Comparison of Serum and Salivary Cotinine Measurements by a Sensitive High-Performance Liquid Chromatography–Tandem Mass Spectrometry Method as an Indicator of Exposure to Tobacco Smoke Among Smokers and Nonsmokers*

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Abstract

Exposure to tobacco smoke, both from active smoking and from passive exposure to environmental tobacco smoke, can be monitored by measuring cotinine, a metabolite of nicotine, in a variety of biological sources including blood, urine, and saliva. Previously, a sensitive atmospheric-pressure ionization, tandem mass spectrometric (LC–API-MS–MS) method for cotinine measurements in serum was developed in support of a large, recurrent national epidemiologic investigation. The current study examined the application of this LC–API-MS–MS method to both serum and saliva cotinine measurements in a group of 200 healthy adults, including both smokers and nonsmokers. The primary objective of this study was to evaluate the relationship between serum and saliva cotinine concentrations to facilitate the linking of results from epidemiologic studies using salivary cotinine measurements to existing national data based on serum cotinine analyses. The results indicate that a simple, linear relationship can be developed to describe serum and saliva cotinine concentrations in an individual, and the expression describing this relationship can be used to estimate with reasonable accuracy (approximately ± 10%) the serum cotinine concentration in an individual given his or her salivary cotinine result. It was further confirmed that saliva cotinine samples are generally quite stable during storage after collection, even at ambient temperatures, and this sample matrix appears to be well-suited to the requirements of many epidemiologic investigations.

Introduction

Cotinine, the major proximate metabolite of nicotine (1,2), is generally regarded as the best biomarker for monitoring tobacco exposure in both actively and passively exposed individuals. A method based on high-performance liquid chromatography–atmospheric-pressure ionization-tandem mass spectrometry (LC–API-MS–MS) suitable for the sensitive, high-speed determination of cotinine in serum samples (3) was developed previously and applied to the analysis of samples from the participants in the Third National Health and Nutrition Examination Survey (NHANES III, 4), as well as to several other studies over the past few years with an emphasis on studies of exposure to environmental tobacco smoke (ETS). Serum cotinine assays are generally regarded as the preferred approach for monitoring exposure to ETS (2,5), but cotinine can be measured in a variety of other matrices including urine, saliva, hair, and other sources, and in some cases an alternative matrix for the analysis of cotinine may be useful or necessary.

The most commonly used sources for cotinine assays are serum, urine, and saliva. Salivary cotinine measurements have been reported in a number of studies with good results (1,2,6–12), and this matrix may have several advantages for epidemiological studies. The collection of saliva is noninvasive, and it can be performed quickly and easily in the field. The noninvasive nature of the assay lends itself well to protocols involving children or multiple collections over time, and in some cases, samples can be collected independently by the participants at remote sites and mailed into a coordinating center for subsequent analysis (13–16), although collections in the presence of the investigator may still be desirable to ensure the timing of the sample collection, the adherence to the collection

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protocol, and the integrity of the sample. The half-life of cotinine in serum and saliva is approximately the same (17), and cotinine concentrations in these two matrices have been found to be correlated, with salivary cotinine concentrations usually reported to be about 10–40% greater than those in serum (1,2).

Although salivary cotinine measurements have several potentially useful characteristics, especially in support of epidemiological investigations, further information is needed to evaluate this approach relative to serum measurements. Several investigators have compared serum and salivary cotinine results in the past (1,18–20), but those studies have not addressed the very low concentration levels that are routinely measured with this MS–MS procedure (3) and that are important in studies of low-level exposure to ETS. In particular, studies of smokers and nonsmokers using both salivary and serum cotinine measurements in the same individuals are needed to establish a valid basis for subsequent comparisons of the results obtained in epidemiological studies using salivary cotinine measurements with large, national survey results for serum cotinine, such as those that were previously reported in NHANES III.

Furthermore, in previous studies, saliva has sometimes been obtained from study participants without the use of defined collection devices, and often information on the means (if any) used to stimulate saliva flow during collection is not available. Opinions vary concerning the preference for the use of stimulated or unstimulated saliva in cotinine measurements (1,20–22), but because both salivary flow and analyte concentrations may vary under different conditions, it is useful to standardize on a defined approach (22). A commercially available device for saliva collection was selected for these studies, providing a defined, integrated mechanism for inducing, collecting, and storing saliva samples. To address the issues of the correspondence between serum and salivary cotinine concentrations in both smokers and nonsmokers at all concentration ranges and the influence of collection and storage conditions on the salivary cotinine assays, simultaneous serum and saliva cotinine measurements were conducted with a modification of the LC–API-MS–MS method on a group of approximately 200 smokers and nonsmokers. The results suggest that salivary cotinine measurements may provide a reliable estimate of blood cotinine concentrations in both smokers and nonsmokers at all concentration levels.

Materials and Methods

Standards and reagents

Native cotinine ((–)-cotinine, 98%) was purchased from Sigma Chemical Co. (St. Louis, MO), and *N*-methyl trideuterated cotinine (DLM-1819) was obtained from Cambridge Isotopes Lab (Andover, MA). Cotinine perchlorate was prepared and purified from stock cotinine as described (23). Ammonium acetate (99.999%) was obtained from Aldrich Chemical Co. (Milwaukee, WI), and potassium hydroxide (85–90% reagent) was purchased from Fisher Scientific (Norcross, GA). Water and other solvents were products of Burdick and Jackson Labs (distributed by Baxter, Stone Mountain, GA), and all solvents were HPLC grade, except for toluene, which was Microsolve VLSI (GC 99.9%) grade, and both methylene chloride and methanol, which were Burdick and Jackson GC[2] grade solvents. The LC–MS nebulizer gas was zero-grade air, and the barrier and collision gases were nitrogen and argon, respectively. All gases were ultra-high-purity grades.

Instrumentation

Analyses were conducted with a PE Sciex API III+ atmospheric-pressure ionization triple-quadrupole MS (Perkin-Elmer Applied Biosystems, Foster City, CA) with the heated nebulizer installed. The MS was interfaced with a short length of fused-silica tubing (~ 0.10 mm × 0.5 m) to a Waters Symmetry C18 column (4.6 × 75 mm, 3.5-µm particle size) mounted in a Hewlett-Packard model 1090L LC. Two Upchurch Scientific A318 Ultralow dead-volume filters were installed in line with 2-µm and 0.5-µm stainless steel frits (in order), followed by a Waters Symmetry precolumn and the analytical column. The system was controlled by using standard Sciex system software.

Sample extraction and cleanup

Serum and saliva samples were prepared by a modification of a previously described liquid–liquid serum extraction method (3) using CE1001 ChemElute columns (Varian, Harbor City, CA). Absorbing the samples onto these columns prior to extraction with methylene chloride enabled us to eliminate the preliminary protein precipitation, centrifugation, and phase-separation steps that were previously required for serum samples (3) while still avoiding any problems with the formation of emulsions. However, the ChemElute columns were also found to make significant contributions to the background cotinine levels when they were used directly. Therefore, all columns were prewashed prior to use by applying 2 mL of 0.5M KOH to the column, followed by two successive elutions with 4 mL of methylene chloride. This wash procedure was automated by using a Hamilton Microlab 2200 programmed to carry out the wash protocol in an unattended, batch manner. The columns were dried under nitrogen for at least 15 min, and then reactivated at 55°C for 48 h. They were then removed from the oven and stored in an air-tight container at room temperature until used. No additional contribution to background cotinine levels from the ChemElute columns could be detected when these washed columns were used in the assays.

For the analysis, the serum or saliva sample (1 mL) was preequilibrated on a shaker with 5 ng of *N*-methyl trideuterated cotinine (10 µL in water) for 20–30 min. The samples were applied and allowed to absorb onto the ChemElute column for about 3 min, and the column was then eluted twice with 4 mL of methylene chloride. The combined eluant was passed through a sodium sulfate column (approximately 1 g, Varian), and taken to dryness in a Savant AES 2010 vacuum evaporator (Savant Instruments, Farmingdale, NY) at ambient temperature and using cryopumping. The residue was dissolved and transferred in a small volume of methylene chloride to prewashed autosampler microvials, and the solvent was allowed to evaporate at room temperature. All unknown samples were prescreened by an enzyme-linked immunoassay as described previously (3), and

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samples with estimated cotinine concentrations greater than 25 ng/mL were diluted prior to analysis.

Assay by LC–MS–MS

Dried samples were recovered in 20 µL of toluene, capped, and placed in the autosampler for analysis. MS analysis was carried out by positive-ion APCI using multiple reaction monitoring essentially as previously described (3) except that the injection volume was 5 µL, and a different column was used for the assays as described. The column was eluted isocratically with a mobile phase of 30% methanol in 9.2mM ammonium acetate (pH 5.0) at a flow rate of 1.1 mL/min. Transition ions were monitored at *m/z* 177 → 80 and *m/z* 177 → 98 for quantitation and confirmation, respectively, and at *m/z* 180 → 80 for the labeled reference compound (internal standard). The total instrumental analysis time in this system was slightly over 2 min/sample.

Study participants

In total, 207 adult volunteers, including both smokers and nonsmokers, were recruited for this study by Tennessee Blood Services (Memphis, TN). All subjects completed informed consent forms, and the study was approved by the CDC Institutional Review Board. Each participant provided a saliva sample by chewing on the sterile swab insert from a Salivette (Sarstedt, Newton, NC) for approximately 2 min. A 10-mL blood sample was then drawn, and a second saliva sample was collected in the same manner as the first. A brief questionnaire concerning cigarette use was completed by each subject, and all subjects were paid a small stipend for their participation in this study. The clotted blood sample and one saliva sample from each participant were centrifuged soon after collection. The saliva samples were centrifuged for 20 min at 2000 × *g*, and the saliva was recovered in the bottom part of the Salivette and frozen for shipment to CDC laboratories in Atlanta. Serum samples were transferred to cryovials after centrifugation and also frozen prior to shipment. The other saliva sample was not further treated, but rather was frozen directly on the swab. The order of selection of saliva samples for immediate centrifugation (i.e., the first or second sample) was alternated for each successive participant.

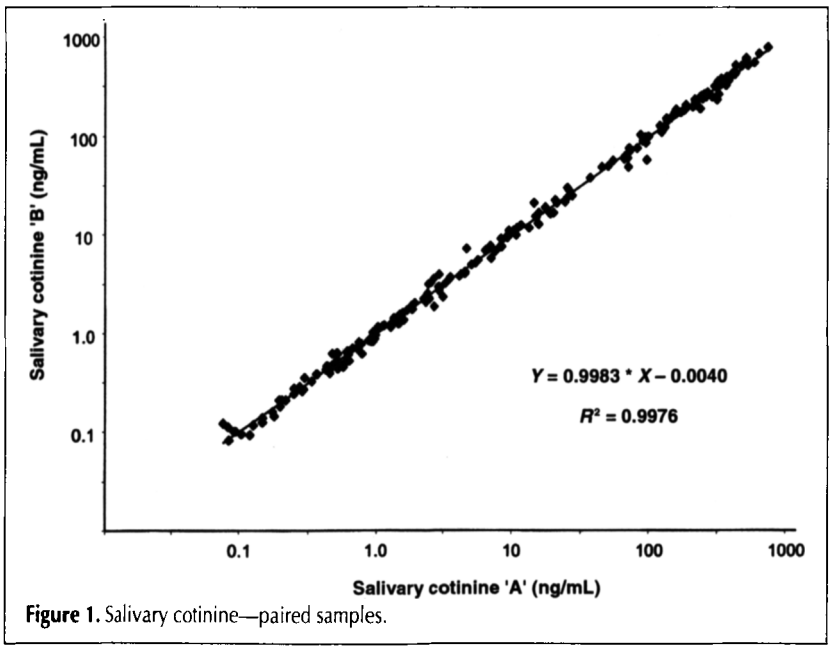

Figure 1. Salivary cotinine—paired samples.

Data analysis

Analytical data were acquired and integrated using Sciex system software, and the data files were then processed using SAS routines (Statistical Analytical System, Cary, NC) as previously described (3). Instrument verification and calibration protocols were also the same as previously described. All subsequent data analyses of the cotinine results were made by using the SAS package (SAS for Windows, Version 6.12). Regression analyses were conducted by using a modified procedure that assumes error in both the *x* and *y* variables (24). Because cotinine is well-known to have a strongly skewed distribution (4), all data were log-transformed before further analyses.

Results

Study participants

In all, 207 adult volunteers were recruited for this study, including both smokers and nonsmokers. According to self-reports made at the time when the participant was selected for inclusion in the study, there were 156 nonsmokers and 51 smokers. However, as noted in the Discussion, these self-reports were not always in agreement with the biomarker results. Approximately 73% of the participants were male, including 46 (90%) of the self-reported smokers and 106 (68%) of the nonsmokers. The ages of smokers and nonsmokers were similar; the nonsmokers averaged 32.5 ± 9.1 (18–57) years, and the smokers averaged 34.9 ± 9.0 (18–61) years.

Participants who smoked cigarettes reported smoking an average of 12.7 ± 6.9 cigarettes per day, with a range of 1–36 cigarettes per day. Of the four subjects who reported smoking 1–2 cigars per day, two smoked only cigars, whereas the other two smoked both cigars and cigarettes. There were two participants who reported chewing tobacco but who did not smoke; they were classified with the smokers throughout this study. All six of the users of cigars and chewing tobacco were male. None of the study participants reported smoking a pipe.

Paired saliva samples

To evaluate the feasibility of using the Salivette device for cotinine assays, and to determine the potential influence of sending intact, frozen samples to a central location for processing, two saliva samples were obtained from each study participant. One of each pair of samples was centrifuged immediately, alternating between the first and second collection in each case. The remaining sample was frozen intact on the swab and sent over dry

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ice, along with the previously centrifuged samples, to CDC laboratories in Atlanta. These samples were subsequently thawed, the intact samples were centrifuged, and volume recoveries were estimated in each case.

There was no difference in the estimated saliva volumes recovered from these samples following immediate centrifugation (1.94 ± 0.42 mL) versus those obtained following frozen storage of the samples on the swab during shipment (1.91 ± 0.51 mL; $p = 0.405$ by a paired t-test): $n = 207$ in each case. Similarly, there was no difference between the mean volumes recovered from the first and second saliva collections from all participants, which were 1.93 ± 0.46 mL and 1.91 ± 0.47 mL, respectively.

A comparison of the cotinine results from the first and second saliva sample from each participant is given in Figure 1. Close agreement was noted between the two samples throughout the salivary cotinine concentration range, with a slope close to 1 and $r^2 = 0.9976$. There are 205 sample pairs included in Figure 1. One sample was lost during processing, and there was insufficient saliva remaining to repeat the assay, so only one salivary cotinine value was available for that individual. The other excluded subject had a highly atypical result, with a second cotinine value approximately threefold higher than the first (1081 vs. 366 ng/mL). This differential was confirmed by a repeat analysis of both samples, and this individual was thus excluded from the paired comparison summarized in Figure 1.

No difference in cotinine values was noted between samples that were centrifuged immediately after collection and those that were stored frozen on the collection swabs for several days before they were further processed. The geometric mean cotinine concentration for the saliva samples processed immediately was 8.19 ± 14.1 ng/mL, whereas the mean value for the samples stored before subsequent processing was 8.28 ± 14.0 ng/mL; $n = 205$ in both cases.

Serum-saliva cotinine comparisons

Figure 2 summarizes the comparison between serum and saliva cotinine measurements in the participants in this study. For this comparison, both of the independent saliva samples collected from each individual were included, for a total of 410 paired samples (from 205 subjects).

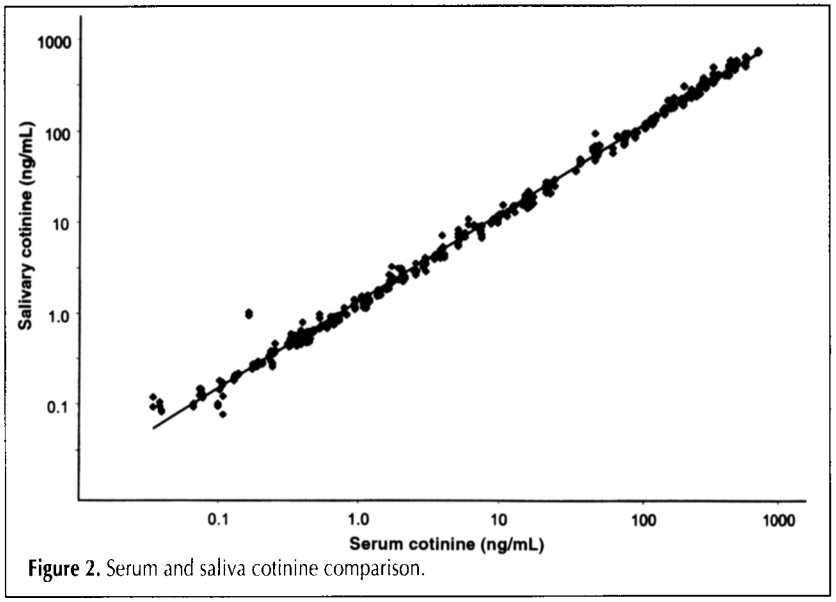

Figure 2. Serum and saliva cotinine comparison.

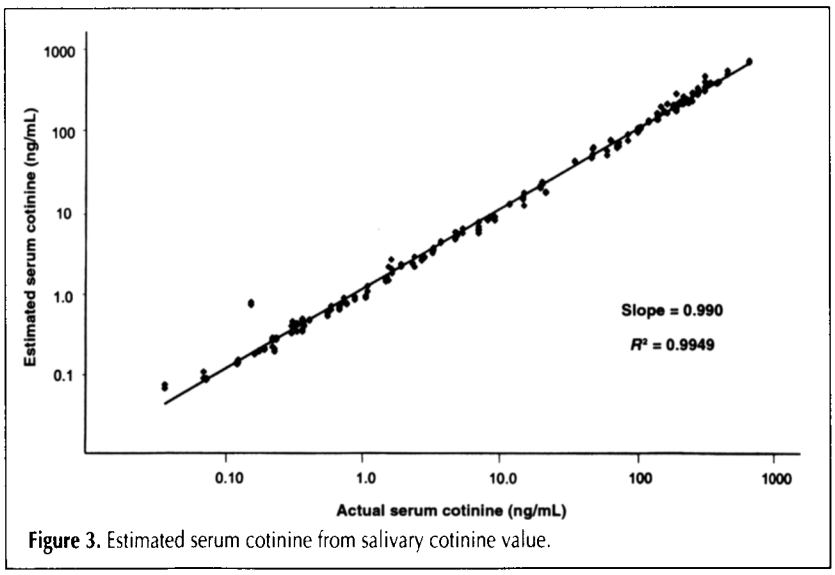

Figure 3. Estimated serum cotinine from salivary cotinine value.

Regression of the salivary cotinine data on the serum cotinine values yielded the following expression: Log_{10}(salivary cotinine) = 0.962817 * Log_{10}(serum cotinine) + 0.127478. For these data, $r^2 = 0.9968$, and the standard error of the estimate (residual standard deviation) was 0.06508 in log space, or 1.16 ng/mL. When the data were subjected to an initial classification on the basis of serum cotinine values into presumed nonsmokers (< 15 ng/mL) and presumed smokers, the corresponding expressions were 0.938152 * Log_{10}(serum cotinine) + 0.124385, and 0.997174 * Log_{10}(serum cotinine) + 0.05953, respectively, with $r^2 = 0.987$ in both cases.

Estimation of serum cotinine

An important aspect of this study was to evaluate the relationship between serum and saliva cotinine concentrations within both the smoker and nonsmoker populations, and to develop an expression that could be used to estimate serum cotinine concentrations in an individual, given his or her salivary cotinine value. Such an evaluation might then be used to compare a group of salivary cotinine results with serum cotinine data from large, national populations such as NHANES. The summarized results suggest that a simple linear relationship could be applied to derive this estimate throughout the entire range of serum and salivary cotinine levels that might be encountered among both smokers and nonsmokers.

To further test this relationship, the subjects in this study were divided into two pop-

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ulations: the first 100 individuals were used as a calibration group to develop a new serum-saliva correlation, and the remaining 105 participants were evaluated by using the resulting regression expression. The predicted and actual serum cotinine concentrations were then compared. For these evaluations the two salivary cotinine values were averaged for the participants in the calibration group, whereas only the first salivary cotinine value was used for the estimates derived for the test population because single cotinine measurements would typically be used in epidemiological studies.

The expression relating serum and salivary cotinine concentrations for the 100 people in the calibration group was very similar to that previously derived for the entire population: Log_{10}(salivary cotinine) = 0.963305 * Log_{10}(serum cotinine) + 0.127211. This relationship was used to predict serum cotinine values from the first salivary cotinine result for the remaining subjects in the study. Figure 3 summarizes the comparison between this estimated value and the actual measured serum cotinine concentrations for the individuals in the evaluation group. Table I provides the mean results from this comparison of actual measured and estimated serum cotinine values for these samples, classified by serum cotinine ranges. For each participant, the percentage bias between the predicted and true serum cotinine concentration was calculated. These values were averaged by group and are also presented in Table I. In most cases, the mean bias was < 10%, although it was somewhat greater in the lowest concentration samples.

Table I. Estimation of Serum Cotinine from Salivary Cotinine Values

Serum cotinine range	N	Mean Values: Serum cotinine (ng/mL)	Mean Values: Calculated serum cotinine* (ng/mL)	% Bias†
< 0.500	24	0.243	0.260	15.9
0.500–0.999	7	0.736	0.690	7.9
1.00–9.99	26	4.01	3.80	10.2
10.0–	48	191	199	9.3

* Calculated as Serum cotinine = $10^{((Log10(Salivary\ Cotinine)-0.127211)/0.963305)}$ (calibration was based on first 100 samples only).
† Percent bias = (ABS[serum cotinine–calc. serum cotine]/serum cotinine) *100.

Table II. Stability of Salivary Cotinine Salmples Maintained at Room Temperature on Salivette Swabs

Days at room temperature	Cotinine (ng/mL)	%	N
0	1.98 ± 0.045	(100)	6
1	1.88 ± 0.036	95	4
3	1.97 ± 0.074	99.5	4
5	1.89 ± 0.104	95.5	4
7	1.98 ± 0.037	100	4
10	1.95 ± 0.035	98.5	4
14	1.97 ± 0.046	99.5	4

Salivary cotinine stability

Comparisons of saliva samples that were centrifuged immediately after collection with those samples that were stored frozen on the swabs for several days before processing indicated that there was no detectable difference either in volumes recovered or in the cotinine results obtained between the two conditions. To further evaluate the stability of samples stored directly on the swabs without freezing, such as might be encountered if samples were collected in the field and transported or mailed back to the laboratory, a saliva pool at a target concentration of approximately 2 ng/mL was prepared by spiking a pooled sample of nonsmoker saliva with cotinine perchlorate. The spiked pool was well-mixed for approximately 2 h, and then individual swabs were briefly immersed in the pool with constant mixing and returned to their holders. The Salivettes were then stored at room temperature (approximately 22°C) for various time periods before being placed in the freezer (–70°C). All samples were centrifuged, extracted, and analyzed together at the end of the study.

The results from this evaluation are given in Table II. There was no change in salivary cotinine concentrations detectable in these samples throughout the two-week storage period. There was concern that even if cotinine were stable under these conditions, some evaporative water losses that could lead to erroneous elevations in the measured concentrations might occur. Therefore, half of the Salivettes in this study were further sealed at the joints with several turns of Teflon tape. However, there was no evidence of any evaporative losses in the Salivettes stored at room temperature with or without additional sealing throughout the two-week period, and it appeared that the seals on the devices themselves were adequate for storage purposes.

In a related analysis, pooled saliva samples were prepared at two low levels, one pool with a cotinine concentration of approximately 0.6 ng/mL and the other with a concentration of about 4 ng/mL. The pools were used to saturate Salivette swabs as before, and two Salivettes from each of the two pools were processed immediately, with the recovered saliva stored frozen at –70°C until analyzed. An additional pair of Salivettes from each pool was mailed by standard U.S. airmail without refrigeration to a collaborating site in Miami, FL, held at room temperature for about two weeks, and then mailed back to CDC laboratories in Atlanta. After the mailed samples were processed, all aliquots were analyzed. The mean cotinine levels measured in the frozen pooled samples were 0.639 and 4.09 ng/mL. For the samples sent and returned through the mail, the measured cotinine concentrations were 0.667 and 4.28 ng/mL, respectively. These results again indicated that the samples had maintained essentially constant concentrations for an extended time period during the ambient temperature storage and mailing process, and are thus in agreement with two previous studies indicating stability of salivary cotinine samples at relatively high (smoker) concentration levels (14,15), and a recent study that found salivary cotinine to be stable at room temperature in samples with concentration levels typical of both smokers and nonsmokers (16).

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Discussion

The volume of saliva collected is an important consideration in these studies. For ETS exposure assessments, at least 1 mL of saliva is required to maintain full sensitivity in the assay. To maximize recoveries during processing of the Salivettes, an integrated centrifugal force that was somewhat greater than that recommended by the manufacturer was used, but that still posed no problems with the devices. Lamey and Nolan (25) also noted improved saliva recoveries from the Salivette when higher speeds were used during centrifugation. Under these conditions of analysis, no difference in either salivary volume or cotinine recoveries was found when either cotton or polyester swabs were used, and the cotton swabs were used for all of these samples.

In this study, in which saliva collections were performed (in duplicate) at one site in the presence and under the guidance of study personnel, good volume recoveries were generally obtained averaging about 1.9 mL overall. Of these samples, 389 (95%) had 1 mL or more available for analysis. The results may vary somewhat according to conditions, however.

In a separate study involving 188 adolescents, in which sample collections were conducted by the participants themselves at remote sites, with the sample subsequently mailed in to the study center, stored (frozen) for a time, and then sent to CDC laboratories in Atlanta for analysis, a lower mean saliva volume was recovered, averaging about 1.5 ± 0.7 mL. In this case, only 71% of the samples had a volume of 1 mL or greater. In another field study of approximately 320 adults in which collections were performed in the presence of study personnel at various work sites, a similar mean saliva volume was obtained of about 1.6 ± 0.6 mL, although in this case, 90% of the samples had a volume of 1 mL or greater. Most samples collected in these various studies had sufficient saliva volumes to be used in cotinine analyses, even at passive exposure levels. Because smaller sample sizes are needed for smokers, essentially all of the samples from smokers had adequate volumes. Very few samples had extremely low saliva volume recoveries of 0.25 mL or less. Whether these samples resulted from xerostomia or simply from poor collection technique could not be established. However, in general, the best results seemed to be obtained when samples were collected in the presence of experienced investigators who could help assure adherence to the proper collection protocol by all participants.

With only one exception, comparison of cotinine concentrations in paired saliva samples collected from each individual within a period of a few minutes showed excellent agreement. The reason for the substantial discrepancy in the paired samples from the remaining person is not known, but this individual was one of the two people in the study who chewed tobacco, and it is possible that salivary cotinine measurements may be adversely influenced in some cases when applied to users of chewing tobacco. Because cotinine is a metabolic product rather than a normal constituent of tobacco, it is unlikely that sample contamination with a small amount of oral tobacco would contribute directly to salivary cotinine measurements in the same manner as it may in the analysis of nicotine; however, we cannot absolutely exclude that possibility. Alternatively, the first-pass metabolism of recent, swallowed tobacco juices might have contributed to the variation. No restrictions on the prior use of tobacco were imposed on the participants of this study, and the time period between the last use of tobacco and the collection of specimens was not recorded. The paired salivary cotinine values for the other user of chewing tobacco in this study appeared to be normal, and his results were retained for the paired sample comparison. However, further evaluations of potential interferences in salivary cotinine measurements when applied to users of chewing tobacco may be indicated.

A close, linear relationship was noted in this study between the (log) serum cotinine and (log) salivary cotinine in all individuals, both smokers and nonsmokers, throughout the range of cotinine concentrations that were encountered. These results suggest that salivary cotinine measurements are capable of providing reasonable estimates of serum cotinine levels. Previous studies have found salivary cotinine concentrations to be generally about 10–40% greater than the corresponding serum cotinine levels in the same individuals (1,2,17–19,26). The mean (± standard deviation) saliva/serum cotinine ratio in this study was 1.27 ± 0.27 overall. However, the ratios were somewhat different between smokers and nonsmokers. The ratio was 1.36 ± 0.303 for those participants classified as nonsmokers based on a serum cotinine concentration < 15 ng/mL, whereas it was 1.14 ± 0.142 for smokers with serum cotinine levels > 15 ng/mL. In fact, from a consideration of the regression expression developed from the data in Figure 2, it is apparent that the saliva/serum ratio would be expected to vary with the cotinine concentration. For example, a salivary cotinine concentration of 500 ng/mL in a heavy smoker would predict a serum cotinine concentration of 469 ng/mL and a ratio of 1.07. However, a nonsmoker with limited ETS exposure and a salivary cotinine concentration of 0.5 ng/mL would have a predicted serum cotinine concentration of 0.359 ng/mL, and a saliva/serum ratio of 1.39. These results suggest that the transport of cotinine from the systemic circulation into saliva may be saturable at higher blood concentration levels, although relative pH effects and limited protein binding in blood probably also influence the final steady-state position in the individual (21). In any case, linearity between serum and salivary cotinine concentrations seemed to be maintained up to the highest cotinine levels examined in this study (approximately 700 ng/mL).

In designing this study, a recruited population that consisted of about one-third smokers and two-thirds nonsmokers was intended. That goal was achieved according to the self-reported smoking status of the participants. However, a rather different classification arose based on the cotinine results. Specifically, a substantial number of self-reported nonsmokers in this study had cotinine levels consistent with active smoking. In each case, excellent agreement was noted among the serum and both saliva sample results from each individual. If we use a serum cotinine cutoff value of 15 ng/mL to mark the demarcation between nonsmokers and smokers (4), 39 of the 156 self-reported nonsmokers, or approximately 25% of this group, would be classified as smokers. The geometric mean serum cotinine concentration for this group of 39 was 65 ± 3.1 ng/mL, with a range of 15 to 456 ng/mL, and more than 40% of this group had a serum cotinine concentration greater than 100 ng/mL.

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These values are consistent with those for active users of tobacco products and suggest that these individuals did not truthfully report their smoking status. This discrepancy may have arisen in many cases because of the compensation offered for participation in this study, coupled with a belief that candidates would be more likely to be included in the study if they reported themselves to be nonsmokers. Whatever the reason for the failure of some people to accurately report their true smoking status, this phenomenon is an additional factor supporting the usefulness of objective biomarkers in the classification of study subjects.

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