Saliva Cotinine and Recent Smoking—Evidence for a Nonlinear Relationship

GARY E. SWAN, PhD
KATHERINE HABINA, AB
BARBARA MEANS, PhD
JARED B. JOBE, PhD
JAMES L. ESPOSITO, PhD

Three of the authors are with SRI International, Menlo Park, CA. Dr. Swan is Director, Health Sciences Program; Ms. Habina, Research Analyst, and Dr. Means, Executive Director, are with the Health and Social Policy Division. Dr. Jobe is Psychologist, National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention, Hyattsville, MD. When this study was conducted, Dr. Esposito was a Service Fellow, NCHS; he is now a Social Psychologist at the Bureau of Labor Statistics, Washington, DC.

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Tearsheet requests to Gary E. Swan, PhD, Health Sciences Program, SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025, telephone 415-859-5322.

Synopsis

Cotinine concentration in various body fluids is considered to be among the most useful markers of nicotine exposure currently available. Despite the prevailing consensus concerning cotinine's usefulness, cotinine's large intrasubject variability has led some to question the value of a single-point measurement. Several individual differences (for example, age, race, sex, and so forth) may affect cotinine excretion, and a peculiar nonlinearity between the number of cigarettes smoked and cotinine concentration has been reported previously in the literature. The purpose of this investigation was to examine the nature of the association between cotinine and reported number of cigarettes smoked after adjustment for the relationship between cotinine and age, a key individual difference known to affect drug absorption, distribution, metabolism, excretion, and tissue sensitivity.

The authors examined the relationship between saliva cotinine and daily cigarette consumption in 116 smokers (mean age = 37.4 years; average number of cigarettes smoked daily = 20.1) who logged each cigarette into a hand-held computer as part of a study on the accuracy of recall. The Pearson correlation between saliva cotinine and the logged number of cigarettes smoked in the previous 17 hours (the time window corresponding to the half-life of cotinine) accounted for significantly more of the variance in cotinine than did the average logged number of cigarettes smoked daily during 5 days. Age was also significantly associated with cotinine levels.

Further examination of the relationship between cotinine and amount smoked in the previous 17 hours revealed evidence for a significant nonlinear component. Inclusion of both age and a cubic nonlinear component of daily cigarette consumption resulted in further significant improvement in the amount of variance accounted for in cotinine levels. These results suggest that adjustments for age and the inclusion of a nonlinear component for cigarette consumption will result in more precise use of cotinine as a validation tool for existing differences in smoking levels.

SEVERAL RECENT investigations have examined the relationship between the number of cigarettes smoked daily and cotinine concentrations in various body fluids. Linear correlations of 0.24 to 0.62 in urine (*1,2*), 0.34 to 0.45 in saliva (*3-6*), and 0.53 in serum have been reported (*2*). Mean differences in cotinine levels between groups of smokers categorized as heavy or light are invariably significant (*1,4*) as are differences between smokers and nonsmokers (*7*). These findings have led some to conclude that cotinine is the most useful marker of nicotine exposure currently available (*2,8*).

Despite the prevailing consensus concerning cotinine's usefulness, some have begun to question the value of a single-point measurement of cotinine as an indication of exposure to nicotine. Idle (*9*), writing from a pharmacogenetic perspective, concluded recently that the epidemiologic use of coti-

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nine to determine exposure to nicotine is seriously in question because little is known about the extent of intersubject variability in human disposition of nicotine and its metabolites. Suspected sources of variability include physiological, environmental, pathological, and genetic differences.

In support of this view, Idle cites the Neurath and Pein study (*10*), in which nine subjects, each smoking 19 cigarettes per day for 6 days, showed plasma cotinine concentrations ranging from 41 to 344 nanograms per milliliter (ng per ml), an eightfold variation. Recent published reports examining the relationship between cotinine and estimated daily nicotine exposure in black (*11*) and Mexican American (*12*) populations obtained results suggestive of genetic differences in nicotine metabolism, or cotinine excretion, or both. These results prompted a call for more basic research in the use of biochemical markers such as cotinine to confirm cigarette smoking (*13*).

Other studies also point to a large amount of unaccounted variance in cotinine levels. Wall and coworkers (*1*), for example, determined that only 6 percent of the variance in urine cotinine levels was accounted for by amount smoked. Abrams and coworkers (*4*) found, in a group of 96 smokers, a correlation of only 0.27 (R^2 = 0.07) between saliva cotinine and amount smoked. Curiously, when smokers were grouped into light (less than 25 cigarettes per day) and heavy (more than 25 cigarettes per day) smokers, the correlation between cotinine and amount smoked was 0.47 in light smokers and only −0.05 (not significant) in heavy smokers, suggesting a nonlinear association that Abrams and his coauthors interpreted as evidence for a "ceiling effect."

The purpose of this investigation was to examine further the relationship between cotinine and reported amount smoked in a sample of 116 smokers who kept daily logs of cigarettes smoked using new self-monitoring technology. Because previous research has determined the half-life of cotinine to be 17 hours (*1*), we were interested to see if the relationship between number of cigarettes smoked in the 17 hours prior to sample collection and cotinine levels in saliva is stronger than that between the more typically reported amount smoked in a 24-hour period. Our primary objective was to examine the nature of the association between cotinine and reported number of cigarettes smoked after adjustment for the relationship between cotinine and age, a key individual difference known to affect drug absorption, distribution, metabolism, excretion, and tissue sensitivity (*14*).

Methods

Subjects. Subjects were recruited to participate in a study designed to investigate whether the accuracy of recent self-reported smoking frequency is affected by the use of different cognitive recall strategies (*15*). Accuracy was determined by comparing self-reports of smoking levels with the cigarettes smoked that the subjects logged into a hand-held computer for a period of 5 days. Subjects were told that the purpose of the study was to examine the relationships among smoking behaviors, nicotine dose, and saliva cotinine. They were asked not to alter their normal smoking habits during the assessment period. Subjects were instructed to log all their cigarettes from the time they left after the first interview until they returned for their second interview—5 days later. Cigarette smokers who smoked an average of at least 5 cigarettes per day, who were not trying to quit, and who had a permanent residence were paid $45 to participate in the 5 days of data collection. The final sample included 43 men and 83 women, with a mean age of 37.4 years.

After answering the questions about smoking behaviors, subjects were shown how to use the hand-held computer (*A*), measuring 3/4 x 3 x 5 1/2 inches. Subjects were instructed to press one button to turn on the computer and another to log in a cigarette just before they smoked it. Pushing this button activated a time and date stamp within the computer. The subject did not see a display of the time or any tally for cigarette consumption. In addition, subjects were required to respond to occasional random prompts (beeps) from the computer to verify that they were carrying it. In the event that the subject logged in a cigarette and then was interrupted before smoking it or smoked a cigarette and forgot to log it in at the time, a 3 ×

Summary of model fitting linear and nonlinear components to the relationship between amount smoked and cotinine with and without age in the model

Components	Linear model		Quadratic model		Cubic model	
	Estimate	P-value	Estimate	P-value	Estimate	P-value
Linear model	10.77	0.0001	7.49	0.3271	74.16	0.0004
Linear model with age	3.54	0.0014	4.10	0.5789	71.29	0.0003
Quadratic model	...	...	0.12	0.6556	−5.13	0.0009
Quadratic model with age	...	...	0.17	0.5072	−5.12	0.0005
Cubic model	...	...	...	...	0.12	0.0006
Cubic model with age	...	...	...	...	0.12	0.0003
Overall model:						
r	0.44		0.45		0.53	
R^2	0.20		0.20		0.28	
Overall model with age:						
r	0.52		0.52		0.59	
R^2	0.27		0.27		0.35	

5 inch card was issued with the computer for entering any such errors.

Subjects practiced using the computer during the initial interview, and they also received a set of written instructions to take with them and a telephone number to call if they had any questions or problems. Subjects signed a form pledging to carry the computer with them and to log in all cigarettes on leaving the interview until they returned for their second interview 5 days later. They were told that their $45 participation fee was contingent on a faithful recording of cigarettes. In addition, subjects were informed that compliance with the computer's periodic prompts would be rewarded with a $5 bonus. This contingency resulted in high compliance with the recording procedure. The mean number of cigarettes subjects said they had smoked without entering them either onto the computer or on the error card was only 0.36. Although error cards appeared to be useful adjuncts, subjects appeared to have no trouble entering most cigarettes on the computer. On average, subjects made a mean of 2.4 entries onto the error cards during a 5-day period.

Sample collection. After logging their smoking for 5 days, subjects returned for a second interview and to provide a saliva sample for cotinine assay. At the end of the interview, each subject was handed an airtight plastic tube containing a dental cotton roll. Subjects held the cotton lightly between two fingers and placed it in their mouth for about 5 minutes to let it absorb as much saliva as possible. Subjects were instructed to let the cotton sit in their mouth without biting or chewing it. At the end of the 5 minutes, subjects pushed the cotton back into the tube with their tongue along with a sample of extra saliva. Once the cotton was back in the tube, subjects put the cap back on, tightened it, and gave it back to the interviewer. After collection, samples were stored in a freezer until they were shipped overnight for assay by the Division of Epidemiology at the University of Minnesota. Levels of cotinine in the saliva were determined using gas chromatography.

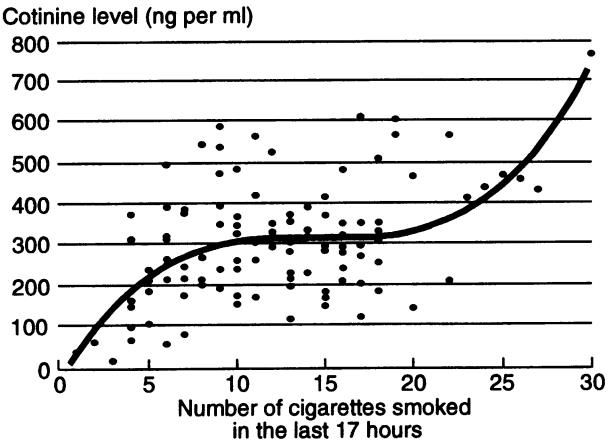
For examining the relationship between amount smoked and cotinine levels, the sample was restricted to 116 subjects. Four subjects had unreliable reporting (for example, they left the computer where they could not get to it, were too busy, or simply forgot to record the cigarette) the day before sample collection; five subjects had insufficient saliva for assay; and the interviewer failed to take a sample from one subject.

Results

Description of the sample. Mean age for the sample was 37.4 years (standard deviation (SD) = 11.0 years). The average number of cigarettes smoked daily, as logged into the hand-held computer, was 20.1 (SD = 8.3). The average number of cigarettes logged into the monitor in the 17 hours preceding the collection of the saliva sample was 12.2 (SD = 5.8). The average cotinine level over all subjects was 297.5 ng per ml (SD = 141.1 ng per ml).

Correlates of cotinine levels. Cotinine was associated significantly with the average number of cigarettes smoked in the 5 days preceding the collection of the saliva sample, r = 0.35, 95 percent confidence interval (CI) = 0.19, 0.55. The correlation was also computed between cotinine

The nonlinear relationship between recent smoking and cotinine levels in saliva



level and the number of cigarettes logged in the 17 hours preceding the interview.

The association between cotinine and the number of cigarettes smoked in the 17 hours prior to sample collection was larger than cotinine's association with the 5-day, 24-hour average number of cigarettes smoked, $r = 0.44$, 95 percent CI = 0.29, 0.65, and accounted for a significantly greater amount of variance in cotinine according to Akiake's information criterion test (*16*), $\chi^2(2) = 10.00$, $P < 0.05$. Age was also significantly associated with cotinine, $r = 0.38$, 95 percent CI = 0.22, 0.58; amount smoked in the previous 17 hours, $r = 0.29$, 95 percent CI = 0.12, 0.48; and the average daily amount smoked, $r = 0.26$, 95 percent CI = 0.09, 0.45.

The suspected nonlinear association between cotinine and the amount smoked in the previous 17 hours was tested using nonlinear modeling that included quadratic and cubic components, both with and without age adjustment. The table summarizes the results of model fitting. The linear relationship between cotinine and amount smoked increased to an R^2 of 0.27 ($r = 0.52$) with the inclusion of age in the model. Using Akiake's information criterion, this represented a significant improvement in total variance accounted for, $\chi^2(2) = 9.0$, $P < 0.05$. The inclusion of a quadratic term in the model did not result in significant improvement in the goodness of fit over that associated with age and the linear component, $\chi^2(2) = 2.0$, not significant. The addition of the cubic component, however, did result in a significant improvement in the fit to the data, $\chi^2(2) = 9.0$, $P < 0.05$, with all nonlinear components being highly significant (overall model $r = 0.53$, $R^2 = 0.28$). For illustrative purposes, this association is presented in the figure. The inclusion of age in the cubic model resulted in the largest overall r (0.59), 95 percent CI = 0.50, 0.86, and R^2 (0.35) observed for the six models tested. The increase in variance accounted for in cotinine levels over that associated with the simple linear correlation with number of cigarettes smoked in the previous 17 hours was highly significant, $\chi^2(2) = 19.0$, $P < 0.001$.

Inspection of the scatter plot in the figure suggested the possibility that the significant cubic fit could have resulted from the influence of one observation, a cotinine value of 768 ng per ml. Review of this value in relation to other values for cotinine in this sample indicated that, although it was a large value, it was not identified as an outlier by the criterion of Tukey (*17*). Moreover, this value was found not to have a uniquely large influence on the relationship between cotinine and average amount smoked in the preceding 17 hours using the COVRATIO statistic (*18*), adjusted for sample size. Finally, the analyses reported previously were repeated after exclusion of this observation. Results of this analysis confirmed the previously observed cubic fit to the data reported in the table.

Discussion

In this analysis, we have demonstrated a significant improvement in the bivariate relationship between cotinine and amount smoked when the recording window is restricted to the 17 hours preceding sample collection and under conditions that emphasize and reward the accuracy of recall. All previous studies, of which we are aware, of the relationship between amount smoked and cotinine have relied on reported amount smoked in a typical day as the measure of smoking. The variance across studies in previously reported associations between cotinine and amount smoked daily may partly be the result of the excessive length of the recording interval (24 hours) relative to the reported half-life of cotinine (17 hours) as well as the known inaccuracy of recall (*15*).

The relationship between age and cotinine in this sample was of a magnitude similar to that seen between amount smoked and cotinine. At a minimum, this finding suggests that age could be one of the important individual characteristics that contribute to the wide intersubject variability noted by Idle (*9*) and others. At this point, we can only speculate as to how age is related to cotinine. The relationship with amount smoked suggests that it could be operating through a behavioral pathway.

On the other hand, age is a known contributor to differences in drug pharmacokinetics (*14*), which may also contribute to variance in cotinine levels.

The linear association between saliva cotinine and amount smoked in the 17 hours preceding sample collection, although significant, obscured a nonlinearity in this relationship that would have been otherwise overlooked. From the present analysis, a midrange of consumption appears to exist for which cotinine levels do not discriminate between different amounts of consumption in the previous 17 hours. To test this observation, the number of cigarettes smoked in the previous 17 hours were divided into tertiles: 1–9 cigarettes ($N = 38$), 10–14 cigarettes ($N = 39$), and 15–30 cigarettes ($N = 39$). The correlation between cotinine and number of cigarettes smoked was then calculated for each level of smoking: $r = 0.53$, 95 percent CI = 0.28, 0.90; $r = -0.06$, 95 percent CI = −0.40, 0.28; $r = 0.50$, 95 percent CI = 0.23, 0.86, respectively.

Using the r to Z transformation to test the difference between the correlations in each of the tertiles, we found that the correlations for the lowest and highest tertiles were, in fact, significantly greater than the correlation in the middle tertile of smoking ($P < 0.01$). It is possible that it is in this midrange of consumption that individual differences in smoking style, dietary habits, metabolism, nicotine clearance, or saliva production exert maximal effect on cotinine, relative to consumption.

Regardless of the interpretation, it is apparent from this analysis and that of Abrams and coworkers (*4*) that, although cotinine levels are sensitive to amount smoked, they are so only in certain ranges of smoking rates. Until a better test is developed, future work should be directed toward understanding the nonlinearity in the relationship between cotinine and amount smoked as seen in this study and in the work of Abrams and colleagues (*4*). Inclusion of both nonlinear components of variance and important individual differences such as age resulted in a significant improvement in prediction over that resulting from a simple bivariate, linear model. Recognition of this possibility could result in an overall improvement in the accuracy of this test to detect differences in smoking behavior and may result in the identification of important confounding influences.

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Equipment

A. PSION Organiser II Model CM, Psion PLC, London, England.