

Combined Analysis of *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-Tetrahydrophenanthrene and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol in Smokers' Plasma

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Abstract

Polycyclic aromatic hydrocarbons (PAH) and tobacco-specific nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), are widely accepted to be two important types of lung carcinogens in cigarette smoke. In this study, we have developed a method to estimate individual uptake of these compounds by quantifying *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in 1 mL of smokers' plasma. PheT and NNAL are biomarkers of PAH and NNK uptake, respectively. [D_{10}]PheT and [pyridine- D_4]NNAL were added to plasma as internal standards. The plasma was treated with β -glucuronidase to release any conjugated PheT and NNAL. The analytes were enriched by solid-phase extraction on a

mixed mode cation exchange cartridge and the PheT fraction was further purified by high-performance liquid chromatography. The appropriate fractions were analyzed by gas chromatography-negative ion chemical ionization-mass spectrometry for PheT and liquid chromatography-electrospray ionization-mass spectrometry for NNAL. The method was sensitive (limits of quantitation: PheT, 13 fmol/mL; NNAL, 3 fmol/mL), accurate, and precise. Levels of PheT and NNAL in plasma from 16 smokers averaged 95 ± 71 and 36 ± 21 fmol/mL, respectively, which are ~1% to 2% of the amounts found in urine. This method should be useful in molecular epidemiology studies of carcinogen uptake and lung cancer in smokers. (Cancer Epidemiol Biomarkers Prev 2006;15(8):1490-4)

Introduction

Lung cancer is the leading cause of cancer death in the world (1). Cigarette smoking causes ~90% of lung cancer (2). Two types of carcinogens that are likely to cause lung cancer in smokers are polycyclic aromatic hydrocarbons (PAH), typified by benzo[*a*]pyrene, and tobacco-specific nitrosamines, typified by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; refs. 3, 4). Our working hypothesis is that the dose of these carcinogens to which smokers are exposed will be related to lung cancer risk, but carcinogen dose is not easily estimated (5). This hypothesis could be tested in nested case control studies using stored plasma samples if appropriate biomarkers and methods for their analysis were available.

PAH require metabolic activation to exert their carcinogenic effects (6, 7). One pathway of metabolic activation proceeds through diol epoxides, which bind to DNA and cause mutations in critical genes (8, 9). However, most of the diol epoxide reacts with water to form tetraols. Thus, tetraols are an indicator of PAH metabolic activation. A practical biomarker for PAH metabolic activation by this pathway is *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), the tetraol resulting from the metabolic activation of phenanthrene by the diol epoxide pathway (10). PheT is a good biomarker of uptake and metabolic activation of phenanthrene, a typical PAH, and the simplest PAH with a bay region, a feature closely associated with carcinogenicity. In previous studies, we have measured PheT in the urine of smokers and nonsmokers (10, 11). However, plasma and

serum are more frequently used than urine in molecular epidemiology studies. Therefore, in this study, we developed a method for quantitation of PheT in smokers' plasma.

The sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides is a useful biomarker of NNK uptake (12). Previous studies have applied this biomarker to estimate lung carcinogen exposure in smokers, smokeless tobacco users, and nonsmokers exposed to environmental tobacco smoke (12). Most of these studies used urine for the analysis, but a method for analysis of NNAL in plasma has also been reported (13). In this study, we report the quantitation of both PheT and NNAL in the same 1-mL plasma sample. This method should be useful for estimating lung carcinogen dose in molecular epidemiology studies of smokers and cancer.

Materials and Methods

Apparatus. For the isolation of the fraction containing PheT, high-performance liquid chromatography (HPLC) was carried out on a Waters Corporation system (Milford, MA) in tandem with a Hewlett Packard 1100 Series autosampler (Agilent, Wilmington, DE). A 150×4.6 -mm ProSphere 300 C18 5- μ m column was used in conjunction with a 7.5×4.6 -mm ProSphere 300 C18 5- μ m guard column (Alltech, Deerfield, IL). The column was eluted with an acetonitrile/1% aqueous formic acid gradient at 1.0 mL/min as follows (percentage aqueous formic acid, time): 95% to 90%, 0 to 20 minutes; 90% to 0%, 20 to 26 minutes; 0%, 26 to 31 minutes. The column temperature was maintained at 30°C and the system was equipped with a Shimadzu SPD-10A UV/Vis detector (Columbia, MD) monitored at 254 nm. Desired eluting fractions were collected with an ISCO Foxy, Jr. (Lincoln, NE) fraction collector.

Gas chromatography-negative ion chemical ionization-mass spectrometry (GC-NICI-MS) for the detection of PheT was carried out with a Hewlett Packard model 5973 system

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(Agilent) using a 0.25 mm (inside diameter) \times 0.15 μ m film thickness \times 30 m DB17-MS column (Agilent) with a 0.53 mm (inside diameter) \times 3 m deactivated fused silica precolumn. The injection port and MS transfer line were kept at 270°C and 320°C, respectively. The oven temperature program was 80°C for 1 minute, then 80 to 190°C at 30°C/min, then 190 to 210°C at 3°C/min, then 210 to 320°C at 30°C/min, then hold for 2 minutes. The carrier gas was He at a flow rate of 1.0 mL/min. The NICI-MS conditions were as follows: collision gas, methane; source temperature, 150°C; quadrupole temperature, 150°C; electron multiplier, 2,000 V; emission current, 49.4 μ A; and electron energy, 235 eV. Selected ion monitoring was used to detect PheT, [D₁₀]PheT, and BaP-T at *m/z* 372, *m/z* 382, and *m/z* 446, respectively.

Analysis of the NNAL fraction by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) was carried out on a Finnigan TSQ Quantum Discovery Max instrument (Thermo Electron Corp., Waltham, MA) interfaced with an Agilent model 1100 capillary HPLC system and a model 1100 micro autosampler (Agilent, Palo Alto, CA). The HPLC system used a 150 \times 0.5 mm (inner diameter) Luna C18(2) 3- μ m column coupled to a Krud-Katcher disposable precolumn (Phenomenex, Torrance, CA). The column was eluted with a methanol/H₂O gradient at a flow rate of 10 μ L/min as follows (percentage H₂O, time): 98% to 58%, 0 to 10 minutes; 58%, 10 to 20 minutes; 58% to 0%, 20 to 22 minutes; 0%, 22 to 28 minutes; 0% to 98%, 28 to 30 minutes. The column was maintained at 25°C. MS/MS variables were as follows: positive ion electrospray mode with selected reaction monitoring for *m/z* 210 \rightarrow 180 for NNAL, *m/z* 214 \rightarrow 184 for [pyridine-D₄]NNAL, and *m/z* 216 \rightarrow 186 for [¹³C₆]NNAL, at 0.05 amu scan width. Argon was the collision gas with a pressure of 1 mTorr, with the collision energy at 12 eV. Quadrupoles 1 and 3 were operated at resolutions of 0.2 and 0.7 amu, respectively. The ion transfer tube was at 250°C, the spray voltage was 2,500 V, and the sheath gas was N₂ at 30 units. The instrument was tuned using [¹³C₆]NNAL infused in 10% methanol in H₂O. The autosampler needle was washed with 80:20:1 acetonitrile/methanol/formic acid between injections.

Chemicals and Enzymes. NNAL was obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). [Pyridine-D₄]NNAL and PheT were synthesized as previously described (10, 13). [¹³C₆]NNAL was prepared by NaBH₄ reduction of [¹³C₆]NNK (Cambridge Isotope Laboratories, Andover, MA). *r*-7,*t*-8,9,*c*-10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BaP-T) was obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository. *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-[D₁₀]1,2,3,4-tetrahydrophenanthrene ([D₁₀]PheT) was prepared by *in vitro* metabolism of [D₁₀]phenanthrene (Cambridge Isotope Laboratories). Briefly, [D₁₀]phenanthrene was incubated with human cytochrome P450 1A1 and cofactors to produce a mixture of diols and phenols, which was separated by HPLC. [D₁₀]Phe-1,2-diol was isolated by HPLC, oxidized to the diol epoxide by treatment with *m*-Cl-peroxybenzoic acid, and the diol epoxide hydrolyzed at pH 7.0 to [D₁₀]PheT, which was purified by HPLC. 2,7-Dihydroxynaphthalene was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO).

β -Glucuronidase type IX-A from *Escherichia coli* was purchased from Sigma-Aldrich. This enzyme was more effective than that from *Helix pomatia* for deconjugation of NNAL; both work well for PheT (14). Oasis MCX mixed mode cation exchange solid-phase extraction cartridges (60 mg) were purchased from Waters. *bis*-Trimethylsilyltrifluoroacetamide plus 1% trimethylchlorosilane (BSTFA-TMCS) was purchased from Regis Technologies, Inc. (Morton Grove, IL).

Subjects. Pooled plasma from 10 smokers was purchased from Biochemed Pharmacologicals (Winchester, VA). Individ-

ual smokers' plasma samples were obtained in EDTA-containing tubes from an ongoing study of longitudinal stability of tobacco carcinogen biomarkers, approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee.

Analysis of NNAL and PheT in Plasma. Plasma (1 mL) was placed in a 10-mL glass conical tube with a Teflon-lined cap. Three milliliters of saline were added to the sample and the pH was adjusted to 6 to 7 if necessary. [Pyridine-D₄]NNAL [10 μ L of 1 pg (4.7 fmol)/ μ L 10% acetonitrile in H₂O] and [D₁₀]PheT [5 μ L of 5 pg (19.5 fmol)/ μ L acetonitrile] were added to the sample as internal standards. β -Glucuronidase (12,000 units in 0.4 mL H₂O) was added and the mixture was incubated at 37°C overnight with shaking. The sample was brought to pH 2 to 3 with 1 N HCl. The mixed mode cation exchange cartridges were placed in a 16-port vacuum manifold and conditioned with 5-mL methanol and 10-mL H₂O. The samples were slowly added to the column and eluted with 5-mL 1 N HCl and 5-mL 1% methanol in H₂O, which were discarded. The PheT-containing fraction was eluted with 5-mL 40% methanol in H₂O and collected in a 10-mL conical tube. The column was further eluted with 5-mL methanol and 5-mL 90:5:5 H₂O/methanol/NH₄OH, which were discarded. The NNAL-containing fraction was eluted from the column with 30:65:5 H₂O/methanol/NH₄OH and collected in a 10-mL glass conical tube. Both of the collected fractions were concentrated to dryness with a Speedvac (Thermo Savant, Milford, MA).

The residue from the PheT-containing fraction was transferred using three 80- μ L aliquots of 5:1 H₂O/methanol to a 0.45- μ m nylon filter HPLC vial (Whatman, Clifton, NJ). To the vial was added 30 μ g of 2,7-dihydroxynaphthalene as an HPLC retention time marker (20.0 minutes). HPLC eluant was collected from 7.5 to 12 minutes. Retention time for PheT and [D₁₀]PheT was 8.8 minutes. The collected HPLC fraction was concentrated to dryness on the Speedvac. The residue was then transferred into a 1.8-mL glass autosampler vial with a 0.2-mL glass insert (SunSRI, Rockwood, TN) by three 60- μ L aliquots of methanol. The solution was concentrated to dryness again. To the residue was added BaP-T (2 μ L of 100 fmol/ μ L acetonitrile) as an injection standard and 12 μ L of BSTFA-TMCS. The mixture was then heated at 60°C for 60 minutes with occasional mixing. Four microliters of the mixture were injected on GC-NICI-MS using the splitless mode.

The residue from the NNAL-containing fraction was reconstituted in 0.5-mL 15 mmol/L ammonium acetate. The solution was extracted with three 1-mL portions of methylene chloride. The methylene chloride layers were combined in a 10-mL glass conical tube and concentrated to dryness. The residue was transferred to 250- μ L polypropylene autosampler vials (Waters) with two 100- μ L portions of methanol and concentrated to dryness again. The sample was reconstituted in 10 μ L of 2% methanol in 15 mmol/L ammonium acetate containing 31 fmol [¹³C₆]NNAL. Four microliters of the mixture were injected on LC-ESI-MS/MS.

The results are expressed as femtomoles of PheT or NNAL per milliliter of plasma. PheT refers to the total of PheT and PheT glucuronides and NNAL refers to the total of NNAL and its glucuronides.

Results

The combined analytic method is outlined in Fig. 1. The internal standards for this analysis were [pyridine-D₄]NNAL and [D₁₀]PheT. They were added and the mixture was incubated with β -glucuronidase to catalyze hydrolysis of NNAL glucuronides (~5% of total) and PheT glucuronides (~15% of total). A mixed mode cation exchange solid-phase extraction cartridge was used to enrich PheT and NNAL from

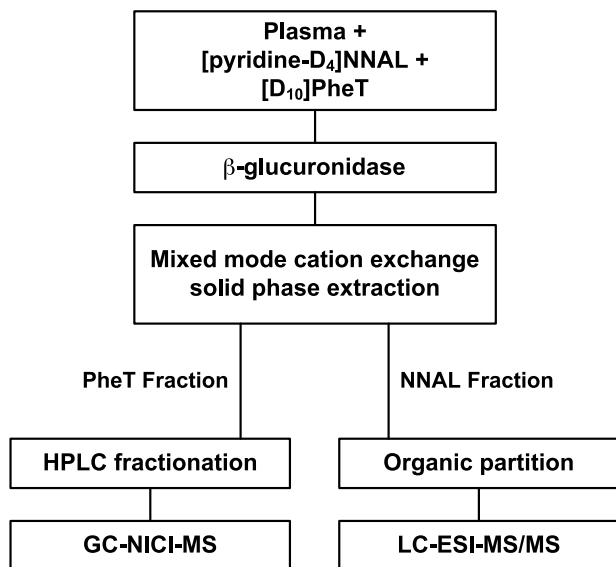


Figure 1. Analytic scheme for PheT and NNAL in human plasma.

other plasma constituents. The eluents containing PheT were further enriched by HPLC. The samples were derivatized with BSTFA-TMCS to produce PheT-tetratrimethylsilyl ethers, and analyzed by GC-NICI-MS, monitoring m/z 372 $[M - (OSi(CH_3)_3 + Si(CH_3)_3)]^-$. A typical chromatogram for the PheT analysis is shown in Fig. 2. The internal standard ($[D_{10}]PheT$) eluted 0.04 minute before PheT. The eluents containing NNAL were directly analyzed by LC-ESI-MS/MS without further enrichment. The transition m/z 210 $[M + H]^+ \rightarrow 180 [(M + H) - NO]^+$ was monitored. A typical chromatogram is shown in Fig. 3. The internal standard eluted 0.1 minute before NNAL.

The on-column detection limit for standard PheT was 0.2 fmol (signal/noise = 3). The limit of quantitation of the

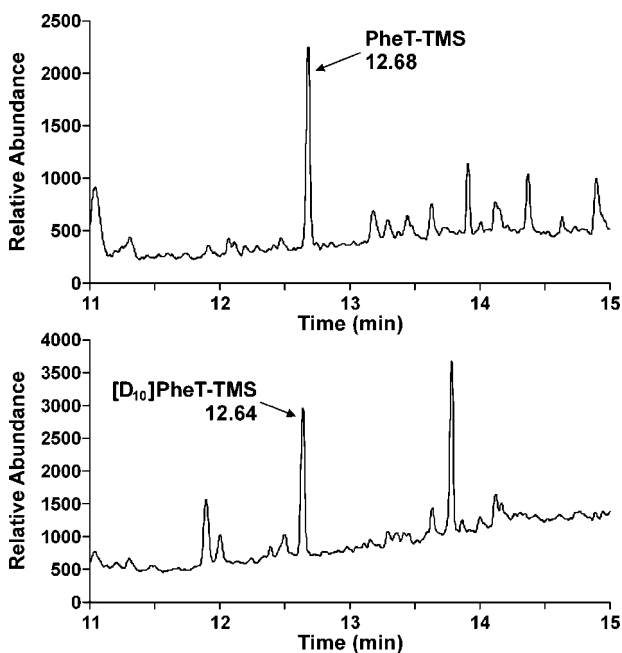


Figure 2. Chromatograms obtained by GC-NICI-MS of PheT in a smoker's plasma. Selected ion monitoring of m/z 372 for PheT-tetratrimethylsilyl ethers (*top*; PheT-TMS) and m/z 382 for $[D_{10}]PheT$ -tetratrimethylsilyl ethers, internal standard (*bottom*).

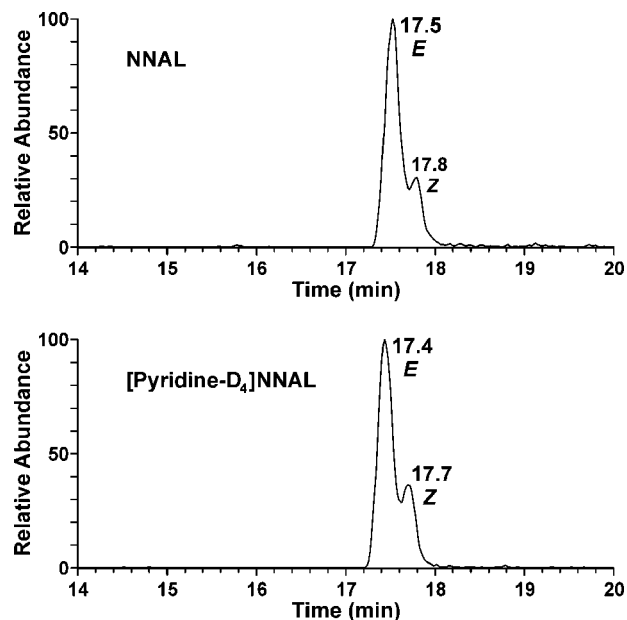


Figure 3. Chromatograms obtained by LC-ESI-MS/MS of NNAL in a smoker's plasma. Selected reaction monitoring of m/z 210 \rightarrow 180 for NNAL (*top*) and m/z 214 \rightarrow 184 for $[pyridine-D_4]NNAL$, internal standard (*bottom*). Peaks with numbers indicating retention times are the E (major) and Z (minor) rotamers of NNAL and $[pyridine-D_4]NNAL$.

assay was ~ 13 fmol PheT/mL plasma. The intraday precision of the assay was determined by analyzing six aliquots of pooled smokers' plasma. The results were 47 ± 3.1 fmol PheT/mL (relative SD, 6.5%). The interday precision based on analyses of the pooled smokers' plasma (2-6 per set as positive controls in three sets of assays) had a relative SD of 13.7%. Assay accuracy was determined by spiking pooled smokers' plasma, which contained 47 fmol/mL PheT, with 50, 100, 150, and 200 fmol/mL PheT. Analysis produced the results illustrated in Fig. 4 ($r = 0.97$; y intercept = 54 fmol/mL). PheT recovery was $26 \pm 11\%$ ($N = 53$).

The detection limit for standard NNAL injected on the LC-ESI-MS/MS system was 0.1 fmol (signal/noise = 3). The limit of quantitation of the assay was ~ 3 fmol NNAL/mL plasma. The intraday precision of the assay was determined by analyzing six aliquots of pooled smokers' plasma. The results were 30 ± 2.3 fmol NNAL/mL (relative SD, 7.7%). The interday precision based on analyses of the pooled smokers' plasma (2-6 per set as positive controls in three sets of assays) had a relative SD of 11.7%. Assay accuracy was determined by spiking pooled smokers' plasma, which contained 30 fmol/mL

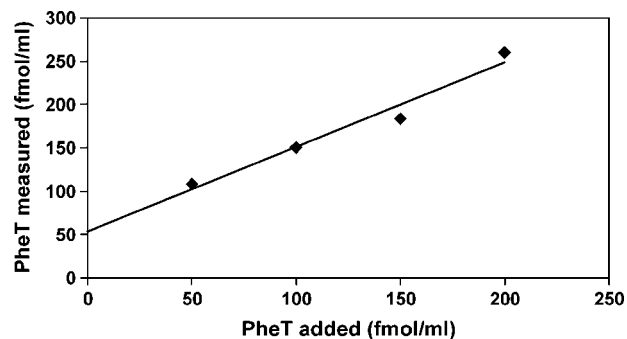


Figure 4. Relationship between levels of PheT added to pooled smokers' plasma and measured amounts ($r = 0.97$). Points, mean of duplicate determinations.

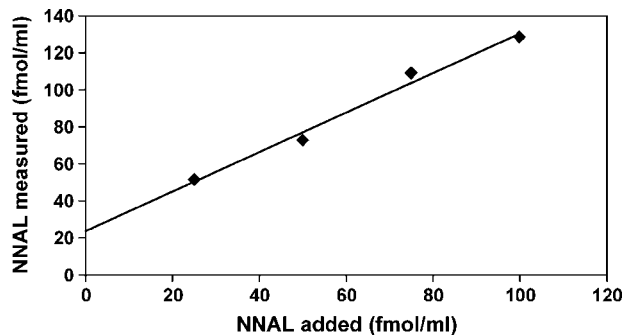


Figure 5. Relationship between levels of NNAL added to pooled smokers' plasma and measured amounts ($r = 0.97$). Points, mean of duplicate determinations.

NNAL, with 25, 50, 75, and 100 fmol/mL NNAL. Analysis produced the results illustrated in Fig. 5 ($r = 0.99$; y intercept = 24 fmol/mL). NNAL recovery was $43 \pm 21\%$ ($N = 46$).

The assay was applied to plasma samples from 16 smokers. Data for PheT and NNAL in smokers' plasma are shown in Table 1. PheT levels ranged from 20 to 272 fmol/mL plasma with a mean of 95 ± 71 fmol/mL. NNAL levels ranged from 13 to 88 fmol/mL plasma with a mean of 36 ± 21 fmol/mL. There was no correlation between levels of PheT and NNAL.

Discussion

We have developed a method to quantify plasma biomarkers of two important classes of lung carcinogens in cigarette smoke: PAH and tobacco-specific nitrosamines. PheT and NNAL, representative biomarkers, were quantified by GC-NICI-MS and LC-ESI-MS/MS, respectively. Advantages of this method include low detection limits for both analytes, high specificity with respect to analyte structure due to the use of MS techniques, and excellent accuracy and precision. A further advantage is the detection of two diverse types of biomarkers in a single 1-mL plasma sample. The method involves analysis of two different fractions from the cation exchange cartridge, one enriched in PheT and the other in NNAL. The HPLC system used for the further enrichment of PheT is markedly improved over the one previously described (10). The NNAL part of this assay is quite similar to that which we reported earlier, and the results are also similar (13).

We have recently completed a study in which we analyzed PheT in the urine of 346 smokers.¹ The mean level was ~ 5.6 pmol/mL urine. The mean amount of PheT detected in plasma samples from smokers in this study was 0.095 pmol/mL, or $\sim 1.7\%$ of the amount found in urine. In a study of 354 smokers, the average amount of total NNAL (referred to as NNAL in this article) was 2.2 pmol/mg creatinine (5). Using a value of 1.3 mg creatinine/mL urine (15), the average amount of NNAL in smokers' urine was 2.9 pmol/mL. The mean amount of NNAL detected in plasma samples from smokers in this study was 0.036 pmol/mL, or $\sim 1.2\%$ of the amount found in urine. Both analytes were readily quantifiable at these levels.

Previous studies have shown that urinary PheT can be a biomarker of PAH uptake, as it varies predictably with exposure and correlates with levels of urinary 1-hydroxypyrene,

Table 1. PheT and NNAL in smokers' plasma

Subject no.	PheT (fmol/mL)	NNAL (fmol/mL)
1	71	25
2	87	88
3	75	13
4	44	24
5	80	38
6	52	18
7	85	60
8	174	38
9	65	54
10	225	16
11	90	41
12	39	52
13	119	16
14	25	40
15	272	35
16	20	13

NOTE: PheT and NNAL refer to the total of free plus glucuronidated forms.

an accepted biomarker of PAH uptake (10, 16). However, PheT has an additional attractive feature, as it also includes a metabolic activation pathway. Thus, PheT can be considered a biomarker of individual uptake *plus* metabolic activation of PAH. NNAL is a well-established biomarker of NNK uptake, but would not be considered as a measure of metabolic activation (12). The metabolic activation of NNK proceeds by α -carbon hydroxylation, producing urinary hydroxy and keto acids identical to metabolites of nicotine, thus precluding their use of specific biomarkers of NNK metabolic activation (17, 18). Thus, PheT and NNAL are somewhat different types of end points and both are related to carcinogen exposure, but they result from different types of metabolic pathways, which probably contributes to their lack of correlation in plasma.

This study has some limitations. First, little is known about the kinetics of NNAL and PheT in human plasma after a cigarette is smoked. Levels of these analytes in plasma will most likely be affected by time since the last cigarette. This requires further study. Second, there are many other metabolites of NNK and phenanthrene that were not measured in this study. Further research is needed to develop a more complete metabolic profile of these compounds in humans. Third, PheT is not a tobacco-specific biomarker, unlike NNAL. Diet and environmental exposures to phenanthrene will also contribute to PheT levels. PheT is found in the urine of both smokers and nonsmokers, although the levels are higher in smokers (10). PheT is also found in the plasma of nonsmokers, unlike NNAL (13).² The lack of correlation between levels of PheT and NNAL is probably due, in part, to the nonspecificity of phenanthrene to cigarette smoke, as well as to metabolic differences noted above.

In summary, we have developed what promises to be a useful method to assess individual uptake of PAH and NNK, important lung carcinogens in cigarette smoke. We plan to apply this method in molecular epidemiology studies investigating the relationship between carcinogen dose and lung cancer in smokers.

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References

1. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
2. International Agency for Research on Cancer. Tobacco smoke and involuntary smoking. IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 83. Lyon (France): IARC; 2004. p. 53–119.
3. Hecht SS. Tobacco carcinogens, their biomarkers, and tobacco-induced cancer. *Nat Rev Cancer* 2003;3:733–44.

¹ S.S. Hecht, S.G. Carmella, A. Yoder, M. Chen, Z. Li, C. Le, R. Dayton, J. Jensen, and D.K. Hatsukami. Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. *Cancer Epidemiol Biomarkers & Prev* 2006; submitted for publication.

² Unpublished data.

4. Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999;91:1194–210.
5. Joseph AM, Hecht SS, Murphy SE, et al. Relationships between cigarette consumption and biomarkers of tobacco toxin exposure. *Cancer Epidemiol Biomarkers Prev* 2005;14:2963–8.
6. Cooper CS, Grover PL, Sims P. The metabolism and activation of benzo[*a*]pyrene. *Prog Drug Metab* 1983;7:295–396.
7. Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. *Cancer Res* 1982;42:4875–917.
8. Szeliga J, Dipple A. DNA adduct formation by polycyclic aromatic hydrocarbon dihydrodiol epoxides. *Chem Res Toxicol* 1998;11:1–11.
9. Pfeifer GP, Denissenko MF, Olivier M, et al. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* 2002;21:7435–51.
10. Hecht SS, Chen M, Yagi H, et al. *r*-1,*t*-2,3,*c*-4-Tetrahydroxy-1,2,3,4-tetrahydrophenanthrene in human urine: a potential biomarker for assessing polycyclic aromatic hydrocarbon metabolic activation. *Cancer Epidemiol Biomarkers Prev* 2003;12:1501–8.
11. Hecht SS, Chen M, Yoder A, et al. Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. *Cancer Epidemiol Biomarkers Prev* 2005;14:2969–74.
12. Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis* 2002;23:907–22.
13. Carmella SG, Han S, Villalta PW, et al. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in smokers' blood. *Cancer Epidemiol Biomarkers Prev* 2005;14:2669–72.
14. Carmella SG, Akerkar S, Richie JP, Jr., et al. Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers' urine. *Cancer Epidemiol Biomarkers Prev* 1995;4:635–42.
15. Barr DB, Wilder LC, Caudill SP, et al. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect* 2005;113:192–200.
16. Kim JY, Hecht SS, Mukherjee S, et al. A urinary metabolite of phenanthrene as a biomarker of polycyclic aromatic hydrocarbon metabolic activation in workers exposed to residual oil fly ash. *Cancer Epidemiol Biomarkers Prev* 2005;14:687–92.
17. Hecht SS. Biochemistry, biology, and carcinogenicity of tobacco-specific *N*-nitrosamines. *Chem Res Toxicol* 1998;11:559–603.
18. Hecht SS, Hatsukami DK, Bonilla LE, et al. Quantitation of 4-oxo-4-(3-pyridyl)butanoic acid and enantiomers of 4-hydroxy-4-(3-pyridyl)butanoic acid in human urine: a substantial pathway of nicotine metabolism. *Chem Res Toxicol* 1999;12:172–9.

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