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Temporal stability of urinary and plasma biomarkers of tobacco smoke exposure among cigarette smokers

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Abstract

Intraindividual variability of measurements of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), nicotine, cotinine, and *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) over time is uncertain. From 70 habitual smokers' plasma and urine sampled bimonthly for a year we analysed plasma for NNAL, cotinine and PheT, and urine for NNAL, cotinine and nicotine. We estimated the intraclass correlation coefficients (ρ_I) for each measurement. Plasma and creatinine-corrected urinary NNAL were stable ($\rho_I \geq 70\%$); plasma PheT and plasma and urinary total cotinine were fairly stable ($\rho_I \geq 50\%$), but urinary nicotine $\rho_I \approx 40\%$ was not. Except for nicotine, single measurements from plasma or urine adequately represent individual mean exposure over time.

Keywords: *Tobacco carcinogens; NNK; NNAL; phenanthrene tetraol; intraclass correlation coefficient*

Introduction

Cigarette smoking is responsible for approximately 90% of lung cancer mortality and 26% of all cancer death in the world (IARC 2004b, Peto et al. 2007). There are over 60 established carcinogens in cigarette smoke, and there can be little doubt that collectively they are the major cause of this massive death toll (IARC 2004a). However, less is known about the roles of individual carcinogens and toxicants in cigarette smoke as causes of cancer. One approach to dissecting the role of individual compounds in tobacco-induced cancer is the use of tobacco carcinogen biomarkers, which are metabolites or adducts that can be quantified in humans and related to specific carcinogens (Hecht 2002, 2003). As an example, these biomarkers could be used in nested case–control studies to examine the relationship between exposure to specific carcinogens or other toxicants and cancer outcome. Ultimately, data from such studies could be used to identify mechanisms of carcinogenesis, and those biomarkers that are significantly more predictive than smoking history alone could be used to develop an algorithm to identify smokers at highest risk for cancer, who could then be targeted for preventive interventions.

Most commonly, samples available from nested case–control studies have been collected at single time points from each subject. Although there may be little technical variability in the laboratory process itself, the actual level in an individual may vary considerably over time. Because the effect of an exposure is usually thought to be related to the average or cumulative level over time and because epidemiological analyses aim to relate differences among individuals in this level to differential disease outcomes, stability within an individual is critical. Here, stability means that the between-individual differences must be large enough relative to within-individual variation to provide a useful distinction between individuals.

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For tobacco biomarkers, little is known about the extent to which a biomarker in this single sample represents its average level over time. In this study, we addressed this problem by determining the longitudinal stability of several tobacco-smoke-related biomarkers in a cohort of smokers over a period of 1 year, sampling every other month. The biomarkers measured in plasma were: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL); *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT); and total cotinine. Total NNAL, total cotinine, and total nicotine were the biomarkers measured in urine. Total NNAL is a biomarker of uptake of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), widely regarded as a causative agent for lung cancer in smokers (Hecht 2002, IARC 2007). PheT is a biomarker of uptake plus metabolic activation of polycyclic aromatic hydrocarbons, important lung carcinogens in cigarette smoke that are believed to play a major causal role in cancer among smokers and occupationally exposed individuals (Hecht et al. 2003). Cotinine is a major metabolite of nicotine, the main known addictive constituent of tobacco products (Hukkanen et al. 2005). Both cotinine and nicotine are glucuronidated in humans: the sum of the free and glucuronidated forms, termed total cotinine and total nicotine, was measured here. These biomarkers were chosen because we assessed their relation to lung cancer in a nested case–control study using single baseline samples from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial sponsored by the National Cancer Institute (Gohagan et al. 1995).

While a number of recent studies have investigated tobacco carcinogen biomarkers in groups of smokers over various time periods (Scherer et al. 2006, Roethig et al. 2007, Mendes 2008, Sarkar 2008), few have specifically addressed the question of biomarker intraindividual variation, even for cotinine, arguably the most widely used biomarker of tobacco and nicotine exposure (Lee 1999). In one study, salivary cotinine in two smokers was followed for 1 month (Haley et al. 1983). In a second, plasma cotinine in 148 smokers was measured at two time points separated by 14 weeks (Kemmeren et al. 1994). A third study measured plasma cotinine in 138 cigarette smokers at two intervals separated by 2–4 weeks (Block et al. 2006). Other studies have examined the longitudinal stability of urinary cotinine in non-smokers exposed to secondhand cigarette smoke (reviewed in Lee 1999). We are not aware of any studies that have specifically investigated the longitudinal stability of cotinine or nicotine in smokers for as long as a year. Two investigations specifically compared levels of total NNAL in the urine of smokers over periods of several days to 6 weeks, and one reported on levels of PheT in the urine of smokers over a 7-week period (Hecht et al. 2004, 2005, Carmella et al. 1995). There are no published reports on the longitudinal stability of total NNAL and PheT in plasma.

Methods

Subjects

The target population comprised long-term smokers of at least ten cigarettes a day who continued their smoking during a year. Subjects were recruited from three sources. Initially, subjects of a genetic study of regular smokers were contacted by mail to assess their interest in a longitudinal assessment of their smoking exposure. Subjects who were interested were asked to call the study office. The second source included subjects who responded to a solicitation for a screening trial but were ineligible due to smoking status. These subjects were contacted by mail and asked to call the study office if interested in the current study. The third source was from flyers and posters placed in conspicuous areas around the campus of the University of Minnesota, with a brief description of the study and a telephone number to call for those interested. Subjects were offered compensation of up to $300 for full participation at all study visits. This study was approved by the University of Minnesota Research Subjects' Protection Program Institutional Review Board Human Subjects Committee and all subjects signed consent forms prior to enrolment.

Enrollment and baseline measures

A screening questionnaire was administered to potential subjects to establish eligibility. Eligible subjects were men and women between the ages of 25 and 74 years who smoked at least ten cigarettes per day. Subjects were excluded if they used other tobacco products (e.g. pipes, cigars); planned to quit smoking or move out of the area within the next year; reported certain chronic health conditions such as type II diabetes, liver disease, cancer; were currently pregnant; or had unstable or untreated psychiatric or chemical dependency problems.

Eligible subjects who were interested in enrolling were invited to attend an orientation to learn more about the study, ask questions and complete the informed consent process. Subjects who enrolled were scheduled for an initial clinic visit. Two weeks before the visit, subjects received a 7-day smoking diary and were asked to complete it for the week prior to the visit. They were also sent a 5-oz (142 ml) urine collection cup and asked to collect their first void of the day the morning of the visit. At the visit, the subjects filled out a baseline questionnaire and smoking history. Carbon monoxide (CO), height and

weight were measured, and blood and urine samples were obtained. A schedule of visits every 2 months for the next year was set up at this time although visits could be rescheduled if necessary. The subjects were paid $40 at the end of the visit. Blood and urine samples were processed at the clinic and transported to the Analytical Biochemistry Shared Resource, Masonic Cancer Center, University of Minnesota, for storage and eventual analysis.

Follow-up visits

For the six follow-up visits, subjects were again sent a 7-day smoking diary to complete and a urine collection cup for their first void of the morning prior to the scheduled visit. At the visit, subjects completed a brief medical history update, submitted their 7-day smoking diary, and were weighed. An alveolar CO measurement was taken using a Bedfont Micro Smokerlyzer and subjects were asked to report the number of cigarettes smoked on the day of the visit to validate their continued smoking. Blood and urine samples were obtained, processed, and stored in the same way as for the baseline visit. Subjects were paid $40 for each clinic visit, with the exception of the final visit when they were paid $60.

Laboratory samples

At each visit, subjects provided blood in one 10-ml purple-top (ethylenediamine tetraacetic acid (EDTA) preservative) tube. Each tube was centrifuged according to protocol at the clinic, refrigerated and transported within 24 h to the Analytical Biochemistry Shared Resource for storage and eventual analysis. Plasma samples were stored in a freezer at –20°C. Each 5-oz (142 ml) urine collection cup was refrigerated upon receipt and processed for storage at the end of the clinic day. Urine samples were aliquoted into four cryovials (4.5 ml), stored in a –20°C freezer until analysis. All samples were analysed within 1 year of receipt.

Laboratory methods

All samples were processed regardless of whether the subject provided a complete series or not. Total nicotine (free nicotine plus nicotine-*N*-glucuronide) and total cotinine (free cotinine plus cotinine-*N*-glucuronide) in urine (Hecht et al. 1999) and total NNAL and PheT in plasma (Carmella et al. 2006) were analysed as described previously. Total cotinine concentration in plasma was quantified by gas chromatography (GC)–mass spectrometry. The method was similar to that used previously to analyse urinary total cotinine (Hecht et al. 1999) with the addition of a solid-phase extraction step carried out on an Oasis MCX column (Waters Corporation, Milford, MA, USA). The MCX column was prepared and the sample eluted as described previously (Murphy et al. 2007).

Analysis of total NNAL in urine

Our current method for total NNAL in urine has not been previously described. Therefore, details are presented here.

Apparatus

An HP 6890 gas chromatograph (Agilent Technologies, Sanat Clara, CA, USA) was interfaced to a model 543 Thermal Energy Analyzer (Orion Research, Beverly, MA, USA). The pyrolyzer and interface temperatures of the Thermal Energy Analyzer were 500°C and 275°C, respectively. The separation was performed on a 15 m × 0.25 mm i.d., 0.25 μm film thickness, DB-1701 column (J&W Scientific, Folsom, CA, USA) attached to a 2 m × 0.53 mm i.d. deactivated fused silica pre-column. The injection port temperature was 225°C. A 4 mm i.d. single gooseneck liner containing 1 cm of deactivated borosilicate glass wool was used in the injection port. The injection was done in the pressure pulsed splitless injection mode (14 psi pressure pulse for 1 min). A ramped pressure program was used to keep the flow of helium at 2.7 ml min^{-1} through the column. The oven temperature program was 80°C for 2 min, then ramped to 190°C at 30°C per min, then to 215°C at 5°C per min, then to 250°C at 30°C per min and held for 3 min.

Chemicals and enzymes

NNAL, *N*-nitrosopentyl-(3-picolyl)amine (NPPA), and 5-(methylnitrosamino)-1-(3-pyridyl)-1-pentanol (C5-NNAL) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). *bis*-Trimethylsilyltrifluoroacetamide plus 1% trimethylchlorosilane (BSTFA-TMCS) was obtained from Regis Technologies, Inc. (Morton Grove, IL, USA). β-Glucuronidase type 1X-A from *Escherichia coli* was procured from Sigma-Aldrich (St Louis, MO, USA). Chem-Elute cartridges (5 ml) were purchased from Varian, Inc. (Walnut Creek, CA, USA). Oasis MCX mixed mode cation exchange solid-phase extraction cartridges (60 mg) were obtained from Waters Corp.

Analysis

Urine (4 ml) was added to a 10 ml conical glass centrifuge tube (Kimble-Kontes, Vineland, NJ, USA). The pH was determined with pH paper and corrected to 6–8 with either 1 N HCl or 1 N NaOH if necessary. Twenty microlitres of 0.1 ng μl^{-1} (2 ng) C5-NNAL was added to the sample using a positive displacement pipettor. β-Glucuronidase (15 000 units) in 0.5 ml of water was added to the sample and it was mixed and incubated at 37°C overnight with shaking. The resulting mixture was added to a 5 ml Chem-Elute cartridge. The sample was

eluted with two 8 ml aliquots of CH_2Cl_2, and the pooled eluents were collected in a 15 ml conical glass centrifuge tube (Kimble-Kontes). The CH_2Cl_2 was removed with a Speedvac (Thermo Savant, Milford, MA, USA). The sample was dissolved in 1 ml of water and acidified with 100 µl of 1 N HCl. The MCX cartridges were placed in a 16-port vacuum manifold and conditioned with 5 ml MeOH and 10 ml H_2O. The sample was added to the column and the column was washed with 5 ml of 1 N HCl, 5 ml MeOH and 5 ml 90:5:5 H_2O/MeOH/NH_4OH. The sample was eluted with 5 ml of 30:65:5 H_2O/MeOH/NH_4OH and collected in a 10 ml conical glass centrifuge tube. The sample was concentrated to dryness in the Speedvac. The residue was transferred to 250 µl polypropylene autosampler vials (Waters) with two 120 µl portions of acetonitrile and concentrated to dryness in a Speedvac. The sample was reconstituted in 5 µl of BSTFA-TMCS containing NPPA (3 µl of 0.125 µg μl^{-1} NPPA in 1 g of BSTFA-TMCS). The vial was capped and heated at 50°C for 1 h with mixing every 15 min. Three microlitres were injected on the GC column from a cooled autosampler (Leap Technologies, Carboro, NC, USA). GC data were collected on a PeakSimple data system (Alltech Associates, Inc., Deerfield, IL, USA).

Intraday and interday precision of the assay were 6.4% and 7.5%, respectively; accuracy was >95%, and the limit of quantitation was 0.15 pmol ml^{-1} urine.

Statistical methods

All laboratory measurement series from each individual were plotted against time to examine the data for trends and variability over time. Means, medians, standard deviations and coefficients of variation (CV) were computed for each time point across individuals; CV were computed for each individual across time. As CV only measures the ratio of the standard deviation of a measurement to its mean value, it is not a measure of reliability (Lachin 2004). To examine the reliability of individual measurements of biomarker levels over time, we estimated the intraclass correlation coefficient for the logarithm of the measurements,

$$\rho_I = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2},$$

where σ_w^2 and σ_b^2 are the within- and between-subject variance, respectively. Because it quantifies the fraction of total variability due to the variation between individuals, ρ_I is a robust measure of the relative stability of the within-subject measurements; the smaller the variance of measurements within subject relative to the variance between subjects, the closer ρ_I will be to 1. ρ_I was estimated for each laboratory measurement by fitting a mixed model with a random intercept for individuals, both with and without fixed time effects, to the log-transformed biomarker values. Because the estimates were nearly identical for the two models, we used the simpler model without fixed time effects.

As a secondary analysis, we also examined the relationship of these biomarkers to known demographic and smoking variables by including these additional variables in the mixed model.

Results

All of the 70 participants made at least one visit to the clinic, and 69 complied with nearly all visits. Only one had strong evidence of smoking cessation early in the follow-up period and was removed from the final analyses. There were 34 men and 35 women, aged 50.8 ± 12.0 (mean ± SD) years (range 25–72); 60.9% were Caucasian, 31.9% African American, 1.5% Asian and 5.8% other ethnicity. They reported smoking 24.1 ± 10.5 (range 10–50) cigarettes per day at enrolment. The average number of cigarettes smoked per day reported for the week prior to each visit is consistent with these self-reported historical patterns (Table 1). CO levels over the follow-up period were also consistent (Table 1). A wide variety of brands of cigarettes were reported to have been smoked by the subjects (data not shown).

Table 2 presents the median, mean, SD and range for each of the biomarkers at each sampling time. These results exhibit consistent typical values for each biomarker and demonstrate the extent of variation between the subjects.

Next we addressed the main topic of this paper: intraindividual variation. The coefficient of variation for each subject for each biomarker was calculated for the initial and six follow-up visits, and these were averaged over the 69 subjects to obtain the overall mean coefficients of variation ± as follows: plasma total NNAL 29.3 ± 15.3, plasma total cotinine 21.4 ± 10.1, plasma PheT 47.4 ± 35.7, urine total NNAL 29.6 ± 15.0, urine total cotinine 35.8 ± 21.5, urine total nicotine 60.1 ± 26.9. Table 3 presents the intraclass correlation coefficients, ρ_I for the plasma and urine biomarkers. Consistent with the mean coefficient of variation data, among the plasma biomarkers, total NNAL and total cotinine were the most stable, with ρ_I values (x 100%) of 70% and 69%, respectively, while PheT was more variable with a value of 56%. Among the urinary biomarkers, total NNAL (76%) was more stable than total cotinine (58%), while total nicotine (40%) was quite variable. When the urine biomarker values were not corrected for creatinine, variability increased with values of 53%, 52% and 33% being obtained for total NNAL, total cotinine and total nicotine, respectively (data not shown). Plasma nicotine was not measured in this study because it was expected to be highly variable due to its short half-life. Urine PheT was not measured because its

Table 1. Cigarettes per day and carbon monoxide levels during the study.

	N	Mean	SD	Minimum	Maximum
Number of cigarettes per day reported at baseline					
	69	24.1	10.5	10.0	50.0
Average number of cigarettes per day for 7 days prior to each visit					
Sampling month					
0	69	23.1	9.9	8.1	52.3
2	64	23.7	10.1	10.3	63.4
4	63	23.6	10.5	9.0	53.6
6	62	23.2	11.1	6.0	60.0
8	60	23.2	9.9	6.9	51.0
10	59	24.3	10.8	4.6	53.1
12	59	23.3	9.7	6.9	52.7
Carbon monoxide measurements (parts per million) at each visit					
Sampling month					
0	69	25.4	10.8	6	58
2	64	22.2	9.6	3	49
4	63	21.4	12.1	4	64
6	62	20.7	11.4	4	67
8	60	20.1	11.1	2	62
10	59	24.0	10.8	1	48
12	60	23.2	9.2	6	55

N, number of subjects with non-missing values.

longitudinal stability was addressed previously (Hecht et al. 2005).

Adding demographic and smoking covariates to the regressions to determine which factors most influence the biomarker levels showed that in nearly all regressions, CO level was statistically significant, while age, sex and race did not have a consistent effect on biomarker levels (data not shown).

Discussion

Being able to measure reliably exposure to cigarette smoke toxicants and carcinogens is an important and necessary step in understanding the causal relation between cigarette smoking and the development of cancer. Many studies have examined biomarkers of cigarette smoke exposure (IARC 2004b). In most instances, a single measurement was taken and the power of the study design to detect associations depends upon that measurement representing the typical level for each individual. This study measured the stability of total NNAL, total cotinine, PheT and total nicotine levels over a full year in the plasma and urine of cigarette smokers whose cigarette consumption remained steady over that time. The study included a spectrum of ages from 25 to 72 years and of smoking intensities from 10 to 50 cigarettes per day. Accurate, validated laboratory methods were used and included quality control at each step. There was a very low rate of drop-out over the 1-year sampling period.

The results indicate that, with the exception of urinary total nicotine, single measurements in plasma and single creatinine-corrected values in urine are reliable indicators of the typical biomarker values over that 1-year period. The intraclass correlation coefficients show that the measurements within an individual vary much less than they do from person to person. Whether measured in urine (relative to creatinine) or in plasma, the intraclass correlation coefficient for total NNAL was at least 70%, and for plasma PheT and both plasma and urinary total cotinine, it exceeded 50%. Only urinary total nicotine had a coefficient less than 50%, indicating that most of the variability in a single measurement comes from time-to-time variability in the individual. This result is consistent with the well-known relatively short half-life of nicotine (100–150 min) compared with cotinine (770–1130 min), and it is for this reason that cotinine and not nicotine is widely used as a biomarker of nicotine and cigarette smoke exposure (Hukkanen et al. 2005). The higher variability of plasma PheT compared with plasma total cotinine or plasma total NNAL undoubtedly is related to multiple other sources of phenanthrene exposure such as polluted air and diet, while nicotine and NNK, the precursors to cotinine and NNAL, are tobacco-specific compounds.

In addition to the relative stability of the measurements over longer periods of time, the results show that levels are highly related to CO levels but not, in general, to age, sex or race. Thus it is not necessary to adjust for these factors when examining biomarkers of exposure. Overall, the results of this study should be reassuring to those who have accepted the studies done to date, not only because the measurements are reliable, but also because they allow correction of the estimates of association derived

Table 2. Biomarker values over the course of the study.

	N	Median	Mean	SD	95% LCL	95% UCL	Minimum	Maximum
Plasma total NNAL concentration (fmol ml^{-1})								
Sampling month								
0	66	31.8	37.8	26.7	31.2	44.4	8.0	173.4
2	64	32.9	38.1	24.9	31.9	44.3	4.5	155.8
4	62	32.4	37.0	22.9	31.2	42.8	2.0	154.6
6	59	26.8	36.8	29.5	29.1	44.5	3.2	162.5
8	59	28.8	35.1	25.3	28.5	41.7	5.8	159.7
10	57	33.9	39.7	22.7	33.7	45.7	0.0	111.7
12	57	36.3	41.3	19.0	36.3	46.4	12.8	99.3
Plasma total cotinine concentration (ng ml^{-1})								
Sampling month								
0	66	211.22	230.02	100.36	205.35	254.69	38.90	549.82
2	64	226.94	239.41	113.86	210.97	267.85	39.43	680.88
4	62	223.35	235.93	110.46	207.88	263.98	44.17	669.85
6	59	222.35	230.56	112.06	201.35	259.76	32.61	581.20
8	59	238.26	233.10	100.85	206.82	259.38	42.50	508.89
10	58	215.24	236.73	108.69	208.15	265.31	8.00	493.51
12	57	234.93	240.79	100.82	214.03	267.54	42.17	482.43
Plasma PheT concentration (fmol ml^{-1})								
Sampling month								
0	66	78.8	109.5	114.8	81.3	137.8	15.4	830.9
2	64	75.9	109.8	130.5	77.2	142.4	19.3	956.8
4	62	74.9	131.5	199.7	80.8	182.2	17.6	1095.2
6	59	74.9	99.1	117.2	68.5	129.6	13.4	885.0
8	59	70.1	96.6	131.3	62.4	130.8	12.9	971.0
10	57	64.2	88.6	63.2	71.9	105.4	26.3	344.1
12	57	77.4	170.3	304.5	89.5	251.1	26.1	2066.5
Urine total NNAL concentration (pmol mg^{-1} creatinine)								
Sampling month								
0	66	1.17	1.39	0.92	1.17	1.62	0.39	5.51
2	62	1.11	1.43	0.96	1.18	1.67	0.44	5.34
4	62	1.17	1.49	1.01	1.23	1.75	0.30	5.80
6	57	1.14	1.38	1.04	1.10	1.65	0.24	5.27
8	57	1.16	1.44	1.18	1.13	1.76	0.18	6.74
10	57	1.23	1.62	1.70	1.17	2.07	0.24	12.06
12	55	1.08	1.57	1.85	1.07	2.07	0.37	13.05
Urine total cotinine concentration (ng mg^{-1} creatinine)								
Sampling month								
0	67	3422	4099	2445	3503	4696	753	17625
2	64	3526	4265	2957	3526	5004	659	17823
4	63	3453	4266	2819	3556	4976	650	13114
6	61	3504	4287	3037	3510	5065	583	17181
8	60	3776	3826	2280	3237	4415	611	13562
10	59	3504	3883	2473	3239	4528	22	14159
12	59	2948	3537	2426	2905	4170	422	12458
Urine total nicotine per concentration (ng mg^{-1} creatinine)								
Sampling month								
0	67	1889	2387	2407	1800	2974	310	17460
2	64	1777	2345	2783	1650	3040	320	21448
4	63	1905	2849	2899	2119	3579	257	14917
6	61	1706	2459	2524	1813	3106	118	14105
8	60	2108	2639	2800	1916	3363	186	19392
10	59	1557	2374	3092	1568	3180	15	20844
12	59	1432	2120	2140	1562	2678	429	13500

LCL, lower confidence limit; UCL, upper confidence limit.

Table 3. Average coefficient of variation (CV) within subjects and within- and between-subject variance and intraclass correlation coefficient for log-transformed biomarkers.

Biomarker in log scale	Average CV within subjects (CV × 100%)	Between subject variance (σ_b^2)	Within subject variance (σ_w^2)	Intraclass correlation coefficient, ($\rho_I \times 100\%$)
Plasma biomarkers				
Plasma total NNAL (ln(fmol ml^{-1}))	29%	0.257	0.111	70%
Plasma total cotinine (ln(ng ml^{-1}))	21%	0.200	0.088	69%
Plasma PheT (ln(fmol ml^{-1}))	47%	0.332	0.261	56%
Urinary biomarkers				
Urine total NNAL (ln(pmol mg^{-1} creat))	30%	0.330	0.105	76%
Urine total cotinine (ln(ng mg^{-1} creat))	36%	0.271	0.195	58%
Urine total nicotine (ln(ng mg^{-1} creat))	60%	0.294	0.448	40%

NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; creat, creatinine.

from those studies, by taking into account the inherent variability of the measures.

One example is our recent study that measured the association of total serum NNAL and serum PheT with lung cancer in a nested case–control study (Church et al. 2009). The estimated odds ratio (OR) for a 1 SD increase in total NNAL was 1.57. Applying a calibration method for measurement error (Armstrong 1990) and assuming that ρ_I for plasma total NNAL and for serum total NNAL are the same, the corrected OR would be 2.24, a 42% increase in the effect size. Although the statistical significance of the estimate is unchanged, this corrected point estimate and confidence interval are less biased, given the assumptions of the model.

The levels of biomarkers reported in this study are quite consistent with results in the literature. Our mean value for plasma total cotinine of 235 ± 106 ng ml^{-1} compares favourably with levels of 200–300 ng ml^{-1} reported in numerous studies (Lee 1999). Our mean values of total cotinine (4030 ± 2646 ng mg^{-1} creatinine) and total nicotine (2455 ± 2669 ng mg^{-1} creatinine) in urine are also consistent with typical levels in the literature (Davis & Curvall 1999, Hecht et al. 2007). Our mean level of total NNAL (1.47 ± 1.26 pmol mg^{-1} creatinine) is somewhat lower than our previously reported mean from 420 smokers of 2.82 ± 1.99 pmol mg^{-1} creatinine (Hecht et al. 2007), but is quite consistent with total NNAL data from a number of recent studies (Melikian 2007, Carmella et al. 2009, Roethig et al. 2009, Scherer et al. 2007, Mendes 2008, Lowe 2009, Sarkar 2008, Kavvadias et al. 2009). Levels of PheT and total NNAL in plasma have been reported only in two previous small studies and are similar to the mean levels of 115 ± 167 and 38 ± 25 fmol ml^{-1}, respectively, observed here (Carmella et al. 2005, 2006).

The study has potential limitations. These subjects were not randomly selected and so cannot be said to represent statistically the entire population of smokers. However, the large spectrum of ages and smoking intensities and the inclusion of both men and women ensure that the results include representatives of most of the smoking population. In addition, the small number of missing data points may not have been at random, so some small bias may be represented by those missing data. However, a comparison of the results of complete datasets to the partial sets gave no indication of detectable bias. Furthermore, the distributions observed in this study sample are consistent with those observed in prior studies, as discussed above. Total nicotine equivalents is considered a superior biomarker to cotinine because it captures 73–96% of the nicotine dose (Hukkanen et al. 2005), but the overall longitudinal stability of cotinine in this study was excellent. Another limitation to this study was the collection of first morning urine samples rather than the more desirable 24-h samples. This was done to maximize subject participation and compliance. Finally, note that these results only apply to smokers, not necessarily to other populations exposed to cigarette smoke.

In summary, the plasma biomarkers total NNAL, total cotinine and PheT can be estimated reliably with a single measurement provided well-established and validated laboratory methods are used. The urinary biomarkers total NNAL and total cotinine can also be reliably estimated with a single measurement after correcting for creatinine concentration. Studies employing single measurements of these biomarkers can, therefore, produce useful estimates of association with other outcomes, including cancer risk.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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