the case subjects reported initiating smoking prior to age 18. These women appeared to be early initiators with higher prevalence of characteristics associated with smoking; the effect of their inclusion would be to inflate the calculated odds ratios.

In summary, it appears that many factors influencing young adult women to begin smoking are similar to those that influence adolescents. Peers, particularly significant others, appear to be an especially important influence. Prevention efforts should target women of lower educational attainment, using approaches that reduce the acceptability of smoking in the social environment. Further research is needed to develop salient messages for young women at risk of becoming regular smokers. □

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ABSTRACT

Cotinine levels in the semen, urine, and blood of 88 male smokers and nonsmokers, aged 18 to 35, were analyzed via radioimmunoassay. Detectable cotinine levels were found in all three body fluids, and cotinine levels in all three fluids were highly correlated. Cotinine levels in semen and blood were of similar magnitude; cotinine levels in urine were an order of magnitude or more higher. In all three fluids, cotinine levels increased with an increase in cigarette smoke exposure. (*Am J Public Health.* 1993; 83:1335–1338)

Cotinine Concentrations in Semen, Urine, and Blood of Smokers and Nonsmokers

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Introduction

Cigarette smoking has been associated with various documented and suspected adverse reproductive outcomes, including reduced sperm density and motility and increased abnormal morphology.[1] The role of cigarette smoking with respect to these effects is unclear. The purpose of this study is (1) to document the presence of cotinine, a metabolite of nicotine and a marker of tobacco smoke exposure, in the semen of male smokers; (2) to correlate the amount of cigarette smoke exposure, as determined by questionnaire, with cotinine concentrations in

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Note. The views expressed here are the authors' and do not necessarily reflect those of the US Environmental Protection Agency.

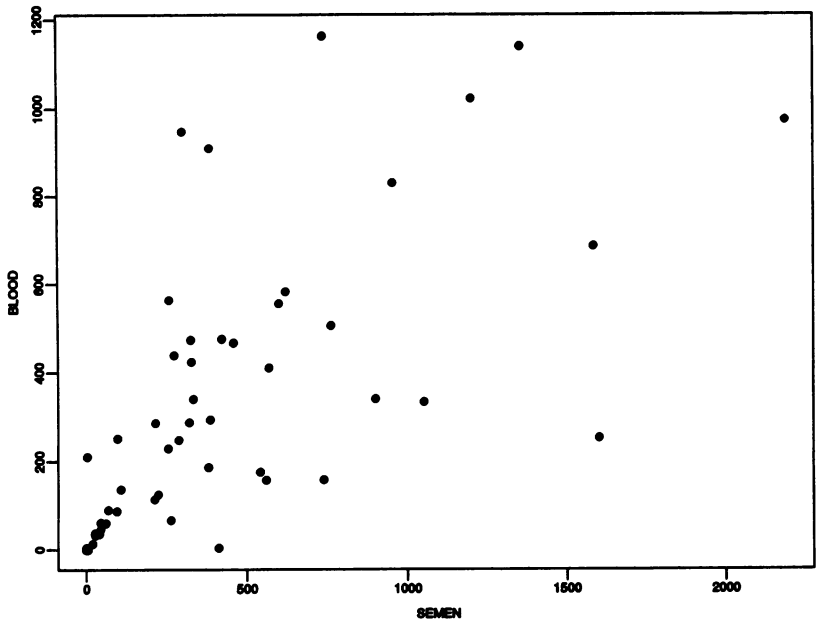
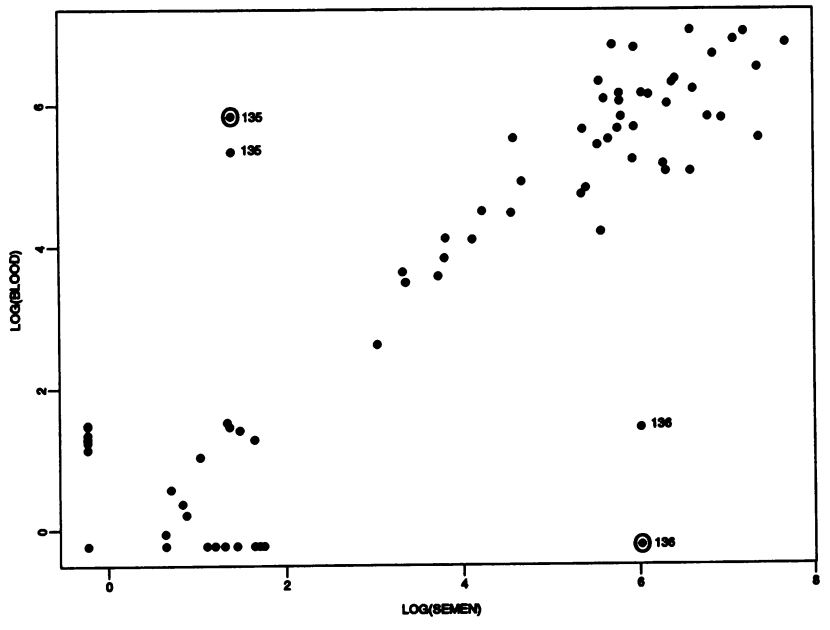


FIGURE 1—Scatter plot comparing the unlogged semen with the unlogged blood cotinine concentrations (ng/mL).



Note. Circles indicate specimens with repeated assays.

FIGURE 2—Scatter plot comparing the logged semen with the logged blood cotinine concentrations (ng/mL).

semen, urine, and blood; and (3) to correlate cotinine concentrations among the three body fluids to determine whether cotinine levels in one fluid can serve as a surrogate for levels in another. Cotinine, with a half-life in various body fluids of 16 to 19 hours,[2] indicates cigarette smoke exposure during the previous 2 to 3 days.

Methods

Study Participants

Participants eligible for inclusion in the study were healthy White men aged 18 to 35. Eighty-eight men (40 nonsmokers, 23 light smokers, and 25 heavy smokers) were recruited into the study via newspaper advertisements between March and October 1989. Participants provided semen, urine, and blood specimens (45 mL) and completed a self-administered questionnaire describing their smoking habits and demographic characteristics. Nonsmokers had not smoked 100 cigarettes in their lifetime. Light smokers currently averaged between 1 and 19 cigarettes per day; heavy smokers, 20 or more cigarettes per day. All smokers had smoked cigarettes for at least 1 year. Light smokers and nonsmokers were selected based on the age distribution of the heavy smokers (18 to 21, 22 to 25, 26 to 29, 30+ years).

Specimen Collection and Storage

Urine and semen specimens were collected into separate polypropylene containers at the Reproductive Endocrinology/Fertility Laboratory at the University of North Carolina School of Medicine. Semen specimens were collected via masturbation after an abstinence period of 3 to 5 days. Trained laboratory personnel drew blood specimens into clean, heparinized tubes via venipuncture. Blood, urine, and semen supernatants were stored at –70 °C for future analyses.

Cotinine Analyses

One-milliliter samples of blood, urine, and semen supernatant for each participant were analyzed for cotinine via radioimmunoassay.[3,4] The level of cross-reactivity of the cotinine antibody used in the assay with other nicotine metabolites, such as 3-hydroxycotinine, is believed to be essentially 0% in blood and about 2% in urine (H. Van Vunakis, personal communication, December 1992). There are no data with respect to seminal fluid. Misclassification that might result from cross-reactivity is likely to be more significant at lower concentrations of cotinine than at higher ones. The limit of detection of the radioimmunoassay for cotinine concentration was 0.8 ng/mL of fluid. For some samples, this includes a dilution factor. The laboratory technician performing the cotinine analyses was blind to smoking status and fluid type. Semen specimens from two participants were not available for analysis, one because of a laboratory accident (a nonsmoker) and one because the participant did not produce enough specimen (a light smoker). One-milliliter samples of urine from each participant were also analyzed for creatinine concentration.

Statistical Analyses

To assess the validity of the individual cotinine values, cotinine concentra-

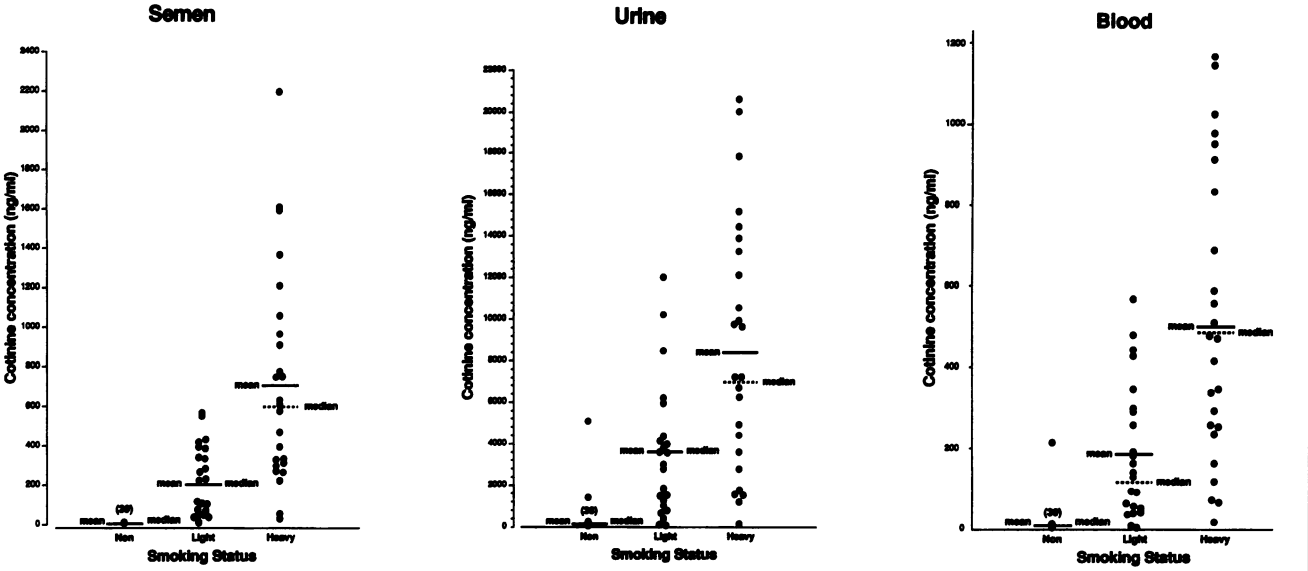


FIGURE 3—Distribution of cotinine concentrations (ng/mL), by smoking status and fluid: semen, urine, and blood.

tions for each fluid were plotted against cotinine concentrations for each of the other fluids. In Figure 1, this procedure is illustrated with the plot of semen versus blood cotinine concentrations. From the plots it can be seen that the response variance increased with an increase in mean cotinine concentration. Cotinine concentrations were then log transformed to produce a more nearly homogeneous variance. The fact that the distribution of cotinine concentrations among participants was skewed provided an additional reason to log the cotinine values.

When the logged cotinine concentrations for each fluid were plotted against the logged concentrations for each of the other fluids, a linear relationship was evident. The increase in homogeneity of variance for the comparisons of logged cotinine concentrations among the three fluids is illustrated in Figure 2 with the plot of semen versus blood. However, cotinine values for five individuals did not seem to fit this linear relationship. Laboratory assays on the specimens with the five seemingly aberrant values (three urine specimens and two blood specimens) were repeated, with the result that four of the five cotinine concentrations remained essentially the same. (Two of the aberrant data points appear in Figure 2.) Therefore, the original laboratory values for the cotinine concentrations for all individuals were retained in the analyses.

Median cotinine values were calculated by amount of smoke exposure. The median was selected as the summary statistic because it is less susceptible to the effects of outliers. Ninety-five percent confidence intervals for the median values were calculated according to Dixon and Massey.[5] Chi-square approximation tests were performed to determine whether differences in median cotinine concentrations by amount of smoke exposure were statistically significant. Pearson correlation coefficients were calculated to determine the correlation of cotinine concentrations among the three fluids. The value 0.8 was used in calculations for men whose cotinine concentrations were below the limits of detection of the assay. Analyses involving urine cotinine measurements were performed both with and without corrections for creatinine concentration to control for the effects of differential urine flow among participants.

Results

Non-, light, and heavy smokers were similar in age, with heavy smokers (mean age: 26.3 ± 4.8 years) being slightly older, on average, than light (23.2 ± 3.7 years) and nonsmokers (24.3 ± 4.6 years). Heavy smokers averaged 26.8 ± 8.0 cigarettes per day while light smokers averaged 10.8 ± 4.9 cigarettes per day.

Results of the cotinine analyses showed that, among the 48 smokers, detectable levels of cotinine were found in 100% of urine and semen specimens and in 98% of blood specimens. Cotinine levels were highest in the urine. For each individual, blood and semen cotinine levels were of similar magnitude; urine cotinine levels were an order of magnitude or more higher.

Figures 3a to c show the distribution of cotinine concentrations by the amount of smoke exposure in the three fluids. Despite the seemingly aberrant values, median cotinine concentrations in blood, urine, and semen increased with an increase in the amount of smoke exposure ($P < .0001$) (Table 1). Removal of the five aberrant values did not significantly affect median cotinine values.

The correlation of cotinine concentrations among the three body fluids for smokers was relatively high, ranging from .63 to .66 unlogged and from .67 to .78 logged (Table 2). Among nonsmokers, correlation coefficients comparing cotinine concentrations among the three fluids were not statistically significant. The correlation of cotinine concentrations with the number of cigarettes smoked per day among smokers was modest, with correlation coefficients approximately equal to .5 (Table 2).

The correlation coefficient for the log of the urine cotinine concentration compared with the log of the urine cotinine concentration divided by the creatinine concentration was .98. Because the correlation coefficient was so high, it was felt that analyses involving urine cotinine need not be corrected for creatinine concentration.

Discussion

Detectable levels of cotinine, a metabolite of nicotine, were found in the semen of healthy male smokers of reproductive age. Cotinine concentrations in semen were of similar magnitude to cotinine con-

TABLE 1—Median Cotinine Concentrations (ng/mL), by Fluid and Number of Cigarettes Smoked per Day

Fluid/Cigarettes Smoked per Day	No. Subjects	Median Cotinine Concentrations (ng/mL)	95% CI	P
Semen				
0 cigarettes	39[a]	0.8	0.8, 2.8	
1–19 cigarettes	22[b]	220.7	61.6, 381.6	
20+ cigarettes	25	602.3	322.4, 901.6	.0001
Urine				
0 cigarettes	40	17.0	13.8, 21.9	
1–19 cigarettes	23	3516.1	1438.6, 4308.1	
20+ cigarettes	25	7179.1	4369.6, 12073.6	.0001
Blood				
0 cigarettes	40	0.8	0.8, 1.8	
1–19 cigarettes	23	137.0	52.6, 287.1	
20+ cigarettes	25	467.6	253.5, 686.6	.0001

Note. Nondetectable values were set at 0.8 ng/mL.
[a]Specimen missing owing to laboratory accident.
[b]Specimen missing owing to insufficient specimen produced.

TABLE 2—Correlation Coefficients for Logged and Unlogged Cotinine Concentrations, by Body Fluid and Number of Cigarettes Smoked per Day (Smokers Only)[a]

	Semen Cotinine		Urine Cotinine		Blood Cotinine	
	Unlogged	Logged	Unlogged	Logged	Unlogged	Logged
Semen, ng/mL	1	1				
Urine, ng/mL	.66 (.0001)	.67 (.0001)	1	1		
Blood, ng/mL	.63 (.0001)	.78 (.0001)	.65 (.0001)	.72 (.0001)	1	1
No. cigarettes per day	.53 (.0001)	.53 (.0001)	.53 (.0001)	.40 (.0044)	.57 (.0001)	.47 (.0008)

Note. Row values are logged or unlogged as the column indicates; *P* values are indicated in parentheses.
[a]Smokers only (n = 48).

centrations in blood. Concentrations in urine were an order of magnitude or more higher than those in semen or blood. A statistically significant increase in median cotinine concentrations with an increase in amount of smoke exposure was noted in all three body fluids. The correlation of cotinine concentrations among the three fluids was high, with logged cotinine values showing greater predictive value than unlogged values. Cotinine concentrations increased with an increase in the number of cigarettes smoked per day, although the correlation between logged cotinine concentrations in the three fluids and the number of cigarettes smoked per day among smokers was modest (r = .40 to .53).

We are not aware of other documentation of the presence of cotinine in the semen of men exposed to tobacco smoke. Earlier studies have reported elevated cotinine concentrations in urine as compared with blood.[6,7]

When cotinine concentrations among the three fluids were compared for each individual, five seemingly aberrant values (three urine specimens and two blood specimens) were noted. Repeated analyses confirmed the cotinine concentrations in these specimens. The reason for the aberrant values is unknown, and conclusions reached in this manuscript are not altered by their presence or absence.

Logged cotinine concentrations were used in these analyses to produce a measure of concentration that was less skewed and had a more nearly homogeneous variance. There is some precedent in the literature for using such values. Thompson et al.[8] recommended using logged cotinine concentrations in their study correlating blood and urine cotinine concentrations in order to make the distributions of cotinine concentrations in the two fluids more symmetrical.

Cotinine in this investigation serves as a marker of tobacco smoke exposure. The high concordance in cotinine concentrations across the three body fluids supports the use of any of these fluids as a surrogate for cigarette smoke exposure in the other fluids.

Smoking has been associated with decreased sperm density and motility and increased abnormal sperm morphology.[1] How cigarette smoke affects semen quality is unclear. Although cotinine is not known to be mutagenic, further research is needed to determine whether potentially harmful substances in tobacco smoke reach the semen and have adverse effects on sperm. □

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