Research Article

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Estimation of Nicotine Dose after Low-level Exposure Using Plasma and Urine Nicotine Metabolites

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Abstract

Background: We sought to determine the optimal plasma and urine nicotine metabolites, alone or in combination, to estimate the systemic dose of nicotine after low-level exposure.

Methods: We dosed 36 nonsmokers with 100, 200, or 400 μg p.o. of deuterium-labeled nicotine (doses similar to exposure to secondhand smoke) daily for 5 days and then measured plasma and urine nicotine metabolites at various intervals over 24 hours.

Results: The strongest correlations with nicotine dose were seen for the sum of four (cotinine + cotinine-glucuronide + trans-3′-hydroxycotinine + 3HC-glucuronide) or six (including also nicotine + nicotine-glucuronide) of the major nicotine metabolites in 24-hour urine collection (r = 0.96), with lesser correlations for these metabolites using spot urines corrected for creatinine at various times of day (r = 0.72-0.80). The sum of plasma cotinine + trans-3′-hydroxycotine was more highly correlated with nicotine dose than plasma cotinine alone (r = 0.82 versus 0.75).

Conclusions: Our results provide guidance for the selection of biomarkers to estimate the dose of nicotine taken in low-level (secondhand smoke) tobacco exposure.

Impact: This is probably relevant to active smoking as well. *Cancer Epidemiol Biomarkers Prev; 19(5); 1160–6.* *©2010 AACR.*

Introduction

Biomarkers of nicotine intake are widely used as indicators of tobacco smoke exposure from active and passive smoking. The most commonly used biomarker is the concentration of cotinine, the major proximate metabolite of nicotine, measured in blood, saliva, or urine (1). As blood cotinine has been considered the most direct measure of nicotine intake, we recently published an analysis of the relationship between urine nicotine metabolites and plasma cotinine concentrations (2). However, the ultimate goal for estimation is not blood cotinine but rather the daily systemic dose (intake) of nicotine, which also reflects exposure to related tobacco smoke toxins. The relationship between the systemic dose of nicotine and the plasma cotinine concentration is known to be variable from person to person as it is influenced by the proportion of nicotine that is metabolized to cotinine and the clearance rate of cotinine, both of which exhibit considerable individual variability (1).

The present study seeks to determine the optimal plasma and urine nicotine metabolites, alone or in combination, to estimate low-level exposure to nicotine. Other researchers have studied nicotine and metabolites in blood and urine in relation to estimated nicotine intake from cigarettes or transdermal nicotine, in relation to cigarettes smoked per day or in relation to concentrations of other tobacco smoke constituents (3-5). However, to the best of our knowledge, the quantitative relationship between known doses of nicotine taken in steady state conditions and concentrations of nicotine and metabolites in blood and urine has not been previously validated. We present data in nonsmokers dosed with nicotine at levels similar to those associated with secondhand smoke exposure.

Materials and Methods

Subjects. The design of the study was described in an earlier publication (2). In brief, the subjects were 36 healthy nonsmokers recruited from flyers at local colleges, newspaper advertisements, and a notice on a local web site. The average age was 32.6 years, ranging from 20 to 61. Race/ethnicity of the subjects was 20 non-Hispanic whites, 5 Hispanics, 8 Asians, and 3 non-Hispanic blacks. Subjects were studied in three groups of 12. All subjects within a group received the same dose of nicotine,

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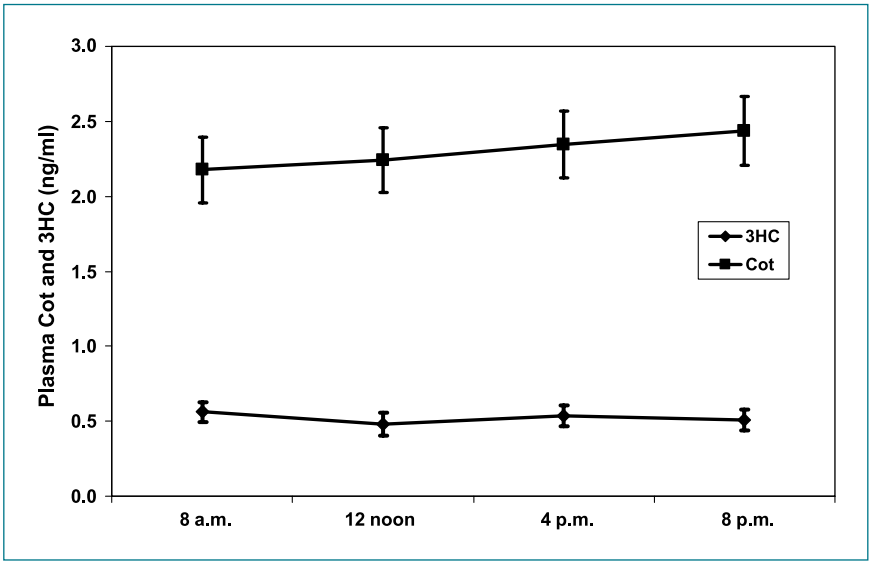

Figure 1. Mean plasma cotinine (Cot) and trans-3′-hydroxycotinine (3HC) concentrations at various times of day in subjects receiving 200 μg/d of nicotine (n = 12). Bars, SEM.

but each group received a different dose of nicotine. Each group included equal numbers of men and women. Subjects were financially compensated for their participation. Written informed consent was obtained from each subject. The study was approved by the University of California, San Francisco committee on human research.

Study design. Groups of subjects received deuterium-labeled nicotine in doses of 100, 200, or 400 μg/d p.o., respectively, for 5 days. Five days was selected as an adequate time for plasma cotinine levels and urine nicotine metabolite levels to reach steady state. The doses of nicotine were selected to be similar to doses of nicotine that might be absorbed systemically during exposure to secondhand smoke (1). The total daily dose was distributed into four equal doses to simulate exposure to nicotine from secondhand smoke over a period of 12 hours each day. Doses were to be ingested every 4 hours between 8 a.m. and 8 p.m. For the first 4 days, subjects took the doses at home. On the final (5th) day, subjects were admitted to the General Clinical Research Center at San Francisco General Hospital Medical Center where oral nicotine was administered on the same schedule as the previous 4 days, and for blood sampling and urine collection over 24 hours.

Deuterium-labeled nicotine (nicotine-3′-3′-d2) was synthesized in our laboratory and prepared for human administration as described previously (6). Deuterium-labeled nicotine was administered so that we could differentiate exposure to nicotine that we provided (in known doses) from exposure to natural nicotine from secondhand smoke or from food. Nicotine was prepared in vials containing 10 mL of water and was stored refrigerated until they were dispensed to subjects to take home. Subjects were instructed to take their nicotine doses at 8 a.m., 12 noon, 4 p.m., and 8 p.m. Subjects were given timers to take home that were programmed to signal the times at which each dose was supposed to be taken.

On the morning of day 5, subjects were admitted to the General Clinical Research Center. Blood was obtained at 8 a.m., 12 noon, 4 p.m., and 8 p.m. for measurement of plasma concentrations of nicotine, cotinine, and trans-3′-hydroxycotinine. Urine was collected in five blocks at intervals of 8 a.m. to 12 noon, 12 noon to 4 p.m., 4 p.m. to 8 p.m., 8 p.m. to 12 midnight, and 12 midnight to 8 a.m. Urine samples were pooled with appropriate weighting of volumes according to the volume collected in particular time intervals, to constitute a sample representing a 24-hour urine.

Analytic chemistry. Concentrations of nicotine, cotinine, and trans-3′-hydroxycotinine in plasma and urine samples were measured by liquid chromatography-tandem mass spectrometry, as described in detail previously (7). Urine samples were assayed before and after deconjugation with a glucuronidase enzyme, as described previously (3). The concentration before deconjugation represents the free (unconjugated) concentration, whereas the concentration after deconjugation represents the total (sum of free and conjugated) metabolite. In addition to nicotine, cotinine, and 3HC and their glucuronides, we also measured nicotine N-oxide, cotinine N-oxide, nornicotine and its glucuronide, and norcotinine and its glucuronide. Abbreviations used for nicotine and metabolites are as follows: nicotine (Nic), nicotine glucuronide (Nic-G), cotinine (Cot), cotinine glucuronide (Cot-G), trans-3′-hydroxycotinine (3HC), and trans-3′-hydroxycotinine glucuronide (3HC-G).

Data analysis. The primary analysis was the Pearson's product moment correlation comparing various plasma and urine analyte concentrations, the latter with and without correction for creatinine, to the daily dose of

Table 1. Mean plasma cotinine and 3HC concentrations (ng/mL) at various times of day with different doses of nicotine (coefficient of variation)

Nicotine dose (μg)	Urine metabolite	8 a.m.	12 noon	4 p.m.	8 p.m.	12-h average
100	Cot	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)
	3HC	0.4 (0.5)	0.3 (0.5)	0.3 (0.5)	0.3 (0.5)	0.3 (0.5)
200	Cot	2.2 (0.3)	2.2 (0.3)	2.3 (0.3)	2.4 (0.3)	2.3 (0.3)
	3HC	0.6 (0.4)	0.5 (0.5)	0.5 (0.5)	0.5 (0.5)	0.6 (0.3)
400	Cot	4.1 (0.5)	4.2 (0.4)	4.4 (0.4)	4.5 (0.4)	4.3 (0.4)
	3HC	1.4 (0.3)	1.3 (0.4)	1.3 (0.3)	1.2 (0.4)	1.3 (0.3)

nicotine. Plasma and urine metabolite analyses included individual analytes as well as combinations of analytes that might be expected to predict nicotine dose. Molar concentrations were used when different analyte concentrations were summed.

Results

Figure 1 shows average plasma concentrations of cotinine and 3HC throughout the day in subjects receiving the 200 μg/d dose of nicotine. Data from the 200 μg dose are shown as a representative dose; other nicotine doses produced similar curves with absolute values that were proportional to the dose (Table 1). Plasma levels of cotinine and 3HC were fairly consistent throughout the day, consistent with having achieved steady state.

Figure 2 shows the urine metabolite profiles for the 200 μg dose of nicotine. Patterns of urine metabolites were similar for other doses. Table 2 presents data on average urine nicotine metabolite concentrations at various times of day in subjects receiving 200 μg nicotine, as well as individual variation (coefficient of variation).

Correlations between plasma cotinine, plasma 3HC, and the sum of Cot + 3HC measured at various times of day and as the 12-hour average value, and nicotine dose are shown in Table 3. Plasma 3HC and the sum of cotinine + 3HC were more highly correlated with nicotine dose than was plasma cotinine alone. Correlations were similar at various times of the day (Table 3).

Correlations between urine nicotine metabolites at various times of day and in the 24-hour collection and the daily dose of nicotine are shown in Table 4. Correlations between nicotine metabolites and nicotine dose were stronger for the 24-hour urine sample than for urine sampled at particular times of day. The strongest

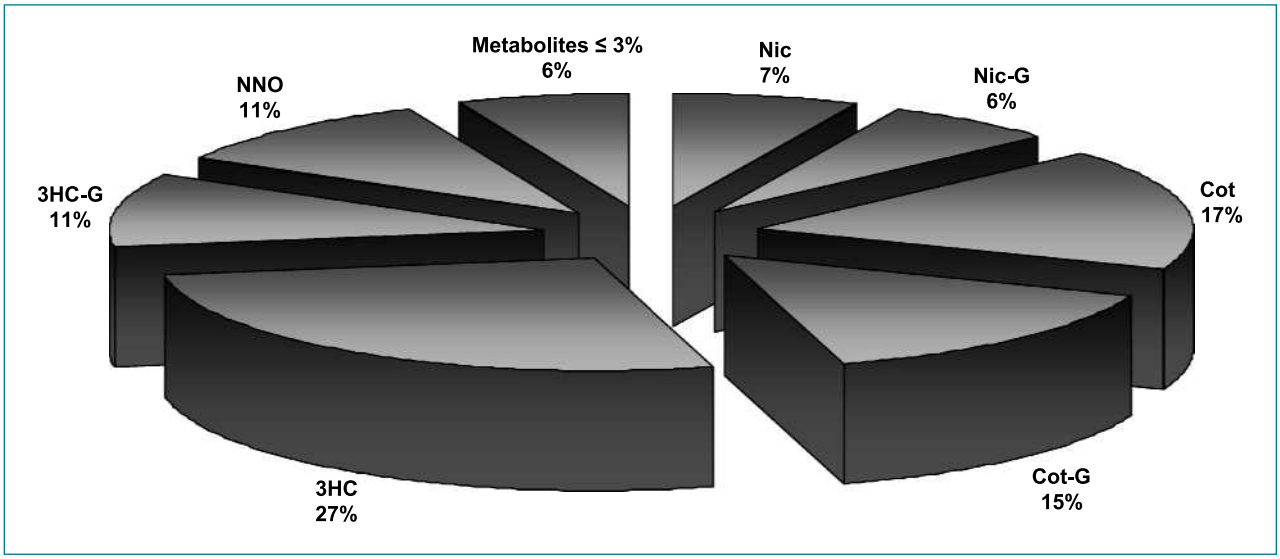

Figure 2. Average nicotine metabolites in a 24-h urine collection expressed as a percentage of total metabolites recovered in subjects receiving 200 μg/d of nicotine (n = 12). Abbreviations are as follows: Nic, nicotine; Nic-G, nicotine glucuronide; Cot, cotinine; Cot-G, cotinine glucuronide; 3HC, trans-3′-hydroxycotinine; 3HC-G, trans-3′-hydroxycotinine glucuronide; NNO, nicotine N-oxide.

Table 2. Average concentration of nicotine metabolites at various times of day, with and without correction for urine creatinine concentration (coefficient of variation)

Urine metabolite	8 a.m. to 12 noon	12 noon to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 12 midnight	12 midnight to 8 a.m.	24 h
Nicotine (pmol/mL)	18.4 (0.8)	21.1 (1.2)	14.5 (1.1)	68.1 (1.1)	34.7 (1.3)	18.3 (0.5)
Nicotine-G (pmol/mL)	10.6 (0.5)	22.6 (1.2)	18.4 (0.9)	39.2 (0.7)	40.7 (1.1)	21.2 (0.5)
Nicotine (pmol/mg creatinine)	45.3 (0.9)	43.3 (1.1)	46.5 (1.0)	84.3 (1.1)	41.3 (1.2)	—
Nicotine-G (pmol/mg creatinine)	32.2 (0.7)	46.5 (0.7)	62.3 (1.0)	53.9 (0.7)	43.1 (0.9)	—
Cotinine (pmol/mL)	58.9 (0.4)	63.8 (0.4)	49.8 (0.6)	81.0 (0.5)	74.2 (0.4)	62.4 (0.5)
Cotinine-G (pmol/mL)	41.5 (0.6)	51.4 (0.4)	33.9 (0.5)	73.7 (0.6)	99.3 (0.9)	66.7 (0.5)
Cotinine (pmol/mg creatinine)	144.9 (0.4)	150.2 (0.5)	139.8 (0.4)	128.6 (0.5)	102.9 (0.7)	—
Cotinine-G (pmol/mg creatinine)	109.4 (0.6)	126.3 (0.6)	121.5 (0.6)	103.1 (0.6)	98.8 (0.5)	—
3HC (pmol/mL)	97.7 (0.9)	104.8 (0.8)	73.0 (0.8)	135.5 (0.8)	209.2 (1.4)	118.7 (0.6)
3HC-G (pmol/mL)	54.6 (1.6)	45.9 (0.9)	30.1 (1.1)	40.3 (0.6)	56.1 (1.2)	47.2 (0.8)
3HC (pmol/mg creatinine)	198.4 (0.5)	210.1 (0.5)	186.7 (0.5)	194.2 (0.6)	193.7 (0.6)	—
3HC-G (pmol/mg creatinine)	97.9 (0.8)	107.9 (1.0)	87.4 (0.8)	71.3 (0.9)	59.3 (1.3)	—
NNO (pmol/mL)	28.1 (0.5)	52.3 (1.7)	36.0 (1.0)	72.2 (0.9)	54.1 (1.3)	30.8 (0.7)
NNO (pmol/mg creatinine)	77.9 (0.9)	92.2 (1.0)	97.3 (1.0)	99.4 (0.7)	49.4 (1.1)	—

correlations were for the sum (Nic + Nic-G + Cot + Cot-G + 3HC + 3HC-G) and for (Cot + Cot-G + 3HC + 3HC-G), both $r = 0.96$, but was also quite strong for (Cot + Cot-G), (Cot + 3HC), and (Nic + Cot + 3HC), all $r > 0.92$.

When sampled at various times of day, correlations with nicotine dose were usually but not always stronger for metabolites normalized for creatinine concentration compared with absolute concentrations. The strength of correlation of various analytes with nicotine dose varied across time of day. The strongest correlations to nicotine dose seemed to be (Nic + Nic-G + Cot + Cot-G + 3HC + 3HC-G; $r = 0.74$-0.80) and (Cot + Cot-G + 3HC + 3HC-G; $r = 0.72$-0.78). Also fairly highly correlated were (Cot + Cot-G)/mg creatinine, (Cot + 3HC)/mg creatinine, and (Nic + Cot + 3HC)/mg creatinine ($r = 0.59$-0.81). Nicotine or nicotine glucuronide alone or the sum combination of the two were poorly correlated with daily nicotine dose.

Table 3. Correlation coefficients for the relationship between plasma cotinine and 3HC with daily dose of nicotine ($n = 36$)

Plasma analyte	8 a.m.	12 noon	4 p.m.	8 p.m.	12-h average
Cot	0.69	0.69	0.76	0.79	0.75
3HC	0.83	0.80	0.82	0.80	0.83
Cot + 3HC	0.79	0.78	0.83	0.84	0.82

NOTE: All values were significant at <0.0001.

Discussion

We present novel data validating the use of various nicotine metabolite biomarkers in plasma and urine in relation to daily dose of nicotine. We have focused on low-level exposure in nonsmokers as would be most relevant to evaluating nonsmokers' exposure to secondhand smoke.

The strongest correlation with nicotine dose was observed for the sum of the four or six of the major nicotine metabolites (Cot + Cot-G + 3HC + 3HC-G, with or without Nic + Nic-G) in a 24-hour urine collection. The correlation coefficient of 0.96 indicates that these biomarker measures account for most of the variance in the estimation of dose. Other combinations of urine metabolites including cotinine and 3HC also correlated exceedingly well with dose of nicotine.

Plasma concentrations of cotinine, 3HC, and the sum of Cot + 3HC were also highly correlated with nicotine dose, but not as strongly as 24-hour urine metabolite measures.

Interestingly, plasma 3HC and the sum of Cot + 3HC in plasma seemed to be more highly correlated with nicotine dose than was plasma cotinine alone. That the sum of Cot + 3HC in plasma is a strong predictor makes sense as the sum accounts for more of the metabolites of nicotine than does cotinine alone. We are hesitant to recommend the use of plasma 3HC alone however, as 3HC is known to be quite low in relation to nicotine intake in people with deficient CYP2A6 activity, resulting in little or no conversion of cotinine to 3HC (7, 8). Presumably, in our study subjects, there were few poor metabolizers, so that 3HC was highly correlated with the nicotine dose.

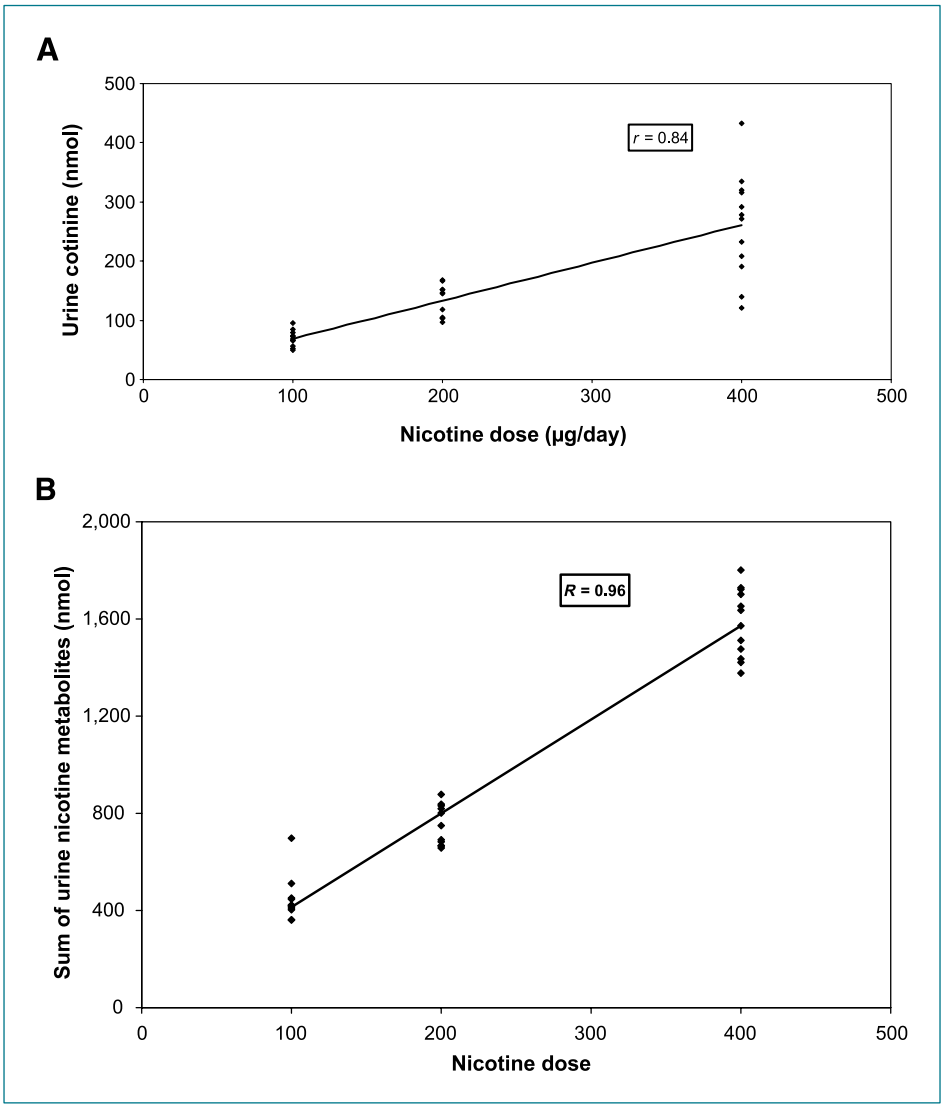

Figure 3. Correlations of cotinine (A) and sum of nicotine metabolites (B) in a 24-h urine collection versus daily dose of nicotine (n = 36).

Not surprisingly, urine nicotine metabolites measured at various time of day did not correlate as highly with nicotine dose as did the 24-hour sample. However, the strength of correlations with nicotine dose for the sum of major metabolites in urine was as strong as those of plasma analytes. The strongest correlations were for the sums of the four or six major metabolites normalized per milligram of creatinine, with r = 0.72 to 0.80 at various times of day. Simpler measures of Cot + Cot-G or Cot + 3HC also correlated well, but were more variable at different times of day (r = 0.58-0.81). Urine nicotine and nicotine glucuronide did not correlate well with nicotine dose, which was expected because the renal clearance of nicotine is highly variable from person to person, depending on urine pH and flow (9).

That the sum of nicotine and its five major metabolites measured in a 24-hour urine collection is highly correlated with dose of nicotine is anticipated. Benowitz et al. (3) and Feng et al. (5) found that 80% to 90% of nicotine taken in from cigarette smoking or transdermal nicotine could be accounted for by the sum of nicotine, cotinine, 3HC, and their glucuronides measured in a 24-hour urine sample. Scherer et al. (10) reported strong correlations between 24-hour nicotine and major metabolites and cigarettes smoked per day. Joseph et al. (4) measured the sum of cotinine and cotinine-glucuronide in spot urines (corrected for creatinine) and found strong correlations with cigarettes smoked per day (r = 0.42) and urine NNAL [4-(methylnitrosamino)-1-(3)pyridyl-1-butanol; a tobacco-specific carcinogen; r = 0.62]. The data in our study are novel in that we examine the strength of correlation between known doses of nicotine administered in steady state conditions with various combinations of nicotine metabolites in plasma and urine. We also

compare urine samples collected at various times of day with 24-hour urine collections.

One potential limitation of our study is the administration of nicotine by the oral route, whereas nicotine from tobacco is absorbed either by inhalation, or in the case of smokeless tobacco, primarily by buccal absorption. Oral dosing could affect the metabolite profile because when taken orally, nicotine undergoes first-pass metabolism as it passes through the liver and before it reaches the systemic circulation. Therefore, one would expect lower systemic exposure to nicotine compared with exposure to its metabolites after oral dosing compared with inhalation. It follows that nicotine as a fraction of total nicotine and metabolites in urine would be lower with oral dosing compared with inhalation. This was the case, with an average of 7% of recovery as nicotine in the present study, compared with 10% to 11% with cigarette smoking or transdermal nicotine in a previous study (3). Nicotine N-oxide recovery was also higher in the present study compared with the findings in smokers, but the recovery of other metabolites was similar in the present study compared with the study in smokers. Another potential limitation is that the present study was conducted in nonsmokers and we are extrapolating the findings to smokers. Smoking could affect the metabolism of nicotine and could alter the pattern of urine nicotine metabolites (11). However, as noted previously, the recovery of cotinine and 3HC and their metabolites as a fraction of total recovered nicotine and metabolites was similar in the present study compared with that observed in smokers.

Our results provide guidance as to the use of biomarkers to estimate the dose of nicotine taken in from secondhand smoke, and is probably relevant to active smoking as well. The gold standard seems to be measurement of the sum of multiple metabolites in a 24-hour urine. The simplest and strongest correlation is with the sum of (Cot + Cot-G + 3HC + 3HC-G). For urine samples measured at various times of the day, we would recommend

Table 4. Correlation between urine nicotine metabolites at various times of day and the daily dose of nicotine (n = 36)

Urine analyte	8 a.m. to 12 noon	12 noon to 4 p.m.	4 p.m. to 8 p.m.	8 p.m. to 12 midnight	12 midnight to 8 a.m.	24 h
Cot (pmol/mL)	0.76	0.70	0.42	0.56	0.74	0.84
Cot (pmol/mg creatinine)	0.58	0.71	0.68	0.52	0.60	—
Cot-G (pmol/mL)	0.67	0.75	0.62	0.53	0.63	0.86
Cot-G (pmol/mg creatinine)	0.66	0.62	0.71	0.66	0.74	—
Cot + Cot-G (pmol/mL)	0.81	0.83	0.59	0.60	0.75	0.94
Cot + Cot-G (pmol/mg creatinine)	0.65	0.73	0.74	0.76	0.81	—
3HC (pmol/mL)	0.65	0.54	0.30	0.40	0.42	0.83
3HC (pmol/mg creatinine)	0.77	0.76	0.58	0.68	0.66	—
3HC-G (pmol/mL)	0.36	0.48	0.50	0.37	0.43	0.67
3HC-G (pmol/mg creatinine)	0.50	0.52	0.71	0.56	0.53	—
Cot + 3HC (pmol/mL)	0.74	0.65	**0.35**	0.46	0.50	0.92
Cot + 3HC (pmol/mg creatinine)	0.75	0.81	0.68	0.66	0.73	—
Cot + Cot-G + 3HC + 3HC-G (pmol/mL)	0.70	0.71	0.48	0.48	0.57	0.96
Cot +Cot-G + 3HC + 3HC-G (pmol/mg creatinine)	0.72	0.77	0.77	0.78	0.78	—
Nic (pmol/mL)	0.39	**0.11**	**0.11**	**0.001**	**0.24**	0.39
Nic (pmol/mg creatinine)	0.35	**0.32**	**0.26**	0.18	**0.28**	—
Nic-G (pmol/mL)	0.70	0.38	0.39	0.46	0.46	0.71
Nic-G (pmol/mg creatinine)	0.57	0.53	0.47	0.66	0.57	—
Nic + Nic-G (pmol/mL)	0.53	**0.26**	**0.29**	**0.17**	0.36	0.57
Nic + Nic-G (pmol/mg creatinine)	0.45	0.48	0.41	0.42	0.43	—
Nic + Cot +3HC (pmol/mL)	0.73	0.63	**0.36**	0.43	0.52	0.94
Nic + Cot +3HC (pmol/mg creatinine)	0.75	0.81	0.68	0.65	0.75	—
Nic + Nic-G + Cot + Cot-G + 3HC + 3HC-G (pmol/mL)	0.71	0.72	0.49	0.48	0.59	0.96
Nic + Nic-G + Cot + Cot-G + 3HC + 3HC-G (pmol/mg creatinine)	0.74	0.77	0.77	0.78	0.8	—

NOTE: Numbers in boldface were not significant, all other correlations were significant at <0.05.

normalization for urine creatinine concentration, as in most cases, this improved correlation with nicotine dose compared with absolute metabolite levels. Although measuring multiple metabolites in urine provides the strongest correlations with nicotine dose, the use of simpler combinations such as (Cot + Cot-G) and (Cot + 3HC) also performs well. For use of plasma levels, we recommend using the sum of (Cot + 3HC), which is more highly correlated with nicotine dose than is cotinine alone.

Disclosure of Potential Conflicts of Interest

N. Benowitz is a paid consultant for pharmaceutical companies that market smoking cessation medications, and has also been a paid expert witness in litigation against tobacco companies.

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