Carcinogen Exposure during Short-term Switching from Regular to ''Light'' Cigarettes

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Abstract

Objectives: **''Light'' cigarettes are extremely popular and are perceived by many smokers as less hazardous than higher-yield cigarettes. The objectives of this study were (a) to assess a battery of biomarkers of tobacco smoke exposure that includes tobacco smoke carcinogens, (b) to examine the behavioral nature of compensation, and (c) to examine the consistency of an individual's tobacco smoke exposure when smoking the same cigarette at different times.**

Methods: **The study was a 3-week crossover study in which smokers smoked their usual cigarettes during weeks 1 and 3, and a light cigarette, with a machine-determined nicotine yield of about 50% of the usual cigarette, during week 2. Blood and urine biomarkers of exposure and subjective questionnaires were collected weekly.**

Results: **Based on cotinine and carboxyhemoglobin levels, compensation averaged 78% and 83%, respectively. Urinary excretion of 4-(methylnitrosamino)-1-(3-pyridyl)-butanol, a metabolite of the tobacco specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-butanone, and a number of polycyclic aromatic hydrocarbon metabolites was similar in all conditions. Compensation was accomplished both by smoking cigarettes more intensively and by smoking more cigarettes per day. Exposures to various tobacco smoke constituents while smoking the usual brand of cigarette in weeks 1 and 3 were highly correlated.**

Conclusion: **Our findings support the idea that smokers compensate to a high degree when switched from their usual brand to a light cigarette. Short-term switching resulted in no significant reduction in carcinogen exposure. Our assessment, based on measures of biochemical exposures, supports the idea that switching to light cigarettes is unlikely to reduce the health risks of cigarette smoking.** (Cancer Epidemiol Biomarkers Prev 2005;14(6):1376–83)

Introduction

Cigarette smoking is sustained by addiction to nicotine (1). Dependent smokers tend to regulate their intake of nicotine from day to day. When switched to cigarettes of lower machine-determined yield, smokers on average increase their intake of nicotine beyond that which would be expected based on the stated (machine tested) yield (2, 3). The process of smoking lower-yield cigarettes more intensively than higher-yield cigarettes is termed compensation. When compensating for lower nicotine yields, smokers also take in larger amounts of tar and other tobacco toxins from each cigarette. For this reason, the reduction of health risk that might be expected from smoking lower-yield cigarettes has not been realized (4).

When smoking lower-yield cigarettes, smokers can compensate by smoking more cigarettes per day (CPD), or by smoking their cigarettes more intensively. The latter occurs by taking more and deeper puffs, puffing with a faster draw rate, and/or by blocking ventilation holes in the cigarette filter (3). Different smokers may compensate in different ways.

The phenomenon of compensation for lower-yield cigarettes has been most extensively investigated in two types of studies (3). The first type of study involves switching smokers from higher- to lower-yield cigarettes as an experimental intervention. A second type of study examines the relationship between machine-determined yields and biomarkers of exposure in cross-sectional studies of smokers smoking their chosen brand of cigarettes. Experimental brand switching studies generally show compensation, although compensation is often incomplete. Cross-sectional studies generally show similar exposure to nicotine (assessed as its metabolite cotinine) and carbon monoxide (CO) across a wide range of machine-determined nicotine yields, indicating nearly complete compensation.

Most studies of brand switching have measured only a few biomarkers of tobacco smoke exposure, usually nicotine and/or cotinine, CO, and sometimes thiocyanate (3). It is important to study a variety of constituents of tobacco smoke because changing the intensity of smoking may change the proportions of various tobacco smoke toxins delivered in the smoke. For example, more intensive smoking increases the tar-to-nicotine ratio, as seen both in machine testing and in human exposure studies (5, 6).

The present study was intended to evaluate a battery of biomarkers of tobacco smoke exposure in a group of smokers switched from their usual regular cigarettes to light cigarettes. We chose light cigarette brands that had machine-determined nicotine deliveries of about half that of the smoker's usual brand, representing the most popular brands of light cigarettes. Many smokers perceive these light cigarettes as less hazardous than their regular brands and smoke them in the hope of reducing the adverse health consequences of smoking (7).

Our study had three main objectives. The first objective was to assess a battery of biomarkers of tobacco smoke exposure that included tobacco alkaloids, a gaseous phase component (CO), 4-(methylnitrosamino)-1-(3-pyridyl)-butanol (NNAL) and NNAL-glucuronide (metabolites of the tobacco smoke

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carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-butanone [NNK]; ref. 8), and metabolites of polycyclic aromatic hydrocarbons (PAH), representing another class of tobacco smoke carcinogens. A second major aim was to examine the behavioral nature of compensation among individuals, specifically looking at the extent to which compensation occurred by smoking a greater number of cigarettes compared with smoking a cigarette more intensively. A third aim of the study was to examine the consistency of an individual's tobacco smoke exposure over time by studying smokers while smoking their usual brand of cigarettes before and after a 1-week period of light cigarette smoking. We also measured cardiovascular and subjective responses and cigarette acceptability in the different brand conditions.

Materials and Methods

This study was a 3-week crossover study in which smokers smoked their usual cigarette for the first week. During the second week, they smoked a light cigarette with a machine-determined nicotine yield targeted to be about 50% of the usual cigarette. For the third week, they were switched back to their usual brand of cigarettes.

Subjects. Sixteen healthy smokers, nine women and seven men, were recruited by newspaper advertisements and were paid for their participation in the study. The subjects averaged 36 years of age with a range of 19 to 56. Subjects were generally well-educated with all but one having some college education. The subjects smoked an average of 19 CPD (range, 10-30) and had smoked for an average of 22 years (range, 3-43). The yields of their usual brand of cigarettes, as determined by the U.S. Federal Trade Commission (FTC) machine test method, averaged 1.06 mg nicotine (range, 0.8-1.5), 14.4 mg tar (range, 9-23), and 14.1 mg CO (range, 10-18). The average score on the Fagerström test for nicotine dependence score was 6.0 with a range of 2 to 10 (9). The Proschaska stage of change for the subjects on entry to the study were 11 in precontemplation, three in contemplation, and two in preparation for quitting (10). On screening, the saliva cotinine averaged 237 ng/mL (range, 90-478) and expired CO averaged 22 ppm (range, 8-58).

Written, informed consent was obtained for each subject. The study was approved by the Institutional Review Boards at the University of California, San Francisco, and the Centers for Disease Control and was in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services.

Study Protocol. Subjects were asked to come to the General Clinical Research Center at San Francisco General Hospital once weekly for 4 weeks. On the first visit, subjects were told the details of the study, filled out baseline questionnaires, and received at no cost a supply of their usual brand of cigarettes for the next week. The baseline questionnaires included the Minnesota Nicotine Withdrawal Scale, the Profile of Mood Scale, and the Center for Epidemiological Studies Depression Scale Depression Questionnaire (11-13).

At the next three visits (the seventh day of weeks 1, 2, and 3), subjects returned questionnaires that they had completed at home and completed additional questionnaires on how they liked their cigarettes (14) as well as the Minnesota Nicotine Withdrawal Scale, the Profile of Mood Scale, and the Center for Epidemiological Studies Depression Scale. At the week 1 visit, subjects were given a supply of light cigarettes to smoke during week 2, and likewise at the week 2 visit, they were given a supply of their usual brand of cigarettes for week 3. Week 3 was the final visit and no cigarettes were provided.

During each of the week 1, 2, and 3 visits, subjects had a catheter placed in a forearm vein for blood sampling. At a minimum of 40 minutes after smoking their last cigarette, subjects were asked to smoke a cigarette as they usually do. Before smoking the cigarette and again 2 minutes after the end of smoking, a blood sample was taken for measurement of nicotine, cotinine, and carboxyhemoglobin (COHb) concentrations. A urine sample was collected for measurement of creatinine, nicotine and metabolites, NNAL and NNAL-glucuronide and metabolites of polycyclic aromatic hydrocarbons. NNAL and NNAL-glucuronide are metabolites of the tobacco-specific nitrosamine, NNK. Both NNK and NNAL are suspected pulmonary carcinogens (8, 15). Polycyclic aromatic hydrocarbons are an important class of tobacco smoke carcinogens. The metabolite of pyrene, 1-hydroxypyrene, has been used in other studies as a biomarker of PAH exposure (15, 16). However, smokers are exposed to many PAHs, and induction of PAH metabolism by smoking may complicate exposure assessment using 1-hydroxypyrene alone. It has been proposed that measurement of metabolites of several PAHs may provide a better measure of exposure (17). Therefore, we have measured urine concentrations of 11 metabolites derived from four major PAHs: pyrene, naphthol, fluorene, and phenanthrene.

Cigarette Selection. The light cigarette brand for each subject was selected to have a nicotine delivery that was ~50% of the usual brand. The average FTC yields of the light cigarettes were nicotine 0.5 mg (range, 0.4-0.9 mg), tar 6.1 mg (range, 4-11), CO 8.1 mg (range, 6.3-11). The average ratio of FTC nicotine yield of light to usual brands was 0.51 (range, 0.36-0.77).

Analytic Chemistry. Plasma concentrations of nicotine and cotinine were measured by gas chromatography with nitrogen phosphorus detection as previously described (18), modified for simultaneous extraction and determination of nicotine and cotinine using a capillary GC column (19).

Urinary NNAL. NNAL was obtained from Toronto Research Chemicals (Toronto, Canada). The internal standard for NNAL assays, [1,2′,3′,4′,5′,6′-13C6]-NNAL with an isotopic purity > 99%, was custom synthesized by Cambridge Isotope Laboratories (Andover, MA). NNAL was assayed in 5-mL aliquots of urine both directly and after hydrolysis with glucuronidase to measure both the free and total content in each sample. For hydrolyzed samples, 10,000 units of *Helix pomatia* was added to 5 mL of urine and the sample was incubated for 48 hours at 37°C. NNAL was recovered in both unincubated and incubated samples by a combination of liquid-liquid and solid-phase extraction.

A 10-μL aliquot (2 ng) of $^{13}C_6$-NNAL was equilibrated with the sample for 30 minutes. An aliquot (100 μL) of 10 N NaOH was then added followed by 8 mL of methylene chloride, and the mixture was vortexed (VWR Multitube vortexer) for 30 minutes. The mixture was centrifuged, the organic layer recovered, and NNAL was back-extracted by adding 3 mL of 0.1 N HCl. After vortexing, the sample was centrifuged and the HCl layer was recovered. After washing the solid-phase extraction column (Waters Oasis HLB 3 cc) with methanol and water, we added 100 μL of 10 N NaOH to the aqueous HCl extract and passed the mixture through the column. Following a brief drying interval, NNAL was eluted with 4 mL of methylene chloride and the eluant was dried in a vacuum evaporator.

Extracts were separated on a Waters Symmetry C18 column (2.1 × 150 mm, 3.5 μm) mounted in an Agilent 1100 liquid chromatograph which was interfaced to an Applied Biosystems API 3000 tandem mass spectrometer. An injection volume of 5 μL was used, and NNAL was eluated at 0.2 mL/min under isocratic conditions using 15% methanol in 10 mmol/L ammonium acetate, 0.1% acetic acid. The eluant was analyzed by using the TurboIonSpray

interface and monitoring transition ions at *m/z* 210 > 180, 210 > 149, and 210 > 93 for native NNAL and *m/z* 216 > 186 and 216 > 155 for the internal standard. Concentrations were determined from the ion ratio 210 > 180/216 > 186 with the use of a standard curve. Short-term precision (percent coefficient of variation, $n = 4$) was 6% at 50 pg/mL and 3% at 200 pg/mL. Long-term precision for a quality control pool was 7.3% at 73.8 pg/mL ($n = 26$) for free NNAL and 4.3% at 146.8 pg/ml ($n = 20$) for total NNAL. Accuracy (percent of expected value) was 71%, 100.4%, 103.5%, and 106.4% at concentrations of 10, 200, 500, and 1,000 pg/mL, respectively. The limit of detection was 4 pg/mL.

Urinary Hydroxylated PAH Metabolites. Concentrations of the following metabolites were determined, standards were obtained commercially: 1-naphthol, 2-naphthol, and 1-hydroxypyrene were obtained from Acros Organics/Fisher Chemical Co. (Pittsburgh, PA). 1-Hydroxyfluorene was obtained from Chem Bridge Corp. (San Diego, CA), 2-hydroxyfluorene and 3-hydroxyfluorene were from Aldrich Chemical Co. (Milwaukee, WI). 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, and 9-hydroxyphenanthrene were purchased from Crescent Chemical Co. (Islandia, NY). Three internal standards were used: 2-naphthol-d_7 (C/D/N Isotopes, Inc., Pointe-Claire, Quebec, Canada) for 1-naphthol and 2-naphthol; 9-hydroxyphenanthrene-d_9 (synthesized from phenanthrene-d_{10} in our laboratory), for the isomeric hydroxyphenanthrenes and for the isomeric hydroxyfluornenes; and 1-hydroxypyrene-$^{13}C_6$ from the National Cancer Institute Chemical Carcinogen Reference Standard Repository/Midwest Research Institute (Kansas City, MO). Pentafluorobenzyl bromide was obtained from Aldrich Chemical.

Urine specimens (2.7 mL) were buffered to pH 7 with 0.3 mL of 1 mol/L phosphate buffer and incubated at 37°C with β-glucuronidase (3,000 units, type 1XA from *Escherichia coli*, Sigma, St. Louis, MO) and sulfatase (0.6 units, type VI from *Aerobacter aerogenes*, Sigma) overnight. The samples were extracted with a 99:1 mixture of toluene/1-butanol (4 mL) by vortex-mixing, the organic layers were separated (freeze/pour) and evaporated using a centrifugal vacuum evaporator. The hydroxylated PAH were converted to the pentafluorobenzyl ethers by treatment with pentafluorobenzyl bromide (50 μL of 5% in acetonitrile) and potassium hydroxide (50 μL of 0.8% in ethanol) in a 13 × 100 mm borosilicate glass culture tube. The tube was capped and heated at 60°C for 30 minutes, after which it was cooled to room temperature and vortexed with 1 mL of 1% ammonia. After adding 0.5 mL of 8 N sulfuric acid, the derivatives were extracted with pentane (3 mL, freeze/pour) and evaporated to dryness. The residues were dissolved in 120 μL methanol and 20 μL were injected into the liquid chromatography tandem mass spectrometry system, which consisted of a Surveyor high-performance liquid chromatography interfaced to a TSQ Quantum Ultra triple-stage quadrupole mass spectrometer (Thermo-Finnigan, San Jose, CA). The analytes were separated on a PAH Green column (4.6 × 150 mm, Thermo-Hypersil-Keystone (Bellefonte, PA), using a gradient of 20% water in methanol to 100% methanol over 5.5 minutes followed by 100% methanol for 3.5 minutes. The mass spectrometer was operated using atmospheric pressure chemical ionization (negative ion), which generates $(M-H)^-$ ions via electron capture and loss of the pentafluorobenzyl radical (''ECAPCI''; ref. 20). Selected reaction monitoring was used to acquire data. The following transitions were monitored and used for quantitation: *m/z* 143 to *m/z* 115 for 1-naphthol and 2-naphthol; *m/z* 181 to *m/z* 153 for 1-hydroxyfluorene, 2-hydroxyfluorene, and 3-hydroxyfluorene; *m/z* 193 to *m/z* 165 for 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, and 9-hydroxyphenanthrene; *m/z* 217 to *m/z* 189 for 1-hydroxypyrene; *m/z* 150 to *m/z* 122 for 2-naphthol-d_7 (internal standard); *m/z* 202 to *m/z* 174 for 9-hydroxyphenanthrene-d_9 (internal standard); and *m/z* 223 to *m/z* 195 for 1-hydroxypyrene-$^{13}C_6$ (internal standard). Calibration curves were generated using the peak area ratio of analyte/internal standard and linear regression (1/X weighting). The following analyte pairs were not resolved and were reported as sums: 1-naphthol and 2-naphthol; 1-hydroxyphenanthrene and 9-hydroxyphenanthrene; 3-hydroxyphenanthrene and 4-hydroxyphenanthrene. Precision and accuracy were evaluated by analysis of nonsmokers' urine added to the analytes at four different concentrations. For 2-naphthol and the isomeric fluorenols, these ranged from 0.5 to 10 ng/mL; the limits of quantitation for these analytes were 0.5 ng/mL. The mean values for precision (percent coefficient of variation) and accuracy (percent of expected value), respectively, were as follows: 2-naphthol, 4.0% and 100.4%; 1-hydroxyfluorene, 6.0% and 117.5%; 2-hydroxyfluorene, 10.1% and 104.9%; 3-hydroxyfluorene, 5.4% and 106.6%. For 1-hydroxypyrene and the isomeric hydroxyphenanthrenes, concentrations ranged from 0.05 to 1 ng/mL; the limits of quantitation for these analytes were 0.05 ng/mL. Mean precision and accuracy was, respectively, 1-hydroxyphenanthrene, 9.1% and 98.6%; 2-hydroxyphenanthrene, 20% and 98.8%; sum of 3-hydroxyphenanthrene and 4-hydroxyphenanthrene, 7.4% and 103.3%; 1-hydroxypyrene, 2.9% and 89.6%.

Data Analysis. The main comparisons were between the usual brand (week 1) versus light cigarettes (week 2) and between the first exposure to the usual brand (week 1) and the second exposure to the usual brand (week 3). Repeated measures ANOVA was done for the subjects across the three conditions. Tukey post hoc tests were done to compare week 1 versus week 2 (to examine the response to brand switching) and week 1 versus week 3 (to examine the consistency of smoking behavior and exposures at different times while smoking the same cigarette). All statistical tests were two sided.

The dependent variables were the questionnaire data, cigarette consumption (CPD), and biochemical exposure measurements. For analysis of blood concentrations before and after cigarette smoking, the difference between the two concentrations was used in the ANOVA.

The PAH excretion data are presented for the four major PAHs, summing their metabolites as appropriate. Thus, naphthol includes the sum of 1-naphthol and 2-naphthol; hydroxyphenanthrene includes the sum of 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, and 9-hydroxyphenanthrene; and hydroxyfluorene includes the sum of 1- hydroxyfluorene, 2-hydroxyfluorene, and 3- hydroxyfluorenes.

Compensation was defined as the degree to which proportional changes in a subject's intake of a smoke constituent make up for the same proportional change in the machine-determined yield of that constituent. Compensation can be expressed mathematically as:

$$C = 1 - \left[\frac{\log(\text{marker}_2) - \log(\text{marker}_1)}{\log(\text{yield}_2) - \log(\text{yield}_1)}\right],$$

where C = the extent of compensation and marker_1 and yield_1 represent the levels of the exposure (i.e., plasma cotinine or blood COHb) and the smoking machine-determined yield (i.e., FTC nicotine or CO) before the brand change, respectively; and marker_2 and yield_2 represent exposure and machine yield after the brand change (21).

Individual patterns of compensation were examined graphically by plotting the change in daily cigarette

consumption versus the change in intensity of smoking each cigarette, comparing the usual brand (week 1) and the light cigarette condition (week 2). The change in intensity of smoking each cigarette was determined by comparing the actual nicotine exposure when smoking the light-yield cigarettes to that which was predicted based on the actual exposure while smoking the usual brand and the machine-determined nicotine yields of the light compared with the usual cigarette. If the intensity of smoking a cigarette was the same while smoking the usual brand and the light cigarette (as is the case when the cigarettes are smoked by the smoking machine), then the nicotine intake per cigarette would change in direct proportion to the change in the nicotine yield. To the extent that nicotine intake declines less than predicted, the light cigarette has been smoked more intensively compared with the higher-yield cigarette. The intensity of smoking was computed mathematically using the cotinine levels as a marker of nicotine intake, and the machine-determined yields as follows. The predicted intake per cigarette,

$$\text{Predicted } \text{COT}_2/\text{CPD}_2 = \frac{\text{COT}_1}{\text{CPD}_1} \times \frac{\text{FTC} - \text{NIC}_2}{\text{FTC} - \text{NIC}_1}$$

where COT and CPD are the plasma cotinine and cigarettes smoked per day in weeks 1 and 2, and FTC-NIC represents the machine-determined yields of the cigarettes smoked in week 1 (usual brand) and week 2 (lights). If the actual $\text{COT}_2/\text{CPD}_2$ were the same as predicted, this would indicate no change in intensity of smoking. To examine the change in intensity, we computed the ratio of:

$$\text{Actual}\frac{\text{COT}_2}{\text{CPD}_2}/\text{Predicted}\frac{\text{COT}_2}{\text{CPD}_2}.$$

This ratio-1 represents the fraction of nicotine intake per cigarette that differs from the predicted nicotine intake per cigarette, which can be used as a measure of change of intensity of smoking each cigarette in response to the brand switch.

Results

Cigarette Consumption and Nicotine and CO Exposure. When switched to light cigarettes, smokers smoked more CPD (average 27.5 versus 22.5, $P < 0.02$; Table 1). The presmoking plasma nicotine and cotinine concentrations and the boost in plasma nicotine concentration after smoking a cigarette were slightly lower in the light cigarette condition, but their differences were not significant compared with the usual brand condition. The baseline and boost in blood COHb levels were similar in the light and usual brand conditions.

There were no significant differences in any measurement comparing the usual brand at week 1 versus week 3. The correlations and percent absolute differences for various measurements within individuals comparing week 1 versus week 3 are shown in Table 2. Correlations of the PAH metabolites between week 1 and week 3 that are not provided in Table 2 were not significant.

Exposure to Tobacco Smoke Carcinogens. No significant differences were observed in the urinary excretion of free (unconjugated) NNAL, or total (free plus conjugated) NNAL, comparing either usual brand versus light conditions or the two usual brand conditions (Table 3). Likewise, no difference was observed in urinary excretion of the metabolites of the four PAHs as shown in Table 3.

Cardiovascular and Subjective Response. No significant treatment effects were observed in blood pressure or heart rate (Table 4). No significant treatment effects were observed in the Minnesota Nicotine Withdrawal Score; the Profile of Mood Scale total mood disturbance score and tension, depression, anger, vigor, or fatigue scales; or in the Center for Epidemiological Studies Depression Scale depression score.

Table 1. Cigarette consumption and nicotine, carbon monoxide exposure while smoking usual brand and light cigarettes

	Week 1 (usual brand)	Week 2 (light)	Week 3 (Usual brand)	Overall (P)	Post hoc P	
					Week 1 versus week 2	Week 1 versus week 3
CPD						
Mean	22.5	27.5	22.9	0.01	0.02	0.97
SD	12.5	17.8	15.1			
95% CI	(15.8-29.1)	(18.0-37.0)	(14.8-30.9)			
Plasma* nicotine (ng/mL)						
Mean	15.0	13.4	15.7	0.35	—	—
SD	7.1	5.6	7.6			
95% CI	(11.3-18.8)	(10.4-16.4)	(11.5-19.9)			
Plasma* cotinine (ng/mL)						
Mean	221	201	247	0.03	0.4	0.3
SD	90	107	117			
95% CI	(174-269)	(144-258)	(183-312)			
Blood COHb* (%)						
Mean	4.5	4.3	4.8	0.12	—	—
SD	1.7	2.0	1.4			
95% CI	(3.6-5.4)	(3.2-5.4)	(4.1-5.6)			
Nicotine boost† (ng/mL)						
Mean	13.8	10.5	14.5	0.11	—	—
SD	7.2	7.6	11.4			
95% CI	(10.0-17.7)	(6.4-14.6)	(8.2-20.9)			
COHb boost† (%)						
Mean	0.69	0.63	0.78	0.08	—	—
SD	0.25	0.2	0.3			
95% CI	(0.55-0.82)	(0.53-0.73)	(0.61-0.96)			

Abbreviation: 95% CI, 95% confidence interval.
*Measurements before smoking the test cigarette.
†Differences in concentrations before and after smoking the test cigarette.

Table 2. Correlations and percent difference in cigarette consumption and biomarkers while smoking usual brand of cigarettes on two occasions

	Correlation coefficient (r)	P	% Difference (95% confidence interval)
Cigarettes per day	0.91	<0.001	0.8 (−8.8 to 10.4)
Baseline plasma nicotine (ng/mL)	0.55	0.034	34.5 (−46 to 115)
Baseline plasma cotinine (ng/mL)	0.81	<0.001	10.9 (−6.0 to 27.9)
Baseline COHb (%)	0.91	<0.001	14.7 (−0.1 to 29.5)
Plasma nicotine boost (ng/mL)	0.46	0.08	8.4 (−25.2 to 42.0)
Free NNAL (pmol/mg creat)	0.83	<0.001	−15.5 (−34.4 to 3.4)
Total NNAL (pmol/mg creat)	0.92	<0.001	−12.7 (−30.7 to 5.2)
1-Hydroxypyrene (pmol/mg creat)	0.78	0.001	12.3 (−23.4 to 48.0)
Sum 1- and 2-naphthols (pmol/mg creat)	0.40	0.15	16.9 (−22.4 to 56.1)
Sum of isomeric hydroxyphenanthrenes (pmol/mg creat)	0.09	0.74	24.9 (−30.8 to 80.5)
Sum of isomeric hydroxyfluorenes (pmol/mg creat)	0.21	0.45	17.4 (−35.6 to 70.5)

Cigarette Acceptability and Craving. Significant differences between light and usual brand cigarette smoking were observed for ratings of strength/mildness, smoothness/harshness, quality of flavor, overall cigarette quality, and satisfaction (Table 5). There were overall significant effects on ANOVA for hotness and amount of flavor, but post hoc comparisons were not significant. Ratings were nearly identical comparing the two usual brand-smoking conditions.

Compensation. Using baseline (presmoking value at week 1 visit) blood cotinine concentration (as the biomarker of exposure) and the machine-determined nicotine yield, smoker compensation averaged 78%. Using baseline blood COHb as the biomarker of exposure and machine-determined CO yield, smoker compensation averaged 83%.

The behavioral mechanism of smoking compensation was examined by looking at the relative changes in overall exposure, cigarette consumption, and intake per cigarette (estimated by daily exposure/CPD; Table 6). With a 50% average reduction in machine-determined nicotine yield, one would expect a 50% reduction in cotinine concentration per cigarette if there was no compensation. Using the baseline cotinine, there was on average a 19% decrease in cotinine concentration per cigarette comparing usual brand and light cigarette conditions, indicating that a substantial degree of compensation occurs by smoking cigarettes more intensively than predicted from smoking machine deliveries. The subjects smoked on average 21% more CPD, which is another mechanism of compensation. Figure 1 shows how individual subjects changed their smoking behavior comparing the usual and light cigarette smoking condition. This figure shows that some subjects compensate exclusively by smoking more CPD (Fig. 1, *top left quadrant*); some compensate exclusively by smoking cigarettes more intensively (*bottom right quadrant*), whereas most employ both mechanisms (*top right quadrant*).

Discussion

Our study provides novel findings in relation to our three objectives. First, we present a battery of biomarkers that can be used to assess exposure to various types of potentially toxic smoke constituents when smoking different cigarettes. The biomarkers include markers of nicotine exposure (nicotine boost and plasma levels of nicotine and its metabolite cotinine), gas-phase exposure (CO), and tar-phase carcinogen exposure (NNAL and PAH metabolites). Some of these biomarkers have been used to assess responses to potential harm reduction interventions (22, 23), but to our knowledge, NNAL and PAH metabolite excretion has not been reported in smokers switching to lower-yield commercial brands of cigarettes. It is necessary to measure a variety of chemical exposures because one cannot extrapolate from smoking machine tests to evaluate human exposure to different chemicals. Smoking cigarettes more or less intensively compared with the standard FTC smoking machine variables affects the relative generation of different chemical constituents of tobacco smoke (5, 6). Furthermore, different levels of inhalation might affect the systemic absorption of different smoke chemicals. Therefore, it is necessary to measure the smoker's actual exposure when studying the effects of smoking different cigarettes.

Our second main finding addresses the mechanism of compensation in smokers who are switched from commercial regular to light cigarettes. Compensation can be accomplished by smoking more CPD and/or by smoking each cigarette more intensively. A number of studies have examined the extent of compensation after experimental brand switching (2, 3). Our study is novel in that we measured multiple chemical exposures, as described above, and have used biomarkers to assess the relative contribution of smoking a greater number of low-yield CPD versus smoking each cigarette more intensively for individual smokers. We have found, as expected, a high degree of compensation for nicotine when switching to lower-yield cigarettes, with insignificant changes in exposure to CO, NNAL, or PAH metabolites. We confirmed previous observations that compensation occurs primarily by smoking each

Table 3. Exposure to tobacco-specific nitrosamines and PAHs while smoking usual brand and light cigarettes

	Week 1 (usual brand)	Week 2 (light)	Week 3 (usual brand)	Overall, P
Total NNAL (pmol/mg creatinine)				
Mean	2.3	2.0	2.1	0.54
SD	1.6	1.6	1.9	
95% CI	(1.4-3.2)	(1.0-3.0)	(0.8-3.3)	
Free NNAL (pmol/mg creatinine)				
Mean	1.1	1.1	1.0	0.81
SD	0.8	0.9	0.9	
95% CI	(0.7-1.6)	(0.6-1.6)	(0.4-1.6)	
1-Hydroxypyrene (pmol/mg creatinine)				
Mean	1.7	2.1	1.9	0.72
SD	0.9	2.8	1.5	
95% CI	(1.2-2.1)	(0.6-3.6)	(1.1-2.7)	
1-Naphthol and 2-naphthol (pmol/mg creatinine)				
Mean	181.5	166.4	178.6	0.56
SD	88.2	70.8	86.9	
95% CI	(134.5-228.6)	(128.6-204.1)	(128.4-228.8)	
Sum of hydroxyfluorenes (pmol/mg creatinine)				
Mean	32.3	30.1	35.4	0.98
SD	11.8	20.7	25.2	
95% CI	(26.0-38.6)	(18.6-41.5)	(21.4-49.4)	
Sum of hydroxyphenanthrenes (pmol/mg creatinine)				
Mean	6.2	8.9	6.5	0.42
SD	2.7	11.9	5.4	
95% CI	(4.7-7.6)	(2.3-15.5)	(3.6-9.5)	

Abbreviation: 95% CI, 95% confidence interval.

Table 4. Cardiovascular and subjective responses while smoking usual brand and light cigarettes

	Week 1 (usual brand)	Week 2 (light)	Week 3 (usual brand)	Overall, *P*
Systolic blood pressure*				
Mean	132	129	128	0.11
SD	14	10	11	
95% CI	(125-140)	(124-134)	(122-134)	
Diastolic blood pressure* (mm Hg)				
Mean	77	79	75	0.34
SD	11	11	12	
95% CI	(71-83)	(74-85)	(68-82)	
Heart rate* (min^{-1})				
Mean	84	84	83	0.93
SD	12	14	11	
95% CI	(78-91)	(76-91)	(77-90)	
Total Minnesota Nicotine withdrawal score				
Mean	173	205	155	0.16
SD	133	152	133	
95% CI	(100-247)	(121-289)	(82-229)	
POMS				
Total mood disturbance score				
Mean	26.4	30.2	24.1	0.86
SD	32.7	29.0	24.7	
95% CI	(7.7-45.2)	(13.5-47.0)	(9.9-38.4)	
Tension score				
Mean	7.8	9.6	7.7	0.56
SD	6.7	6.1	5.3	
95% CI	(4.0-11.7)	(6.1-13.0)	(4.6-10.7)	
Depression score				
Mean	9.5	8.5	7.4	0.50
SD	12.8	9.7	10.9	
95% CI	(2.2-16.9)	(2.9-14-1)	(1.1-13.7)	
Anger score				
Mean	4.95	6.4	4.1	0.36
SD	4.9	5.6	3.0	
95% CI	(2.1-7.8)	(3.1-9.6)	(2.4-5.9)	
Vigor score				
Mean	9.5	9.0	8.0	0.63
SD	5.0	5.5	4.7	
95% CI	(6.6-12.4)	(5.8-12.1)	(5.2-10.7)	
Fatigue score				
Mean	7.6	8.3	6.9	0.84
SD	4.9	6.4	4.8	
95% CI	(4.7-10.4)	(4.6-12.0)	(4.2-9.7)	
CES-D				
Mean	14.3	14.0	14.6	0.86
SD	11.7	12.3	12.8	
95% CI	(8.1-20.6)	(7.5-20.5)	(7.8-21.4)	

Abbreviations: 95% CI, 95% confidence interval; POMS, Profile of Mood Scale; CES-D, Center for Epidemiological Studies Depression Scale.
*Before test cigarette.

cigarette more intensively, but we also found that there is considerable individual variability in the mechanism of compensation by individual smokers; that is, how much occurs by smoking more intensively versus by consuming more CPD.

The third finding of our study is the consistency of smoking behavior and a variety of chemical exposures in individuals smoking the same brand of cigarettes measured on two occasions, 2 weeks apart. Despite having an interval of smoking a different type of cigarette for 1 week in between, the level of exposure to various smoke chemicals while smoking their usual brand was highly correlated within individuals on the two occasions.

The cigarettes that were smoked by our subjects were popular commercial cigarettes in the full flavor (about 1 mg nicotine) and light (about 0.5 mg nicotine) categories. For each subject, we tried to find a low-yield cigarette that was ~50% of the usual machine-determined nicotine delivery. Using blood cotinine concentrations as a marker of nicotine exposure, we calculated an average compensation of 78%, which is consistent with findings of other experimental brand switching studies (3). However, cross-sectional studies of smokers smoking self-selected brands indicate that cotinine levels are quite similar for individuals smoking 1- versus 0.5-mg nicotine cigarettes, showing virtually complete compensation (3). Thus, experimental switching studies such as ours do not perfectly reflect the real world situation of smokers smoking cigarettes that they choose and find satisfying.

Whereas the average extent of compensation for nicotine in our study was 78%, exposure to NNAL and various PAHs were on average similar while smoking usual brand and light cigarettes. That incomplete compensation for nicotine does not result in lower exposure to other tobacco smoke toxicants can be explained by differences in how different cigarettes are smoked, as discussed recently by Harris (24). The ratio of various toxicants to nicotine in tobacco smoke increases when cigarettes are smoked more intensely compared with the standard FTC method. This has been shown both in smoking machine studies and in human exposure studies, the latter using urine mutagenicity as an index of tar exposure (5, 6). The 1999 Massachusetts Benchmark Study tested cigarettes using a smoking paradigm that is more intensive than that used in the FTC method but closer to how cigarettes are actually smoked (24). Using this method, the ratio of a number of smoke constituents to nicotine in smoke is substantially greater for

Table 5. Cigarette acceptability and craving while smoking usual brand and light cigarettes

	Week 1 (usual brand)	Week 2 (light)	>Week 3 (usual brand)	Overall, *P*	Post hoc *P*	
					Week 1 versus week 2	Week 1 versus week 3
Strength/mildness*	4.0	5.4	3.9	<0.001	<0.001	0.9
Smoothness/harshness	4.0	3.2	4.3	0.002	0.02	0.5
Hotness	4.1	4.7	3.8	0.014	0.1	0.6
Amount of flavor	2.9	2.6	3.4	0.023	0.5	0.2
Quality of flavor	1.7	3.6	1.9	<0.001	<0.001	0.8
Overall cigarette quality	4.1	2.9	4.3	<0.001	<0.001	0.8
Satisfaction	2.3	4.3	2.0	<0.001	<0.001	0.8
Strongest craving last 24 h	6.2	7.3	6.6	0.08	—	—

NOTE: Smoothness or harshness was rated 1 to 7, where 1 = much too smooth and 7 = much too harsh for me.
Hotness was rated 1 to 7, where 1 = much too hot and 7 = much too cool for me.
Amount of flavor was rated 1 to 5, where 1 = no flavor at all and 5 = very noticeable flavor.
Quality of flavor was rated 1 to 5, where 1 = absolutely right for me and 5 = not what I would choose.
Overall cigarette quality was rated 1 to 5, where 1 = a very poor cigarette and 5 = a very good cigarette.
Satisfaction was rated 1 to 6, where 1 = extremely satisfying and 6 = not satisfying at all.
Strongest craving last 24 hours was rated 1 to 10, with 10 representing the highest level of craving.
The Cigarette Acceptability Questionnaire was adapted from Rose and Behn (14).
*Strength or mildness was rated 1 to 7, where 1 = much too strong and 7 = much too mild for me.

Table 6. Per cigarette nicotine and CO exposure while smoking usual brand and light cigarettes

	Week 1 (usual brand)	Week 2 (light)	Week 3 (usual brand)	Overall, *P*	Post hoc *P*	
					Week 1 versus week 2	Week 1 versus week 3
CPD	22.5	27.5	22.9	0.01	0.02	0.97
Plasma nicotine/CPD (ng/mL)						
Mean	0.84	0.68	0.82	0.11	—	—
SD	0.49	0.43	0.46			
95% CI	(0.58-1.10)	(0.45 0.90)	(0.57 1.08)			
Plasma cotinine/CPD (ng/mL)						
Mean	11.9	9.6	13.0	0.03	0.19	0.53
SD	6.2	5.4	6.6			
95% CI	(8.6 15.2)	(6.7 12.5)	(9.4 16.7)			
COHb/CPD						
Mean	0.24	0.18	0.27	0.002	0.03	0.46
SD	0.12	0.09	0.13			
95% CI	(0.18 0.31)	(0.14 0.23)	(0.20 0.34)			

Abbreviation: 95% CI, 95% confidence interval.

low-tar compared with high-tar cigarettes. Using data from the Massachusetts Benchmark Study, Harris estimated that a person has to compensate only 73% for nicotine to obtain the same dosage of NNK from a light Marlboro cigarette compared with a regular Marlboro cigarette. These observations highlight the importance of measuring actual exposure to various tobacco smoke toxins in people when comparing potential risks of different types of cigarettes.

We determined the mechanism of compensation for switching to low-yield cigarettes by examining the observed versus machine-predicted cotinine levels per cigarette smoked, total cigarette consumption, and total nicotine exposure. Using this type of analysis, we found an average increase in cigarette consumption of 21% and an average increase in nicotine intake per cigarette (compared with FTC yield predictions) of 58%. Thus, we estimate that on average 74% of compensation occurs by smoking cigarettes more intensively and 26% occurs by smoking more CPD. The idea that smoking cigarettes more intensely accounts for a greater proportion of compensation than smoking more CPD has been previously noted (3, 25). Individual differences in how smokers compensate were observed, as shown in Fig. 1. Behavioral differences in how smokers compensate are important to recognize when trying to assess the biological effects of smoking new types of cigarettes and in doing epidemiologic studies of health consequences of smoking different cigarettes.

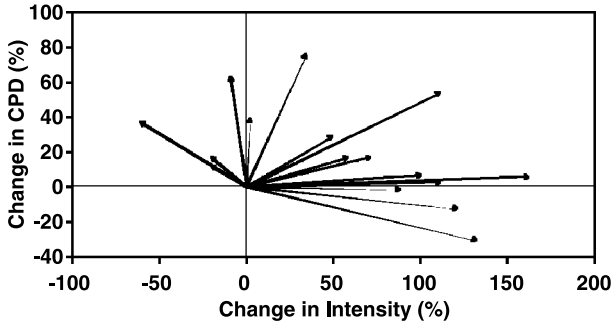

Figure 1. Individual mechanisms of compensation when smoking light compared to usual brand of cigarettes. Behavioral response of an individual smoker (*arrow*). The arrow depicts the vector showing both percent change in cigarettes smoked per day and the percent change in intensity of smoking. The (0, 0) value represents the usual brand condition; the arrowhead represents the change when switching to light cigarettes. The percent change in intensity of smoking is based on the change in the cotinine/cigarette ratio when smoking light cigarettes compared with the predicted ratio based on cotinine intake from the usual brand and the relative change in machine nicotine yields. This calculation is described in detail in Materials and Methods. Subjects with vectors that point to the top left quadrant have compensated by smoking more cigarettes per day exclusively. Subjects with arrows in the bottom right quadrant have compensated by smoking cigarettes more intensively only. Subjects in the top right quadrant have compensated by the combination of mechanisms.

We examined cigarette acceptability and satisfaction. We found significant differences in ratings of strength/mildness, smoothness/harshness, quality of flavor, overall cigarette quality, and satisfaction. Not surprisingly, subjects found their usual brand more satisfying than the low-yield brand. Possibly, had the smokers been smoking low-yield cigarettes for a longer period of time than 1 week, their taste would have changed and the low-yield cigarettes would have been more satisfying (perhaps associated with more complete compensation for nicotine).

A comment on the interpretation of levels of NNAL in the urine is warranted. NNAL is reported to have a long elimination half-life (40 days), presumably related to extensive distribution in and slow release from body tissues (8). Because of the long half-life, it would take several months to reach a new steady-state level in the body once the daily level of exposure has changed. At 1 week, steady state will not be achieved. However, when exposure to tobacco-specific nitrosamines is changed abruptly, such as when a smoker quits smoking, there is a rapid initial decline in NNAL excretion, dropping to about 35% of baseline in 1 week (26). Because of the rapid initial decline, it is expected that if NNAL exposure was substantially reduced when switching to light cigarettes, evidence of this reduction would be seen by 1 week. We did not see a significant difference in excretion of NNAL at 1 week after switching from regular to light cigarettes, indicating that there was no significant reduction in exposure. The elimination rate of PAHs is more rapid than that of NNAL. Elimination half-life based on urine excretion rates of 1-hydroxypyrene average 10 to 15 hours (16, 27). Thus, 1 week is more than sufficient time in which a new steady state of excretion in relation to exposure will be reached.

We measured biological responses to nicotine (heart rate and blood pressure), as well as nicotine withdrawal symptoms and mood and depression scores, when smokers were switched from regular to low-yield cigarettes. No differences

were observed in heart rate and blood pressure. We found no change in withdrawal score, mood, or depression in our study. The lack of change in nicotine-related biological responses is not surprising in view of the similar levels of nicotine in the different treatment conditions.

Our data on biochemical exposures in smokers smoking the same cigarettes measured at two different times indicate a high degree of consistency of smoking behavior. A high level of within-subject correlation was observed for cigarette consumption, plasma cotinine, COHb, and NNAL, and PAHs. This confirms prior observations that smokers smoke in a consistent way and take in similar amounts of nicotine tobacco-specific nitrosamines over time (28-32). Murphy et al. recently reported within-subject correlations for a number of urine biomarkers of tobacco smoke exposure, measured on four occasions over 8 weeks, while subjects were smoking the same brand of cigarettes (32). These investigators found high within-subject correlations for urine cotinine, NNAL, and 1-hydroxypyrene, similar to the findings of the present study. In addition, we report high within-subject correlations for other PAH metabolites, as well as for plasma cotinine and blood COHb. These data on within-individual variability of several biomarkers can be useful in the design of future studies of within-subject changes in smoke exposure. Of note, however, was that plasma nicotine concentration and nicotine boost from smoking individual cigarettes in our study and urine nicotine excretion in the Murphy et al. study (32) did not show as strong within-subject correlations as did other measures. This presumably reflects the short half-life of nicotine and differences in how smokers smoke individual cigarettes in response to the desire for nicotine at the particular time the cigarette is smoked compared with how consistently they regulate nicotine intake on a daily basis.

In summary, our study confirms prior research indicating a high degree of compensation for nicotine when smokers switch for brief periods of time from regular to light cigarettes. There was no significant reduction in exposure to CO or the tobacco smoke carcinogens NNAL or PAH metabolites. Likewise, there were no differences in cardiovascular measurements, withdrawal symptoms, or mood while smoking light compared with regular cigarettes. Subjects compensated by a combination of smoking cigarettes more intensively and smoking more cigarettes, with considerable individual variation in how compensation was accomplished. Exposure to various biochemical markers of tobacco smoke was fairly consistent when a smoker smoked the same brand of cigarettes measured at different points in time. Our findings are consistent with the idea that switching to popular light cigarettes will be associated with no different health risks than smoking regular cigarettes (4).

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Carcinogen Exposure during Short-term Switching from Regular to "Light" Cigarettes

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