Validation of Self-Reported Smoking Behavior: Biochemical Analyses of Cotinine and Thiocyanate

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Abstract: Biochemical determinations of plasma and salivary cotinine and thiocyanate were used to differentiate smokers from non-smokers and to follow daily smoking patterns in smokers. Results indicate that cotinine is better suited than thiocyanate to determine smoking status in large scale epidemiologic studies and to follow alterations in smoking behavior over periods of time. Salivary cotinine is a reliable alternative to plasma for validation of smoking status and for following changes in daily smoking patterns. (*Am J Public Health* 1983; 73:1204–1207.)

Introduction

Smoking control research has generally relied upon self-report for information concerning smoking status, but the validity of this measure is severely limited. Denial and minimizing the extent of cigarette smoking are common practices among youth and announced quitters.[1-3] Therefore, investigators[4,5] recognize the need for biochemical validation of smoking behavior. There is controversy, however, concerning the most appropriate measuring devices for smoking status. Salivary sampling has been suggested as an alternative to invasive venipuncture, but the relation of salivary levels to plasma levels of cigarette smoke metabolites remain imprecise. Thiocyanate measurement has been used in large scale epidemiologic studies.[6,7]

We investigated the question of whether cotinine or thiocyanate measurements should be used to separate smokers from non-smokers, to follow changes in daily cigarette smoke absorption, and to investigate smoker compensation. We also attempted to validate analysis of these components in saliva.

Methods

Experiment 1

Thirty individuals were asked to volunteer both blood and saliva samples. The participants were 12 smokers and 18 nonsmokers. Blood was collected into vacutainers containing EDTA as the anticoagulant and resulting plasma was frozen.

Saliva was collected at the same time. Participants were instructed to deposit saliva directly into a vial marked at the one ml level. Control studies on this method showed routine recovery of more than 95 per cent of both exogenous thiocyanate and ^{3}H-cotinine.

Participants then completed a questionnaire on smoking behavior as well as a 24-hour dietary recall.

Experiment 2

Two smokers* and two non-smokers were asked for a saliva sample each morning for two or four weeks. Samples were collected directly into premarked vials and then frozen. Dietary recalls were requested from both smokers and non-smokers.

Analytical Techniques

Cotinine was quantitated by a modification of the radioimmunoassay (RIA) as developed by Langone, *et al.*[8] This method uses a specific antiserum produced by injection of trans-4-carboxycotinine bound to albumin into rabbits. The inter- and intra-assay variations are less than 6 per cent. Approximately 60 samples plus standards and controls can be analyzed per day with this RIA methodology. Results compare well with those obtained by GLC.

Plasma thiocyanate was determined by an automated procedure following the method of Butts, *et al.*[9] Saliva was analyzed for thiocyanate content following suitable dilution. Samples were run in duplicate with excellent reproducibility (±1.5 per cent).

Results

Table 1 displays the mean thiocyanate and cotinine values for plasma and saliva. No cotinine was detected in non-smokers. Plasma thiocyanate was increased in smokers compared to non-smokers, reinforcing the consensus of other researchers that plasma thiocyanate levels greater than 100 μM/l can serve as indicators of regular smoking behavior.[10,11]

Salivary analysis for cotinine showed high levels present in smokers (361 ng/ml) with no cotinine being detected in non-smokers. Comparisons of salivary thiocyanate in these groups showed a difference in mean values, but a loss of resolution in the standard deviations. This is better illustrated in Figures 1A and 1B where individual values can be compared. In both plasma and saliva, cotinine analysis could distinguish between smokers and non-smokers with a high degree of accuracy, while thiocyanate determinations provide a less clear-cut answer. The "grey area" resulted from smokers reporting 10 or less cigarettes smoked per day as well as from 20 per cent of the non-smoking group. Cotinine in plasma and saliva is lightly correlated (0.90) while the correlation for thiocyonate in plasma and saliva is less than 0.40.

Thiocyanate levels are influenced by daily food consumption, but dietary recalls for the collection periods showed no abnormal consumption of vegetables or products known to influence the dietary background for thiocyanate.

Daily salivary analyses of cotinine and thiocyanate in nonsmokers gave the results for thiocyanate shown in Figure

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*Smokers were instructed to collect the sample prior to the first cigarette of the day.

TABLE 1—Average Values for Cotinine and Thiocyanate

	Smokers (n = 12)	Nonsmokers (n = 18)
Plasma Cotinine (ng/ml)	246 ± 92	N.D.*
Plasma Thiocyanate (μM/l)	157 ± 34	57 ± 22
Salivary Cotinine (ng/ml)	361 ± 80	N.D.*
Salivary Thiocyanate (μM/l)	3339 ± 1117	1293 ± 652

* N.D. = Not detected. The limits of reliability for this assay are generally placed at 1 ng/ml.

2. Values varied, ranging from a low of 700 μM/l to a high of 2400 μM/l. Daily variation over the two-week period was generally ± 600 μM/l. Cotinine was not detected in the saliva on any day. These nonsmokers reported no exposure to sidestream smoke at home or work. To confirm the reliability and limits of sensitivity of our assay, one individual who had not smoked for two years smoked a single cigarette of medium tar:nicotine yield. Four hours later, 32 ng/ml of cotinine was detected in her saliva and 26 ng/ml was present in plasma.

Figure 3 illustrates the same experimental protocol carried out for one month on two smokers of 10–15 cigarettes per day. Both participants are employed in an area where smoking is not permitted during work hours.

At first glance, the cotinine values showed variability over the month-long period. Closer inspection, however, pointed out that the maximum values coincided with weekends or holidays when the individual's smoking behavior was not restricted. The parallel analyses of salivary thiocyanate also showed variability, but no correlation with the days of peak cigarette consumption. Even if we assume a longer or shorter time for appearance of thiocyanate after periods of increased cigarette use, no significant trend is noted.

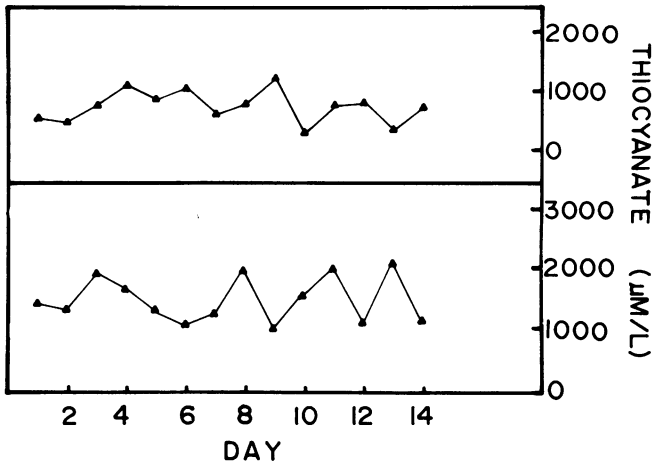

FIGURE 2—Levels of Thiocyanate in the Saliva of Two Non-smokers from Samples Collected Daily for Two Weeks. [Cotinine was not detected in any of the samples obtained from non-smokers.]

Discussion

Results of our research[12,13] and the work of others[2,8] have shown that the longer-lived metabolites of inhaled tobacco smoke are more suitable for validation of smoking behavior than are actual absorbed smoke constituents. The biological half-lives of nicotine and carbon monoxide limit their effectiveness in determining smoking status and in evaluating compensatory mechanisms.[14] The use of thiocyanate and cotinine as indices of cigarette smoke exposure is becoming increasingly more popular.[10,15]

In this study, we have demonstrated that cotinine exhibits a greater specificity in separating smokers from nonsmokers and in evaluating day to day smoking behavior. In addition, we have validated the use of saliva as an acceptable biological fluid for analysis of cigarette smoke metabolites. We believe our results have significant applications to sever-

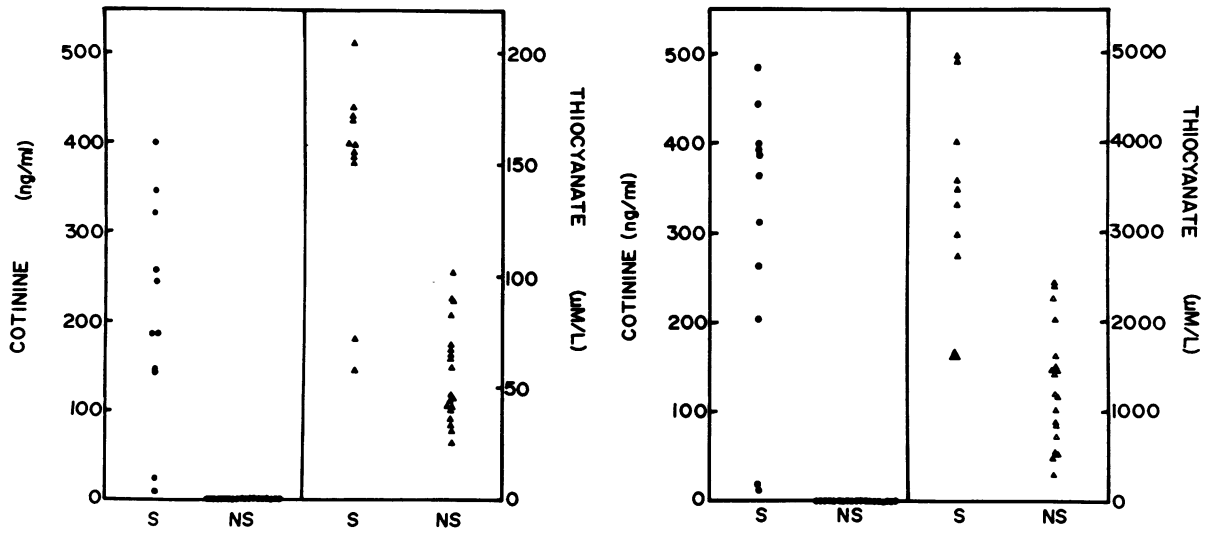

FIGURE 1—Distribution of Cotinine and Thiocyanate in the Plasma (left) and in the Saliva (right) of Smokers and Non-smokers. [S = smokers; NS = non-smokers. Cotinine was not detected in the plasma or saliva of any of the non-smokers.]

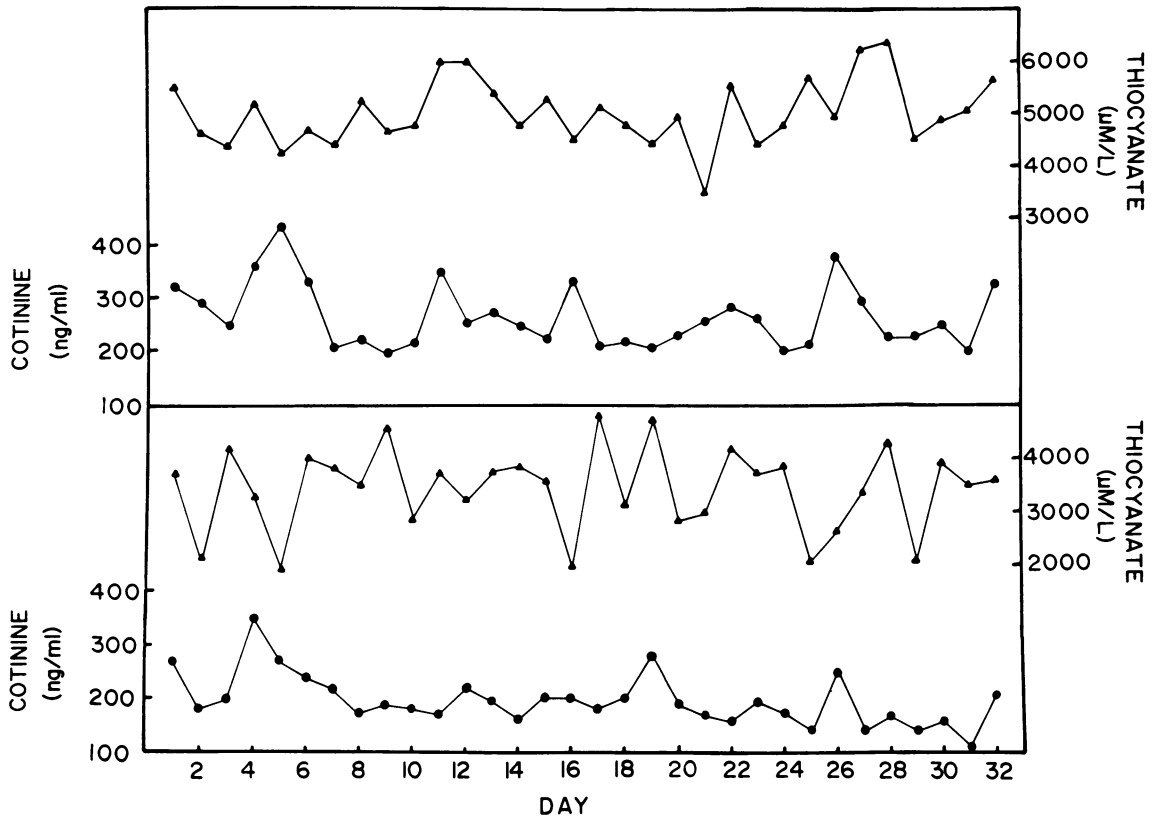

FIGURE 3—Levels of Cotinine and Thiocyanate in the Saliva of Two Smokers from Samples Collected Daily for One Month.

al kinds of smoking research. Cotinine is superior to thiocyanate in validating the cigarette smoking in an adolescent population or a group of announced quitters. Salivary thiocyanate can detect smoking behavior in habitual smokers, but the two aforementioned groups will probably fall into the "grey area" of analysis. At levels of less than 20 cigarettes per week, thiocyanate measures can be greatly complicated by dietary influences. In fact, diets enriched in certain foods can produce thiocyanate levels as high as those found in habitual smokers.[16]

Following changes in smoking patterns as individuals switch from high to low yield cigarettes has recently been a major focus in the study of addictive behaviors. Smokers tend to compensate for altered yields by increasing the number of cigarettes smoked each day or by changing how they smoke each cigarette.[12,17,18] Hill and Marquardt[19] demonstrated that when smokers switch to lower yield products, their plasma cotinine values tend to stay about the level they were at with their customary brand, although their carboxyhemoglobin levels rise. To follow the rate of compensation with analyses of cotinine is a current area of research in our laboratory.[12,13]

Salivary collection is preferable to venipuncture in many situations, and can provide reliable data on smoking status when it is followed by biochemical determination of cotinine content. Analysis of salivary cotinine, especially when measured by RIA, can provide an effective epidemiological tool in evaluating smoking prevalence as well as behavior patterns.

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The Variability of Blood Pressure Measurements In Children

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Abstract: This study examined the sources and amount of variation present in the blood pressures of 99 third grade children, using data collected from three repeated measurements made on three separate visits. The results highlight factors to consider when planning or evaluating studies designed to relate children's blood pressure levels to environmental conditions. Factors include variability both between and within children by visit and between observers; periodic restandardization of observers during the course of a study is desirable. (*Am J Public Health* 1983; 73:1207–1210.)

Introduction

Studies of blood pressure variation in adult populations[1–3] indicate that variation is a function of the time interval in which observations are made and is greater between than within examinations. This pattern of variation may be the same in children, but data to evaluate this hypothesis are not generally available.

In this study we estimated the variation present in blood pressures using data collected from repeated measurements made on third grade school children on three separate visits and examined possible sources of this variation.

Methods

The study population consisted of all third grade students at one elementary school in the Forest Hills School District in suburban Cincinnati, Ohio. The third grade was chosen because of the virtual absence of adult hypertensive levels in children 6–9 years[4] and the absence of confounding variables, e.g., smoking, that may be present in older children.

Study procedures were explained and demonstrated to the students prior to distribution of parental permission slips. Parents of participating children completed a questionnaire supplying information on the child's age, general health, and medication use. No child was taking medication known to influence blood pressure. Of 113 students, 109 (96 per cent) participated in the study.

Students came to the screening area in groups of six. The procedures were explained again and a short questionnaire was completed on each student. The children were acclimated for 10 minutes by observing the screening of their classmates. Each child passed through three stations at each of which a 30-second pulse and right arm blood pressure were taken. Blood pressure was measured by three registered nurses (observers) with mercury sphygmomanometers using the first (systolic) and fifth (diastolic) Korotkoff sounds. The rate of descent of the mercury column was controlled at 2–3 mmHg/second. Observers were blind to each others' measurements and to previous measurements. After the third set of measurements were taken, the student's height and weight were recorded. These study procedures were conducted at three screening visits over a five-week period. Each visit consisted of two consecutive days of screening with half of the children screened each day. The time of day a child was screened remained constant throughout the study.

All blood pressures were taken by the same three observers who underwent testing and special training, including audiovisual recordings of Korotkoff sounds.

Results

Descriptive Statistics

Ten students who were absent for one or more screenings were excluded from the analysis. The means and confidence intervals for the average blood pressure, height, weight, and pulse for the remaining 99 study participants* are shown in Table 1. There were no significant sex differences in the levels of systolic or diastolic pressure, pulse, or height. This is consistent with previous reports[4,5] that sex differences are generally small at this age. Correlation coeffi-

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*93 White, 2 Black, 4 Hispanic or Oriental. There were no significant differences in BP by race.