Only active cigarette smokers were included. Individuals using nicotine replacement therapy, those with cotinine concentration under 10 ng/mL, and those from whom saliva samples could not be obtained were excluded. In addition, individuals who did not smoke at least one cigarette per day during the month prior to the interview and those who did not smoke in the previous 24 hours were excluded.

The total random sample included 2,393 subjects, representing a response rate of 96.5%. Subjects were interviewed in the households by trained interviewers after obtaining their informed consent. Information was collected on age, gender, education, smoking history and smoking behavior variables using a standard questionnaire. The latter included average number of cigarettes smoked daily, number of cigarettes smoked in the previous 24 hours, duration of smoking, depth and frequency of inhalation and type of cigarette. The Fagerström Test for Nicotine Dependence index (FTND)[12] and whether their first cigarette was smoked within five minutes after waking up were both used as dependence indicators. Weight was measured in kilograms using a portable scale. Height was measured without shoes in meters using a tape mounted on the wall. Body mass index (BMI) was calculated as weight divided by the square of the height. Stringent quality assurance and control procedures were followed.

Saliva samples were collected from smokers in the households shortly after the interviews. Subjects were asked to spit approximately six milliliters of saliva in a test tube. Specimens were frozen, stored at –20ºC, and shipped for cotinine measurements to the Clinical Pharmacology Laboratory at San Francisco General Hospital, University of California, San Francisco. Cotinine concentration was determined using gas chromatography with nitrogen-phosphorus detection.[18] Cotinine concentrations were determined using standard curves ranging from 10 to 5,000 ng/mL. The lower limit of the test's sensitivity was 10 ng/mL.

Regarding data analysis, first frequency distributions were assessed. The shape of the function expressing the relationship between number of cigarettes smoked in the last 24 hours and cotinine levels was examined by means of a nonparametric fitting technique of robust locally weighted regression (LOWESS).

Crude and adjusted means of salivary cotinine concentration were calculated for categories of selected variables, separately for those smoking 1–20 and those smoking 21–40 cigarettes in the last 24 hours. Linear regression was used for multiple adjustment using mean cotinine ng/mL as the outcome. Covariates included demographic and socioeconomic variables (age, gender, educational level), BMI, and smoking behavior variables. Variables for which effect modification was suspected upon stratification were included in the regression models as interaction terms. For the multivariable models it was used as a marker of dependence the amount of time subjects wait to smoke their first cigarette after waking up.

Cotinine concentrations greater than 40 ng/mL per cigarette smoked in the previous 24 hours (cot>40) are believed to be unlikely from a biological viewpoint, possibly resulting from information or measurement bias. The rationale for excluding values >40 ng/mL per cigarette is based on studies of how much nicotine a person can take in from a cigarette and nicotine and cotinine metabolism rate. Commercial cigarettes typically contain 10–15 mg nicotine per tobacco rod. The usual systemic absorption of nicotine from a cigarette is 1–1.5 mg but can be as high as three milligrams per cigarette with very intense smoking.[6] In experimental studies in which cigarette smoking is measured and in which nicotine and cotinine pharmacokinetic parameters are characterized, the typical cotinine concentration per cigarette is 12 ng/mL.[5] Assuming this reflects an intake of one milligram of nicotine, one can estimate a cotinine level of 36 ng/mL for a person taking in 3 mg nicotine from each cigarette. To be conservative it was set a limit of 40 ng/mL per cigarette as a limit above which a level would be considered biologically implausible. Thus, in supplementary sensitivity analyses, subjects with such values were excluded.

Analyses were performed using Stata Statistical Software package, release 8.0.

The study was approved by the institutional review boards of *Instituto Nacional do Câncer* (Brazilian National Cancer Institute), and Johns Hopkins Bloomberg School of Public Health. Subjects' informed consents were obtained.

RESULTS

Of a total of 2,393 subjects, 513 (21.4%) were current active smokers. Overlapping reasons for exclusion were use of nicotine replacement therapy (13 subjects), smoking cigars or pipes (17), cotinine concentration

Table 1. Characteristics of all interviewees and current active smokers. Rio de Janeiro, Southeastern Brazil, 2001.

Variable	All interviewees* (N=2,393)		Active smokers** (N=401)***	
Individual characteristic	N	%	N	%
Age (in years)				
15–34	995	41.7	111	27.7
35–54	892	37.4	218	54.4
55–89	498	20.9	72	17.9
Gender				
Males	1,032	43.1	194	48.4
Education				
Up to high school	1,246	52.3	244	61.1
BMI****				
< 24	1,154	48.2	201	50.1
Smoking prevalence				
Current	513	21.4	401	100.0
Former	416	17.4	-	-
Cigarettes smoked daily on average*****				
1–10	-	-	190	47.4
11–20	-	-	161	40.1
21–30	-	-	28	7.0
31	-	-	22	5.5
Cigarettes smoked in the previous 24 hours*****				
1–10	-	-	143	35.8
11–20	-	-	189	47.3
21–30	-	-	33	8.3
31	-	-	35	8.8
When do you have your first cigarette after waking up*****				
Within 5 minutes	-	-	142	35.4
6–30 minutes	-	-	124	30.9
31–60 minutes	-	-	39	9.7
1 hour	-	-	96	27.0
Frequency of inhaling*****				
< 1/2 the time	-	-	51	12.7
1/2 the time	-	-	23	5.7
> 1/2 the time	-	-	15	3.7
Always	-	-	312	77.8
Depth of inhaling*****				
Never	-	-	26	6.0
Lightly	-	-	164	40.9
Moderately	-	-	139	34.7
Deeply	-	-	74	18.4
Duration (years)*****				
≤ 25	-	-	202	50.4
> 25	-	-	199	49.6
Fagerstrom Index*****				
Very low	-	-	123	30.7
Low	-	-	93	23.2
Medium	-	-	58	14.5
High	-	-	90	22.4
Very high	-	-	37	9.2
Type of cigarette*****				
Light	-	-	282	70.3
Regular	-	-	119	29.7

* The following information was missing (number of subjects): education (11) and age (1)
** 112 current active smokers were excluded for various reasons (see text)
*** Information was missing for 2 subjects on education, and for 1 subject on number of cigarettes smoked in the previous 24 hours
**** BMI = body mass index (weight/height^2 in kg/m^2)
***** Current smokers

Table 2. Crude and adjusted* mean cotinine concentration in subjects who reported smoking 20 cigarettes or less and 21 to 40 cigarettes. Rio de Janeiro, Southeastern Brazil, 2001.

Individual characteristic	Cigarettes smoked in the last 24 hours					
	1–20				21–40	
	Salivary cotinine level per cigarette >0		0 < Salivary cotinine level per cigarette ≥ 40		Salivary cotinine level per cigarette > 0	
	N=332** Mean value (SD)	Adjusted mean value****	N= 297**,*** Mean value (SD)	Adjusted mean value****	N=58 Mean value (SD)	Adjusted mean value****
Age (in years)						
15–34	184.3 (125.4)	196.6	172.9 (123.2)	176.6	194.7 (134.1)	183.7
35–54	215.9 (141.1)	214.3	203.2 (134.3)	204.5	293.4 (133.4)	293.9
55–89	226.1 (150.9)	208.8	221.1 (143.3)	210.7	295.0 (121.6)	302.7
Gender						
Female	199.6 (129.0)	202.6	186.9 (124.7)	192.3	266.8 (145.5)	264.2
Male	217.7 (148.9)	214.1	208.7 (142.1)	202.7	284.8 (125.9)	286.9
Schooling*****						
Complete high school or more	197.3 (136.2)	197.0	194.9 (135.1)	194.5	250.9 (114.4)	298.0
Up to high school	216.1 (140.4)	215.1	199.9 (132.5)	199.2	329.7 (157.9)	232.9
BMI******						
≥ 24	193.9 (132.6)	194.1	182.5 (124.1)	181.6	255.9 (143.2)	248.2
< 24	221.9 (143.7)	221.7	211.9 (140.9)	**212.9**	302.3 (119.9)	311.8
When do you have your first cigarette after waking up						
≥ 6 minutes	181.5 (124.3)	190.7	171.4 (118.1)	186.0	219.2 (116.6)	230.4
"within 5 minutes"	273.2 (151.2)	**250.4**	261.5 (147.5)	**225.1**	311.9 (133.3)	**304.9**
Duration (years)						
≤ 25	182.6 (126.8)	193.1	171.5 (122.0)	191.6	259.7 (135.9)	294.1
> 25	236.3 (146.3)	221.3	226.3 (139.9)	203.5	290.5 (133.2)	262.1
Depth of inhaling						
Never/ lightly	199.6 (130.8)	213.5	183.7 (121.1)	200.9	297.8 (148.9)	268.9
Moderately/ deeply	216.5 (146.2)	202.5	210.0 (143.2)	193.7	265.7 (126.4)	291.5
Frequency of inhaling						
≤ 1/2 the time	177.8 (144.2)	174.3	168.5 (137.3)	173.6	367.8 (164.9)	344.6
> 1/2 the time	215.4 (136.7)	**216.2**	204.4 (131.6)	203.2	262.2 (124.4)	265.8
Type of cigarette						
Light	203.3 (137.5)	207.5	195.6 (135.6)	201.8	253.7 (125.4)	262.8
Regular	221.2 (142.2)	209.5	201.8 (127.0)	183.4	303.2 (141.2)	292.6

Bold values: difference significant at α=0.05
* Multiple linear regression was used in the analyses
** 181 current active smokers were excluded for various reasons (see text)
*** 216 current active smokers were excluded for various reasons (see text)
**** Each variable adjusted simultaneously for all other variables shown in the table and number of cigarettes smoked in the past 24 hours
***** Information was missing for 2 subjects on education for those who smoked 1–20 cigarettes in the past 24 hours
****** BMI: body mass index (=weight/height^2 in kg/m^2)

under 10 ng/mL (42), failure to collect saliva (9), not having smoked at least one cigarette per day in the last month or in the 24 hours preceding the interview (87), and discrepant values between average number of cigarettes and number of cigarettes smoked in the last 24 hours (one subject). After these exclusions, there remained 401 current active smokers. For sensitivity analyses, an additional 35 subjects who reported smoking 1–20 cigarettes in the past 24 hours were excluded because of cotinine values greater than 40 ng/dL per cigarette smoked. These unlikely values were not seen in those smoking more than 20 cigarettes.

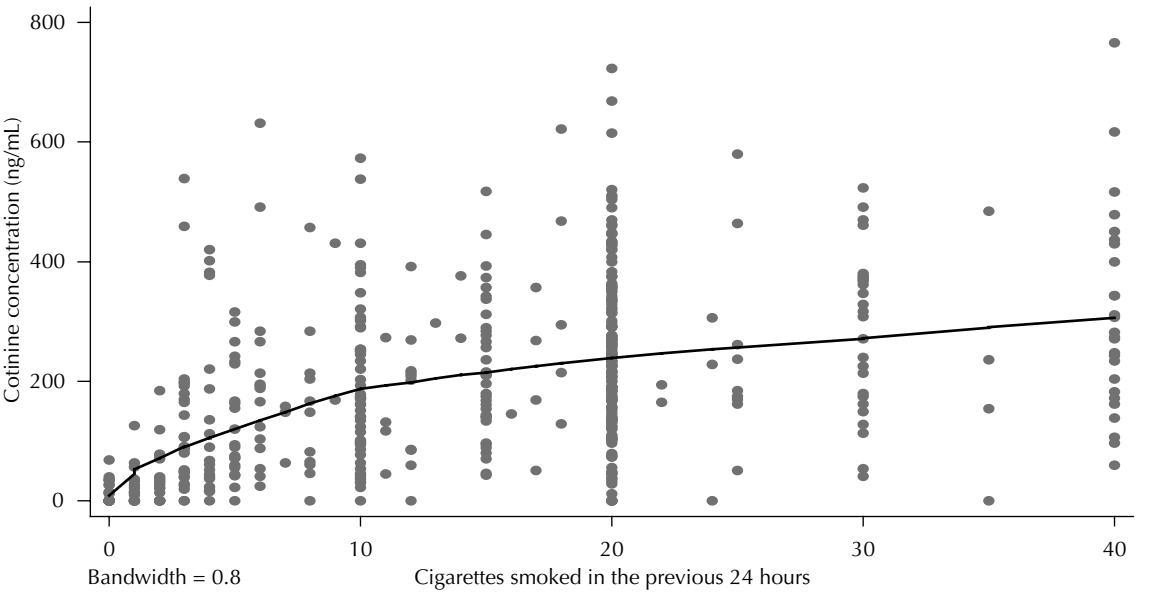

Figure. Cotinine increase per cigarette smoked in the previous 24 hours. Rio de Janeiro, Southeastern Brazil, 2001.

For the total population sample, 39% reported current or past smoking. Compared with the total random sample, current smokers were more likely to be older, male and less educated (Table 1). Among current smokers, the proportions smoking a daily average of 20 or fewer cigarettes, or smoking this same amount in the 24 hours prior to saliva collection were 87.5% and 83.1%, respectively. Approximately 1/3 reported smoking their first cigarette within 5 minutes after waking up. About 78% reported always inhaling but only around 18% were deep inhalers. Half of current smokers reported smoking for 25 years or longer. About 1/3 had high or very high FTND index scores, and close to 70% smoked "light" cigarettes.

Two approximately straight lines with an inflection point at about 20 cigarettes characterized the relationship between number of cigarettes smoked in the prior 24 hours and salivary cotinine concentration (Figure). The predicted slope was much steeper for subjects who reported smoking 1–20 cigarettes (8.0 ng/mL) than for those reporting more than 20 cigarettes (1 ng/mL).

Table 2 shows mean salivary cotinine concentrations according to selected variables, for two categories of number of cigarettes (1–20 and 21–40), and both before and after excluding those smoking 1–20 cigarettes with cotinine levels greater than 40 ng/mL (cot>40). The adjusted values reflect simultaneous adjustment for the number of cigarettes as well as for all other variables shown in Table 2.

Although not statistically significant at α=0.05, higher cotinine levels were consistently found for older compared to younger subjects and for men, compared to women. No consistent or significant differences were found for educational level.

Subjects with lower BMI (<24) had higher cotinine concentrations than those with higher BMI, and the adjusted difference was found to be significant ($p<0.05$) after excluding smokers of 1–20 cigarettes with cot>40. Those who reported smoking their first cigarette within five minutes after waking up had higher levels of salivary cotinine than those who waited longer than five minutes before starting to smoke, and these differences were significant for the adjusted values both before and after excluding persons with cot>40, and regardless of the category of number of cigarettes smoked (1–20 or >20).

Duration of smoking and depth of inhaling were not significantly related to cotinine concentrations, but those subjects in the 1–20 cigarettes category who reported inhaling more frequently (>1/2 time) had a higher cotinine concentration than their counterparts, and the adjusted difference was found to be significant before excluding those with cot>40. No consistent or significant differences were seen with regard to type of cigarette (light vs. regular).

Because the slope for those smoking more than 20 cigarettes was very small and most subjects reported smoking 1–20 cigarettes, interactions were explored only for the 1–20 cigarettes category. Before excluding subjects with salivary cotinine >40 ng/mL per cigarette, statistically significant interactions were seen between number of cigarettes and both waiting time after waking up until first cigarette is smoked, and cigarette type. Not shown in the Table, the predicted increases in cotinine

concentration per cigarette smoked (ng/mL) were found to be for the former variable: within five minutes, 1.5; more than five minutes, 8.3 (p for interaction =0.02); and for the latter variable: "light", 7.9; regular, 2.6 (p for interaction=0.009). However, after exclusion of subjects whose salivary cotinine concentrations were greater than 40 ng/mL per cigarette, no significant interactions or important heterogeneities remained.

DISCUSSION

In the present and previous studies, the increase in cotinine per cigarette smoked in the past 24 (or, in some studies, 48) hours seems to be particularly evident up to an inflection point of about 20 cigarettes. Over this amount the slope noticeably decreases, which would indicate that cotinine is a weak predictor of the number of cigarettes in those individuals who smoke more than one pack per day.[10,11,17] This finding suggests that smokers satisfy their dependence-related craving with around 20 cigarettes per day. Also, corroborating previous studies,[8,9,13] marked inter-individual variability was found in cotinine levels for the same number of cigarettes smoked (Figure).

A direct relationship of salivary cotinine was found with age, regardless of number of cigarettes, although it was not statistically significant. Two previous studies have similarly found a weak association between age and cotinine levels.[11,17] A weak or no association with age found in the present and previous studies suggests that cotinine metabolism does not seem to change as individuals' age.[13,19]

Mean cotinine levels were found to be higher in men, although not significantly at α=0.05. Prior research has been inconsistent with regard to the relationship between cotinine and gender. In a recent study by Etter et al,[11] (2000) males were found to have higher mean concentration of salivary cotinine than women, regardless of the number of cigarettes, duration of smoking, nicotine yield, FTND index score, attempts to stop smoking in the past year, perceived difficulty to quit smoking, and stage of change with regard to intention to quit smoking. Benowitz et al[2] recently reported that nicotine and cotinine are metabolized more rapidly in women than men. More rapid metabolism may explain, at least in part, the lower levels of cotinine seen in women compared to men in the present study. However, other studies have found that cotinine concentration per cigarette smoked was similar in men and women.[4]

The concentration of cotinine was higher in subjects with lower BMI, even after multiple adjustment. The concentration of cotinine in the body varies according to the daily intake of nicotine, the extent of conversion of nicotine to cotinine, and the rate of cotinine metabolism.[5] That salivary cotinine appears to increase in individuals who lose weight even while they continue smoking the same number of cigarettes suggests that either a great amount of nicotine is converted to cotinine or that the rate of cotinine metabolism is slower in individuals with lower BMI.[16,21]

In the present study, after controlling for the number of cigarettes smoked, the mean level of salivary cotinine was higher in subjects who reported smoking within five minutes after waking up than in those who reported waiting for a longer period. This finding and the positive correlation between Fagerström Tolerance Questionnaire (FTQ) or FTND[12] score and biochemical measures of nicotine intake – like saliva or blood cotinine or blood nicotine – seen in other studies[14,23] indicate that higher levels of nicotine dependence can be associated with greater daily intake of nicotine, and therefore higher cotinine levels.

For the 1–20 cigarettes category, higher concentration of cotinine was seen in those who reported inhaling >1/2 time, with the adjusted difference found to be significant – probably reflecting increased absorption of nicotine – before excluding those with cot>40. However, no significant associations were found between cotinine concentration and depth of inhaling. A possible misclassification bias related to depth of inhaling may explain this apparently inconsistent result.

No significant association was found between cotinine concentration and duration, although among those reporting 1–20 cigarettes in the past 24 hours, a pattern of higher mean levels of cotinine with longer duration of smoking (>25 years) was seen. In Etter et al[11] study (2000) they reported a positive association with duration, which, however, disappeared after multiple adjustment.

The authors failed to find differences in cotinine levels between those who reported smoking either "light" or regular yield cigarettes. Changes in cigarette design – such as ventilation holes in cigarette filters, more efficient filters, or more rapidly burning cigarette paper – are designed to decrease machine-determined measures of tar and nicotine. They result in changes in conscious or unconscious smoking behavior. These changes in smokers of "light" nicotine yield cigarettes include increased volume and frequency of puffs, and attempts at covering ventilation holes with finger or lips.[20,*] Benowitz & Jacob[7] (1984) noted that low-yield cigarette smokers smoked 25% more cigarettes, and absorbed 60% more nicotine per cigarette than pre-

* National Institutes of Health. National Cancer Institute. Risks associated with smoking cigarettes with Low Machine-Measured Yields of Tar and Nicotine. Smoking and Tobacco Control. Rockville: National Institutes of Health; 2001. (Monograph, 13). Available from: http://cancercontrol.cancer.gov/tcrb/monographs/13/m13_complete.pdf

dicted by the Federal Trade Commission. According to Benowitz et al[8] (1983), only about 4% to 5% of the total serum cotinine variance can be explained by nicotine yield. Results of previous studies are, therefore, consistent with the authors' failure to find a difference in cotinine concentration between smokers of "light" and smokers of regular cigarettes.

For the analysis of interactions, there were excluded subjects smoking more than 20 cigarettes, in whom the increase in mean cotinine with increasing number of cigarettes was found to be negligible. In those smoking 1–20 cigarettes, two modifiers of the effect of number of cigarettes on cotinine concentration were found. The first one was a marker of addiction, which is the length of time individuals waits before smoking their first cigarette; and the second modifier was the type of cigarette ("light" vs. regular cigarette). Stronger associations were seen in those who waited more than five minutes before smoking their first cigarette and in those who reported smoking "light" cigarettes. These findings, which are consistent with a study conducted in China,[17] suggest that these individuals adopt an adaptive behavior, such as increasing the number of puffs or depth of inhaling, so as to reach a "satisfactory" level of nicotine while at the same time avoiding serious side effects. However, it is possible that the interactions found resulted from measurement error as they disappeared with the exclusion of subjects with cotinine levels greater than 40 ng/mL per cigarette.

The present study's limitations included its reliance on information provided by subjects with regard to the independent variables. Whenever possible, further studies should try to validate subject information with objective measures, such as determination of nicotine yield using smoking machines. The study's strengths included its population-based design, high participation rate, and stringent quality control measures.

In conclusion, the present study was the first to assess determinants of cotinine concentration in a Brazilian population. Of practical public health importance, the marked racial admixture in Brazil and the consistency between this study's findings and those reported in populations with different ethnic backgrounds suggest that predictors of cotinine levels, especially number of cigarettes, are similar across different populations. The results of the present study showed that self-reports of smoking five minutes after waking up, and inhaling more than ½ the time are consistent with higher cotinine levels and therefore are likely accurate markers of dependence and higher nicotine intake. Accurate information on nicotine intake provided by salivary cotinine in the present study is useful for better assessment of recent smoking not only in epidemiological studies but also as a therapeutic guide in smoking cessation programs.

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