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Smokers' Behaviour and Exposure According to Cigarette Yield and Smoking Experience

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HEE, J., F. CALLAIS, I. MOMAS, A. M. LAURENT, S. MIN, P. MOLINIER, M. CHASTAGNIER, J. R. CLAUDE AND B. FESTY. *Smokers' behaviour and exposure according to cigarette yield and smoking experience.* PHARMACOL BIOCHEM BEHAV 52(1) 195–203, 1995.—The influence of cigarette yield and length of smoking experience on smoking behaviour and biomarker levels was sought in 108 smokers who have never changed cigarette class. Smoking parameters, carboxyhaemoglobin percentage (COHb), urinary nicotine, and its metabolites, mutagens, and thioethers were measured. Cigarette yield does not affect daily consumption or smoke volume puffed per cigarette. But the inhalation depth increases with decreasing cigarette yield and with length of smoking habit. The COHb level after the first cigarette in the morning increases significantly with CO cigarette yield and length of smoking experience. In the evening, only the cigarette yield has an effect on COHb level. Biomarker levels excreted in urine are generally lower for females than for males. They tend to increase with smoking history. Only COHb level and total urinary nicotine metabolites (Barlow index) are weakly correlated with cigarette yield. The absence of significant differences due to cigarette class in urinary biomarkers can be explained by changes in inhalation depth, individual differences of metabolism, and limited specificity of some markers (mutagens, thioethers).

Smoking behaviour Cigarette yield Biomarkers Carboxyhaemoglobin Nicotine Cotinine Mutagens Thioethers

CIGARETTE manufacturers are marketing increasingly milder cigarettes, especially in France where 34% of smokers smoke mild cigarettes. The question as to whether these milder cigarettes are safer is still controversial. Most published studies concentrate on smoking behaviour and the effects associated with changing from cigarettes of one yield to another. These studies generally show that switching from high- to low-yield cigarettes induces an increase of cigarette consumption (33), of the smoke volume per cigarette (3,10), and/or of smoke inhalation (13,34), resulting in little or no reduction in toxic uptake (21,35). All these observed changes in smoking behaviour could be due to a transitional effect that decreases in the long term (28).

To avoid the risk of this bias, this study compares the smoking behaviour and smoke uptake of subjects who have never changed their class of cigarette throughout their smoking life ("steady-state smokers"). We have examined the influence of cigarette yield and length of smoking habit on consumption, smoking behaviour, depth of inhalation, and levels of biomarkers that are more or less specifically associated with smoking.

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METHOD

Experimental Plan

For both controlled factors, three levels have been defined. The limits of cigarette classes were fixed according to tar yield: less than 4.5 mg (extra-mild cigarettes), from 4.5 to 12 mg (mild cigarettes), and more than 12 mg (full-flavor cigarettes). To appreciate the early development of smoking behaviour and consumption, the limits of length of smoking habit classes were fixed at less than 1 year, 1-3 years, and more than 3 years.

To assess the influence of these factors and their interactions, nine groups of 12 subjects were selected as a complete 3 × 3 plan.

Subjects

One hundred and eight volunteers (43 males, 65 females) were recruited by a specialised pharmaco-clinical centre ("Therapharm Recherches") according to the following main criteria: healthy people using no drugs, always smoking cigarettes of the same class with a daily consumption of at least five bright tobacco filter cigarettes, and inhaling while smoking. Inhalation was verified by measuring the CO concentration in end tidal air (>8 ppm, which is equivalent to a carboxyhaemoglobin level of at least 2%) in the afternoon (4 to 6 h p.m.). The proportion of each sex was similar for each smoking history but was different for cigarette classes: proportions of males were 31%, 28%, and 61% for lowest, middle, and highest yield cigarette classes, respectively. The mean age of the smokers was 22.7 ± 4.2 years. The only difference observed was that the mean age of smokers with less than 1 year smoking experience (23.8 ± 5.4 years) was higher than that for 1-3 years group (21.4 ± 2.0 years).

Procedure

The subjects visited the centre twice. On the first time (D1) in the afternoon, the inclusion criteria were verified, the selected subjects filled in a questionnaire on their smoking habits and familiarised themselves with the smoking parameter analyser. Their carboxyhaemoglobin level was evaluated. During the second visit (D2) in the morning, before smoking the first cigarette, the following parameters were determined: smoking parameters, CO levels before and 5 min after smoking the first cigarette.

Measurement of Smoking Parameters

Smoking characteristics were measured with a specially designed analyser that includes a cigarette holder with an associated flowmeter (13).

The parameters measured were puff number, mean puff duration, mean puff volume (excluding the first and the last puff, which are atypical)—later referred as puff volume, total smoke volume, mean puff interval (excluding the last interval), and total smoking duration.

Carboxyhaemoglobin Evaluation

COHb was measured indirectly from the carbon monoxide concentration analysed by infrared spectrometry in end tidal air, after 20-s apnea and throwing out the first liter of breathed out air. The equivalent carboxyhaemoglobin level is calculated by the following equation: COHb (%) = 0.174 FE_{CO} + 0.726, where FE_{CO} is the end tidal CO concentration expressed in ppm (25). Three measurements were considered: in the evening on day 1 (COHbe), before the first cigarette on day 2 (COHbb), and after smoking this first cigarette (COHba).

Inhalation Index

We have attempted to calculate an inhalation index as the ratio between the quantity of CO retained by the subject and that produced by the cigarette smoked.

The quantity of CO retained by the subject is calculated from the increase in carboxyhaemoglobin percentage (dCOHb = COHba − COHbb) produced by the first cigarette of the day, the standard CO capacity value of haemoglobin (1.39 ml CO/g haemoglobin), the haemoglobin mean concentration (Hb) for each sex (159 g/l in male, 143 g/l in female), and the blood volume according to body weight (65.6 ml/kg). The quantity of CO retained was given by the following equation: CO (ml) = 65.6 × body weight × Hb × 1.39 × dCOHb/10^5.

The volume (ml) of CO produced by the cigarette smoked was calculated from the mean volume (ml) of CO per ml of smoke under standardised conditions (ISO 3308) and the total smoke volume drawn by the smoker.

Urinary Biomarkers

They were evaluated without knowledge of the reported smoking status and were expressed on a 24-h basis.

The urine samples were collected over 24 h in a polyethylene flask and kept at 4°C during the collection period. They were brought to the pharmaco-clinical centre, at the time of the second visit. The volume was measured and the urine was divided into duplicate aliquots of 100, 20, 10, and 15 ml for measurement of mutagens, thioethers, Barlow index, nicotine, and cotinine respectively. Aliquots were kept at −20°C until analysis. Then the samples were thawed at +4°C, homogenized, and centrifuged at 3000 × g for 2 min.

The urinary pH was measured and creatinine was determined according to the Jaffe method (20).

Nicotine and its pyridinic metabolites measurement by colorimetry: Barlow index. Nicotine and its pyridinic metabolites were evaluated by a simple colorimetric assay, based on the Koënig reaction, using barbituric acid as reactant and cotinine as standard (4). Then all results were expressed as "cotinine equivalent."

Nicotine and cotinine measurement by gas chromatography. A urine sample, adjusted to pH 11, was passed through a C18 silica cartridge (Sep-Pak C18, Millipore Waters) previously rinsed successively with deionized water, ethylacetate, and water (pH 11). The cartridge was eluted with ethylacetate spiked with an internal standard (quinoline). The extract was analysed using a gas chromatograph fitted with a fused silica capillary column (CW 57 CB, KOH treated) and a thermoionic selective detector (NPD).

Thioethers measurement. Thioethers were measured by colorimetry (37) following an adapted procedure for smokers (16).

Mutagens measurement. Urine samples were concentrated on XAD-2 columns (41). The 500-fold concentrate of urine was obtained by redissolving the dried acetone eluate with DMSO.

The mutagenic activity of urine sample extracts was determined by a microsuspension assay (18). Tester strain TA 98, kindly provided by Dr. B. N. Ames, was used. All samples were tested in duplicate with metabolic activation (S9 fraction of rat liver extract induced with aroclor 1254). The mean val-

TABLE 1

MEAN ± SD TAR, NICOTINE, AND CO YIELDS (mg/cig) ACCORDING TO CIGARETTE CLASS

	Extra-Mild (n = 36)	Mild (n = 36)	Full Flavor (n = 36)
Tar	3.38 ± 0.92	8.95 ± 1.41	14.72 ± 1.02
Range	0.9-3.9	4.9-10.8	12.7-18.4
Nicotine	0.35 ± 0.10	0.72 ± 0.10	1.08 ± 0.07
Range	0.09-0.40	0.49-0.86	0.97-1.19
Carbon monoxide	3.24 ± 0.64	9.09 ± 1.82	13.60 ± 1.50
Range	1.1-4.2	4.6-11.5	10.4-15.0

ues were considered. Several concentrations of urine extract were tested, 1.25–10 μl corresponding to 0.625–5 ml equivalent urine, respectively.

4-Nitroquinoline-oxide and benzo[*a*]pyrene were used as positive control. Mean spontaneous values in all experiments were 45 ± 6 revertants in the presence of S9 mix.

The urine samples were regarded as mutagenic when at least one dose doubled the number of spontaneous revertant colonies in untreated plates. The method of least-square regression analysis was used in the linear portion of the dose-response curves to determine the mutagenicity of samples expressed as His^+ revertants/24 h.

Statistical Analysis

The BMDP statistical software (University of California, 1990) was used in all analyses with a significant alpha level of 0.05.

In spite of a two-factorial design (yield, history), a three-way analysis of variance (ANOVA) was used because more women than men were recruited: the factors were gender, class of cigarettes, and length of smoking habit. A transformation (square-root or logarithm) of dependent variables was attempted when the hypothesis of normality was not valid and to stabilize variances. Interactions were tested. Upon obtaining a significant F from the ANOVA, the Newman-Keuls multiple range test was performed to determine where the differences lay.

Correlations between variables were measured according to Pearson; the null hypothesis that the population correlation is zero was tested.

Multiple regression models were used to determine in a stepwise manner the potential predictors of the smoking biomarkers levels from four variables: number of cigarettes per day, total volume puffed per cigarette, inhalation index, and tar, nicotine, or carbon monoxide yields. These models give multiple R^2, which is the proportion of the total variation in the dependent variable accounted for by the predictors.

RESULTS

Cigarette Classes and Smoking Behaviour

The mean yields of cigarettes (mg/cig.) smoked by the subjects of each class are given in Table 1. They were not significantly different for the three groups of smoking history.

The mean values of consumption, smoking parameters, and inhalation index for the first cigarette in the morning are presented by sex, cigarette class, and smoking history in Table 2. This table also gives results from the three-way ANOVA. There were no significant interactions.

Cigarette yields and smoking history do not significantly influence cigarette consumption or any of the measured smoking parameters, except the mean puff volume, which is smaller

TABLE 2

SMOKING PARAMETERS (MEAN RAW VALUES ± SD) ACCORDING TO CIGARETTE CLASS AND SMOKING HISTORY

			Cigarette Class (Mean Tar Yield)				Smoking History			
		Sex Effect F Signif.	3.38 mg (M + F = 36)	8.95 mg (M + F = 36)	14.72 mg (M + F = 36)	Cig. Class Effect F	< 1 Year (M + F = 36)	1-3 Years (M + F = 36)	> 3 Years (M + F = 36)	History Effect F
Consumption	M		13.6 ± 7.5	11.9 ± 3.7	12.6 ± 6.1		10.2 ± 4.9	14.5 ± 7.4	14.1 ± 3.8	
(cig./day) (ln)	F		12.6 ± 5.7	10.1 ± 6.6	15.0 ± 6.4		13.7 ± 6.4	11.8 ± 7.6	12.8 ± 4.3	
Puff number (ln)	M	4.40	9.9 ± 2.3	12.7 ± 4.0	12.1 ± 4.5		13.4 ± 4.8	10.6 ± 3.0	10.4 ± 3.0	
	F	*	13.6 ± 4.6	13.0 ± 4.9	12.6 ± 2.6		13.6 ± 4.4	12.7 ± 3.7	12.8 ± 3.7	
Puff duration	M	4.30	2.04 ± 0.60	2.06 ± 0.57	2.24 ± 0.86		2.04 ± 0.83	2.08 ± 0.69	2.40 ± 0.64	
(s) (ln)	F	*	1.88 ± 0.53	1.70 ± 0.33	2.07 ± 0.81		1.93 ± 0.45	1.72 ± 0.38	1.89 ± 0.70	
Puff volume (ml)	M	7.46	61.0 ± 20.2	53.4 ± 14.7	55.1 ± 19.3		49.3 ± 18.6	57.9 ± 18.3	64.6 ± 15.4	4.10
	F	†	54.3 ± 12.5	46.0 ± 13.6	53.4 ± 13.6		49.6 ± 13.6	49.0 ± 13.5	54.2 ± 13.3	*
Inter puff interval	M		35.2 ± 11.2	26.5 ± 11.3	28.3 ± 16.1		25.0 ± 12.9	34.9 ± 13.6	29.5 ± 15.0	
(s) (ln)	F		25.6 ± 14.1	27.3 ± 12.4	27.7 ± 9.7		25.4 ± 12.3	28.3 ± 10.2	27.3 ± 14.5	
Smoking duration	M		329 ± 89	301 ± 66	293 ± 70		294 ± 65	328 ± 51	287 ± 108	
(s)	F		298 ± 81	307 ± 73	328 ± 73		309 ± 86	327 ± 78	300 ± 69	
Total volume	M		563 ± 169	653 ± 202	635 ± 236		634 ± 248	583 ± 203	652 ± 167	
smoke (ml)	F		732 ± 265	598 ± 204	667 ± 223		698 ± 270	611 ± 204	682 ± 243	
Inhalation index	M	4.85	0.88 ± 0.56	0.67 ± 0.44	0.57 ± 0.29	6.93	0.50 ± 0.30	0.78 ± 0.44	0.79 ± 0.49	5.61
(SR)	F	*	0.79 ± 0.30	0.57 ± 0.32	0.45 ± 0.21	†	0.56 ± 0.37	0.68 ± 0.24	0.66 ± 0.34	†

M = males, F = females.

Three-way variance analysis (no interaction). Variable transformation: (ln) = logarithm; (SR) = Square root.

*$0.01 < p < 0.05$, †$0.001 < p < 0.01$.

TABLE 3

BIOMARKER LEVELS (MEAN RAW VALUES ± SD) ACCORDING TO CIGARETTE CLASS AND SMOKING HISTORY

		Sex Effect *F* Signif.	Cigarette Class (Mean Tar Yield) 3.38 mg (M + F = 36)	8.95 mg (M + F = 36)	14.72 mg (M + F = 36)	Cig. Class Effect *F*	Smoking History <1 Year (M + F = 36)	1-3 Years (M + F = 36)	>3 Years (M + F = 36)	History Effect *F*
% COHb before	M	10.34	2.57 ± 0.61	2.74 ± 0.97	2.97 ± 0.96		2.42 ± 0.75	2.85 ± 0.63	3.38 ± 1.09	
1st cig (SR)	F	†	2.13 ± 0.61	2.14 ± 0.97	2.54 ± 0.49		2.26 ± 0.60	2.02 ± 0.77	2.34 ± 0.91	
% COHb after	M		3.08 ± 0.67	3.83 ± 1.01	4.33 ± 1.33	13.90	3.28 ± 0.91	4.03 ± 1.09	4.65 ± 1.41	4.43
1st cig	F		2.96 ± 0.80	3.40 ± 1.17	4.42 ± 0.97	‡	3.25 ± 0.87	3.09 ± 0.80	3.66 ± 1.37	*
COHb increment	M	4.09	0.51 ± 0.26	1.09 ± 0.54	1.36 ± 0.69	18.58	0.86 ± 0.46	1.18 ± 0.73	1.27 ± 0.80	3.85
with 1st cig (SR)	F	*	0.83 ± 0.39	1.26 ± 0.75	1.89 ± 0.83	‡	0.98 ± 0.65	1.07 ± 0.56	1.32 ± 0.74	*
Evening %	M		4.80 ± 1.86	5.23 ± 1.80	5.74 ± 2.26	3.82	5.13 ± 2.43	5.25 ± 1.77	5.93 ± 1.85	
COHb (ln)	F		4.82 ± 2.34	5.94 ± 2.56	6.73 ± 2.04	*	5.41 ± 1.71	4.58 ± 1.71	6.43 ± 3.07	
Barlow index	M		35.6 ± 13.8	54.5 ± 30.6	62.7 ± 31.4		40.2 ± 26.2	59.8 ± 27.8	66.9 ± 30.1	
(μmol/24/h)	F		42.1 ± 25.3	41.1 ± 38.5	51.5 ± 29.7		43.0 ± 26.0	38.5 ± 32.5	48.5 ± 34.5	
(SR)										
Urinary nicotine	M	8.76	4.87 ± 6.71	5.05 ± 4.90	6.25 ± 3.92		2.86 ± 3.56	7.06 ± 5.31	7.91 ± 4.38	4.61
(μmol/24h) (ln)	F	†	4.60 ± 4.50	2.12 ± 2.18	3.70 ± 2.77		3.71 ± 4.57	2.75 ± 3.27	3.94 ± 2.90	*
Urinary cotinine	M	6.28	2.93 ± 2.29	3.65 ± 2.80	3.70 ± 2.44		2.66 ± 2.38	4.26 ± 2.35	3.73 ± 2.54	
(μmol/24h) (SR)	F	*	2.55 ± 1.90	1.95 ± 1.85	2.97 ± 1.80		2.40 ± 1.37	1.97 ± 2.08	2.76 ± 2.16	
Urine mutagens	M	5.17	19122 ± 12083	14899 ± 15598	23269 ± 15437		11602 ± 9522	23894 ± 11851	28692 ± 18663	3.10
(revert/24h) (SR)	F	*	7506 ± 7703	23092 ± 38480	14775 ± 24432		10858 ± 9887	8694 ± 11383	24241 ± 41665	*
Urine thioethers	M	10.49	69.0 ± 23.7	78.7 ± 18.9	79.3 ± 38.4		71.7 ± 27.2	90.8 ± 31.8	64.5 ± 31.0	
(μmol/24h) (ln)	F	†	69.3 ± 37.5	45.1 ± 26.7	55.9 ± 30.1		67.2 ± 31.5	47.6 ± 36.5	58.9 ± 32.4	

M = males, F = females.

Three-way variance analysis (no interaction). Variable transformation: (ln) = logarithm; (SR) = Square root.

*$0.01 < p < 0.05$, †$0.001 < p < 0.01$, ‡$p < 0.001$.

TABLE 4

SIGNIFICANT CORRELATIONS BETWEEN BIOMARKERS ($p < 0.05$)

	COHbb	COHba	COHbe	Barlow	Nicotine	Cotinine	Mutagens
COHba	0.789						
COHbe	0.528	0.594					
Barlow	0.644	0.560	0.621				
Nicotine	0.541	0.490	0.395	0.666			
Cotinine	0.603	0.534	0.525	0.688	0.726		
Mutagens	0.266	0.280	0.289	0.331	0.362	0.367	
Thioethers	0.256		0.206	0.266	0.373	0.340	0.257

in less than 1 year smokers. Nevertheless some differences are observed between sexes: compared to females, males take less puffs, but the duration and volume of their puffs are greater.

The inhalation index is significantly influenced by the three factors. It is higher in males than in females and increases with decreasing cigarette yield and with longer smoking experience. The inhalation index for the lowest yield cigarette is significantly higher than that of the other two cigarette classes. "Less than 1 year" smokers inhale less smoke than the others.

Biomarkers

The results and statistical analysis are summarized in Table 3.

Carboxyhaemoglobin. Before the first cigarette in the morning, the carboxyhemoglobin level is globally higher in males (2.81 ± 0.88%) than in females (2.22 ± 0.77%), irrespective of cigarette class. Although this level tends to increase with cigarette yield and the length of smoking history, the differences are not significant.

After the first cigarette of the day, the observed COHb percentage increases significantly with the cigarette yield. The effect of smoking history appears, with the difference between "less than 1 year" and "more than 3 years" smokers being significant. There is no difference between sexes.

The COHb increment produced by the first cigarette is significantly influenced by cigarette class, smoking history, and sex. This increment increases with the cigarette yield and the length of smoking habit: the "less than 1 year" smokers absorb less CO than the others. It is globally greater for females (1.23 ± 0.76%) than for males (1.08 ± 0.66%).

In the evening the COHb percentage is significantly affected by the cigarette yield but not by the other factors. The COHb level is significantly lower for extra-mild cigarette smokers than for full-flavor cigarette smokers.

Urinary parameters. Mean pH value was 6.5 ± 0.6. Mean 24-h creatinine excretion was different according to sex: 12.95 ± 4.16 mmol/24 h for men and 8.14 ± 2.25 mmol/24 h for women. All biomarkers except the Barlow index are significantly higher in men than in women.

The urinary biomarkers levels are not statistically related to the cigarette yields. However, there is a nonstatistically significant increase of the Barlow index with nicotine yield.

On the contrary, Barlow index, nicotine, cotinine, and mutagens increase with length of smoking habits. This evolution is statistically significant for nicotine and mutagens, with their levels being higher among the most experienced smokers.

Correlations

Neither cigarette consumption nor smoking parameters are correlated with cigarette yields. Inhalation index is negatively related to these yields; for the 108 subjects, the correlation coefficients with the tar, nicotine, and CO yields are, respectively, $r = -0.318$, -0.313, and -0.370 ($p < 0.05$).

Contrary to the total volume of smoke, the mean puff volume is weakly related to the consumption ($r = 0.226$, $p < 0.05$). As expected, the total volume is related ($p < 0.05$) positively to the number of puffs ($r = 0.659$), the mean puff volume ($r = 0.442$), and negatively to the interval between puffs ($r = -0.617$).

All biomarkers are correlated with each other (Table 4), except thioethers with COHb level after the first cigarette in the morning. Apart from the obvious relationships between

TABLE 5

SIGNIFICANT CORRELATIONS BETWEEN BIOMARKERS AND CIGARETTE YIELDS OR SMOKING PATTERN ($p < 0.05$)

	Tar Yield	Nicotine Yield	CO Yield	Daily Consump.	Puff Volume	Smoke Volume	Inhalat. Index
COHbb	0.226	0.214	0.218	0.384	0.272		
COHba	0.450	0.455	0.437	0.403	0.324		0.216
dCOHb	0.481	0.501	0.461		0.225	0.232	0.362
COHbe	0.231	0.223	0.238	0.488	0.362	0.295	
Barlow	0.220	0.215	0.209	0.505	0.314	0.215	
Nicotine				0.476	0.420	0.268	
Cotinine				0.437	0.482	0.303	
Mutagens		0.217			0.325		
Thioethers					0.201		

the nicotine indicators, the strongest correlation is observed between Barlow index and COHb percentage, whatever the time of the day.

The correlations between biomarkers and cigarette yields or smoking parameters are summarized in Table 5. Cigarette yield significantly influences the COHb level and, to a lesser extent, the Barlow index, but not the other indicators. The biomarkers, except thioethers and mutagens, are more generally affected by consumption and smoking parameters. The only significant effects of inhalation index are of course on the COHb increment with the first cigarette in the morning (r = 0.362) and on the COHb level after this cigarette (r = 0.216).

Referring to the stepwise multiple regressions (Table 6) used to assess the respective influence of cigarette yields, consumption, and smoking parameters on biomarker levels, the consumption is generally the most important factor. Cigarette yield has an effect on COHb levels and Barlow index but not on urinary nicotine or cotinine. The total volume of smoke affects a greater number of biomarkers. The urinary thioethers and mutagens are not significantly dependent on any of the factors that theoretically affect the absorbed dose.

DISCUSSION

Smoking Habits

Cigarette consumption. On average, the studied subjects smoked 12.8 cigarettes daily, which is comparable to the mean consumption (13.5 cigarettes/day) of French smokers of this age bracket.

Contrary to that usually reported when smokers switch to lower-yield cigarettes (33), we have not observed a higher consumption in subjects who have always smoked mild cigarettes compared with subjects smoking full-flavor cigarettes. This confirms the other results obtained in "steady-state smokers" (5,40) and is in good agreement with French statistical data: the mean consumption of "extra-mild," "mild," and "full-flavor" smokers is 13.8, 13.9, and 14.9 cigarettes/day, respectively (SOFRES periodical survey, 1992).

It is likely that the increase in consumption sometimes observed after switching to lower yield cigarettes is due to a transitional effect. This could depend on the difference in cigarette yields, the acceptability of the new cigarettes, and the time interval between the switch and the measurements. In support of this hypothesis, one notices that the greatest increases in consumption were observed with the shortest delays after switching (1,12,31,36). For longer delays, 6 months or more, the consumption variation is nullified (11) or even reversed (26).

Smoking parameters. The apparatus and method we used to measure smoking parameters are comparable to those employed by other authors (13,26). Despite the care taken to familiarize the subjects to smoking with the cigarette holder, this method imposes certain constraints, which tend to induce a substantial increase in puff number and volume, compared with natural smoking (34). Furthermore, the flowmeter is necessarily calibrated with the unlit cigarette and the sensitivity of the flowmeter is slightly increased when the cigarette is lit, due to the temperature (14) and/or aerosol density. All these phenomena tend to overestimate the total volume of smoke produced by the smokers under these conditions. So the measurements of smoking parameters are not absolute values, but, as the conditions are the same for all the subjects, we can assume the comparisons are valid.

We have not observed differences in smoking parameters according to the class of cigarette yields. This is confirmed by the absence of correlation between smoking parameters and cigarette tar, nicotine, or CO yields. As for the consumption, the change in smoking parameters sometimes observed after switching could depend on the delay: 10 weeks or more after switching to milder cigarette, no increase in puff number and volume could be demonstrated (15,28).

In "steady-state" smokers, a substantial increase of total volume puffed per cigarette was reported for lower nicotine yield cigarettes: 0.28–0.43 mg/cig. (6). However this result was obtained in only five subjects, and, in the same study, there is no significant difference in total smoke volume between the other five class ranking from 0.5 to 1.6 mg of nicotine per cigarette.

The mean puff volume tends to increase with the smoking experience, but this trend is not confirmed on total smoke volume because of a slight decrease in puff number. Also, we

TABLE 6

MULTIPLE STEPWISE REGRESSION ANALYSIS (R^2) BETWEEN BIOMARKERS AND THE MAIN SMOKING BEHAVIOUR PARAMETERS

Variables	Step 1	Step 2	Step 3	Step 4
COHbb	Consumption	CO yield		
(R^2)	(0.15)	(0.18)		
COHba	CO yield	Inhalation	Total smoke	Consumption
(R^2)	(0.20)	index (0.36)	volume (0.45)	(0.51)
dCOHb	CO yield	Inhalation	Total smoke	
(R^2)	(0.21)	index (0.54)	volume (0.69)	
COHbe	Consumption	Total smoke	CO yield	
(R^2)	(0.24)	volume (0.28)	(0.34)	
Barlow index	Consumption	Nicotine yield	Total smoke	
(R^2)	(0.26)	(0.30)	volume (0.33)	
Nicotine	Consumption	Total smoke		
(R^2)	(0.23)	volume (0.28)		
Cotinine	Consumption	Total smoke		
(R^2)	(0.19)	volume (0.27)		

observed differences between sexes in some smoking parameters: compared with males, females took more puffs of smaller volume, so that there is no significant difference in total smoke volume. We can therefore consider that none of the three factors controlled in our study has a clear effect on the main smoking parameter.

The inhalation index, although approximative because it is based on a number of hypotheses, is the smoking behaviour parameter that is the most sensitive to the controlled factors. It is increased by cigarette yield reduction and by the longer smoking experience, and is greater for males than for females. This estimate should be improved and considered together with cigarette consumption, cigarette yield, and smoking parameters when trying to explain biomarkers levels.

Biomarkers

Carboxyhaemoglobin. Although CO is not a specific component of the cigarette smoke, carboxyhaemoglobin level is often used to evaluate the smoke uptake by the smokers. For that reason, the indirect evaluation of COHb, which has been validated in our precise conditions (25), was prefered to simple CO concentration in expired air, which is often measured with different methods of air sampling and analysis.

Our results clearly show that COHb level increases with CO yield of cigarettes and consumption. This increase is more evident and the differences between the subjects groups are more significant for the first cigarette of the day rather than for the evening measure. This is mainly due to the fact that the CO uptake is also dependent on alveolo-capillary gradient of CO partial pressure. Everything else being equal, the CO absorption is lower when the initial COHb level is higher (13). So, apart from possible changes in smoking behaviour during the day—which cannot be dismissed a priori—the increase of COHb percentage due to the smoking of a cigarette tends to decrease as the initial COHb level increases. Consequently, even if the COHb percentage increases with the quantity of smoke inhaled, this relationship is not linear. This explains why the differences are attenuated for the evening measurements. On the other hand, if the inhalation depth is to be evaluated from the COHb increment due to one cigarette, this evaluation should be done when the COHb level is the lowest in the day: hence, the relevance of our measurements before and after the first cigarette of the day. It also follows that the differences observed between cigarette classes in CO uptake with the first cigarette are reduced because the initial COHb levels are higher in "full-flavor" cigarette smokers than in subjects smoking lower-yield cigarettes.

In switching experiments, the benefits associated with milder cigarettes are sometimes observed, as shown by decrease of COHb level (15,38,42), but not always (28), suggesting some compensation occurs. The increase of inhalation depth, observed for extra-mild cigarette smokers in this study, is one possible explanation. However, this compensation is incomplete when cigarette yield differences are large (39). In "steady-state smokers," we confirm the decrease of COHb level with milder cigarettes (40).

If we consider the COHb level after the first cigarette, this study shows, for the first time, the influence of smoking history on smoke uptake. As attested by the inhalation index, the increase of COHb with the length of smoking experience is only due to the increase in inhalation depth, as there is no change in smoking parameters. As the mean smoking history of the most experienced smokers is only 5.1 years, it would be interesting to study this trend for subjects with longer smoking experiences.

The differences between sexes reveal pecularities in the kinetics of CO absorption and elimination. Even though, in the evening, the COHb level tends to be slightly lower for males than for females, the level before the first cigarette, in the morning, is significantly higher for males. This suggests that CO elimination during the night is slower for men, probably due to their greater muscle mass, which retains a larger fraction of CO.

Urinary biomarkers. The 24-h creatinine excretion is in agreement with the usual values, according to the well-known physiological variations between men and women. This suggests a correct collection of the urinary samples.

Our results allow us to compare the actual 24-h biomarkers excretion between subjects without referring to creatinine excretion, which is unstable (7). They cannot be compared to findings published in the literature except one (22), as the other authors (19,23,24,34) analysed only fractional urinary samples and so had to express their results with reference to creatinine.

This study reveals higher values of all the urinary biomarkers in men than in women, the consumption being equal. Other authors have not reported this observation, but they selected either a male population (19) or a mixed population without discerning between men and women (35). Moreover, as mentioned above, they used fractional urinary samples. Considering indicator ratios to creatinine, these authors might have failed to show a significant difference by gender (24) if the biomarkers levels vary in the same way as creatinine levels.

The influence of cigarette class on urinary biomarkers is much less evident than for carboxyhaemoglobin percentage. Other authors (9) have also recently found weak correlations between cigarette yields (tar and CO) and bioindicators. Contrary to the urinary nicotine and cotinine, the Barlow index tends to increase with the cigarette yield, but the differences between cigarette classes are not significant. This result might be explained by the excretion of metabolites other than those we determined by gas chromatography: 3-hydroxy-cotinine is known to represent a high proportion of excreted metabolites (17). The absence of any clear influence of nicotine yield on urinary nicotine indicators could result from the deeper inhalation observed in our extra-mild cigarette smokers than in full-flavor cigarette smokers, as also suggested by other authors (28). On the other hand, it has been demonstrated that a large proportion of the nicotine absorbed while smoking can be accounted for as nicotine glucuronide conjugates and that the observed differences reflect individual responses in nicotine metabolism (8,27,29): the analytical methods used in this study do not take into account conjugated forms.

The comparison with literature data is rather difficult because the markers are measured in different biological liquids. Generally, differences in nicotine and its metabolites have only been observed in plasma or urine, when the differences in cigarette yields are large, in both switching studies (3,15,42) and for "steady-state smokers" (5).

A decrease of urinary mutagens in ultra-low tar cigarette smokers was observed once (23). However, most of the authors, like us, have not demonstrated a clear effect of tar yield on urinary mutagens or thioethers (32,35). As generally reported, neither of these nonspecific biomarkers can be considered as a good indicator of smoke absorption, because they could depend on other factors like diet, exposure, etc. (2,30).

The urinary biomarkers are more sensitive to the length of smoking habits than to the cigarette yields. The increase of the urinary nicotine metabolites with smoking history could be partly due to the increase of the inhalation depth, and partly

to a modification of metabolism because of either a slower nicotine transformation into its metabolites or an increase of the nonconjuguated form of nicotine. Like nicotine, the free mutagens fraction would be higher in the most experienced smokers, which could be confirmed by the treatment of urine samples by beta-glucuronidase and arylsulfatase.

General Considerations on Biomarkers Validity

In theory, the quantity of smoke products absorbed by smokers depends on four factors: the daily consumption, the total volume puffed per cigarette, the cigarette yield, and the inhalation depth, because most of the compounds are absorbed via the lung. Surprisingly, the multiple regression analysis shows that the COHb level after the first cigarette of the day is the only parameter influenced by all the four factors, accounting for 51% of the variance. The influence of inhalation index is not observed on evening COHb or Barlow index, for which the three other factors explain only 34% and 33% of the variance, respectively. Even more surprising, the excreted nicotine and cotinine are only affected by consumption and total volume of smoke (28% and 27% of the total variance). No factor has a significant effect on the urinary mutagens and thioethers, as discussed above. Although all the biomarkers are correlated with each other, their evaluation of smoke exposure is not equivalent.

If we refer to the fraction of variance explained by the smoking habit factors, the best marker is the COHb level after the first cigarette, although the influence of consumption is minimized at this time of the day. Conversely, evening COHb and Barlow index are well related to the consumption, but not to the inhalation depth. The absence of influence of this last factor on COHb comes from the saturation phenomenon already discussed. For Barlow index, it could be partly explained by absorption of nicotine before the smoke reaches the lung, and also by an enhancement of metabolism during the day. Finally, the amazing absence of correlation between urinary nicotine and cotinine with the nicotine yield of cigarettes could mainly result from the individual variation of the metabolism and/or excretion.

All these observations (lack of specificity, variations of metabolism, saturation phenomena) lead to bring into question the biomarkers generally used as representative of absorbed doses. As long as the linearity of the relation between biomarkers and doses is not demonstrated, the absence of an influence of cigarette yield on most biomarkers, except COHb, does not mean that absorbed doses of other smoke components are equivalent whatever the cigarette class may be.

CONCLUSION

According to the two best markers (COHb and Barlow index), it has been possible to confirm the decrease of smoker's exposure due to lower-yield cigarettes, even if the decrease is not as large as expected from the yield values. In the absence of differences in smoking behaviour (daily consumption and smoking parameters), the apparent partial compensation can be explained by deeper smoke inhalation in subjects smoking milder cigarettes, and probably by the limited representativeness of biomarkers generally used.

The influence of sex and, to a lesser extent, of length of smoking history has been demonstrated for the first time.

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