Relationships between Cigarette Consumption and Biomarkers of Tobacco Toxin Exposure

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Abstract

Epidemiologic studies show a dose-response relationship between cigarettes per day and health outcomes such as heart and lung disease, and health outcomes are related to some biomarkers of tobacco exposure. The objective of this study was to examine the relationships between cigarettes per day and levels of selected biomarkers of tobacco toxin exposure: carbon monoxide (CO), metabolites of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and polycyclic aromatic hydrocarbons [total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 1-hydroxypyrene (1-HOP), respectively], and total cotinine (cotinine plus cotinine-*N*-glucuronide). We did a cross-sectional analysis of merged data from (*a*) two clinical trials and (*b*) two cohorts of light smokers (total *n* = 400). The mean age of participants was 50.4 years and the range of cigarette consumption was 1 to 100/d; however, few subjects smoked >45 cigarettes/d (*n* = 12). Results show that levels of the biomarkers CO, total NNAL, and total cotinine increase with an increase in the number of cigarettes smoked per day, but not in a linear fashion. 1-HOP is a less discriminating biomarker as levels are relatively stable regardless of the number of cigarettes smoked per day. There is considerable variability in toxin measurement, especially at high levels of smoking. There was a significant correlation between cigarettes per day and total NNAL, 1-HOP, total cotinine, and CO. Total NNAL was highly significantly correlated with total cotinine and CO and also significantly correlated with 1-HOP. These findings suggest that the number of cigarettes smoked per day is not necessarily a reliable measure of toxin exposure and may underestimate tobacco toxin exposure at low levels of smoking or overestimate exposure at high levels of smoking. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2963–8)

Introduction

Epidemiologic studies have established a strong dose-response relationship between the amount and duration of smoking and various health effects, such as lung cancer and heart disease (1). These data show that those who smoke fewer cigarettes per day have a lower risk of disease than those who smoke a higher number of cigarettes per day, but that there is no ''safe'' level of smoking.

Biomarkers of tobacco toxin exposure are also related to heart disease (2, 3) and lung cancer (although less reliably; ref. 4), which make them attractive surrogate end points to evaluate the effectiveness of smoking reduction interventions at improving health. For example, the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and polycyclic aromatic hydrocarbons (PAH) present in tobacco smoke are believed to be the major causative agents for lung cancer in smokers (5-7). NNK metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), its glucuronide (NNAL-Gluc), and a PAH metabolite, 1-hydroxypyrene (1-HOP), are biomarkers of carcinogen exposure (8, 9). 1-HOP is the major metabolite of the abundant but noncarcinogenic PAH pyrene, which is always present in mixtures of PAH (10). 1-HOP is not tobacco specific, unlike total NNAL.

Because biomarkers and health outcomes are related and the amount of smoking and health outcomes are related, it is conceptually sound to hypothesize that there is a consistent relationship between the amount of smoking and biomarkers. It is not clear, however, whether there is a linear (or other) relationship between cigarette consumption and toxin exposure or whether it is necessary to reduce cigarette consumption below a specific threshold to accomplish exposure reduction.

This cross-sectional study merged the relevant data from four projects to investigate the relationships between behavioral measures of tobacco exposure (reported cigarettes per day) and biochemical measures of tobacco exposure. We compared the number of cigarettes smoked to four groups of tobacco toxins, carbon monoxide (CO), total NNAL (NNAL plus NNAL-glucuronides), 1-HOP, and total cotinine (cotinine plus cotinine-*N*-glucuronide), in a diverse population of cigarette smokers and over a broad range of smoking. It was hypothesized that there was a dose-response relationship between cigarette exposure and tobacco toxin exposure (the toxin levels would increase as the amount of cigarette exposure increased) but that it would not be linear across a broad range of smoking. We considered, however, that the relationships might vary depending on the toxin measured.

These results are relevant to the ongoing debate about the potential of smoking reduction to reduce health risks from tobacco use. Although abstinence confers known health benefits, and therefore is the optimal goal, some smokers are either unable or unwilling to stop smoking completely. If smoking fewer cigarettes confers health benefits, without detracting from motivation to stop smoking, then reduction may be advantageous. Unfortunately, measuring potential risk reduction associated with smoking reduction is challenging because many smoking-related diseases, such as malignancy, chronic lung disease, and cardiovascular disease, develop over an extended period of time and reduction of risk is likely to take years to become evident. Whereas it is critical to measure smoking-related disease outcomes, it may not always be feasible. The clinical goals of smoking reduction interventions are, to date, not based on evidence that a specific lowered level

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of smoking can predict improved health outcomes or even lower exposure to toxins found in tobacco smoke. Data from this cross-sectional analysis might prove useful by suggesting specific goals of smoking reduction interventions if smoking levels reliably predict toxin exposure.

Materials and Methods

Study Design. The smoking history and biomarker data from four populations of smokers were merged to achieve a broad range of cigarette exposure. Two of the groups, cohorts 1 and 2, were participants in clinical trials. Only baseline data were used for the analysis. The other two groups, cohorts 3 and 4, were recruited specifically to examine the relationship between stable low smoking rates and toxin exposure. This is a cross-sectional study; the amounts of cigarette exposure and tobacco toxin exposure were measured at one point in time. This is not a population-based study and the study participants were not intended to be representative of the general population who smoke cigarettes.

Setting and Participants. Cohort 1 included participants from a clinical trial, the Tobacco Reduction Intervention Program (TRIP) Study, which recruited cigarette smokers, ages 18 to 70 years, who were interested in reducing, but not quitting, cigarette use in the next 30 days. They were screened to ensure they met the following eligibility criteria: (*a*) smoking 15 to 45 cigarettes/d for the past year, (*b*) apparent good health with no unstable medical condition, (*c*) no contraindications to nicotine replacement use, (*d*) no history of schizophrenia or unstable depressive disorder, (*e*) not using other tobacco products, and (*f*) not pregnant or nursing. The average number of cigarettes per day was calculated from a daily diary.

Baseline levels of CO, carcinogen biomarkers, and total cotinine were determined at two points, 1 week apart, and averaged. This study has been described in detail and the results are available in an earlier publication (8).

Cohort 2 included participants from a clinical trial, the Reduction of Smoking in Cardiac Patients (ROSCAP) Study. This study includes cigarette smokers, ages 18 to 80 years, with at least one diagnosis of heart disease who were interested in reducing smoking but not quitting in the next 30 days. Eligibility criteria included (*a*) smoking ≥15 cigarettes/d; (*b*) having at least one of the following diagnoses: coronary artery disease, arrhythmia, congestive heart failure, peripheral vascular disease, or history of cerebrovascular disease; (*c*) no unstable angina in the past 2 weeks; (*d*) no unstable mental health or substance use diagnoses; and (*e*) no contraindications to nicotine replacement therapy (including pregnancy or intention to become pregnant). The average number of cigarettes per day was calculated from recall of the smoking level in the past week. CO, carcinogen biomarkers, and total cotinine were measured once at baseline.

Examination of the full spectrum of the relationship between the number of cigarettes smoked per day and toxin exposure required the recruitment of additional cohorts of low-level smokers from the Minneapolis-Saint Paul metropolitan area [Adult Cross-sectional Study (ACSS), cohort 3] and the Minneapolis VA Medical Center [Low Level Smoking Study (LLSS), cohort 4]. Eligibility criteria for the ACSS Study were the same as for cohort 1, however, participants had to smoke <15 cigarettes/d at a stable level for a minimum of 1 year. Eligibility criteria for the LLSS were the same as for ROSCAP, however, participants also had to smoke <15 cigarettes/d at a stable level for a minimum of 1 year. These smokers were seen once to obtain a history and biomarker specimens.

Measures. Smokers in all four groups provided demographic information and completed a tobacco use questionnaire that included the number of cigarettes smoked per day in the past week and the Fagerstrom Test for Nicotine Dependence (FTND; ref. 11). Exhaled CO was measured using a Bedfont Micro Smokerlyzer.

We quantified total NNAL (12, 13), 1-HOP (14), and total cotinine (12, 15) as previously described. Creatinine was assayed by Fairview-University Medical Center Diagnostic Laboratories (Minneapolis) using Vitros CREA slides and the Minneapolis VA Medical Center laboratory.

Statistical Analysis. TRIP and ROSCAP were randomized clinical trials. Only the baseline data from participants in these two studies who were assigned randomly to treatments were used. No randomization was involved in the cross-sectional ACSS and LLSS studies. Participants from the TRIP study had two pretreatment baseline visits and those from the ACSS study also had two visits. For these two cohorts, the number of cigarettes per day and levels of CO, total cotinine, total NNAL, and 1-HOP from the two visits were averaged. If the measurement of one visit was missing, then the nonmissing measurement of the other visit was used. ROSCAP and LLSS participants each had data from one baseline visit.

Total NNAL, total 1-HOP, and total cotinine were divided by creatinine to normalize for urinary concentration. Thus, the units of total NNAL, 1-HOP, and total cotinine used for analysis were pmol/mg creatinine, pmol/mg creatinine, and nmol/mg creatinine, respectively.

After merging the data, the descriptive statistics of age, gender, and FTND scores were calculated, as well as the number of cigarettes per day and tobacco toxins including CO, total NNAL, 1-HOP, and total cotinine. The relationships between the number of cigarettes per day and tobacco toxin variables were described using two-dimensional scatter plots. Cases with extreme values in either or both dimensions were identified visually and then excluded from the plotting (five cases). In each scatter plot, a Loess smoothing curve with 2 degrees of freedom is presented to show the data patterns. We chose 2 degrees of freedom because we hypothesized before the merging that the pattern would show a slowly climbing upward curve at low doses, a sharp increase at middle doses, and a plateau at high dose of cigarettes per day in which biomarkers reach a threshold.

In addition, the number of cigarettes was stratified with an interval of 5 cigarettes/d. Within each stratum, the descriptive statistics of tobacco toxin variables were calculated. The correlations among exposure variables were computed using the nonparametric Spearman's method because most variables did not follow a normal distribution. The test of the significance of correlation coefficients was two sided at a significance level of $\alpha = 0.05$.

S-PLUS 6.2 (Lucent Technologies, Inc., Murray Hill, NJ) was used to draw scatter plots with Loess smoothing curves. SAS v8.2 (SAS Institute, Inc., Cary, NC) was used for all other analyses.

Human Subjects Approval. All studies were reviewed and approved by the University of Minnesota Institutional Review Board. The ROSCAP Study and LLSS were also approved by the Minneapolis VA Institutional Review Board.

Results

There were 400 participants in the analysis; 151 (37.8%) participants were from TRIP, 152 (38.0%) from ROSCAP, 86 (21.5%) from ACSS, and 11 (2.8%) from LLSS.

The demographic characteristics and smoking and biomarker profiles of the participants are shown in Table 1. A total of 391 participants contributed age and gender information. The overall mean age was 50.4 years (SD, 12.2 years). Participants from TRIP and ACSS were younger, with a mean

Table 1. Characteristics of participants and biomarker levels

	Cohort 1 (TRIP)	Cohort 2 (ROSCAP)	Cohort 3 (ACSS)	Cohort 4 (LLSS)	Total
	Adult ''healthy'' smokers, 15-45 cigarettes/d	Adults with heart disease, >15 cigarettes/d	Adult ''healthy'' smokers, <15 cigarettes/d	Adults with heart disease, <15 cigarettes/d	
N	151	152	86	11	400
Age (y)	44.7 (10.5)	57.9 (9.1)	45.1 (11.5)	67.9 (7.0)	50.4 (12.2)
Gender (M/F)	69:82	135:17	34:46*	8:0†	246:145
Cigarettes/d	24.2 (6.4)	27.5 (11.6)	9.2 (8.0)	8.5 (4.0)	21.9 (11.5)
FTND score	6.1 (1.9)	6.0 (2.0)	2.3 (2.3)	2.5 (1.9)	5.2 (2.5)
CO (ppm)	23.7 (10.3)	23.9 (11.8)	10.0 (7.9)	11.1 (3.1)	20.2 (12.0)
Total NNAL (pmol/mg creatinine)	2.2 (1.1)	2.7 (2.3)	1.2 (1.1)	2.2 (1.5)	2.2 (1.8)
Total 1-HOP (pmol/mg creatinine)	1.6 (1.1)	2.2 (4.7)	1.6 (1.1)	1.0 (0.6)	1.8 (3.2)
Total cotinine (nmol/mg creatinine)	26.4 (13.3)	25.1 (17.4)	12.2 (11.1)	17.4 (9.5)	22.2 (15.7)

NOTE: Cell values for age, cigarettes/d, FTND score, CO, total cotinine, total NNAL, and total 1-HOP were mean (SD). Cell values for gender were counts.
*ACSS has six participants with missing values for gender.
†LLSS has three participants with missing values for gender.

age of 44.7 (SD, 10.5) and 45.1 (SD, 11.5) years, respectively; participants from ROSCAP were older, with a mean age of 57.9 (SD, 9.1) years; and those from LLSS were the oldest, with a mean age of 67.9 (SD, 7.0) years. Of 391 participants, 246 (62.9%) were male.

There were 374 participants with values of cigarette smoking and FTND scores. The number of cigarettes smoked per day ranged from 1 to 100 with an overall mean of 21.9 cigarettes/d (SD, 11.5). Compared with participants from TRIP (mean, 24.2 cigarettes/d) and ROSCAP (mean, 27.5 cigarettes/d), those from ACSS (mean, 9.2 cigarettes/d) and LLSS (mean, 8.5 cigarettes/d) smoked considerably fewer cigarettes per day (due to the different eligibility criteria of the four studies). The overall mean FTND score was 5.2 (SD, 2.5). Participants from ACSS (mean score, 2.3) and LLSS (mean score, 2.5) had lower mean FTND scores than those from TRIP (mean score, 6.1) and ROSCAP (mean score, 6.0).

Descriptive statistics about levels of CO, total NNAL, 1-HOP, and total cotinine are shown in Table 2, with cigarettes per day divided into strata including intervals of 5 cigarettes/d. The relationships between biomarker levels and cigarettes per day are shown in scatter plots with Loess smoothing curves in Figs. 1-4.

The range of CO measurements was 1 to 70 ppm and the overall mean was 20.2 ppm (SD, 12.0; Tables 1 and 2). CO measures increased steadily with increased smoking up to ~45 cigarettes/d and then declined. The scatter plot, excluding outliers, suggests similar results (Fig. 1). Of note, however, there were relatively few participants who smoked >45 cigarettes/d (n = 12) and, therefore, these measures are relatively unstable.

The range of total NNAL measurements was 0 to 23.9 pmol/mg creatinine. The overall mean total NNAL was 2.2 (SD, 1.8) pmol/mg creatinine (Tables 1 and 2). There is a steady rate of increase when the data are examined by strata up to 40 cigarettes/d, and then a modest decrease with the exception of the 55 to 60 cigarette/d stratum (n = 1). There are relatively few samples from participants smoking >40 cigarettes/d. Examination of the continuous distribution, excluding outliers, suggests a steady rate of increase in total NNAL up to ~15 cigarettes/d and then a slower rate of increase (Fig. 2).

The range of 1-HOP measurements was 0.1 to 53.9 pmol/mg creatinine and the overall mean was 1.8 (SD, 3.2) pmol/mg creatinine (Tables 1 and 2). 1-HOP levels seem to be relatively flat between 10 and 55 cigarettes/d (Fig. 3), particularly in comparison with CO and total NNAL. This may be explained by the fact that there are multiple sources of 1-HOP in the environment other than cigarette smoke that contribute to the values observed.

The range of total cotinine measurements was 0.2 to 159.4 nmol/mg creatinine and the overall mean was 22.2 (SD, 15.7) nmol/mg creatinine (Tables 1 and 2). Strata-specific means increase fairly consistently through the 40 to 45 cigarettes/d stratum and then are less stable. The scatter plot (Fig. 4) shows a similar trend. The rate of increase is greatest between 1 and 25 cigarettes/d.

Spearman correlation coefficients between cigarettes per day and various biomarkers were calculated. There was a significant

Table 2. Descriptive statistics of CO, total NNAL, 1-HOP, and total cotinine stratified by cigarettes per day

Strata, cigarettes/d	*N*	Carbon monoxide* (ppm)		Total NNAL† (pmol/mg creatinine)		Total 1-HOP‡ (pmol/mg creatinine)		Total cotinine§ (nmol/mg creatinine)	
		Mean (SD)	Min-max	Mean (SD)	Min-max	Mean (SD)	Min-max	Mean (SD)	Min-max
(0-5)	40	5.6 (4.8)	1-19	0.8 (1.0)	0-3.8	1.2 (0.9)	0.1-4.2	7.0 (7.9)	0.2-29.5
(5-10)	32	10.0 (5.4)	2.5-26	1.2 (0.9)	0.2-3.6	1.5 (1.0)	0.5-5.7	13.3 (8.1)	3.5-38.8
(10-15)	37	15.4 (7.3)	3-31	2.8 (3.9)	0.2-23.9	2.1 (1.5)	0.3-5.7	24.2 (26.0)	1.7-159.4
(15-20)	99	19.9 (8.6)	5-45	2.1 (1.2)	0.5-7.0	2.3 (6.0)	0.1-53.9	23.4 (13.2)	0.5-70.0
(20-25)	63	21.7 (7.5)	6-48	2.2 (1.0)	0.5-6.4	1.6 (1.2)	0.4-5.3	27.0 (12.9)	8.2-68.9
(25-30)	69	25.9 (11.6)	8-55	2.6 (1.3)	0.6-6.2	1.9 (1.5)	0.33-8.0	27.5 (14.1)	3.6-71.2
(30-35)	17	27.9 (13.9)	8-70	2.1 (1.0)	0.8-3.9	2.1 (1.4)	0.9-5.3	22.9 (12.8)	5.0-45.6
(35-40)	28	30.2 (12.5)	12-64	3.7 (2.0)	0.8-8.3	1.7 (1.2)	0.4-4.8	27.0 (14.2)	2.4-57.8
(40-45)	3	38.3 (23.5)	18-64	2.7 (1.7)	1.4-4.5	1.6 (0.8)	0.6-2.1	33.4 (16.1)	22.8-52.0
(45-50)	10	29.1 (15.4)	13-56	2.3 (1.0)	1.1-4.0	1.7 (1.1)	0.8-4.1	17.6 (11.3)	3.6-40.5
(55-60)	1	18.0 (—)	—	4.2 (—)	—	1.1 (—)	—	32.3 (—)	—
(95-100)	1	25.0 (—)	—	1.8 (—)	—	0.9 (—)	—	26.2 (—)	—

*Fifty-six missing values.
†Forty-six missing values.
‡Thirty-seven missing values.
§Thirty-seven missing values.

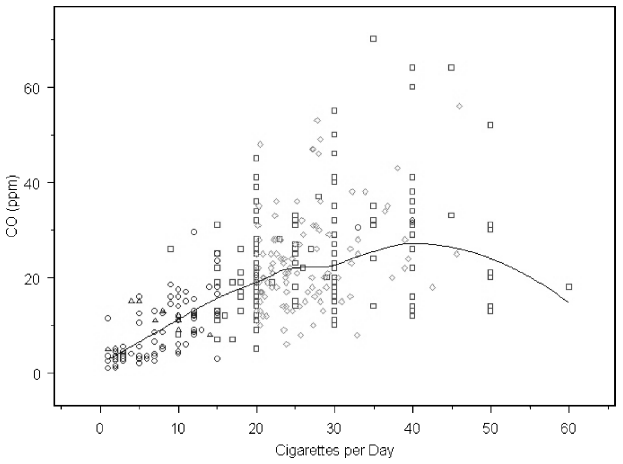

Figure 1. TRIP (◇), ROSCAP (□), ACSS (○), and LLSS (△).

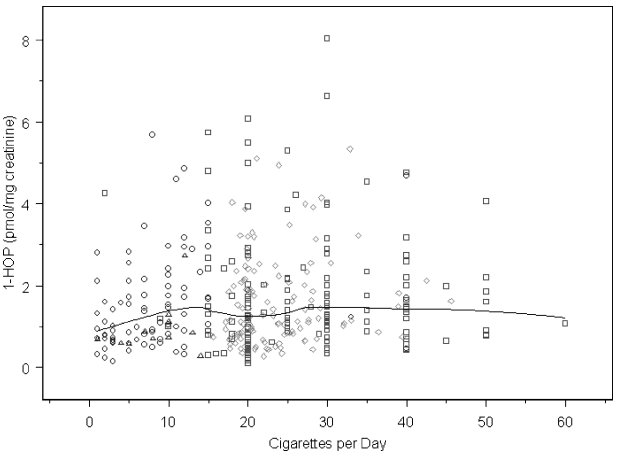

Figure 3. TRIP (◇), ROSCAP (□), ACSS (○), and LLSS (△).

correlation with total NNAL (r = 0.478, P < 0.0001), 1-HOP (r = 0.126, P < 0.05), total cotinine (r = 0.426, P < 0.0001), and CO (r = 0.631, P < 0.0001). In addition, total NNAL was highly significantly correlated with total cotinine (r = 0.619, P < 0.0001) and CO (r = 0.586, P < 0.0001) and it was also significantly correlated with 1-HOP (r = 0.183, P = 0.0006; Table 3). Separate analyses were conducted for males and females and for each of the four cohorts and showed similar results. Total NNAL and total cotinine were highly and significantly correlated in three of the four cohorts (r = 0.417-0.768).

Discussion

These cross-sectional data show that levels of the biomarkers CO, total NNAL, and total cotinine increase with an increase in the number of cigarettes smoked per day, but not in a linear fashion, particularly at higher amounts of cigarette consumption. In general, 1-HOP seems to be a less discriminating biomarker than CO, total NNAL, or total cotinine as exposure levels are relatively stable regardless of the number of cigarettes smoked. Each of these measures seems to plateau around 25 to 35 cigarettes/d; however, there is considerable variability in toxin measurement at high levels and analyses are less robust because of the low number of study participants in these strata of smoking. The highest rates of increase in toxin exposure appear between 1 and 10 cigarettes/d, with the exception of 1-HOP. The data are consistent with previously published reports about CO and cotinine; however, prior work has concentrated on the utility of these measures to discriminate smokers from nonsmokers and to validate nonsmoking status (16-18).

Although asking the number of cigarettes smoked per day is currently regarded as the gold standard measure of exposure, these results suggest that this statistic may not be a good indicator of toxin exposure. As noted, we observe considerable variability in exposure at all levels of smoking, but particularly at high levels. There are several potential sources of variability. Toxin delivery may vary with the type of cigarette. The amount of NNK per cigarette has been observed to vary over a wide range (19). Levels may also vary with gender or may be affected by prior reduction and potential compensation.

Another potential source of variability in biomarker levels is lack of precision in reporting the number of cigarettes per day. Inaccuracy could be due to faulty recall or rounding errors. Many participants reported round numbers of cigarettes smoked per day, such as 20 or 30. This potential error is a variant of terminal digit preference that has been previously described (20). We therefore consider that biomarkers such as expired CO, total cotinine, or carcinogen uptake may be more meaningful markers of exposure than recall of cigarettes smoked per day, although the requirement for biological specimens to use alternative measures and their cost may preclude adopting this practice. Tobacco-specific

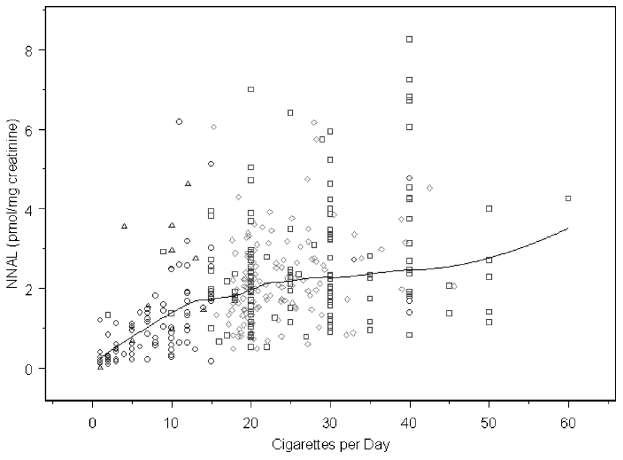

Figure 2. TRIP (◇), ROSCAP (□), ACSS (○), and LLSS (△).

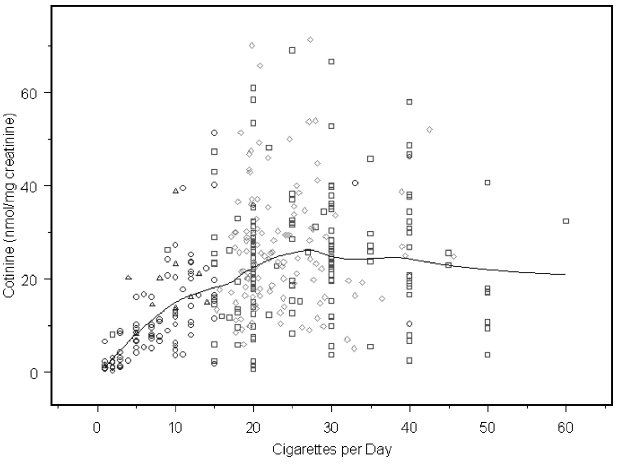

Figure 4. TRIP (◇), ROSCAP (□), ACSS (○), and LLSS (△).

Table 3. Spearman correlation coefficients of biomarkers with cigarettes per day and other biomarkers

	Age (y)	Cigarettes/d	Total NNAL	Total 1-HOP	Total cotinine	CO
Age (y)	1	0.124	0.149	−0.002	0.131	0.046
	—	0.0143	0.0056	0.9668	0.0138	0.4013
Cigarettes/d		1	0.478	0.126	0.426	0.631
		—	<0.0001	0.0168	<0.0001	<0.0001
Total NNAL			1	0.183	0.619	0.586
			—	0.0006	<0.0001	<0.0001
Total 1-HOP				1	0.220	0.182
				—	<0.0001	0.0011
Total cotinine					1	0.535
					—	<0.0001
CO						1
						—

NOTE: Each cell contains two values: (*a*) Spearman correlation coefficient; (*b*) *P* value of testing if the correlation is significant.

biomarker data such as total NNAL and cotinine (in the absence of nicotine replacement therapy) may be particularly helpful in measuring toxin exposure from environmental tobacco smoke. In addition to these biomarkers, CO may be helpful in measuring toxin exposure among those who reduce smoking.

There are several limitations to these data. The aggregated sample of smokers is not representative of all smokers, and therefore the results may not apply to the general population of smokers. In addition, the study is composed of four different samples of smokers from different studies with various enrollment criteria. Some of the smokers were healthy and others had medical problems, especially heart disease. There is little information about the effect of these medical conditions on the pharmacokinetics of the biomarkers measured, but there are no a priori hypotheses about their effect on biomarker metabolism, and correlations between cigarettes per day and the various biomarkers were consistent across cohorts. There was also some variability in study methods between the four cohorts that might affect the stability of the measures; e.g., participants in cohorts 1 and 3 had two baseline measures that were averaged whereas participants in cohorts 2 and 4 had only one measure. A study by Murphy et al. (21) showed significant intraindividual differences, suggesting that a single determination of biomarker level may not be optimal. Finally, the cohort included relatively few low-level and high-level smokers and, therefore, conclusions about these strata are less definitive.

Although these data suggest that smoking fewer cigarettes, at least fewer than 20-25 cigarettes/d, is associated with lower levels of total NNAL, CO, and total cotinine, it cannot be inferred that an individual smoker who reduced the amount smoked below this level will achieve lower toxin exposure and a lower level of risk. Smokers who reduce smoking may exhibit compensatory smoking behavior that results in failure to reduce tobacco toxin exposure. In a smoking reduction study by Hecht et al. (8, 9), the reduction in carcinogen exposure was not proportional to the reduction in smoking, probably secondary to compensatory smoking.

This analysis does suggest that reducing from a very large number of cigarettes to a moderate number (e.g., from 50 to 30) may not have much potential to reduce toxin exposure because a reduction is not shown in these cross-sectional data. Whereas lower levels of toxin uptake were observed in smokers who smoked few cigarettes, it is unclear if these low levels confer any health benefits. Furthermore, the data suggest there is no threshold for reduction that can be expected to protect against toxin exposure; i.e., advice to ''just get below 5 cigarettes a day'' is not supported. This is consistent with epidemiologic data that show there is no safe level of smoking and with data about the risk of exposure to environmental tobacco smoke. Studies of second-hand smoke exposure show a significant risk of heart disease and cancer in spite of low levels of toxin exposure (22).

The proportion of increase in toxin exposure compared with cigarettes smoked per day varies between CO, total NNAL, 1-HOP, and total cotinine, and therefore may also vary for other harmful exposures. This suggests that if smoking reduction does accomplish a reduction in exposure to a specific tobacco toxin, it cannot be assumed that other toxins will be reduced in a similar way.

We conclude, therefore, that although there is a strong trend for the number of cigarettes smoked per day to be associated with exposure to total NNAL, CO, and total cotinine, the relationship varies depending on the biomarker measured. There is sufficient variability that it may be difficult to predict toxin exposure for an individual based on the number of cigarettes they smoke per day. Reported high levels of smoking (e.g., >35 cigarettes/d) may not increase toxin exposure levels proportionally or at the rate observed at lower levels of smoking. Smoking abstinence is an important goal because even low levels of smoking are associated with significant toxin exposure.

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